**Higher early pregnancy plasma *myo*-inositol associates with increased post-prandial glycemia later in pregnancy: secondary analyses of the NiPPeR Randomized Controlled Trial**

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**Abstract**

**Aim:** *Myo*-inositol supplementation from ~13-weeks’ gestation reportedly improves glycemia regulation in metabolically at-risk women, with speculation that earlier supplementation might bring further improvement. However, the NiPPeR trial of a *myo*-inositol-containing supplement starting preconception did not lower gestational glycemia in generally healthy women. We postulated that the earlier timing of supplementation influences the maternal metabolic adaptation for gestational glycemia regulation.

**Methods:** In 585 NiPPeR study women recruited from Singapore, UK and New Zealand, we examined associations of plasma *myo*-inositol concentrations at 7-weeks’ and 28-weeks’ gestation with 28-weeks plasma glucose (PG; fasting, 1h, 2h in 75g oral glucose tolerance test) and insulin indices using linear regression adjusting for covariates.

**Results:** Higher 7-week-*myo*-inositol, but not 28-week-*myo*-inositol, associated with higher 1h-PG (βadj [95%CI] 0.05 [0.01, 0.09] loge mmol/L per loge µmol/L, p=0.022) and 2h-PG (0.08 [0.03, 0.12], p=0.001); equivalent to 0.39mmol/L increase in 2h-PG for an average 7-week-*myo*-inositol increase of 23.4umol/L with *myo*-inositol supplementation. Higher 7-week-*myo*-inositol associated with a lower 28-week Stumvoll index (1st phase), an approximation of insulin secretion (−0.08 [−0.15, −0.01], p=0.020) but not with 28-week Matsuda insulin sensitivity index. However, the clinical significance of a 7-week-*myo*-inositol-related increase in glycemia was limited since there was no association with gestational diabetes risk, birthweight and cord C-peptide levels. *In-silico* modelling found higher 28-week-*myo*-inositol was associated with lower gestational glycemia in White, but not Asian, women after controlling for 7-week-*myo*-inositol effects.

**Conclusion:** Our study provides the first evidence that increasing first trimester plasma *myo*-inositol may slightly exacerbate later pregnancy post-challenge glycemia, indicating that the optimal timing for starting prenatal *myo*-inositol supplementation needs further investigation.

**Keywords**: glucose, gestational diabetes, inositol, insulin, pregnancy, supplementation

**Introduction**

Higher gestational glycemia is linked to higher clinical risk across a continuum for both the woman and her offspring, including hypertensive disorders of pregnancy, cesarean section delivery, fetal macrosomia, shoulder dystocia, and neonatal hypoglycemia.1 *In-utero* exposure to increasing maternal glycemia also associates with long-term adverse cardiometabolic health in offspring.2 Thus, many different strategies have been trialed attempting to optimize gestational glycemia.3-5

One approach is through prenatal supplementation with *myo*-inositol, an endogenously synthesized polyol, also enriched in dietary grains, fruit and vegetables.6 It is a precursor of phosphoinositides and other key secondary messengers for hormone signal transduction including that of insulin, and is derivatized with different compounds to regulate many cellular functions, and form signaling agents, including insulin-mimetics.7

Several trials of antenatal *myo*-inositol supplementation in White Caucasian Italian women with risk factors for gestational diabetes (e.g. family history of diabetes,8,9 high BMI,10,11 polycystic ovary syndrome12) have reported reduced gestational glycemia. A meta-analysis of 6 Italian studies (n=995 women) which started *myo*-inositol supplementation mostly from the end of the first trimester reported glycemia reductions at all three timepoints of a classic 2-hour 75g oral glucose tolerance test (OGTT) conducted at 24-28 weeks’ gestation.13 Additionally, these trials reported accompanying reductions in gestational diabetes incidence and insulin resistance (HOMA-IR). With these trials showing that *myo*-inositol supplementation at a daily dose of 4g is safe and tolerable in pregnancy, some have advocated starting *myo*-inositol supplementation earlier in gestation or even preconception to further improve gestational glycemia regulation.

However, these trials lack generalizability to other populations. Indeed, some trials conducted elsewhere reported different results. An Irish trial in predominantly White women with a family history of diabetes reported that a combined *myo*-inositol and *D-chiro*-inositol supplement made no difference to gestational glycemia.14 Another trial, the NiPPeR (Nutritional Intervention Preconception and During Pregnancy to Maintain Healthy Glucose Metabolism and Offspring Health) study reported no reduction in the primary outcome of gestational glycemia overall, with a marginal increase in 2h glycemia among women with overweight/obesity.15 NiPPeR differed from the other trials in several ways: it was double-blinded, supplementation commenced preconception, recruited many women with no metabolic risk factors, included different ethnicities from three continents with a significant proportion of Asians, and administered *myo*-inositol in a preparation enriched with other micronutrients and probiotics.

Here we have utilized data from the NiPPeR study to explore possible reasons for the inconsistent findings observed among trials of antenatal *myo*-inositol supplementation in relation to gestational glycemia. If *myo*-inositol supplementation is to be considered more widely for optimizing glycemia regulation in pregnancy, it is important to understand which subpopulations may derive most benefit, the potential impact of co-supplementation with other micronutrients, and the optimal timing for commencement of supplementation.

We postulated that an early pregnancy timing of supplementation may unfavorably alter the maternal metabolic adaptation for gestational glycemia regulation. This study aimed to investigate the associations of plasma *myo*-inositol concentrations at 7-weeks (early pregnancy) and 28-weeks (late pregnancy) gestation with glycemia at 28-weeks, as well as explore the potential influences of ethnicity, pre-existing metabolic risk factors, variations in inositol metabolism and excretion, and by co-variation in other supplement components. Besides gestational diabetes risk, we also assessed association with the related outcomes of birthweight16 and cord C-peptide,21 serving as indicators of clinical impact on the offspring. Exploratory analyses were conducted to elucidate potential underlying mechanisms of effect, with *in-silico* modeling to mimic conditions in other trials to investigate if increasing *myo*-inositol later in pregnancy could still benefit glycemia regulation.

**Materials and Methods**

***Study design and participants***

Approval was granted by research ethics services at each site and written informed consent obtained from participants.20 This study is a secondary analysis of data collected in the NiPPeR international multi-center, double-blind randomized controlled trial (ClinicalTrials.gov NCT02509988), which recruited from the community 1729 UK, Singapore, and New Zealand women aged 18–38 years who were planning conception in 2015-2017. Women were randomized into control and intervention arms (1:1 ratio), with supplements commenced preconception and continued throughout pregnancy. Supplements for both arms contained folic acid, iron, calcium, iodine and β-carotene; the intervention additionally included *myo*-inositol (4g daily), vitamin D, riboflavin, vitamin B6, vitamin B12, zinc and probiotics (Lactobacillus rhamnosus and Bifidobacterium animalis sp. lactis).15,17 Following preconception randomization, women were given up to a year to conceive before withdrawal from the study. The current sub-study included 585 participants who conceived and provided *myo*-inositol and gestational glycemia data (Supplementary Figure 1). Adherence was determined by supplement counting; 96.6% reported rates above the pre-specified good compliance threshold of 60% averaged from recruitment to delivery.15

***Procedures and Laboratory analyses***

At recruitment, height, weight, waist circumference and blood pressure were measured and questionnaires ascertained maternal age, ethnicity, household income, parity, previous history of gestational diabetes and family history of diabetes. Preconception body mass index (BMI) was calculated. During pregnancy, participants reported their smoking status. Offspring birthweights were extracted from medical records and birthweight centiles standardized for gestational age and sex.18

A 75g OGTT was conducted at preconception baseline (fasting, 30, 120 min) and again at 28-weeks’ gestation (range 24-32 weeks; fasting, 30, 60, 90, 120 min), with plasma glucose (PG) processed using a standardized protocol across sites.17 Gestational diabetes was defined by the International Association of Diabetes and Pregnancy Study Group (IADPSG) criteria.19 Serum insulin concentrations at these same OGTT timepoints, and fasting plasma triglycerides and high-density lipoproteins cholesterol (HDL-C) were batch-analyzed (Roche Cobas). The Stumvoll 1st phase index,20 the homeostasis model assessment for insulin resistance (HOMA2-IR; [www.OCDEM.ox.ac.uk](http://www.OCDEM.ox.ac.uk))21 and Matsuda index (<http://mmatsuda.diabetes-smc.jp/xpoints.html>)22 were calculated.

Plasma and urine samples were collected preconception at recruitment, in early pregnancy (7-weeks; median 7.4 weeks [IQR 7.1, 7.9]), and late pregnancy (28-weeks; median 27.7 weeks [27.2, 28.3]). We used ultra-high-performance-liquid chromatography tandem mass spectrometry (UHPLC-MS/MS; Neotron, Italy, in collaboration with Nestlé Research, Switzerland) to quantify the concentrations of plasma and urinary *myo*-inositol and *scyllo*-inositol.23 Urinary *myo*-inositol was corrected for dilution using urinary creatinine. Plasma concentrations of folate and vitamins B12 (cobalamin) were measured by microbiological assay, and D (25(OH)D) by LC-MS/MS (Bevital, Bergen, Norway). Umbilical cord venous serum samples collected at delivery were batch-analyzed for C-peptide concentrations (electrochemiluminescence immunoassay, Roche Cobas).

***Statistical Analysis***

Loge transformation of skewed plasma *myo*-inositol and glycemia data achieved approximately Normal distributions. Linear regressions were conducted in a single combined group of control and intervention cases to associate plasma *myo*-inositol concentrations (7-weeks, 28-weeks) with gestational glycemia (fasting, 1h, 2h PG at 28-weeks), adjusting for recruitment site, and relevant covariates based on literature, including ethnicity (non-Asian/Asian), preconception BMI (continuous), parity (nulliparous/parous), maternal age (continuous), household income level (decile for country), family history of diabetes and smoking during pregnancy (never/passive/active). Resulting β coefficients (with 95%CI) represent % change in glycemia for each % increase in *myo*-inositol; β equivalents in mmol/L per µmol/L were calculated using the anti-loge mean of glycemia and *myo*-inositol of the combined control-intervention group. The predicted effect of 7-week-*myo*-inositol on 28-week-glycemia was represented by residuals computed using linear regression. Pearson’s correlation was used to evaluate relations between loge plasma *myo*-inositol and 28-week-glycemia (adjusted for the above covariates).

Since specific preconception maternal risk factors are known to relate to gestational glycemia, we stratified the study population by these factors to detect potentially different associations between *myo*-inositol and glycemia, reporting any statistical interactions. Preconception baseline risk factors studied were ethnicity (non-Asian/Asian), family history of type 2 diabetes (present/absent), and metabolic risk using the 5 criteria defined by the International Diabetes Federation (IDF; low: no risk factors, moderate: with risk factors but did not fulfil metabolic syndrome criteria, and high: metabolic syndrome having central obesity with two other risk factors; Supplementary Table 1).

In sensitivity analyses, we additionally adjusted for the following covariates in regression models: (i) plasma concentrations of other supplement components (folate, vitamins B12 and D, which are thought to influence gestational glycemia24,25); (ii) inherent variations in baseline inositol processing represented by inositol metabolism (plasma *scyllo*-inositol:*myo*-inositol ratio) and urinary excretion (urine:plasma *myo*-inositol ratio).

Causal mediation analysis examined if plasma *myo*-inositol concentration could explain the overall NiPPeR intervention effect on gestational glycemia, adjusting for covariates listed above. Analysis was performed under the assumption of sequential ignorability, reporting the average causal mediation effect (mediation package of statsmodels, Python). To elucidate a potential underlying mechanism, we examined associations between plasma *myo*-inositol concentrations and the three insulin parameters of Stumvoll 1st phase index (approximate measure of insulin secretion in response to a glucose load), HOMA2-IR (insulin resistance) and Matsuda index (insulin sensitivity) at 28-weeks. The adjusted association between 7-week-*myo*-inositol and gestational diabetes was assessed by Poisson regression, while non-standardized birthweight and cord C-peptide (indicator of fetal insulin response to transplacental glucose transfer) linear regressions were additionally adjusted for gestational age and sex.

There was no imputation of missing data. Statistical analyses were carried out using STATA 15 (Stata Corp., College Station, Texas) and Python version 3.9.7. All statistical tests were two sided, with *p*<0.05 considered statistically significant.

***In-silico modeling***

To investigate if previous *myo*-inositol trial results could be replicated within a similar subpopulation of the NiPPeR dataset, we used Monte Carlo simulation with bootstrapping (100 iterations; i.e. resampling with replacement for 100 times with each time ran with a selection of 90% of the population). First, we only used data from a population similar to previous *myo*-inositol trials, specifically White Caucasian women with a family history of diabetes or with BMI≥25 kg/m2. During resampling, similar ratios of UK and NZ participants, and of control and intervention were maintained. For each resampled test, the association of late-pregnancy-*myo*-inositol (representing supplementation after the first trimester) with 28-week-glycemia was examined, with adjustment for previously listed co-variates, the predicted effect of 7-week-*myo*-inositol on 28-week-glycemia (to control for impact of early *myo*-inositol supplementation) and intervention group (to account for all other intervention components). If the confidence interval (CI) of the pooled iteration results did not cross zero, it was considered statistically significant.26 We also conducted similar *in-silico* modelling in other subpopulations to identify potentially different effects.

**Results**

Among the 585 women in this study, 290 took the control supplement and 295 the intervention supplement containing *myo*-inositol. Plasma *myo*-inositol concentrations in early and late pregnancy were higher in the intervention group than in controls (Table 1). Overall, the population mean age was 30.3 years, with average BMI 23.7 kg/m2 (Table 1). Non-Asian participants comprised 64.3%, mostly White Caucasian (92.2%; 59.3% of total cohort). The majority of Asian women were Chinese (87.4%; 24.8% of total cohort). Most were nulliparous (63.4%), did not smoke during pregnancy (96.6%), came from high-income households (66.2% from top two quintiles) and did not have a family history of type 2 diabetes (76.9%). The population was generally metabolically healthy at preconception baseline: median fasting glucose 4.9 mmol/L, mean HbA1C 5.2%, and median HOMA2-IR 0.9.

In combined control-intervention group analyses, a higher 7-week plasma *myo*-inositol was associated with a higher 28-week 1h-PG (βadj [95%CI] 0.05 [0.01, 0.09] loge mmol/L per loge µmol/L, p=0.022) and 2h-PG (0.08 [0.03, 0.12], p=0.001) but not fasting glycemia (Figure 1A). This equates to 0.29 mmol/L and 0.39 mmol/L increases in 1h-PG and 2h-PG, respectively, for the average 7-week-*myo*-inositol increase of 23.4 µmol/L (the mean difference in anti-loge plasma *myo*-inositol between control and intervention) with a daily 4g *myo*-inositol supplement. Mean 1h-PG and 2h-PG of the combined control-intervention group were 7.91 (SD 1.28) and 6.61 (SD 1.28) mmol/L, respectively. However, 28-week plasma *myo*-inositol was not associated with 28-week glycemia (Figure 1B). Adjusting for the predicted effect of 7-week-*myo*-inositol, 28-week-*myo*-inositol still showed no association with 28-week-glycemia (Figure 1C). The association between 7-week-*myo*-inositol and 2h-PG were similar in control and intervention groups (*myo*-inositol\*group interaction-p=0.967). These associations were confirmed by positive correlations between 7-week-*myo*-inositol and adjusted 28-week 1h-PG (Figure 1D) and 2h-PG (Figure 1E) concentrations. Linear relationships held across the 7-week-*myo*-inositol range, where a substantial overlap between control and intervention groups was noted.

To uncover potentially different *myo*-inositol-glycemia associations in subgroups defined by preconception risk factors for hyperglycemia, we stratified the population by non-Asian/Asian ethnicity, with/without family history of diabetes, and by IDF-defined metabolic risk. Following stratification by each risk factor, similar associations were observed between 7-week-*myo*-inositol and 28-week 1h-PG and 2h-PG in the subgroups (Figure 3A-C); all interaction-p-values for 7-week-*myo*-inositol\*each risk factor were not significant (p>0.05), suggesting these risk factors do not modulate/influence *myo*-inositol-glycemia associations. Of note, those classically considered of lower risk demonstrated notable 7-week-*myo*-inositol-glycemia associations (Figures 2A-C): those with no family history of diabetes and non-Asians showed increased 2h-PG (βadj 0.06 [0.01, 0.11], p=0.012; and 0.08 [0.02, 0.13], p=0.007, respectively), while those with no IDF risk factors showed increased 1h-PG (0.07 [0.01, 0.13], p=0.040) and 2h-PG (0.12 [0.05, 0.18], p=0.001). In those with a family history of diabetes, associations of 7-week-*myo*-inositol with 1h-PG (0.18 [0.09, 0.27], p<0.001) and 2h-PG (0.14 [0.04, 0.24], p=0.007) were particularly marked (Figure 2A). Conversely, in a small subgroup of 30 women with metabolic syndrome, higher 7-week-*myo*-inositol associated with a decrease in 2h-PG (−0.16 [−0.31, −0.01], p=0.033) instead (Figure 2C) but this should be interpreted with caution (7-week-*myo*-inositol\*IDF-risk-category interaction term non-significant p=0.075).

Results and strength of associations were largely unchanged with additional adjustment for 7-week plasma concentrations of folate, vitamins B12 and D (1h-PG: 0.04 [−0.01, 0.09], p=0.077; 2h-PG: 0.08 [0.03, 0.13], p=0.001; Figure 2D) and with preconception plasma-*scyllo:myo*-inositol and urine:plasma *myo*-inositol ratios (1h-PG: 0.05 [0.01, 0.09], p=0.024; 2h-PG: 0.07 [0.03, 0.12], p=0.001; Figure 2E).

We previously reported that compared to control, the NiPPeR intervention marginally increased the 28-week-2h-PG concentration from a median (IQR) of 6.49 (5.51-7.70) mmol/L in control to 6.60 (5.84-8.02) mmol/L in the intervention group (adjusted mean difference 0.29 [0.04, 0.55] mmol/L), not reaching the pre-specified statistical significance of p<0.017 for the trial.15 Causal mediation analysis suggested that 7-week-*myo*-inositol could explain the NiPPeR intervention effect on 28-week-2h-PG (mediation co-efficient 0.05 [0.01, 0.11], p=0.02).

In exploratory analyses to identify a possible mechanism for the 7-week-*myo*-inositol-glycemia association, higher 7-week-*myo*-inositol was found to be associated with a lower 28-week Stumvoll index (βadj −0.08 [−0.15, −0.01], p=0.02) but not associated with HOMA2-IR (−0.07 [−0.14, 0.01], p=0.101) or Matsuda index (0.04 [−0.05, 0.12], p=0.398).

Among short-term clinical outcomes, there were no associations between 7-week-*myo*-inositol and gestational diabetes (adjusted relative risk 1.35 [0.98, 1.84] per loge µmol/L, p=0.063), birthweight (βadj 0.02 [−0.05, 0.09] kg per loge µmol/L, p=0.635), birthweight centile (βadj 0.01[−0.03, 0.06] centile per loge µmol/L, p=0.576), and cord C-peptide concentrations (βadj −0.02 [−0.13, 0.08] loge ng/ml per loge µmol/L, p=0.681).

*In-silico*modelingmimicking circumstances of previous *myo*-inositol trials showed that among White women with a family history of diabetes or a high BMI (n=168), a higher 28-week-*myo*-inositol associated with lower fasting, 1h and 2h PG at 28-weeks, equivalent to reductions of 0.09, 0.07 and 0.10 mmol/L, respectively, for an average increase in 28-week-*myo*-inositol of 10.1 µmol/L with 4g daily *myo*-inositol supplementation (Table 2). Glycemia reductions were also observed in subpopulations of White women with and without IDF risk factors, and in non-Asian women. However, among Asian women, a higher 28-week-*myo*-inositol associated with overall increased 28-week glycemia, especially among those with an IDF risk factor, although effect sizes were small (Table 2).

**Discussion**

Secondary analyses of the NiPPeR trial data showed that a higher plasma *myo*-inositol concentration very early in gestation (~7-weeks) associated with higher post-prandial glucose concentrations later in pregnancy (~28 weeks), regardless of ethnicity and in those with and without risk factors for hyperglycemia. Additional analyses suggest a lowered insulin secretory capacity as a possible underlying mechanism. However, *in silico* modeling indicated that, accounting for the glucose-raising influence of early pregnancy *myo*-inositol, higher plasma *myo*-inositol concentration later in pregnancy may still be associated with slightly reduced gestational glycemia in White Caucasian women, but not in Asian women. Our findings may explain why the NiPPeR supplement containing *myo*-inositol did not reduce gestational glycemia like previous trials, with demonstration that the 7-week-*myo*-inositol concentration could account for the non-statistically significant modest increase in 2h-PG in the intervention group compared with control. We conclude that *myo*-inositol supplementation that increases plasma *myo*-inositol in the early first trimester of pregnancy may impair later pregnancy post-prandial glycemic regulation.

NiPPeR was the only trial of *myo*-inositol supplementation aimed at optimizing gestational glycemia that started supplementation preconception and continued through pregnancy, resulting in higher plasma *myo*-inositol concentrations from the very beginning of pregnancy. Other trials have not reported plasma *myo*-inositol levels to allow comparison with our findings. We speculate that in previous trials an early pregnancy plasma *myo*-inositol increase would not have happened since supplementation commenced typically at 8-13 weeks’ gestation10,11,27 with one stretching to 26 weeks,28 thus they unintentionally avoided the unfavorable impact on glycemia. In an observational study, higher urinary concentrations of *myo*-inositol and *D-Chiro*-inositol in the first trimester, likely reflecting correspondingly higher plasmainositol concentrations, were predictive of later gestational diabetes development,29 supporting our findings.

We found an association between higher 7-week-*myo*-inositol and lower 28-week Stumvoll index of acute insulin secretion. One possible mechanism for the observed increase in gestational glycemia could therefore be through *myo*-inositol limiting beta-cell expansion in early pregnancy (Figure 3). Pancreatic beta-cells are thought to undergo hyperplasia or increase insulin secretory capacity as part of normal physiological maternal adaptation starting from early pregnancy to prepare for later pregnancy changes, including higher maternal insulin resistance requiring increased insulin production, to support increasing fetal nutritional demand.30-32 Studies in cultured rat pancreatic islets suggest that *myo*-inositol promotes beta-cell responses to a glucose load, but high levels of *myo*-inositol suppressed beta-cell proliferation.33 It is thus possible that higher plasma *myo*-inositol in early pregnancy in humans could limit physiological beta-cell expansion, resulting in a lasting defect for the remainder of pregnancy in insulin secretory capacity in response to glucose challenge. This postulation is also consistent with the lack of association between 7-week-myo-inositol and 28-week fasting glycemia.

This postulation may also explain the NiPPeR subpopulation differences in *myo*-inositol-glycemia associations (Figure 3). The only subgroup displaying a possible reduction in post-prandial glycemia in association with higher 7-week-*myo*-inositol were those who met criteria for metabolic syndrome preconception. This result on a small sample needs to be interpreted with caution, but we speculate that such women may already be near their maximal beta-cell secretory capacity preconception,34 hence, a higher 7-week-*myo*-inositol has little impact on beta-cell expansion, if any. Instead, the reduction in 28-week-2h-PG may simply reflect the insulin sensitizing action of a higher *myo*-inositol concentration through pregnancy.

Overall, the magnitude of glycemia increase associated with early pregnancy *myo*-inositol supplementation was small with no apparent short-term clinical consequences (no associated increases in gestational diabetes, birthweight or cord C-peptide concentrations). However, given the documented continuum of risk with increasing gestational glycemia across the glycemia range,1 there may be more subtle impacts on offspring, which may emerge with our ongoing follow-up. Of some reassurance, we have previously demonstrated that higher placental inositol may suppress the adiposity-generating effects of maternal glucose in neonates,35 with multiple actions of inositol potentially acting collectively to neutralize maternal glycemia-associated alterations in the offspring.

While most other trials either provided supplements containing a combination of inositol isomers or *myo*-inositol with folic acid, the NiPPeR intervention also contained other micronutrients and probiotics. Our analyses accounting for folate, vitamins B12 and D did not alter the 7-week-*myo*-inositol-glycemia association. However, we cannot exclude the possibility that other supplement components may weaken 28-week-*myo*-inositol-glycemia associations, and underlie the relatively modest *in-silico*-estimated supplement-induced reductions in fasting, 1h and 2h PG of 0.09, 0.07 and 0.10 mmol/L, respectively, in White women; being less than the 0.23, 0.49 and 0.48 mmol/L equivalents reported in a meta-analysis of 6 Italian trials.13

Unlike in White Caucasian women, higher 28-week-*myo*-inositol associated with slightly increased gestational glycemia in Asian women in our *in-silico* modeling. This suggests that genetic or lifestyle factors may influence *myo*-inositol action or *myo*-inositol-micronutrient interactions in glycemia regulation. Indeed, gestational hyperglycemia in Asians, particularly East Asians (the predominant Asian ethnic group in the NiPPeR trial), is more heavily driven by pancreatic beta-cell/insulin insufficiency rather than peripheral insulin resistance.36 As we postulate that *myo*-inositol limits early pregnancy beta-cell expansion, Asian women could therefore be disproportionately affected by a higher early pregnancy plasma *myo*-inositol concentration (Figure 3).

A strength of our study is the robust measurement of the *myo*-inositol isomer in plasma which confirmed that higher concentrations were achieved in early and late gestation with overall good adherence to the NiPPeR supplement.15 Blood sample collection and processing were strictly standardized and batch-analyzed (except glucose) in accredited laboratories, minimizing technical variabilities and imprecision. However, only free *myo*-inositol was quantified while conjugated *myo*-inositol, including the inositol-phosphoglycans, which are insulin-mimetics, were not measured. Without a full representation of *myo*-inositol and its derivatives in the circulation, interpretation is limited. Also, with the lack of *myo*-inositol measurements between 7-weeks’ and 28-weeks’ gestation, we could not determine more precisely the optimal gestational timing for commencement of *myo*-inositol supplementation to achieve good glycemia outcomes. Gold-standard hyperinsulinemic-euglycemic glucose clamp studies would be needed to confirm our postulation of a *myo*-inositol-induced impairment in insulin secretory capacity but this has not been done.

While the NiPPeR supplement containing *myo*-inositol yielded some benefits including lower risks of preterm birth and postpartum hemorrhage as secondary outcomes,15 our present study indicates that periconception *myo*-inositol supplementation which increases early pregnancyplasma *myo*-inositol concentration may slightly increase later post-prandial glycemia. Further research is required to replicate these findings, identify mechanisms, and investigate the potential long-term implications for the offspring. Future studies should investigate the optimal timing for starting *myo*-inositol supplementation aimed at regulating gestational glycemia, and evaluate the benefit-risk ratio of prenatal *myo*-inositol supplementation.

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**Conflict of interest**

SC, WC and KG report grants from Société Des Produits Nestlé S.A. during the conduct of the study, and are co-inventors on patent filings by Nestlé S.A. relating to the NiPPeR intervention or its components. SC, SB, WC and KG are part of an academic consortium that has received grants from Nestlé S.A. and Benevolent AI Bio Ltd outside the submitted work. KG has received reimbursement for speaking at conferences sponsored by companies selling nutritional products. SC has received reimbursement from the Expert Group on Inositol in Basic and Clinical Research (EGOI; a not-for-profit academic organization) and Nestlé Nutrition Institute for speaking at conferences. LL, JMRN, JPG and ISZ are employees of Société des Produits Nestlé SA. All other authors declare no competing interests.

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**Figure legends**

**Figure 1. Associations between plasma *myo*-inositol in early (7-weeks; A) or late (28-weeks; B and C) pregnancy with gestational glycemia at 28-weeks.** Gestational glycemia (fasting, 1h, 2h) was assessed by a 3-timepoint 75g oral glucose tolerance test (OGTT) at 28-weeks. Plasma *myo*-inositol (µmol/L) and glycemia (mmol/L) were loge transformed and adjusted for study site, Asian ethnicity, preconception BMI, parity, maternal age, household income level, family history of diabetes, and smoking during pregnancy in linear regression models. (C) Additionally, adjusted for the predicted effect of early pregnancy plasma *myo*-inositol on glycemia. β coefficient represents the % change in loge-transformed glycemia relative to each % change in loge-transformed early pregnancy plasma *myo*-inositol. Pearson correlation between plasma *myo*-inositol in early pregnancy (7-weeks, loge transformed) and adjusted (for above covariates) 1h (D) and 2h (E) gestational glycemia at 28-weeks. The overlap in plasma *myo*-inositol concentrations in the control and intervention (taking supplement containing *myo*-inositol) groups are indicated. R2 provides an estimate of how much of the variance in glycemia is explained by plasma *myo*-inositol after accounting for the listed covariates. N (number) indicates those with available data. Abbreviations: CI, confidence interval. \*p<0.05, \*\*\*p=0.001.

**Figure 2.** **Associations between early pregnancy plasma *myo*-inositol and gestational glycemia at 28-weeks in subgroups or with additional adjustments.** Stratified by preconception risk factors: (A) Ethnicity (Non-Asian, Asian); (B) Family history of type 2 diabetes mellitus37 (No FHx, With FHx); (C) Metabolic risk defined by the International Diabetes Federation (IDF) (Low – no risk factor, Moderate – at least one risk factor but did not fulfil criteria for MetS, High - fulfils criteria for Metabolic Syndrome [MetS; central obesity with 2 other risk factors]; the five IDF factors are: Central obesity defined as a waist circumference ≥88 cm for non-Asian, ≥80 cm for South and East Asian women, hyperglycemia defined as fasting plasma glucose ≥100 mg/dL (5.6 mmol/L), hypertriglyceridemia defined as triglycerides ≥150 mg/dL, HDL cholesterol <50 mg/dL, Hypertension with systolic blood pressure > 130 mmHg or diastolic blood pressure > 85 mmHg, or on anti-hypertensive treatment; see Supplementary Table 1). Additional adjustment for potential confounders: (D) other components of the NiPPeR intervention known to influence gestational glycemia (folate, vitamins B12 and D); (E) inherent variations in inositol processing (pre-intervention plasma *scyllo*-inositol:*myo*-inositol ratio and urine:plasma *myo*-inositol ratio). Plasma *myo*-inositol and gestational glycemia (fasting, 1h, 2h in a 75g oral glucose tolerance test) were loge-transformed and analyzed by linear regression adjusted for study site, Asian ethnicity, preconception BMI, parity, maternal age, household income level, family history of diabetes, and smoking during pregnancy. β coefficient represents the % change in loge-transformed glycemia (mmol/L) relative to each % change in loge-transformed early pregnancy plasma *myo*-inositol (µmol/L). \*p<0.05, \*\*p<0.01, \*\*\*p=0.001. N (number) indicates number of women with available data. Abbreviations: CI, confidence interval; FHx, family history of type 2 diabetes mellitus.

**Figure 3. Schematic diagram of the postulated underlying mechanism for the role of early and late pregnancy plasma *myo*-inositol in gestational glycemia regulation.** Early pregnancy (7-week) *myo*-inositol may limit physiological beta-cell expansion in early pregnancy (reducing beta-cell hyperplasia alongside inducing a more efficient beta-cell response that reduces the stimulus for physiological beta-cell expansion) leaving a lasting defect which compromises pancreatic response to a glucose challenge later in pregnancy. However, in women with metabolic syndrome (MetS; red lines) where beta-cell capacity is already near maximal preconception, there is limited potential for further beta-cell expansion early in pregnancy anyway, hence a muted impact of early pregnancy *myo*-inositol. Late pregnancy (28-week) *myo*-inositol promotes beta-cell response (i.e. insulin secretion) to a glucose load as well as increases peripheral insulin sensitivity to promote good glycemia regulation. In Asian women (blue lines) where beta-cell insufficiency is a greater contributor to poor glycemia regulation,36 early pregnancy *myo*-inositol-induced suppression of beta-cell expansion would have a disproportionately greater adverse effect on glycemia regulation than in White women (black lines) where peripheral insulin resistance is the more predominant contributor to impaired glycemia control.

**Table 1. Characteristics of participants who provided inositol and gestational glycemia data**

|  |  |  |
| --- | --- | --- |
| **Characteristics** |  | **N=585** |
| **Age (years), mean ± SD** |  | 30.3 ± 3.4 |
| **BMI (kg/m2), median (IQR)** |  | 23.7 (21.3 to 27.0) |
|  | Overweight†, n (%)  Obese†, n (%) | 157 (26.9)  101 (17.3) |
| **Ethnic origin, n (%)** | Non-Asian  White Caucasian  Polynesian  Other  Asian  Chinese  South Asian (Indian, Pakistani, Bangladeshi)  Malay  Other | 376 (64.3)  347 (59.3)  16 (2.7)  13 (2.3)  209 (35.7)  145 (24.8)  30 (5.1)  23 (3.9)  11 (1.9) |
| **Site, n (%)** | United Kingdom  Singapore  New Zealand | 190 (32.5)  166 (28.4)  229 (39.1) |
| **Nulliparous, n (%)** |  | 371 (63.4) |
| **Smoking in pregnancy, n (%)** | Passive  Active | 69 (11.9)  20 (3.4) |
| **Household income quintile, n (%)** | 1 (lowest)  2  3  4  5 (highest)  Not available | 7 (1.2)  44 (7.6)  123 (21.0)  204 (34.9)  183 (31.3)  24 (4.0) |
| **Family history of T2DM, n (%)** |  | 135 (23.1) |
| **Previous GDM (% of parous only), n (%)** |  | 16 (7.5) |
| **Preconception baseline parameters** |  | |
| Fasting glucose (mmol/L), median (IQR) |  | 4.9 (4.5 to 5.2) |
| 2-hour glucose (mmol/L) in OGTT, median (IQR) |  | 5.5 (4.5 to 6.4) |
| HbA1C (%), mean ± SD |  | 5.2 ± 0.3 |
| HOMA2-IR, median (IQR) |  | 0.9 (0.6 to 1.3) |
| Plasma *myo*-inositol (µmol/L), median (IQR) |  | 21.9 (19.1 to 25.5) |
| **Post-supplementation parameters in pregnancy, median (IQR**) | | |
| Plasma *myo*-inositol in early pregnancy (7-weeks) (µmol/L) | All (N=564)  Control (N=274)  Intervention (N=290) | 29.1 (21.6 to 48.8)  21.8 (19.0 to 25.3)  48.5 (35.3 to 60.2) |
| Plasma *myo*-inositol in late pregnancy (28-weeks) (µmol/L) | All (N=581)  Control (N=289)  Intervention (N=292) | 20.4 (17.0 to 28.4)  17.4 (15.5 to 19.7)  28.3 (22.4 to 34.7) |

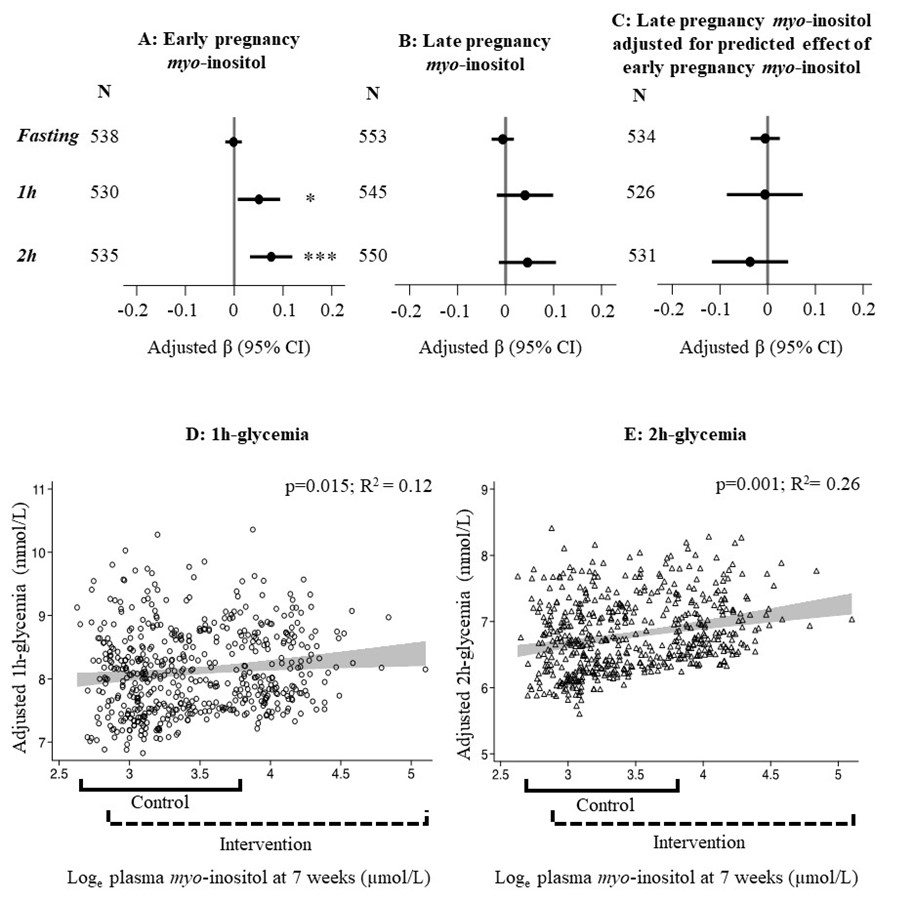
†Defined using ethnic-specific thresholds for overweight and obesity: ≥23 to <27.5 and ≥27.5 kg/m2, respectively, for Asians including Chinese, Indians, Pakistani, Bangladeshi, Malay, mixed Asian; ≥25 to <30 and ≥30 kg/m2, respectively, for non-Asians including White Caucasian, Polynesian, Black, mixed Asian-non-Asian.38 Abbreviations: BMI, body mass index; GDM, gestational diabetes; HOMA2-IR, homeostasis model assessment for insulin resistance version 2; IQR, interquartile range; N, number; OGTT, 75g oral glucose tolerance test; SD, standard deviation; T2DM, type 2 diabetes mellitus.

**Table 2. *In-silico* bootstrapping model of the association between late pregnancy plasma *myo*-inositol and gestational glycemia at 28-weeks in different subpopulations**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Subpopulation** | **Total N** | **N per site UK/NZ/SG** | **N per resampling** | **Adjusted**  **β coefficient (95% CI)** | **Estimated difference in**  **28-week glycemia (mmol/L) with**  **4g daily *myo*-inositol supplementation**†**(95% CI)** |
| **All Whites** | 318 | 163/155/0 | 286 | F: -0.014 (-0.015, -0.012)  1h: -0.076 (-0.080, -0.072)  2h: -0.101 (-0.105, -0.098) | -0.03 (-0.03, -0.02)  -0.2 (-0.27, -0.25)  -0.29 (-0.30, -0.28) |
| **Whites sharing similar characteristics to previous trials** | 168 | 94/74/0 | 152 | F: -0.041 (-0.043, -0.039)  1h: -0.019 (-0.024, -0.014)  2h: -0.032 (-0.038, -0.026) | -0.09 (-0.09, -0.08)  -0.07 (-0.09, -0.05)  -0.10 (-0.12, -0.08) |
| **Whites without IDF risk factors** | 128 | 53/75/0 | 115 | F: -0.027 (-0.028, -0.025)  1h: -0.130 (-0.138, -0.123)  2h: -0.131 (-0.136, -0.125) | -0.05 (-0.05, -0.05)  -0.40 (-0.42, -0.37)  -0.34 (-0.36, -0.33) |
| **Whites with any one IDF risk factor** | 190 | 110/80/0 | 171 | F: -0.007 (-0.009, -0.005)  1h: -0.049 (-0.054, -0.043)  2h: -0.088 (-0.093, -0.084) | -0.01 (-0.02, -0.01)  -0.18 (-0.20, -0.16)  -0.27 (-0.28, -0.26) |
| **All Non-Asians** | 340 | 168/172/0 | 306 | F: -0.021 (-0.023, -0.020)  1h: -0.075 (-0.079, -0.071)  2h: -0.095 (-0.099, -0.092) | -0.04 (-0.05, -0.04)  -0.26 (-0.27, -0.25)  -0.27 (-0.29, -0.27) |
| **All Asians** | 184 | 4/30/150 | 165 | F: 0.019 (0.017, 0.020)  1h: 0.104 (0.100, 0.108)  2h: 0.053 (0.049, 0.058) | 0.04 (0.04, 0.04)  0.41 (0.40, 0.43)  0.18 (0.16, 0.19) |
| **Asians without IDF risk factors** | 91 | 1/13/77 | 82 | F: -0.065 (-0.068, -0.062)  1h: -0.073 (-0.080, -0.066)  2h: -0.055 (-0.063, -0.048) | -0.13 (-0.14, -0.12)  -0.28 (-0.30, -0.25)  -0.17 (-0.20, -0.15) |
| **Asians with any one IDF risk factor** | 93 | 3/17/73 | 84 | F: 0.067 (0.065, 0.070)  1h: 0.188 (0.183, 0.193)  2h: 0.085 (0.079, 0.091) | 0.14 (0.14, 0.15)  0.78 (0.76, 0.80)  0.30 (0.28, 0.32) |

Plasma *myo*-inositol and gestational glycemia (F, 1h, 2h PG) were loge-transformed and analyzed by linear regression adjusted for site, preconception BMI, parity, family history of type 2 diabetes mellitus, maternal age, household income level, smoking during pregnancy, and the predicted effect of early pregnancy plasma *myo*-inositol on glycemia. Monte Carlo simulation with bootstrapping (100 iterations; i.e. resampling with replacement for 100 times with each time ran with a selection of 90% of the population). If the confidence interval does not cross zero, it is regarded as statistical significance of p<0.05. β coefficients represent the % change in loge-transformed glycemia (mmol/L) relative to each % change in loge-transformed late pregnancy plasma *myo*-inositol (µmol/L). †Applicationof each β co-efficient to the general average increase in 28-week plasma *myo*-inositol with 4g daily *myo*-insoitol supplementation of 10.1 µmol/L [SD 0.17] (calculated as the mean difference in anti-loge plasma *myo*-inositol between control and intervention at 28-weeks) to derive an estimated difference in glycemia with supplementation. Abbreviations: CI, confidence interval; F, fasting; N, number; NZ, New Zealand; PG, plasma glucose; SG, Singapore; 1h, one-hour; 2h, two-hour; SD, standard deviation.

**Figure 1**

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**Figure 2**

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**Figure 3.**

