**Myo-inositol, Probiotics and Micronutrient Supplementation from Preconception for Glycemia in Pregnancy: NiPPeR International Multi-center Double-blind Randomized Controlled Trial**

**Short running title:** NiPPeR supplement: preconception and pregnancy

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**Abstract**

**Objective:** Better preconception metabolic and nutritional health are hypothesized to promote gestational normoglycemia and reduce preterm birth, but evidence supporting improved outcomes with nutritional supplementation starting preconception is limited.

**Research Design and Methods:** Double-blind randomized controlled trial recruited from the community 1729 UK, Singapore and New Zealand women aged 18-38 years planning conception. We investigated if a nutritional formulation containing myo-inositol, probiotics and multiple micronutrients (intervention), compared with a standard micronutrient supplement (control), taken preconception and throughout pregnancy, could improve pregnancy outcomes. The primary outcome was combined fasting, 1-hour and 2-hour post-load glycemia (28 weeks’ gestation oral glucose tolerance test).

**Results**: Between 2015-2017, participants were randomized to control (n=859) or intervention (n=870); 585 conceived within 1-year and completed the primary outcome (295 intervention, 290 control). In an intention-to-treat analysis adjusting for site, ethnicity and preconception glycemia with pre-specified p<0.017 for multiplicity, there were no differences in gestational fasting, 1-hour and 2-hour glycemia between groups (β [95%CI] loge mmol/L intervention versus control: -0·004 [-0·018, 0·011], 0·025 [-0·014, 0·064], 0·040 [0·004, 0·077], respectively). Between the intervention and control groups there were no significant differences in gestational diabetes (24·8% versus 22·6%, adjusted risk ratio aRR=1·22 [0·92, 1·62]), birthweight (adjusted β=0·05kg [-0·03, 0·13]), or gestational age at birth (mean 39.3 versus 39.2 weeks, adjusted β=0·20 [-0·06, 0·46]), but there were fewer preterm births (5·8% versus 9·2%, aRR=0·43 [0·22, 0·82]) adjusting for pre-specified covariates.

**Conclusions:** Supplementation with myo-inositol, probiotics and micronutrients preconception and in pregnancy did not lower gestational glycemia, but did reduce preterm birth.

Sub-optimal metabolic and nutritional health around conception and during pregnancy have important implications for pregnancy outcomes, fetal growth, adiposity and long-term offspring health (1). Adverse effects of higher maternal glucose concentrations increase across the continuum of maternal glycemia (2,3) and micronutrient insufficiency is highly prevalent in women. Interventions that optimize glycemia and nutritional status are thought to improve pregnancy and offspring outcomes but supportive evidence from intervention studies is sparse.

Pregnancy is a state of relative maternal insulin resistance, promoting glucose transfer to the fetus (4). Physiological insulin resistance and impaired insulin secretion can be accentuated by individual genetic and environmental vulnerabilities, and lead to gestational diabetes mellitus (GDM) (5). The global GDM incidence is rising, recently estimated at 14% (6). Following GDM diagnosis, lifestyle changes, oral hypoglycemic drugs and insulin can improve some short-term obstetric outcomes (7) but cannot fully mitigate pregnancy and offspring adversity (8). Risk reduction strategies have thus shifted towards GDM prevention. However, population trials of dietary, physical activity or combined lifestyle measures, mostly beginning in the first half of gestation, have had limited impact on preventing GDM (9,10). This has led to postulations that preconception interventions could be more effective and that alternative approaches are required.

Small clinical trials suggest that supplementation with myo-inositol or probioticsfrom early pregnancy may be beneficial; myo-inositol is a naturally-occurring 6-carbon polyol with insulin sensitizing actions arising from functions relating to many second messenger signaling pathways and endogenous insulin-mimetic factors (11). Meta-analysis of women given myo-inositol supplementation from the end of the first trimester reported reductions in GDM, gestational glycemia and preterm birth (12). Similarly, meta-analysis of studies of probiotics (Lactobacillus and/or Bifidobacterium species) from early pregnancy showed improved insulin sensitivity (13). One trial of probiotics taken from the first trimester reported improved glucose toleranceand reduced GDM (14).Low intakes and insufficiencies of several micronutrients (vitamins B6, B12, riboflavin, zinc) are prevalent in pregnancy and have been linked with glucose intolerance and pregnancy outcomes (15-17), but there are few intervention studies (18). Vitamin D deficiency has also been linked with GDM and preterm birth (19), but a trial of Vitamin D supplementation starting in early pregnancy showed no preventive effects on pregnancy complications (20). Another trial of a nutritional supplement (containing protein, polyunsaturated fatty acids and micronutrients without inositols or probiotics) in low resource settings showed improved birth length but no difference in preterm birth compared with no supplementation, with no difference between the group starting supplementation preconception compared with those starting in early pregnancy; glycemia outcomes were, however, not reported (21).

Dysglycemia and maternal micronutrient insufficiency preconception or in early pregnancy are common in the general population and thought to influence the risk of adverse pregnancy outcomes (1,5,17,22). We hypothesized that a myo-inositol, probiotic and micronutrient nutritional supplement commencing before pregnancy could collectively lower maternal glycemia and improve pregnancy outcomes across the general population. We therefore undertook an international multi-center, double-blind randomized controlled trial [Nutritional Intervention Preconception and During Pregnancy to Maintain Healthy Glucose Metabolism and Offspring Health (NiPPeR) study (23)] to investigate whether intervention with a nutritional supplement containing myo-inositol, probiotics and additional micronutrients (vitamins D, B6, B12, riboflavin and zinc), compared with a standard preconception micronutrient supplement, taken prior to and during pregnancy, would promote improved maternal pregnancy glycemia and outcomes.

**Research Design and Methods**

This international multi-center, double-blind randomized controlled trial recruited women who were planning to conceive within the next 6 months. Women were recruited in Singapore, Auckland (New Zealand) and Southampton (UK) primarily from the community (Figure 1). Our trial was approved by the United Kingdom, Singapore and New Zealand research ethics services at each site (Southampton: Health Research Authority NRES Committee South Central Research Ethics Committee (REC) reference 15/SC/0142, the National Healthcare Group Domain Specific Review Board (NHG DSRB) Singapore reference 2015/00205, and the Health and Disability Ethics Committee (HDEC) New Zealand reference 15/NTA/21), with confirmation from the relevant regulatory authorities that the formulation was not an investigational medicinal product. Trial oversight and monitoring were provided by an independent data and safety monitoring committee. This trial was prospectively registered at ClinicalTrials.gov NCT02509988, UTN U1111-1171-8056.

***Participants***

Based on our previous population-based Southampton Women’s Survey our initial target was 1800 recruits to have 600 established pregnancies to study; in the event pregnancy rates were higher and recruitment was stopped at 1729 women when it was clear that the projected number of pregnancies would exceed our target (final conceptions n=725, Figure 1).

Women were eligible for trial enrolment if they were aged 18-38 years, planning to conceive within 6 months and have future maternity care at the recruiting centers. In Singapore, women had to be of homogeneous or mixed Chinese, Malay or Indian ethnicity. *A priori*, women conceiving within a year were followed through pregnancy and beyond. Women were excluded if they were pregnant or lactating at recruitment, undergoing assisted conception (apart from taking clomiphene or letrozole alone), had known serious food allergy or pre-existing type-1 or type-2 diabetes, were using oral, implanted or intra-uterine contraception or taking metformin, systemic steroids, anticonvulsants or treatment for HIV, Hepatitis B or C in the past month. Participants provided written informed consent.

***The Formulation***

The intervention and control formulations were packaged as a powder in sachets and stored at 2-6°C until made up in water and taken twice daily, with similar sensory characteristics. Formulations were produced by SIIT (Italy). Ingredients common to control and intervention formulations were folic acid 400 µg/day, iron 12 mg/day, calcium 150 mg/day, iodine 150 µg/day and β-carotene 720 µg/day; the intervention additionally included myo-inositol 4 g/day, vitamin D 10 µg/day, riboflavin 1.8 mg/day, vitamin B6 2.6 mg/day, vitamin B12 5.2 µg/day, zinc 10 mg/day and probiotics [Lactobacillus rhamnosus NCC 4007 (CGMCC 1.3724; LPR) and Bifidobacterium animalis sp. lactis NCC 2818 (CNCM I-3446; Bl818); average survival counts remained within target over the shelf-life of the refridgerated product] (24).Quantities were either standard amounts based on previous trials (myo-inositol, probiotics) (12,14), amounts enhanced above those available in over the counter products (vitamins B6, B12, riboflavin), UK recommended daily allowance amounts for pregnant women (vitamin D, zinc, folic acid, iodine) or minimal amounts for micronutrients linked with potential detrimental effects at higher doses (iron, β-carotene, calcium).

***Randomization and Procedures***

Participants were randomly assigned in a 1:1 ratio to the control or intervention groups via the electronic study database (23),with minimization by site and ethnicity to ensure balanced allocation to the groups. Throughout the trial participants, investigators, clinicians, and fieldworkers were unaware of the trial-group assignments.

Following a baseline 75g oral glucose tolerance test (OGTT), anthropometric measurements and questionnaire ascertainment of the woman’s characteristics, trial formulations were initiated before conception and continued until the end of pregnancy. Participants were instructed to contact the trial team as soon as they had a positive urinary pregnancy test, which was then confirmed by an ultrasonographic examination at 6-8 weeks’ gestation. Once pregnant, the women were followed-up with questionnaires, for resupply of trial formulations, a 20-week fetal anomaly scan and a 28-week OGTT. Plasma glucose was collected in anti-glycolytic-buffered tubes and transported on an ice slurry to the laboratory within 30 minutes using standardized protocols across sites. Glucose measurements using the glucose oxidase method were undertaken by a single laboratory at each site, with uniform external quality assurance in the Royal College of Pathologists of Australasia (RCPA) Quality Assurance Program. Plasma 25-hydroxyvitamin D, and serum insulin concentrations (fasting, 30-min, 2-hour at preconception baseline; fasting, 30-min, 1-hour, 90-min, 2-hour at the 28-weeks’ OGTT), were batch-analyzed by liquid chromatography-tandem mass spectrometry (Bevital, Bergen, Norway) and an electrochemiluminescence immunoassay (Roche Cobas), respectively. The homeostasis model assessment for insulin resistance (HOMA2-IR; [www.OCDEM.ox.ac.uk](http://www.OCDEM.ox.ac.uk)) (25) and Matsuda index measure of insulin sensitivity (<http://mmatsuda.diabetes-smc.jp/xpoints.html>.) (26) were calculated.

Antenatal, peri-partum and neonatal outcomes were ascertained from medical records. Adherence to the trial formulation was ascertained by sachet counting. Good adherence was defined *a priori* as at least 60% of the sachets taken.

***Outcomes***

The primary outcome was fasting and/or 1-hour and/or 2-hour plasma glucose concentrations following a 75g OGTT at 28 weeks’ gestation (*a priori* specification included all conducted between 24-32 weeks). Based on prior systematic reviews, principal pre-specified secondary outcomes were GDM (defined by IADPSG criteria (27)), large size-for-gestational age at birth (using sex- and gestational age- specific RCPCH 2009 UK-WHO growth charts (28)) and preterm birth; other secondary outcomes are shown in Table 3, and Supplemental Table S2. Gestational age was determined using a pre-specified algorithm (29) using menstrual data (date of last menstrual period (LMP), self-reported cycle regularity, mean cycle lengths in last 3 months), with first trimester fetal ultrasonographic crown-rump length measurement used if >7 days’ discrepancy between LMP and scan dates, uncertain LMP date, irregular cycles or hormonal contraception-use within last 3 months.

***Statistical Analysis***

Considering the composite multiple endpoint primary outcome of plasma glucose at three 75g OGTT time points, the pre-specified level of statistical significance was set as p<0.017 (i.e. 0.05 divided by 3). With a sample size of 600 (300 in each group), a two-sided test with alpha 0.017 and 80% power, the detectable differences in fasting, 1-hour and 2-hour glucose concentrations between groups were 0.12, 0.45 and 0.34 mmol/L, respectively (each with a standardized effect size of 0.265 standard deviations using values reported in the HAPO study (30)). Such magnitudes of glycemic change are expected to have clinically appreciable effects on neonatal size and adiposity, and longer term offspring health (2, 3).

Glucose values were loge transformed to achieve approximately Normal distributions before using these values for analysis. Analysis of the primary outcome used linear regression on the intention-to-treat dataset (all randomized participants who provided an OGTT at 24-32 weeks). Group (control or intervention) was included as a predictor and regressions adjusted for site, ethnicity and corresponding preconception glycemia to account for potential imbalance between treatment arms amongst pregnancies that reached 24-32 weeks’ gestation. Subsequent regression models were additionally adjusted for pre-specified factors thought to be important predictors of outcomes and for other factors not balanced across control and intervention groups and believed to be prognostic; these are listed in the relevant tables of results. Likewise, HOMA2-IR and Matsuda indices were loge transformed and comparisons at 28 weeks’ were similarly adjusted but with corresponding preconception values instead of preconception glycemia. Estimates of differences (beta) between the groups are presented with 95% CI. T-tests on loge glucose were also conducted. Group comparisons were performed in two pre-specified special interest subgroups: women who were overweight or obese (defined using ethnic-specific thresholds of BMI >23 kg/m2 for Asians including Chinese, Indians, Pakistani, Bangladeshi, Malay, mixed Asian; >25 kg/m2 for non-Asians including White Caucasian, Polynesian, Black, mixed Asian-non-Asian), and women with documented evidence of dysglycemia prior to conception defined as at least one of the following: GDM in a previous pregnancy, preconception baseline first visit raised HbA1C (≥5·7% (39 mmol/mol) or impaired fasting glucose (5·6 to 6·9 mmol/L) or impaired glucose tolerance (2-hour glucose 7·8 to 11·0 mmol/L) (31).

The statistical analysis plan did not include correction for multiple comparisons for secondary or other outcomes. Therefore, results for these outcomes are reported as point estimates and 95%CI, and should not be used to infer definitive treatment effects. Analyses were performed using Stata software v15.1 (StataCorp, College Station, TX, USA).

**Results**

Between 3rd August 2015 and 12th May 2017, 1729 women were recruited and randomly assigned to either control (n=859) or intervention (n=870) groups. Pregnancies fulfilling the study criteria and reaching 28 weeks’ gestation were achieved in 588 women, 292/859 (34%) and 296/870 (34%) in the control and intervention groups, respectively (Figure 1); 585/588 (99·5%) had an OGTT and provided the primary outcome of glycemia at 28 weeks’ gestation [median (interquartile range) 27·7 (27·2 to 28·3) weeks]. Median BMI and other baseline characteristics were similar in the two study groups providing the primary outcome, except fewer women in the intervention group were obese, nulliparous or had a family history of diabetes (Table 1).

Comparisons of unadjusted plasma glucose values at the three OGTT time points between the control and intervention groups were not significantly different (Table 2). In the primary outcome intention-to-treat analysis adjusting for site, ethnicity and matched preconception glucose values (where available), plasma glucose values did not differ between study groups at each of the three time points (p>0·017) (Table 2). Full adjustment as pre-specified provided similar results (Table 2). The incidence of GDM was similar between study groups (Table 3). Sensitivity analyses excluding 32 participants who were subsequently found not to fulfil the eligibility criteria or did not have good adherence gave similar results (Supplemental Table S1).

Glycemia outcomes were examined in two special interest subgroupsspecified *a priori* where it was hypothesised that the intervention could have a greater effect*.* Among womenwho were overweight or obese prior to conception (n=258), intervention did not alter fasting and 1-hour glycemia; 2-hour glycemia was higher in the intervention group (adjusted β=0·076 [95%CI 0·020, 0·131] loge mmol/L; equivalent to 0·53 mmol/L glucose) but with no increased risk of GDM (Supplemental Table S1).In women with documented dysglycemia prior to conception (n=94), glycemia at 28 weeks and GDM incidences were similar between study groups (Supplemental Table S1). Interaction terms in the fully adjusted models including all women showed no evidence of differential effects on 28-week glycemia in response to the intervention between the three study sites and between ethnicities. As measures of insulin resistance and insulin sensitivity, respectively, HOMA2-IR (adjusted β -0·022 [95%CI -0·090, 0·046]) and Matsuda index (adjusted β 0·001 [95%CI -0·068, 0·070] at 28 weeks were also similar between study groups.

Adjusting for covariates, there was a lower incidence of preterm birth (<37 weeks’ gestation) in the intervention group (adjusted risk ratio aRR 0·43 [0·22, 0·82]) (Table 3). There were similar trends in both spontaneous and iatrogenic preterm births. The effect of the intervention was principally observed for late preterm births (34-36 completed weeks’ gestation, aRR 0·41 [0·20, 0·85]) and preterm births associated with preterm pre-labor rupture of membranes (PPROM, aRR 0·21 [0·06, 0·69]), with the incidence of PPROM itself also reduced (aRR 0·39 [0·16, 0·97]) (Table 3). There were no differences in mean gestational age at delivery, neonatal unit admissions and neonatal septicemia (Table 3).

The incidence of major post-partum hemorrhage (>1 liter blood loss) was lower in the intervention group (aRR 0·44 [0·20, 0·94]); this reduction was not explained by cesarean section delivery rates or birthweight, which were similar between study groups (Table 3). There were no differences between groups for the secondary outcomes of miscarriage, congenital anomaly, severe nausea and vomiting of pregnancy, hypertensive disorders of pregnancy, intrauterine death, neonatal death, neonatal hypoglycemia and other neonatal complications (Table 3, Supplemental Table S2).

Among women who provided the primary and birth outcomes, overall supplement adherence was good, with 80·7% having 80-100% adherence, 15·9% 60-80% adherence, and only 3·4% below 60% adherence averaged from recruitment to delivery. Adherence was similar in the control and intervention groups. As a further indication of good adherence, 25-hydroxyvitamin D concentrations in the intervention and control group were similar at the preconception baseline but higher in the intervention compared with the control group at the 28-week OGTT (median 92.8 versus 63.0 nmol/L). Among all randomized women, withdrawals due to perceived minor side-effects from the supplement were similar in both groups (8·3% control, 7·5% intervention), as were other serious adverse events (2·3% control, 2·8% intervention) (Supplemental Table S3).

**Conclusions**

In this international multi-center randomized controlled trial, a nutritional formulation enriched with myo-inositol, probiotics and multiple micronutrients, commenced preconception and continued throughout pregnancy, did not result in lowered maternal glycemia at 28 weeks’ gestation. There were no significant effects on the incidence of GDM and large-for-gestational-age infants. Intervention reduced preterm birth, affirming findings from previous myo-inositol trials. We also found a reduction in the incidence of major post-partum hemorrhage.

Three previous trials of open label myo-inositol taken from early pregnancy to prevent GDM in women in Italyfocused on discrete high risk groups for dysglycemia, namely those who were overweight or obese, or with a family history of type 2 diabetes mellitus (32). All showed a similar reduction in GDM, with an overall odds ratio (OR) of 0.34, as well as lower fasting, 1-hour- and 2-hour glycemia in a 24-28-week OGTT (32). A further small trial among women with impaired fasting glycemia in early pregnancy reported a large reduction in GDM risk (RR 0.127) alongside lower fasting and 1-hour glycemia (33). These observations contrast with the finding of no difference in glycemia at 28 weeks’ gestation with our intervention. However, our study intervention was administered double-blinded over two important periods, preconception and pregnancy, in a general population of women planning pregnancy across multiple centers and ethnicities, excluding those with existing or newly-diagnosed type 1 or 2 diabetes mellitus preconception. Subgroup analysis of overweight and obese women or those with documented dysglycemia did not show any benefit of our intervention on glycemia, although the trial was not powered to do so. Our results, however, are consistent with an Irish trial of a lower dose of myo-inositol combined with D-chiro-inositol in women with a family history of type 1 or 2 diabetes, which showed no impact on glycemia (34).

In another small trial, dietary counselling and probiotics in pregnant women improved glycemia (OR for elevated glucose 0.31) and insulin sensitivity (14); these findings are discordant with ours despite using the same probiotic combination. However, a meta-analysis of ten trials of probiotic supplementation in pregnancy found no difference in fasting glycemia (despite a reduction in HOMA-IR) (13) and a recently completed trial showed no difference in GDM rates, with slightly higher fasting glycemia (35). Inconsistent findings may be attributable to different populations and concurrent use of different combinations of prenatal supplements.

Meta-analysis of the group of three Italian myo-inositol studies found a reduction in fetal macrosomia (OR 0.38) and large-for-gestational age (OR 0.52) (32), in contrast to the finding of no difference with our intervention. The same meta-analysis also found a reduction in preterm birth (OR 0.44) (32), with the separate Irish inositol trial of a lower myo-inositol dose observing a non-statistically significant trend of fewer preterm births in the intervention group (2% versus 7%, p=0.11) (34). Another meta-analysis of trials of multiple micronutrient supplements concluded they probably also lead to a slight reduction in preterm birth [aRR 0.95 (0.90-1.01)] (36).In contrast, none of the probiotic trials reported a change in preterm birth rates. Nonetheless, findings of a meta-analysis of myo-inositol trials (12) are consistent with our demonstration of a reduction in preterm birth. Furthermore, our finding of a reduction particularly in PPROM and PPROM-associated preterm births in the intervention group indicates that this is the likely explanation for reduced prematurity. Approximately 30% of preterm births are preceded by PPROM, of which 60-70% occur late preterm after 34 weeks’ gestation (37). PPROM is postulated to break down the barrier to ascending pathogens resulting in intrauterine infection, increased inflammation and the triggering of preterm labor. In our trial there was only a reduction in preterm births with intervention without any associated difference in clinically detectable infections between study groups. Potential mechanisms for a preventive effect on PPROM-associated preterm births in our study may include anti-inflammatory effects of myo-inositol (38) and a contribution from the potential synergistic effect of micronutrients including zinc and vitamin D (39). Our results of specifically a reduction in late preterm births is still clinically significant since prematurity survivors in this group constitute the majority of cases of neurodevelopmental disability associated with preterm delivery (40), thus, the supplement could potentially be impactful.

Our observation that intervention was associated with a reduction in major post-partum hemorrhage is novel and has not previously been reported with myo-inositol, probiotics or the micronutrients enriched in the supplement used. Since this observation is not explained by differences in cesarean section rates, parity or birth size, this effect may be mediated by other factors such as length of labour, myometrial contractility or blood coagulation, which remains to be examined. Of note, our study found no difference in hypertensive disorders of pregnancy, which is in contrast to a probiotic trial reporting an increased trend of pre-eclampsia (35), possibly due to counteraction by other components in our intervention. However, our result of a lack of effect on hypertensive disorders is consistent with the myo-inositol trials and a vitamin D trial (20) that also reported no difference.

Collectively available data suggest further studies are required to determine if there are sub-populations, dose regimens or intervention commencement time points when myo-inositol and probiotics may lower maternal glycemia. Conversely, there appear to be potential benefit of myo-inositol containing supplements in reducing preterm birth. Whether the other components of our intervention could play an additive role in preterm birth reduction is unclear. Assessment of longitudinal changes in levels of myo-inositol and the other components may shed further light on potential pathways of effect, which may pave the way for the design of more definitive trials in future.

In contrast to most previously published myo-inositol and probiotic trials, major strengths of our study are its double-blind design, and inclusion of multi-ethnic women from three different continents. Nevertheless, generalizability is limited by the lack of Latina and native American Indians, and only a few Black and Polynesian participants, and with less than half of participants being overweight/obese unlike typical US and Western populations, alongside our trial being conducted in high resource settings. Microbiome data were not available to confirm viability of the probiotic in participant samples and sachet counts provide a limited measure of adherence to the intervention; good adherence is, however, suggested by higher plasma 25-hydroxyvitamin D concentrations in the intervention group at 28-weeks’ gestation. Another limitation is that we studied a combination of myo-inositol and probiotics with micronutrients. Previous studies have generally examined these individually or as a less complex formulation (e.g. myo-inositol with vitamin D) (12). We cannot exclude the possibility that constituents of the supplement may have moderated individual effects in lowering maternal glycemia, or that intervening in the general population (versus a high risk population), or commencing intervention preconception (versus early pregnancy) altered the impact on gestational glycemia.

In conclusion, our trial showed that supplementation with myo-inositol, probiotics and multiple micronutrients preconception and in pregnancy did not lower gestational glycemia, but did reduce preterm birth.

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**Table 1:** **Baseline pre-conception characteristics of women who provided a primary outcome.**

|  |  |  |
| --- | --- | --- |
|  | **Control (N=290)** | **Intervention (N=295)**  |
| **Age** (years)  | 30·14 (3·30) | 30·53 (3·40) |
| **BMI** (kg/m2) | 23·75 ( 21·34 to 27·5) | 23·65 (21·16 to 26·23) |
| *Overweight\** | 68 (23·5%) | 89 (30·3%) |
| *Obese\** | 61 (21·0%) | 40 (13·6%) |
| **Ethnic origin** | … | … |
| *White Caucasian* | 167 (57·6%) | 180 (61·0%) |
| *Chinese* | 73 (25·2%) | 72 (24·4%) |
| *South Asian (Indian, Pakistani, Bangladeshi)* | 15 (5·2%) | 15 (5·1%) |
| *Malay* | 12 (4·1%) | 11 (3·7%) |
| *Other (Mixed, Black, Polynesian)* | 23 (7·9%) | 17 (5·8%) |
| **Site** | … | … |
| *New Zealand* † | 116 (40·0%) | 113 (38·3%) |
| *Singapore* | 82 (28·3%) | 84 (28·5%) |
| *United Kingdom* ‡ | 92 (31·7%) | 98 (33·2%) |
| **Nulliparous** | 200 (69·0%)  | 171 (58·0%)  |
| **Smoker** | 12 (4·2%)  | 12 (4·1%)  |
| **Family history of Type 2 diabetes** | 79 (27·2%)  | 56 (19·1%)  |
| **Household Income quintile**  | … | … |
| *5 (lowest)* | 5 (1·7%) | 2 (0·7%) |
| *4* | 20 (6·9%) | 24 (8·1%) |
| *3* | 69 (23·8%) | 54 (18·3%) |
| *2* | 95 (32·8%) | 109 (37·0%) |
| *1 (highest)* | 91 (31·4%) | 92 (31·2%) |
| *Not available*  | 10 (3·5%) | 14 (4·8%) |
| **Preconception plasma glucose (OGTT)** | … | … |
| *Fasting (mmol/L)* | 4·85 (4·52 to 5·18) | 4·85 (4·63 to 5·18) |
| *30 minute (mmol/L)* | 7·81 (6·71 to 8·90) | 7·70 (6·60 to 9·01) |
| *2 hour (mmol/L)* | 5·40 (4·41 to 6·38) |  5·51 (4·63 to 6·27) |

Data are mean (sd), median (lower quartile to upper quartile), or number of women (%)

\*defined using ethnic-specific thresholds for overweight and obesity: BMI ≥23 to <27·5 and ≥27·5 kg/m2, respectively, for Asians including Chinese, Indians, Pakistani, Bangladeshi, Malay, mixed Asian; BMI ≥25 to <30 and ≥30 kg/m2, respectively, for non-Asians including White Caucasian, Polynesian, Black, mixed Asian-non-Asian.

† 72.9% White Caucasian, 16.6% any Asian, 10.5% other

‡ 94.8% White Caucasian, 2.6% any Asian, 2.6% other

**Table 2. Primary outcome of maternal oral glucose tolerance test (OGTT) plasma glucose values at 28 (24-32) weeks’ gestation.**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  |  | **Control** |  | **Intervention** | **Adjusted beta (95% CI)** | **Fully adjusted beta (95% CI)** |
| **OGTT time point** | **N** | **Plasma glucose (mmol/L)** | **N** | **Plasma glucose (mmol/L)** | **for loge glucose\* (loge mmol/L)** | **for loge glucose**†**(loge mmol/L)** |
| **Fasting** | 290 | 4·41 | 295 | 4·30 | -0·004 | 0·0002 |
|  |  | (4·08 to 4·63) |  | (4·08 to 4·63) | (-0·018 to 0·011) | (-0·014 to 0·014)  |
| p value |  | … |  | p=0.55 | p=0.63 | p=0.98 |
| **1-hour** | 283 | 8·02 | 294 | 8·24 | 0·025 | 0·036  |
|  |  | (6·60 to 9·23) |  | (6·93 to 9·45) | ( -0·014 to 0·064) | (-0·003 to 0·074)  |
| p value |  | … |  | p=0.26 | p=0.22 | p=0.07 |
| **2-hour** | 287 | 6·49 | 295 | 6·60 | 0·040 | 0·043 |
|  |  | (5·51 to 7·70) |  | (5·84 to 8·02) | (0·004 to 0·077) | (0·006 to 0·081) |
| p value |  | … |  | p=0.03 | p=0.03 | p=0.02 |

Plasma glucose data are median (lower quartile to upper quartile (IQR); unadjusted).

\*loge glucose at 24-32 weeks adjusted for site, ethnicity and baseline loge glucose (for fasting and 2-hour only, baseline 1-hour glucose not available); N=584 and 578 for fasting and 2-hour glucose respectively, as a result of missing values for corresponding preconception glucose.

†loge glucose at 24-32 weeks adjusted for site, ethnicity, maternal age, pre-pregnancy BMI, preconception smoking, parity, family history of diabetes and baseline loge glucose (for fasting and 2-hour only, baseline 1-hour glucose not available); N=581, 574 and 575 for fasting, 1-hour and 2-hour glucose respectively, as a result of missing data.

All p values (T-tests on loge transformed glucose and linear regressions) were not significant ≥0·017 (a priori statistical significance is p<0·017).

IQR, interquartile range; OGTT, oral glucose tolerance test

**Table 3.** **Secondary outcomes of pregnancy complications, delivery events and neonatal outcomes with the NiPPeR intervention compared with control**

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Control** | **Intervention** | **Effect of Intervention** |
| **PREGNANCY COMPLICATIONS** | … | … | **Risk ratio (95% CI)**† |
| Gestational Diabetes Mellitus\* *(denominator: all those who completed OGTT at 24-32 weeks)* | 64/283 (22·6%) | 73/294 (24·8%) | 1·22 (0·92 to 1·62; N=545) |
| Miscarriages <24 weeks’ gestation *(denominator: all those who became pregnant after the second preconception visit)*  | 51/359 (14·2%) | 50/366 (13·7%) | 0·91 (0·62 to 1·33; N=688) |
| Congenital abnormalities‡ *(denominator: all reaching 7 weeks)* | 16/314 (5·1%) | 15/330 (4·5%) | 0·83 (0·35 to 1·96; N=557) |
| Severe nausea and vomiting of pregnancy§ *(denominator: all reaching 7 weeks)* | 51/305 (16·7%) | 43/322 (13·4%) | 0·86 (0·57 to 1·30; N=553) |
| Hypertensive disorders of pregnancy, both pre-eclampsia|| and pregnancy-induced hypertension *(denominator: all pregnancies reaching 24 weeks or beyond)* | 14/292 (4·8%) | 12/294 (4·1%) | 1·19 (0·55 to 2·59; N=557) |
| **DELIVERY OUTCOMES** *(denominator: all live births ≥24 weeks unless otherwise stated)* | … | … | **Mean difference (95% CI)**# **or Risk ratio (95% CI)**# |
| Gestational age at birth in decimal weeks | 39·2 (1·74) | 39·3 (1·78) | 0·20 (-0·06 to 0·46; N=553) |
| All preterm deliveries (<37 weeks) [Spontaneous labor-onset : Iatrogenic, N:N] | 27/292 (9·2%) [12:15]†† | 17/293 (5·8%) [8:9]‡‡ | 0·43 (0·22 to 0·82; N=553) |
| Late preterm deliveries (34+0 to 36+6 weeks) [Spontaneous labor-onset : Iatrogenic, N:N] | 22/292 (7·5%) [11:11] | 13/293 (4·4%) [6:7] | 0·41 (0·20 to 0·85; N=553) |
| Preterm pre-labor rupture of membranes (PPROM) | 19/280 (6·8%) | 8/277 (2·9%) | 0·39 (0·16 to 0·97; N=526) |
| Preterm deliveries associated with PPROM [Spontaneous labor-onset : Iatrogenic, N:N] | 17/280 (6.1%) [8:9] | 5/277 (1·8%) [2:3] | 0·21 (0·06 to 0·69; N=526) |
| Cesarean section delivery [Elective : Emergency, N:N]  | 85/292 (29·1%) [41:44] | 84/293 (28·7%) [34:50] | 0·99 (0·76 to 1·28; N=553) |
| Major post-partum hemorrhage (>1 liter blood loss, *denominator: all pregnancies reaching ≥24 weeks*) | 24/292 (8·2%) | 9/294 (3·1%) | 0·44 (0·20 to 0·94; N=554) |
| **NEONATAL OUTCOMES** *(denominator: all live births ≥24 weeks)* | … | … | **Mean difference (95% CI)**# **or Risk ratio (95% CI)**# |
| Birthweight (kg) | 3·30 (0·54) | 3·33 (0·55) | 0·05 (-0·03 to 0·13; N=553) |
| Large-for-gestational-age (LGA >90th *centile adjusted for sex and gestational age*\*\*) | 22/292 (7·5%) | 21/293 (7·2%) | 0·94 (0·54 to 1·63; N=555) |
| Small-for-gestational-age (SGA <10th *centile adjusted for sex and gestational age*\*\*) | 21/292 (7·2%) | 24/293 (8·2%) | 1·34 (0·79 to 2·29; N=555) |
| Admission to neonatal unit | 19/290 (6·6%) | 24/293 (8·2%) | 1·11 (0·57 to 2·17; N=550) |
| Neonatal hypoglycemia requiring dextrose treatment | 24/292 (8·2%) | 19/293 (6·5%) | 0·79 (0·43 to 1·48; N=553) |
| Neonatal septicemia (positive blood culture) | 0/287 (0%) | 2/288 (0·7%) | Insufficient to analyze |

Data are mean (sd), or number of women (%), where data is available.

†Adjusted for site, ethnicity, maternal age, preconception BMI, household income level, parity, preconception smoking, preconception baseline fasting glucose, family history of diabetes and offspring’s sex (not applicable for miscarriages).

\*according to IADPSG criteria (fasting glucose ≥5·1 mmol/L or 1-hour glucose ≥10·0 mmol/L or 2-hour glucose ≥8·5 mmol/L) (24); includes only women with complete OGTT data at all 3 time points.

‡includes anomalies in the following categories: in control group 4 cases of karyotypic/multiple anomalies, 2 cardiovascular, 6 genitourinary, 2 respiratory, 2 musculoskeletal; in intervention group 5 cases of karyotypic/multiple anomalies, 3 cardiovascular, 4 genitourinary, 3 musculoskeletal.

§requiringadmission to hospital for intravenous rehydration, with or without significantly deranged biochemistry or weight loss.

||pre-eclampsia defined as hypertension in pregnancy associated with significant proteinuria or evidence of multisystem disorder.

¶pregnancy-induced hypertensiondefined as isolated non-proteinuric hypertension in a previously normotensive woman or aggravation of hypertension during pregnancy.

|| and ¶ There were no differences in incidence for either of these between study groups.

#Adjusted for site, ethnicity, maternal age, preconception BMI, household income level, parity, smoking during pregnancy, offspring sex (except for LGA and SGA) and (where data was available) 28 weeks’ gestation fasting glucose.

\*\*by RCPCH 2009 UK-WHO growth charts(25). Use of respective local population charts, Fenton growth charts, WHO INTERGROWTH-21st charts did not materially alter results.

†† Iatrogenic preterm births include casesof induction of labor and non-labor cesarean section. Indications for iatrogenic delivery in the control group: 5 for PPROM alone, 4 for PPROM plus another indication (previous cesarean section, vasa previa, breech presentation, maternal medical condition), 5 for placental-associated conditions (intrauterine growth restriction (IUGR) with or without pre-eclampsia or placental abruption), 1 maternal medical condition.

‡‡ Indications for iatrogenic delivery in the intervention group: 3 for PPROM alone, 4 for placental-associated conditions (IUGR with or without pre-eclampsia or placental abruption), 1 maternal medical condition, 1 fetal anomaly with breech presentation.

**Figure 1: Consort diagram outlining participant flow.**

\*premature ovarian failure; †new onset Graves’ disease, hemoglobinopathy with iron overload, prolactinoma, endometrial polyp, endometrial atypia, breast cancer; ‡withdrew as product may contain animal remnants, no storage space in fridge, participant suspicion of product-related symptoms; § includes 2 cases of Trisomy 21, Klinefelter Syndrome; ¶ includes hypoplastic left heart, unknown reason in private clinic; # includes one stillbirth and one neonatal death. T2D, Type 2 diabetes mellitus.