**A daily snack containing green leafy vegetables, fruit and milk before and during pregnancy prevented gestational diabetes in a randomized controlled trial in Mumbai, India**

Sirazul A Sahariah, Ramesh D Potdar, Meera Gandhi, Sarah H Kehoe, Nick Brown, Harshad Sane, Patsy J Coakley, Ella Marley-Zagar, Harsha Chopra, Devi Shivshankaran, Vanessa A Cox, Alan A Jackson, Barrie M Margetts, Caroline HD Fall

**Author affiliations:** 1) Centre for the Study of Social Change, Mumbai, India (SAS, RDP, MG, HS, HC, DS); 2) MRC Lifecourse Epidemiology Unit, University of Southampton, Southampton, UK (SK, NB, PJC, EM-Z, VAC, CHDF); 3) NIHR Southampton Biomedical Research Centre, University of Southampton, Southampton, UK (AAJ); 4) Public Health Nutrition, Faculty of Medicine, University of Southampton, Southampton, UK (BMM).

**List of authors as per Pubmed indexing:** Sahariah SA, Potdar RD, Gandhi M, Kehoe SH, Brown N, Sane H, Coakley PJ, Marley-Zagar E, Chopra H, Shivshankaran D, Cox VA, Jackson AA, Margetts BM, Fall CHD.

**Author for correspondence:** Caroline HD Fall, MRC Lifecourse Epidemiology Unit, University of Southampton, Southampton General Hospital, Tremona Road, Southampton, UK, SO16 6YD. Tel: 00 44 2380 777624; Fax: 00 44 2380 704021; e-mail: chdf@mrc.soton.ac.uk

**Word count (abstract through references): 5,343**

**Figures (not OSM): 1**

**Tables (not OSM): 3**

**OSM Tables: 4**

**OSM Figures: 1**

**Running title:** Daily micronutrient-rich snack prevents gestational diabetes

**Conflicts of interest:** None of the authors has any conflicts of interest to declare

**Funding disclosure:** The trial was funded by the Wellcome Trust, the Medical Research Council (UK), the Department for International Development, UK; the Parthenon Trust, Switzerland, and ICICI Bank Ltd. Social Initiatives Group, Mumbai, India. None of the funders played a role in the design, conduct or analysis of the data.

**Footnote to the title:** Supplemental Tables 1-4, Supplemental Figure 1 and the trial protocol are available from the “Online Supporting Material” link in the online posting of the article and from the same link in the online table of contents at [jn.nutrition.org](http://jn.nutrition.org/).

The MMNP Study Protocol is not included in the manuscript text due to length but is available as a supplemental file for review purposes.

**Abbreviations used:**

**GDM** Gestational diabetes

**IADPSG** International Association of Diabetes and Pregnancy Study Groups

**ISRCTN** International Standard Randomized Controlled Trial Registry Number

**LMP** Last menstrual period

**OGTT** Oral glucose tolerance test

**SLI** Standard of Living Index

**WHO** World Health Organization

**Abstract**

**Background:** Prospective observational studies suggest that maternal diets rich in green leafy vegetables and fruit may help to prevent gestational diabetes (GDM).

**Objective:** Our objective was to test whether increasing women’s dietary intakes of green leafy vegetables, fruit and milk before conception and throughout pregnancy reduced their risk of GDM.

**Methods:** Project SARAS (2006-2012) was a non-blinded individually randomized controlled trial among women living in slums in the city of Mumbai, India. The interventions were a daily snack made from (treatment group) green leafy vegetables, fruit and milk or (controls) low micronutrient vegetables (potato, onion) in addition to usual diet. Results for the primary outcome, birth weight, have been reported. Women were invited for an oral glucose tolerance test (OGTT) at 28-32 weeks gestation to screen for GDM (WHO 1999 criteria). The prevalence of GDM was compared between intervention and control groups, and Kernel density analysis was used to compare distributions of 120-minute plasma glucose concentrations between groups.

**Results:** Of 6513 women randomized, 2291 became pregnant, of whom 2028 reached 28 weeks gestation, 1008 (50%) attended for an OGTT, and 100 (9.9%) had GDM. In an intention to treat analysis, the prevalence of GDM was reduced in the treatment group (7.3% v 12.4% among controls, OR 0.56 [95%CI 0.36, 0.86], p=0.008). The reduction in GDM remained significant after adjusting for pre-pregnancy adiposity and fat or weight gain during pregnancy. Kernel density analysis showed that it was explained by fewer women in the treatment group having a 2-hour glucose concentration in the range 7.5-10.0 mmol/L.

**Conclusions:** In low-income settings, where women have low intakes of micronutrient-rich foods, improving dietary micronutrient quality by increasing intakes of green leafy vegetables, fruit and/or milk may have an important protective effect against the development of gestational diabetes.The clinical trial registration number wasISRCTN 62811278.

**Key words:** Randomized controlled trial, Food-based supplement; Green leafy vegetables; Fruit; Milk; Micronutrients; Pregnancy; Gestational diabetes; India

**INTRODUCTION**

Gestational diabetes mellitus (GDM) is a common disorder of pregnancy associated with increased risks for the mother (obstructed labour and later type 2 diabetes) and baby (congenital malformations, macrosomia and neonatal hypoglycemia). Offspring of mothers with GDM have an increased risk of developing adult obesity and type 2 diabetes (T2DM) (1). Risk factors for GDM are similar to those for T2DM (older age and greater adiposity) and the prevalence is rising everywhere (2). The Hyperglycemia and Adverse Pregnancy Outcomes (HAPO) study showed that the complications of GDM increase linearly across the range of plasma glucose values, leading to debate about the best clinical criteria for diagnosing GDM (3,4). Although improved management of GDM reduces obstetric complications (5), treatment can be onerous, and it is unknown whether long-term complications in the offspring are reduced (6). Strategies to prevent the disease are therefore needed.

Observational studies suggest that maternal diet may influence GDM risk (7-9). Lower intakes of saturated fat, red or processed meat, refined grains and sweets, and higher intakes of fiber, fruit, vegetables, poultry and fish, before or during pregnancy, are associated with a lower prevalence (7,8). Higher plasma vitamin B-12, C and D concentrations have also been associated with lower risk (7,9). There is similar evidence for T2DM (10,11), and recent prospective studies have shown that higher intakes of dairy products (12) and green leafy vegetables (13,14) predict lower risk. Observational studies are subject to confounding and these findings need to be tested in randomized intervention studies. However, most dietary trials to prevent GDM have focused on weight gain reduction in pregnancy, with little impact (15,16).

South Asians are at high risk of GDM and studies in India have recorded prevalence rates of 6-17% in urban populations (17). “Project SARAS” (“excellent”) was a randomized controlled trial in India, in which women were supplemented with a daily snack made from green leafy vegetables, fruit and milk, pre-conceptionally and throughout pregnancy. The primary objective was to increase birth weight. In the intention to treat analysis, there was no overall increase in birth weight; however, there was an interaction (p<0.001) with maternal pre-pregnant body mass index (BMI) such that birth weight increased by 63g (95% CI 11,115) in the treatment group compared with controls among mothers of normal of high pre-pregnancy BMI (>18.5 kg/m2) (18). Women were offered an oral glucose tolerance test (OGTT) at 28-32 weeks gestation, because, although not a primary outcome, GDM status was an important co-variate for the interpretation of supplementation effects on birth weight. The OGTT data enabled us to test whether the intervention benefited maternal metabolism as assessed by glucose tolerance.

**METHODS**

*Setting and participants*

The trial took place from 2006 to 2012 in slums in the city of Mumbai, India (18). Women were eligible if aged <40 years, married, non-pregnant, not sterilized, planning to have more children and intending to deliver in Mumbai.

*Intervention*

The intervention was a daily snack resembling local street foods like samosas and fritters, prepared fresh each day and fried in sunflower oil. Treatment snacks contained green leafy vegetables in fresh (~30g) or dried (~7.5g) form, full-fat milk powder (12-16g) and dried fruits (4-60g) (**Supplemental Tables 1 and 2**). Control snacks were made from low-micronutrient vegetables such as potato and onion. To avoid monotony, we created multiple recipes from these foods (**Supplemental Table 3**). On average, treatment snacks contained 10-23% of the WHO/FAO recommended Reference Nutrient Intake for β-carotene, riboflavin, folate, vitamin B-12, calcium and iron; they contained 0.69MJ of energy and 6.4g of protein compared with 0.37MJ and 2.4g in control snacks (Supplemental Table 2).

*Recruitment, baseline investigations and randomization*

Women were screened for eligibility and individual written informed consent was obtained. We recorded education, occupation, and socio-economic status using the Standard of Living Index (SLI) (21). Tobacco use was recorded. Diet was assessed using a food frequency questionnaire, with the reference period the preceding week (22). Weight, height, and triceps and subscapular skinfolds were measured using standardized techniques. Women were individually randomized, stratified by age and BMI (3 groups for each) (18).

*Blinding*

Full blinding is impossible in a food-based trial. Although treatment and control snacks were outwardly similar, their contents looked different. To obscure allocation, we created two treatment and two control groups, each with its own recipes, which were merged for the analysis. Four different snacks were therefore produced daily. Staff who measured outcomes were blind to the women’s allocation group.

*Supplementation*

Snacks were produced daily except on holidays, packaged in color-coded bags, and transported to 61 supplementation centres. Women were asked to maintain their usual diet, and snacks were available 3-6pm, to interfere least with meals. Women were offered one snack per day, and consumption was observed and recorded. Centre staff recorded women’s serial last menstrual period (LMP) dates. Compliance was defined as an average of >3 snacks/week from 90 days before the LMP date until delivery.

*Pregnancies and oral glucose tolerance tests*

Women who became pregnant were prescribed iron (100mg) and folic acid (500μg) according to national guidelines. At 9-13 weeks gestation, blood was collected for hemoglobin and plasma vitamin B-12 and folate. Plasma cobalamin (B-12) and folate were measured using microbiological assays (23-26). At 28-32 weeks gestation, women were offered an oral glucose tolerance test (OGTT). Venous blood was collected after an overnight fast and 120 minutes after 75 g anhydrous glucose orally in water. Glucose samples were collected into fluoride tubes and analysed by autoanalyzer (ERBA EM200, Transasia) in a single Mumbai laboratory within 6 hours of venesection. Fasting insulin samples were placed on ice and centrifuged within 3 hours; plasma aliquots were stored at -80OC until analysis using ELISA kits (Mercodia Ultrasensitive Insulin kits, Mercodia AB, SE-754 50 Uppsala, Sweden) with inter- and intra-assay coefficients of variation <7%. GDM was diagnosed according to WHO 1999 criteria (fasting glucose >7.0 mmol/L (126 mg/dl) and/or 120-minute glucose >7.8 mmol/L (140mg/dl)) (27). Women were referred to their own obstetricians for further GDM management.

*Deliveries*

Trained research nurses measured birth weight. Gestational age was calculated from the LMP date unless different by >+14 days from that estimated by <20 week ultrasound scan (9%), when the latter was used (28).

*Outcomes*

Outcomes for this analysis were GDM, fasting and 120-minute glucose concentrations and fasting insulin concentration. During the trial we used the 1999 WHO definition of GDM (27). In 2013, the definition changed (29) to match the International Association of Diabetes and Pregnancy Study Groups (IADPSG) Consensus Panel recommendations (30) as follows: fasting glucose 5.1-6.9 mmol/L (92-125 mg/dl) and/or a 120-minute glucose of 8.5-11.0 mmol/L (153-199 mg/dL) and a category of ‘diabetes in pregnancy’ was introduced (fasting glucose >7.0 (126 mg/dL) and 120-minute glucose >11.1 mmol/L (200 mg/dL)). We report results for both 1999 (27) and 2013 (29) criteria.

*Ethical approval and governance*

The trial (ISRCTN 62811278) was approved by the ethics committees of BYL Nair and TN Medical College, Grant Medical College, and Sir JJ Group of Hospitals, Mumbai, and Southampton and SW Local Research Ethics Committees. An independent Data Monitoring Committee reviewed data on compliance, completeness of follow-up, pregnancy outcomes and adverse events 6-monthly for the first three years of the trial and then 12-monthly.

*Statistical methods*

We compared baseline measurements between women who did and did not have an OGTT, and between allocation groups. We compared outcomes between allocation groups in all women who were randomized, became pregnant and had an OGTT (intention to treat analysis) and limited to women who started supplementation >90 days before their last menstrual period date (per protocol analysis) (**Supplemental** **Figure 1**). We tested for interactions between allocation group and maternal age, BMI, height and parity. Small-for-gestational age and large-for-gestational age births were defined according to Oken et al. (31) and also ‘within-cohort’ as <10th and >90th percentile based on singleton live births without major congenital abnormalities. Pre-term birth was defined as gestation <37 weeks. T-tests, Mann-Whitney U tests and Chi-Squared or Fisher’s Exact tests were used to compare groups for normally distributed continuous, non-parametric and categorical variables respectively; further comparisons of glucose concentrations between groups were made using Kernel density estimates. Main results are reported unadjusted; we then used multiple regression to assess intervention effects on GDM adjusting for maternal age, adiposity (subscapular skinfold thickness and/or weight at recruitment and subscapular skinfold gain and/or weight gain from recruitment to 28 weeks gestation), height, parity, socio-economic status and compliance. Analysis was performed using STATA (v13.0, StataCorp, Texas).

**RESULTS**

A total of 6513 non-pregnant women participated in the trial, of whom 2291 became pregnant (Supplemental Figure 1). Of these 241 had either an abortion or termination of pregnancy before 28 weeks gestation, and we lost contact with 22 women. Of the remaining 2028, 1008 (50%) attended for an OGTT at a median (IQR) gestation of 29.7 (29.3, 30.4) weeks. Women who did not have an OGTT were younger and of lower socio-economic status and parity than women who attended (**Table 1**). However, there were only small differences in characteristics between allocation groups; among women who had an OGTT, those in the treatment group had a lower baseline weight, BMI and subscapular skinfold thickness than controls, and thinner skinfolds at visit 1, but greater pregnancy weight gain (Table 1).

The prevalence of GDM (WHO 1999 criteria) (27) was 9.9%. Both in the intention to treat and per protocol analyses, the prevalence was lower in the treatment group (intention to treat: 7.3% v 12.4%, p for difference=0.008, OR 0.56 [95%CI 0.36,0.86]; per protocol: 7.5% v 13.1%, p=0.01, OR 0.54 [95%CI 0.33,0.86]) (**Table 2**). This effect was independent of baseline and 28 week skinfold measurements (**Table 3**) or baseline and 28-week weight, or all of these measures combined.

There was no difference between treatment and control groups when using the WHO 2013 GDM criteria (29) (intention to treat analysis: 8.9% v 11.1%, p=0.27, OR 0.79 [95%CI 0.52,1.20]; per protocol: 9.1% v 11.2%, p=0.32, OR 0.79 [95%CI 0.50,1.26) or diabetes in pregnancy criteria (Table 2). Nor were there significant differences between allocation groups in mean fasting or 120-minute glucose concentrations, or fasting insulin concentration. A Kernel density analysis (**Figure 2**) explained these findings, and the discrepancy between 1999 (27) and 2013 (29) criteria. There were more control women than treatment women with 120-minute glucose concentrations in the range 7.5-10 mmol/L (p=0.06 for heterogeneity in frequencies among three glucose groups <7.5; 7.5-10.0; >10.0). Frequencies of normal or very high glucose concentrations were similar in both allocation groups (Table 2)

There were no significant interactions between allocation group and maternal age, BMI, height or parity in relation to any outcome.

Women who developed GDM were older and more adipose than women who did not (**Supplemental Table 4**, 1999 criteria). They had similar pre-pregnancy intakes of green leafy vegetables, fruit and milk. Overall, 25% of women were vegetarian (ate no meat or fish); women who developed GDM ate non-vegetarian foods more frequently than women who did not develop GDM. One third of women were anemic in early pregnancy and 17% were vitamin B-12 deficient, while only 1% were folate deficient; there were no differences in the prevalence of anemia, or B-12 or folate deficiency between women who did and did not develop GDM. There were more pre-term births in the GDM group (p=0.002) and fewer small-for-gestational-age births, and more congenital anomalies and emergency Cesarean sections (all borderline significant ~p=0.1). Findings were similar for the 2013 criteria.

**DISCUSSION**

In a large randomized controlled trial among women living in Mumbai slums, a daily micronutrient-rich snack starting pre-conceptionally and continuing throughout pregnancy almost halved the prevalence of gestational diabetes according to WHO 1999 criteria (27) (OR 0.56 [95%CI 0.36, 0.86]). The effect was independent of maternal adiposity. There was no effect on GDM diagnosed according to WHO 2013 criteria (29).

Strengths of the trial were individual randomization, supervised supplementation, LMP and ultrasound dating of pregnancies, and standardized OGTTs. There were a number of important limitations to the GDM data. Only half the women chose to have an OGTT. This resulted in a fairly small sample size (100 cases of GDM) and could have biased the results. The main reason for women not attending for the OGTT was that blood testing is greatly disliked, and while many obstetricians were pleased to accept our OGTT results, others preferred to carry out their own, in which case women were understandably reluctant to have another OGTT with our research team. We did not use the results of other OGTTs, because of the variety of protocols and laboratories used. We do not have reliable data on history of GDM in earlier pregnancies; it is possible that women with a prior history of GDM were more likely to attend for an OGTT than those with no previous GDM history. We did not take blood one hour after the glucose load, and so it is possible that we missed some cases of GDM according to the 2013 diagnostic criteria (29). However, all these issues would be expected to affect both allocation groups equally, and so we think they are unlikely to have created spurious or biased results. The prevalence of GDM in our study was well within the range expected for an urban Indian population (2,17). Women who had an OGTT were older, and of higher parity and socio-economic status than women who did not, and women in the treatment group were slightly lighter and less adipose pre-conceptionally than controls, but gained more weight during pregnancy. However, adjusting for these factors did not alter our findings. Because it was food-based, full blinding of our intervention was not possible. However, laboratory staff were blind to the women’s allocation, and it is difficult to see how lack of blinding could alter the women’s behaviour in ways that would reduce GDM so markedly. Due to funding constraints, we had limited information on the women’s micronutrient status, which limits our ability to suggest mechanisms for the reduction in GDM. We measured only vitamin B-12 and folate, which have been linked to birth weight in Indian populations (32-34). We did, however, carry out a separate study among non-pregnant women in a similar slum community in Mumbai, using the same supplements, specifically to measure a range of micronutrients before and after 3 months of supplementation (vitamin C, β-carotene, retinol, ferritin, folate, vitamin B-12, and homocysteine) and found that, of these, only β-carotene increased (35). It would also have been useful to record women’s physical activity to determine if this was part of the mechanism for the reduction of GDM. We have to consider the possibility that the intervention did not prevent GDM in the treatment group, but that the control snacks, which were lower in protein, increased the risk of GDM. This seems unlikely, because the control snacks contained less energy than the intervention snacks, and control women did not gain more fat than intervention women. Our results, in a predominantly vegetarian population, with very low baseline intakes of green leafy vegetables, fruit and milk, and in which the daily snack made a substantial difference to intakes of these foods, may not be generalizable to more affluent, non-vegetarian populations with a more diverse habitual diet. Neither was our trial designed to determine whether starting supplementation before conception rather than during pregnancy was important.

As far as we know, this is the first randomized trial in which it was possible to examine the effect of micronutrient-rich foods on GDM risk, albeit as a secondary outcome. The effect was large, translating to a number needed to treat of 20 (intention to treat analysis, 1999 criteria). Observational studies have shown that higher polyunsaturated to saturated dietary fat ratio, higher carbohydrate relative to fat intake, and higher vitamin B-12, C and D status are associated with a lower risk. The US Nurse’s Study found that higher ‘prudent diet’ scores (higher intakes of fruit, green leafy vegetables, poultry and fish) predicted lower risk (relative risk in lowest intake quartile=1.37 [95%CI 1.09, 1.72]) (36). There is similar prospective evidence of protection against T2DM (10-14,37,38). Two reviews of specific food groups found no relationship of *total* fruit or vegetable intake with T2DM risk, but a sub-group of studies that gave separate information for green leafy vegetable intakes showed an approximately 14% lower risk of developing T2DM in highest compared with lowest intake categories (13,14).

We do not know which constituent(s) of the snacks produced the effect. The main differences between the snacks were the fillings (green leafy vegetables, fruit and milk in the intervention snacks, compared with low micronutrient vegetables in the controls). Other nutrients in the snacks came from the covering/binding ingredients and the cooking oil, which were similar in both groups, though the former were greater in quantity in the intervention snacks, resulting in 0.32 MJ more energy on average and 4g more protein per snack. Green leafy vegetables contain anti-oxidants beta-carotene, vitamin C and polyphenols. However these have not prevented T2DM in randomized trials (39). They are rich in magnesium, higher intakes of which have been associated with a lower risk of T2DM (40) and reduce fasting glucose in trials (41). The effect may be from fatty acids; green leafy vegetables are a rich source of long-chain polyunsaturated omega-3 fatty acids, which may improve insulin sensitivity by influencing the properties of cell membranes (42). Green leafy vegetables also contain nitrates, which increase thermogenesis, oxygen consumption and β-oxidation in rat adipocytes (43). The association of higher dairy intakes with lower risk of future T2DM has been attributed to calcium, vitamin D or whey protein (12). The snack format for this trial was chosen for pragmatic reasons after piloting various preparations of the key foods. The fried snacks could be individually packaged, preventing contamination, and remained palatable after transportation to the supplementation centres. If green leafy vegetables, fruit and milk, or any one of these foods, were the effective agent(s), we would not necessarily advocate their delivery in the form of a fried snack.

The different results from the 1999 (27) and 2013 (29) diagnostic criteria were explained by fewer women in the treatment group having 120-minute glucose values in a middle ‘impaired glucose tolerance’ range than controls (Figure 1). Glucose measurements below and above this range, and mean glucose values, were similar in both allocation groups. The WHO 1999 criteria defined GDM using the same glucose cut-offs as for impaired glucose tolerance in the non-pregnant state (27). The WHO 2013 criteria were based on 1) plasma glucose cut-off values associated with an odds ratio of 1.75 (compared with mean values) for birth weight, newborn adiposity and cord blood C-peptide above the >90th percentile, and 2) a simulation exercise suggesting that smaller numbers would need to be screened to prevent adverse outcomes (29). However, it is recognized that the cut-offs are still to some extent arbitrary (29,44) because the adverse maternal and neonatal outcomes increase linearly across the range of glucose concentrations, with no apparent thresholds (3,4). Our interpretation of our findings is that the supplement had no effect on glucose concentrations in women with normal glucose tolerance or in those with established diabetes in pregnancy, but that there was an intermediate group of women who were vulnerable to diabetes and whose metabolic competence was improved by the supplement. Our findings add to the debate about diagnostic cut-offs, and perhaps make a case for maintaining the impaired glucose tolerance range within the criteria for GDM.

We measured maternal vitamin B-12 and folate status because of data showing a high prevalence of vitamin B-12 deficiency among pregnant Indian women, especially in rural communities, thought to result from vegetarianism (45,46), and an association between vitamin B-12 deficiency and GDM (9). There was no difference, however, in vitamin B-12 status in early pregnancy between the intervention and control groups, and no association between vitamin B-12 status and GDM in our study.

In conclusion, the results of this randomized controlled trial suggest that improving women’s dietary micronutrient quality may have important protective effects against GDM. Because GDM was not the trial’s primary outcome and because of 50% non-participation for the OGTT, the findings would need to be replicated. However, they are consistent with observational research showing a lower risk of GDM and T2DM in association with the foods contained in the trial supplements.

**ACKNOWLEDGEMENTS**

We thank DMC members Suhas Otiv (Chair), Christopher Roberts (Statistician), Armida Fernandez, Lakshmi Lingam and Hemu Adikari; Steering Committee chairman Harsh Pal Singh Sachdev and Jeya Henry (supplement development) for their expert advice; and Chittaranjan Yajnik and DS Bhat in the Diabetes Unit, KEM Hospital, Pune for B-12, folate and insulin assays. We are grateful to the late Professor David Barker for his advice to and support for the study. We acknowledge the academic support of the Women of India Network and Sneha-India.

*Author contributions:* RDP, CHDF, NB, BMM, and AAJ designed the research. SAS, MG, RDP, DS, SHK, HC, HS, PJC and VAC conducted the research. EM-Z analysed the data. SAS and CHDF wrote the paper; all authors have read and approved the final manuscript. CHDF had primary responsibility for the final content.

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**Figure Legend**

**Figure 1:** Kernel density plot of 120-minute plasma glucose concentrations in the treatment and control groups (intention to treat analysis) and WHO cut-off values for gestational diabetes

**Table 1: Comparison of baseline characteristics between women who did and did not attend for a glucose tolerance test and between treatment and control groups**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | *n* | ATTENDED FOR OGTT*n* = 1008 |  | *n* | DID NOT ATTEND FOR OGTT*n* = 1020 | p |  | ATTENDED FOR OGTT |  | DID NOT ATTEND FOR OGTT |
|  |  |  | TREATMENT*n*=492 | CONTROL*n*=516 |  | TREATMENT*n*=497 | CONTROL*n*=523 |
|  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| At recruitment (pre-pregnancy) |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Age, years | 1008 | 24.0 [21.0, 27.0] |  | 1020 | 23.0 [21.0, 26.0] | <0.001 |  | 24.0 [21.0, 27.0] | 25.0 [22.0, 27.0] |  | 23.0 [21.0, 26.0] | 23.0 [21.0, 26.0] |
| Weight, kg | 1008 | 45.6 [40.0, 51.9] |  | 1019 | 45.4 [40.3, 51.1] | 0.78 |  | 45.1 [39.3, 51.3]\* | 46.1 [40.8, 52.7] |  | 45.5 [40.4, 51.7] | 45.4 [40.3, 50.8] |
| Height, cm1 | 1008 | 151.3 + 5.4 |  | 1019 | 151.4 + 5.5 | 0.59 |  | 151.3 + 5.6 | 151.3 + 5.2 |  | 151.5 + 5.5 | 151.4 + 5.5 |
| Body mass index, kg/m2 | 1008 | 19.8 [17.8, 22.6] |  | 1018 | 19.7 [17.9, 22.3] | 0.71 |  | 19.6 [17.7, 22.3]\* | 20.1 [17.9, 22.8] |  | 19.8 [17.7, 22.6] | 19.6 [17.9, 22.0] |
| Subscapular skinfold, mm | 1008 | 21.3 [15.2, 29.1] |  | 1020 | 21.0 [15.3, 27.6] | 0.30 |  | 20.7 [14.4, 27.6]\* | 21.6 [16.2, 30.2] |  | 21.4 [15.4, 28.4] | 20.4 [15.3, 27.2] |
| Parity2 0 | 1008 | 304 (30.2) |  | 1020 | 364 (35.7) | 0.01 |  | 155 (31.5) | 149 (28.9) |  | 197 (39.6) | 167 (31.9) |
|  1 |  | 513 (50.9) |  |  | 451 (44.2) |  |  | 253 (51.4) | 260 (50.4) |  | 208 (41.9) | 243 (46.5) |
|  >1 |  | 191 (18.9) |  |  | 205 (20.1) |  |  | 84 (17.1) | 107 (20.7) |  | 92 (18.5) | 113 (21.6) |
| Tobacco user2 | 1008 | 85 (8.4) |  | 1020 | 93 (9.1) | 0.59 |  | 45 (9.1) | 40 (7.8) |  | 40 (8.0) | 53 (10.1) |
| Standard of living index | 978 | 25.0 [21.0, 30.0] |  | 990 | 25.0 [21.0, 29.0] | 0.004 |  | 26.0 [21.0, 30.0] | 25.0 [21.0, 29.0] |  | 25.0 [21.0, 29.0] | 25.0 [20.0, 29.0] |
| Religion3 Hindu | 1008 | 732 (72.6) |  | 1020 | 707 (69.3) | 0.22 |  | 355 (72.2) | 377 (73.1) |  | 353 (71.0) | 354 (67.7) |
|  Muslim |  | 241 (23.9) |  |  | 278 (27.3) |  |  | 117 (23.8) | 124 (24.0) |  | 131 (26.4) | 147 (28.1) |
|  Other |  | 35 (3.5) |  |  | 35 (3.4) |  |  | 20 (4.1) | 15 (2.9) |  | 13 (2.6) | 22 (4.2) |
| Education2  | 1006 |  |  |  | 1020 |  |  | 0.07 |  |  |  |  |  |  |  |  |  |  |
|  Primary or less |  | 84 (8.3) |  |  | 116 (11.4) |  |  | 45 (9.2) | 39 (7.6) |  | 63 (12.7) | 53 (10.1) |
|  Secondary |  | 867 (86.2) |  |  | 853 (83.6) |  |  | 420 (85.5) | 447 (86.8) |  | 409 (82.3) | 444 (84.9) |
|  Graduate |  | 55 (5.5) |  |  | 51 (5.0) |  |  | 26 (5.3) | 29 (5.6) |  | 25 (5.0) | 26 (5.0) |
| Occupation2  | 1008 |  |  |  | 1020 |  |  | 0.001 |  |  |  |  |  |  |  |  |  |  |
|  Semi-skilled/Unskilled |  | 194 (19.2) |  |  | 135 (13.2) |  |  | 95 (19.3) | 99 (19.2) |  | 62 (12.5) | 73 (14.0) |
|  Skilled/self-employed |  | 34 (3.4) |  |  | 28 (2.7) |  |  | 13 (2.6) | 21 (4.1) |  | 14 (2.8) | 14 (2.7) |
|  Professional |  | 23 (2.3) |  |  | 16 (1.6) |  |  | 12 (2.4) | 11 (2.1) |  | 6 (1.2) | 10 (1.9) |
|  Not working/Other |  | 757 (75.1) |  |  | 841 (82.5) |  |  | 372 (75.6) | 385 (74.6) |  | 415 (83.5) | 426 (81.5) |
| First language2  | 1005 |  |  |  | 1019 |  |  | <0.001 |  |  |  |  |  |  |  |  |  |  |
|  Marathi |  | 588 (58.5) |  |  | 501 (49.2) |  |  | 290 (58.9) | 298 (58.1) |  | 245 (49.4) | 256 (48.9) |
|  Hindi |  | 324 (32.2) |  |  | 412 (40.4) |  |  | 158 (32.1) | 166 (32.4) |  | 196 (39.5) | 216 (41.3) |
|  Other |  | 93 (9.3) |  |  | 106 (10.4) |  |  | 44 (8.9) | 49 (9.6) |  | 55 (11.1) | 51 (9.8) |
| Dietary intake (frequency/week)2 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Milk <1 |  | 475 (47.1) |  |  | 514 (50.4) | 0.18 |  | 241 (49.0) | 234 (45.3) |  | 249 (50.1) | 265 (50.7) |
|  1-6 |  | 396 (39.3) |  |  | 360 (35.3) |  |  | 191 (38.8) | 205 (39.7) |  | 169 (34.0) | 191 (36.5) |
|  ≥7 |  | 137 (13.6) |  |  | 146 (14.3) |  |  | 60 (12.2) | 77 (14.9) |  | 79 (15.9) | 67 (12.8) |
| Green leafy vegetables <1 |  | 236 (23.4) |  |  | 252 (24.7) | 0.76 |  | 121 (24.6) | 115 (22.3) |  | 124 (24.9) | 128 (24.5) |
|  1-6 |  | 746 (74.0) |  |  | 740 (72.5) |  |  | 357 (72.6) | 389 (75.4) |  | 358 (72.0) | 382 (73.0) |
|  ≥7 |  | 26 (2.6) |  |  | 28 (2.7) |  |  | 14 (2.8) | 12 (2.3) |  | 15 (3.0) | 13 (2.5) |
| Fruit <1 |  | 161 (16.0) |  |  | 176 (17.3) | 0.64 |  | 73 (14.8) | 88 (17.1) |  | 84 (16.9) | 92 (17.6) |
|  1-6 |  | 692 (68.7) |  |  | 681 (66.8) |  |  | 335 (68.1) | 357 (69.2) |  | 333 (67.0) | 348 (66.5) |
|  ≥7 |  | 155 (15.4) |  |  | 163 (16.0) |  |  | 84 (17.1) | 71 (13.8) |  | 80 (16.1) | 83 (15.9) |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| At visit 1 (median [IQR] gestation 10.1 [9.4, 12.0] weeks) |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |
| Weight, kg | 861 | 46.9 [41.3, 53.5] |  | 625 | 46.2 [40.7, 52.3] | 0.21 |  | 46.4 [40.1, 53.1] | 47.5 [41.9, 53.8] |  | 46.2 [40.9, 53.0] | 46.2 [40.6, 51.5] |
| Triceps skinfold, mm | 898 | 13.9 [9.6, 18.2] |  | 656 | 13.1 [9.2, 17.4] | 0.04 |  | 13.3 [9.2, 18.0]\* | 14.2 [10.4, 18.2] |  | 13.4 [9.4, 17.3] | 12.7 [9.0, 17.4] |
| Subscapular skinfold, mm | 898 | 21.6 [15.7, 28.6] |  | 656 | 20.7 [14.8, 27.5] | 0.04 |  | 21.2 [15.2, 28.5]\* | 22.6 [16.4, 28.7] |  | 21.7 [15.2, 27.6] | 20.0 [14.5, 27.3] |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| At Visit 3 (median [IQR] gestation 29.7 [29.3, 30.7] weeks) |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |
| Weight, kg | 957 | 52.7 [47.5, 59.1] |  | 379 | 51.8 [46.9, 58.5] | 0.24 |  | 52.4 [46.6, 59.1] | 52.8 [48.0, 59.0] |  | 52.5 [46.9, 59.4] | 51.7 [47.0, 57.8] |
| Weight gain from recruitment, kg1 | 957 | 7.2 + 3.9 |  | 379 | 7.3 + 3.9 | 0.74 |  | 7.4 + 3.9\* | 6.9 + 3.9 |  | 7.2 + 3.6 | 7.3 + 4.1 |
| Triceps skinfold, mm | 991 | 14.4 [10.6, 19.3] |  | 399 | 13.3 [10.2, 18.2] | 0.007 |  | 14.2 [10.3, 19.1] | 14.7 [11.0, 19.4] |  | 13.4 [10.1, 18.2] | 13.3 [10.3, 18.1] |
| Triceps gain from recruitment, mm1 | 991 | 0.5 + 4.7 |  | 399 | -0.1 + 4.5 | 0.05 |  | 0.7 + 4.4 | 0.3 + 4.8 |  | -0.5 + 4.5 | 0.3 + 4.6 |
| Triceps gain from visit 1, mm1 | 885 | 1.0 + 3.6 |  | 321 | 0.3 + 3.8 | 0.007 |  | 1.0 + 3.4 | 0.9 + 3.7 |  | 0.0 + 3.6 | 0.6 + 4.0 |
| Subscapular skinfold, mm | 991 | 23.4 [17.8, 29.4] |  | 399 | 21.7 [17.0, 28.5] | 0.03 |  | 22.9 [17.5, 28.5] | 23.6 [18.4, 29.7] |  | 21.7 [17.0, 29.4] | 21.7 [17.1, 27.1] |
| Subscapular gain from recruitment, mm1 | 991 | 1.2 + 7.5 |  | 399 | 0.8 + 7.2 | 0.43 |  | 1.4 + 7.5 | 1.0 + 7.4 |  | 1.0 + 7.1 | 0.6 + 7.2 |
| Subscapular gain from visit 1, mm1 | 885 | 1.6 + 5.6 |  | 321 | 0.8 + 5.3 | 0.03 |  | 1.5 + 5.6 | 1.6 + 5.5 |  | 0.9 + 5.4 | 0.7 + 5.2 |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |

Values are medians [IQR]; 1 Values are mean + SD; 2 Values are n (%). \* Different from control, P<0.05. IQR=inter-quartile range. OGTT=oral glucose tolerance test.

**Table 2: Prevalence of gestational diabetes and mean glucose and insulin concentrations according to allocation group**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  |  TREATMENT GROUP |  |  CONTROL GROUP |  |  |
|  | *n* |  |  | *n* |  |  | P |
|  |  |  |  |  |  |  |  |  |  |
| Intention to treat analysis (all women who became pregnant and attended for an OGTT) |
|  |
| ALL WOMEN |  |  |  |  |  |  |  |  |  |
| Gestational diabetes, WHO 1999 (27)1 | 492 | 36 (7.3) |  | 516 | 64 (12.4) |  | 0.007 |
| Gestational diabetes, WHO 2013 (29)1 | 492 | 44 (8.9) |  | 516 | 57 (11.1) |  | 0.27 |
| Diabetes in pregnancy, WHO 2013 (29)1 | 492 | 6 (1.2) |  | 516 | 3 (0.6) |  | 0.33 |
| Fasting glucose, mmol/L | 492 | 4.21 [3.99, 4.55] |  | 516 | 4.20 [3.95, 4.58] |  | 0.40 |
| Fasting insulin, IU/L | 481 | 6.00 [4.00, 9.10] |  | 508 | 6.05 [4.15, 9.10] |  | 0.61 |
| 120-minute glucose, mmol/L | 484 | 5.66 [4.98, 6.54] |  | 512 | 5.73 [5.01, 6.70] |  | 0.14 |
|  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |
| Per protocol analysis (sub-group of women who started supplementation at least 3 months before conception) |
|  |  |  |  |  |  |  |  |  |  |
| ALL WOMEN |  |  |  |  |  |  |  |  |  |
| Gestational diabetes, WHO 1999 (27)1 | 375 | 28 (7.5) |  | 420 | 55 (13.1) |  | 0.01 |
| Gestational diabetes, WHO 2013 (29)1 | 375 | 34 (9.1) |  | 420 | 47 (11.2) |  | 0.32 |
| Diabetes in pregnancy, WHO 2013 (29)1 | 375 | 4 (1.1) |  | 420 | 3 (0.7) |  | 0.71 |
| Fasting glucose, mmol/L | 375 | 4.19 [3.97, 4.51] |  | 420 | 4.20 [3.95, 4.58] |  | 0.97 |
| Fasting insulin, IU/L | 365 | 6.00 [4.00, 8.90] |  | 412 | 6.00 [4.20, 9.20] |  | 0.43 |
| 120-minute glucose, mmol/L | 370 | 5.64 [4.95, 6.59] |  | 416 | 5.73 [5.01, 6.73] |  | 0.16 |
|  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |

Values are medians [IQR]; 1 Values are *n* (%).). IQR=inter-quartile range. WHO=World Health Organization.

**Table 3: Multiple logistic regression analysis for the effect of supplementation on gestational diabetes (intention to treat analysis)**

|  |  |  |  |
| --- | --- | --- | --- |
| Variables1 | Effect on GDM (Normal 0, GDM 1)(Odds ratio) | 95% confidence intervals | P |
| WHO 1999 criteria (27) |  |  |  |
| Effect of intervention |  |  |  |
|  Control group | (ref) | (ref) | (ref) |
|  Treatment group | 0.6 | 0.4, 0.9 | 0.02 |
| Maternal  Baseline subscapular skinfold, mm (logged) | 1.9 | 1.0, 3.4 | 0.05 |
|  Subscapular gain from registration to visit 3, mm | 1.0 | 1.0, 1.0 | 0.90 |
|  Height, cm | 1.0 | 1.0, 1.1 | 0.75 |
|  Age, years (logged) | 16.6 | 3.3, 83.2 | 0.001 |
|  Standard of Living Index, score (logged) | 2.0 | 0.7, 5.4 | 0.19 |
|  Parity 0 | (ref) | (ref) | (ref) |
|  1 | 1.4 | 0.8, 2.5 | 0.23 |
|  >1 | 1.1 | 0.5, 2.3 | 0.82 |
| Compliance2 |  |  |  |
|  Non-compliant | (ref) | (ref) | (ref) |
|  Compliant | 0.8 | 0.5, 1.3 | 0.39 |
|  |  |  |  |
| Gestational age at visit 3, weeks (logged) | 0.9 | 0.0, 46.6 | 0.95 |
| Intercept | 0.0 | 0.0, 2.8 | 0.07 |
|  |  |  |  |
| WHO 2013 criteria (29) |  |  |  |
| Effect of intervention |  |  |  |
|  Control group | (ref) | (ref) | (ref) |
|  Treatment group | 0.8 | 0.5, 1.3 | 0.39 |
| Maternal  Baseline subscapular skinfold, mm (logged) | 1.5 | 0.8, 2.7 | 0.22 |
|  Subscapular gain from registration to visit 3, mm | 1.0 | 1.0, 1.0 | 0.43 |
|  Height, cm | 1.0 | 1.0, 1.1 | 0.27 |
|  Age, years (logged) | 1.0 | 0.2, 4.9 | 0.97 |
|  Standard of Living Index, score (logged) | 1.4 | 0.5, 3.6 | 0.50 |
|  Parity 0 | (ref) | (ref) | (ref) |
|  1 | 1.5 | 0.9, 2.6 | 0.13 |
|  >1 | 1.0 | 0.5, 2.2 | 0.97 |
| Compliance2 |  |  |  |
|  Non-compliant | (ref) | (ref) | (ref) |
|  Compliant | 0.9 | 0.6, 1.4 | 0.53 |
|  |  |  |  |
| Gestational age at visit 3, weeks (logged) | 1.9 | 0.0, 123.5 | 0.76 |
| Intercept | 0.0 | 0.0, 732.0 | 0.23 |
|  |  |  |  |

1 All variables shown were included in the model together, based on (*n*=837) pregnancies with complete data for all variables. 2 Compliance was a categorical variable, which equalled 1 if [the total number of supplements consumed in the 90 days before the LMP date up to delivery/total number it was possible to have eaten in that time] was 0.5 or more (compliant), and otherwise 0 (non-compliant). GDM=gestational diabetes. Ref=reference group. WHO=World Health Organization.

**Supplemental Table 1: Ingredients of the treatment and control snacks at each stage of the trial**

|  |  |  |  |
| --- | --- | --- | --- |
|  | Treatment |  | Control |
|  |  |  |  | Fruit bar |  |  |
|  | Jan 2006 to Oct 2006 | Oct 2006 to Jun 2007 | Jun 2007 to May 2012 | Jan 2010 to May 2012 |  | Jan 2006 to May 2012 |
| Ingredients |  |  |  |  |  |  |
|  Dry GLV powder, g1 | 7.5 | 3.8 | 0 | 0 | 0 |
|  Milk powder, g | 16 | 12 | 12 | 0 | 0 |
|  Fruit powder, g | 4 | 4 | 0 | 0 | 0 |
|  Fresh GLV, g | 0 | 29 | 30 | 0 | 0 |
|  Dried fruit, g | 0 | 0 | 4 | 60 | 0 |
|  Chickpeas, g | 0 | 0 | 0 | 2 | 0 |
|  Sesame seeds, g | 0 | 0 | 0 | 3 | 0 |
|  Low-micronutrient vegetables2 | 0 | 0 | 0 | 0 | 18 |
|  Binding ingredients, g3 | 30 | 28 | 30 | 0 | 22 |
|  Spices, g | 2 | 2 | 2 | 2 |  | 2 |

1 GLV: green leafy vegetable; GLVs included spinach, colocasia, amaranth, fenugreek, coriander, shepu, onion stalk and curry leaves. Dried GLVs were air-dried at room temperature and supplied as powders or flakes. Dried fruits included figs, dates, raisins, mango, apple, gooseberry and guava.

2 Low micronutrient vegetables included potato and onion.

3 Binding ingredients used were wheat flour, rice flour, chickpea flour or semolina. The treatment snacks changed during the course of the trial in order to improve the palatability of the snacks, hence the four columns for the treatment snacks in the table (18). The nutrient content remained similar (Supplemental Table 2).

**Supplemental Table 2: Mean nutrient composition and percentage contribution to nutrient requirements of the treatment and control snacks at each stage of the trial**

|  |  |  |  |
| --- | --- | --- | --- |
|  | Treatment |  | Control |
|  |  |  |  | Fruit bar | All treatment snacks Jan 2006-Jun 20121 |  | All control snacks Jan 2006-Jun 20121 |
|  | Jan 2006 to Oct 2006 | Oct 2006 to Jun 2007 | Jun 2007 to May 2012 | Jan 2010 to May 2012 |  |
|  |  |  |
| Micronutrient content/ snack |  |  |  |  |  |  |  |  |  |  |  |
|  ß-Carotene, RE | 114 + 26 | 200 + 23 | 141 + 85 | 353 + 180 | 159 + 55  | [21-595] |  | 2 + 1 | [0-3] |
|  Riboflavin, mg | 0.20 + 0.01 | 0.21 + 0.02 | 0.15 + 0.03 | 0.04 + 0.02 | 0.16 + 0.04 | [0.00-0.22] |  | 0.01 + 0.01 | [0.00-0.02] |
|  Folate, μg2 | 26.0 + 5.7 | 50.8 + 19.5 | 67.5 + 30.6 | 40.2 + 35.9 | 58.5 + 14.6 | [5.2-93.0] |  | 6.1 + 4.6 | [2.7-12.1] |
|  Vitamin C, mg | <1 + 0.0 | 0.5 + 0.6 | 2.1 + 3.0 | 8.7 + 12.7 | 2.1 + 1.8 | [0.0-36.6] |  | 0.0 + 0.0 | [0.0-0.6] |
|  Vitamin B-12, μg | 0.64 + 0.05 | 0.58 + 0.16 | 0.31 + 0.13 | 0.14 + 0.15 | 0.38 + 0.14 | [0.00-0.74] |  | 0.18 + 0.25 | [0.00-0.60] |
|  Calcium, mg | 210 + 14 | 275 + 66 | 194 + 35 | 76 + 16 | 200 + 42 | [52-356] |  | 25 + 35 | [8-87] |
|  Iron, mg | 6.85 + 1.07 | 5.90 + 1.58 | 3.93 + 1.26 | 1.75 + 0.49 | 4.42 + 1.27 | [1.22-7.59] |  | 0.90 + 0.26 | [0.65-1.28] |
| Macronutrient content/ snack3 |
|  Energy, MJ | 0.74 + 0.09 | 0.70 + 0.06 | 0.61 + 0.07 | 0.92 + 0.04 | 0.69 + 0.08 | [0.56-0.92] |  | 0.37 + 0.05 | [0.27-0.66] |
|  Protein, g | 7.3 + 0.9 | 6.9 + 0.7 | 6.4 + 1.0 | 2.7 + 0.3 | 6.4 + 1.0 | [2.7-7.9] |  | 2.4 + 0.6 | [1.0-3.3] |
| % of RNI |
|  ß-Carotene, RE | 14 |  | 25 |  | 18 |  | 44 |  | 20 |  |  | <1 |  |
|  Riboflavin, mg | 14 |  | 15 |  | 11 |  | 3 |  | 11 |  |  | <1 |  |
|  Folate, μg | 4 |  | 8 |  | 11 |  | 7 |  | 10 |  |  | 1 |  |
|  Vitamin C, mg | <1 |  | 1 |  | 4 |  | 16 |  | 4 |  |  | <1 |  |
|  Vitamin B-12, μg | 25 |  | 22 |  | 12 |  | 5 |  | 15 |  |  | 7 |  |
|  Calcium, mg | 18 |  | 23 |  | 16 |  | 6 |  | 17 |  |  | 2 |  |
|  Iron, mg | 35 |  | 30 |  | 20 |  | 9 |  | 23 |  |  | 5 |  |

Values are mean + SD; 1Values are weighted mean + SD [range] for the nutrient content of the snacks, given in the units shown, and represent the weighted average based on the number of days that the different snack recipes were distributed over the study period, and the lowest and highest nutrient content measured in a sample of an individual snack recipe; 2 Total folate; 3 Macronutrient content calculated from Indian Food Tables (19). RE=retinol equivalents; RNI= FAO/WHO recommended reference nutrient intake (RNI) during the first trimester of pregnancy, except calcium, for which only a third trimester value is available (20).

**Supplemental Table 3: Examples of treatment and control recipes.**

**Treatment Recipes Control Recipes**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Ingredient | Weight, g |  | Ingredient | Weight, g |
| RECIPE 1 |  |  | RECIPE 1 |  |
| Fresh spring onion stalk | 15 |  | Tapioca | 30 |
| Whole milk powder | 12 |  | Potato | 10 |
| Dried raisins and figs | 4 |  | Corn flour | 2.5 |
| Wheat flour | 7 |  | Wheat flour | 2.5 |
| Rice flour | 6 |  | Mixed spices | 1 |
| Sorghum flour | 0.5 |  |  |  |
| Pearl millet flour | 4 |  | RECIPE 2 |  |
| Mixed spices | 1 |  | Potato | 45 |
|  |  |  | Wheat flour | 5 |
| RECIPE 2 |  |  | Corn flour | 2 |
| Fresh onion stalk | 10 |  | Mixed spices | 1 |
| Fresh coriander | 10 |  |  |  |
| Whole milk powder | 12 |  | RECIPE 3 |  |
| Dried apricot | 4 |  | Potato | 25 |
| Wheat flour | 19 |  | Semolina | 5 |
| Pearl millet flour | 5 |  | Rice flour | 15 |
| Mixed spices | 1 |  | Mixed spices | 1 |
|  |  |  |  |  |
| RECIPE 3 (fruit bar) |  |  | RECIPE 4 (‘chikki’) |  |
| Mango | 30 |  | Puffed rice | 7 |
| Indian gooseberry | 17 |  | Jaggery | 2 |
| Raisins | 5 |  | Sugar | 4 |
| Chickpea | 10 |  |  |  |
| Sesame seed | 3 |  |  |  |

All treatment and control snacks except the fruit bar and chikki were cooked by deep-frying in sunflower oil.

**Supplemental Table 4: Anthropometry and nutritional status in early and late pregnancy among women with and without gestational diabetes (WHO 1999 criteria (27))**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | GESTATIONAL DIABETES |  | NO GESTATIONAL DIABETES |  |  |
|  | *n* |  |  | *n* |  |  | *P* |
|  |  |  |  |  |  |  |  |  |  |
| At recruitment, pre-pregnancy |  |  |  |  |  |  |  |  |  |
| Age, years | 100 | 26.0 [24.0, 29.0] |  | 908 | 24.0 [21.0, 27.0] |  | <0.001 |
| Weight, kg | 100 | 48.8 [42.2, 55.6] |  | 908 | 45.2 [39.9, 51.5] |  | 0.001 |
| Height, cm1 | 100 | 151.1 + 5.0 |  | 908 | 151.3 + 5.4 |  | 0.73 |
| Body mass index, kg/m2 | 100 | 21.7 [18.4, 24.2] |  | 908 | 19.7 [17.8, 22.4] |  | <0.001 |
| Triceps skinfold, mm | 100 | 16.8 [12.2, 21.3] |  | 908 | 13.4 [9.6, 18.5] |  | <0.001 |
| Subscapular skinfold, mm | 100 | 25.1 [19.0, 34.8] |  | 908 | 20.7 [14.9, 28.5] |  | <0.001 |
| Parity2 0 | 100 | 20 (20.0) |  | 908 | 284 (31.3) |  | 0.07 |
|  1 |  | 58 (58.0) |  |  | 455 (50.1) |  |  |
|  >1 |  | 22 (22.0) |  |  | 169 (18.6) |  |  |
| Standard of living index (score) | 100 | 26.0 [21.5, 29.5] |  | 878 | 25.0 [21.0, 30.0] |  | 0.26 |
| Education2  | 100 | 6 (6.0) |  | 906 | 78 (8.6) |  | 0.64 |
|  Primary or less |  | 89 (89.0) |  |  | 778 (85.9) |  |  |
|  Secondary |  | 5 (5.0) |  |  | 50 (5.5) |  |  |
|  Graduate |  |  |  |  |  |  |  |  |  |
| Dietary intake, frequency/week2 |  |  |  |  |  |  |  |  |  |
| Milk <1 |  | 47 (47.0) |  |  | 428 (47.1) |  | 0.49 |
|  1-6 |  | 43 (43.0) |  |  | 353 (38.9) |  |  |
|  ≥7 |  | 10 (10.0) |  |  | 127 (14.0) |  |  |
| Green leafy vegetables <1 |  | 20 (20.0) |  |  | 216 (23.8) |  | 0.68 |
|  1-6 |  | 77 (77.0) |  |  | 669 (73.7) |  |  |
|  ≥7 |  | 3 (3.0) |  |  | 23 (2.5) |  |  |
| Fruit <1 |  | 14 (14.0) |  |  | 147 (16.2) |  | 0.38 |
|  1-6 |  | 66 (66.0) |  |  | 626 (68.9) |  |  |
|  ≥7 |  | 20 (20.0) |  |  | 135 (14.9) |  |  |
| Meat and fish <1 |  | 29 (29.0) |  |  | 224 (24.7) |  | 0.02 |
|  1-6 |  | 54 (54.0) |  |  | 600 (66.1) |  |  |
|  ≥7 |  | 17 (17.0) |  |  | 84 (9.3) |  |  |
|  |  |  |  |  |  |  |  |  |  |
| Visit 1, median [IQR] gestation 10.1 [9.4, 12.0] weeks |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
| Weight, kg | 92 | 50.3 [43.5, 56.9] |  | 769 | 46.6 [41.0, 53.0] |  | 0.004 |
| Triceps skinfold, mm | 94 | 15.6 [12.5, 19.4] |  | 804 | 13.5 [9.4, 17.6] |  | <0.001 |
| Subscapular skinfold, mm | 94 | 25.3 [18.6, 32.5] |  | 804 | 21.4 [15.5, 28.3] |  | 0.001 |
| Hemoglobin, g/dL | 88 | 11.4 [10,7, 12.1] |  | 719 | 11.3 [10.6, 12.1] |  | 0.84 |
| Anemia2  | 88 | 29 + 33.0 |  | 719 | 260 + 36.2 |  | 0.55 |
| Vitamin B-12, pmol/L | 82 | 216.0 [180.0, 282.0] |  | 602 | 223.0 [171.0, 290.0] |  | 0.92 |
| Vitamin B-12 deficiency2 | 82 | 9 + 11.0 |  | 602 | 106 + 17.6 |  | 0.13 |
| Plasma folate, nmol/L | 82 | 33.5 [17.6, 67.6] |  | 603 | 30.2 [17.5, 64.2] |  | 0.69 |
| Folate deficiency2 | 82 | 2 + 2.4 |  | 603 | 8 + 1.3 |  | 0.34 |
|  |  |  |  |  |  |  |
| Visit 3, median [IQR] gestation 29.7 [29.3, 30.7] weeks |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
| Weight, kg | 92 | 56.0 [50.3, 63.0] |  | 865 | 52.4 [47.4, 58.8] |  | 0.004 |
| Weight gain from registration, kg1 | 92 | 6.6 + 3.8 |  | 865 | 7.2 + 3.9 |  | 0.16 |
| Triceps skinfold, mm | 98 | 17.5 [12.5, 21.2] |  | 893 | 14.3 [10.5, 19.0] |  | <0.001 |
| Triceps gain from registration, mm1 | 98 | 0.1 + 5.0 |  | 893 | 0.5 + 4.6 |  | 0.38 |
| Triceps gain from visit 1, mm1 | 92 | 0.7 + 4.1 |  | 793 | 1.0 + 3.5 |  | 0.37 |
| Subscapular skinfold, mm | 98 | 26.6 [19.7, 34.3] |  | 893 | 23.1 [17.6, 28.6] |  | 0.003 |
| Subscapular skinfold gain from registration, mm1 | 98 | -0.2 + 7.3 |  | 893 | 1.3 + 7.5 |  | 0.05 |
| Subscapular gain from visit 1, mm1 | 92 | 1.0 + 5.6 |  | 793 | 1.6 + 5.6 |  | 0.35 |
| Hemoglobin, g/dL | 99 | 10.9 [10.2, 11.7] |  | 886 | 10.7 [9.8, 11.5] |  | 0.04 |
| Anemia2  | 99 | 54 (54.5) |  | 886 | 518 (58.5) |  | 0.45 |
|  |  |  |   |  |  |  |  |  |  |
| Birth outcomes |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |
| Birth weight, g1 | 86 | 2606.0 + 351.8 |  | 729 | 2620.5 + 381.2 |  | 0.74 |
| Low birth weight2 | 86 | 32 (37.2) |  | 729 | 246 (33.7) |  | 0.52 |
| Small for gestational age, Oken2 | 86 | 53 (61.6) |  | 720 | 511 (71.0) |  | 0.07 |
| Small for gestational age, within cohort2 | 84 | 3 (3.6) |  | 716 | 67 (9.4) |  | 0.10 |
| Large for gestational age, Oken2 | 86 | 0 (0.0) |  | 720 | 2 (0.3)  |  | 1.00 |
| Large for gestational age, within cohort2 | 84 | 9 (10.7) |  | 716 | 61 (8.5) |  | 0.50 |
| Gestation, weeks | 100 | 39.0 [37.2, 39.7] |  | 892 | 39.1 [38.1, 40.0] |  | 0.04 |
| Pre-term2 | 100 | 20 (20.0) |  | 892 | 87 (9.8) |  | 0.002 |
| Major congenital anomalies2 | 100 | 2 (2.0) |  | 908 | 4 (0.4) |  | 0.11 |
| Elective cesarean section birth2 | 87 | 8 (9.2) |  | 734 | 69 (9.4) |  | 0.95 |
| Emergency cesarean section birth2 | 87 | 15 (17.2) |  | 734 | 83 (11.3) |  | 0.11 |
| Forceps/Ventouse delivery2 | 87 | 1 (1.1) |  | 734 | 6 (0.8) |  | 0.55 |
| Perinatal death2 | 98 | 3 (3.1) |  | 897 | 14 (1.6) |  | 0.41 |

Values are median [IQR]; 1 Values are mean + SD; 2 Values are *n* (%). Small for gestational age (Oken) and large for gestational age (Oken) were defined using reference 31. Small for gestational age (within cohort) were defined as <10th percentile and >90th percentile respectively, based on sex-specific gestation-adjusted birth weights among all live singleton newborns without major congenital anomalies. IQR=interquartile range.

**Supplemental Figure 1: CONSORT diagram showing participant flow in the trial**

**PREGNANCIES**

***n* = 1106**

**PREGNANCIES**

***n* = 1185**

**STARTED SUPPLEMENT >90 DAYS BEFORE**

**LMP DATE**

***n*=857**

**STARTED SUPPLEMENT <90 DAYS BEFORE**

**LMP DATE**

***n*=249**

**STARTED SUPPLEMENT >90 DAYS BEFORE**

**LMP DATE**

***n*=969**

**STARTED**

**SUPPLEMENT <90**

**DAYS BEFORE**

**LMP DATE**

***n*=216**

**PREGNANCY REACHED AT LEAST 28 WEEKS GESTATION**

***n*=765**

**PREGNANCY REACHED AT LEAST 28 WEEKS GESTATION**

***n*=224**

**PREGNANCY REACHED AT LEAST 28 WEEKS GESTATION**

***n*=854**

**PREGNANCY REACHED AT LEAST 28 WEEKS**

**GESTATION**

***n*=185**

Natural abortion 17

Termination 11 Unknown outcome 3

**ATTENDED CLINIC FOR GTT**

***n*=375 (49%)**

**ATTENDED CLINIC FOR GTT**

***n*=117 (52%)**

**ATTENDED CLINIC FOR GTT**

***n*=420 (49%)**

**ATTENDED CLINIC FOR GTT**

***n*=96 (52%)**

Natural abortion 60

Termination 47

Unknown outcome 8

Natural abortion 48

Termination 39

Unknown outcome 5

Natural abortion 9

Termination 10

Unknown outcome 6

**TOTAL WOMEN**

**RECRUITED**

***n*=6513**

**RANDOMIZED TO TREATMENT GROUP**

***n* = 3205**

**RANDOMIZED TO**

 **CONTROL**

**GROUP**

***n* = 3308**

**STAYED IN THE STUDY BUT NEVER**

**BECAME PREGNANT**  755

**DROPPED OUT OF THE STUDY BEFORE BECOMING PREGNANT**

Moved away 500

Declined further follow-up 481

Centre closed 48

Became sterilized 54

Separated from husband 4

Died 10

Husband died 11

**BECAME PREGNANT TOO EARLY**

**EXCLUDED AND NOT FOLLOWED FURTHER**

Started supplementation <90 days

Before LMP (up to Dec 2008) 156

Started supplementation after LMP

(after Dec 2008) 104

**STAYED IN THE STUDY BUT NEVER**

**BECAME PREGNANT** 692

**DROPPED OUT OF THE STUDY BEFORE**

**BECOMING PREGNANT**

Moved away 476

Declined further follow-up 547

Centre closed 40

Became sterilized 53

Separated from husband 6

Died 7

Husband died 6

**BECAME PREGNANT TOO EARLY**

**EXCLUDED AND NOT FOLLOWED FURTHER**

Started supplementation <90 days

before LMP (up to Dec 2008) 171

Started supplementation after LMP

(after Dec 2008) 101