

## PRiDE study protocol

**Title: Micronutrients in Pregnancy as a Risk Factor for gestational Diabetes and Effects on mother and baby**

**Short title: PRiDE study**

Sponsor: University of Warwick

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## 1. SYNOPSIS

<b>Study Title</b>	A prospective multi-ethnic early pregnancy cohort study, ‘Micronutrients in Pregnancy as a Risk Factor for gestational Diabetes and Effects on mother and baby’ (PRiDE study), will be conducted to determine the relationship between early pregnancy B12 and folate levels and their impact on hyperglycaemia and GDM status at 26-28 weeks of gestation
<b>Study Design</b>	Longitudinal cohort study
<b>Trial Participants</b>	<p>The study will identify pregnant women (n=4500) at less than 16 weeks’ gestation who have risk factors for GDM according to the NICE criteria and who will therefore be referred for an oral glucose tolerance test (OGTT) at 26-28 weeks’ gestation. Blood test for B12, folate and related biomarkers will be done at first trimester along with anthropometric measurements. These anthropometric measurements will be repeated during the OGTT visit and glucose data collected.</p> <p>Women with pre-gestational diabetes mellitus, a previous pregnancy with a neural tube defect, multiple gestation, severe anaemia (Hb&lt;10 g/dL), confirmed vitamin B12 or folate deficiency or having received B12 injections within the last six months were excluded</p>
<b>Planned Sample Size</b>	4500 participants
<b>Follow-up duration</b>	Until delivery
<b>Planned Trial Period</b>	5 years
<b>Primary Objective</b>	To determine differences in the risk of GDM in women with and without early pregnancy B12 insufficiency.
<b>Secondary Objectives</b>	<ol style="list-style-type: none"> <li>1. To study the predictors of maternal B12, folate and tHcy in early pregnancy</li> </ol> <p>To determine associations between the following:</p> <ol style="list-style-type: none"> <li>1. B12, folate and glycaemia (FPG and 2hr-PG as continuous variables)</li> <li>2. tHcy and glycaemia/GDM risk</li> <li>3. ‘Low B12-high folate’ status and glycaemia/GDM risk</li> <li>4. Ethnic differences between B12, folate and tHcy and glycaemia/GDM risk.</li> </ol>

## 2. ABBREVIATIONS

T2D: type 2 diabetes

CVD: cardiovascular disease

GDM: gestational diabetes mellitus

B12: vitamin B12

tHcy: total homocysteine

BMI: body mass index

OGTT: oral glucose tolerance test

FPG: fasting plasma glucose

2hr-PG: 2-hour plasma glucose

NICE: The National Institute for Health and Care Excellence

IADPSG: The International Association of Diabetes and Pregnancy Study Groups

### 3. BACKGROUND AND RATIONALE

There is an escalating epidemic of type 2 diabetes (T2D) across the world in all ethnic groups, in particular, people of South Asian origin(1). Current strategies for preventing T2D rely mainly on altering risk factors such as obesity, sedentary lifestyle and healthy eating. Though this is important, it is unlikely to make a big impact on this escalating epidemic, which consumes significant proportion of health care budgets, world-wide(2). Some of the risk of developing metabolic diseases such as T2D and cardiovascular disorders (CVD) are evident even at birth(3-5). Indeed, recent research suggests that factors acting during early development, including foetal growth restriction and exposure to gestational diabetes (GDM) in the mother increases diabetes and obesity risk in later life(6-8). Our recent work has shown that maternal vitamin B12 insufficiency in combination with normal/high maternal folate status, is associated with an increased risk of GDM in the mothers, growth restriction in the foetus, and adiposity and insulin resistance in the children (5, 8).

GDM is described as glucose intolerance first recognised in pregnancy. Similar to T2D, GDM is also increasing at an alarming rate, attributed partly to increasing maternal age and body weight(9, 10). The current estimate is around 4% of all pregnancy(11) though this could increase to as much as 20% if the new IADPSG guidelines are implemented(12). Women who develop GDM are at 7-8 times higher risk of T2D(13) and the children born to them are at higher risk of developing T2D, abdominal obesity and metabolic syndrome later in life thus increasing their risk of CVD in adulthood(6, 7, 14, 15). Though the exact etiological processes of such increased risk not clear, some of these effects could act through epigenetic mechanisms (intra-uterine programming) (16, 17).

The study by Krishnaveni et al raises an intriguing possibility for vitamin B12 deficiency in the pathogenesis of GDM(8). It is believed that the high prevalence of B12 insufficiency in India was attributed to vegetarianism(5). As the majority of the UK population is non-vegetarian, it is conceivable that B12 deficiency is unlikely during pregnancy. However, we have recently shown: a) B12 deficiency is not uncommon in women of childbearing age (~14%) and pregnant women (15% at 16-18 weeks of pregnancy)(18, 19) and b) B12 deficiency in GDM is associated with higher BMI and higher birth weight in a clinic population(20).

#### *Psychological impact of GDM:*

Mothers who are screened for GDM have lower perceived well-being(21, 22). Diagnosis of GDM during pregnancy often comes as a shock and causes significant distress to expectant mothers. There is some evidence that such distress improves after treatment, presumably due to education and support(21, 23). These limited data come from women of Caucasian origin and no such data exists in women of South Asian origin.

To confirm these observations in the UK multi-ethnic population, we plan to carry out an adequately powered longitudinal cohort study to examine: a) the influence of early pregnancy B12 and folate and homocysteine levels on the risk of GDM; b) to determine the association of the abovementioned factors on offspring's risk of future metabolic disorders by; and c) the psychological impact of diagnosis of GDM by measuring the anxiety and distress by questionnaires.

### 4. AIMS AND OBJECTIVES

#### **Hypothesis:**

Imbalance of B12 and folate in early pregnancy is associated with the risk of hyperglycaemia and GDM subsequently in pregnancy

#### **Aims:**

The main aim of the study is to determine whether early pregnancy B12 and folate levels (and/or imbalance of the two micronutrients) is independently associated with the onset of GDM

#### **Objectives:**

##### **Primary objectives:**

Primary:

Differences in the risk of GDM in women with and without early pregnancy B12 insufficiency.

Secondary:

1. To investigate predictors of maternal B12, folate and tHcy in early pregnancy

To determine associations between the following:-

2. B12, folate and glycaemia (FPG and 2hr-PG as continuous variables)
3. tHcy and glycaemia/GDM risk
4. 'Low B12-high folate' status and glycaemia/GDM risk
5. Ethnic differences between B12, folate and tHcy and glycaemia/GDM risk

6. Early pregnancy B12, folate and tHcy levels and offspring birth weight and adiposity
7. To investigate differences in offspring's birth weight and adiposity between GDM and non-GDM controls in each ethnic group
8. To determine predictors of abnormal post-natal oral glucose tolerance test (OGTT) from variables at first trimester
9. To study differences in intra-uterine growth pattern of South Asian and Caucasian women with GDM
10. To study differences in B12 levels and offspring birth weight in women with GDM treated with and without metformin

## 5. STUDY DESIGN

A prospective observational cohort study, 'Micronutrients in Pregnancy as a Risk Factor for gestational Diabetes and Effects on mother and baby' (PRiDE study), will be conducted to determine the relationship between early pregnancy B12 and GDM (clinicaltrials.gov number: NCT03008824). All participants provided written informed consent.

Approximately 8-12 study sites will be invited to enable achievement of the recruitment target and sampling of several ethnic groups in the UK. These will be primarily based around the West Midlands region. Several study launches are planned to raise the awareness among the public, midwives and general practitioners in the region. The research networks (Comprehensive Local - CLRN, Primary Care – PCRN and Diabetes - DRN) will be involved to raise awareness and for direct involvement in the study.

Most potential participants will be given the participant information sheet (PIS) when they are seen for their booking visit by the community midwife. The midwife will assess their eligibility for the study and hand out the PIS if they fit the inclusion criteria. The midwife will inform the research team of the potential participants' details via a Reply Slip if she has no objection to be contacted. The research team will then contact the woman and if possible, the recruitment visit will be planned to coincide with her booking bloods or dating scan appointment (usually around 12 weeks of gestation) to minimise the study visits.

In the situation where a woman has been identified by her midwife as fitting the inclusion criteria for the study (i.e., she has high-risk factors for GDM and is therefore referred for a GTT), but is not given a PIS at the booking visit, the research team will send this out by post or give it to her in person during the recruitment visit.

Feedback from women at the recruitment visit (i.e. dating scan) suggests that they would have been happy to partake in the study at the same visit as receiving the PIS for 4 main reasons: 1) the study (and the PIS) is simple to understand; 2) they spend a significant amount of time awaiting scan and midwife appointment during this visit; 3) they want to have their blood sample taken at the same visit as they routinely have a blood test at this time; and 4) they prefer not to come back for a separate visit. We also believe this is reasonable as this is a non-interventional study and provides opportunity for more women to participate in the study. Therefore, if a potential participant has had enough time to read and understand the PIS and provides consent, she could be recruited straightaway and blood tests done together with her routine bloods on the same day. However, if she prefers to take the PIS away and have more time to decide, the research team will see her at a future date. Such a strategy can also be replicated in the community booking visit to facilitate participation in the study.

## 6. STUDY PARTICIPANTS

Women in early pregnancy will be recruited as per the inclusion and exclusion criteria with an aim to have equal numbers of GDM in White British and South Asian women, in line with the ethnic mix of women seeking maternity care in the study sites.

Inclusion criteria:

- Pregnant women less than 16 weeks' gestation
- Age 18 – 45 years
- High-risk group for GDM (at least 1 of the following risk factors): BMI >30 kg/m<sup>2</sup>; previous GDM; previous unexplained still birth or baby >4.5kg; first degree relative with diabetes, ethnic minority group (South Asians, Middle Eastern, Afro-Caribbean), age >35years, polycystic ovarian syndrome. (or other local criteria followed by the participating centres to comply with routine practise)

Exclusion

- Pre-gestational diabetes mellitus (Type 1 or 2)
- Diagnosis of Vitamin B12 or folate deficiency in current pregnancy
- Previous pregnancy (-ies) with neural tube defect
- Diagnosis of severe anaemia in current pregnancy (Hb<10 g/dL)
- On Vitamin B12 injections within last 6 months

### **Ethnicity definitions:**

Women's self-reported ethnicity was recorded using the Office of National Statistics Census of England and Wales, UK(24). For the statistical analyses, they are combined in the following categories:

- White: English, Welsh, Scottish, Northern Irish, British, Irish, Any other White background
- South Asian: Indian, Pakistani, Bangladeshi, Sri Lankan, Nepalese, Any other South Asian background
- Others: Black, African, Caribbean, East Asian (e.g., Chinese, Japanese, Korean), South East Asian (e.g., Malaysian, Thailand, Philippines), Arabic (or Middle Eastern)

## 7. STUDY PROCEDURES

### *Visit 1 (Recruitment /1<sup>st</sup> trimester)*

The participants will have basic demographic information collected and anthropometric measurements done during the recruitment visit and be asked to fill out physical activity and psychological well-being questionnaires. Non-fasting blood, (approximately 15-20ml in addition to routine booking bloods), urine and stool samples (optional) will then be collected from the participants.

### *Visit 2 (OGTT)*

Women will have their OGTT at 24-28 weeks' gestation (or earlier if she had GDM in a previous pregnancy, in line with NICE guidelines). Fasting blood tests will be taken followed by the consumption of 75g of anhydrous glucose over a maximum of two minutes and glucose measured again at two hours. Further blood, urine and stool (optional) samples will be taken at this time for study purposes. Initially, the modified WHO and NICE 2015 criteria was used in all our study centres for deciding if a woman is classified as gestational diabetes or not (i.e., fasting plasma glucose  $\geq 6.0$  mmol/l OR fasting plasma glucose  $\geq 5.6$  mmol/l and/or 2-hour plasma glucose  $\geq 7.8$ mmol/l on 75g glucose tolerance test). The diagnostic criteria was changed in March 2015 due to the publication of the NICE 2015 guidelines (fasting plasma glucose  $\geq 5.6$  mmol/l and/or 2 hour plasma glucose  $\geq 7.8$ mmol/l). The women will be asked to fill out further psychological well-being questionnaires during the waiting time during the OGTT. The remainder of a participant's antenatal care will then carry on in the joint antenatal-diabetes clinic (if she has been diagnosed with GDM) or in the community (for non-GDM women, unless she has any other indication to be referred to the obstetric clinic).

## 8. SAMPLE COLLECTION AND HANDLING

All blood samples will be centrifuged within 30 minutes of collection or if this is not possible, be stored in a 4°C fridge for a maximum of 4 hours before centrifuging. Serum and plasma samples will be separated into 1ml aliquots after centrifuging and additional aliquots of buffy coat and whole blood from the EDTA bottle will be extracted and stored. All the above and urine samples will be stored in -80°C freezers until analysis. All samples will be transferred in batches on dry ice to the main study site in George Eliot Hospital for analysis. The samples will be stored for future analyses under the guardianship of Department of Pathology, George Eliot Hospital NHS Trust (Host site) and University of Warwick during and indefinitely after the end of the study where future analysis could happen. All of the analyses will be carried out ONLY on pseudo-anonymised samples/data (i.e., only the study ID number will be labelled on the samples).

Analysis of serum glucose will be done by a hexokinase enzymatic method using a Synchron CX7 auto-analyser (Beckman Coulter, Fullerton, CA, USA) and reported in mmol/l. Serum B12 and folate will be analysed by an electrochemiluminescent immunoassay using a Roche Cobas immunoassay analyser (Roche Diagnostics UK, Burgess Hill, UK) and reported in pmol/l and nmol/l respectively. The measuring range of the B12 kit is 37 - 1476 pmol/L and all the measured values of B12 in this cohort were within this range. The measuring range of the folate kit is 1.4 - 45.4 nmol/L, but in the first 1000 samples of folate measurement, it was noted that >25% of serum folate were >45.4 nmol/L. Therefore, samples with folate concentrations above the measuring range were diluted manually with Diluent Universal. The recommended dilution by the kit manufacturer was 1:3. After manual dilution, the results were multiplied by the dilution factor 3. Plasma tHcy will be analysed by stable isotopic dilution method using Shimadzu HPLC system with an auto-sampler coupled to a detection system of API 6500 QTrap® tandem mass spectrometer (LCMS) (Applied Biosystems, Warrington, UK) using a protocol described previously (25). It will be reported in  $\mu\text{mol/l}$ . A calibration curve and QC samples (low, medium and high concentrations within the limits of quantitation; Waters, UK) were set up for each sample batch that was analysed.

## 9. OUTCOMES

### **Primary outcome:**

Differences in the risk of GDM in women with and without early pregnancy B12 insufficiency.

## Secondary outcomes:

Associations between

1. B12, folate and glycaemia (B12, folate, FPG and 2hr-PG as continuous variables)/GDM risk
2. tHcy and glycaemia/GDM risk
3. 'Low B12-high folate' status and glycaemia/GDM risk and
4. Ethnic differences between B12, folate and tHcy and glycaemia/GDM risk
5. To assess the role of BMI on these associations

## 10. STATISTICS

There is no published data on the rate of B12 deficiency in GDM women in the UK. Preliminary results from our group have shown that approximately 15% insufficiency in non-GDM and around 20%-25% in GDM women of Caucasian origin. To detect a 5% difference in the prevalence of GDM between normal and B12 insufficiency with 90% power at the 5% significance level, 3822-4322 women will be required. Therefore we will recruit 4500 women in early pregnancy, allowing for a 10% drop-out rate.

Continuous variables will be reported as mean and standard deviation (SD) and categorical variables using percentages. B12, folate and tHcy will be summarised using median and interquartile range (IQR) as they are expected not to be normally distributed. Chi-square and t-tests will be used to assess differences in categorical and continuous variables, respectively.

Logistic regression models will be used to estimate the relative risk (and 95%CI) for developing GDM among B12 insufficient compared to sufficient woman, and for one standardised increase in B12, folate and tHcy levels. Multiple linear regression analyses will be used to test the associations of early pregnancy micronutrients with FPG and 2hr-PG at OGTT. The micronutrients and the other continuous covariates will be standardized to aid comparison. Ethnic specific associations of the micronutrients on glucose levels and the risk of GDM will be tested using similar logistic and linear regression models.

The covariates adjusted for will include age, parity, family history, smoking status, ethnicity, household income (SES), BMI and respective micronutrient status (B12 for folate, folate for B12 and B12 and folate for tHcy). From published studies, while the causal direction is clear between BMI and glucose, this is not clear between BMI and B12 and folate. In addition, the association between B12 and GDM seems to be mediated by BMI. Therefore, to understand this complex interplay, two different models will be used, a priori, for the regression analyses while adjusting for possible confounders. Model 1 will include age, parity, marital and smoking status, ethnicity, family history, household income, gestational weight gain and respective micronutrient status (B12 for folate, folate for B12 and B12 and folate for tHcy). Model 2 will include model 1 plus BMI.

To investigate whether BMI is a confounder or mediator of the relationships between glucose levels and B12 and folate, structural equation modelling analyses by bootstrapping method with 1000 simulations will be conducted. Interaction function will be used between B12 and folate to investigate the association of low B12-folate imbalance on glycaemia and the risk of GDM, both as continuous variables and after categorizing them by tertiles. Multiple imputation technique will be used to deal with missing data.

## 11. SAFETY REPORTING

Since this is an observational study, we anticipate very minimal adverse events. However, the process for reporting of Adverse Events (AEs) and Serious Adverse Events (SAEs) will be followed for all study sites.

In research other than CTIMPs, a SAE is defined as an untoward occurrence that:

- a) results in death
- b) is life-threatening
- c) requires hospitalisation or prolongation of existing hospitalisation
- d) results in persistent or significant disability or incapacity
- e) consists of a congenital anomaly or birth defect
- f) is otherwise considered medically significant by the investigator

In determining the severity of an SAE, the following definitions are used:

- a) Related – i.e., it resulted from administration of any research procedure
- b) Unexpected – i.e., it was not listed in the study protocol as an expected occurrence

Process of Notification of SAEs

All SAEs (refer to criteria above) should be reported by to the trial sponsor immediately (i.e., within 24 hours). The following are some AEs which **do not need** to be reported to the trial sponsor (but should be recorded in the Trust site file):-

- a) Miscarriages
- b) Pregnancy –related hospitalisations – e.g., hyperemesis gravidarum, pregnancy-induced hypertension, fetal malposition
- c) Ante-partum haemorrhage
- d) Post-partum haemorrhage of <500mls if normal vaginal delivery or <750mls if Caesarean section

Research Team members should fill in the form titled ‘Report of Serious Adverse Event (non-CTIMP), ver. 3.0, April 2007’ (available from the NRES website <http://www.nres.nhs.uk/applications/after-ethical-review/safetyreports/safety-reports-for-all-other-research/> ) and send it to the PRiDE Study team, George Eliot Hospital NHS Trust. The CI should submit reports of all ‘Related’ and ‘Unexpected’ SAEs within 15 days to the main REC (i.e., the REC which gave a favourable opinion of the study). The coordinator of the main REC will acknowledge receipt of the safety reports within 30 days

## 12. CODES OF PRACTICE AND REGULATIONS – to include Ethics, Sponsor SOP, Approvals, Participant Confidentiality

The study is funded by a grant has been obtained from the Indian Council for Medical Research and Medical Research Council UK (ICMR-MRC Joint Initiative: Chronic Non-Communicable Diseases Research Funding) for a four-year study period. This was further extended by 18 months. Ethics approval has been obtained from the National Research Ethics Committee (South Birmingham) (REC Reference number: 12/WM/0010) and permissions from the local Research and Development departments of the individual study sites were obtained prior to commencement of the study. All participants will provide written informed consent. The study sponsor is University of Warwick, who will act as the overall guardian of all data emerging from the study. The study will comply with SOPs in accordance with the Study sponsor and participating NHS Trusts to ensure that it conforms to all relevant legislation and guidelines.

The study will ensure that the participants’ anonymity is maintained. The participants will be identified only by initials and a unique participant’s ID number on the CRF and any electronic database. All documents and patient identifiable information will be stored securely in locked cabinets in the individual study sites and on password protected computers (according to the sponsors SOPs). Access will be granted to authorised members of the research study teams. The study will comply with the data protection regulations which requires data to be anonymised as soon as it is practical to do so.

## 13. DATA HANDLING AND RECORD KEEPING

Data will be treated as confidential and stored securely. A validated electronic study database was created by the Clinical Trials Unit in Warwick Medical School for electronic capturing of the data. All data from the CRF will be entered on to the database by the participating study sites and transferred electronically to the data coordinating centre at the end of the study. Only the unique participant ID number will be included in the study database and this will be linked to the independent personal details database to arrange clinic visits and follow-up. However, the linked information will remain with the clinical research team in the individual study sites only. Participants data will not be used or given to any other third party without written permission of the participant, as defined in their consent form. The name and any other identifying detail will NOT be included in any study data electronic file. This unidentifiable data will be stored for 10 years and then archived according to the sponsor’s SOP.

Any identifiable personal data will be permanently deleted from the servers once the final report has been submitted or within 5 years if the participant has agreed to be contacted for other studies. Biological samples (bloods) taken for the study will be destroyed once analysed in a Human Tissue Act compliant site. Blood samples will be sent for analysis to the primary study site (George Eliot Hospital NHS Trust) and any other specialist labs as required and the results fed back to the research team to be entered in the CRF. Every effort will be made by the investigators to adhere to the ethical principles described by the UK Good Governance Procedures and as enshrined in the Declaration of Helsinki.

Any hard copies of the data (questionnaires, CRFs etc.) will be kept in a locked filing cabinet at the respective study sites. Only named members of the research team will have access to the filing cabinet. These will be kept for 5 years following the end of the study and further archived according to the sponsors SOP’s. Due to blood samples being stored for future research use, consent forms will be stored in secure filing cabinets for the duration the samples are stored.

#### 14. REFERENCES

1. Hadden D. Gestational Diabetes: What Every Mother Needs to Know. *Diabetes Voice* 2002;47:18-21.
2. International Diabetes Federation. Internet: <http://www.idf.org/diabetes-prevention/health-economics> (accessed 03/12/2011 2011).
3. Whincup PH, Kaye SJ, Owen CG, Huxley R, Cook DG, Anazawa S, Barrett-Connor E, Bhargava SK, Birgisdottir BsE, Carlsson S, et al. Birth Weight and Risk of Type 2 Diabetes: A Systematic Review. *JAMA* 2008;300(24):2886-97.
4. Saravanan P, Yajnik CS. Role of maternal vitamin B12 on the metabolic health of the offspring: a contributor to the diabetes epidemic? 2010.
5. Yajnik CS, Deshpande SS, Jackson AA, Refsum H, Rao S, Fisher DJ, Bhat DS, Naik SS, Coyaji KJ, Joglekar CV, et al. Vitamin B12 and folate concentrations during pregnancy and insulin resistance in the offspring: the Pune Maternal Nutrition Study. *Diabetologia* 2008;51:29-38.
6. Clausen TD, Mathiesen ER, Hansen T, Pedersen O, Jensen DM, Lauenborg J, Damm P. High Prevalence of Type 2 Diabetes and Pre-Diabetes in Adult Offspring of Women With Gestational Diabetes Mellitus or Type 1 Diabetes. *Diabetes Care* 2008;31(2):340-6.
7. Clausen TD, Mathiesen ER, Hansen T, Pedersen O, Jensen DM, Lauenborg J, Schmidt L, Damm P. Overweight and the Metabolic Syndrome in Adult Offspring of Women with Diet-Treated Gestational Diabetes Mellitus or Type 1 Diabetes. *J Clin Endocrinol Metab* 2009;94(7):2464–70.
8. Krishnaveni GV, Hill JC, Veena SR, Bhat DS, Wills AK, Karat CLS, Yajnik CS, Fall CHD. Low plasma vitamin B12 in pregnancy is associated with gestational ‘diabesity’ and later diabetes. *Diabetologia* 2009;52:2350–8.
9. Hedderson MM, Gunderson EP, Ferrara A. Gestational Weight Gain and Risk of Gestational Diabetes Mellitus. *Obstet Gynecol* 2010;115(3):597-604.
10. Ferrara A, Kahn HS, Quesenberry CP, Riley C, Hedderson MM. An Increase in the Incidence of Gestational Diabetes Mellitus: Northern California, 1991–2000. *Obstet Gynecol* 2004;103(3):526-33.
11. Metzger B, Coustan D, Dyer A, Hadden D, Hod M, Lowe L, Oats J, Persson B, Trimble E. New findings in gestational diabetes – the HAPO Study. *Diabetes Voice* 2005;54:25-8.
12. Ryan EA. Diagnosing gestational diabetes. *Diabetologia* 2011;54:480-6.
13. Bellamy L, Casas J-P, Hingorani AD, Williams D. Type 2 diabetes mellitus after gestational diabetes: a systematic review and meta-analysis. *Lancet* 2009;373:1773–79.
14. Boney CM, Verma A, Tucker R, Vohr BR. Metabolic Syndrome in Childhood: Association With Birth Weight, Maternal Obesity, and Gestational Diabetes Mellitus. *Pediatrics* 2005;115(3):e290-6.
15. Pirkola J, Pouta A, Bloigu A, Hartikainen A-L, Laitinen J, Jarvelin M-R, Vaarasmaki M. Risks of Overweight and Abdominal Obesity at Age 16 Years Associated With Prenatal Exposures to Maternal Prepregnancy Overweight and Gestational Diabetes Mellitus. *Diabetes Care* 2010;33(5):1115-21.
16. Lawlor D, Lichtenstein P, Langstrom N. Association of Maternal Diabetes Mellitus in Pregnancy With Offspring Adiposity Into Early Adulthood. *Circulation* 2011;123:258-65.
17. Yajnik CS. Fetal Programming of Diabetes: Still So Much to Learn! *Diabetes Care* 2010;33(5):1146-8.
18. Saravanan P, Wood C, Anderson N. B12 deficiency is more common than folate deficiency in early pregnancy: do we need to consider B12 fortification? *Diabetologia Suppl EASD* 2010;53(Suppl. 1):S151-S2.
19. Farrar D, Simmonds M, Griffin S, Duarte A, Lawlor DA, Sculpher M, Fairley L, Golder S, Tuffnell D, Bland M, et al. The identification and treatment of women with hyperglycaemia in pregnancy: an analysis of individual participant data, systematic reviews, meta-analyses and an economic evaluation. *Health Technol Assess* 2016;20(86):1-348. doi: 10.3310/hta20860.
20. Sukumar N, Bawazeer N, Patel V, Saravanan P. Low B12 Level is Associated with Maternal Obesity and Higher Birthweight in Gestational Diabetes. *J Dev Orig Health Dis* 2011;2(Suppl. 1):S128-9.
21. Crowther CA, Hiller JE, Moss JR, McPhee AJ, Jeffries WS, Robinson JS. Effect of Treatment of Gestational Diabetes Mellitus on Pregnancy Outcomes. *N Engl J Med* 2005;352(24):2477-86.
22. Rumbold A, Crowther C. Women's experiences of being screened for gestational diabetes mellitus. *Aust N Z J Obstet Gynaecol* 2002;42(2):131-7.
23. Langer N, Langer O. Emotional Adjustment to Diagnosis and Intensified Treatment of Gestational Diabetes. *Obstetrics & Gynecology* 1994;84(3):329-34.
24. GOV.UK. Internet: <https://www.ethnicity-facts-figures.service.gov.uk/style-guide/ethnic-groups#list-of-ethnic-groups> (2018).
25. Adaikalakoteswari A, Webster C, Goljan I, Saravanan P. Simultaneous detection of five one-carbon metabolites in plasma using stable isotope dilution liquid chromatography tandem mass spectrometry. *Journal of chromatography B, Analytical technologies in the biomedical and life sciences* 2016;1012-1013:186-92. doi: 10.1016/j.jchromb.2016.01.026.