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Maternal vitamin D and neonatal anthropometrics and markers of neonatal glycaemia: Belfast HAPO study

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Abstract

Vitamin D deficiency is a common occurrence globally, and particularly so in pregnancy. There is conflicting evidence regarding the role of vitamin D during pregnancy on non-skeletal health outcomes for both the mother and the neonate. The aim of this study was to investigate the associations of maternal total 25-hydroxy vitamin D (25OHD) with neonatal anthropometrics, and markers of neonatal glycaemia in the Belfast centre of the Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study. Serological samples (n=1585) were obtained from pregnant women in the Royal Jubilee Maternity Hospital, Belfast, Northern Ireland between 24-32 weeks gestation as part of the HAPO study. 25OHD concentrations were measured by liquid chromatography tandem mass spectrometry (LC-MS/MS). Cord blood and neonatal anthropometric measurements were obtained within 72h of birth. Statistical analysis was performed. Following adjustment for confounders, birth weight standard deviation scores (SDS) and birth length SDS were significantly associated with maternal total 25OHD. A doubling of maternal 25OHD at 28 weeks' gestation was associated with mean birth weight SDS and mean birth length SDS higher by 0.05 and 0.07, respectively (both, p=0.03). There were no significant associations with maternal 25OHD and other measures of neonatal anthropometrics or markers of neonatal glycaemia. In conclusion, maternal total 25OHD during pregnancy was independently associated with several neonatal anthropometric measurements; however, this association was relatively weak.

Introduction

Vitamin D deficiency in pregnancy is a common occurrence globally[1]. Adequate vitamin D intake during pregnancy is required to address the ongoing demand for calcium for fetal growth and development [2]. In addition, vitamin D has been associated with a number of neonatal outcomes including neonatal birth length and weight [3],[4]. Pérez-López and colleagues [3] conducted a systematic review and meta-analysis of randomised controlled trials (RCT) which examined vitamin D supplementation during pregnancy with various neonatal outcomes. They observed that neonatal birth weight and birth length were significantly greater in the vitamin D intervention groups than the placebo group. However, evidence surrounding a possible beneficial relation between vitamin D and birth weight is conflicting. Harvey and colleagues [5] carried out a comprehensive systematic review on the relationship of vitamin D with maternal and neonatal health outcomes. They observed a modest relationship between maternal 25OHD status and offspring birth weight and bone mass in observational studies[5]. Observational studies which directly measured maternal serum 25OHD, reported no association of serum 25OHD with low birth weight [6]. The lack of clear evidence suggests a need for larger observational and potentially, intervention trials, to further investigate this relation.

It has been hypothesised that destruction of beta cells in the development of type 1 diabetes may occur before birth [7] and, if correct, then early identification of environmental determinants in utero which could affect beta cell function is of particular relevance. Preliminary reports have suggested that vitamin D deficiency is associated with decreased beta cell function [8],[9], and as neonates derive vitamin D from their mother, it is crucial to confirm or refute these findings.

The aim of this study was to investigate the associations of maternal total 25-hydroxy vitamin D (25OHD) with neonatal anthropometrics and markers of neonatal glycaemia in the Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study at the Belfast centre.

Subjects and methods

The methods for the HAPO study have been published in detail elsewhere [10],[11]. Briefly, the HAPO study was a 15-center multicultural and multinational study designed to examine the association between maternal hyperglycemia and adverse pregnancy outcomes in singleton pregnancies whose results on oral glucose tolerance testing (OGTT) were below the traditional thresholds for overt diabetes. All pregnant women at a given centre were eligible to participate unless they had one or more of the following exclusion criteria: age younger than 18 years, a plan to undergo delivery at another hospital, an uncertain date of last menstrual period and no ultrasonographic estimation between 6 and 24 weeks of gestational age, inability to complete the oral glucose-tolerance test within 32 weeks of gestation, multiple pregnancy, conception by means of gonadotropin ovulation induction or in vitro fertilization, glucose testing before recruitment or a diagnosis of diabetes during the current pregnancy, diagnosis of diabetes before the current pregnancy and requiring treatment with medication, participation in another study that could interfere with the HAPO study, infection with the human immunodeficiency virus or hepatitis B or C virus, previous participation in the HAPO study, or inability to converse in the languages used on center forms without the aid of an interpreter.

Each participant underwent a standard 75g OGTT between 24-32 weeks gestation (average 28 weeks), with sampling of plasma glucose fasting and at one hour and two hours. OGTT results were blinded to the clinician responsible for the care of the pregnant woman unless the fasting plasma glucose level exceeded 5.8 mmol/L or the 2-hour post-load level exceeded 11.1 mmol/L. Additional blood samples were collected concurrently for storage and future biomarker analysis. A number of standardised questionnaires were used to determine information about the mother including age at OGTT, pre-pregnancy BMI, family history of diabetes, parity and years in education. Maternal height, weight and blood pressure were measured at the OGTT. The number of cigarettes smoked during pregnancy per day and the number of alcoholic drinks taken during pregnancy per day were collected using a standardised questionnaire at the time of the OGTT. This information was used to derive smoking status (≥1 cigarette per day) and alcohol use during pregnancy (≥1 drink/per day). In addition, Belfast centre participants at their OGTT visit completed a semi-quantitative validated food-frequency questionnaire (FFQ) which was used to assess usual dietary intake [12]. Mean dietary vitamin D intake was calculated from the FFQ using the nutritional software package Q-Builder (Questionnaire Design System), version 2.0 (Tinuviel Software, Anglesey, UK) which uses United Kingdom (UK) food composition tables to quantify nutrient intakes [13]. Quantification of dietary intake of vitamin D was based on food sources alone, as the FFQ was not designed to ascertain the quantification of vitamin D entering the diet via food fortification or vitamin supplementation.

A random blood sample was also collected between 34 and 37 weeks' gestation to identify woman with undiagnosed diabetes in late gestation. If the plasma glucose equalled or exceeded 8.9 nmol/L or was less than or equal to 2.5 nmol/L, the result was unblinded to the medical caregivers responsible for the pregnant woman.

Cord blood specimens were collected at delivery for the analysis of serum C-peptide and plasma glucose. Outcome measures included delivery method (including any adverse outcomes such as shoulder dystocia and birth injury); birth weight, birth length, head circumference and neonatal skin fold thickness measurements. All neonatal anthropometric measurements were obtained within 72 hours by trained HAPO personnel, and a detailed description has been published elsewhere[11]. Birth weight was obtained without a nappy using a calibrated electronic scale. Length was measured on a standardized plastic length board constructed for use in the HAPO Study. Head circumference was measured across the occipital fontanel (standard plastic measuring tape). Skin fold thickness was measured with skin fold calipers (Harpenden, Baty, U.K.). Triceps, subscapular, and flank skin fold thicknesses were measured twice, and if results differed by more than 0.5 mm, a third measurement was made.

Overall, 23316 blinded participants successfully completed the study. Of the participating 1677 women from the Belfast centre, 37 women were removed from the study due to glucose intolerance and being unblinded [n=1640 (98%)]. A further 28 women were of non-white European ethnicity and were removed from the analysis due to the relationship between GDM and ethnicity [14] [n=1612 (96%)]. Of these 1612 women, six had antepartum fetal deaths and two had neonatal deaths. Serological samples for the measurement of vitamin D were available for 1585 women.

Laboratory analysis

25OHD$_2$/D$_3$ and 3-epi-25hydroxyvitamin D$_2$/D$_3$ (3-epi-25OHD$_2$/ D$_3$) in serum samples were measured using a liquid chromatography tandem-mass spectrometry (LC-MS/MS) method [Waters® Xevo TQ-S® & ACQUITY UPLC (Waters Corporation, UK)].

For 25OHD$_2$/D$_3$, calibration was achieved using commercially available 25OHD$_2$/D$_3$ bi-level (level I and level II) serum controls (Chromsystems, Germany) diluted in horse serum (Sigma-Aldrich Co Ltd., UK). Low, medium and high quality control (QC) samples were prepared by diluting 25OHD$_2$/D$_3$ level I and II serum controls in horse serum. For 3-epi-25OHD$_2$/D$_3$, calibration was achieved using commercially available 3-epi-25OHD$_2$/D$_3$ (Sigma-Aldrich Co Ltd., UK) diluted in methanol (Fisher Scientific; UK) to make a stock solution of 270 nmol/L. Extra low, low, medium and high QC samples were prepared by diluting 3-epi-25OHD$_2$/D$_3$ stock solution in horse serum (Invitrogen Life Technologies, UK). The final concentrations of the extra low, low, medium and

high QC samples were 8.438, 16.875, 67.5 and 135 nmol/L, respectively, for both 3-epi-25OHD$_2$ and 3-epi-25OHD$_3$.

A liquid-liquid extraction method was used to extract serum samples for 25OHD$_2$/D$_3$ and 3-epi-25OHD$_2$/D$_3$. Hexadeuterated 25OHD$_3$ (d6-25OHD3; internal standard; Synthetica AS, Norway) and trideuterated 3-epi-25OHD$_3$ (d3-3-epi-25OHD$_3$; internal standard, Sigma-Aldrich Co Ltd., UK) were added to calibrator, quality control and participant's serum samples to correct for variability during sample preparation.

The extracted sample (20 µL) was injected onto an Agilent Zorbax SB-CN column (2.1 x 50 mm; 1.8 µm particle size). A Waters Xevo TQ-S Tandem Quadrupole Mass Spectrometer was used to quantify the amount of 25OHD and 3-epi-25OHD in samples. Instrument analysis time was 17.5 minutes per sample.

The inter-assay coefficients of variation (CVs) of the method for 25OHD$_2$ and 25OHD$_3$ were 4.4% and 3.4% at concentration 16.1 nmol/L, respectively, while the intra-assay CVs were 2.7 and 2.3%, respectively. The interassay CVs of the method for 3-epi-25OHD$_2$ and 3-epi-25OHD$_3$ were 2.3 and 2.6% at concentration 8.4 nmol/L, respectively, while the intra-assay CV were 5.5 and 4.5%, respectively. The quality and accuracy of serum 25OHD analysis using the LC-MS/MS method in our laboratory was monitored on an ongoing basis by participation in the Vitamin D External Quality Assessment Scheme (Charing Cross Hospital); however, this scheme was for total serum 25OHD and does not take into consideration 3-epi-25OHD. Commercially available quality control samples (Chromsysytems, Germany) were extracted and analyzed in parallel to the serum samples, and were strategically placed close to the beginning, middle and end of the analysis on the LC–MS/MS instrument, in addition, a number of patient samples were also routinely re-analyzed on a daily basis to ensure accuracy and precision of the method.

The measurement of all maternal and cord glucose samples were done at the HAPO Central Laboratory (Belfast, Northern Ireland, U.K.). Aliquots of maternal fasting, 1-hour and 2-hour OGTT specimens and serum cord were analyzed for glucose using a chemical analyser (Vitros 750; OrthoClinical Diagnostics, Rochester, NY) by an oxidase/peroxidase method. Cord c-peptide was measured only in non-haemolysed samples by a two-way immunometric assay on an Autodelfia instrument (Waltham, MA).

Statistical analysis

Statistical analysis in the Belfast HAPO cohort was carried out using SPSS version 21 (IBM Corp, Armonk, NY, USA). Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) and Homeostatic Model Assessment-Beta (HOMA-beta) were calculated using the HOMA2 calculator [15].

Birth weight, birth height and head circumference were converted to standard deviation scores (SDS) using the 1990 British Growth Standard which takes into consideration the offspring gender and gestational age [16]. Neonates born before 36 weeks gestation were removed for analysis with fat mass, as the equation does not apply to those neonates born <36 weeks gestation. Season of maternal OGTT was defined as winter/spring (November, December, January, February, March, April) or summer/fall (May, June, July, August, September, October) for regression analysis. Total 25OHD was comprised of $25OHD_2$ and $25OHD_3$, of which $25OHD_3$ is the main constituent. Total 25OHD was split into quintiles for certain analysis (≤25 nmol/L, 25.01 to 49.9 nmol/L, 50 to 74.9 nmol/L, 75-99.9 nmol/L and ≥100 nmol/L).

Variables were examined for a normal distribution using normality plots and serum 25OHD, cord HOMA-IR, and cord HOMA-beta were logarithmically transformed to the base 2 because their distributions were positively skewed. Pearson's correlation coefficient was used to assess the association between total 25OHD concentrations and continuous variables. Independent samples t-tests and one-way analysis of variance (ANOVA) were used to compare total 25OHD concentrations between groups defined by variables with two and three or more categories, respectively.

Multiple linear regressions were used to determine the independent association of total 25OHD with a number of neonatal anthropometric measurements, cord HOMA-IR and cord HOMA-beta. Analyses were adjusted for a number of variables: season of sampling, maternal age at OGTT, body mass index (BMI) at OGTT, smoker during pregnancy, alcohol user during pregnancy, family history of diabetes, gestational age at delivery, gender of neonate, parity, systolic blood pressure at OGTT, maternal height, fasting plasma glucose (excluded in cord HOMA-IR/beta analysis), and maternal education.

Results are presented as means (geometric means if the variable was log transformed) and 95% confidence intervals (CIs). Regression coefficients were back transformed if the dependent variable was logged. A p value less than or equal to 0.05 was considered statistically significant.

Ethics- Written informed consent was obtained from all study participants. Ethical approval was obtained from the Northern Ireland Regional Ethics Committee and the research adhered to the tenets of the Declaration of Helsinki.

188 Results

189 Neonatal birth weights were obtained in 1605 neonates, and other neonatal anthropometric
190 measurements were obtained in smaller numbers (1507-1601). Cord measurements of insulin
191 resistance and beta cell function were available in 1150 neonates born to Belfast HAPO mothers.

192 Descriptive statistics for the mother at 28 weeks gestation and the neonate are shown in Table 1.
193 The mean ± SD age and body mass index (BMI) at the OGTT of participants in the HAPO study at
194 the Belfast centre were 29.7 ±5.5 years, and 28.3 ±4.6 kg/m^2, respectively. Women had blood
195 samples taken on average at 29 weeks gestation. The prevalence of cigarette smoking and alcohol
196 use during pregnancy was relatively high (24.1% and 26.9%, respectively). The mean ± SD
197 maternal 25OHD concentration was 46.3 ±30.3 nmol/L. No 3-epi-25OHD$_2$ was present in
198 participant samples. 3-epi-25OHD$_3$ concentrations were low (2.9 ±1.9 nmol/L) and present in 95%
199 of all participants sampled. Dietary vitamin D as estimated from the FFQ was low (3.3 ±2.5
200 µg/day), and below the recommended nutrient intake (RNI) of 10 µg/day for pregnant women in the
201 UK[17], however, it should be again noted, that the FFQ did not quantify vitamin D from nutritional
202 supplements and fortified foods. The prevalence of vitamin D deficiency was high, with 26.7% of
203 women having 25OHD concentrations less than 25 nmol/L. Neonates at the Belfast HAPO Centre
204 were born at an average of 40 weeks gestation. The average birth weight was 3,402 ± 517 g and the
205 average birth weight SDS was -0.1 ± 1.0. The average birth length was 50.8 ± 2.5 cm and birth
206 length SDS was 0.2 ± 1.1.

207 Circulating concentrations of maternal 25OHD were not significantly correlated with maternal age
208 or maternal BMI, however, 25OHD was significantly and positively correlated with years of
209 education ($p \leq 0.001$) (data not shown). Serum 25OHD was significantly lower in those women who
210 smoked during pregnancy ($p \leq 0.001$). There were no significant differences in 25OHD
211 concentrations between alcohol use and non-alcohol use during pregnancy. There was evidence of
212 seasonal variation in 25OHD concentrations. Maternal 25OHD concentrations were lower in the
213 winter/spring (29.3 nmol/L) compared to summer/fall (47.6 nmol/L) (data not shown).

214 Total 25OHD was split into quintiles (≤25 nmol/L, 25.01-49.99 nmol/L, 50-74.99 nmol/L, 75-99.99
215 nmol/L and ≥100 nmol/L) and neonatal anthropometric outcomes were compared. There were no
216 significant differences between the quintiles of total 25OHD and birth weight, birth length, neonatal
217 fat mass, neonatal subscapular skinfold, neonatal flank skinfold thickness and neonatal triceps'
218 skinfold thickness (data not shown). In addition, no significant differences were observed between
219 quintiles of total 25OHD and markers of neonatal glycaemia (data not shown).

Table 2 shows the associations between maternal 25OHD at 28 weeks gestation and neonatal anthropometric measurements adjusted for confounders. The regression analysis found that the doubling of maternal 25OHD gave rise to a birth weight higher by 0.05 and birth length SDS higher by 0.07 (both, p=0.03). No associations were found for neonatal measures of skinfold thickness with maternal 25OHD (Table 2).

No significant associations were observed between maternal 25OHD concentrations at 28 weeks' gestation and cord HOMA-IR and cord HOMA-beta in both the unadjusted analysis and adjusted analysis (Table 3).

Discussion

Maternal vitamin D deficiency was common in participants in the Belfast HAPO study. After adjustment for confounding variables, maternal serum 25OHD was associated only with neonatal birth weight SDS and birth length SDS. However, the contribution of maternal 25OHD to these neonatal anthropometric outcomes appears to be limited. No associations were observed with maternal 25OHD and markers of neonatal beta-cell function and insulin resistance.

There is conflicting evidence regarding the relationship between vitamin D and neonatal and maternal outcomes. A number of studies have found associations of maternal vitamin D status with a number of neonatal outcomes, including birth weight [18], pre-term birth [4], asthma and allergies [19]. The conflicting results are most likely due to low numbers of participants in the studies, and failure to control for relevant confounding variables. Well-designed longitudinal observational studies are required to investigate thoroughly maternal-neonatal associations with vitamin D. These might in turn point to the need for large-scale vitamin D intervention trials to investigate the association of 25OHD with maternal and neonatal outcomes. In the current study, maternal 25OHD was significantly associated with birth weight SDS in adjusted analysis. Further, there was a lack of association between maternal 25OHD with measures of neonatal adiposity. One small study reported greater neonatal fat mass with higher cord 25OHD concentrations [20] and that cord 25OHD was directly related to maternal 25OHD concentrations [21]. While we did not measure cord 25OHD, our data pertain to a much larger cohort of mother-neonate pairs, and our results have been adjusted for a considerable number of confounding variables. The lack of significant associations in the present study between maternal 25OHD and neonatal adiposity is also concordant with the results from a sub sample of a North American HAPO cohort which also found no significant association between cord 25OHD and neonatal adiposity [21].

Previous studies which have examined the association between maternal vitamin D and birth weight have reached conflicting conclusions. In an observational study, Gernard and colleagues assessed the 25OHD concentrations of 2146 pregnant women at 26 weeks gestation and reported mean maternal 25OHD concentrations of 51.3 nmol/L similar to findings in the current study (46.3 nmol/L). The authors observed a non-linear relationship between 25OHD concentrations and birth weight. When adjusted for several confounders (trimester at maternal blood draw, maternal race (white/other), pre-pregnancy BMI, height, smoking, season, and study site) maternal 25OHD concentrations higher by 1 nmol/L were associated with mean neonatal birth weight higher by 3.6g [6]. A prospective cohort study preformed in a number of cities in Spain among 2,382 pregnant women during the early second trimester reported a median circulating 25OHD level of 73.4 nmol/L. No association was found between maternal 25OHD concentrations and neonatal birth

weight in either the unadjusted or adjusted analysis. In addition, maternal 25OHD was not associated with low birth weight (<2,500g) [22]. It is possible that the association of vitamin D with low birth weight is only observed in vitamin D deficient mothers. A prospective study by Chen and colleagues in 3,658 pregnant women reported a significant positive correlation between maternal 25OHD and birth weight ($r=0.477$; $p<0.001$), with evidence of a threshold of 100 nmol/L for maternal 25OHD concentrations below which vitamin D was an important predictor of neonatal birth weight but above which there was no association [23]. The evidence, therefore, regarding the relationship between vitamin D and birth weight is inconclusive. There are data which suggest an association between maternal glucose and fatty acid metabolism and therefore the transfer of energy to the foetus, and consequently increase neonatal birth weight[6]. The relationship with vitamin D and birth weight could also be simply explained by a healthy maternal diet, which in turn increases 25OHD concentrations and gives rise to an increased birth weight in the neonate.

A significant association was observed in the current study between maternal 25OHD concentrations and birth length SDS. This is in line with a large mother-neonate study in the US (n=2473)[24]. In this latter study, maternal 25OHD at 26 weeks gestation was 58.9 nmol/L, and a significant positive association was observed between 25OHD and birth length Z-score in adjusted analysis. The authors also observed that this association was sustained from birth to 12 months [24], which would suggest that skeletal growth deficits in infants with low maternal 25OHD may be difficult to recover. This is concordant with a previous study which suggested maternal vitamin D status is associated with reduced bone mass accrual during childhood up to 9 years of age [25]. The effect of maternal vitamin D on birth length, and perhaps bone mass, could be linked to the maternal-foetal transfer of calcium during pregnancy. Javaid and colleagues observed that umbilical-venous concentrations of calcium were significantly associated with bone mass accrual in offspring [25]. The main role of vitamin D in pregnancy is to escalate calcium absorption and placental calcium transport [26]. It is possible that vitamin D deficiency may reduce the capacity of maternal transfer of calcium to the foetus and therefore decrease bone mass, and bone length.

25OHD concentrations above the threshold of deficiency have been associated with improved beta cell function in various population groups [8],[9], however, this was not observed in the current study. Vitamin D actions are mediated by vitamin D receptors (VDRs), which undergo several processes to activate metabolic regulatory factors, including insulin. VDRs are present in beta cells, which suggest vitamin D may have a direct involvement in regulating beta cell function [27]. Information on the impact of maternal vitamin D deficiency on the incidence of type 1 diabetes in the offspring is limited. A meta-analysis on the association between maternal vitamin D intake and the risk of type 1 diabetes was conducted by Dong and colleagues. Three studies were included in the analysis

297 and the pooled odds-ratio (OR) was 0.95 (95% CI, 0.66–1.36) indicating no association between
298 maternal intake of vitamin D and risk of type 1 diabetes in the offspring [28]. Due to limited
299 literature on the impact of maternal vitamin D concentration on neonatal beta cell function, it is
300 difficult to assess if further investigation is warranted regarding maternal vitamin D and the risk of
301 diabetes in the offspring.

302 The strengths of this study include the large number of participants, the rigorous nature of the
303 methodology, the detailed neonatal endpoints and the methodology for vitamin D measurement
304 during pregnancy. In addition, exploration of the association between maternal vitamin D and
305 neonatal outcomes was controlled for relevant confounding variables. The limitations include the
306 observational nature of the study and the use of HOMA equations to assess beta cell function and
307 insulin resistance. Information on cord HOMA-beta and HOMA-IR was only available for 75% of
308 the total study population which reduced the sample size for analysis of cord HOMA-beta and
309 HOMA-IR. In addition, we did not measure 25OHD in cord blood which would have provided
310 further information on the relation of maternal 25OHD to neonatal adiposity.

311 In future studies, we plan to examine the associations of maternal 25OHD on offspring bone mass
312 and bone length in childhood years. In summary, the present study has shown a positive association
313 between maternal 25OHD and birth weight SDS and birth length SDS controlled for confounding
314 variables, however, the contribution of maternal 25OHD in regression models appears to be limited.
315 No other significant associations were found for other measures of neonatal anthropometry. In
316 general, it would seem wise to encourage pregnant women to maintain 25OHD concentrations ≥25
317 nmol/L for skeletal health and to prevent the neonate being vitamin D deficient.

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Conflict of interest- No potential conflicts of interest relevant to this article were reported.

Author's contribution- DRM designed the project; CC and AMcG conducted the research; CC, CCP, ISY and DMC analyzed data; CC, VAH and DRM wrote the paper. DRM has primary responsibility for the final content. All authors read and approved the final manuscript.

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Table 1 Neonatal anthropometric outcomes and biochemical insulin indices in neonates born to Belfast HAPO mothers

Variable	N (%)	Range	Mean	Standard deviation
Mother at OGTT				
Age (years)	1612	18.1 to 43.3	29.7	5.5
Weight (kg)	1611	46.9 to 137.3	75.0	12.8
Height (cm)	1612	139.8 to 183.1	162.9	6.3
BMI (kg/m^2)	1611	18.1 to 50.3	28.3	4.6
Gestational age (weeks)	1612	23.9 to 33.1	29.0	1.2
Education (years)	1601	10 to 27	15	3
Ethnicity		-	-	
Caucasian	1612/1612 (100%)			
Smoker during pregnancy	388/1612 (24.1%)	-	-	
Alcohol use during pregnancy	434/1612 (26.9%)	-	-	
Fasting plasma glucose (mmol/L)	1612	3.6 to 6.2	4.6	0.3
1-hour glucose (mmol/L)	1611	2.8 to 10.2	7.4	1.7
2-hour glucose (mmol/L)	1612	2.4 to 10.2	6.0	1.2
Total 25OHD (nmol/L)[b]	1585	3.1 to 266.1	38.6	24.1, 60.7
3-epi-25OHD$_3$ (nmol/L)	1512	2.7 to 20.0	2.9	1.9
Dietary vitamin D (μg/day)	1568	0.0 to 29.8	3.3	2.5
Vitamin D deficient (≤25 nmol/L)	423/1585 (26.7%)	-	-	
Neonatal measurements				
Birth weight (g)	1605	1070 to 5000	3402	517
Birth weight SDS	1605	-3.1 to 3.5	-0.1	1.0
Birth length (cm)	1593	41.3 to 60.5	50.8	2.5
Birth length SDS	1593	-4.3 to 5.2	0.1	1.1
Head circumference (cm)	1601	25.6 to 39.7	35.0	1.5
Head circumference SDS	1601	-3.4 to 3.6	0.2	1.0
Fat mass (g)[a]	1490	1.9 to 1070.9	420.3	168.6
Subscapular skinfold thickness (cm)	1510	2.1 to 8.1	4.4	1.0
Flank skinfold thickness (cm)	1510	1.8 to 8.1	4.0	0.9
Triceps skinfold thickness (cm)	1507	2.0 to 8.1	4.1	0.9
Cord glucose (mmol/L)	1352	2.1 to 12.7	4.5	1.1
Cord HOMA-beta[b]	1150	17.1 to 412.7	101.2	69.0, 142.2
Cord HOMA-IR[b]	1150	0.4 to 3.9	0.7	0.5, 1.0

25OHD, 25-hydroxy vitamin D HAPO, Hyperglycemia and Adverse Pregnancy Outcome Study HOMA-beta, Homeostasis model assessment-of beta cell function HOMA-IR, Homeostasis model assessment-of insulin resistance SDS, Standard deviation score.

a- Neonates born <36 weeks gestation were removed from analysis.

b- Values expressed as median and interquartile range

Table 2 Unadjusted and adjusted associations of maternal 25OHD at 28 weeks' gestation with neonatal anthropometric measurements

		Unadjusted			Adjusted [b]		
Dependent variables	**n**	**Coefficient [a] (95% CI)**	**p-value**	**R^2**	**Coefficient [a] (95% CI)**	**p-value**	**R^2**
Birth weight SDS	1555	0.08 (0.03 to 0.13)	**0.002**	0.01	0.05 (0.00 to 0.10)	**0.03**	0.21
Birth length SDS	1543	0.08 (0.02 to 0.14)	**0.007**	0.01	0.07 (0.01 to 0.13)	**0.03**	0.12
Head circumference SDS	1551	0.06 (0.01 to 0.12)	**0.02**	0.00	0.05 (-0.00 to 0.11)	0.08	0.11
Fat mass (g) [c]	1442	11.91 (2.90 to 20.92)	**0.01**	0.01	7.95 (-0.80 to 16.70)	0.08	0.24
Subscapular skinfold (cm)	1462	0.06 (0.00 to 0.11)	**0.03**	0.00	0.04 (-0.01 to 0.10)	0.11	0.13
Flank skinfold (cm)	1462	0.06 (0.01 to 0.11)	**0.02**	0.00	0.03 (-0.02 to 0.08)	0.25	0.13
Triceps skinfold (cm)	1459	0.06 (0.01 to 0.11)	**0.01**	0.00	0.04 (-0.01 to 0.09)	0.13	0.13

25OHD, 25-hydroxyvitamin D CI, confidence intervals SDS, Standard deviation score

a- Regression coefficients represent the additive effect on the dependent variable associated with a doubling in maternal serum 25OHD level (due to 25OHD being logged to the base 2).

b- Adjusted for season of sampling, maternal age at OGTT, maternal BMI at OGTT, smoker during pregnancy, alcohol use during pregnancy, family history of diabetes, gestational age at delivery, gender of neonate, parity, systolic blood pressure at OGTT, maternal height, fasting plasma glucose and maternal education

c- Neonates born <36 weeks gestation were excluded from analysis.

Table 3-Summary table of the relationship of maternal 25OHD at 28 weeks' gestation to markers of neonatal glycaemia

		Unadjusted			**Adjusted**[b]		
Dependent variables	**n**	**Coefficient(95% CI)**	**p-value**	**R^2**	**Coefficient(95% CI)**	**p-value**	**R^2**
Cord glucose (mmol/L)	1314	-0.02 (-0.08 to 0.04)	0.55	0.00	-0.05 (-0.11 to 0.02)	0.16	0.09
Cord HOMA-Beta [a]	1105	1.03 (1.00 to 1.06)	0.10	0.00	1.03(1.00 to 1.06)	0.08	0.10
Cord HOMA-IR [a]	1105	1.03(1.00 to 1.06)	0.05	0.00	1.01(0.98 to 1.04)	0.58	0.06

25OHD, 25-hydroxyvitamin D CI, confidence intervals HOMA-beta, homeostatic model of assessment-beta HOMA-IR, homeostatic model of assessment-insulin resistance.

a-Regression coefficients have been antilogged to represent the multiplicative effect on the dependent variable associated with a doubling in maternal serum 25OHD level (due to 25OHD being logged to the base 2).

b- Adjusted for season of sampling, maternal age at OGTT, maternal BMI at OGTT, smoker during pregnancy, alcohol use during pregnancy, family history of diabetes, gestational age at delivery, gender of neonate, parity, systolic blood pressure at OGTT, maternal height, and maternal education