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Vitamin D insufficiency is common in Indian mothers but is not associated with gestational diabetes or variation in newborn size

Hannah JW Farrant[1], **Ghattu V Krishnaveni**[2], **Jacqueline C Hill**[1], **Barbara J Boucher**[3], **David J Fisher**[1], **Kate Noonan**[4], **Clive Osmond**[1], **Sargoor R Veena**[2], and **Caroline HD Fall**[1]

1 *MRC Epidemiology Resource Centre, University of Southampton, Southampton General Hospital, Southampton, SO16 6YD*

2 *Holdsworth Memorial Hospital, Mysore, Karnataka, India*

3 *Centre for Diabetes and Metabolic Medicine, Institute for Cellular and Molecular Science, Barts and the London School of Medicine and Dentistry, London, E1 2AT*

4 *Department of Clinical Biochemistry, Barts and the London NHS Trust, London, E1 2AD*

Abstract

Background/objectives: Vitamin D is required for bone growth and normal insulin secretion. Maternal hypovitaminosis D may impair fetal growth and increase the risk of gestational diabetes. We related maternal vitamin D status in pregnancy to maternal and newborn glucose and insulin concentrations, and newborn size, in a South Indian population.

Subjects/methods: Serum 25 hydroxy vitamin D (25(OH)D) concentrations, glucose tolerance, and plasma insulin, proinsulin and 32-33 split proinsulin concentrations were measured at 30 weeks gestation in 559 women who delivered at the Holdsworth Memorial Hospital, Mysore. The babies' anthropometry and cord plasma glucose, insulin and insulin precursor concentrations were measured.

Results: 66% of women had hypovitaminosis D [25(OH)D concentrations <50 nmol/l] and 31% were below 28 nmol/l. There was seasonal variation in 25(OH)D concentrations ($P<0.0001$). There was no association between maternal 25(OH)D and gestational diabetes (incidence 7% in women with and without hypovitaminosis D). Maternal 25(OH)D concentrations were unrelated to newborn anthropometry or cord plasma variables. In mothers with hypovitaminosis D, higher 25(OH)D concentrations were associated with lower 30-minute glucose concentrations ($p=0.03$) and higher fasting proinsulin concentrations ($p=0.04$).

Conclusions: Hypovitaminosis D at 30 weeks gestation is common in Mysore mothers. It is not associated with an increased risk of gestational diabetes, impaired fetal growth, or altered neonatal cord plasma insulin secretory profile.

Keywords

Vitamin D; Prenatal nutrition; Pregnancy outcome; Birth weight; India; Gestational diabetes; Glucose tolerance; Insulin secretion; Cord blood insulin

Correspondence and reprint requests to: Dr Caroline HD Fall, MRC Epidemiology Resource Centre, University of Southampton, Southampton General Hospital, Temona Road, Southampton, SO16 6YD, UK. Tel: 0044 (0)23 8077 7624, Fax: 0044 (0)23 8070 4021, Email: E-mail: chdf@mrc.soton.ac.uk..

INTRODUCTION

The best-known function of vitamin D is the maintenance of calcium homeostasis through its actions on phosphate and calcium handling in the intestine, kidney and bone. Vitamin D is required throughout life for the normal growth and maintenance of bone. Its role in fetal skeletal growth is well established, and maternal vitamin D supplementation has been shown to increase birth weight and length in human studies. The most effective interventions, in terms of increased newborn size, have been in South Asian mothers with a high prevalence of vitamin D insufficiency (Brooke *et al.* 1980; Marya *et al.* 1981).

Vitamin D is also required for the normal production and secretion of insulin by the endocrine pancreas, and possibly for normal insulin sensitivity (Boucher *et al.* 1995, Clark *et al.* 1981, Pittas *et al* 2007). In experimental animals vitamin D deficiency impairs insulin release and causes glucose intolerance (Clark *et al* 1980, Bikle *et al* 1992). In humans, it predicts increased glycaemia, and reduced insulin sensitivity and secretion in normoglycaemic subjects (Chiu *et al* 2004, Hypponen and Power 2006). Serum 25 hydroxy vitamin D concentrations correlated positively with 30-minute insulin concentrations, and negatively with glycaemia, during an oral glucose tolerance test in UK South Asians (Boucher *et al* 1995) and it has been proposed that vitamin D deficiency may contribute to the increasing prevalence of type 2 diabetes, especially in South Asian populations (Boucher 1998).

Insulin is a growth-promoting hormone during fetal life, acting directly, mainly on fetal adipogenesis, and indirectly (on all body tissues) through stimulation of the insulin-like growth factors and their binding proteins (Fowden 2003). Maternal vitamin D status may therefore influence fetal growth partly through the insulin axis.

We have assessed maternal vitamin D status by measuring serum 25(OH)D in samples collected during a study of pregnant women in South India; the Parthenon Study. The original aim of this study was to determine the incidence of gestational diabetes and its effects on fetal growth. Stored serum samples were used to assess vitamin D status in this population, its seasonal variation, and to examine whether maternal hypovitaminosis D at 30 weeks gestation is associated with i) altered maternal glucose and insulin concentrations or an increased risk of gestational diabetes, ii) reduced fetal growth, and iii) altered neonatal insulin concentrations. Because of recent interest in threshold effects of vitamin D (Hollis 2005) these associations were examined in the whole sample of women and then separately in those with hypovitaminosis D (serum 25(OH)D concentrations below 50 nmol/l). This value was chosen because although it is currently suggested that vitamin D depletion is defined by concentrations below 70 or 75 nmol/l (Vieth *et al.* 2007), concentrations are known to be lower in pregnancy (Bouillon *et al.* 1981).

SUBJECTS AND METHODS

Subjects

In 1997, the Mysore Parthenon Study recruited pregnant women attending the antenatal clinic at the Holdsworth Memorial Hospital (HMH), Mysore, South India (Hill *et al* 2005, Krishnaveni *et al* 2005). All women booking into the clinic were invited to take part. They were eligible for the study if they were not diabetic before pregnancy, planned to deliver at HMH, had a singleton pregnancy and were less than 32 weeks gestation, determined by the last menstrual period (LMP) or by a first trimester ultrasound scan if the LMP was uncertain. Of 1,539 women interviewed, 10 were carrying twins, 198 planned delivery elsewhere and 98 booked too late, leaving 1,233 eligible women, of whom 830 (67%) participated. Ethical permission for the study was granted by HMH Research Ethics Committee and informed consent was obtained.

Clinic investigations

At 30 +/− 2 weeks gestation, maternal weight, height and skinfold thicknesses (biceps, triceps, subscapular and suprailiac) were measured using standardised methods by one of four trained observers. Socio-economic status was assessed using a standard questionnaire method, based on education, occupation and income (Kuppuswamy 1962).

Fasting blood was taken for measurement of plasma glucose, insulin and insulin precursor concentrations. A 100g oral glucose load was administered and blood taken 30, 60, 120 and 180-minutes later for plasma glucose and insulin concentrations. The glucose tolerance test (OGTT) was discontinued in 37 women due to vomiting, 4 did not wish to complete the OGTT and in 5 some samples were haemolysed. OGTT data were complete for 784 women. Plasma glucose concentrations were measured in Mysore using a standard hexokinase method. Gestational diabetes was diagnosed using the Carpenter and Coustan criteria (Carpenter and Coustan 1982), the standard method in clinical use in HMH. Aliquots of plasma were stored at −80°C until the end of the study (up to 18 months) and transported in dry ice to the department of Clinical Biochemistry, Addenbrooke's Hospital, Cambridge, UK, where glucose was re-measured using a standard hexokinase method, specific intact human insulin was measured by enzyme immunoassay (Medgenix, Florius, Belgium; CV <10%, cross reactivity with proinsulin and 32-33 split proinsulin 0%), and proinsulin and 32-33 split proinsulin concentrations were measured by immunoradiometric assay (CV<7.5%) (Alpha *et al.* 1992, Sobey *et al.* 1989).

Deliveries

674 women delivered in HMH (81% of participants). Umbilical cord venous blood was sampled for plasma glucose, insulin, proinsulin and 32-33 split proinsulin assay. Neonatal anthropometric measurements were made immediately after birth by one of four trained observers, using standardised methods. Weight was measured on digital weighing scales (Seca, Germany, CMS Instruments Ltd, London) and crown-heel length on a Harpenden neonatal stadiometer (CMS Instruments Ltd). Head, chest (xiphisternum), abdomen (umbilicus) and mid-upper-arm circumferences (MUAC) were measured with blank anthropometric tape, marked and measured against a fixed ruler. Triceps and subscapular skinfold thicknesses were measured 3 times using Harpenden callipers (CMS Instruments Ltd) and averaged.

Calcium and vitamin D supplements

At the time of the study, it was routine practice for general practitioners and obstetricians to prescribe calcium and vitamin D supplements to pregnant women from the beginning of the 2nd trimester. A range of products was prescribed, according to each practitioner's preference, containing 500-1250mg of calcium carbonate and 2.5 μg (100 IU) - 7.5 μg (250 IU) of vitamin D3. Medications and supplements being taken by the women were recorded at recruitment, but not subsequently, and no information was therefore available on their use at 30 weeks gestation, when blood samples were taken, or at term.

Vitamin D analysis

Vitamin D status was assessed using stored serum samples from mothers who delivered at HMH, had a term delivery (≥37 weeks gestation) and full OGTT data (n=574). Adequate samples were available for 559. Maternal serum 25(OH)D concentrations were measured using radioimmunoassay (IDS Immunodiagnostics Ltd, Boldon, Tyne and Wear UK; intra- and inter-assay coefficients of variation 8.8% and 10.8% respectively). Mothers were defined as having hypovitaminosis D at serum concentrations of 25(OH)D <50 nmol/l (Malabanan *et al.* 1998).

Statistical analysis

The distributions of maternal body mass index (BMI), 25(OH)D concentrations, placental weight, and maternal and neonatal skinfold thicknesses, glucose, insulin and insulin precursor concentrations were skewed; these data were log-transformed for analysis. The 30-minute insulin increment (30 minute insulin - fasting insulin/30 minute glucose) was used as an index of maternal insulin secretion (Wareham *et al.* 1995). Insulin resistance was estimated using the Homeostasis Model Assessment equation (HOMA) (Matthews *et al.* 1985). Maternal fat mass was calculated using the four skinfolds (Van Raaij *et al.* 1988). Birth measurements were adjusted to a gestational age of 40 weeks, for the sexes separately, using linear regression. The significance of seasonal variations in 25(OH)D concentrations and other variables was tested by Fourier analysis, using the sum of sinusoidal curves of periodicity one year, six months and four months, to obtain the best fit. Relationships between maternal 25(OH)D concentrations and other maternal and neonatal variables were examined using multiple linear and logistic regression, using variables as continuous where appropriate. Analysis was carried out using STATA version 7.

RESULTS

The median (IQR) maternal serum 25(OH)D concentration was 37.8 (24.0, 58.5) nmol/l. Sixty-six percent of women had hypovitaminosis D (25(OH)D concentrations below 50 nmol/l) and 31% were below 28 nmol/l. Concentrations were not related to the mother's socio-economic status or religious group (Hindus, 57% of population, median 25(OH)D = 40.0 nmol/l, Muslims 34%, 37.5 nmol/l, Christians 9%, 41.0 nmol/l). Of the 559 mothers, 156 were taking calcium and vitamin D at recruitment. They were of higher socio-economic status than women not taking supplements (p=0.02), but their 25(OH)D concentrations at 30 weeks gestation were lower: median 34.5 nmol/l compared with 39.3 nmol/l (p=0.04).

Seasonal variation

There was marked seasonal variation in maternal serum 25(OH)D concentrations (P<0.0001, Figure 1), accounting for 25% of the variation. Concentrations were highest during September-February and lowest during March-August. There was no seasonal variation in maternal or newborn glucose and insulin variables.

Maternal outcomes

There were no associations between 25(OH)D concentrations and maternal age, height, BMI or fat mass (Table 1). Concentrations were similar in the 34 mothers with gestational diabetes (median 38.8 nmol/l) to those in mothers with normal glucose tolerance (37.8 nmol/l; p=0.8). Percentages of women with gestational diabetes were similar in women with and without hypovitaminosis D (7% in both groups). After adjustment for maternal age, fat mass and diabetes status, 25(OH)D concentrations were positively related to fasting 32-33 split proinsulin concentrations. There were no other associations between 25(OH)D concentrations and maternal outcomes; however among women with hypovitaminosis D, 30-minute glucose concentrations were inversely associated, and fasting proinsulin concentrations positively associated, with 25(OH)D concentrations.

Newborn outcomes

Mean birthweight (adjusted to 40 weeks gestation) was 3.0 kg, and was positively related to maternal weight, height, BMI and fat mass at 30 weeks gestation (p<0.001, 0.06, <0.001, <0.001). Gestational diabetes was associated with an increase in all neonatal measurements (mean (SD) birthweight 3262 (430)g compared with 2889 (414)g for babies of mothers with normal glucose tolerance; p<0.001). None of the neonatal body measurements, or cord plasma

concentrations of glucose, insulin, proinsulin or 32-33 split proinsulin, were related to maternal 25(OH)D concentration (Table 1).

DISCUSSION

A high proportion (66%) of mothers recruited from the antenatal clinic, and giving birth to full-term babies, in one large maternity unit in the city of Mysore, India, had hypovitaminosis D, according to recognised criteria (Malabanan *et al* 1998) at 30 weeks gestation, despite many women being prescribed calcium and vitamin D supplements earlier in pregnancy. There was marked seasonal variation in 25(OH)D concentrations. There were no significant associations between maternal vitamin D status and risk of gestational diabetes, newborn size or cord insulin concentrations. In women with hypovitaminosis D, 25(OH)D concentrations were inversely related to 30-minute glucose concentrations and positively related to fasting proinsulin concentrations.

Our study was limited to mothers booking into one hospital and was not population-based. Maternity services are not centralised in India, and in cities there is a wide choice of facilities. HMH charges patients but offers concessions for the poorest, providing a niche between free government hospitals and private nursing homes. Women who deliver there are of all religions, and mainly of middle and lower socio-economic status. The women in the original study were unselected consecutive attenders in the ante-natal clinic, and the only selection for the vitamin D study was on the basis of full term delivery at HMH, complete OGTT data and adequacy of stored serum samples.

The high frequency of maternal hypovitaminosis D, assessed by 25(OH)D values <50 nmol/l is consistent with findings in other studies (Sachan *et al.* 2005). South Asians, both in their country of origin and after migration to Europe or the USA, have lower serum 25(OH)D concentrations than white Caucasians (Goswami *et al.* 2000, Awumey *et al.* 1998, Hamson *et al.* 2003) due to skin pigmentation, covered-up clothing (especially common in women) and low dietary vitamin D intake. There may also be differences in vitamin D metabolism in South Asians; in vitro studies have shown that tissue fibroblasts have increased 25-hydroxy-24-hydroxylase activity, leading to increased catabolism of 25(OH)D and of activated vitamin D, $1,25(OH)_2D$ (Awumey *et al.* 1998). The expression of this enzyme is dose-related to the habit of betel-nut chewing (Ogunkolade *et al.* 2006), but we did not record betel-chewing in Mysore, where it is generally practiced by women only on special occasions.

Because our study was not originally designed to examine vitamin D status, we did not collect data on the use of supplements at 30 weeks gestation, but only at recruitment. Women not on supplements at recruitment may have been prescribed them later in pregnancy, whilst those prescribed supplements in early pregnancy may have stopped taking them by 30 weeks. Paradoxically, mothers taking supplements at recruitment had lower 25(OH)D concentrations at 30 weeks, suggesting that information collected at recruitment probably bore little relationship to supplement usage in late pregnancy. We can, however, conclude that despite the frequent prescription of calcium and vitamin D during the second trimester (generally 250 IU or less), many mothers had hypovitaminosis D in late pregnancy.

Seasonal variation in serum 25(OH)D concentrations in Mysore is probably related to sunlight exposure. Low concentrations in winter are well described in European and US populations (Van der Wielen *et al.* 1995, Sherman *et al.* 1990, Kuoppala *et al.* 1986) and concentrations vary with season and correlate with sun exposure in India and other Asian countries (Goswami *et al.* 2000, Kim *et al.* 2000, Nakamura *et al.* 2000). We have no sunlight exposure data for our women, but it is likely to have been lowest during the rains (June-August) and hottest

summer months when people avoid the sun (March-May). 25(OH)D concentrations were highest in the sunny, but relatively cool, months of September-February.

We found no relationship in Mysore between maternal vitamin D status at 30 weeks and neonatal size. Trials of vitamin D supplementation in pregnant Asian women have shown improvements in maternal and neonatal biochemical indices of calcium and vitamin D status, and higher birthweight and length (Marya *et al.* 1988). Canadian women with higher milk and/or vitamin D intakes had heavier babies (Mannion *et al.* 2006). Australian women with higher 25(OH)D concentrations in late pregnancy had babies with longer knee-heel length (Morley *et al.* 2006). In contrast, an early study of UK Asian women showed no correlation between maternal 25(OH)D concentrations and birthweight (Dent *et al.*1975). The discrepancy between our data and reported interventional data may reflect the timing of our 25(OH)D measurements. In trials, supplementation has usually been given throughout the last trimester, whereas vitamin D status was assessed at the beginning of the last trimester in our study. Alternatively, vitamin D concentrations in Mysore may all have been below those required for optimal fetal growth (Hollis and Wagner 2006). Our mothers may have lacked other nutrients relevant to vitamin D function, such as vitamin A, often deficient in pregnancy in India (Singh and Toteja 2003), since activated vitamin D exerts its main effects through complexation of the activated vitamin D receptor (VDR) with the retinol-X receptor (Colston 1993). Additionally, low serum 25(OH)D concentrations may be compensated for by placental synthesis of $1,25(OH)_2D$ (Seely *et al.* 1997).

The absence of associations between maternal vitamin D status at 30 weeks gestation and neonatal anthropometry in our study does not rule out other important effects on fetal development since maternal deficiency leads to infantile rickets (Pawley and Bishop 2004) and postnatal growth failure (Brooke *et al.* 1981). Variations in maternal vitamin D status are related to neonatal and childhood bone mineral content (Namgung *et al.* 1994, Javaid *et al.* 2006).

Pancreatic β cells express the VDR, and insulin secretion in South Asians is impaired during vitamin D deficiency and improved by cholecalciferol administration (Boucher *et al.* 1995). Thus the high prevalence of vitamin D insufficiency in South Asians, could contribute to their high incidence of type 2 diabetes (Boucher 1998). There is little information on vitamin D status and gestational diabetes, but maternal hypovitaminosis D has been reported in diabetic pregnancies in Spain (Martinez *et al.* 1991), and fasting glycaemia fell with vitamin D supplementation in one small study of women with gestational diabetes (Rudnicki and Molstead-Pedersen 1997). Our data did not show increased gestational diabetes in women with hypovitaminosis D. The inverse association between 25(OH)D and 30-minute glucose concentrations among women with hypovitaminosis D was in the expected direction. The positive associations between 25(OH)D and 32-33 split proinsulin after adjustment and (in mothers with hypovitaminosis D) proinsulin concentrations may indicate subtle effects of vitamin D status on insulin secretory profiles, but could have arisen by chance.

This is the first study in South Asians to measure neonatal insulin and its precursors in relation to maternal 25(OH)D concentrations. There were no associations. A recent study of white caucasian UK neonates showed an inverse association between 34-week maternal 25(OH)D concentrations and cord proinsulin concentrations (Boucher *et al* 2003).

In conclusion, our findings did not support our starting hypotheses, and showed no evidence that hypovitaminosis D is an important contributing factor to gestational diabetes at 30 weeks gestation, or impaired fetal growth in this population. Further prospective studies are required in this area, including studies in the later stages of pregnancy, since fetal insulin responses to glucose normally develop late in pregnancy (Hellerstrom and Swenne 1991). The results of

randomised controlled trials of vitamin D supplementation in pregnancy, now in progress, are awaited with interest (Hollis and Wagner 2006).

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Table 1

Characteristics of the mothers and babies according to 25(OH)D concentrations

	All (N=559)				25(OH)D <50nmol/l (N=372)			
			p-value				p-value	
	Geometric mean	IQR	(1)	(2)	Geometric mean	IQR	(1)	(2)
MOTHERS								
Age (years)	**23.7**	(20.0, 26.0)	0.2	0.2	**23.5**	(20.0, 26.0)	0.7	0.6
Height (cm)	**154.5**	(151.0, 158.1)	0.9	0.8	**154.8**	(151.2, 158.4)	0.1	0.2
Body mass index (kg/m2)	**23.4**	(21.0, 26.0)	0.6	0.9	**23.4**	(21.1, 26.0)	0.8	0.4
Fat mass (kg)	**17.7**	(14.0, 22.6)	0.5	0.4	**17.9**	(14.3, 22.8)	0.5	0.4
Gestational diabetes (N, %)†	**39, 7.0%**†		0.8	0.9	**26, 7.0%**†		0.9	0.9
Glucose (mmol/l)								
Fasting	**4.5**	(4.3, 4.8)	0.3	0.2	**4.5**	(4.3, 4.8)	0.6	0.5
30 minutes	**7.2**	(6.5, 8.1)	0.4	0.5	**7.2**	(6.4, 8.0)	0.03−	0.01−
120 minutes	**5.9**	(5.2, 6.7)	0.7	0.5	**5.9**	(5.2, 6.7)	0.6	0.6
Insulin (pmol/l)								
Fasting	**32.9**	(22.0, 48.0)	0.6	0.3	**32.7**	(22.0, 46.5)	0.6	0.8
30 minutes	**329.7**	(220.0, 541.5)	0.7	0.8	**327.5**	(213.0, 547.0)	0.4	0.3
120 minutes	**251.5**	(166.0, 451.0)	0.7	0.8	**248.4**	(160.0, 446.0)	0.9	0.8
Proinsulin (pmol/l)	**1.7**	(1.3, 2.0)	0.7	0.5	**1.7**	(1.3, 2.0)	0.04+	0.04+
32-33 Split proinsulin (pmol/l)	**4.0**	(2.5, 6.1)	0.09	0.02+	**3.9**	(2.5, 6.0)	0.3	0.3
Insulin resistance (HOMA)	**1.2**	(0.8, 1.7)	0.4	0.1	**1.1**	(0.8, 1.7)	0.6	0.7
Insulin increment	**146.5**	(125.6, 166.8)	0.6	0.7	**146.4**	(125.7, 167.6)	0.4	0.3
BABIES								
Birthweight (g)	**2.9**	0.4	0.3	0.3	**2.9**	0.4	0.9	0.8
Triceps skinfold (mm)	**4.2**	(3.7, 4.8)	0.8	0.8	**4.2**	(3.6, 4.7)	0.4	0.5
Subscap. skinfold (mm)	**4.4**	(3.9, 5.0)	0.3	0.3	**4.4**	(3.9, 5.0)	0.9	0.9
Crown-heel length (cm)	**48.9**	2.2	0.6	0.7	**48.9**	2.0	0.9	0.9
Placental weight (g)	**408**	(360, 465)	0.2	0.2	**404**	(360, 465)	0.5	0.5
Insulin (pmol/l)	**22.8**	(13.0, 40.0)	0.9	0.9	**23.4**	(13.0, 41.0)	0.1	0.1
Proinsulin (pmol/l)	**7.8**	(5.9, 11.0)	0.1	0.1	**8.0**	(6.0, 11.0)	0.9	0.8
32-33 Split proinsulin (pmol/l)	**9.4**	(5.9, 14.0)	0.7	0.6	**9.7**	(5.9, 15.0)	0.7	0.8
Glucose (mmol/l)	**6.1**	(5.0, 7.4)	0.08	0.08	**6.2**	(5.0, 7.6)	0.7	0.6

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	All (N=559)				25(OH)D <50nmol/l (N=372)			
	Geometric mean	IQR	p-value (1)	p-value (2)	Geometric mean	IQR	p-value (1)	p-value (2)

p-values are for associations between log25(OH)D concentration (predictor) and each variable, derived from linear regression (logistic regression for gestational diabetes; p1 unadjusted; p2 adjusted for maternal age, fat mass and diabetes status.

† data presented as N (%)

+ significant positive association

– significant negative association.