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ARTICLE

Low plasma vitamin B_{12} in pregnancy is associated with gestational 'diabesity' and later diabetes

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Abstract
Aims/hypothesis This study was designed to test the hypothesis that low plasma vitamin B_{12} concentrations combined with high folate concentrations in pregnancy are associated with a higher incidence of gestational diabetes (GDM) and later diabetes.
Methods Women (N=785) attending the antenatal clinics of one hospital in Mysore, India, had their anthropometry, insulin resistance (homeostasis model assessment-2) and glucose tolerance assessed at 30 weeks' gestation (100 g oral glucose tolerance test; Carpenter–Coustan criteria) and at 5 years after delivery (75 g OGTT; WHO, 1999). Gestational vitamin B_{12} and folate concentrations were measured in stored plasma samples.
Results Low vitamin B_{12} concentrations (<150 pmol/l, B_{12} deficiency) were observed in 43% of women and low folate concentrations (<7 nmol/l) in 4%. B_{12}-deficient women had higher body mass index (p<0.001), sum of skinfold thickness (p<0.001), insulin resistance (p=0.02) and a higher incidence of GDM (8.7% vs 4.6%; OR 2.1, p=0.02; p=0.1 after adjusting for BMI) than non-deficient women. Among B_{12}-deficient women, the incidence of GDM increased with folate concentration (5.4%, 10.5%, 10.9% from lowest to highest tertile, p=0.04; p for interaction= 0.2). Vitamin B_{12} deficiency during pregnancy was positively associated with skinfold thickness, insulin resistance (p<0.05) and diabetes prevalence at 5 year follow-up (p=0.009; p=0.008 after adjusting for BMI). The association with diabetes became non-significant after excluding women with previous GDM (p=0.06).
Conclusions/interpretation Maternal vitamin B_{12} deficiency is associated with increased adiposity and, in turn, with insulin resistance and GDM. Vitamin B_{12} deficiency may be an important factor underlying the high risk of 'diabesity' in south Asian Indians.

Keywords Adiposity · Diabesity · Folate · Gestational diabetes · India · Insulin resistance · Vitamin B_{12}

Abbreviations

DBP	Diastolic blood pressure
GDM	Gestational diabetes mellitus
HMH	Holdsworth Memorial Hospital
HOMA	Homeostasis model assessment
IDF	International Diabetes Federation
IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
PMNS	Pune Maternal Nutrition Study
SBP	Systolic blood pressure
SES	Socioeconomic status

Introduction

The prevalence of type 2 diabetes is increasing rapidly in countries such as India. Type 2 diabetes tends to appear at a younger age in Indians than in white populations in high-

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income countries [1]. One consequence of this is a high incidence of gestational diabetes (GDM), reaching 10–20% in some urban studies [2]. A major contributory factor to this is the characteristic phenotype of Indians, which includes lower muscle mass, a higher percentage of body fat and higher insulin resistance compared with other populations [3].

Although the cause of this adverse phenotype is unknown, a role for intrauterine undernutrition has been suggested [3]. Specifically, in the Pune Maternal Nutrition Study (PMNS), children born to mothers with low plasma vitamin B_{12} concentrations were more adipose and insulin resistant than children born to mothers with normal vitamin B_{12}, especially if the mother had normal or high folate concentrations [4]. Folate and vitamin B_{12} are essential for several important methylation processes involving synthesis, repair and/or regulation of nucleic acids, proteins, membrane phospholipids, neurotransmitters and epigenetic modifications. Deficiency of these vitamins is associated with wide-ranging multi-system abnormalities, including megaloblastic anaemia, neurological disorders and birth defects [5]. The PMNS authors hypothesised a general phenomenon whereby the susceptibility of Indians to adiposity and diabetes ('diabesity') might result from widespread vitamin B_{12} deficiency, which may be further exacerbated by recent improvement in folate nutrition [4]. Indeed, a high prevalence of vitamin B_{12} deficiency has been demonstrated in various subgroups of the Indian population [4, 6–9]. We tested this hypothesis in pregnant women who participated in the Parthenon Study, which investigated the effect of GDM on newborn size and future risk of diabetes [10, 11]. We hypothesised that maternal vitamin B_{12} deficiency is associated with increased adiposity, insulin resistance and impaired glucose tolerance in the mother herself, and that these outcomes are exaggerated by adequate or high folate status.

Methods

A scheme of the number of participants and the investigations done at different time points is illustrated in Fig. 1.

Pregnancy During 1997–1998, 830 women booking consecutively into the antenatal clinic of the Holdsworth Memorial Hospital (HMH) in Mysore, south India, and matching our eligibility criteria (no known history of diabetes, intention to deliver at HMH, singleton pregnancy) had a 100 g 3 h OGTT at 30±2 weeks' gestation after an overnight fast [10]. Weight, height and skinfold thickness (biceps, triceps, subscapular and suprailiac) were measured using standardised methods [12]. Socioeconomic status

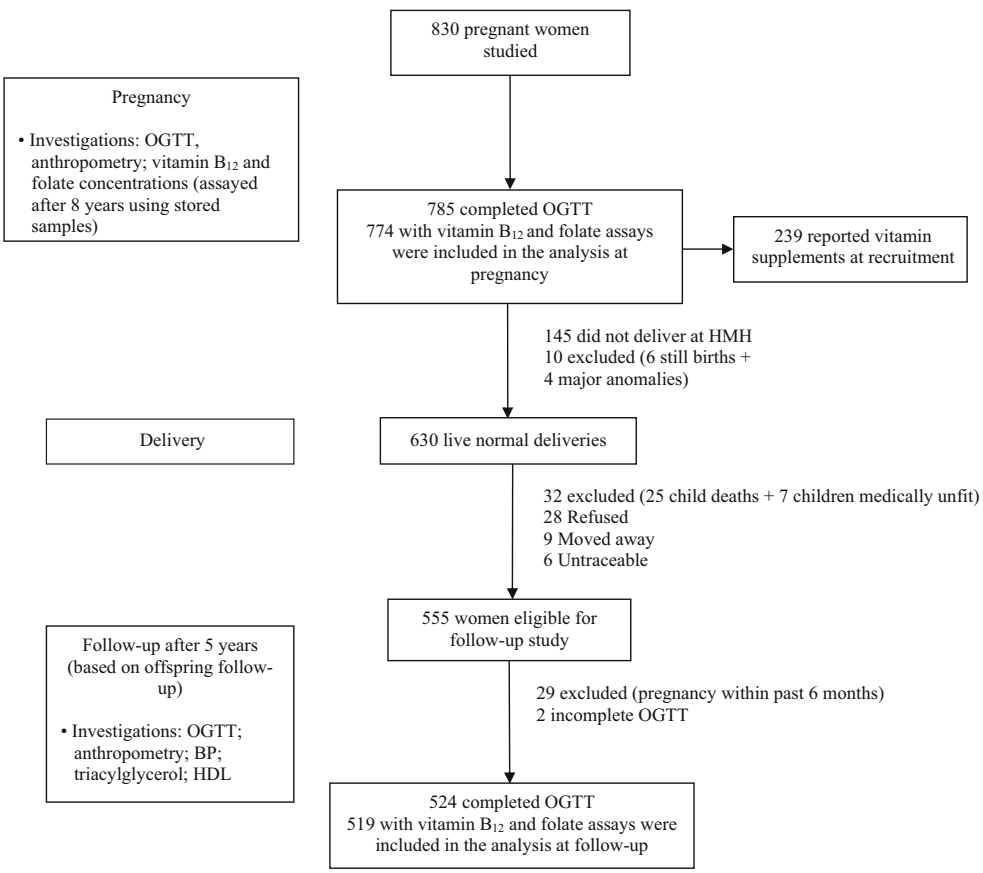

Fig. 1 Flow diagram illustrating the number of participants and investigations carried out at the various stages of the study. HMH, Holdsworth Memorial Hospital

(SES) was assessed using the Kuppuswamy score, a questionnaire which derives a score based on the education, occupation and the income of the head of the family [13]; a higher score indicates higher SES. The majority of our women belonged to middle or lower-middle social classes (score: 5–25).

Plasma glucose was measured using a standard hexokinase method, and insulin using a one-step chemiluminescent immunoenzymatic assay (Sanofi Pasteur Diagnostics, Marnes la Coquette, France). Complete OGTT results were available for 785 women. GDM was diagnosed in 49 women (6.2%) using the Carpenter–Coustan criteria [14], the standard method in clinical use in the HMH. Insulin resistance was estimated using the updated homeostasis model assessment equation (HOMA-2) from an online HOMA calculator [15]. The women's own consultant obstetricians managed their further clinical care.

A total of 630 women delivered live, normal babies in HMH. There were 41 women diagnosed with GDM, ten of whom received insulin treatment during pregnancy, but none of whom was on insulin or oral hypoglycaemic agents at the time of discharge from the hospital.

The hospital ethical committee approved the study, and informed verbal consent was obtained from all women.

Vitamin B_{12} and folic acid supplements It was routine for general practitioners and obstetricians to prescribe folic acid and/or multivitamin supplements to pregnant women. Supplements taken by the women were recorded at recruitment but not subsequently at 30 weeks' gestation, when blood samples were taken, or at term.

Vitamin B_{12} and folate during pregnancy We used stored fasting plasma samples to measure vitamin B_{12} and folate in 774 of the 785 women who had complete OGTT data in pregnancy. The samples had been stored in the freezer (−80°C for 8 years) within 1 h of sampling, and were transferred on dry ice for laboratory analysis at the Diabetes Research Centre, KEM Hospital, Pune, India, using microbiological assays [16, 17]. Intra- and interassay coefficients of variations were <8% for both assays. As there is no specified cut-off for pregnancy, we defined vitamin B_{12} deficiency as a concentration <150 pmol/l and folate deficiency as a concentration <7 nmol/l based on the values generally used in a normal population [9, 18].

Follow-up Follow-up of the women was based on the follow-up of their offspring [11]. Twenty-five children died between birth and 5 years, seven children were excluded after birth because of medical reasons, and 43 families either refused follow-up or moved away from Mysore. Five years later, we were able to follow 555 women, 29 of whom were excluded from the current study because of recent pregnancy (within the previous 6 months). The remaining 526 women had a 2 h, 75 g OGTT; 519 of these women, for whom vitamin B_{12} and folate concentrations were measured (35 met criteria for GDM), completed the study. Detailed anthropometry was performed and systolic (SBP) and diastolic blood pressures (DBP) were measured using an automated BP monitor (CRITIKON, DINAMAP model 8100, Tampa, FL, USA). Plasma glucose (glucose oxidase–peroxidase method), triacylglycerols (glycerol 3 phosphate oxidase-peroxidase method) and HDL-cholesterol (direct HDL-cholesterol method) were measured on an autoanalyser (Alcyon 300; Abbott Laboratories, Abbott Park, IL, USA), and insulin was measured using a time-resolved, fluoroimmunoassay (DELFIA) method (PerkinElmer Life and Analytical Sciences, Wallac Qy, Turku, Finland) at the Diabetic Research Centre, Pune, India.

Diabetes was defined as a fasting glucose concentration ≥7.0 and/or 120 min glucose ≥11.1 mmol/l [19]. Women were also classified as having diabetes if they had been diagnosed by a doctor as having diabetes since the index pregnancy. Impaired glucose tolerance (IGT) was a fasting glucose concentration <7.0 mmol/l and 120 min glucose ≥7.8 mmol/l but <11.1 mmol/l. Impaired fasting glucose (IFG) was defined as fasting glucose ≥6.1 mmol/l but <7.0 mmol/l [19].

Metabolic syndrome was defined by the International Diabetes Federation (IDF) criteria recommended for south Asian women [20]. Waist circumference ≥80 cm, and any two of the following: triacylglycerol ≥1.7 mmol/l; HDL-cholesterol <1.29 mmol/l; SBP ≥130 or DBP ≥85 or having treatment for hypertension; fasting glucose ≥5.6 mmol/l; or type 2 diabetes.

Statistical methods The distributions of HOMA-2 and vitamin B_{12} concentrations were skewed; these data were log-transformed for analysis where required. The main exposures of interest were the vitamin B_{12} deficiency (yes/no), and plasma vitamin B_{12} and folate concentrations. Other confounding exposures such as maternal age, parity, religion, family history of diabetes and SES were used as covariates in the multiple regression models. The outcomes of interest were anthropometry, insulin resistance and the incidence of GDM during pregnancy, and anthropometry, insulin resistance and the prevalence of diabetes and metabolic syndrome at follow-up. Associations of maternal vitamin B_{12} and folate concentrations with anthropometry and HOMA-2 during pregnancy and at follow-up were examined using linear regression analysis, and with the incidence of GDM, and the prevalence of diabetes and metabolic syndrome at follow-up, using logistic regression analysis. Interaction terms were used to test for modification by folate status of associations between vitamin B_{12} status and the several diabetes-related outcomes by using

vitamin B_{12} as two groups (deficiency and normal groups), and tertiles of folate concentrations. p values <0.05 were considered significant. All statistical analyses were performed using SPSS V16.

Results

Three-hundred and thirty-five women (43%) had vitamin B_{12} deficiency, while only 34 (4%) had folate deficiency (Table 1). Vitamin B_{12} deficiency was higher among Hindu women (50.7%, median B_{12}=148.5 pmol/l) than Muslims (31.8%, 180.0 pmol/l) and others (35.6%, 186.0 pmol/l); folate concentrations were lowest among Muslim women (24.2 nmol/l; Hindus 37.5 mmol/l; others 46.9 nmol/l). Vitamin B_{12} concentrations were unrelated to SES, parity or family history of diabetes. Higher SES (p<0.001), presence of a family history of diabetes (p=0.003), and lower parity (p<0.001) were associated with higher folate concentrations.

At recruitment, 239 (31%) women reported taking multivitamin supplements containing both vitamin B_{12} and folic acid, and 60 (8%) reported taking folic acid alone. Supplement use was not significantly associated with vitamin B_{12} and folate concentrations.

Vitamin B_{12}, folate and outcomes during pregnancy Vitamin B_{12} concentrations were inversely associated with the women's BMI and sum of skinfold thickness (Table 2).

Table 1 Characteristics of the study women during pregnancy (n_{max}=774) and at 5 year follow-up (n_{max}=519)

Characteristic	Followed-up after 5 years (n=519)		Lost to follow-up (n=255)	
	Median or n	IQR or %	Median or n	IQR or %
Pregnancy				
Age (years)	24.0	21.0, 26.0	22.0	20.0, 26.0
Religion				
Hindu (n)	298	57.4	162	63.6
Muslim (n)	174	33.5	81	31.8
Other (n)	47	9.1	12	4.7
Socioeconomic status (score)	14.0	11.0, 18.0	13.0	10.0, 17.0
Family history of diabetes, yes	102	29	44	17
Parity, 2+	88	17.0	29	11.4
Anthropometry				
Weight (kg)	55.5	50.0, 62.5	55.0	48.5, 60.0
Height (cm)	154.2	150.9, 57.9	154.8	151.0, 158.5
BMI (kg/m^2)	23.4	21.1, 26.3	22.6	20.6, 25.2
Sum of four skinfold thicknesses (mm)	84.9	61.5, 111.6	79.9	60.0, 102.2
Micronutrient concentrations				
Plasma vitamin B_{12} (pmol/l)	162.0	123.0, 221.0	159.0	119.0, 215.0
Vitamin B_{12} deficiency (<150 pmol/l)	223	42.6	112	42.9
Plasma folate (nmol/l)	35.4	17.7, 51.4	29.0	15.8, 48.0
Folate deficiency (<7 nmol/l)	22	4.2	12	4.6
Insulin resistance (HOMA-2)	0.6	0.4, 0.9	0.5	0.4, 0.7
Gestational diabetes	35	6.7	14	5.4
Follow-up				
Anthropometry				
Weight (kg)	56.0	48.0, 64.0	–	–
Height (cm)	154.1	151.0, 57.9	–	–
BMI (kg/m^2)	23.5	20.5, 26.9	–	–
Sum of four skinfold thicknesses (mm)	96.5	64.8, 127.8	–	–
Diabetes	21	4	–	–
IGT	46	8.9	–	–
IFG	40	7.7	–	–
Insulin resistance (HOMA-2)	0.9	0.6, 1.3	–	–

Data shown are median (interquartile range [IQR]) or n (%)

Table 2 Indicators of diabetes risk of the study women during pregnancy according to vitamin B_{12} and folate concentrations during pregnancy

B_{12}/folate status	*n*	BMI (kg/m^2)	Sum of skinfold thickness (mm)	Insulin resistance (HOMA-2)	GDM, *n* (%)
Vitamin B_{12} deficiency					
Yes	335	24.2 (3.6)	93.7 (33.9)	0.61 (0.4, 0.9)	29 (8.7)
No	439	22.9 (3.4)	83.0 (32.1)	0.56 (0.4, 0.8)	20 (4.6)
Effect size (95% CI)[a]		1.4 (0.9, 1.8)	10.7 (6.0, 15.4)	0.08 (−0.01, 0.2)	2.0 (1.1, 3.6)
p^1		<0.001	<0.001	0.07	0.02
p^2		<0.001	<0.001	0.02	0.02
p^3		–	–	0.6	0.1
Vitamin B_{12} (quartiles)[b]					
1 (lowest)	196	24.3 (3.6)	94.6 (33.4)	0.61 (0.4, 0.9)	17 (8.7)
2	190	24.0 (3.6)	90.9 (34.9)	0.60 (0.4, 0.8)	15 (7.9)
3	194	23.0 (3.4)	84.0 (31.7)	0.57 (0.4, 0.8)	7 (3.6)
4 (highest)	194	22.7 (3.5)	81.0 (31.7)	0.55 (0.4, 0.8)	10 (5.2)
Effect size (95% CI)[a]		−0.004 (−0.01, −0.002)	−0.03 (−0.06, −0.01)	−0.0001 (−0.001, 0.0003)	1.0 (0.99, 1.0)
p^1		0.002	0.009	0.6	0.4
p^2		0.002	0.002	0.3	0.5
p^3		–	–	0.8	0.9
Folate (quartiles)[b]					
1 (lowest)	193	23.3 (3.5)	83.1 (31.2)	0.62 (0.4, 0.9)	7 (3.6)
2	193	23.7 (3.8)	88.8 (34.5)	0.55 (0.4, 0.8)	11 (5.7)
3	195	23.3 (3.7)	88.4 (34.6)	0.58 (0.4, 0.8)	20 (10.3)
4 (highest)	193	23.6 (3.2)	90.2 (32.5)	0.59 (0.4, 0.9)	11 (5.7)
Effect size (95% CI)[a]		0.01 (−0.01, 0.02)	0.1 (−0.003, 0.2)	−0.001 (−0.003, 0.002)	1.0 (0.99, 1.0)
p^1		0.4	0.06	0.6	0.08
p^2		0.8	0.7	0.1	0.3
p^3		–	–	0.2	0.3

Values presented are mean (SD) for BMI and sum of skinfold thickness, median (interquartile range [IQR]) for HOMA-2, and *n* (%) for GDM

p values were derived by linear regression for continuous variables (BMI, sum of skinfold thickness and HOMA-2) and logistic regression for GDM

[a] Expressed as the regression coefficient (β) for continuous variables (BMI, sum of skinfold thickness, HOMA-2) and the OR for GDM. For HOMA-2 (logged variable) the β value indicates the percentage change in the outcome per unit change in the predictor

[b] *p* values derived using B_{12} and folate variables as continuous

p^1 unadjusted

p^2 adjusted for age, SES, religion, parity and family history of diabetes

p^3 adjusted additionally for gestational BMI

Vitamin B_{12} deficiency was associated with higher BMI, larger sum of skinfold thickness, higher HOMA-2 and an increased risk of GDM after adjusting for age, religion, SES, parity and family history of diabetes. Gestational diabetes was two times more frequent in vitamin B_{12}-deficient women compared with non-deficient women. The associations with GDM became non-significant after adjusting for BMI (OR 1.1, p=0.1) or sum of skinfold thickness (p=0.09).

Vitamin B_{12}-deficient women were also more likely to be in the highest quartile for measures of adiposity (BMI: OR 1.9, 95% CI 1.4–2.6, p<0.001, sum of skinfold thickness: OR 1.6, 95% CI 1.2–2.3, p=0.004). Folate concentrations were not related to the women's adiposity, insulin resistance or the incidence of GDM (Table 2).

In women with vitamin B_{12} deficiency, the incidence of GDM increased across tertiles of the folate distribution (5.4% (n=7), 10.5% (n=12), 10.9% (n=10)) from lowest to highest tertile (p=0.1; p=0.04 after adjusting for age, religion, SES, parity and family history of diabetes). There was no significant interaction between vitamin B_{12} deficiency and folate concentrations for the incidence of GDM (p=0.2). Insulin resistance decreased in non-deficient women (β=−0.09, p=0.02) from the lowest to highest tertiles of folate concentrations, there was no significant association between folate and insulin resistance in B_{12}-deficient women (β=0.04, p=0.4, p for interaction=0.06). There was no interaction between vitamin B_{12} deficiency and folate concentrations for any other outcomes of interest.

Table 3 Indicators of diabetes risk of the study women at 5 year follow-up according to vitamin B_{12} and folate concentrations during pregnancy

Vitamin B_{12}/ folate status	*n*	BMI (kg/m^2)	Sum of skinfold thickness (mm)	Insulin resistance (HOMA-2)	DM, *n* (%)	Metabolic syndrome, *n* (%)
Vitamin B_{12} deficiency						
Yes	223	24.7 (4.4)	103.5 (38.6)	0.90 (0.6, 1.5)	16 (7.2)	73 (33.2)
No	296	23.0 (4.4)	91.5 (39.3)	0.78 (0.6, 1.2)	5 (1.7)	73 (24.7)
Effect size (95% CI)[a]		1.7 (0.9, 2.4)	12.0 (5.2, 18.8)	0.1 (0.03, 0.3)	4.5 (1.6, 12.5)	1.5 (1.0, 2.2)
p^1		<0.001	0.001	0.02	0.004	0.04
p^2		<0.001	<0.001	0.02	0.003	0.02
p^3		–	–	0.2	0.009	0.5
p^4		<0.001	0.001	0.07	0.06	0.1
Vitamin B_{12} (quartiles)[b]						
1 (lowest)	124	24.9 (4.4)	105.4 (37.5)	0.89 (0.5, 1.5)	7 (5.6)	38 (31.1)
2	130	24.4 (4.5)	101.0 (40.2)	0.91 (0.6, 1.5)	10 (7.7)	48 (37.2)
3	131	22.5 (4.2)	90.0 (39.4)	0.77 (0.6, 1.1)	3 (2.3)	34 (26.0)
4 (highest)	134	23.1 (4.4)	90.7 (38.6)	0.77 (0.6, 1.2)	1 (<1)	26 (19.5)
Effect size (95% CI)[a]		−0.004 (−0.01, −0.0003)	−0.02 (−0.1, 0.02)	−0.0004 (−0.001, 0.0002)	0.99 (0.98, 0.998)	1.0 (0.996, 1.001)
p^1		0.03	0.3	0.2	0.02	0.2
p^2		0.02	0.2	0.2	0.02	0.1
p^3		–	–	0.4	0.03	0.5
p^4		0.03	0.3	0.4	0.2	0.3
Folate (quartiles)[b]						
1 (lowest)	124	23.9 (4.5)	98.5 (41.2)	0.84 (0.6, 1.4)	5 (4.0)	37 (30.1)
2	121	23.8 (4.9)	94.9 (41.1)	0.79 (0.6, 1.2)	4 (3.3)	24 (20.0)
3	138	23.4 (4.5)	94.1 (40.1)	0.87 (0.6, 1.5)	7 (5.1)	39 (28.3)
4 (highest)	136	23.8 (4.1)	98.1 (36.7)	0.83 (0.6, 1.2)	5 (3.7)	46 (34.3)
Effect size (95% CI)[a]		−0.01 (−0.03, 0.02)	−0.06 (−0.2, 0.1)	−0.0002 (−0.003, 0.003)	0.999 (0.98, 1.0)	1.01 (0.996, 1.02)
p^1		0.6	0.5	0.9	0.9	0.3
p^2		0.3	0.2	0.4	0.7	0.3
p^3		–	–	0.5	0.98	0.2
p^4		0.2	0.2	0.6	0.8	0.4

Values presented are mean (SD) for BMI and sum of skinfold thickness, median (interquartile range [IQR]) for HOMA-2, and *n* (%) for DM and metabolic syndrome

p values are derived by linear regression for continuous variables (BMI, sum of skinfold thickness and HOMA-2) and logistic regression for DM and metabolic syndrome

[a] Expressed as the regression coefficient (β) for continuous variables (BMI, sum of skinfold thickness, HOMA-2) and the OR for DM and metabolic syndrome. For HOMA-2 (logged variable) the β value indicates the percentage change in the outcome per unit change in the predictor

[b] *p* values derived using vitamin B_{12} and folate variables as continuous

p^1 unadjusted

p^2 adjusted for SES, religion, parity and family history of diabetes

p^3 adjusted additionally for gestational BMI

p^4 after excluding women with GDM and adjusted for age, SES, religion, parity and family history of diabetes

DM, Diabetes mellitus

Vitamin B_{12}, folate and outcomes at follow-up Women who were lost to follow-up after 5 years (n=255) were significantly younger, lighter, thinner and less adipose during pregnancy (Table 1). They also had lower HOMA-2, folate concentrations and lower SES. There were no significant differences in vitamin B_{12} concentrations and the prevalence of vitamin B_{12} deficiency.

Twenty-one women acquired diabetes after pregnancy, including 12 with previous GDM. Two of them were diagnosed between pregnancy and follow-up, and 19 more were diagnosed from OGTT at follow-up.

Of the components of metabolic syndrome, 297 (57%) met the IDF criteria for high waist circumference, 26 (5%) for high BP, 207 (40%) for increased glucose variables, 87 (17%) for

elevated triacylglycerols and 393 (76%) women for low HDL-cholesterol. One-hundred and forty-six women (28%) were classified as having metabolic syndrome.

Gestational vitamin B_{12} deficiency was significantly positively associated with maternal adiposity 5 years later, HOMA-2 and the prevalence of diabetes and metabolic syndrome (Table 3). The association with diabetes remained significant even after adjusting for gestational BMI (p=0.009) or sum of skinfold thickness (p=0.006). No significant associations were seen with the prevalence of IGT or IFG. There were no associations between plasma folate concentrations and 5 year outcomes, and no significant interactions between B_{12} deficiency and folate concentrations.

After excluding the 35 women who had GDM, gestational vitamin B_{12} deficiency was significantly associated with higher BMI (p<0.001) and sum of skinfold thickness (p=0.001). There was a non-significant association between vitamin B_{12} deficiency and higher insulin resistance (0.87 vs 0.78, p=0.08), and a higher prevalence of diabetes (3.0% vs <1%; OR 5.2, 95% CI 0.95–28.2, p=0.06) and metabolic syndrome (28.9% vs 23.7%; OR 1.4, 95% CI 0.9–2.2, p=0.1). Among women who had GDM, ten (50%) of those with vitamin B_{12} deficiency during pregnancy were diagnosed with diabetes at follow-up, compared with three (20%) with normal vitamin B_{12} concentrations.

Discussion

We observed a high prevalence of vitamin B_{12} deficiency in pregnant women attending one maternity hospital in south India. Folate deficiency was rare; plasma folate concentrations as a group were normal to high. Vitamin B_{12} deficiency was associated with adiposity, insulin resistance and diabetes both during pregnancy and 5 years later. The associations with gestational diabetes appeared to be explained by increased adiposity, as indicated by BMI and sum of skinfold thickness. Although the risk of GDM was highest among women with B_{12} deficiency and adequate folate concentrations, the test for interaction did not support our hypothesis that high/adequate folate status exacerbates the effects of B_{12} deficiency on the diabetes risk.

Earlier studies have observed a high prevalence of vitamin B_{12} deficiency among Indians [6–9]. Consistent with these results, more than 40% of our women were vitamin B_{12} deficient while only 4% had low plasma folate concentrations. Hindu women, who are likely to be mostly lacto-vegetarian with infrequent meat consumption in our population, had the lowest vitamin B_{12} concentrations; Muslim women, who are mainly non-vegetarian with minimal consumption of vegetables, had the lowest folate concentrations. As our study was not originally designed to examine vitamin B_{12} and folate status, we recorded supplement use only at recruitment. Women not on supplements at recruitment may have been prescribed them later in pregnancy, while those prescribed supplements in early pregnancy may have stopped taking them by 30 weeks. This may be the reason for no significant association between supplement intake and vitamin concentrations.

The associations of vitamin B_{12} deficiency with insulin resistance and GDM were reduced after adjusting for BMI, suggesting a mediating role for adiposity. This is the first description of an association between vitamin B_{12} deficiency, adiposity and its associated disorders ('diabesity'). One interpretation could be that vitamin B_{12} deficiency promotes adiposity. Another possibility is that obesity/adiposity lowers plasma vitamin B_{12} concentrations. Several studies have reported decreased bio-availability of micronutrients, especially fat-soluble vitamins and folate, in obese/overweight individuals [21–23]. The mechanisms suggested are decreased absorption or dietary intake, increased catabolism, and sequestration in adipose tissue. A poor-quality dietary intake, rich in energy content but low in micronutrients, may result in both adiposity and low B_{12} levels. If this was the explanation for the association we observed between maternal adiposity and vitamin B_{12} deficiency, we would also expect to see an association between adiposity and folate concentration. Moreover, vitamin D, a fat-soluble vitamin that is commonly decreased in obese individuals, was not associated with adiposity in our women [24].

The association between B_{12} deficiency and diabetes at follow-up was independent of adiposity, but not of GDM. As the association between B_{12} deficiency and GDM was related to adiposity, the above association may be due to residual confounding. As the prevalence of diabetes was very low in our young non-GDM women, a statistical association with B_{12} deficiency was difficult to show. However, even in women without GDM, the incidence of diabetes tended to increase with vitamin B_{12} deficiency. We speculate that there may be additional mechanisms, apart from adiposity, for the association between low vitamin B_{12} and diabetes.

Insulin resistance and the prevalence of GDM were highest among women with a combination of vitamin B_{12} deficiency and adequate/high folate concentrations. As discussed in the PMNS paper there are plausible biochemical reasons why vitamin B_{12} deficiency could cause increased adiposity, especially in the presence of high folate [4]. In vitamin B_{12} deficiency cellular folate is trapped as inactive 5-methyltetrahydrofolate [25]. This results in impaired methionine, and thus protein synthesis, which may hinder lean tissue deposition. Decreased conversion of methylmalonic acid to succinyl coA, for which vitamin B_{12} acts as a rate-limiting coenzyme, results in the accumulation of methylmalonic acid, and may increase lipogenesis and insulin resistance [4]. Although

folate does not participate in these pathways, it has recently been shown that in vitamin B_{12} deficiency higher folate is associated with high concentrations of homocysteine and methylmalonic acid [26].

In our study, insulin resistance was positively related to folate concentration in B_{12}-deficient women (non-significant association), and negatively related to folate in non-deficient women. This is analogous to data from a recent study among elderly people in the USA showing that in the presence of vitamin B_{12} deficiency, a high folate concentration was associated with anaemia and cognitive impairment, but with protection against cognitive impairment when vitamin B_{12} status was normal [27]. The possible implications are that folate may have adverse effects in vitamin B_{12} deficiency, or vitamin B_{12} status may need to be normal for optimum folate actions. Our study and the Pune study suggest that there may be a similar phenomenon with insulin resistance. However, there was no statistically significant interaction between vitamin B_{12} and folate in either study.

The major strength of this large prospective cohort study was the availability of both nutritional and metabolic measurements during pregnancy, and follow-up measurements after 5 years. Although the micronutrient assays were performed using stored samples, vitamin B_{12} and folate have been shown to be stable in long-term storage at lower temperatures [28]. The study was based in one single hospital; however, the women belonged to different religions, and had different socio-cultural backgrounds and dietary habits that were representative of the Mysore population. The dietary preferences of the majority of Hindu women were as described in other studies, thus rendering our data relevant to other parts of India [4]. Though vitamin B_{12} concentrations are difficult to interpret in pregnancy, because of haemodilution and raised glomerular filtration rate, and there is no agreed cut-off for deficiency; a similar definition has been used in other studies [29]. Concentrations of homocysteine and methylmalonic acid, more specific and sensitive indicators of vitamin B_{12} deficiency, were not measured in our study and thus we may have underestimated the prevalence of deficiency. Vitamin status at follow-up, which might have important effects on adiposity, insulin resistance and the prevalence of diabetes, was not measured. Another limitation was that information on exposures such as dietary intake and physical activity level, which may have significant impact on plasma vitamin levels, and adiposity and insulin resistance was not collected.

Conclusion

Our findings suggest a link between vitamin B_{12} deficiency and adiposity and diabetes (‘diabesity’) in pregnant women. Further studies are needed to confirm our findings.

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Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

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