Original Article

Association between Vitamin D Insufficiency and the Risk for Gestational Diabetes Mellitus in Pregnant Chinese Women*

WANG Ou[1,^], NIE Min[1,^], HU Ying Ying[1], ZHANG Kui[1], LI Wei[1], PING Fan[1], LIU Jun Tao[2], CHEN Li Meng[3,#], and XING Xiao Ping[1,#]

1. Department of Endocrinology, Peking Union Medical College Hospital, Peking Union Medical College, Chinese Academy of Medical Sciences; Key Laboratory of Endocrinology, Ministry of Health, Beijing 100730, China; 2. Department of Obstetrics & Gynecology, Peking Union Medical College Hospital, Peking Union Medical College, Chinese Academy of Medical Sciences, Beijing 100730, China; 3. Department of Nephrology, Peking Union Medical College Hospital, Peking Union Medical College, Chinese Academy of Medical Sciences, Beijing 100730, China

Abstract

Objective To investigate the association between vitamin D deficiency and risk of gestational diabetes mellitus (GDM) in pregnant Chinese women.

Methods A nested case-control study was conducted. Clinical and biochemical data were analyzed for 200 subjects with GDM and 200 subjects with normal glucose tolerance (NGT).

Results The median (interquartile range) serum 25-hydroxyvitamin D (25OHD) levels were 22.39 (17.67, 29.38) and 25.86 (19.09, 34.88) nmol/L in the GDM and NGT groups, respectively. Rates of 25OHD deficiency or insufficiency were significantly higher in the GDM group than in the NGT group. Subjects with 25OHD levels <25 nmol/L had a 1.8-fold higher risk of GDM compared with subjects with higher vitamin D levels. In the GDM group, serum 25OHD was independently associated with HbA1c and insulin resistance after adjusting for confounding factors. In the NGT group, serum 25OHD was independently associated with fasting plasma glucose and systolic blood pressure after adjusting for maternal age and other confounding factors.

Conclusion 25OHD insufficiency is very common in Chinese women. Low 25OHD status may be associated with insulin resistance and act as a risk factor for GDM.

Key words: Gestational diabetes mellitus; Vitamin D insufficiency; Chinese women

Biomed Environ Sci, 2012; 25(4):399-406 doi: 10.3967/0895-3988.2012.04.004 ISSN:0895-3988

www.besjournal.com(full text) CN: 11-2816/Q *Copyright ©2012 by China CDC*

INTRODUCTION

Vitamin D is mainly synthesized in the skin following exposure to sunlight, and is then hydroxylated in the liver and kidney. The circulating concentrations of 25-hydroxyvitamin D (25OHD) reflects the nutritional status of vitamin D, while 1,25-dihydroxyvitamin D [1,25$(OH)_2$D] is the active hormonal form. Besides its classical roles in calcium and bone metabolism, vitamin D also has several "non-classical" effects on modulating the functions of a wide variety of cell types through the

*This study was partially supported by grants from the National Natural Science Foundation Project of China (grants 30971369, 81170674, and 81070630).

^WANG Ou and NIE Min contributed equally to this work.

#Correspondence should be addressed to Prof. XING Xiao Ping, MD and Prof. CHEN Li Meng, MD. Tel: 86-10-65295084. Fax: 86-10-69155084. E-mail: xiaopingxing@126.com and climeng2000@yahoo.com.cn

Biographical note of the first author: WANG Ou, female, born in 1973, MD, majoring in endocrinology and metabolism. NIE Min, female, born in 1970, MD, majoring in endocrinology and metabolism.

Received: December 20, 2011; Accepted: June 4, 2012

ubiquitous expression of vitamin D receptor. Accumulating evidence has linked vitamin D deficiency with increased risk for several components of metabolic syndrome, including abnormal glucose metabolism, obesity, hypertension, and cardiovascular disease[1-2].

Type 2 diabetes mellitus (T2DM) is characterized by a combination of insulin resistance and impaired β cell function. Some studies have also suggested that vitamin D deficiency plays a role in the pathogenesis of T2DM via direct effects on pancreatic β cell function or insulin sensitivity, or via indirect effects involving immunologic disturbances that occur in vitamin D deficiency[3-5]. Several epidemiologic studies have revealed a high prevalence of vitamin D deficiency in patients with T2DM, and an inverse correlation between serum 25OHD and glucose levels in people with or without T2DM[6-9].

Gestational diabetes mellitus (GDM) is defined as glucose intolerance occurring in or is detected during pregnancy. The pathophysiology of GDM parallels that of T2DM in many aspects, including insulin resistance and relative insulin insufficiency. Women with previous GDM are at increased risk for developing T2DM in their later life[10]. Therefore, vitamin D deficiency may also play a role in the pathogenesis of GDM.

Vitamin D deficiency has reappeared as an important public health problem in developed and developing countries, and vitamin D deficiency during pregnancy is becoming increasingly common[1]. In recent studies in the United States (US), Canada, Australia, Iran, Sweden, and Pakistan, 24%-95.8% of pregnant women were deficient and 54%-100% were insufficient for vitamin D, with a general trend towards increased prevalence of vitamin D deficiency or insufficiency in women with darker skin pigmentation or who regularly wear veils[11-17]. Serum 25OHD levels were negatively correlated with fasting glucose and insulin levels[18-19]. Furthermore, maternal plasma 25OHD concentrations in early pregnancy were reported to be associated with an increased risk of GDM[20], but no significant association between 25OHD levels and GDM risk was observed in an Indian population[21]. Results from these studies point towards an inverse association between vitamin D status and the risk of hyperglycemia or insulin resistance, but are inconclusive. The present study was designed to evaluate the association between serum 25OHD levels and the risk for GDM in pregnant Chinese women.

METHODS

Study Population

This was a nested case-control study based on a prospective cohort study of pregnant women-the GDM Study. In this cohort, participants were recruited from among pregnant women attending the endocrinology clinic of Peking Union Medical College hospital. Women with a history of DM, metabolic bone diseases, abnormal liver function, or impaired kidney function were excluded. The analytical population was selected from among the pregnant women who were enrolled in the GDM Study between January 2007 and December 2008. There were approximately 2500 deliveries annually at our hospital in this period, and a total of 1231 pregnant women provided blood samples. Of these, we identified 200 women who developed GDM and we established a random sample of 200 women who were not diagnosed with GDM as controls. The control subjects were frequency matched to the cases for the estimated season of conception of the index pregnancy (i.e., spring, summer, autumn, winter). The study protocol was approved by the ethics committee of Peking Union College hospital. All participants provided written informed consent.

Diagnosis of GDM

All pregnant women without a previous diagnosis of glucose intolerance were routinely screened for GDM at weeks 26-28 of gestation with a 50-g glucose challenge test (GCT) in our obstetric clinic. Women with plasma glucose concentration >7.8 mmol/L a 1 h after the glucose load were referred to an endocrinologist and underwent a 100-g oral glucose tolerance test (OGTT). GDM was defined according to American Diabetes Association criteria[22]. The threshold glucose values were as follows: fasting glucose 5.3 mmol/L, 1 h post-load 10.0 mmol/L, 2 h post-load 8.6 mmol/L, and 3 h post-load 7.8 mmol/L. If two or more of the glucose values met or exceeded the threshold value, a diagnosis of GDM was made. Normal glucose tolerance (NGT) was accepted if all of the plasma glucose values were below the threshold values. Subjects enrolled in this study attended an endocrine clinic and all underwent blood sampling for the purpose of the 100-g OGTT.

Data Collection

Clinical data were collected for all participants, and included maternal age, height, pre-pregnancy

body weight, systolic blood pressure (SBP) and diastolic blood pressure (DBP) at 26-28 weeks gestation, family history of T2DM, parity, and gravidity. Pre-pregnancy body mass index (BMI) was calculated as pre-pregnant body weight in kilograms divided by the square of height in meters.

Measurements

Plasma glucose levels were measured using the glucose oxidase method. Insulin concentrations were measured by an enzyme-linked immunosorbent assay (ELISA)[23]. Inter-and intra-assay coefficients of variation were 4.1% and 7.0%, respectively. Serum calcium (Ca), phosphorus (P), and lipid [triglyceride (TG), high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C), and cholesterol (CHO)] levels were measured using an Olympus AU5400 Autoanalyzer (Dallas, Texas, USA). Homeostasis model assessment (HOMA-IR) was used as an index of insulin resistance and was determined as the product of fasting plasma glucose (FPG) and fasting plasma insulin (FPI) divided by 22.5. HOMA-IR values <3.0 are considered normal, while values $\geqslant$ 3.0 indicate severe insulin resistance. HOMA-B was calculated as (FPI in mU/mL × 20) / (FPG in mmol/L − 3.5), as previously reported[24]. Total insulin area under the concentration curve was calculated using the trapezoidal method[25].

Serum samples were obtained at weeks 26-28 of gestation and used to measure 25OHD and parathyroid hormone (PTH). Samples were stored at -80 °C until analysis. Serum 25OHD levels were measured by ELISA (IDS Immunodiagnostics Ltd., Boldon, Tyne and Wear, UK). The intra- and inter-assay coefficients of variation were 4.6% and 5.3%, respectively. Vitamin D sufficiency, insufficiency, and deficiency were defined as serum 25OHD concentrations $\geqslant$50, 25-50 and <25 nmol/L, respectively[18,26]. Serum PTH levels were measured by a chemiluminoimmunoassay (DPC Immulite 2000 Immunoassay System; Siemens, New York city, New York, USA). The intra- and inter-assay coefficients of variation were 5.7% and 6.3%, respectively.

Statistical Analysis

Normally distributed data are presented as means±standard deviation, and between-group comparisons were made by one-way analysis of variance (ANOVA). Non-normally distributed data are presented as medians and interquartile ranges. Since serum PTH and insulin levels were non-normally distributed, we used natural log-transformed serum PTH and insulin levels in the analysis. Univariate comparisons of dichotomous data were made using the χ^2 or Fisher's exact test, as appropriate. Odds ratios (ORs) were calculated by the Mantel-Haenzel test and logistic regression models were used to adjust for confounding factors. Pearson's or Spearman's correlation coefficient was used to examine univariate associations between serum 25OHD as the predictor variable and other clinical characteristics. Multiple linear regression analysis with a stepwise selection method was then applied to determine whether serum 25OHD was an independent predictor of glucose and lipid metabolism indices in the GDM and NGT groups. Values of P<0.05 (two tailed) were considered statistically significant. SPSS software version 10.0 for Windows 0 (SPSS, Chicago, IL, USA) was used for all data analyses.

RESULTS

The anthropomorphic and biochemical data for the GDM and NGT group are listed in Table 1. Serum 25OHD concentrations were significantly lower in the GDM group [22.39 (17.67, 29.38) nmol/L] than in the NGT group [25.86 (19.09, 34.88) nmol/L; P<0.001]. The difference was attenuated but remained statistically significant after adjusting for age and pre-pregnancy BMI (P=0.039). According to the definition of vitamin D status based on serum 25OHD levels, 122 (61.0%), 78 (39.0%), and 0 (0%) subjects in the GDM group were classified with vitamin D deficiency, insufficiency, and sufficiency, respectively, compared with 93 (46.5%), 92 (46.0%), and 15 (7.5%) subjects in the NGT group, respectively. The distribution of vitamin D status was significantly different between the two groups (P<0.001). Since the 25OHD levels in this study were very low, <25 nmol/L in about half of the subjects, we re-analyzed the data with a cutoff 25OHD level of 25 nmol/L; the results are shown in Table 2. The proportion of subjects with low 25OHD levels was still significantly higher in the GDM group than in the NGT group (P=0.004). Subjects with low vitamin D levels had a 1.8-fold higher risk of GDM as compared with those with higher vitamin D levels (OR: 1.800, 95% CI: 1.209-2.678, P=0.004). After adjusting for maternal age, family history of T2DM, and TG, subjects with 25OHD levels <25 nmol/L had a 1.588-fold increased risk of GDM (adjusted OR: 1.588, 95% CI: 1.034-2.441, P=0.035). We also performed

Table 1. Characteristics of Pregnant Women with GDM and NGT

Variable	GDM Group	NGT Group	*P* Value
n	200	200	—
Age (years)	32.0[29.0,35.0]	31.0[28.0,34.0]	0.010
Gravidity	2.1±1.1	1.9±1.0	0.083
Parity	1.1±0.3	1.1±0.2	0.499
Family history of T2DM	75/176	50/139	0.140
Pre-BMI (kg/cm^2)	21.9[19.9,24.4]	20.6[19.1,22.9]	0.001
SBP (mmHg)	111.0[107.0,122.0]	110.0[100.0,118.0]	0.009
DBP (mmHg)	70.0[64.0,75.0]	68.0[60.0,72.0]	0.021
25OHD (nmol/L)	22.4[17.7,29.4]	25.9[19.1,34.9]	<0.001
PTH (pg/mL)	27.0[19.0,34.8]	22.4[16.5,32.8]	0.016
OGTT			
0h Glucose (mmol/L)	4.8[4.5,5.2]	4.50[4.3,4.7]	<0.001
1h Glucose (mmol/L)	10.4±1.5	8.2±1.2	<0.001
2h Glucose (mmol/L)	9.5[8.8,10.3]	7.3[6.6,8.0]	<0.001
3h Glucose (mmol/L)	8.2±1.4	6.3±1.0	<0.001
0h Insulin (mU/L)	7.6[5.8,10.7]	6.6[4.6,9.1]	<0.001
1h Insulin (mU/L)	63.5[45.0,95.0]	62.0[39.3,90.6]	0.148
2h Insulin (mU/L)	85.7[57.5,136.5]	60.5[38.4,92.8]	<0.001
3h Insulin (mU/L)	70.2[46.9,106.6]	44.1[29.5,71.5]	<0.001
HbA1c (%)	5.4[5.2,5.7]	5.2[5.1,5.5]	<0.001
HOMA-IR	1.7[1.2,2.2]	1.3[0.9,1.8]	<0.001
HOMA-B	121.7[83.9,190.9]	141.8[94.8,182.8]	0.498
AUC of insulin	198.1[138.5,287.4]	152.9[103.9,217.2]	<0.001
Cr (umol/L)	62.0[59.0,66.0]	62.0[58.0,66.0]	0.588
CHO (mmol/L)	6.06[5.30,6.76]	5.85[5.26,6.51]	0.127
TG (mmol/L)	2.58[2.09,3.12]	2.21[1.73,2.65]	<0.001
HDL-C (mmol/L)	2.07±0.42	2.05±0.43	<0.001
LDL-C (mmol/L)	3.28[2.70,3.89]	3.25[2.77,3.80]	0.987
Ca (mmol/L)	2.31±0.08	2.27±0.09	<0.001
P (mmol/L)	1.32±0.13	1.22±0.14	<0.001

Note. GDM, gestational diabetes mellitus; NGT, normal glucose tolerance; DM, diabetes mellitus; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; 25OHD, 5-hydroxyvitamin D; PTH, parathyroid hormone; OGTT, oral glucose tolerance test; HOMA-IR, omeostasis model assessment of insulin resistance; HOMA-β, homeostasis model assessment of β cell function; AUC, area under the curve; Cr, creatinine; CHO, cholesterol; TG, triglyceride; HDL-C, high density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol; Ca, calcium; P, hosphorus; SD, standard deviation. Normally distributed data are presented as means±SD. Non-normally distributed data are presented as medians [interquartile range].

Table 2. Odds Ratios and 95% Confidence Intervals for GDM according to Serum 25OHD Concentrations in Pregnant Women

Vitamin D Status	GDM Group	NGT Group	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
Vitamin D sufficiency (25OHD ⩾ 25nmol/L)	78 (42.2%)	107 (57.8%)	1.00 (referent)	1.00 (referent)
Vitamin Ddeficiency (25OHD <25 nmol/L)	122 (56.7%)	93 (43.3%)	1.800 (1.209-2.678)	1.588 (1.034-2.441)
P for trend	-	-	0.004	0.035

analysis in which serum 25OHD was included as a continuous variable; each 1 nmol/L decrease in serum 25OHD concentration increased the risk for GDM by 1.025-fold (95% CI: 1.002-1.049, *P*=0.035) after adjusting for maternal age, family history of T2DM, and TG, in logistic regression analysis.

Univariate linear correlation analysis showed that serum 25OHD levels in the GDM group were inversely correlated with several variables, including pre-pregnancy BMI (*r*=-0.203, *P*=0.007), HbA1c (*r*=-0.185, *P*=0.018), and TG (*r*=-0.151, *P*=0.038) (Table 3). In multivariate linear regression analysis, serum 25OHD was independently associated with HbA1c (β=-0.171, *P*=0.036) after adjusting for pre-pregnancy BMI, FPI, family history of T2DM, and TG. To further evaluate the relationship between 25OHD and insulin resistance, the proportion of subjects with HOMA-IR ⩾3 was calculated[19], and was significantly higher in subjects with 25OHD <25 nmol/L than in subjects with 25OHD ⩾25 nmol/L (18.3% *vs.* 7.8%, *P*=0.041). Logistic regression analysis confirmed that serum 25OHD levels were associated with reduced risk of insulin resistance defined as HOMA-IR ⩾ 3 after adjusting for maternal age, pre-pregnancy BMI, family history of T2DM, and AUC-insulin (adjusted OR 0.935, 95% CI 0.877-0.996, *P*=0.039).

In the NGT group, simple linear correlation analysis revealed that 25OHD levels were inversely correlated with FPG (*r*=-0.184, *P*=0.009) and SBP (*r*=-0.215, *P*=0.015). Multivariate linear regression analysis confirmed that serum 25OHD levels were independently associated with FPG (β=-0.147, *P*=0.036) after adjusting for maternal age, FPI, and

Table 3. Correlation between Serum 25OHD Levels and Subject Characteristics

	r	*P* Value	*r* of NGT Group	*P* Value of NGT Group	*r* of GDM Group	*P* Value of GDM Group
n	400		200		200	
Age (years)	-0.090	0.077	-0.092	0.212	-0.021	0.765
Pre-BMI (kg/cm^2)	-0.187	0.001*	-0.147	0.089	-0.203	0.007*
SBP (mmHg)	-0.174	0.003*	-0.215	0.015*	-0.086	0.271
DBP (mmHg)	-0.129	0.027*	-0.131	0.140	-0.091	0.240
PTH (pg/mL)	-0.133	0.009*	-0.131	0.073	-0.081	0.256
OGTT						
0h Glucose (mmol/L)	-0.194	<0.001*	-0.184	0.009*	-0.100	0.162
1h Glucose (mmol/L)	-0.179	<0.001*	-0.056	0.430	-0.031	0.663
2h Glucose (mmol/L)	-0.169	0.001*	0.011	0.872	-0.021	0.772
3h Glucose (mmol/L)	-0.107	0.034*	0.011	0.875	0.114	0.113
0h Insulin (mU/L)	-0.101	0.047*	-0.035	0.632	-0.101	0.161
1h Insulin (mU/L)	-0.109	0.033*	-0.082	0.258	-0.113	0.121
2h Insulin (mU/L)	-0.083	0.105	-0.022	0.760	-0.032	0.662
3h Insulin (mU/L)	-0.120	0.018*	-0.075	0.301	-0.041	0.569
HbA1c (%)	0.079	0.161	0.030	0.710	-0.185	0.018*
HOMA-IR	-0.135	0.008*	-0.058	0.424	-0.126	0.081
HOMA-B	0.037	0.469	0.103	0.158	-0.016	0.830
AUC of insulin	-0.123	0.017*	-0.077	0.294	-0.072	0.321
Cr (umol/L)	0.089	0.104	0.109	0.178	0.071	0.340
CHO (mmol/L)	-0.029	0.575	-0.036	0.625	0.034	0.639
TG (mmol/L)	-0.091	0.077	0.012	0.874	-0.151	0.038*
HDL-C (mmol/L)	0.006	0.913	0.011	0.884	0.019	0.796
LDL-C (mmol/L)	-0.008	0.883	-0.052	0.479	0.077	0.294
Ca (mmol/L)	-0.129	0.033*	-0.022	0.792	-0.137	0.115
P (mmol/L)	-0.130	0.032*	-0.053	0.532	-0.033	0.702

Note. * $P<0.05$.

LDL-C. Interestingly, multiple linear regression analysis also suggested that serum 25OHD levels were independently associated with SBP (β=-0.234, *P*=0.016) after adjusting for maternal age, pre-pregnancy BMI, HbA1c, HOMA-IR, and LDL-C. No association was found between 25OHD levels and HOMA-IR (Table 3).

DISCUSSION

It has been reported that vitamin D insufficiency is very prevalent in different ethnic groups, particularly in pregnancy. This is the first study to evaluate vitamin D status in pregnant Chinese women. The rate of vitamin D insufficiency was very high, being 96.25% among women with serum 25OHD levels <50 nmol/L and 53.75% among women with serum 25OHD <25 nmol/L, in this cohort of pregnant women with GDM living in Beijing. Several previous studies have also document a high prevalence of vitamin D insufficiency in pregnant women, ranging from 33% in Australia[27] to 70.6% in Iran[19]. Data from different ethnic groups suggest that 25OHD levels during pregnancy are lower in Asian women than in European and American women[18,20]. Zhang et al. and Maghbooli et al. reported a higher prevalence of low vitamin D levels in patients with GDM compared with control subjects in the second trimester (week 16; 25OHD <50 nmol/L: 33% *vs*. 14%) and third trimester (week 24-28; 25OHD <12.5 nmol/L: 44.2% *vs*. 23.5%).

The vitamin D receptor is expressed in a large number of tissues, including those involved in the regulation of glucose metabolism, such as muscle and pancreatic β cells. Vitamin D is also required for normal insulin secretion[1,5]. A number of studies revealed that 25OHD levels in non-pregnant women were positive correlated with insulin sensitivity and that higher 25OHD levels were associated with a lower risk of IGT and T2DM[4,6,28]. In the present

study, 25OHD levels were lower in the GDM group than in the NGT group, and low vitamin D levels increased the risk of GDM. Moreover, we found an inverse association between serum 25OHD and HbA1c levels in patients with GDM. These findings are generally consistent with those of studies in other ethnic populations, except for a study in Indian subjects, in which no significant association between 25OHD concentrations at week 30 of gestation and GDM was observed[18-21]. Unlike Clifton-Bligh's results, 25OHD was not significantly associated with FPG or FPI in the present study. There may be several reasons for these differences. First, ethnicity is a risk factor for altered vitamin D status and may independently affect the association between 25OHD levels and the risk of diabetes[18,20]. In the Third National Health and Nutrition Examination Survey (NHANES III) and a meta-analysis of cross-sectional studies, serum 25OHD levels were inversely associated with the risk of diabetes in non-Hispanic whites and Mexican Americans, but not in non-Hispanic blacks[6,29]. Second, the present study was conducted as a nested case-control study, in which we enrolled a large cohort of women with GDM. The previous studies were mostly cross-sectional in design, and the differences in study design may influence the results. Third, the median serum 25OHD level [22.39, interquartile range (17.67, 29.38) nmol/L] was relatively lower and vitamin D deficiency was more frequent in patients with GDM group in our study than in Clifton-Bligh's study (48.6±24.9 nmol/L). This might also influence the relationship between vitamin D and FPG or FPI.

Vitamin D can promote insulin sensitivity by stimulating the expression of insulin receptors and enhancing insulin-dependent glucose transport. Chiu et al. determined the insulin sensitivity index (ISI), and the first- and second-phase insulin responses during hyperglycemic clamps in 126 healthy non-pregnant subjects. They found a positive correlation between 25OHD levels and insulin sensitivity[4]. Meanwhile, Maghbooli et al. found that 25OHD levels were inversely associated with HOMA-IR, a marker of insulin resistance, in a cross-sectional study involving 741 pregnant women. Similar to these studies, we found that low 25OHD levels were a risk factor for HOMA-IR $\geqslant$3 in patients with GDM[19]. The results of these results suggest that subjects with vitamin D insufficiency/deficiency are at increased risk of developing insulin resistance and metabolic syndrome in non-pregnant individuals and in patients with GDM.

Borissova et al. evaluated the effects of vitamin D3 supplementation on insulin secretion and insulin resistance in 10 women with T2DM. Administration of 1332 IU/day cholecalciferol for 1 month significantly increased plasma 25OHD levels and increased first-phase insulin secretion during an intravenous glucose tolerance test. They also observed a decrease in insulin resistance of 21.4%, although this was not statistically significant[30]. In another prospective study, the association between vitamin D and the risk of T2DM was examined in 83 779 women without history of diabetes at baseline. During 20 years of follow-up, the relative risk of T2DM was 0.87 (95% CI 0.75-1.00, *P*=0.04) for the highest versus lowest level of supplemental vitamin D intake[31]. These results suggest that vitamin D supplementation may have beneficial effects in terms of reducing the risk of T2DM. However, a clinical study on the role of vitamin D supplementation on the risk of GDM is still lacking.

Several *in vivo* studies have shown that $1,25(OH)_2D$ has an important role on the regulation of plasma renin activity[32]. A large epidemiologic survey of non-hypertensive Caucasians in the US revealed that mean SBP was lower in the highest quantile of 25OHD levels than in the lowest quantile, and that 25OHD levels were inversely associated with SBP[33]. Meanwhile, a randomized, placebo-controlled study in which serum 25OHD levels were measured in 145 elderly women showed that vitamin D supplementation for 8 weeks significantly increased serum 25OHD by 72% and decreased SBP by 9.2%[34]. Our present study was the first to show an inverse association between SBP and 25OHD levels in pregnant women with NGT, even after adjusting for age, pre-pregnancy BMI, FPG, and FPI. Since this the subjects in this case-control study were classified by GDM and NGT, it is necessary to conduct large prospective epidemiologic studies to confirm our results.

Unlike other studies[18], we did not find an independent association between serum 25OHD and PTH/lnPTH levels using linear correlation analysis in the GDM or NGT groups. It was unfortunate that we did not measure PTH-related peptide (PTHrP) levels in the present study. Ardawi et al. reported that maternal serum PTH levels tended to decrease, whereas PTHrP levels, which are usually undetectable in non-pregnant women, increased gradually during pregnancy[35]. PTHrP can also bind to the PTH/PTHrP receptor, which might mirror the relationship between 25OHD and PTH levels. In our

study, PTH levels were detectable in the third trimester, when serum PTHrP might reach a relative high level. Therefore, parallel measurements of serum PTH and PTHrP levels may help to explain the changes in calcium-regulating hormones during pregnancy.

Our study has some limitations. First, a single measurement of serum 25OHD levels during the third trimester does not reflect vitamin D status throughout gestation. Longitudinal analysis of serial measurements of serum 25OHD may provide us with a more comprehensive understanding of vitamin D status, and provide a more reliable conclusion on the relationship between vitamin D and glucose metabolism during pregnancy. Second, more indices of insulin sensitivity and more information on the medical history and lifestyle (e.g., intake of vitamin D and calcium supplements) are needed to exclude potential confounding factors. In fact, most of the pregnant women in this cohort did not take or took very low doses of vitamin D (<400 IU/day) during pregnancy. Third, we used an IDS enzyme immunoassay to measure serum 25OHD. According to information from the Vitamin D External Quality Assessment Scheme (DEQAS, www.DEQAS.org.uk), the mean annual DEQAS bias ranged from +5.7 to +23% between 2004 and 2006, suggesting that 25OHD levels are overestimated by established assays. Although this positive bias was reduced to around +5% after re-calibration in 2006, 25OHD levels may still be overestimated, meaning vitamin D deficiency/insufficiency might be even more common and severe in this group of subjects[36]. Fourth, our study was designed as a nested case-control study. A sampling bias may exist because only ~50% of the pregnant women followed at our clinic provided informed content and subjects with family history of T2DM or a history of abnormal delivery were more likely to be included in this study. Thus larger and prospective studies are needed to confirm the results.

In conclusion, our study provides preliminary data showing that vitamin D insufficiency was very common in the third trimester of pregnancy in a group of Chinese women with GDM. Low vitamin D status may be correlated with insulin resistance and act as a risk factor for GDM in pregnant Chinese women. Since circulating vitamin D levels can be modified conveniently by consuming foods with high vitamin D levels, the use of vitamin supplements, and exposure to sunlight, prospective or intervention studies are needed to assess the effects of vitamin D supplements on glucose metabolism and blood pressure during pregnancy.

CONFLICT OF INTEREST DISCLOSURE

The authors declare that they have no conflicts of interest.

REFERENCES

1. Holick MF. Vitamin D deficiency. N Engl J Med, 2007; 357(3), 266-81.
2. Liu S, Song Y, Ford ES, et al. Dietary calcium, vitamin D, and the prevalence of metabolic syndrome in middle-aged and older U.S. women. Diabetes Care, 2005; 28(12), 2926-32.
3. Ayesha I, Bala TS, Reddy CV, et al. Vitamin D deficiency reduces insulin secretion and turnover in rats. Diabetes Nutr Metab, 2001; 14(2), 78-84.
4. Chiu KC, Chu A, Go VL, et al. Hypovitaminosis D is associated with insulin resistance and beta cell dysfunction. Am J Clin Nutr, 2004; 79(5), 820-5.
5. de Souza SR, Vianna LM. Effect of cholecalciferol supplementation on blood glucose in an experimental model of type 2 diabetes mellitus in spontaneously hypertensive rats and Wistar rats. Clin Chim Acta, 2005; 358(1-2), 146-50.
6. Scragg R, Sowers M, Bell C. Serum 25-hydroxyvitamin D, diabetes, and ethnicity in the Third National Health and Nutrition Examination Survey. Diabetes Care, 2004; 27(12), 2813-8.
7. Isaia G, Giorgino R, Adami S. High prevalence of hypovitaminosis D in female type 2 diabetic population. Diabetes Care, 2001; 24(8), 1496.
8. Boucher BJ, Mannan N, Noonan K, et al. Glucose intolerance and impairment of insulin secretion in relation to vitamin D deficiency in east London Asians. Diabetologia, 1995; 38(10), 1239-45.
9. Forouhi NG, Luan J, Cooper A, et al. Baseline serum 25-hydroxy vitamin d is predictive of future glycemic status and insulin resistance: the Medical Research Council Ely Prospective Study 1990-2000. Diabetes, 2008; 57(10), 2619-25.
10. Damm P, Kuhl C, Bertelsen A, et al. Predictive factors for the development of diabetes in women with previous gestational diabetes mellitus. Am J Obstet Gynecol, 1992; 167(3), 607-16.
11. Li W, Green TJ, Innis SM, et al. Suboptimal vitamin D levels in pregnant women despite supplement use. Can J Public Health, 2011; 102(4), 308-12.
12. Al Kalbani M, Elshafie O, Rawahi M, et al. Vitamin D status in pregnant Omanis: a disturbingly high proportion of patients with low vitamin D stores. Sultan Qaboos Univ Med J, 2011; 11(1), 52-5.
13. Sääf M, Fernell E, Kristiansson F, et al. Severe vitamin D deficiency in pregnant women of Somali origin living in Sweden. Acta Paediatr, 2011; 100(4), 612-4.
14. Asemi Z, Taghizadeh M, Sarahroodi S, et al. Assessment of the relationship of vitamin D with serum antioxidant vitamins E and A and their deficiencies in Iranian pregnant women. Saudi Med J, 2010; 31(10), 1119-23.
15. Johnson DD, Wagner CL, Hulsey TC, et al. Vitamin D deficiency and insufficiency is common during pregnancy. Am J Perinatol, 2011; 28(1), 7-12.
16. Hossain N, Khanani R, Hussain-Kanani F, et al. High prevalence of vitamin D deficiency in Pakistani mothers and their newborns. Int J Gynaecol Obstet, 2011; 112(3), 229-33.

17.Teale GR, Cunningham CE. Vitamin D deficiency is common among pregnant women in rural Victoria. Aust N Z J Obstet Gynaecol, 2010; 50(3), 259-61.
18.Clifton-Bligh RJ, McElduff P, McElduff A. Maternal vitamin D deficiency, ethnicity and gestational diabetes. Diabet Med, 2008; 25(6), 678-84.
19.Maghbooli Z, Hossein-Nezhad A, Karimi F, et al. Correlation between vitamin D3 deficiency and insulin resistance in pregnancy. Diabetes Metab Res Rev, 2008; 24(1), 27-32.
20.Zhang C, Qiu C, Hu FB, et al. Maternal plasma 25-hydroxyvitamin D concentrations and the risk for gestational diabetes mellitus. PLoS One, 2008; 3(11), e3753.
21.Farrant HJ, Krishnaveni GV, Hill JC, et al. Vitamin D insufficiency is common in Indian mothers but is not associated with gestational diabetes or variation in newborn size. Eur J Clin Nutr, 2009; 63 (5), 646-52.
22.Soheilykhah S, Rashidi M, Mojibian M, et al. An appropriate test for diagnosis of gestational diabetes mellitus. Gynecol Endocrinol, 2011; 27(10), 785-8.
23.Li M, Wu C, Song A, et al. Development and preliminary application of enzyme linked immunosorbent assay for human net insulin in serum. Chin J Endocrinol Metab, 1997; 13(11), 214-7. (In Chinese)
24.Matthews DR, Hosker JP, Rudenski AS, et al. Homeostasis model assessment: insulin resistance and b-cell function from fasting plasma glucose and insulin concentrations in man. Diabetes, 1985; 28(7), 412-9.
25.Purves RD. Optimum numerical integration methods for estimation of area-under-the-curve (AUC) and area-under-the-moment-curve (AUMC). J Pharmacokin Biopharm, 1992; 20(3), 211-27.
26.Ramos-Lopez E, Kahles H, Weber S, et al. Gestational diabetes mellitus and vitamin D deficiency: genetic contribution of CYP27B1 and CYP2R1 polymorphisms. Diabetes Obes Metab, 2008; 10(8), 683-5.
27.Bowyer L, Catling-Paull C, Diamond T, et al. Vitamin D, PTH and calcium levels in pregnant women and their neonates. Clin Endocrinol (Oxf), 2009; 70(3), 372-7.
28.Scragg R, Holdaway I, Singh V, et al. Serum 25-hydroxyvitamin D3 levels decreased in impaired glucose tolerance and diabetes mellitus. Diabetes Res Clin Pract, 1995; 27(3), 181-8.
29.Pittas AG, Lau J, Hu FB, et al. The role of vitamin D and calcium in type 2 diabetes. A systematic review and meta-analysis. J Clin Endocrinol Metab, 2007; 92(6), 2017-29.
30.Borissova AM, Tankova T, Kirilov G, et al. The effect of vitamin D3 on insulin secretion and peripheral insulin sensitivity in type 2 diabetic patients. Int J Clin Pract, 2003; 57(4), 258-61.
31.Pittas AG, Dawson-Hughes B, Li T, et al. Vitamin D and calcium intake in relation to type 2 diabetes in women. Diabetes Care, 2006; 29(3):650-6.
32.Li YC, Kong J, Wei M, et al. 1,25-Dihydroxyvitamin D(3) is a negative endocrine regulator of the renin-angiotensin system. J Clin Invest, 2002; 110(2), 229-38.
33.Judd SE, Nanes MS, Ziegler TR, et al. Optimal vitamin D status attenuates the age-associated increase in systolic blood pressure in white Americans: results from the third National Health and Nutrition Examination Survey. Am J Clin Nutr, 2008; 87(1), 136-41.
34.Pfeifer M, Begerow B, Minne HW, et al. Effects of a short-term vitamin D(3) and calcium supplementation on blood pressure and parathyroid hormone levels in elderly women. J Clin Endocrinol Metab, 2001; 86(4), 1633-7.
35.Ardawi MS, Nasrat HA, BA'Aqueel HS. Calcium-regulating hormones and parathyroid hormone-related peptide in normal human pregnancy and postpartum: a longitudinal study. Eur J Endocrinol, 1997; 137(4), 402-9.
36.Wallace AM, Gibson S, de la Hunty A, et al. Measurement of 25-hydroxyvitamin D in the clinical laboratory: current procedures, performance characteristics and limitations. Steroids, 2010; 75(7), 477-88.