รายงานวิจัยฉบับสมบูรณ์

โครงการ

ระดับวิตามินดี โพลีมอร์ฟิซมของวิตามินดีไบนดิงโปรตีนยีน และความเสี่ยงต่อการเกิดโรคเบาหวานระหว่างการตั้งครรภ์

โดย

ผศ.พญ.ณัฏฐิณี จรัสเจริญวิทยา

วันที่เสร็จโครงการ 31 มกราคม 2557

สัญญาเลขที่ MRG5480244

รายงานวิจัยฉบับสมบูรณ์

โครงการ

ระดับวิตามินดี โพลีมอร์ฟิซมของวิตามินดีไบนดิงโปรตีนยีนและความเสี่ยงต่อการเกิดโรคเบาหวานระหว่างการตั้งครรภ์

ผู้วิจัย	สังกัด
1. ผศ.พญ. ณัฏฐิณี จรัสเจริญวิทยา	คณะแพทยศาสตร์มหาวิทยาลัยธรรมศาสตร์
2. ศ.นพ. บุญส่ง องค์พิพัฒนกุล	คณะแพทยศาสตร์โรงพยาบาลรามาธิบดี
3. รศ.พญ. จรินทร์ทิพย์ สมประสิทธิ์	คณะแพทยศาสตร์มหาวิทยาลัยธรรมศาสตร์
4. รศ.พญ. อธิตา จันทเสนานนท์	คณะแพทยศาสตร์มหาวิทยาลัยธรรมศาสตร์
5. ดร. ละออ ชัยเลอกิจ	คณะแพทยศาสตร์โรงพยาบาลรามาธิบดี
6. นางสาว สุวรรณี ชาญประเสริฐโยธิน	คณะแพทยศาสตร์โรงพยาบาลรามาธิบดี

สนับสนุนโดยสำนักงานคณะกรรมการการอุดมศึกษา สำนักงานกองทุนสนับสนุนการวิจัย
และมหาวิทยาลัยธรรมศาสตร์

(ความเห็นในรายงานนี้เป็นของผู้วิจัย สกอ. และ สกว. ไม่จำเป็นต้องเห็นด้วยเสมอไป)

บทคัดย่อ

รหัสโครงการ : MRG5480244

ชื่อโครงการ : ระดับวิตามินดี โพลีมอร์ฟิซมของวิตามินดีไบนดิงโปรตีนยีนและความเสี่ยงต่อการเกิดโรคเบาหวานระหว่างการตั้งครรภ์

ชื่อนักวิจัย : ผศ.พญ.ณัฏฐิณี จรัสเจริญวิทยา

อีเมลล์ : natthineei@yahoo.com

ระยะเวลาโครงการ : 2 ปี และขอขยายเวลาต่ออีก 10 เดือน

บทคัดย่อ:

ที่มาของการวิจัย: ยังไม่ทราบแน่ชัดว่าระดับวิตามินดีที่ไม่เพียงพอเพิ่มความเสี่ยงต่อการเกิดโรคเบาหวานระหว่างการตั้งครรภ์หรือไม่ การตรวจวัดระดับวิตามินดีเพียงครั้งเดียวอาจจะไม่สะท้อนถึงระดับวิตามินดีตลอดการตั้งครรภ์และอาจจะเป็นสาเหตุของผลการศึกษาที่แตกต่างกันได้ นอกจากนี้ปัจจัยทางพันธุกรรมที่เกี่ยวข้องกับวิตามินดีไบนดิงโปรตีนอาจจะมีความสัมพันธ์กับระดับวิตามินดีและความเสี่ยงต่อการเกิดโรคเบาหวานระหว่างการตั้งครรภ์

วัตถุประสงค์: 1. เพื่อศึกษาถึงความแตกต่างของระดับวิตามินดีระหว่างหญิงตั้งครรภ์ที่มีโรคเบาหวานระหว่างการตั้งครรภ์และหญิงตั้งครรภ์ปกติ 2. เพื่อศึกษาถึงความสัมพันธ์ระหว่างโพลีมอร์ฟิซมที่ตำแหน่ง 2282679 ในวิตามินดีไบนดิงโปรตีนยีนและความเสี่ยงต่อการเกิดโรคเบาหวานระหว่างการตั้งครรภ์

วิธีการวิจัย: เป็นการศึกษาแบบ nested case control ในหญิงตั้งครรภ์ที่เข้าร่วมโครงการตรวจคัดกรองโรคเบาหวานระหว่างการตั้งครรภ์ อาสาสมัครได้รับการเก็บข้อมูลทางคลินิกและตัวอย่างเลือด 2 ครั้ง ระหว่างไตรมาสที่ 1 ของการตั้งครรภ์ และระหว่างอายุครรภ์ 24-28 สัปดาห์ หญิงตั้งครรภ์ที่มีโรคเบาหวานระหว่างการตั้งครรภ์ 80 ราย ได้รับการจับคู่กับหญิงตั้งครรภ์ปกติจำนวน 80 ราย ที่มีช่วงอายุและน้ำหนักตัวก่อนตั้งครรภ์อยู่ในช่วงเดียวกัน อาสาสมัครได้รับการตรวจวัดระดับวิตามินดี อินซูลิน ฮอร์โมนพาราไทรอยด์ และชนิดของยีน ที่ตำแหน่ง 2282679 ในวิตามินดีไบนดิงโปรตีนยีน

ผลการศึกษา: ระดับวิตามินดีในทั้ง 2 กลุ่มไม่มีความแตกต่างกันทั้งในไตรมาสที่ 1 ของการตั้งครรภ์ (27.2±6.9 ng/ml vs. 28.7±6.7 ng/ml; p=0.16) และ ระหว่างอายุครรภ์ 24-28 สัปดาห์ (35.3±9.3 vs. 36.5±7.6; p=0.39) มีแนวโน้มแสดงให้เห็นว่าหญิงที่มี G allele (ยีนชนิด TG และ GG) มีความเสี่ยงต่อการเกิดโรคเบาหวานระหว่างการตั้งครรภ์สูงกว่าหญิงที่ไม่มี G allele (OR 1.8, 95%CI: 0.94-3.43, p=0.07) ซึ่งความสัมพันธ์ชัดเจนยิ่งขึ้นเมื่อคัดหญิงจำนวน 6 รายที่มีภาวะขาดวิตามินดีระหว่างอายุครรภ์ 24-28 สัปดาห์ออกจากการวิเคราะห์ทางสถิติ (OR 2.14, 95%CI: 1.1-4.2; p=0.03)

สรุป: การศึกษานี้ไม่พบหลักฐานที่แสดงให้เห็นว่าระดับวิตามินดีมีความสัมพันธ์กับการเกิดโรคเบาหวานระหว่างการตั้งครรภ์ ทั้งในช่วงไตรมาสแรกของการตั้งครรภ์และระหว่างอายุครรภ์ 24-28 สัปดาห์ หญิงที่มี G allele (ยีนชนิด TG และ GG) ที่ตำแหน่ง 2282679 ในยีน GC มีแนวโน้มว่าจะมีความเสี่ยงต่อการเกิดโรคเบาหวานระหว่างการตั้งครรภ์เพิ่มขึ้น ถึงแม้จะมีระดับวิตามินดีที่เพียงพอระหว่างอายุครรภ์ 24-28 สัปดาห์ เป็นไปได้ว่าโพลิมอร์ฟิซมในวิตามินดีไบนดิงโปรตีนยีนอาจจะมีความสัมพันธ์กับการเกิดโรคเบาหวานระหว่างการตั้งครรภ์

คำหลัก: วิตามินดี โรคเบาหวานระหว่างการตั้งครรภ์ โพลีมอร์ฟิซมของวิตามินดีไบนดิงโปรตีนยีน

Abstract

Project Code : MRG5480244

Project Title : Maternal vitamin D status, polymorphism of vitamin D binding protein gene and risk for gestational diabetes

Investigator : Assistant professor Natthinee Charatcharoenwitthaya

E-mail Address : natthineei@yahoo.com

Project Period : 2 years with 10 months extension

Abstract:

Background: It is unclear whether low maternal 25-hydroxyvitamin D (25OHD) level is associated with gestational diabetes mellitus (GDM). The single measurement of 25OHD levels may not reflect vitamin D status throughout pregnancy and may be associated with the contradictory findings in previous studies. Genetic factors associated with vitamin D binding protein may be associated with 25OHD levels and risk of GDM.

Objectives: (1) To prospectively examine the differences in 25OHD concentrations between pregnant women with GDM and normal controls; (2) to examine the relationship between SNP at positions 2282679 in the GC gene and the risk of GDM. The relationships between 25OHD levels and parameters of glucose homeostasis were also examined.

Methods: A nested case-control study was conducted among pregnant women who participated in GDM screening project. Clinical data and blood samples were obtained during the first trimester of pregnancy and during 24-28 weeks' gestation on the same day of blood glucose testing. Eighty women with GDM according to IADPSG criteria were matched with 80 normal pregnancies who had the same age range (< 25 years or $\geq$ 25 years) and the same pre-pregnancy body mass index (BMI) range (< 25 kg/m^2 or $\geq$ 25 kg/m^2). The 25OHD, fasting insulin, and parathyroid hormone were measured. Individual genotyping of rs2282679 in the GC gene was performed using real-time PCR.

Results: The 25OHD levels were not different between women with GDM and normal pregnancies both during the first trimester (27.2$\pm$6.9 ng/ml vs. 28.7$\pm$6.7 ng/ml; p=0.16) and during 24-28 weeks' gestation (35.3$\pm$9.3 vs. 36.5$\pm$7.6; p=0.39). There was a trend that women carrying the G allele (TG and GG genotypes) had a higher risk of GDM than women without G allele (OR 1.8, 95%CI: 0.94-3.43, p=0.07). In multiple logistic regression model adjusting for age, log pre-pregnancy BMI, and a family history of diabetes, there was a trend that women carrying the G allele increased risk of GDM (OR 1.88, 95%CI: 0.97-3.66; p=0.06). The association between the G allele and GDM was stronger after excluding 6 women with vitamin D deficiency during 24-28 weeks' gestation (OR 2.14, 95%CI: 1.1-4.2; p=0.03).

Conclusions There is no strong evidence suggesting an independent association between GDM and 25OHD concentrations both during the first trimester of pregnancy and during 24-28 weeks' gestation. Carrying the G allele (TG and GG genotypes) at positions 2282679 in the GC gene may be associated with the increased risk of GDM even had adequate vitamin D levels during 24-28 weeks' gestation. The GC polymorphism may have a role in GDM development.

Keywords : vitamin D, gestational diabetes mellitus, polymorphism of vitamin D binding protein gene

บทสรุปผู้บริหาร (Executive Summary)

โครงการวิจัย “ระดับวิตามินดี โพลีมอร์ฟิซมของวิตามินดีไบนดิงโปรตีนยีนและความเสี่ยงต่อการเกิดโรคเบาหวานระหว่างการตั้งครรภ์” จัดทำขึ้นเพื่อศึกษาถึงความสัมพันธ์ระหว่างระดับวิตามินดี โพลีมอร์ฟิซมของวิตามินดีไบนดิงโปรตีนยีน และความเสี่ยงต่อการเกิดโรคเบาหวานระหว่างการตั้งครรภ์ ผลการศึกษาแสดงให้เห็นว่า ผู้ที่มี G allele (TG และ GG genotypes) ที่ตำแหน่ง 2282679 ในวิตามินดีไบนดิงโปรตีนยีนมีความเสี่ยงต่อการเกิดโรคเบาหวานระหว่างการตั้งครรภ์เพิ่มขึ้น โดยไม่ขึ้นกับระดับวิตามินดี ที่ตรวจวัดจากระดับของ 25-hydroxyvitamin D (25OHD) ระหว่างสัปดาห์ที่ 24-28 ของการตั้งครรภ์ ซึ่งแสดงให้เห็นว่าวิตามินดีน่าจะมีบทบาทเกี่ยวข้องกับการเกิดโรคเบาหวานระหว่างการตั้งครรภ์จริง แต่อย่างไรก็ตาม การศึกษาก่อนหน้านี้พบว่าผลการศึกษาถึงความสัมพันธ์ระหว่างระดับวิตามินดีและความเสี่ยงต่อการเกิดโรคเบาหวานระหว่างการตั้งครรภ์ไม่ได้ไปในทิศทางเดียวกันทั้งหมด ซึ่งอาจเนื่องมาจากโพลีมอร์ฟิซมของวิตามินดีไบนดิงโปรตีนยีน ซึ่งนอกจากมีผลต่อระดับ 25OHD แล้ว อาจจะมีผลต่อ binding affinity ระหว่าง 25OHD และ vitamin D binding protein ซึ่งมีผลต่อระดับของ free 25OHD และ bioavailable 25OHD ซึ่งอาจจะเป็น markers ที่มีความสัมพันธ์กับความเสี่ยงต่อการเกิดโรคเบาหวานระหว่างการตั้งครรภ์ที่ดีกว่าการตรวจระดับ total 25OHD ได้ จึงควรทำการศึกษาเพิ่มเติมถึงความสัมพันธ์ระหว่างระดับ free 25OHD และ/หรือ bioavailable 25OHD ต่อการเกิดโรคเบาหวานระหว่างการตั้งครรภ์เพิ่มเติมในอนาคตต่อไป

Introduction

Gestational diabetes mellitus (GDM), defined as glucose intolerance with onset or first recognition during pregnancy[1], is one of the most common medical complications of pregnancy.[2] The reported prevalence ranges from 2-25% depending on the diagnostic criteria and ethnicity.[3] GDM is not only associated with adverse pregnancy outcomes, but also increases future risk for development of type 2 diabetes mellitus (T2DM) in both mother and offspring.[4] Identification of modifiable risk factors for GDM may contribute to the prevention of GDM.

Vitamin D inadequacy is prevalent in pregnant women in many countries, even in sun-rich areas.[5] Accumulating evidence suggests that vitamin D deficiency might be a modifiable risk factor for the development of GDM.[6] However, the observational evidence investigating the impact of sub-optimal vitamin D status on the development of GDM, and on glucose homeostasis during pregnancy showed inconsistent results. While several studies found that women who developed GDM had significantly lower mean or median 25OHD concentrations, and had a higher risk of having vitamin D deficiency than normal pregnancies[7-13], some studies with adequate power and well control for confounding factors did not find any important association between vitamin D status and GDM.[14,15] The possible thresholds for the negative effects of low vitamin D status on glucose homeostasis were proposed differently. Parlea et al.[10] suggested that even a mild decrease in vitamin D concentrations (25OHD < 29.4 ng/ml) may increase risk of glucose intolerance during pregnancy, while Zuhur et al.[16] suggested that only severe vitamin D deficiency (25OHD < 5 ng/ml) was associated with an elevated relative risk of GDM in multivariate analysis. The mechanisms by which vitamin D deficiency is associated with increased risk of GDM are also inconsistent. Some studies suggested an association between low 25OHD levels and insulin resistance.[7,11,13] However, McLeod et al. found an association between 25OHD and pancreatic beta-cell function as estimated by HOMA-B.[16] It is possible that single measurement of 25OHD levels may not reflect vitamin D status throughout pregnancy and may be associated with the contradictory findings in previous studies. A prospective study design with serial measurements of 25OHD concentrations and parameters of glucose metabolism may provide new information related to the patho-physiologic consequences of low maternal vitamin D status on glucose homeostasis during pregnancy and the development of GDM. Also studies of the genetic variants that influence circulating 25OHD levels may identify individuals at risk for vitamin D deficiency and enhance the understanding of the observed associations between low maternal vitamin D status and GDM. If lower maternal vitamin D status is causally related to risk

of developing GDM, a genetic variant associated with lower 25OHD concentrations should be associated with a higher risk of GDM.

The vitamin D binding protein (DBP), formerly known as group-specific component (GC), is the main transporter of vitamin D and its metabolites.[17] The GC gene is localized on chromosome 4 (4q12-q13). Genotypic variations in the GC gene may be associated with changes in serum concentrations of DBP, and/or the binding affinity. A genome-wide association study found that the single nucleotide polymorphism (SNP) at position rs2282679 was associated with vitamin D status and the concentrations of DBP.[18] A recent study suggested that the rs2282679G allele (TG genotype) was associated with increased risks of vitamin D deficiency and vitamin D insufficiency in comparison with the TT genotype.[19] Currently, there is no study examining the relationship between SNP at positions 2282679 in the GC gene and the risk of GDM.

The objectives of the present study are (1) to prospectively examine the differences in 25OHD concentrations (during the first trimester of pregnancy and during 24-28 weeks' gestation) between pregnant women with GDM and normal controls; (2) to examine the relationship between 25OHD concentrations and parameters of glucose metabolism as follows: first trimester 25OHD and first trimester glucose parameters, first trimester 25OHD and 24-28 weeks' glucose parameters, and 24-28 weeks' 25OHD and 24-28 weeks' glucose parameters; (3) to examine the relationship between SNP at positions 2282679 in the GC gene and the risk of GDM.

Materials and Methods

A nested case-control study was conducted among pregnant women who participated in GDM screening project at Thammasat University Hospital, Pathumthani, Thailand. The inclusion criteria were singleton pregnancies, age 18-40 years, gestational age less than or equal to 14 weeks, and having at least 1 risk factors for GDM including age $\geq$ 25 years, pre-pregnancy body mass index (BMI) $\geq$ 25 kg/m^2, previous history of GDM, previous delivery of a baby weighted $\geq$ 4,000 grams, a family history of diabetes in first degree relatives, and glycosuria at the first prenatal visit. The exclusion criteria were a history of pre-gestational diabetes, first trimester fasting plasma glucose (FPG) $\geq$ 126 mg/dl, chronic medical conditions, taking medication known to affect glucose metabolism, and twin pregnancy.

Through the interviews and medical record reviews, we collected information on demographic data, medical and obstetrical history, a family history of diabetes, current medication, prenatal vitamin supplements, consumption of vitamin-fortified milk and vitamin D-containing diet, and sunlight exposure. Clinical data and fasting blood samples were obtained during the first

trimester of pregnancy and during 24-28 weeks' gestation on the same day of blood glucose testing. Fasting plasma glucose was measured during the first trimester of pregnancy, and a 75-g 2-hour glucose tolerance test (75-g OGTT) was performed during 24-28 weeks' gestation. Women with FPG $\geq$ 126 mg/dl were diagnosed with overt diabetes and were excluded from the study. The diagnosis of GDM was based on the International Association of Diabetes and Pregnancy Study Group (IADPSG) criteria: either the first trimester FPG between 92-125 mg/dl or at least 1 abnormal 75-g OGTT glucose concentrations (FPG $\geq$ 92 mg/dl, 1-hour plasma glucose $\geq$ 180 mg/dl, or 2-hour plasma glucose $\geq$ 153 mg/dl). From an overall cohort of 355 women, 80 cases of GDM were matched by age range (<25 years or $\geq$ 25 years), and pre-pregnancy BMI range (<25 kg/m^2 or $\geq$25 kg/m^2) with 80 pregnant women with normal glucose levels. Assuming a 15% differences in the 25OHD levels between women with GDM and controls, a standard deviation of 10, and α of 0.05, we needed a sample size of 80 women per group (80 cases and 80 controls) to achieve 80% power. All blood samples were fractionated by using standard procedure and stored at -80^{o}c until analysis. All participants were provided the same standard antenatal care as other women attending the antenatal clinic at Thammasat University Hospital. The research protocol was approved by the Ethics Committee of the Faculty of Medicine, Thammasat University. All participants provided written informed consent

Laboratory Methods

Serum 25OHD2 and 25OHD3 concentrations were measured by liquid chromatography coupled with mass spectrometry (LC-MS/MS) using MassChrom® 25-OH-Vitamin D3/D2 in serum/plasma reagent kit (Chromsystems Instrument & Chemicals GmbH, Munich, Germany) with intra- and inter-assay precision of 5.0 % and 6.3 %, respectively. The total 25OHD concentrations were summations of serum 25OHD2 and 25OHD3 concentrations. Vitamin D status was defined based on the Endocrine Society recommendations: 25OHD < 20 ng/ml as vitamin D deficiency, 25OHD 20-29.9 ng/ml as vitamin D insufficiency, and $\geq$ 30 ng/ml as vitamin D sufficiency. The levels of 25OHD < 30 ng/ml was defined as vitamin D inadequacy. Serum insulin and plasma intact parathyroid hormone (PTH) were determined by electrochemiluminescence immunoassay on a Cobas e411 (Roche Diagnostic GmbH, Mannheim, Germany). The assays have an intra-assay precision of 1.9% and 2.7%, respectively. Plasma glucose concentrations were measured using Glucose Flex® reagent cartridge, Dade Behring Inc., DE, USA.

Insulin resistance was estimated using the homeostasis model assessment of insulin resistance (HOMA-IR) equation: HOMA-IR = [FPG (mg/dl) x fasting serum insulin (μIU/ml)] / 405. Pancreatic beta-cell function was estimated using the homeostasis model assessment of beta-cell function (HOMA-B) equation: HOMA-B = [360 X fasting serum insulin (μIU/ml)] / [FPG (mg/dl) - 63].

SNP Genotyping

Genotyping was based on SNP rs2282679 in the GC gene. DNA was extracted from a 200 μl serum sample using a QIAamp® DNA Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Individual genotyping of rs2282679 in the GC gene was performed using real-time PCR (TaqMan® MGB probes): 20 ng of DNA was added into the PCR reaction, consisting of TaqMan® Universal Master Mix (1x), and TaqMan® MGB probes for intronic G/T SNP rs2282679 (1x) in a total volume of 20 μl. The real-time PCR reaction protocol was 10 minutes at 95° C, 40 cycles of 15 seconds at 92° C, and 1 minute at 60° C using a 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA).

Statistical Analyses

Statistical analyses were performed using JMP 8.0 (SAS Institute, NC, USA) and MedCalc 13.0 (MedCalc software bvba, Belgium). Data were presented as mean ± standard deviation (SD) for normally distributed variables, median (interquartile range) for non-normally distributed variables, and absolute numbers (proportions) for categorical variables. The differences in variables between cases and controls were examined using Student's t tests and analysis of variance (ANOVA) for normally distributed variables; Wilcoxon rank sum test and Kruskal-Wallis test for non-normally distributed variables; and Chi-squared test for categorical variables. The independent predictors of GDM were examined using multiple logistic regressions. Covariates included in the model 1 for examining the association between first trimester 25OHD concentrations and GDM were age, pre-pregnancy BMI, a family history of diabetes in first degree relatives, and first trimester 25OHD concentrations. Covariates included in the model 2 for examining the associations between 25OHD concentrations during 24-28 weeks' gestation and GDM were age, BMI at the time of 75-g OGTT, a family history of diabetes in first degree relatives, and 25OHD concentrations at the time of 75-g OGTT. Non-normal data including pre-pregnancy BMI, and BMI at the time of 75-g OGTT were log-transformed to make these data adequately follow a Gaussian distribution. Spearman's rank correlation analyses were used to examine the correlations between 25OHD concentrations and parameters of glucose metabolism (glucose levels, insulin levels, HOMA-IR, and HOMA-B); PTH

concentrations and 25OHD concentrations; and PTH concentrations and glucose parameters. The independent predictors of glucose parameters were separately examined using 3 multiple linear regressions models. Covariates included in Model 1 for examining the independent predictors of the first trimester glucose parameters were age, log pre-pregnancy BMI, and first trimester 25OHD concentrations. Covariates included in Model 2 and Model 3 for examining the independent predictors of 24-28 weeks' glucose parameters were age, log BMI at 24-28 weeks' gestation, and first trimester 25OHD concentrations (for Model 2) or 24-28 weeks' 25OHD concentrations (for Model 3). The associations between the G allele and GDM were examined using multiple logistic regressions. Covariates included in the model were age, log pre-pregnancy BMI, a family history of diabetes, and G allele. All statistical tests were considered significant if the two-tailed p-value was < 0.05.

Results

Study Population

The clinical characteristics, biochemical parameters, and genotyping distribution at positions 2282679 in the GC gene of pregnant women according to GDM case-control status are presented in Table 1. Blood samples were collected at the median gestational age 12 weeks (9-14 weeks) for the first blood collection, and 26 weeks (25-28 weeks) for the second blood collection. There were no significant differences in age (p=0.66), pre-pregnancy BMI (p=0.09), pregnancy weight gain (p=0.93), and a family history of diabetes in first degree relatives (p=0.33) between women with GDM and healthy controls. Thirty women (37.5%) in each group had pre-pregnancy BMI $\geq$ 25 kg/m^2. Women with GDM had higher median BMI at the time of 75-g OGTT than controls (median BMI 27 kg/m^2 vs. 25.5 kg/m^2, p=0.04). One woman in the GDM group had a previous history of GDM. No one had a previous delivery of a baby weighted $\geq$ 4,000 grams.

The 25OHD concentrations and maternal vitamin D status

The mean 25OHD concentrations and maternal vitamin D status according to GDM case-control status are presented in Table 1. There were no significant differences in mean 25OHD levels between women with GDM and healthy controls both during the first trimester of pregnancy (mean 25OHD levels 27.2$\pm$6.9 ng/ml vs. 28.7$\pm$6.7 ng/ml, p=0.16), and during 24-28 weeks' gestation (mean 25OHD levels 35.3$\pm$9.3 ng/ml vs. 36.5$\pm$7.6 ng/ml, p=0.39). The prevalence of first-trimester vitamin D deficiency (25OHD $<$ 20 ng/ml), and the prevalence of first-trimester vitamin D inadequacy (25OHD $<$ 30 ng/ml) were not different between women with GDM and controls (prevalence 13.7% vs. 12.5%, p=0.81 for vitamin D deficiency; and prevalence 68.7% vs. 60%, p=0.25 for vitamin D inadequacy).

One hundred and forty five women (90.6%) were taking vitamin D supplementations through prenatal vitamins and/or vitamin fortified milk (73 women in the GDM group and 72 women in the control group, p=0.79). The median doses of vitamin D supplementations were 600 units/day (400-800 units/day) in both groups (p=0.59). Changes in mean 25OHD concentrations between 2 blood collections were not different between GDM women and controls (mean change 8.2±6.7 ng/ml vs. 7.8±7.0 ng/ml, p=0.72). Of 103 women with vitamin D inadequacy in the first trimester, 64 women (62.1%) had vitamin D adequacy (25OHD > 30 ng/ml) during 24-28 weeks' gestation. There were no differences in the proportion of women with changes in vitamin D status from vitamin D inadequacy in the first trimester to vitamin D sufficiency during 24-28 weeks' gestation between 2 groups (33 of 55 women (60%) in the GDM group vs. 31 of 48 women (64.6%) in the control group; p=0.51). There were no differences in the proportion of women with changes in vitamin D status from vitamin D deficiency in the first trimester to vitamin D sufficiency during 24-28 weeks' gestation between 2 groups (3 of 11 women (27.3%) in the GDM group, and 2 of 10 women (20%) in the control group; p=0.70). The prevalence of vitamin D inadequacy, and the prevalence of vitamin D deficiency during 24-28 weeks' gestation were not different between women with GDM and controls (prevalence 30% vs. 23.7%, p=0.37 for vitamin D inadequacy; and 6.2% vs. 1.2%, p=0.10 for vitamin D deficiency).

Multiple logistic regression models for examining the association between 25OHD concentrations and GDM are presented in Table 2. Neither the first trimester 25OHD concentrations nor 24-28 weeks' 25OHD concentrations were significant predictors of GDM in multivariate analyses.

Parameters of glucose metabolism

The parameters of glucose metabolism including plasma glucose levels, fasting insulin levels, HOMA-IR and HOMA-B according to GDM case-control status are presented in Table 1. Women with GDM had higher plasma glucose levels, higher fasting insulin levels and higher HOMA-IR than controls both during the first trimester of pregnancy, and during 24-28 weeks' gestation. HOMA-B was not different between cases and controls during the first trimester of pregnancy, but was lower in cases during 24-28 weeks' gestation.

The correlations between 25OHD concentrations and parameters of glucose metabolism are presented in Table 3. The first trimester 25OHD concentrations were not associated with neither the first trimester glucose parameters (including FPG, fasting insulin levels, HOMA-IR, and HOMA-B) nor the 24-28 weeks' glucose parameters (including 75-g OGTT glucose levels, fasting insulin levels, HOMA-IR and HOMA-B). The 24-28 weeks' 25OHD concentrations were not associated with any glucose parameters during 24-28 weeks' gestation.

The independent predictors of glucose parameters during the first trimester of pregnancy and at 24-28 weeks' gestation were separately examined using multiple linear regressions. Covariates included in the models for examining the independent predictors of the first trimester glucose parameters were age, log pre-pregnancy BMI, and first trimester 25OHD concentrations. Log FPG was positively associated with log pre-pregnancy BMI (p=0.006), and negatively associated with the first trimester 25OHD concentrations (p=0.03). Log first trimester fasting insulin levels was positively associated with age (p=0.02), and log pre-pregnancy BMI (p <0.0001), but was not associated with the first trimester 25OHD levels (p=0.28). Log first trimester HOMA-IR was positively associated with age (p=0.02), and log pre-pregnancy BMI (p <0.0001), but was not associated with first trimester 25OHD levels (p=0.16). Log first trimester HOMA-B was positively associated with log pre-pregnancy BMI (p <0.0001), but was not associated with either age (p=0.08) or first trimester 25OHD levels (p=0.58).

There were 2 models for examining the independent predictors of 24-28 weeks' glucose parameters. Covariates included in model 1 were age, log BMI at 24-28 weeks' gestation, and first trimester 25OHD levels. Covariates included in model 2 were age, log BMI at 24-28 weeks' gestation, and 25OHD levels at 24-28 weeks' gestation. For model 1, log BMI at 24-28 weeks' gestation was the only significant predictor of log glucose concentrations at 0 hr (p=0.007), log fasting insulin levels (p <0.0001), log HOMA-IR (p <0.0001), and log HOMA-B (p=0.0002). Age and log BMI at 24-28 weeks gestation were independent predictors of log glucose concentrations at 1 hr (p=0.03 for age, and p=0.003 of log BMI), and log glucose concentrations at 2 hr (p=0.02 for age, and p=0.004 for log BMI). For model 2, log BMI at 24-28 weeks' gestation was the only significant predictor of log glucose concentrations at 0 hr (p=0.005), log fasting insulin levels (p <0.0001), log HOMA-IR (p <0.0001), and log HOMA-B (p=0.0002). Age and log BMI at 24-28 weeks gestation were independent predictors of log glucose concentrations at 1 hr (p=0.02 for age, and p=0.004 of log BMI), and log glucose concentrations at 2 hr (p=0.02 for age, and p=0.005 for log BMI). Neither first trimester 25OHD concentrations nor 24-28 weeks' 25OHD concentrations were predictors of glucose parameters at 24-28 weeks' gestation. The adjusted P-values for 25OHD concentrations are presented in Table 3.

The median changes in glucose parameters between 2 blood collections according to GDM case-control status are presented in Table1. Women with GDM had lesser median change in HOMA-B than controls (median change 113% vs. 212%, p<0.0001). There were no differences in median changes in fasting insulin (p=0.51) and HOMA-IR (p=0.71) between cases and controls. The

correlations between changes in 25OHD concentrations and changes in glucose parameters (values during 24-28 weeks' gestation – values during the first trimester of pregnancy) are presented in Table 4. There were no associations between changes in 25OHD concentrations and changes in any parameters of glucose metabolism including fasting glucose levels, fasting insulin levels, HOMA-IR, and HOMA-B.

Parathyroid hormone

Parathyroid hormone (PTH) concentrations according to GDM case-control status are presented in Table 1. There were no significant differences in median PTH concentrations between women with GDM and controls both during the first trimester of pregnancy (median PTH concentrations 25.3 pg/ml vs. 22.2 pg/ml, p=0.10), and during 24-28 weeks' gestation (median PTH concentrations 26.5 pg/ml vs. 24.6 pg/ml, p=0.23). There were no significant correlations between 25OHD concentrations and PTH concentrations both during the first trimester of pregnancy (r -0.04, p=0.64); and during 24-28 weeks' gestation (r -0.15, p=0.06). Changes in PTH concentrations between the first trimester and 24-28 weeks' gestation was not correlated with changes in 25OHD concentrations between the first trimester and 24-28 weeks' gestation (r -0.03, p=0.70).

The relationships between PTH concentrations and parameters of glucose metabolism are presented in Table 5. There were significant correlations between first trimester PTH concentrations and first trimester fasting insulin concentrations (r 0.17, p=0.03); first trimester PTH concentrations and first trimester HOMA-IR (r 0.18, p=0.02); 24-28 weeks' PTH concentrations and 1-hour plasma glucose concentrations (r 0.17, p=0.03); and 24-28 weeks' PTH concentrations and 2-hour plasma glucose concentrations (r 0.16, p=0.04). In multivariate analyses adjusting for age and log pre-pregnancy BMI, log first trimester PTH levels was not a significant predictor of any first trimester glucose parameters including log FPG, log fasting insulin, log HOMA-IR, and log HOMA-B. In another models adjusting for age and log BMI at 24-28 weeks' gestation, log PTH levels at 24-28 weeks' gestation was a significant predictor of log glucose levels at 2 hr (p=0.04), but was not a significant predictor of any other 24-28 weeks' glucose parameters including log glucose levels at 0 hr, log glucose levels at 1 hr, log fasting insulin levels, log HOMA-IR, and log HOMA-B. The adjusted P-values for PTH concentrations are presented in Table 5.

Genotyping distributions of the GC rs2282679 SNP

The genotyping distributions of the GC rs2282679 SNP according to GDM case-control status are presented in Table 1. There were no significant differences in the genotyping distributions at positions 2282679 in the GC gene between women with GDM and controls (p=0.20). The 25OHD

concentrations, vitamin D status, and GDM according to GCrs2282679 genotypes are presented in Table 6. Women with the TG genotype had lower first trimester 25OHD concentrations than women with the TT genotype (mean 25OHD concentrations 26.3±6.5 ng/ml vs. 28.8±7.0 ng/ml, p=0.03). In multivariate analysis adjusting for log gestational age, log pre-pregnancy BMI, and log sunlight exposure time, the TG genotype was the independent predictor of first trimester 25OHD concentrations (p=0.01).

There was a trend that women carrying the G allele (TG and GG genotypes) had a higher risk of GDM than women without G allele (OR 1.8, 95%CI: 0.94-3.43, p=0.07). In multiple logistic regression model adjusting for age, log pre-pregnancy BMI, and a family history of diabetes, there was a trend that women carrying the G allele increased risk of GDM (OR 1.88, 95%CI: 0.97-3.66; p=0.06). The association between the G allele and GDM was stronger after excluding 6 women with vitamin D deficiency during 24-28 weeks' gestation (OR 2.14, 95%CI: 1.1-4.2; p=0.03).

Discussion

The main findings in the present study included maternal 25OHD concentrations either during the first trimester of pregnancy or during 24-28 weeks' gestation were not a significant predictor of GDM after adjusting for known risk factors of GDM including maternal age, pre-pregnancy BMI (for first trimester 25OHD concentrations) or BMI at the time of OGTT (for 24-28 weeks' 25OHD concentrations), and a family history of diabetes in first degree relative. There was a negative association between first trimester 25OHD concentrations and log first trimester FPG in multivariate analysis. SNP at positions 2282679 in the GC gene was associated with first trimester 25OHD concentrations. There was a trend that women carrying G allele (TG and GG genotypes) at positions 2282679 in the GC gene increased risk of GDM in comparison with the TT genotype. The GC polymorphism may have a role in GDM development.

Measurements of 25OHD concentrations and glucose parameters both during the first trimester of pregnancy and during 24-28 weeks' gestation provided information on prospective changes in vitamin D status and glucose metabolism during the course of pregnancy that gave us an opportunity to examine the association between first trimester 25OHD concentrations and first trimester glucose parameters; first trimester 25OHD concentrations and 24-28 weeks' glucose parameters; 24-28 weeks' 25OHD concentrations and 24-28 weeks' glucose parameters; and changes in 25OHD concentrations and glucose parameters between first trimester of pregnancy and 24-28 weeks' gestation.

We did not find evidence suggesting an independent association between 25OHD concentrations and GDM. The 25OHD concentrations, the prevalence of vitamin D inadequacy, and the prevalence of vitamin D deficiency were not significantly different between women with GDM and normal controls both during the first trimester of pregnancy and during 24-28 weeks' gestation. The finding that the proportions of women with changes in vitamin status from vitamin D inadequacy during the first trimester of pregnancy to vitamin D sufficiency during 24-28 weeks' gestation were not significantly different between cases and controls suggested that the improvement in vitamin D status during the course of pregnancy did not decrease the risk of developing GDM in pregnancies in this cohort. This finding was consistent with the result from a combined analysis of 2 randomized vitamin D supplementation trials during pregnancy that demonstrated no association between GDM and final 25OHD levels of < 32 ng/ml vs. ≥32 ng/ml (OR 1.04 per 10 ng/ml increase in 25OHD, 95%CI 0.82-1.33, p=0.75).[20] It is unclear why first trimester 25OHD concentrations was negatively associated with first trimester FPG, while there were no associations between first trimester 25OHD concentrations and the remaining first trimester glucose parameters including fasting insulin, HOMA-IR, and HOMA-B. Currently, there is no published study examining the association between first trimester 25OHD levels and first trimester parameters of glucose metabolism. The findings that first trimester 25OHD levels were not associated with any 24-28 weeks' glucose parameters as well suggested that first trimester 25OHD levels alone might not have any effects on glucose metabolism during pregnancy and also the risk of developing GDM. Our findings were consistent with previous studies that did not find an association between early-pregnancy vitamin D status and the development of GDM.[15,21,22] Due to 90% of women in this study were taking vitamin D supplementation, vitamin D deficiency at the time of OGTT was found in only 6 women who did not take vitamin D supplementation (5 GDM women and 1 control). Therefore, we could not confidently draw a conclusion about the effects of vitamin D deficiency at the time of OGTT on glucose homeostasis and risk of GDM.

We found the evidence suggesting that the GC polymorphism at positions 2282679 may have a role in GDM development. There was a trend that women carrying the G allele (TG and GG genotypes) increased the risk of GDM in comparison with the TT genotype after adjusting for known risk factors of GDM. And the association was stronger after excluding women with vitamin D deficiency during 24-28 weeks' gestation. These findings suggested that carrying the G allele at positions 2282679 in the GC gene may be associated with the increased risk of GDM even had adequate vitamin D levels during 24-28 weeks' gestation. Genotypic variations in the GC gene may

be associated with not only the concentrations of DBP, but also its binding affinity.[17] The differences in the binding affinity may be associated with the differences in the levels of free 25OHD and/or bioavailable 25OHD even had the same total 25OHD levels.[23] The free or bioavailable 25OHD levels may be better correlate with glucose parameters and the risk of developing GDM than the total 25OHD levels. A recent study suggested that DBP concentrations were regulated by total 25OHD levels to maintain adequate concentrations of bioavailable 25OHD.[24] It is possible that the contradictory findings observed in previous studies may be, in some part, associated with the concentrations of free or bioavailable 25OHD and the genotypic variations in the GC gene. Further studies examining the effects of the GC polymorphism on free 25OHD and/or bioavailable 25OHD levels, and the risk of GDM are required.

The relationships between PTH levels and 25OHD levels in pregnant women were inconsistent. In this study, we did not find any relationships between PTH levels and 25OHD levels both during the first trimester of pregnancy and during 24-28 weeks' gestation. Previous studies found both no relationships[11,13] and a negative correlation between PTH levels and 25OHD levels.[7,25,26] The other calcium-regulating hormone, PTH-related peptide (PTHrP), may be more correlate with 25OHD levels than PTH during pregnancy.[28] The genotypic variations in the GC gene may be associated with these inconsistent findings observed in previous studies.

The parameters of glucose metabolism between 2 blood collections suggested that women with GDM had significantly higher insulin resistance (suggesting by higher HOMA-IR, higher fasting insulin levels and higher FPG) than normal pregnancies since the first trimester of pregnancy. The magnitudes of increase in HOMA-IR were comparable between 2 groups. The higher HOMA-IR during 24-28 weeks' gestation in women with GDM was likely due to higher baseline HOMA-IR. Pancreatic beta cell function as estimated by HOMA-B was not sensitive enough to detect the differences in beta-cell function between women who subsequently developed GDM and normal women during the first trimester of pregnancy. The defect in beta cell function was clearly demonstrated by the difference in 24-28 weeks' HOMA-B, and the difference in magnitude of change in HOMA-B between cases and controls. These findings were consistent with previous results from hyperinsulinemic-euglycemic clamp.[27] It is likely that women with GDM have both insulin resistance and a beta-cell defect that start early since the first trimester of pregnancy, or may present before pregnancy. Since the known risk factors of GDM were comparable between cases and controls in this study, it is likely that there were some unknown confounding factors that may be associated with the increased risk of GDM.

The present study was conducted in Pathum Thani province, which is located in central Thailand at a latitude of 14° 01' N. Based on the Endocrine Society recommendations, 65% of women in this cohort had inadequate vitamin D levels during the first trimester of pregnancy. Vitamin D supplementation at the median dose of 600 units/day could effectively prevent vitamin D deficiency and raise the 25OHD to sufficient levels in 73% of pregnancies in this cohort. These findings were consistent with our previous study that found that for areas with abundant sun exposure like Thailand, a dose of vitamin D of 400 IU/day is high enough to prevent vitamin D deficiency in pregnant women.[29] Although the result of a combined analysis of 2 randomized vitamin D supplementation trials during pregnancy did not find that the final 25OHD levels $\geq$ 32 ng/ml could decrease risk of developing GDM, this final levels could decrease the rates of infection, hypertensive disorders of pregnancy, preterm birth without preeclampsia, and combined comorbidities.[20] Further studies are needed to confirm the benefits of vitamin D supplementation in pregnancies and the optimal daily doses required to achieve adequate vitamin D levels for different regions.

The strengths of the present study included a prospective, nested case-control study design with 2 times measurements of 25OHD levels and glucose parameters, which allowed us to better assess the relationship between vitamin D status and glucose metabolism during the course of pregnancy, and was able to control for known risk factors of GDM including maternal age and pre-pregnancy BMI. FPG was measured in all women during the first trimester of pregnancy, so we could identify and exclude women with undiagnosed pre-gestational diabetes from the study. The 25OHD concentrations were measured using a gold-standard method. The limitations included a small sample size and low prevalence of vitamin D deficiency at the time of OGTT. The possibility of a type II error cannot entirely exclude.

Conclusions

There is no strong evidence suggesting an independent association between GDM and 25OHD concentrations both during the first trimester of pregnancy and during 24-28 weeks' gestation. Carrying the G allele (TG and GG genotypes) at positions 2282679 in the GC gene may be associated with the increased risk of GDM in comparison with the TT genotypes even had adequate vitamin D levels during 24-28 weeks' gestation. The GC polymorphism may have a role in GDM development.

Table1 Clinical characteristics, biochemical parameters, and genotyping distributions of the GC rs 2282679 SNP of pregnant women according to GDM case-control status

Characteristics	GDM Cases (n=80)	Controls (n=80)	P-value
Age			
- Mean±SD (years)	33.0±4.6	32.7±4.3	0.66
- Age ≥ 25 years (number, %)	78 (97.5%)	78 (97.5%)	1.0
Multiparous (number, %)	54 (67.5%)	54 (67.5%)	1.0
Pre-pregnancy BMI (kg/m^2)			
- Median (interquartile range)	24.0 (21.1-28.1)	22.4 (20.1-25.8)	0.09
- BMI ≥ 25 kg/m^2 (number, %)	30 (37.5%)	30 (37.5%)	1.0
BMI at the time of 75-g OGTT (kg/m^2)	27 (24.7-30.8)	25.5 (23.1-28.6)	0.04
Weight gain between 2 blood collections (kg)	7.0 (5.6-9.0)	7.0 (5.0-10.0)	0.93
Previous history of GDM (number, %)	1 (1.3%)	0 (0%)	-
Diabetes in first degree relative (number, %)	14 (17.5%)	19 (23.8%)	0.33
GC rs2282679 polymorphism			
- TT (number, %)	44 (55%)	55 (68.8%)	0.20
- TG (number, %)	33 (41.3%)	23 (28.7%)	
- GG (number, %)	3 (3.7%)	2 (2.5%)	
25OHD concentration (ng/ml)			
- First trimester	27.2±6.9	28.7±6.7	0.16
- 24-28 weeks' gestation	35.3±9.3	36.5±7.6	0.39
- Change from first trimester	8.2±6.7	7.8±7.0	0.72
First trimester vitamin D status			
- 25OHD < 20 ng/ml (number, %)	11 (13.7%)	10 (12.5%)	0.81 OR^a 1.11 (0.44-2.80) OR^b 1.13 (0.44-2.87)
- 25OHD < 30 ng/ml (number, %)	55 (68.7%)	48 (60%)	0.25 OR^a 1.47 (0.76-2.81) OR^b 1.52 (0.78-2.95)
Vitamin D status during 24-28 weeks' gestation			
- 25OHD < 20 ng/ml (number, %)	5 (6.2%)	1 (1.2%)	0.10

- 25OHD < 30 ng/ml (number, %)	24 (30%)	19 (23.7%)	OR[a] 5.27 (0.60-46.1) OR[c] 4.81 (0.53-43.29) 0.37 OR[a] 1.38 (0.68-2.78) OR[c] 1.31 (0.63-2.69)
Glucose concentration (mg/dl)			
- First trimester FPG	94 (88-98)	85 (81-87)	<0.0001
- 75-g OGTT			
0-h plasma glucose (mg/dl)	80 (75-88)	73 (69-76)	<0.0001
1-h plasma glucose (mg/dl)	163 (136-183)	132 (114-144)	<0.0001
2-h plasma glucose (mg/dl)	144 (124-159)	114 (104-126)	<0.0001
Fasting insulin concentration (μIU/ml)			
- First trimester	8.6 (5.4-12.5)	6.2 (4.3-9.9)	0.004
- 24-28 weeks' gestation	11.3 (7.7-16.1)	8.7 (6.6-13.2)	0.02
- Change from first trimester	2.0 (0.2-4.5)	2.8 (0.3-4.5)	0.51
HOMA-IR			
- First trimester	2.0 (1.2-2.8)	1.3 (0.9-2.0)	0.0002
- 24-28 weeks' gestation	2.2 (1.5-3.5)	1.6 (1.1-2.3)	0.0009
- Change from first trimester	0.2 (-0.4-0.8)	0.3 (-0.2-0.7)	0.71
HOMA-B (%)			
- First trimester	112.2 (62.8-155.4)	101.2 (76.8-194)	0.50
- 24-28 weeks' gestation	213.5 (163.7-317.2)	331.1 (217.9-593.1)	0.0001
- Change from first trimester	113 (43-190)	212 (133-427)	<0.0001
PTH (pg/ml)			
- First trimester	25.3 (19.2-30.6)	22.2 (18.0-27.3)	0.10
- 24-28 weeks' gestation	26.5 (19.6-34.1)	24.6 (18.5-31.4)	0.23

SNP, single nucleotide polymorphism; BMI, body mass index; OGTT, oral glucose tolerance test; 25OHD, 25-hydroxyvitamin D; FPG, fasting plasma glucose; HOMA-IR, homeostasis model assessment of insulin resistance; HOMA-B, homeostasis model assessment of beta-cell function; PTH, parathyroid hormone; OR, odds ratio.

Data are presented as means±SD, median (interquartile range), or number (%).

Calculated using Student's T-test for continuous variables normally distributed, Wilcoxon rank sum test for continuous variables not normally distributed, or Chi-square for categorical variables.

[a] Unadjusted odds ratio, [b] Adjusted odds ratio, adjusted for age, log pre-pregnancy BMI, and a family history of diabetes, [c] Adjusted odd ratios, adjusted for age, log BMI at the time of OGTT, and a family history of diabetes.

Table 2 Multiple logistic regression models

Independent variable	Coefficient	Standard error	P-value
Model 1: Examining the association between first trimester 25OHD levels and GDM (p=0.12)			
Intercept	-5.11	3.10	0.10
Age	0.03	0.04	0.47
Log pre-pregnancy BMI (kg/m^2)	1.62	0.82	0.049
Family history of DM in first degree relative (yes)	-0.18	0.20	0.38
First trimester 25OHD levels (ng/ml)	-0.04	0.02	0.13
Model 2: Examining the association between 24-28 weeks' 25OHD levels and GDM (p=0.20)			
Intercept	-6.7	3.54	0.06
Age	0.03	0.04	0.42
Log BMI at 24-28 weeks' gestation (kg/m^2)	1.85	0.92	0.04
Family history of DM in first degree relative (yes)	-0.15	0.20	0.46
24-28 weeks' 25OHD levels (ng/ml)	-0.01	0.02	0.51

BMI, body mass index; 25OHD, 25-hydroxyvitamin D.

Calculated using spearman's rank correlation analyses.

Table 3 Correlations between 25OHD concentrations and parameters of glucose metabolism

Parameters of glucose metabolism	25OHD concentration (ng/ml)					
	First trimester			24-28 weeks' gestation		
	r	P-value	Adjusted P-value*	r	P-value	Adjusted P-value*
First trimester FPG (mg/dl)	-0.14	0.09	0.03	NA	NA	NA
75-g Oral glucose tolerance test (mg/dl)						
- Glucose at 0 hr	-0.07	0.39	0.76	0.03	0.70	0.15
- Glucose at 1 hr	-0.13	0.10	0.16	-0.12	0.12	0.23
- Glucose at 2 hr	-0.14	0.08	0.38	-0.08	0.30	0.70
Fasting insulin concentration (μIU/ml)						
- First trimester	-0.03	0.73	0.28	NA	NA	NA
- 24-28 weeks' gestation	-0.06	0.46	0.08	-0.05	0.57	0.66
HOMA-IR						
- First trimester	-0.05	0.54	0.16	NA	NA	NA
- 24-28 weeks' gestation	-0.06	0.48	0.10	-0.03	0.74	0.99
HOMA-B (%)						
- First trimester	0.06	0.44	0.58	NA	NA	NA
- At 24-28 weeks' gestation	-0.01	0.90	0.72	-0.05	0.50	0.13

Calculated using Spearman's rank correlation analyses.

*Adjusted for age, and pre-pregnancy BMI (for first trimester glucose parameters) or BMI at the time of OGTT (for 24-28 weeks' glucose parameters).

Table 4 Correlations between changes in 25OHD concentrations and changes in glucose parameters

Changes in glucose parameters (values during 24-28 weeks' gestation – values during the first trimester)	Changes in 25OHD concentrations (ng/ml) (values during 24-28 weeks' gestation – values during the first trimester)	
	r	P-values
Fasting plasma glucose (mg/dl)	0.07	0.36
Fasting insulin concentration (μIU/ml)	0.10	0.20
HOMA-IR	0.12	0.13
HOMA-B (%)	-0.06	0.41

HOMA-IR, homeostasis model assessment of insulin resistance; HOMA-B, homeostasis model assessment of beta-cell function

Calculated using spearman's rank correlation analyses.

Table 5 Correlations between PTH concentrations and parameters of glucose metabolism

First trimester				24-28 weeks' gestation			
Parameter of glucose metabolism	PTH			Parameter of glucose metabolism	PTH		
	r	P-value	Adjusted P-value*		r	P-value	Adjusted P-value*
Fasting plasma glucose (mg/dl)	0.12	0.13	0.21	75-g OGTT			
				Glucose at 0 hr (mg/dl)	0.10	0.21	0.21
				Glucose at 1 hr (mg/dl)	0.17	0.03	0.11
				Glucose at 2 hr (mg/dl)	0.16	0.04	0.04
Fasting insulin concentration (μIU/ml)	0.17	0.03	0.23	Fasting insulin concentration (μIU/ml)	0.01	0.86	0.18
HOMA-IR	0.18	0.02	0.17	HOMA-IR	0.03	0.69	0.35
HOMA-B (%)	0.08	0.30	0.99	HOMA-B (%)	-0.08	0.31	0.40

Calculated using Spearman's rank correlation analyses.

*Adjusted for age, and pre-pregnancy BMI (for first trimester values) or BMI at the time of OGTT (for 24-28 weeks' values).

Table 6 The 25OHD concentrations, vitamin D status, and GDM according to GCrs2282679 genotypes

	Genotypes			P-value	
	TT (n=99)	TG (n=56)	GG (n=5)	All	TT vs. TG
25OHD concentrations (ng/ml)					
- First trimester	28.8±7.0	26.3±6.5	29.3±4.4	0.09	0.03
- 24-28 weeks' gestation	36.8+8.8	34.4+7.8	36.0+9.7	0.26	0.10
Vitamin D status in first trimester (number, %)					
- 25OHD < 20 ng/ml	11 (11.1%)	10 (17.9%)	0 (0%)	0.33	0.24
- 25OHD < 30 ng/ml	60 (60.6%)	40 (71.4%)	3 (60%)	0.39	0.18
Vitamin D status during 24-28 weeks' gestation (number, %)					
- 25OHD < 20 ng/ml	4 (4%)	2 (3.6%)	0 (0%)	0.89	0.88
- 25OHD < 30 ng/ml	26 (26.3%)	16 (28.6%)	1 (20%)	0.89	0.76
Gestational diabetes (number, %)	44 (44.4%)	33 (58.9%)	3 (60%)	0.20	0.08

References

1. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care 2012; 35(suppl 1): S64-71.
2. Buchanan TA, Xiang AH, Page KA. Gestational diabetes mellitus: risks and management during and after pregnancy. Nat Rev Endocrinol 2012;8(11):639-49.
3. Donovan L, Hartling L, Muise M, Guthrie A, Vandermeer B, Dryden DM. Screening tests for gestational diabetes: a systematic review for the u.s. Preventive services task force. Ann Intern Med 2013;159(2):115-22.
4. Damm P. Future risk of diabetes in mother and child after gestational diabetes mellitus. Int J Gynaecol Obstet 2009;104 (Suppl 1):S25-6.
5. van Schoor, N.M., Lips, P. (2011) Worldwide vitamin D status. Best Pract Res Clin Endocrinol Metab, 25, 671-680.
6. Alzaim M, Wood RJ. Vitamin D and gestational diabetes mellitus. Nutr Rev. 2013;71(3):158-67.
7. Maghbooli Z, Hossein-Nezhad A, Karimi F, Shafaei AR, Larijani B. Correlation between vitamin D3 deficiency and insulin resistance in pregnancy. Diabetes Metab Res Rev. 2008 Jan-Feb;24(1):27-32.
8. Zhang C, Qiu C, Hu FB, David RM, van Dam RM, Bralley A, Williams MA. Maternal plasma 25-hydroxyvitamin D concentrations and the risk for gestational diabetes mellitus. PLoS One. 2008;3(11):e3753.
9. Soheilykhah S, Mojibian M, Rashidi M, Rahimi-Saghand S, Jafari F. Maternal vitamin D status in gestational diabetes mellitus. Nutr Clin Pract. 2010 Oct;25(5):524-7.
10. Parlea L, Bromberg IL, Feig DS, Vieth R, Merman E, Lipscombe LL. Association between serum 25-hydroxyvitamin D in early pregnancy and risk of gestational diabetes mellitus. Diabet Med. 2012 Jul;29(7):e25-32.
11. Wang O, Nie M, Hu YY, Zhang K, Li W, Ping F, Liu JT, Chen LM, Xing XP. Association between vitamin D insufficiency and the risk for gestational diabetes mellitus in pregnant Chinese women. Biomed Environ Sci. 2012 Aug;25(4):399-406.
12. Burris HH, Rifas-Shiman SL, Kleinman K, Litonjua AA, Huh SY, Rich-Edwards JW, Camargo CA Jr, Gillman MW. Vitamin D deficiency in pregnancy and gestational diabetes mellitus. Am J Obstet Gynecol. 2012 Sep;207(3):182.e1-8.
13. Lacroix M, Battista MC, Doyon M, Houde G, Ménard J, Ardilouze JL, Hivert MF, Perron P. Lower vitamin D levels at first trimester are associated with higher risk of developing gestational diabetes mellitus. Acta Diabetol. 2014 Feb 14. [Epub ahead of print] PubMed PMID: 24526261.

14. Whitelaw DC, Scally AJ, Tuffnell DJ, Davies TJ, Fraser WD, Bhopal RS, Wright J, Lawlor DA. Associations of circulating calcium and 25-hydroxyvitamin D with glucose metabolism in pregnancy: a cross-sectional study in European and South Asian women. J Clin Endocrinol Metab. 2014 Jan 1:jc20132896. [Epub ahead of print] PubMed PMID: 24423329.
15. Makgoba M, Nelson SM, Savvidou M, Messow CM, Nicolaides K, Sattar N. First-trimester circulating 25-hydroxyvitamin D levels and development of gestational diabetes mellitus. Diabetes Care. 2011 May;34(5):1091-3.
16. Zuhur SS, Erol RS, Kuzu I, Altuntas Y. The relationship between low maternal serum 25-hydroxyvitamin D levels and gestational diabetes mellitus according to the severity of 25-hydroxyvitamin D deficiency. Clinics (Sao Paulo). 2013 May;68(5):658-64.
17. Speeckaert M, Huang G, Delanghe JR, Taes YE. Biological and clinical aspects of the vitamin D binding protein (Gc-globulin) and its polymorphism. Clin Chim Acta. 2006 Oct;372(1-2):33-42.
18. Wang TJ, Zhang F, Richards JB, Kestenbaum B, van Meurs JB, Berry D, Kiel DP, Streeten EA, Ohlsson C, Koller DL, Peltonen L, Cooper JD, O'Reilly PF, Houston DK, Glazer NL, Vandenput L, Peacock M, Shi J, Rivadeneira F, McCarthy MI, Anneli P, de Boer IH, Mangino M, Kato B, Smyth DJ, Booth SL, Jacques PF, Burke GL, Goodarzi M, Cheung CL, Wolf M, Rice K, Goltzman D, Hidiroglou N, Ladouceur M, Wareham NJ, Hocking LJ, Hart D, Arden NK, Cooper C, Malik S, Fraser WD, Hartikainen AL, Zhai G, Macdonald HM, Forouhi NG, Loos RJ, Reid DM, Hakim A, Dennison E, Liu Y, Power C, Stevens HE, Jaana L, Vasan RS, Soranzo N, Bojunga J, Psaty BM, Lorentzon M, Foroud T, Harris TB, Hofman A, Jansson JO, Cauley JA, Uitterlinden AG, Gibson Q, Järvelin MR, Karasik D, Siscovick DS, Econs MJ, Kritchevsky SB, Florez JC, Todd JA, Dupuis J, Hyppönen E, Spector TD. Common genetic determinants of vitamin D insufficiency: a genome-wide association study. Lancet. 2010 Jul 17;376(9736):180-8.
19. Foucan L, Vélayoudom-Céphise FL, Larifla L, Armand C, Deloumeaux J, Fagour C, Plumasseau J, Portlis ML, Liu L, Bonnet F, Ducros J. Polymorphisms in GC and NADSYN1 Genes are associated with vitamin D status and metabolic profile in Non-diabetic adults. BMC Endocr Disord. 2013 Sep 29;13(1):36.
20. Wagner CL, McNeil RB, Johnson DD, Hulsey TC, Ebeling M, Robinson C, Hamilton SA, Hollis BW. Health characteristics and outcomes of two randomized vitamin D supplementation trials during pregnancy: a combined analysis. J Steroid Biochem Mol Biol. 2013 Jul;136:313-20.

21. Savvidou MD, Akolekar R, Samaha RB, Masconi AP, Nicolaides KH. Maternal serum 25-hydroxyvitamin D levels at 11(+0) -13(+6) weeks in pregnant women with diabetes mellitus and in those with macrosomic neonates. BJOG. 2011 Jul;118(8):951-5.
22. Baker AM, Haeri S, Camargo CA Jr, Stuebe AM, Boggess KA. First-trimester maternal vitamin D status and risk for gestational diabetes (GDM) a nested case-control study. Diabetes Metab Res Rev. 2012 Feb;28(2):164-8.
23. Chun RF, Peercy BE, Orwoll ES, Nielson CM, Adams JS, Hewison M. Vitamin D and DBP: The free hormone hypothesis revisited. J Steroid Biochem Mol Biol. 2013 Oct 4. pii: S0960-0760(13)00186-6. doi: 10.1016/j.jsbmb.2013.09.012. [Epub ahead of print] PubMed PMID: 24095930.
24. Ashraf AP, Huisingh C, Alvarez JA, Wang X, Gower BA. Insulin resistance indices are inversely associated with vitamin D binding protein concentrations. J Clin Endocrinol Metab. 2014 Jan;99(1):178-83.
25. Clifton-Bligh RJ, McElduff P, McElduff A. Maternal vitamin D deficiency, ethnicity and gestational diabetes. Diabet Med. 2008 Jun;25(6):678-84.
26. Parildar H, Dogruk Unal A, Aksan Desteli G, Cigerli O, Guvener Demirag N. Frequency of Vitamin D deficiency in pregnant diabetics at Baskent University Hospital, Istanbul. Pak J Med Sci. 2013 Jan;29(1):15-20.
27. 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 Oct;137(4):402-9.
28. Catalano PM, Huston L, Amini SB, Kalhan SC. Longitudinal changes in glucose metabolism during pregnancy in obese women with normal glucose tolerance and gestational diabetes mellitus. Am J Obstet Gynecol. 1999 Apr;180(4):903-16.
29. Charatcharoenwitthaya N, Nanthakomon T, Somprasit C, Chanthasenanont A, Chailurkit LO, Pattaraarchachai J, Ongphiphadhanakul B. Maternal vitamin D status, its associated factors and the course of pregnancy in Thai women. Clin Endocrinol (Oxf). 2013 Jan;78(1):126-33.

ภาคผนวก

1. อยู่ในระหว่างแก้ไข Manuscript สำหรับส่งตีพิมพ์ในวารสารวิชาการระดับนานาชาติ
2. ได้รับการคัดเลือกให้นำเสนอผลงานวิจัยแบบ oral presentation งานประชุมระดับนานาชาติ The joint meeting of the International Society of Endocrinology and The Endocrine Society: ICE/ENDO 2014 ที่เมืองชิคาโก มลรัฐอิลลินอยส์ ประเทศสหรัฐอเมริกา ระหว่างวันที่ 21-24 มิถุนายน 2557