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Original Research

The Relationship between 25-hydroxyvitamin D Levels, Insulin Sensitivity and Insulin Secretion in Women 3 Years after Delivery

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Key Messages

- There is a direct correlation between 25-hydroxyvitamin D (25[OH]D) levels and insulin sensitivity.
- Women with gestational diabetes mellitus (GDM) may have lower levels of 25(OH)D compared to controls. We proved a positive association of vitamin D and insulin sensitivity in women 3 years after delivery; however, prior GDM status was not associated with vitamin D levels.
- Our findings suggest that vitamin D supplementation may be useful for prior GDM women; nevertheless, intervention studies are required to elucidate this statement.

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ABSTRACT

Objectives: There is a direct correlation between 25-hydroxyvitamin D (25[OH]D) levels and insulin sensitivity. Furthermore, women with gestational diabetes (GDM) may have lower levels of 25(OH)D compared to controls. The present study intended to investigate 25(OH)D levels and their association with insulin sensitivity and insulin secretion in women with prior GDM and in controls 3.2 years after delivery.
Methods: A total of 87 patients with prior GDM and 45 randomly selected controls (age range, 22 to 44 years) with normal glucose tolerance during pregnancy nested within a cohort of all deliveries at Saint Margit Hospital, Budapest, between January 1 2005, and December 31 2006, were examined. Their 25(OH) D levels were measured by radioimmunoassay. Insulin sensitivity and fasting insulin secretion were estimated using the homeostasis model asssessment (HOMA) calculator and early insulin secretion by the insulinogenic index based on a 75 g oral glucose tolerance test.
Results: There was no significant difference in 25(OH)D levels between cases and controls (27.2±13.1 [±SD] vs. 26.9±9.8 ng/L). There was a positive association between HOMA insulin sensitivity and 25(OH)D levels (beta = 0.017; 95% CI 0.001 to 0.034/1 ng/mL) that was robust to adjustment for age and body mass index. There was a nonsignificant association between HOMA insulin secretion and 25(OH)D (p=0.099), while no association was found with the insulinogenic index.
Conclusions: Prior GDM status was not associated with 25(OH)D levels; however, 25(OH) D levels were associated with HOMA insulin sensitivity. It is hypothesized that the association between HOMA insulin secretion and 25(OH)D levels is related to the autoregulation of fasting glucose levels because no association between 25(OH)D and insulinogenic index was found.

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RÉSUMÉ

Objectifs : Une corrélation directe a été établie entre les concentrations de 25-hydroxyvitamine D (25[OH]D) et la sensibilité à l'insuline. De plus, les femmes atteintes de diabète gestationnel peuvent présenter des concentrations inférieures de 25(OH)D comparativement à celles du groupe témoin. La présente étude visait à mesurer les concentrations de 25(OH)D et leur lien avec la sensibilité à l'insuline et la sécrétion d'insuline chez des femmes ayant des antécédents de diabète gestationnel et des femmes d'un groupe témoin 3,2 années après l'accouchement.
Méthodologie : L'examen a porté sur 87 patientes ayant des antécédents de diabète gestationnel et 45 femmes qui ont présenté une tolérance normale au glucose durant leur grossesse, choisies au hasard pour constituer le groupe témoin parmi une cohorte de toutes les femmes ayant accouché à l'hôpital Saint Margit de Budapest au cours de la période allant du 1er janvier 2005 au 12 décembre 2006. L'âge de la population à l'étude variait de 22 à 44 ans. Les concentrations de 25(OH) D ont été mesurées par radioimmunodosage. La sensibilité à l'insuline et la sécrétion d'insuline à jeun ont été estimées au moyen du modèle HOMA (évaluation du modèle d'homéostasie) et la sécrétion précoce d'insuline, par l'indice insulinogénique établi par l'épreuve de tolérance orale à 75 mg de glucose.
Résultats : On n'a observé aucune différence significative entre les concentrations de 25(OH)D des femmes ayant des antécédents de diabète gestationnel et celles du groupe témoin (27,2±13,1 [±É.-T.] comparativement à 26,9±9,8 ng/L). Une association positive a été établie entre la sensibilité à l'insuline selon l'indice HOMA et les concentrations de 25(OH)D (bêta=0,017; IC à 95 %, de 0,001 à 0,034/1 ng/mL) et s'est maintenue après correction en fonction de l'âge et de l'indice de masse corporelle. On a constaté un lien non significatif entre la sécrétion d'insuline selon l'indice HOMA et les concentrations de 25(OH)D (p=0,099), mais aucune association avec l'indice insulinogénique.
Conclusions : On n'a établi aucune association entre les antécédents de diabète gestationnel et les concentrations de 25(OH)D; cependant, il existe un lien entre les concentrations de 25(OH)D et la sensibilité à l'insuline selon l'indice HOMA. On a émis l'hypothèse que l'association entre la sécrétion d'insuline selon l'indice HOMA et les concentrations de 25(OH)D s'explique par l'autorégulation de la glycémie à jeun en l'absence d'association entre les concentrations de 25(OH)D et l'indice insulinogénique.

Introduction

The role of 25-hydroxyvitamin D (25[OH]D) in calcium and bone metabolism has been known for decades. Recent evidence suggests more complex involvement of 25(OH)D in health and disease. Osteopathy and other chronic conditions, such as autoimmune diseases, diabetes, metabolic syndrome, multiple sclerosis and certain malignancies, are associated with low levels of 25(OH)D (1–5). 25(OH)D deficiency is rather common; recent studies have reported a prevalence of 77% in the United States, 37% in Canada and 50% to 70% in European populations.(6,7)

An association between 25(OH)D levels and insulin sensitivity, regardless of body mass index (BMI) and obesity, is suggested by several studies, mostly in populations with increased risks for diabetes (2,8–11). In a randomized, controlled trial in subjects with high risks for diabetes, oral supplementation of vitamin D prevented the deterioration of insulin sensitivity in the short term (12). Furthermore, it is suggested that levels and supplementation of 25(OH)D could have a bidirectional association with adiposity because supplementation of vitamin D as well as 25(OH)D levels were inversely associated with visceral adiposity and adipocyte size in women who underwent abdominal gynecologic surgery (13).

An association between vitamin D deficiency and type 2 diabetes is supported by cross-sectional studies (8,14,15). A similar longitudinal association was found between low serum 25(OH)D levels and risk for the incidence of type 2 diabetes (16). In addition, randomized trials have demonstrated a direct link between increased vitamin D intake and a reduced risk for type 2 diabetes (14,15,17).

Although the analogy between gestational diabetes (GDM) and type 2 diabetes is well accepted and is supported by a number of common risk factors, little is known about the association between serum 25(OH)D levels and the risk for GDM (18). Cross-sectionally, some studies have reported lower levels of 25(OH)D in women with GDM (19–21), while others have not (22–24). In a prospective study, lower first-trimester 25(OH)D levels were related to incident GDM (25). It is of note that equivocal evidence for an association between 25(OH)D levels and insulin sensitivity during pregnancy was found in both cross-sectional and prospective studies (22,23,25–27). Furthermore, 2 experiments (1 randomized trial) suggest that vitamin D supplementation (with rather high dosages) may improve insulin sensitivity in women with GDM while pregnant and shortly after delivery (28,29).

Limited information is available concerning 25(OH)D levels after delivery. Between 6 weeks and 12 months after delivery, 25(OH)D levels probably increase compared to midpregnancy levels, although it appears that they may remain lower in women with prior GDM compared to those who had non-GDM pregnancies (30). We hypothesized that 1) 25(OH)D levels would remain lower in women with prior GDM compared to controls, even years after delivery and that 2) 25(OH)D levels would be related to insulin sensitivity and, consequently, with fasting insulin secretion. Thus, in this study, we examined the levels of 25(OH)D in women with prior GDM compared to a group of control women, and we interrogated the association between insulin resistance, beta-cell function and 25(OH)D levels in study participants.

Methods

Setting

We report the results of a case-control study (performed between 2008 and 2010) nested within the cohort of all women delivered at Saint Margit Hospital, Budapest, between January 1, 2005, and December 31, 2006. Saint Margit Hospital serves a mostly urbanized population of 235,000 people.

All pregnant women took part in a 2-step GDM screening program:

- First, a 75 g 2-hour oral glucose tolerance test (OGTT) was performed at 16 to 18 weeks of gestation and was evaluated using

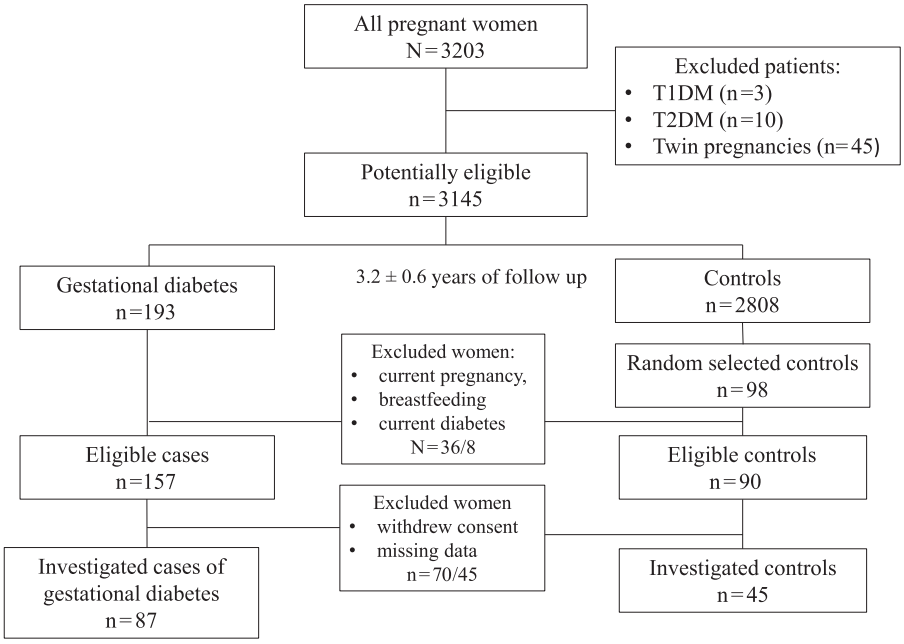

Figure 1. Study design and flow chart of participants. *T1DM,* type 1 diabetes; *T2DM,* type 2 diabetes.

the World Health Organization 1999 criteria (fasting glucose ≥7 mmol/L and/or after 75 g OGTT 120' min glucose level ≥7.8 mmol/L) (31).

- Participants with normal glucose tolerance at the first test subsequently had second OGTTs at 24 to 28 weeks of gestation, evaluated according to the same diagnostic criteria.

All women with GDM were referred to a dietitian and were given standardized dietary and lifestyle advice. If fasting and 1-hour postprandial glucose targets (<5.3 mmol/L and <7.0 mmol/L, respectively) were not achieved, insulin therapy was initiated according to the recommendations of the Hungarian Diabetes Association with cut-offs similar to those of the Canadian guidelines (32,33).

Informed consent was obtained from all patients, and the study was performed after obtaining approval of Semmelweis University Regional and Institutional Committee of Science and Research Ethics (#124/2007) in accordance with the Helsinki Declaration of the World Medical Association.

Participants

During the study period, 3203 deliveries were recorded in Saint Margit Hospital. Thirteen women were excluded because of known pregestational diabetes or overt diabetes (based on fasting glucose measurements at the first prenatal visit), and 45 women were excluded due to twin pregnancies. GDM was diagnosed in 193 cases (6.03%), a figure that is somewhat lower than the recently published GDM prevalence of 7.7% to 8.7% for the Hungarian population (34,35).

All women with GDM (n=193) and a randomly selected control group of women with normal glucose tolerance during pregnancy (n=98) were invited for follow-up investigations 3.2±0.6 years after delivery. All study participants were Caucasians. Of these potentially eligible women, 36 prior GDM, 8 control women were excluded due to current pregnancy, breastfeeding or known diabetes. Of 157 prior GDM, and 90 control eligible women, 87 (55.4%) with prior GDM and 45 (50.0%) controls participated in the follow-up investigation (Figure 1).

Study design

As the first step of the follow-up examination, questionnaires were sent to all potentially eligible women. Based on information collected via these questionnaires, women with current pregnancy or lactation or with known diabetes were excluded.

Eligible participants were invited to a detailed interview using a structured questionnaire concerning maternal sociodemographic characteristics, lifestyle habits (smoking, caffeine and alcohol consumption, physical activity, use of dietary supplements, nutrition) as well as medical and reproductive histories and family histories of diabetes. In addition, physical examinations, including anthropometrics and blood pressure, were performed. Study participants also underwent a 2-hour 75 g oral glucose tolerance test. Fasting blood samples were collected for other laboratory parameters.

Covariates and outcomes

Using questionnaire data, ages at follow up were determined. Smoking status was coded as never, ex-smoker or current smoker (≥5 cigarette/day). Physical activity was assessed by the answers to questions about the frequency and duration of participation in moderate or vigorous physical activity. Physical-activity levels were classified as active (≥2.5 hours/week of moderate or ≥1 hour/week of vigourous physical activity); inactive (≤1 hour/week of moderate and ≤1 hour/week of vigourous physical activity); or moderately active (if not active or inactive) (36). Positive family histories of diabetes were defined as having a first-degree relative with diabetes.

The use of vitamin D supplementation was defined as reports of regular intake of either vitamin D tablets or multivitamins, based on questionnaire data. Most multivitamin compounds in Hungary contain 400 to 600 IU of vitamin D.

25(OH)D levels show clear seasonal variations, so we analyzed the dates of blood sampling, divided into autumn/winter (October to March) and spring/summer (April to September), as previously described (37).

Regular sunbathing or sun-bed use was defined as once a week outdoors (≥30 minutes midsummer midday sun exposure) or 20 minutes or more of sun-bed use per week.

Body weights and heights were measured in light clothing, without shoes, on a calibrated digital scale. Weights were rounded to the nearest 0.1 kg, heights to the nearest centimetre. Waist circumferences were measured at the height of the navel. BMIs were calculated as weight (kg)/height^2 (m^2).

Blood pressures were measured 3 times using a calibrated digital blood-pressure meter (OMRON M4-I, Omron Electronics, Budapest, Hungary) on the upper arm, with adequately sized (to upper-arm circumference) cuffs, after 5 minutes of rest in the sitting position. The average of the second and third measurements was used in the analysis. Hypertension was defined as blood pressures of 140/90 mm Hg or higher or the regular use of antihypertensive medication.

All laboratory measurements were performed by the Central Laboratory of Semmelweis University following standardized protocols (38). OGTTs were performed in the morning (before 9 AM) after an overnight fast. Venous blood samples were drawn for measurement of glucose and insulin levels at fasting and at 120 minutes after ingestion of the glucose load. Serum glucose levels were measured using a glucose oxidase method on an AU 680 Beckman Chemistry System (Beckman Coulter Magyarország, Budapest, Hungary); insulin levels were measured by electrochemiluminescence immunoassay (ECLIA) on a Cobas e601 automated system (Roche Diagnostics Magyarország, Budaörs, Hungary).

Based on the glucose values during the 2-hour OGTTs, we defined glucose intolerance (impaired fasting glucose, impaired glucose tolerance or diabetes mellitus) as fasting glucose levels of 6.1 mmol/L or above and/or 2-hour glucose levels of 7.8 mmol/L or above (31).

To estimate insulin resistance, we used the homeostasis model assessment (HOMA2 Calculator v. 2.2, Diabetes Trials Unit, University of Oxford, Oxford, United Kingdom, which can be accessed at https://www.dtu.ox.ac.uk/homacalculator/download.php) (39). Insulin sensitivity was characterized by HOMA2-S, beta-cell function HOMA2-B. To characterize insulin secretion, we calculated the insulinogenic index: II=(insulin$_{30\ minutes}$–insulin$_{fasting}$)/(glucose$_{30\ minutes}$–glucose$_{fasting}$) where insulin is entered in μIU/mL and blood glucose in mmol/L (40). Fasting samples were used to determine glycated hemoglobin (A1C) levels (high-performance liquid chromatography; Bio-Rad Magyarország, Budapest, Hungary). Serum lipids (cholesterol, high-density lipoprotein-cholesterol, low-density lipoprotein-cholesterol, triglyceride) and gamma-glutamyltransferase (gammaGT) were determined on an AU 680 Beckman Chemistry System (Beckman Coulter Magyarország, Budapest, Hungary).

The levels of 25(OH)D were determined by the chemiluminescent immunoassay (CLIA) method by the LIAISON 25(OH)D total assay (DiaSorin, Biomedica, Budapest, Hungary). The 25(OH)D intra- and interassay coefficients of variation were 4.1% to 7.7% and 7.7% to 10.9%, respectively, at concentrations of 24 and 8 ng/mL. Functional sensitivity was defined at 2.2 ng/mL with a total allowable relative error of less than 20% (41).

Statistical analysis

All analyses were conducted using SPSS 13.0 for Windows statistical software (IBM, Armonk, New York, United States). Statistical significance was inferred by a 2-sided p value <0.05. Continuous variables were presented in arithmetic mean (±SD), categorical variables as n (percentage rate).

To compare prior GDM and control women, we used independent sample t tests (continuous variables) and chi-square or Fischer exact tests (categorical variables). The distribution of continuous variables was tested for normality, and variables tested by a skewed distribution were natural-log transformed.

Table 1
Baseline characteristics of study participants by prior GDM status

	Prior GDM (mean ± SD) or n (%)	Control (mean ± SD) or n (%)	p
N (number of cases)	87	45	
Age (year)	34.8±4.4	33.8±3.6	0.187
BMI (kg/m^2)	25.9±5.9	24.3±4.4	0.119
Waist circumference (cm)	84.4±13	81.3±9.4	0.183
Physical activity n (%)			0.025
Active	12 (14.6%)	15 (34.9%)	
Moderately active	26 (31.7%)	12 (27.9%)	
Inactive	44 (53.7%)	16 (37.2%)	
Smoker, n (%)	16 (20.0%)	8 (19.0%)	0.900
Systolic blood pressure (mm Hg)	122±17	116±14	0.031
Diastolic blood pressure (mm Hg)	78±11	72±11	0.004
Hypertension, n (%)	22 (25.3%)	4 (8.9%)	0.0036
Glucose intolerance, n (%)	24 (28%)	1 (2.2%)	<0.0001
A1c (%)	5.6±0.4	5.4±0.3	0.009
Fasting glucose (mmol/L)	5.7±1.,1	5.2±0.4	<0.0001
120-minute glucose(mmol/L)	6.6±2.1	5.3±1.4	0.001
Fasting insulin (μIU/mL)	13.6±14.5	9.6±6.4	0.039
120-minute insulin (μIU/mL)	69.2±61	37.3±27.3	<0.0001
HOMA2-S	108±132	127±104	0.427
HOMA2-B	99.6±42.6	97.9±41.4	0.842
Insulinogenic index (mIU/mmol)	14±23	31±37	0.012
Cholesterol (mmol/L)	4.8±0.8	4.8±0.9	0.783
HDL-cholesterol (mmol/L)	1.50±0.34	1.53±0.23	0.607
LDL-cholesterol (mmol/L)	2.98±0,72	2.80±0.79	0.208
Triglyceride (mmol/L)	1.4±1.2	1.3±0.9	0.695
GammaGT (IU/L)	19±11	18±13	0.824
25-hydroxivitamin D (ng/mL)	27.2±13.1	26.9±9.8	0.888
25-hydroxivitamin D insufficiency (<20 ng/mL) n(%)	23 (28.4%)	8 (20.0%)	0.381
Blood draw in spring/summer, n (%)	28 (32.2%)	20 (44.4%)	0.185
Sunbathing, sun-bed use, n (%)	22 (26.5%)	13 (30.2%)	0.679
Use of vitamin D supplement, n (%)	4 (4.6%)	12 (26.7%)	<0.0001

A1C, glycated hemoglobin; *BMI,* body mass index; *gammaGT,* gamma-glutamyltransferase; *GDM,* gestational diabetes; *HDL,* high-density lipoprotein; *HOMA2-B,* homeostasis model assessment insulin secretion; *HOMA2-S,* homeostasis model assessment insulin sensitivity; *LDL,* low-density lipoprotein.

To examine the association between insulin resistance (HOMA2-S) or beta-cell function (II and HOMA2-B) and 25(OH)D levels, we used 2 approaches. First, we tested for univariate associations using linear regression of insulin resistance/insulin secretion (outcomes) and 25(OH)D as a predictor. If a significant association was found, we further adjusted for age and measures of obesity (BMI or waist circumference).

Second, we looked for independent determinants of insulin resistance and insulin secretion using 25(OH)D levels and the variables (Table 1) univariately associated with these outcomes in a multiple logistic regression with a stepwise backward method. We selected 1 variable (with the strongest association with the outcome) of those highly correlated with each other.

Results

Baseline characteristics

In total, 87 patients with prior GDM and 45 controls with normal glucose tolerance during pregnancy were examined. No significant differences in age, BMI, waist circumference or the frequency of smokers was found between the prior GDM and control groups. Women with prior GDM were less physically active and had higher systolic and diastolic blood pressure values as well as an increased prevalence of hypertension compared to controls (Table 1).

Glucose intolerance was present in 24 women (28%) in the prior GDM group compared to 1 woman (2.2%) in the control group (p<0.0001). Women with prior GDM had significantly higher A1C,

Table 2
(A) Association between 25-hydroxyvitamin D levels and homeostasis model asssessment insulin sensitivity. (B) Independent determinants of insulin sensitivity.

Independent variables	Beta	95% CI	p
A			
25-hydroxyvitamin D (ng/mL)	0.017	0.001 to 0.034	0.04
+ age, BMI	0.016	0.002 to 0.030	0.031
+ age, waist circumference	0.017	0.003 to 0.031	0.02
B			
25-hydroxyvitamin D (ng/mL)	0.013	−0.0007 to 0.026	0.047
Age (year)	0.056	0.012 to 0.099	0.011
BMI (kg/m^2)	−0.054	−0.018 to 0.089	0.004
120-minute insulin (μIU/mL)	−0.203	−0,046 to −0.35	0.013
GammaGT (IU/L)	−0.019	−0.003 to −0.034	0.024
A1C (%)	−0.47	−1.00 to 0.582	0.085

A1C, glycated hemoglobin level; *BMI,* body mass index; *gammaGT,* gamma-glutamyl-transferase; HOMA2-S, homeostasis model asssessment insulin resistance; HOMA2-B, homeostasis model asssessment beta-cell function.
Notes: Multiple linear regression with log-transformed HOMA2-S was the outcome variable. Independent determinants of HOMA2-S were selected using backward stepwise elimination of nonsignificant terms. Other parameters available for the model were: C-reactive protein, triglyceride, season of blood draw and diastolic blood pressure (r^2 = 0.671 for the model).

Table 3
(A) The association between 25-hydroxyvitamin D levels and homeostasis model asssessment insulin secretion and homeostasis model asssessment beta-cell function. (B) Independent determinants of HOMA insulin secretion.

Output index HOMA2-B	Beta	95% CI	p
A			
25-hydroxyvitamin D (ng/mL)	−0.009	0.002 to 0.019	0.099
+ age, BMI	−0.008	0.002 to 0.018	0.106
+ age, waist circumference	−0.009	0.001 to 0.018	0.085
B			
Age (years)	−0.032	−0,003 to 0,061	0.032
BMI (kg/m^2)	0.018	−0.005 to 0.042	0.018
GammaGT (IU/L)	0.012	0.0002 to 0.024	0.044
120-minute insulin (μIU/mL)	0.158	0.05 to 0.266	0.005

BMI, body mass index; *gammaGT,* glutamyl-transferase; *HOMA2-S,* homeostasis model asssessment insulin resistance; *HOMA2-B,* homeostasis model asssessment beta-cell function.
Notes: Multiple linear regression with log-transformed HOMA2-B was the outcome variable. Independent determinants of HOMA2-B were selected using backward stepwise elimination of nonsignificant terms. Other parameters available for the model were triglyceride and systolic blood pressure (r^2 = 0.541 for the model).

fasting glucose, 120-minute postload glucose and insulin levels and insulinogenic indexes compared to controls (all p<0.05). No significant between-group differences were found for estimated insulin sensitivity and insulin secretion based on fasting measurements (HOMA2-S, HOMA2-B). There was no difference between the 2 groups in other metabolic parameters examined, such as serum lipids and gammaGT (Table 1).

The 25(OH) D levels as well as the frequency of 25(OH)D insufficiency were similar in women with prior GDM and controls. Women with normal glucose tolerance or glucose intolerance also had similar 25(OH)D values (24.6±10.2 vs. 28.1±12.6 ng/L). Although the times of blood draws and sunbed use were similarly prevalent among the prior GDM and control groups, vitamin D supplementation was reported more often in the control group (Table 1).

Associations between insulin sensitivity and 25(OH)D levels and independent determinants of insulin sensitivity

We found a univariately significant association between insulin sensitivity based on fasting measurements (HOMA2-S) and 25(OH)D levels. Furthermore, this association remained statistically significant and did not attenuate substantially after further adjustments for age and measures of obesity (either BMI or waist circumference) (Table 2A).

Using stepwise linear regression with backward elimination, the independent determinants of HOMA insulin sensitivity were higher 25(OH)D levels, older age, lower BMI, postload insulin, gammaGT and lower A1C levels. The full model explained 67% of the variations in insulin sensitivity (r^2=0.671) (Table 2B).

Associations between insulin secretion and 25(OH)D levels and independent determinants of insulin secretion

There was a nonsignificant association between fasting insulin secretion (HOMA-2B) and 25(OH)D levels (p=0.099) that was unaffected by adjustment for obesity (either BMI or waist circumference) (Table 3A).

Using a similar stepwise linear regression with backward elimination, 25(OH)D levels were not among the independent determinants of HOMA2-B. Independent determinants of HOMA2-B were younger age and higher BMI, gammaGT and postload insulin. These variables explained 54% of the variations in insulin secretion (r^2=0.541) (Table 3B). Vitamin D levels were not related to insulinogenic index (beta 0.058; p=0.55).

Discussion

In the present study, 25(OH)D levels were similar in women with prior GDM and control women as well as between glucose-intolerant and normal glucose-tolerant women 3.2 years after delivery. We found a positive association between insulin sensitivity and 25(OH)D levels that remained significant after adjustment for age and measures of obesity. There was a nonsignificant association between fasting insulin secretion and 25(OH)D levels. Furthermore, we found no association between the insulinogenic index and 25(OH)D levels.

Increased prevalence of glucose intolerance and other abnormal glycemic measures and blood pressure levels have been well described following pregnancies involving GDM. Our current results strengthen our previous findings and correspond with other observations in the literature (18,42).

Maternal vitamin D deficiency has been linked to elevated risks for GDM, both cross-sectionally and longitudinally (19–21). Low 25(OH)D levels were significantly associated with elevated risks for GDM, independently of maternal ages, family histories of diabetes and BMIs in a nested case-control study from the United States (21). However, some studies did not find an association between 25(OH) D levels and the risk for GDM (22–24). Our study extends these observations; we found no difference in 25(OH)D levels between women with previous GDM and controls. The interpretation of these findings is further complicated by the fact that 25(OH)D levels are lower in pregnancy compared to nonpregnancy levels (43).

We could not find any associations between present glucose tolerance status and 25(OH)D levels. It should be mentioned that our study included only a limited number of cases of diabetes, and it would be absolutely necessary to raise this number prior to drawing any conclusions about the prevalence of diabetes and the 25(OH)D levels (8,15,16). A recent prospective observational study from the United States of adults at high risk for diabetes found that higher plasma 25(OH)D levels (assessed repeatedly during follow up) were related to lower risk for diabetes (44). On the other hand, low 25(OH)D status was independently associated with incident diabetes and unfavourable longitudinal changes in continuous markers of glucose homeostasis in a Danish population-based observational study (45). A meta-analysis of prospective cohort and case-control studies based on 76,220 participants showed a significant inverse association between 25(OH)D levels and risk for incident type 2 diabetes (46).

Much of the literature suggests that optimal 25(OH)D homeostasis is essential for insulin action and secretion (46). Using the

gold-standard hyperglycemic clamp to measure insulin sensitivity, an association between 25(OH)D levels and insulin sensitivity was found, even after controlling for BMIs. There was also an inverse association between first- and second-phase insulin response and serum 25(OH)D concentrations, although it became nonsignificant after adjustment for covariates (9). Using the other gold-standard method (intravenous glucose tolerance test) Gulseth et al did not find any relationship between these parameters after adjustment for obesity (2,9). In a large case-control study of adults without diabetes but with high diabetes risk and also in a population sample, the association between insulin sensitivity measured by HOMA and 25(OH)D levels was independent of BMIs and other well-known diabetes risk factors (10,11).

In GDM, 25(OH) levels were associated with HOMA insulin sensitivity, with fasting glucose, postload glucose and A1C levels and with fasting insulin levels (22,23,26–28). It is possible that, due to a generally decreased insulin sensitivity associated with pregnancy, glucose levels are more sensitive indicators of changes in insulin sensitivity related to 25(OH)D levels. In the present study, the close association between 25(OH)D levels and HOMA insulin sensitivity was independent of measures of obesity that corresponds closely to previous observations and broadens them to the women with prior GDM.

In the fasting state, there is a close relationship between insulin sensitivity and insulin secretion that is also taken into account when calculating HOMA values (39). Our observation that early insulin secretion (the insulinogenic index) was not related to 25(OH)D levels but that it had a nonsignificant association with HOMA insulin secretion suggests that the connection of HOMA insulin secretion and 25(OH)D levels indicates homeostatic changes in healthy people. Accordingly, we suspect that 25(OH)D levels are related primarily to insulin sensitivity. This hypothesis is supported by 2 experiments in which vitamin D supplementation was associated with improvements in insulin sensitivity among women with GDM and in nondiabetic adults with high risks for diabetes (12,28).

Limitations and strengths

Several limitations of the present study should be mentioned. First, the measures of insulin sensitivity and insulin secretion were based on fasting insulin and glucose values. Although there is a close relationship between HOMA-based insulin sensitivity and the parameters based on the gold-standard clamp method, HOMA insulin secretion values are less well validated. Second, because of the small sample size of our study, our results are considered to be hypothesis generating. Third, the cross-sectional nature of our study makes it impossible to investigate the temporal sequence prior to the abnormalities. Fourth, the method used for the measurement of 25(OH)D levels has known limitations, although the quality-control measures in our laboratory show acceptable performance. A strength of our study is the fact that controls were selected from the same cohort of pregnant women as the cases, thus minimizing the potential bias related to control selection. We would like to emphasise that, to our knowledge, the connection between 25(OH)D levels and insulin sensitivity after a GDM pregnancy has not yet been described in the literature.

Conclusions

In summary, although women with prior GDM have higher incidences of glucose intolerance at follow up, their vitamin D levels are similar to those of women in the control group. On the other hand, we found a negative association between HOMA insulin sensitivity and 25(OH)D levels that was independent of age and BMI or waist circumference. Intervention studies are required to elucidate whether the supplementation with vitamin D for women with prior GDM has any beneficial effects on incident diabetes.

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