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Glucose homeostasis, beta cell function, and insulin resistance in relation to vitamin D status after gestational diabetes mellitus

Running headline: Vitamin D and glucose metabolism after GDM

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Abstract

Introduction: We wanted to determine vitamin D status after gestational diabetes mellitus (GDM) and to evaluate whether levels of 25-hydroxyvitamin D₃ (25OHD₃) are associated with beta cell function, insulin resistance, or a diagnosis of diabetes after GDM. *Material and methods:* Glucose homeostasis was assessed during a 75-g oral glucose tolerance test 1–2 years after delivery in 376 women with previous GDM (287 European and 78 non-European, including 33 Arab and 35 Asian women). Insulin resistance was estimated using homeostasis model assessment of insulin resistance (HOMA-IR). The insulinogenic index (I/G30) and the disposition index [(I/G30)/HOMA-IR] were used to calculate insulin secretion. Concentrations of serum 25OHD₃ were determined. *Results:* Mean (\pm SD) 25OHD₃ concentration was 50.0 ± 22.3 nmol/L and differed significantly among subgroups of body mass index (BMI), ethnicity, and glucose tolerance status; 53% had 25OHD₃ levels <50 nmol/L and 87% had 25OHD₃ levels <75 nmol/L. There was a negative correlation between 25OHD₃ concentration and HOMA-IR ($p < 0.001$) and a positive correlation between 25OHD₃ and disposition index ($p = 0.002$) in univariable regression analysis. Correlations attenuated after adjustment for BMI. In univariable regression analysis, 25OHD₃ concentrations were significantly associated with diabetes after GDM ($p = 0.004$). However, in a multivariable model non-European origin, HOMA-IR, and insulinogenic index were significantly associated with post-partum diabetes whereas 25OHD₃ concentrations were not. *Conclusion:* Vitamin D deficiency/insufficiency in previous GDM cases appears to be associated with beta cell dysfunction and insulin resistance, but not with post-partum diabetes when factors well known to influence type-2 diabetes were adjusted for.

Key words

Disposition index; Ethnicity; Gestational diabetes; HOMA-IR; Insulinogenic index; OGTT; Post-partum diabetes; Vitamin D

Abbreviations

BMI, body mass index; CI, confidence interval; GDM, gestational diabetes mellitus; 25OHD₃, 25-hydroxyvitamin D₃; HOMA-IR, homeostasis model assessment of insulin resistance; OGTT, oral glucose tolerance test; OR, odds ratio; PTH, parathyroid hormone; SD, standard deviation.

Key Message

Vitamin D deficiency/insufficiency is prevalent in previous GDM cases. 25OHD₃ levels correlate negatively with insulin resistance and post-partum diabetes, and positively with insulin secretion. These associations are interrelated and are influenced by other factors, such as BMI and ethnicity.

Introduction

Gestational diabetes mellitus (GDM) and type-2 diabetes have many risk factors in common and they share the same metabolic characteristics. Both are characterized by insulin resistance and an inability of the beta cells to compensate by a sufficient increase in insulin secretion (1). Serum 25-hydroxyvitamin D₃ (25OHD₃) is considered to be the most appropriate indicator of overall vitamin D status (2). Preliminary data suggest that vitamin D is involved in glucose homeostasis. The mechanism possibly involves both insulin secretion and insulin action (3). Lower levels of 25OHD₃ have been associated with higher risk of type-2 diabetes and also the metabolic syndrome (4, 5). Moreover, a recent meta-analysis revealed that maternal vitamin D insufficiency is associated with an increased risk of GDM (6). However, the available evidence is limited and it is not clear whether the relation between vitamin D status and impairment of glucose metabolism is causal or whether it is related to confounding factors.

We have previously shown that Arab women with GDM are more insulin-resistant during pregnancy than Scandinavian women (7). Furthermore, we recently reported from the prospective Mamma Study that women of non-European origin had an increased frequency of diabetes 1–2 years after GDM, which was associated with increased insulin resistance—differently modulated by body mass index (BMI) in Arab and Asian women (8).

The objective of the present study was to determine vitamin D status in women at the 1- to 2-year follow-up in the Mamma Study, and to evaluate whether levels of 25OHD₃ are associated with insulin resistance, beta cell dysfunction, and a diagnosis of diabetes after GDM. We hypothesized that ethnicity and BMI would account for part of the assumed associations, and that adjustment for these factors would attenuate the results.

Material and methods

The design of the Mamma Study has been described in detail elsewhere (9). Briefly, pregnant women who gave birth in southern Sweden during the years 2003–2005 were followed prospectively to detect the development of diabetes. A 75-g OGTT was offered to all women in the twenty-eighth week of gestation, and also in gestational week 12 if they had had a history of GDM in previous pregnancies, or a first-degree relative with diabetes. The

diagnostic criteria for GDM were those recommended by the World Health Organization (WHO) in 1999, defining GDM as a joint category of diabetes and impaired glucose tolerance (IGT) based on the 2-h plasma glucose concentration (10). The 1- to 2-year examination included a standard 75-g OGTT with measurements of both glucose and insulin concentrations at 0, 30, and 120 min to enable calculation of indices of beta cell function and insulin resistance, as previously reported (8). In conjunction with the OGTT at the 1- to 2-year follow-up a blood sample for determination of 25OHD₃ concentration was collected and serum was stored at -20°C until analysis. In all, measurements of 25OHD₃ concentrations were successful in 376 women who had previously had GDM and who formed the basis of the present evaluation.

Based on the stated country of origin of at least three grandparents, women were grouped according to whether they were of European or non-European origin. From the definition used, 11 women with GDM were unclassifiable. The non-European group included the subgroups of Arab women (n = 33; Iraq, Lebanon, Morocco, Palestine, Somalia, and Syria), Asian women (n = 35; Afghanistan, China, India, Iran, Kurdistan, Pakistan, the Philippines, South Korea, Thailand, Turkey, and Vietnam), and women of other origins (n = 10; Berber, Bolivia, Brazil, Chile, Colombia, Eritrea, Ghana, Israel, Uganda, and Uruguay).

The WHO 1999 diagnostic criteria were used for classification of women during follow-up into having normal glucose tolerance, impaired fasting glucose, impaired glucose tolerance, or diabetes (10). All women gave written informed consent and the Ethics Committee of Lund University approved the study (LU 259-00, 2002-04-17), which was performed in accordance with the Declaration of Helsinki.

The HemoCue Glucose 201+ system (HemoCue AB, Ängelholm, Sweden) was used for immediate measurement of plasma glucose concentrations (in mmol/L). Serum insulin concentrations (mU/L) were measured with an enzyme-linked immunosorbent assay (Dako, Glostrup, Denmark). Homeostasis model assessment was used to estimate insulin resistance (HOMA-IR), that is, (fasting serum insulin × fasting plasma glucose)/22.5 (11, 12). Beta cell function was estimated using the insulinogenic index (I/G30), which is the ratio of the incremental insulin to glucose during the first 30 min of the OGTT, that is, (insulin_{30 min} - insulin_{0 min})/(glucose_{30 min} - glucose_{0 min}) (13). As insulin resistance modulates insulin

secretion, the disposition index was used to adjust insulin secretion for the degree of insulin resistance, obtained by dividing I/G30 by HOMA-IR (14).

The serum concentration of 25OHD₃ (in nmol/L), which is stable in stored serum (15), was determined by liquid chromatography mass spectrophotometry (LC-MS/MS). Quality controls in two levels were analyzed at the start and end of every run with the following inter-assay coefficient of variation: 4.4% at 36 nmol/L 25OHD₃, and 3.7% at 134 nmol/L 25OHD₃. The LC-MS method was calibrated with Chromsystems (Munich, Germany) calibrators, which are directly traceable to the US National Institute of Standards and Technology. Assays were performed according to accredited methods at the Department of Clinical Chemistry, Skåne University Hospital, Malmö, Sweden, which also participates in DEQAS, a vitamin D external quality assessment scheme.

Statistical analyses

Categorical variables are shown as n (%). Continuous variables are summarized as mean \pm standard deviation (SD) if normally distributed or as median and interquartile range if skewed. Indices of insulin secretion and insulin resistance were log-transformed to obtain normality. Frequencies were compared between groups using Fisher's exact test. Analysis of variance (ANOVA) was used to test for differences between group means, and the Mann-Whitney *U* test or the Kruskal Wallis test was used to test for differences in medians. Women were classified into groups according to vitamin D status according to the Endocrine Society guidelines: deficiency (< 50 nmol/L), insufficiency 50–74 nmol/L, and sufficiency \geq 75 nmol/L (16). Depending on the date of sample collection for vitamin D analysis, the results were grouped into months and seasons (winter, December–February; spring, March–May; summer, June–August; autumn, September–November). The association between 25OHD₃ levels and measures of glucose homeostasis obtained during the OGTT was examined by univariable linear regression analysis, adjusted for BMI in the final model. Multivariable logistic regression analysis was used to estimate the association between 25OHD₃ and diabetes after GDM, using normal glucose tolerance/impaired fasting glucose/impaired glucose tolerance as reference, while adjusting for confounders that were associated with diabetes in univariable analysis ($p < 0.05$). In addition to 25OHD₃, variables tested for associations with diabetes after GDM were age (years), BMI (kg/m²), non-European vs. European origin (yes/no), insulinogenic index, and HOMA-IR. We used IBM SPSS Statistics

version 22 for Windows (IBM Corporation, Armonk, NY, USA) for analysis. Two-sided p -values of less than 0.05 were considered statistically significant.

Results

The mean (\pm SD) age of participating women was 34.3 ± 4.8 years; 79% were of European origin (79% of whom were Swedish) and 21% were of non-European origin.

Levels of 25OHD₃ were compared among different subgroups (Table 1). The overall mean 25OHD₃ level was 50.0 ± 22.3 nmol/L, but the levels differed significantly between subgroups of BMI, ethnicity, glucose tolerance status, and by season.

Of all 376 women, 53% had vitamin D deficiency (25OHD₃ < 50 nmol/L), 33% had vitamin D insufficiency (25OHD₃ 50–74 nmol/L), and 13% had vitamin D sufficiency (25OHD₃ \geq 75 nmol/L). BMI, HOMA-IR, and disposition index differed significantly according to the level of 25OHD₃, whereas insulinogenic index did not differ significantly (Table 2). All the women with vitamin D sufficiency were of European origin. However, 82% of the European women had 25OHD₃ levels < 75 nmol/L and 42% had 25OHD₃ levels < 50 nmol/L. Of the 198 women with vitamin D deficiency, 51 had 25OHD₃ levels below 25 nmol/L, and 80% of them were of non-European origin.

Snuff was used in 1% of the women at follow-up, whereas 12% smoked. There were no significant differences in the frequencies of tobacco use between women in the different subgroups of glucose tolerance or vitamin D levels.

In univariable linear regression analysis, 25OHD₃ levels correlated negatively with HOMA-IR ($p < 0.001$) and positively with disposition index ($p = 0.002$), but not with insulinogenic index or fasting and 2-h glucose levels during the OGTT. However, when adjusting for BMI, 25OHD₃ levels were not statistically significantly associated with HOMA-IR or disposition index, but they approached significance for HOMA-IR ($p = 0.053$).

Alltogether 26 of 376 women were diagnosed with diabetes at the 1- to 2-year follow-up. In univariable logistic regression analysis, assessing 25OHD₃ as a continuous variable, 25OHD₃

levels were significantly associated with diabetes after GDM ($p = 0.004$). In the multivariable model, the relationship between 25OHD₃ levels and diabetes was not significant whereas non-European origin, HOMA-IR, and insulinogenic index were significantly associated with postpartum diabetes (Table 3).

Discussion

This study confirmed what is known about the high prevalence of low levels of 25OHD₃ in women of childbearing age worldwide (17); 87% of the women had 25OHD₃ levels < 75 nmol/L and 53% had 25OHD₃ levels < 50 nmol/L. Overall, women of non-European origin had lower levels of 25OHD₃ after pregnancy than women of European origin. Furthermore, 80% of the women with extremely low vitamin D levels (25OHD₃ < 25 nmol/L) were of non-European origin, whereas all women with vitamin D sufficiency were of European origin. These observations are supported by previous reports of poor vitamin D status in many immigrant populations in Europe relative to the indigenous populations (18). Interestingly, Clifton-Bligh et al. reported figures similar to ours in a cohort of 307 pregnant women living in Sydney, Australia. Vitamin D deficiency (25OHD₃ < 50 nmol/L) was found in 48%. The reported mean 25OHD₃ concentrations (in nmol/L) were 47.2 ± 18.2 in South-East Asian women, 34.4 ± 19.6 in Asian women, and 29.9 ± 16.3 in Middle Eastern women, as compared to 62.3 ± 23.3 in European women (19). There are several possible explanations for the variation in vitamin D levels between ethnic groups, such as clothing habits, diet, vitamin D supplementation, and differences in melanin production when exposed to sunlight (20).

When stratified by ethnicity, 82% of European women had vitamin D levels < 75 nmol/L and 42% had vitamin D deficiency. This contrasts with the findings of Hedlund et al., who reported corresponding figures of 69% and 23%, respectively, when they evaluated determinants of vitamin D status in a group of 84 non-pregnant, non-lactating, healthy, fair-skinned women of childbearing age living in the city of Gothenburg in Sweden (21). This discrepancy may reflect differences in study group characteristics, and also differences in laboratory methodology for 25OHD₃ assessment. As pointed out by the authors, the electrochemiluminescent immunoassay used by them tends to give higher concentrations than the liquid chromatography mass spectrometry used in the present study (21).

To the best of our knowledge, this is the first study evaluating indices of insulin resistance and beta cell function during OGTT after GDM in relation to vitamin D status. Insulin resistance and impaired beta cell function contribute to the development of type-2 diabetes (1).

Therefore, quantifying insulin sensitivity and insulin secretion are of great importance for the understanding of the disease. Indirect measurements using the HOMA-IR and the insulinogenic index are often used in epidemiological studies and have shown reasonable correlations with direct methods of measurements, which are more complex, labor- and time-consuming (11-13).

As expected, we found a significant association between 25OHD₃ levels and HOMA-IR. However, the association attenuated after adjustment for BMI. Low serum concentrations of 25OHD₃ have been associated with reduced insulin sensitivity, impaired glucose metabolism, and the metabolic syndrome (3-5). The mechanism by which vitamin D affects insulin sensitivity is still unknown. Vitamin D may stimulate expression of insulin receptors in peripheral tissues, and thereby increase glucose transport (22). Insulin-mediated glucose uptake is calcium-dependent, and therefore vitamin D status may indirectly influence glucose uptake (23). Insulin sensitivity, measured by the hyperglycemic clamp technique, has been shown to correlate inversely with plasma parathyroid hormone (PTH) levels in healthy subjects (24). Thus, it could be that ambient variation in PTH production is the key mediator of the insulin resistance associated with poor vitamin D status (25). Unfortunately, we did not investigate PTH levels in the present study.

There is evidence to suggest that vitamin D affects insulin secretion (3). The influence of vitamin D deficiency on insulin secretion could be mediated by the effect on calcium concentrations, as calcium ion fluxes have an essential role in insulin secretion from beta cells (23). Alternately, it may relate to a secondary increase in PTH levels (26). Fadda et al. examined the direct effect of PTH on insulin release from pancreatic islets in normal rats and found that PTH stimulated glucose-induced insulin release in a dose-dependent manner but higher doses inhibited glucose-induced insulin release (27). In the present study, we used the insulinogenic index and the disposition index—which are surrogate measures of first-phase insulin secretion—to evaluate whether vitamin D was associated with impaired insulin secretion. The disposition index, which adjusts insulin secretion for the degree of insulin resistance, was significantly associated with vitamin D levels, supporting the observation that vitamin D appears to affect insulin response upon glucose stimulation (28).

Finally, we examined whether 25OHD₃ levels were associated with diabetes after GDM and found a significant association by univariable logistic regression analysis ($p = 0.004$). However, in the multivariable model, the relation between 25OHD₃ levels and diabetes was not significant, whereas HOMA-IR and insulinogenic index were highly significantly associated with diabetes after GDM. Since insulin resistance and impaired beta cell function are closely linked to the pathophysiology of type-2 diabetes, this could be expected, and a larger sample size is needed to rule out the possibility of an effect of vitamin D status on these measurements.

According to the Endocrine Society guidelines pregnant and lactating women are candidates for screening for vitamin D deficiency. To satisfy the requirements to maintain a 25OHD₃ level above 30 ng/ml (74 nmol/L) it is recommended that their daily regimen should include a multivitamin containing 400 IU vitamin D with a supplement that contains at least 1000 IU vitamin D (16). It is obvious from the literature that these guidelines are not followed by different countries in the world (17). In Sweden pregnant women are not generally screened for vitamin D status, or routinely prescribed vitamin D supplementation. Intervention studies have shown conflicting results regarding the beneficial effect of vitamin D supplementation on insulin secretion and insulin sensitivity (3). Large well-controlled randomized studies are needed to evaluate the effect of adequate doses of vitamin D on glucose homeostasis and the overall health outcomes in pregnant women.

To our knowledge, there have been no studies investigating the possible effect of vitamin D deficiency/insufficiency during pregnancy with GDM on manifest post-partum diabetes. Kramer et al. studied 524 women with a full spectrum of glucose tolerance in pregnancy, ranging from normal glucose tolerance to GDM. They found that increased PTH, rather than vitamin D deficiency/insufficiency, was independently associated with impaired glucose tolerance in pregnancy. Furthermore, vitamin D status was not found to be associated with insulin sensitivity, beta cell function, or gestational glucose tolerance. The authors highlighted the need for consideration of the PTH/25OHD axis when studying the effect of vitamin D status on glucose homeostasis (29).

One strength of this study was that measures of beta cell function were derived from the OGTT. Assessment of beta cell function in the basal state by HOMA does not take first-phase

insulin release into consideration, and therefore gives a less reliable measure of beta cell function than the insulinogenic index and the disposition index (13).

The present study also had some limitations. Although the number of study subjects was relatively large compared to other studies, it still lacked enough power to detect small potential effects of vitamin D on glucose homeostasis, insulin secretion, and insulin sensitivity. Another limitation was that vitamin D was not measured during pregnancy, but 1–2 years after pregnancy on the same occasion as the diagnostic OGTT. Moreover, PTH was not analyzed.

In conclusion, low levels of 25OHD₃ were common in the present group of women of different ethnicity 1–2 years after GDM. Our results indicate that vitamin D deficiency/insufficiency appears to be associated with beta cell dysfunction and insulin resistance. However, no association was found between vitamin D levels and post-partum diabetes when adjusted for factors well known to influence the development of type-2 diabetes, such as BMI, ethnicity and indices of beta cell function and insulin resistance.

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Table 1. Serum 25OHD₃ concentrations in different subgroups of women with a previous history of gestational diabetes

Variable	Sample size	25OHD ₃ , nmol/L	<i>p</i> -value
All women	376 (100)	50.0 ± 22.3	
BMI, kg/m ²			< 0.001
< 25	222 (59)	54.5 ± 22.3	
25–29.9	88 (23)	44.4 ± 20.8	
≥ 30	65 (17)	41.1 ± 19.8	
European origin	287 (79)	54.0 (41.0–70.0)	< 0.001 ^a
Non-European origin	78 (21)	24.0 (16.0–37.0)	
Arab	33 (9)	17.0 (15.0–26.0)	
Asian	35 (10)	26.0 (17.0–47.0)	
Glucose tolerance			0.008
Normal	226 (60)	47.5 (36.0–64.3)	
Pre-diabetes	123 (33)	50.0 (33.0–66.0)	
Diabetes	26 (7)	34.0 (17.8–51.5)	
Season			< 0.001
Winter	101 (27)	43.0 (32.0–54.5)	
Spring	98 (26)	41.5 (32.5–53.3)	
Summer	76 (20)	64.5 (45.3–80.8)	
Autumn	101 (27)	54.0 (35.0–70.0)	

Data are given as n (%), mean ± SD, or median (interquartile range). Differences in means were tested by ANOVA and differences in medians were tested by the Mann-Whitney *U* test or the Kruskal Wallis test.

^a *p*-value for European vs. non-European origin and for European vs. Asian or Arab origin.

Table 2. Clinical phenotypes stratified according to three different levels of 25-hydroxyvitamin D3 (25OHD3)

Variable	All women	25OHD ₃ , nmol/L			<i>p</i> -value
		< 50	50–74	≥ 75	
n (%)	376 (100)	198 (53)	125 (33)	53 (13)	
25OHD ₃ , nmol/L	50.0 ± 22.3	32.9 ± 11.2	60.8 ± 7.1	88.1 ± 11.2	< 0.001
BMI, kg/m ²	25.2 ± 5.2	26.2 ± 5.6	24.7 ± 5.0	23.0 ± 3.1	< 0.001
HOMA-IR ^a	1.6 (1.0–2.5)	1.8 (1.1–2.7)	1.6 (1.0–2.3)	1.1 (0.8–1.8)	0.001
		(173) ^b	(110) ^b	(49) ^b	
Insulinogenic index ^a	12.2 (8.2–19.6)	12.1 (7.7–20.4)	12.4 (8.5–18.9)	11.1 (8.4–18.2)	0.730
		(164) ^b	(103) ^b	(47) ^b	
Disposition index ^a	8.5 (5.3–13.3)	8.1 (4.9–12.3)	8.5 (5.4–14.0)	10.1 (5.4–14.6)	0.035
		(158) ^b	(101) ^b	(47) ^b	

Data are given as mean ± SD or median (interquartile range). Differences in means were tested by ANOVA.

^a Log-transformed in main analyses.

^b Number of samples available.

Table 3. Multivariable model assessing the association between 25OHD₃ and diabetes after gestational diabetes

Variable	OR	95% CI	<i>p</i> -value
25OHD ₃	1.0	1.0–1.1	0.130
BMI	1.1	1.0–1.3	0.055
Non-European origin	15.4	2.3–103.7	0.005
HOMA-IR	16.2	3.6–74.0	< 0.001
Insulinogenic index	0.1	0.0–0.3	< 0.001

BMI, body mass index; CI, confidence interval; HOMA-IR, homeostasis model assessment of insulin resistance; OR, odds ratio.