TITLE

The relationship between 25-hydroxyvitamin D levels, insulin sensitivity, and insulin secretion in women 3 years after delivery

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ABSTRACT

Aim – 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 controls 3.2 years after delivery.
Methods – In total 87 patients with prior GDM and 45 randomly selected controls (age range: 22-44 years) with normal glucose tolerance during pregnancy nested within a cohort of all deliveries at Saint Margit Hospital, Budapest between 01/01/2005-31/12/2006 were examined. 25(OH) D levels were measured by radioimmunoassay. Insulin sensitivity and fasting insulin secretion were estimated using the HOMA calculator, early insulin secretion by the insulinogenic index based on a 75g 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 (β=0.017, 95% CI: 0.001-0.034 / 1 ng/ml) that was robust to adjustment for age and BMI. 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 as no association between 25(OH)D and insulinogenic index was found.

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TEXT

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-hydroxyvitamin 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-hydroxyvitamin D.[1-5] 25-hydroxyvitamin D deficiency is rather common, recent studies reported a prevalence of 77% in the United States, 37% in Canada, and 50-70% in European populations.[6, 7]

An association between 25-hydroxyvitamin D levels and insulin sensitivity regardless of body mass index (BMI)/obesity is suggested by several studies, mostly in populations with an increased risk of diabetes.[2, 8-11] In a randomized, controlled trial among subjects with a high risk of diabetes, oral supplementation of vitamin D prevented the deterioration of insulin sensitivity in the short term.[12] Further, it is suggested that levels and supplementation of 25(OH)D could have a bidirectional association with adiposity, as 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 gynaecological 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-hydroxyvitamin D levels and risk of incident of type 2 diabetes.[16] In addition,

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randomized trials demonstrated a direct link between increased vitamin D intake and a reduced risk of type 2 diabetes.[14, 15, 17]

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

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

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Materials and 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 01/01/2005 and 31/12/2006. Saint Margit Hospital serves a mostly urbanized population of 235 thousand people.

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

- First, a 75 gram 2-hour oral glucose tolerance test (OGTT) was performed at 16-18 weeks of gestation and evaluated using the WHO 1999 criteria (fasting glucose ≥7mmol/l and/or after 75g OGTT glucose level ≥ 7.8mmol/l).[31]
- Those participants with normal glucose tolerance at the first test, subsequently had a second OGTT at 24-28 weeks of gestation evaluated according to the same diagnostic criteria.

All GDM women 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 Recommendation of the Hungarian Diabetes Association with cut-offs similar to the Canadian guideline.[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 (Licence number: 124/2007) in accordance with the Helsinki Declaration of the World Medical Association.

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Participants

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

All GDM women (n=193) and a randomly selected control group of women with normal glucose tolerance during pregnancy (n=98) were invited for a follow-up investigation 3.2±0.6 years after delivery. All study participants were Caucasians. Of these potentially eligible women 36/8 were excluded due to current pregnancy, breastfeeding, or known diabetes. Of 157/90 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 questionnaire women with current pregnancy, lactation, or with known diabetes were excluded.

Eligible participants were invited to a detailed interview using a structured questionnaire on maternal sociodemographic characteristics, lifestyle habits (smoking, caffeine and alcohol consumption, physical activity, use of dietary supplements, nutrition), as well as

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medical and reproductive history and family history of diabetes. In addition a physical examination including anthropometrics and blood pressure was performed.

Study participants underwent a 2-hour 75g oral glucose tolerance test. Fasting blood was collected for other laboratory parameters.

Covariates and outcomes

Using questionnaire data *age* at follow-up was determined. *Smoking* status was coded as never, ex-smoker or current smoker (≥5 cigarette/day). *Physical activity* was assessed by the answers to questions on 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 vigorous physical activity), inactive (≤ 1 hour/week of moderate and ≤ 1 hour/week of vigorous physical activity), or moderately active (if not active or inactive).[36] Positive *family history of diabetes* was defined as having a first degree relative with diabetes.

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

As 25-hydroxyvitamin D levels show a clear *seasonal variation*, we analysed date 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 sun bed use per week.

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Body weight and *height* were measured in light clothing without shoes on a calibrated digital scale. Weight was rounded to the nearest 0.1 kg, height to the nearest centimetre. Waist circumference was measured at the height of the navel. BMI was calculated as weight (kg) /(height (m) * height (m)).

Blood-pressure was measured three times using calibrated, digital blood-pressure meter (OMRON M4-I, Omron Electronics Kft., Budapest, Hungary), on the upper arm, with adequate sized (to upper arm circumference) cuff, after five minutes rest in the sitting position. The average of the second and third measurements was used in the analysis. *Hypertension* was defined as a blood pressure ≥ 140/90 mmHg 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 9:00 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 was measured using a glucose oxidase method on an AU 680 Beckman Chemistry System **(**Beckman Coulter Magyarország Kft. Budapest, Hungary), insulin by electrochemiluminescence immunoassay (ECLIA) on a Cobas e601 automated system (Roche Diagnostics Magyarország Kft., Budaörs, Hungary).

Based on the glucose values during the 2-hour OGTT we defined glucose intolerance (impaired fasting glucose, impaired glucose tolerance, or diabetes mellitus) as a fasting glucose a ≥6.1 mmol/l and/or 2-h glucose ≥7.8 mmol/l.[31]

To estimate insulin resistance we used the homeostasis model assessment (HOMA2 Calculator v.2.2, Diabetes Trials Unit, University of Oxford, Oxford, UK, can be accessed

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at https://www.dtu.ox.ac.uk/homacalculator/download.php).[39] Insulin sensitivity was characterised by HOMA2-S, beta-cell function HOMA2-B. To characterise 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, 41] Fasting samples were used to determine *HbA1c* (high-performance liquid chromatography, Bio-Rad Magyarország Kft., Budapest Hungary). Serum lipids (*cholesterol*, *High-density lipoprotein (HDL)-cholesterol, Low-density lipoprotein* (*LDL)-cholesterol, triglyceride*), *gamma-glutamyl-transferase* (γGT) were determined on an AU 680 Beckman Chemistry System **(**Beckman Coulter Magyarország Kft., Budapest, Hungary).

The level of 25-hydroxyvitamin D was determined by chemiluminescent immunoassay (CLIA) method with LIAISON 25-OH-vitamin D total assay (DiaSorin, Biomedica Kft., Budapest, Hungary). The 25(OH)D intra- and inter-assay coefficients of variation (CVs) were 4.1–7.7% and 7.7–10.9%, respectively, at concentrations (24 and 8 ng/ml). Functional sensitivity was defined at 2.2 ng/ml with a total allowable relative error of less than 20%.[42]

Statistical analysis

All analyses were conducted using SPSS13.0 for Windows statistical software. Statistical significance was inferred at a two-sided p value <0.05.

Continuous variables were presented in arithmetic mean (±SD), categorical variables as n (percentage rate).

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

To examine the association between insulin resistance (HOMA2-S) or beta-cell function (II and HOMA2-B) and 25-hydroxyvitamin D levels we used two approaches.

First, we tested for univariate associations using linear regression of insulin resistance/insulin secretion (outcomes) and 25-hydroxyvitamin 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-hydroxyvitamin D levels and those variables (listed in **Table 1**.) univariately associated with these outcomes in a multiple logistic regression with stepwise backward method. We selected one 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 difference in age, body mass index, waist circumference, or the frequency of smokers was found between the prior GDM and control groups. Prior GDM women were less physically active, had higher systolic and

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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 one woman (2.2%) in the control group ($p<0.0001$). Prior GDM women had significantly higher HbA1c, fasting glucose, 120-minute post load glucose and insulin levels, and insulinogenic index compared to controls (all $p<0.05$). No significant between-group difference was found for estimated insulin sensitivity and insulin secretion based on fasting measurements (HOMA2-S, HOMA2-B). There was no difference between the two groups in other metabolic parameters examined, such as serum lipids and γGT. (**Table 1**)

25-hydroxyvitamin D levels as well as frequency of 25(OH)D insufficiency were similar in prior GDM and control women. 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). While the time 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-hydroxyvitamin 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-hydroxyvitamin D levels. Further, this association remained statistically significant and did not attenuate substantially after

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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 IS were higher 25-hydroxyvitamin D levels, older age, lower BMI, postload insulin, γGT, and lower HbA1c levels. The full model explained 67% of the variation in insulin sensitivity (r^2=0.671). (**Table 2B**)

Associations between insulin secretion and 25-hydroxyvitamin D levels and independent determinants of insulin secretion

There was a non-significant association between fasting insulin secretion (HOMA-2B) and 25-hydroxyvitamin 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-hydroxyvitamin D level was not among the independent determinants of HOMA 2B. Independent determinants of HOMA-2B were younger age, higher BMI, γGT, and post load insulin. These variables explained 54% of the variation in insulin secretion (r^2=0.541). (**Table 3B**).

Vitamin D levels were not related to insulinogenic index (β 0.058; p=0.55).

Discussion

In the present study 25-hydroxyvitamin D levels were similar in 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-

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hydroxyvitamin D levels that remained significant after adjustment for age and measures of obesity. There was a non-significant association between fasting insulin secretion and 25-hydroxyvitamin D levels. Furthermore, we found no association between the insulinogenic index and 25-hydroxyvitamin D levels.

Our results in context

Increased prevalence of glucose intolerance and other abnormal glycaemic measures and blood pressure levels are well described following a GDM pregnancy. Our current results strengthen our previous findings and correspond to other literary observations [18, 43].

Maternal vitamin D deficiency has been linked to an elevated risk for GDM both cross-sectionally and longitudinally.[19-21] Low 25-hydroxyvitamin D levels were significantly associated with an elevated risk for GDM independently of maternal age, family history of diabetes, and BMI in a nested case-control study from the US.[21] However, some studies did not find an association between 25-hydroxyvitamin D levels and the risk of GDM.[22-24]. Our study extends these observations, as we found no difference in 25-hydroxyvitamin D levels between previous GDM and control women. The interpretation of these findings is further complicated by the fact that 25-hydroxyvitamin D levels are lower in pregnancy compared to non-pregnant levels.[44]

We could not find any associations between present glucose tolerance status and 25-hydroxyvitamin D levels. It should be mentioned that our study included only a limited number of diabetes cases and it would be absolutely necessary to raise this number prior to drawing any conclusions about the prevalence of diabetes and the 25-hydroxyvitamin D levels.[8, 15, 16] A recent prospective observational study of adults at high risk of

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diabetes from the US has found that higher plasma 25-hydroxyvitamin D levels (assessed repeatedly during follow-up) were related to lower risk of diabetes.[45] On the other hand low 25-hydroxyvitamin D status was independently associated with incident diabetes and unfavourable longitudinal changes of continuous markers of glucose homeostasis in a Danish population-based observational study.[46] A meta-analysis of prospective cohort and case-control studies, based on 76,220 participants showed an significant inverse association between 25-hydroxyvitamin D level and risk of incident type 2 diabetes.[47]

Much of the literature suggests that optimal 25-hydroxyvitamin D homeostasis is essential for insulin action and secretion.[47] Using the gold standard hyperglycaemic clamp to measure insulin sensitivity, an association between 25-hydroxyvitamin D levels and insulin sensitivity was found even after controlling for BMI. 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 nondiabetic adults with high diabetes risk and also in a population sample the association between insulin sensitivity measured by HOMA and 25-hydroxyvitamin D levels was independent of BMI and other well-known diabetes risk factors.[10, 11]

In gestational diabetes, 25-hydroxyvitamin D level was associated with HOMA insulin sensitivity, with fasting glucose, post load glucose, HbA1c and with fasting insulin level.[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

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insulin sensitivity related to 25-hydroxyvitamin D levels. In the present study the close association between 25-hydroxyvitamin D level and HOMA insulin sensitivity was independent of measures of obesity that corresponds closely to previous observations and broadens them to the prior GDM women.

In the fasting state, there is a close relation 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-hydroxyvitamin D levels, while it had a non-significant association with HOMA insulin secretion, suggests that the connection of HOMA insulin secretion and 25-hydroxyvitamin D levels indicates homeostatic changes in healthy people. Accordingly, we suspect that 25-hydroxyvitamin D levels are primarily related to insulin sensitivity. This hypothesis is supported by two experiments where vitamin D supplementation was associated with improvements in insulin sensitivity among GDM women and in nondiabetic adults with a high risk 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 relation between HOMA-based insulin sensitivity and the parameters based on the gold standard clamp method, HOMA insulin secretion values are less validated. Second, because of the small sample size of our study, our results are considered hypothesis generating. Third, the cross-sectional nature of our study makes it impossible to investigate the temporal sequence prior to the abnormalities. Fourth, the

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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-hydroxyvitamin D levels and insulin sensitivity after a GDM pregnancy has not yet been described in the literature.

Conclusions

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

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Abbreviations: BMI: Body Mass Index; CRP: C-reactive protein; γGT: γ-glutamyl-transferase; GDM: gestational diabetes mellitus; HDL: High-density lipoprotein; HOMA: Homeostasis Model Assessment; HOMA2-B: HOMA insulin secretion; HOMA2-S: HOMA insulin sensitivity; HPLC: high-pressure liquid chromatography; II: Insulinogenic index; LDL: Low-density lipoprotein; OGTT: oral glucose tolerance test; 25(OH)D: 25-hydroxyvitamin D

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Abbreviations: BMI: Body Mass Index; CRP: C-reactive protein; γGT: γ-glutamyl-transferase; GDM: gestational diabetes mellitus; HDL: High-density lipoprotein; HOMA: Homeostasis Model Assessment; HOMA2-B: HOMA insulin secretion; HOMA2-S: HOMA insulin sensitivity; HPLC: high-pressure liquid chromatography; II: Insulinogenic index; LDL: Low-density lipoprotein; OGTT: oral glucose tolerance test; 25(OH)D: 25-hydroxyvitamin D

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Table 1 – Baseline characteristics of study participants by prior GDM status.

Table 2– The association between (A) 25-hydroxyvitamin D levels and HOMA insulin sensitivity (HOMA2-S) and (B) independent determinants of insulin sensitivity.

Table 3 – The association between (A) 25-hydroxyvitamin D levels and HOMA insulin secretion (HOMA2-B) and (B) independent determinants of HOMA insulin secretion.

TITLES OF FIGURES

Figure 1 – Study design and flow chart of participants

Abbreviations: BMI: Body Mass Index; CRP: C-reactive protein; γGT: γ-glutamyl-transferase; GDM: gestational diabetes mellitus; HDL: High-density lipoprotein; HOMA: Homeostasis Model Assessment; HOMA2-B: HOMA insulin secretion; HOMA2-S: HOMA insulin sensitivity; HPLC: high-pressure liquid chromatography; II: Insulinogenic index; LDL: Low-density lipoprotein; OGTT: oral glucose tolerance test; 25(OH)D: 25-hydroxyvitamin D

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/m²)	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 (mmHg)	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
HbA1c (%)	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
γGT[•] (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

[†]:Body mass index,[‡]: Homeostasis Model Assessment insulin sensitivity, [§]: Homeostasis Model Assessment insulin secretion, [•]: γ-glutamyl-transferase

Table 2 –Association between (A) 25-hydroxyvitamin D levels and HOMA insulin sensitivity (HOMA2-S) and (B) independent determinants of insulin sensitivity.

A

Independent variables	β	95 %CI	P
25-hydroxyvitamin D (ng/ml)	0.017	0.001-0.034	0.04
+age, BMI†	0.016	0.002-0.030	0.031
+age, waist circumference	0.017	0.003-0.031	0.02

B

Independent variables	β	95 % CI	P
25-hydroxyvitamin D (ng/ml)	0.013	-0.0007-0.026	0.047
Age (year)	0.056	0.012-0.099	0.011
BMI† (kg/m²)	-0.054	-0.018--0.089	0.004
120-minute insulin (µIU/ml)	-0.203	-0,046- -0.35	0.013
γGT• (IU/L)	-0.019	-0.003- -0.034	0.024
HbA1c (%)	-0.47	-1.00-0.582	0.085

†: Body Mass Index, •: γ-glutamyl-transferase

Multiple linear regression with log-transformed HOMA2-S as the outcome variable.

Independent determinants of HOMA2-S were selected using backward stepwise elimination of non-significant 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 – The association between (A) 25-hydroxyvitamin D levels and HOMA insulin secretion (HOMA2-B) and (B) independent determinants of HOMA insulin secretion.

A

Output index HOMA2-B	β	95% CI	P
25-hydroxyvitamin D (ng/ml)	-0.009	0.002—0.019	0.099
+age, BMI[†]	-0.008	0.002—0.018	0.106
+age, waist circumference	-0.009	0.001—0.018	0.085

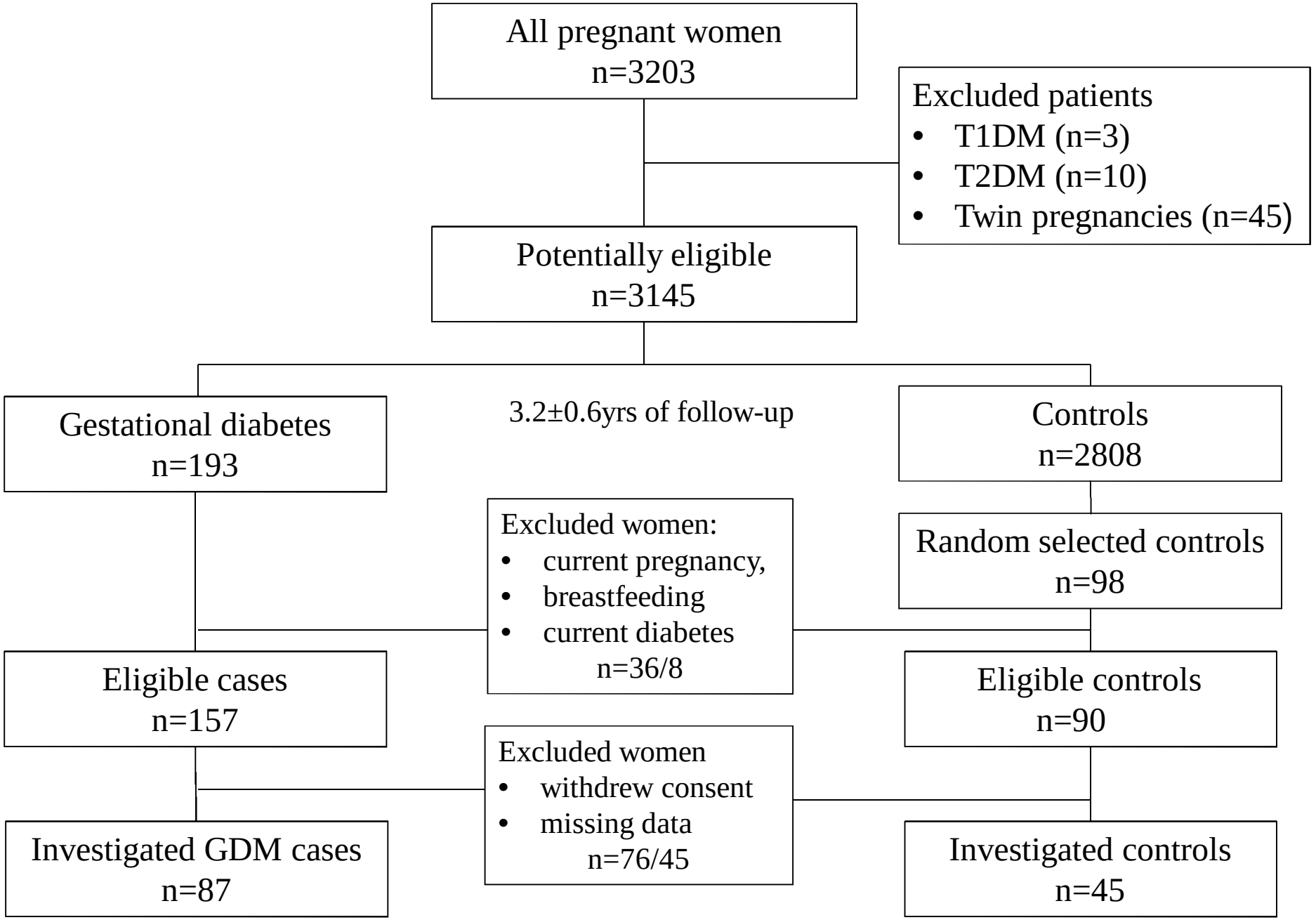
B

Output index HOMA2-B	β	95% CI	P
Age (year)	-0.032	-0,003--0,061	0,032
BMI[†] (kg/m²)	0.018	-0.005-0.042	0.018
γGT[•] (IU/L)	0.012	0.0002-0.024	0.044
120-minute insulin (μIU/ml)	0.158	0.05-0.266	0.005

[†]: Body Mass Index, [•]: γ-glutamyl-transferase

Multiple linear regression with log-transformed HOMA2-B as outcome variable.

Independent determinants of HOMA2-B were selected using backward stepwise elimination of non-significant terms. Other parameters available for the model were: triglyceride and systolic blood pressure (r^2=0.541 for the model).


All pregnant women
n=3203
Excluded patients
• T1DM (n=3)
• T2DM (n=10)
• Twin pregnancies (n=45)
Potentially eligible
n=3145
Gestational diabetes
n=193
3.2±0.6yrs of follow-up
Controls
n=2808
Excluded women:
• current pregnancy,
• breastfeeding
• current diabetes
n=36/8
Random selected controls
n=98
Eligible cases
n=157
Eligible controls
n=90
Excluded women
• withdrew consent
• missing data
n=76/45
Investigated GDM cases
n=87
Investigated controls
n=45