Effect of 1,25-dihydroxycholecalciferol on glucose metabolism in gestational diabetes mellitus

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Summary The effect of 1,25-dihydroxyvitamin D supplement on glucose metabolism was evaluated in 12 women with gestational diabetes mellitus (GDM). All women had an oral glucose tolerance test (OGTT) performed at inclusion in the study. Thereafter, each patient was instructed to continue their normal diet during the following 2 days, after which they received $2 \mu g/m^2$ Etalpha i. v. 2 h prior to the second OGTT. During the next 14 days each patient received $0.25 \mu g$ Etalpha orally, after which a third OGTT was performed. On average the level of 1,25-dihydroxyvitamin D_3 at inclusion at baseline significantly increased after i. v. 1,25-dihydroxyvitamin D_3 (from 92 to 138 ng/l [$p < 0.001$]), but returned to the baseline level after 2 weeks of oral Etalpha (85 ng/l). Simultaneously, the glucose level decreased from 5.6 to 4.8 mmol/l ($p < 0.01$) after i. v. treatment with 1,25-dihydroxyvitamin D_3, but did not differ significantly from inclusion following 2 weeks of oral Etalpha. There was no difference between the glucose concentrations during OGTT prior to and after i. v. or oral 1,25-dihydroxyvitamin D_3, in contrast to the insulin levels which tended to be lower after both i. v. and oral supplementation. In a multiple regression model including 1,25-dihydroxyvitamin D_3, insulin , weight and height against glucose, only 1,25-dihydroxyvitamin D_3 and insulin were selected for the final model ($r^2 = 0.73$, $p < 0.0001$). Our results suggest that supplements of 1,25-dihydroxyvitamin D_3 influence glucose metabolism in patients with GDM probably by increasing the insulin sensitivity. [Diabetologia (1997) 40: 40–44]

Keywords Gestational diabetes mellitus, cholecalciferols.

Several studies have demonstrated specific receptors for 1,25-dihydroxyvitamin D in pancreatic beta cells suggesting a role for 1,25-dihydroxyvitamin D in the regulation of insulin secretion [1, 2]. Others have shown that vitamin D depleted animals exhibited impaired glucose clearance and insulin secretion, which were abolished by vitamin D supplement [3–6]. This beneficial effect appeared early after i. v. injection of 1,25-dihydroxyvitamin D.

In humans the number of studies evaluating the effect of vitamin D on glucose metabolism are increasing. Recently, studies have shown that vitamin D depleted humans have reduced insulin secretion [7, 8], but this was reversed after vitamin D treatment for 3–6 months. In diabetic patients bone disease is often observed suggesting that this group also suffers from impaired vitamin D metabolism. So far, however, conflicting results have been reported on the level of 1,25-dihydroxyvitamin D in diabetic patients [9, 10]. This probably explains the diverging results of vitamin D treatment on glucose tolerance in this group [11, 12].

Others have evaluated the effect of vitamin D treatment in renal failure [13, 14]. In end-stage renal failure the circulating level of 1,25-dihydroxyvitamin D decreases and at the same time impaired glucose

tolerance arises [15]. Mak [13] demonstrated that i. v. administration of 2 μg/m² of 1,25-dihydroxyvitamin D reversed glucose intolerance in such patients. Similarly, Quesada et al. [14] showed that 2 weeks of treatment with oral calcitriol 0.5 μg/day significantly increased serum insulin concentration.

The presence of insulin resistance in late human pregnancy is well-known [16]. During this period the production of 1,25-dihydroxyvitamin D increases [17]. In gestational diabetes mellitus (GDM) and especially in insulin-dependent diabetic pregnant women the concentration of 1,25-dihydroxyvitamin D remains low in late pregnancy compared to normal pregnant women [18, 19].

It is not known whether 1,25-dihydroxyvitamin D increases insulin secretion in GDM. We therefore undertook a study in order to evaluate whether 1,25-dihydroxyvitamin D_3 supplement could improve glucose metabolism in such patients.

Subjects, materials and methods

Twenty consecutive women admitted to hospital because of suspicion of GDM were invited to participate in the study. Of these, 12 had an abnormal glucose tolerance test defined as two or more serum glucose values 3 SD above normal average and without abnormal glucose tolerance or glucosuria after pregnancy. The gestational age at inclusion (assessed by ultrasound measurements of the biparietal diameter in week 16 of pregnancy) was on average 27 weeks (range 17–33) and height and weight were 165 cm (163–8 cm) and 80 kg (53–102 kg), respectively. All were nulliparous and all delivered healthy children appropriate for gestational age. None of the patients received insulin preparations, supplements of vitamin D or calcium during pregnancy. Written informed consent was obtained from all subjects. The study was approved by the local ethical committee.

An oral glucose tolerance test (OGTT) was performed in the morning at the same time for each group after an overnight fast using an oral dose of 75 g glucose. Blood samples were taken at baseline (–5 min) and after 30, 60, 90, 120, 150 and 180 min for measurements of glucose and insulin. Concentrations of triglycerides, total cholesterol, non-esterified fatty acids (NEFA), parathyroid hormone, 1,25-dihydroxyvitamin D_3, ionized calcium adjusted to pH 7.40, magnesium and phosphate were measured only at baseline, –5 min. Each patient was instructed to continue their normal diet during the following 2 days, after which they underwent a second OGTT after an overnight fast. Two hours prior to this OGTT the patients received 2 μg/m² Etalpha i.v. During the following 14 days each patient received daily 0.25 μg Etalpha orally after which a third OGTT was performed following an overnight fast. The final drug dose was taken the day the OGTT was performed. During the latter period the patients received a restricted diet until 3 days prior to the third OGTT. The patients initially received 20 tablets and had to return any remaining tablets after the 2-week treatment period.

Methods. Insulin was measured by an enzyme linked immonosorbent assay (ELISA) as described previously [20]. The intra- and interassay CVs were 6.4 and 5.0 %, respectively. Detection limit was 3 pmol/l.

Table 1. Baseline fasting levels of measured variables in gestational diabetic women prior to, and after intravenous and oral treatment with 1,25-dihydroxyvitamin D

Serum/plasma parameter	Inclusion	2 h after i. v. vitamin D	14 days after oral vitamin D
Glucose (mmol/l)	5.6 ± 0.8	4.8 ± 0.6^a	5.3 ± 0.8
Insulin (pmol/l)	70 ± 34	65 ± 24	62 ± 22
Triglycerides (mmol/l)	2.96 ± 1.12	2.85 ± 1.04	2.69 ± 1.20
Cholesterol (mmol/l)	6.3 ± 1.0	6.2 ± 1.1	6.1 ± 1.4
NEFA (mmol/l)	0.92 ± 0.34	0.97 ± 0.43	0.76 ± 0.46
1,25-dihydroxy-vitamin D_3 (ng/l)	92 ± 27	138 ± 27^b	85 ± 19
Parathyroid hormone (ng/l)	35 ± 14	37 ± 16	35 ± 14
Ionized calcium (mmol/l)	1.24 ± 0.06	1.26 ± 0.06	1.25 ± 0.05
Magnesium (mmol/l)	0.74 ± 0.05	0.74 ± 0.03	0.72 ± 0.07
Phosphate (mmol/l)	1.09 ± 0.12	1.07 ± 0.13	1.06 ± 0.08

Values are mean $\pm$ SD
[a] $p < 0.01$; [b] $p < 0.001$

The active form of vitamin D $(1,25(OH)_2D_3)$ was measured by radioimmunoassay [21]. 1,25-dihydroxyvitamin D_3 was extracted by incubation with a highly specific solid phase monoclonal anti-1,25D and reconstituted eluates were incubated overnight with a highly specific 1,25D sheep anti-1,25D to which ^{125}I-1,25D is added. The intra- and interassay CVs were 7 and 14 %, respectively; the detection limit of the assay was 3 ng/l.

Intact parathyroid hormone was measured by an immunoradiometric assay [22]. The intra- and interassay CVs were 5 and 6 %, respectively. The detection limit of the assay was 3 ng/l.

Plasma glucose, triglycerides, total cholesterol and NEFA were measured using enzymatic colorimetric tests. Ionized calcium was measured at pH 7.40 using an ICA 2 (Radiometer, Denmark). Serum concentrations of phosphate were measured by a multichannel analyser (Technicon SMAC 3 Analyzer; Terrytown, N.Y., USA). Serum magnesium was measured by atomic absorption spectrophotometry.

Statistical analysis

All data are presented as mean $\pm$ SD. Analysis of variance was used for evaluation of time-related changes. Wilcoxon signed rank test was used for comparison of groups. Correlations were calculated by linear regression analysis by the least square method (Statgraphics Inc., version 2.6). A p-value less than 0.05 was considered statistically significant.

Results

From Table 1 it appears that the serum level of 1,25-dihydroxyvitamin D_3 at inclusion significantly increased after i. v. 1,25-dihydroxyvitamin D_3 from 92 to 138 ng/l ($p < 0.001$), but did not differ significantly from the inclusion level after oral supplement of 1,25-dihydroxyvitamin D_3 (85 ng/l). Simultaneously,
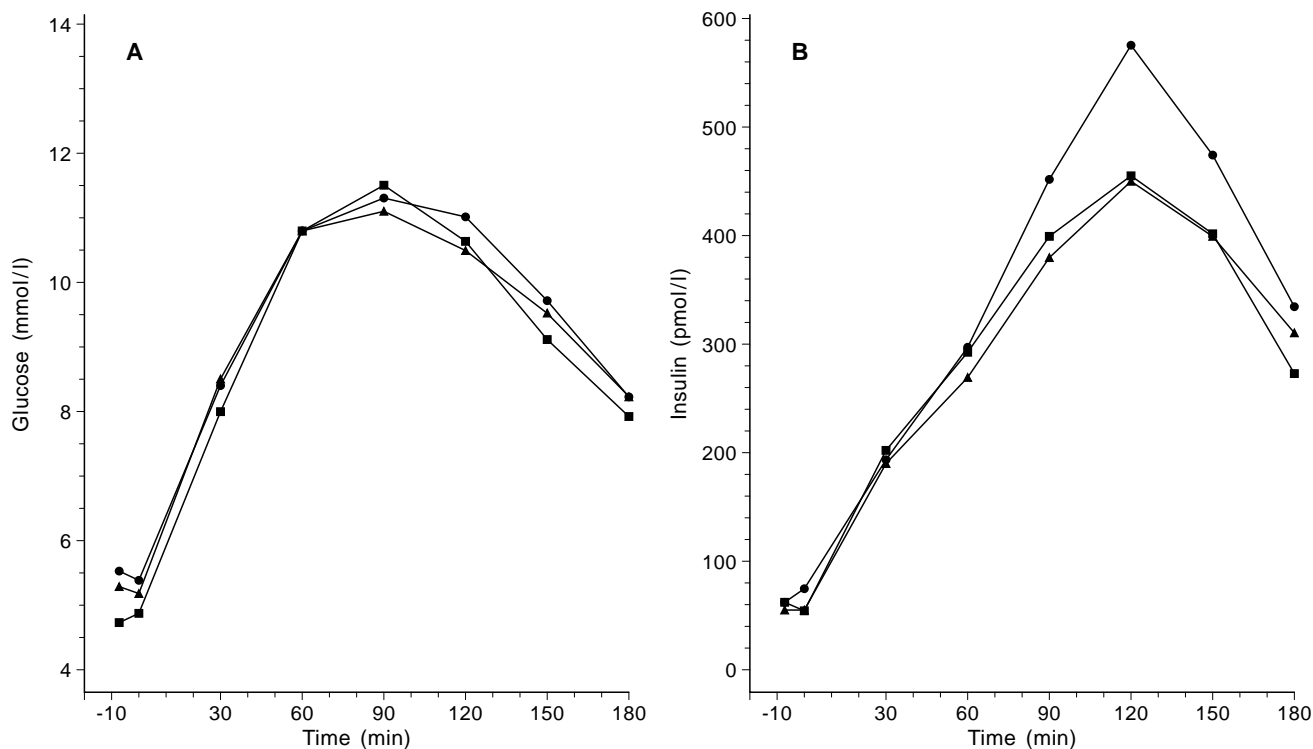
Fig. 1 A, B. Plasma glucose (**A**) and serum insulin (**B**) levels in response to oral glucose tolerance test (75 g) in pregnant women with gestational diabetes mellitus without supplementation of 1,25-dihydroxyvitamin (●), and after intravenous injection (■) and oral treatment with 1,25-dihydroxyvitamin D (▲)

Table 2. Changes in insulin concentrations during OGTT in percent of baseline values

Time (min)	Inclusion	2 h after i. v. vitamin D	14 days after oral vitamin D
Baseline = 0	100	100	100
30	355 ± 165	333 ± 127	353 ± 141
60	572 ± 208	479 ± 221	483 ± 150
90	779 ± 209	625 ± 216	657 ± 232
120	926 ± 306	723 ± 292	780 ± 346
150	743 ± 209	640 ± 157	717 ± 367
180	587 ± 231	451 ± 166	542 ± 264

Values are mean ± SD

the level of glucose decreased from 5.6 to 4.8 mmol/l ($p < 0.01$) after i. v. treatment with 1,25-dihydroxyvitamin D_3, but did not differ significantly from inclusion level after 2 weeks of oral 1,25-dihydroxyvitamin D_3. No other parameter measured at baseline was influenced by treatment with 1,25-dihydroxyvitamin D_3, either i. v. or orally.

Figure 1 presents plasma concentrations of glucose and insulin during OGTT at inclusion, 2 h after i. v. 1,25-dihydroxyvitamin D_3 and after 2 weeks of daily oral 1,25-dihydroxyvitamin D_3. There was no statistically significant difference between the curves regarding glucose levels, whereas the levels of insulin

tended to be lower at 120 min after both i. v. and oral treatment. Calculating insulin changes in percent of baseline values the difference became statistically significant comparing before and after i. v. 1,25-dihydroxyvitamin D_3 ($645 ± 296$ vs $543 ± 234\%$, $p < 0.05$, mean ± SD), whereas the difference between the mean values before and after oral 1,25-dihydroxyvitamin D_3 did not reach statistical significance ($645 ± 296$ vs $588 ± 293\%$, $p = 0.13$) (Table 2).

There was a significant negative relationship between the concentrations of glucose and 1,25-dihydroxyvitamin D_3 ($r = -0.41$, $p < 0.01$) and a positive relationship between concentrations of glucose and parathyroid hormone ($r = 0.31$, $p = 0.07$), when all measurements were included in the analysis. The concentrations of glucose correlated significantly with insulin ($r = 0.51$, $p < 0.002$). In a multiple regression analysis including concentrations of 1,25-dihydroxyvitamin D_3, insulin, parathyroid hormone, weight and height as independent parameters and glucose as dependent, only 1,25-dihydroxyvitamin D_3 and insulin turned out to be included in the final model ($r^2 = 0.73$, $p < 0.0001$).

Discussion

There are several reports on the beneficial effects of 1,25-dihydroxyvitamin D on glucose tolerance. However, the dose given and the administration route differ markedly between the studies. In vitamin D depleted humans supplements of 2000 IU vitamin D_3 per day [7] or a single i. m. dose of 100 000 IU were

given [8]. Non-insulin-dependent diabetic subjects received orally 2 µg 1α (OH) D₃ [11]. In renal failure other regimes were used [13, 14]. These differences make any comparison of the studies difficult. Furthermore, it is difficult to select the optimal treatment regimen.

In the present study, we evaluated the effect of i. v. 1,25-dihydroxyvitamin D_3 using a regimen previously described [13]. The pregnant women received a lower oral dose than that used by others [11, 14] to avoid hypercalcaemia in pregnancy.

Our study shows that i. v. 1,25-dihydroxyvitamin D_3 significantly decreased fasting glucose level in GDM. However, this effect was short-lived since we could not demonstrate any effect on plasma glucose levels during the following OGTT. Neither could daily oral supplementation of Etalpha prescribed for 2 weeks after the initial i. v. bolus of 1,25-dihydroxyvitamin D_3 prevent the initially observed effect on fasting glucose. In contrast the level of insulin apparently decreased after i. v. and oral treatment.

The explanation for the lower glucose level prior to OGTT after i. v. injection of 1,25-dihydroxyvitamin D_3 in our study could not be attributed to an increased insulin level. This is in contrast to most other studies suggesting that the effect of 1,25-dihydroxyvitamin D on glucose metabolism may be through influence on insulin secretion from the pancreatic beta cells [1, 2, 8, 11, 13, 23]. However, in these studies the results are difficult to interpret, since they either report no or minimal changes in glucose or insulin levels [11, 13] or that both variables increased during vitamin D treatment [8].

Another mode of action of 1,25-dihydroxyvitamin D_3 on glucose metabolism could be increasing the cellular absorption of glucose, either directly or by enhancing the effect of insulin. In support of this hypothesis is our observation of lower levels of insulin during the OGTT after i. v. and oral treatment and no change in the level of glucose. The presence of a positive relationship between the concentrations of glucose and 1,25-dihydroxyvitamin D_3 further substantiates this hypothesis. Also, 1,25-dihydroxyvitamin D_3 was included in the regression analysis explaining 73 % of the variation in fasting plasma glucose together with insulin. However, precise knowledge of the mechanism by which 1,25-dihydroxyvitamin D_3 interferes with glucose metabolism must await further studies.

We did not observe any influence of oral 1,25-dihydroxyvitamin D_3 on fasting levels of glucose and 1,25-dihydroxyvitamin D_3. Since all patients had to return the remaining Etalpha tablets after the end of the study, we do not think that the low level of 1,25-dihydroxyvitamin D_3 was due to poor compliance, rather it may have been due to our use of a low dose (0.25 µg/day) compared to other studies using either 2 µg or 0.5 µg per day [11, 14]. On the other hand, this regimen decreased insulin levels to the same extent as i. v. administration. Since the patients received a restricted diet during the oral treatment study we cannot exclude that the change in diet may, in part, be responsible for the observed change in insulin levels.

We expected only minor changes in calcium homeostasis following i. v. 1,25-dihydroxyvitamin D_3 taking the short duration from injection and blood sampling into account. On the other hand, we expected that oral 1,25-dihydroxyvitamin D_3 would increase the intestinal calcium absorption. Since we used a rather low oral dose, this is probably the major reason for the lack of change in calcium-related parameters.

In conclusion, our results demonstrate that administration of 1,25-dihydroxyvitamin D_3 tends to decrease fasting glucose concentration in GDM. This may be due to increased insulin sensitivity, according to our findings of lower levels of insulin during OGTT. However, the exact mechanism by which 1,25-dihydroxyvitamin D_3 acts on glucose metabolism is not yet clear.

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