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# Effect of large doses of parenteral vitamin D on glycaemic control and calcium/phosphate metabolism in patients with stable type 2 diabetes mellitus: a randomised, placebo-controlled, prospective pilot study

*Sigrid Jehle[a], Alessia Lardi, Barbara Felix[a], Henry N. Hulter[b], Christoph Stettler[c], Reto Krapf[a]*

[a] Department of Medicine, Kantonsspital Bruderholz, University of Basel, Switzerland
[b] Department of Medicine, University of California, San Francisco, USA
[c] Division of Endocrinology and Diabetology, Insel University Hospital, Berne, Switzerland

## Summary

OBJECTIVE: Vitamin D ($D_3$) status is reported to correlate negatively with insulin production and insulin sensitivity in patients with type 2 diabetes mellitus (T2DM). However, few placebo-controlled intervention data are available. We aimed to assess the effect of large doses of parenteral $D_3$ on glycosylated haemoglobin ($HbA_{1c}$) and estimates of insulin action (homeostasis model assessment insulin resistance: HOMA-IR) in patients with stable T2DM.

MATERIALS AND METHODS: We performed a prospective, randomised, double-blind, placebo-controlled pilot study at a single university care setting in Switzerland. Fifty-five patients of both genders with T2DM of more than 10 years were enrolled and randomised to either 300,000 IU $D_3$ or placebo, intramuscularly. The primary endpoint was the intergroup difference in $HbA_{1c}$ levels. Secondary endpoints were: changes in insulin sensitivity, albuminuria, calcium/phosphate metabolism, activity of the renin-aldosterone axis and changes in 24-hour ambulatory blood pressure values.

RESULTS: After 6 months of $D_3$ supply, there was a significant intergroup difference in the change in $HbA_{1c}$ levels (relative change [mean ± standard deviation] +2.9% ± 1.5% in the $D_3$ group vs +6.9% ± 2.1% the in placebo group, p = 0.041) as HOMA-IR decreased by 12.8% ± 5.6% in the $D_3$ group and increased by 10% ± 5.4% in the placebo group (intergroup difference, p = 0.032). Twenty-four-hour urinary albumin excretion decreased in the $D_3$ group from 200 ± 41 to 126 ± 39, p = 0.021). There was no significant intergroup difference for the other secondary endpoints.

CONCLUSIONS: $D_3$ improved insulin sensitivity (based on HOMA-IR) and affected the course of $HbA_{1c}$ positively compared with placebo in patients with T2DM.

Clinical trial registration number at ClinicalTrials.gov: NCT01585051

***Key words:*** *Diabetes mellitus; vitamin D; FGF-23; insulin sensitivity*

**Abbreviations**

| | |
|---|---|
| CHD | = coronary heart disease |
| FGF-23: | fibroblast growth factor 23 |
| $HBA_{1c}$: | glycosylated haemoglobin |
| HOMA: | homeostasis model assessment |
| HOMA-IR: | homeostasis model assessment insulin resistance |
| hsCRP: | high-sensitivity C-reactive protein |
| PTH: | parathyroid hormone |
| T2DM: | type 2 diabetes mellitus |
| T1DM: | type 1 diabetes mellitus |

## Introduction

Epidemiological and observational evidence suggests that vitamin D ($D_3$) supply inversely correlates with the risk for T2DM and, once diabetic, serum 25(OH)-$D_3$ levels correlate inversely with impaired glucose tolerance [1, 2]. Since cardiovascular events are greatly increased in T2DM, it has been suggested that $D_3$ status measured as serum 25(OH)-$D_3$ levels might be a modifiable risk factor for cardiovascular events in T2DM patients, as well as in the general population [1, 2]. $D_3$ is required for and improves the production of insulin, and is also implicated in the mechanism of insulin action [3, 4]. However, in both nondiabetic and diabetic patients, the clinical associations of $D_3$ with insulin resistance and beta-cell function are inconsistent [2, 5, 6], and reported intervention studies employing $D_3$ either as 25(OH)-$D_3$ (e.g., cholecalciferol) or as 1,25$(OH)_2$-$D_3$ (e.g., calcitriol) have yielded conflicting results that are difficult to interpret owing to lack of placebo control [7, 8].

Supplementation of $D_3$ and calcium (400 IU $D_3$ and 1000 mg calcium daily) did not reduce the risk of developing diabetes in the Women' s Health Study over 7 years of

follow-up [7] and supplementation of $D_3$ in normal subjects (20,000 IU $D_3$ orally twice weekly for 6 months) did not affect insulin secretion nor sensitivity [8], whereas supplementing 700 IU $D_3$ daily over 3 years was found to attenuate the increases in glycaemia and insulin resistance in elderly subjects with impaired fasting glucose at baseline [9]. In a short-term study (4 weeks) in nondiabetic subjects with $D_3$ deficiency (25(OH)-$D_3$ <25 nmol/l) two oral doses of $D_3$ (100,000 IU $D_3$ 2 weeks apart) had no significant effect on serum glucose, insulin concentration and insulin sensitivity assessed with an oral glucose tolerance test [10]. However, in a study of subjects at risk for T2DM, oral supplementation of $D_3$ (2,000 IU $D_3$ daily for 4 months) was shown to improve beta-cell function, but not insulin sensitivity [11].

Results from randomised controlled trials that evaluated the specific effects of 25(OH)-$D_3$ or 1,25$(OH)_2$-$D_3$ (without also adding calcium to $D_3$) on glucose and insulin homeostasis in T2DM patients have been conflicting [2, 12–20]. A recently published systematic review examining the effect of vitamin D supplementation in 15 newer published studies [21] again found discrepancies in outcomes, which may be due to heterogeneous study populations (number of patients included, stage of diabetes, gender, age, oral or insulin treatment) or to heterogeneous interventions (oral, parenteral, dose, duration).

In view of the suggestive but inconclusive evidence for a clinically important effect of exogenous $D_3$ supplementation on glucose and insulin homeostasis in both normal and diabetic subjects and as few placebo-controlled intervention data are available, we wished to assess insulin sensitivity to large doses of $D_3$ in a double-blinded, randomised, placebo-controlled trial in stable T2DM patients. In addition, in view of the lack of information on responses of calcium/phosphate metabolism, calci-/phosphotropic hormones and 24-hour ambulatory blood pressures to large doses of $D_3$ in T2DM patients, this study also explored these data.

## Methods

### Study design and treatment protocol

This prospective, randomised, double-blind, placebo-controlled pilot study was performed at a single university care setting in Switzerland. The study was approved by the local internal review board (EKBB, University of Basel), the study subjects gave written, informed consent and were paid CHF 50.00 for each office visit. Patients were recruited from the ambulatory care facilities (diabetology and cardiology) of the hospital. Randomisation was performed by a pharmacist using a computer program. He provided the randomisation codes and vials containing $D_3$ or 0.9% NaCl. A nurse not involved in the study administered the injections, either $D_3$ (cholecalciferol, 300,000 IU, 1 ml intramuscularly, vitamin D3 Streuli Inc., Switzerland), or placebo (0.9% NaCl, 1 ml intramuscularly) in a blinded way. After 3 months, all patients received a blinded repeat injection which contained either 0.9% NaCl 0.5 ml (placebo arm or for $D_3$ replete patients in the $D_3$ arm) or $D_3$ 150,000 IU, 0.5 ml (only patients in the $D_3$ arm when serum 25(OH)-$D_3$ levels were below 80 nmol/l in the $D_3$ arm and hypercalcaemia of any degree and hypercalciuria [≥8 mmol/24 hours] remained absent). An independent (non-study) physician evaluated the serum 25(OH)-$D_3$ and calcium results and allocated $D_3$/NaCl administration via the hospital pharmacist who had provided the randomisation code.

### Inclusion criteria

Men and nonpregnant women aged ≥18 years, with T2DM, living independently at home with stable blood glucose control for the past 2 months (less than 2 hypoglycaemic episodes in the past 2 months, unchanged doses of anti-hyperglycaemic agents in the last 3 months and stable glycosylated haemoglobin [$HbA_{1c}$] levels for the past 6 months [variation by less than ±0.7%]). Blood pressure was to be stable below 145/85 mm Hg during the past 2 months under a fixed current regimen of blood pressure medications (if any) and/or potassium supplements (if any). Both diabetic and blood pressure therapies had to be judged as unlikely to require change in the subsequent 6 months by the referring diabetologist and cardiologist. It was prespecified that if changes in these medication regimens were needed during the study, these subjects would be regarded as drop-outs and not included into the analysis.

### Exclusion criteria

- Patients with type 1 diabetes mellitus (T1DM) or insulin-requiring diabetes of undetermined type
- Patients on haemodialysis, with hyperparathyroidism or active cancer disease
- Patients with known metabolic bone disease
- Laboratory evidence of kidney (estimated glomerular filtration rate <60 ml/min) or liver disease
- Dietary calcium intake exceeding 1,500 mg/d (estimated from diet history)
- 25(OH)-$D_3$ levels at baseline ≥70 nmol/l
- Hypercalciuria (>8 mmol/24 hours, measured by means of 24-hour urine collections)
- Hypo- and hypercalcaemia and hypo- and hyperphosphatemia of any cause
- Drugs that affect $D_3$ metabolism (e.g., antiepileptic drugs, calcimimetics, 1-34 PTH, bisphosphonates, calcitonin, $D_3$ therapy over and above 400 IU orally daily) 6 months prior to enrolment and during the study
- History of binge eating or weight gain or loss exceeding 6 kg in past 18 months
- Patients taking any type of coagulation inhibitors (i.e., coumadin, heparin, etc.)

### Biochemical assays and blood pressure measurements

All biochemical analyses were performed in duplicate. All baseline measurements were done twice, 1 week apart and the baseline values reported are the means of these two measurements. Standard biochemical parameters in blood and 24-hour urine collections were determined by the hospital department of clinical chemistry using standard, state-of-the-art methodology as described in reference [22]. All subjects fasted overnight for 9 am blood draws. In insulin treated-patients, no insulin was administered after the final

prescribed dose on the prior day. All oral medications were withheld until after the fasting blood draw. $HbA_{1c}$ was determined by means of high performance liquid chromatography (HPLC). Homeostasis model assessment insulin resistance (HOMA-IR) was calculated using the published HOMA formula [23]. The following endocrine analyses were made with enzyme-linked immunosorbent assays: insulin, proinsulin, C-peptide, intact PTH, C-terminal FGF-23, plasma renin activity and plasma aldosterone. 25(OH)-$D_3$, 1,25$(OH)_2$-$D_3$ and tetrahydro-aldosterone (urine) were determined by means of HPLC. Twenty-four-hour blood pressure readings were recorded using Cardioline® equipment. The equipment was used by an experienced study nurse.

### Statistical analysis

Randomisation was unstratified and unblocked. All analyses are based on the intention-to-treat population, comprising all randomised subjects. Intragroup comparisons (to own group baseline) and intergroup comparison (between the groups) were carried out using the paired t-test for biochemical data, and results are reported as arithmetic means and 95% confidence intervals. The Wilcoxon signed rank test was applied for the analysis of biochemical data that were not normally distributed ($HbA_{1c}$ and HOMA-IR) and results are reported as geometric means. The effect of treatment was evaluated by calculating the percentage change from baseline for all variables studied for all analysis, a two-tailed p-value <0.05 was considered to indicate statistical significance. For analysis of the potential for differing treatment effects in patients with and without insulin treatment, two-way analysis of variance was used. Statistical analysis was performed using SSPS for Windows NT, version 20.0 (SSPS Inc., Chicago, IL).

### Primary endpoint

Change in $HbA_{1c}$ levels at 6 months.

### Secondary, exploratory and safety endpoints

Changes in HOMA-IR (calculated as described in reference [23]) at 3 and 6 months. Changes in calcium/phosphate metabolism, calci-/phosphotropic hormones. Changes in proinsulin levels, renin/aldosterone activity/concentration, 24-hour aldosterone excretion rate, 24-hour urinary albumin excretion, mean systolic and diastolic 24-hour blood pressure values, all at 3 and 6 months.

## Results

A total of 142 patients with T2DM were recruited between October and December 2009, 77 were screened and 55 fulfilled the entry criteria, consented and were enrolled into the study (n = 29 to $D_3$, n = 26 to the placebo group, (fig. 1). Baseline characteristics of the study subjects are summarised in table 1. There were no significant differences between the two treatment groups at baseline (table 1, p-values). All of the 55 study participants completed the study (fig. 1) and there was no change in either antihyperglycaemic drugs (insulin requirements) nor in the number and dose of antihypertensive drugs.

**Table 1:** Baseline characteristics of the participants, according to the study groups (mean ± standard deviation).

| Parameter | Vitamin D ($D_3$) (n = 29) | Placebo (n = 26) | p-value |
|---|---|---|---|
| Age (y) | 66.9 ± 3.1 | 63.7 ± 3.5 | 0.367 |
| Gender | 10 M (34.5%), 19 F (65.5%) | 10 M (38.5%), 16 F (61.5%) | |
| BMI (kg/m²) | 28.9 ± 4.3 | 28.1 ± 3.8 | 0.169 |
| Current smokers | 4 (13.8%) | 4 (15.4%) | |
| Ex-smokers | 10 (34.5%) | 12 (38.5%) | |
| Alcohol, drinks per day | 0.7 ± 0.2 | 1.0 ± 0.3 | 0.124 |
| Mean SBP (mm Hg)/24h | 130.7 ± 14.7 | 136.3 ± 14.4 | 0.849 |
| Mean DBP (mm Hg)/24h | 82.3 ± 6.0 | 82.4 ± 11.3 | 0.912 |
| Duration of diabetes (years) | 12.7 ± 1.7 | 12.6 ± 1.9 | 0.764 |
| $HbA_{1c}$ (%) | 7.0 ± 1.1 | 7.2 ± 0.9 | 0.440 |
| Creatinine clearance (ml/min) | 101 ± 41 | 114 ± 46 | 0.321 |
| Urinary albumin excretion (mg/24h) | 200 ± 41 | 84 ± 21 | 0.045 |
| 25(OH)-$D_3$ (nmol/l) | 36.0 ± 18.1 | 28.1 ± 14.4 | 0.612 |
| Antihyperglycaemic drugs | | | |
| Metformin | 20 (77%) | 23 (79%) | 0.698 |
| Sulfonylureas | 10 (34%) | 4 (15%) | 0.047 |
| Pioglitazone | 6 (23%) | 4 (14%) | 0.401 |
| GLP-1 receptor signalling | 5 (17%) | 1 (4%) | 0.041 |
| Insulin | 12 (41%) | 17 (65%) | 0.249 |
| Statins | 17 (59%) | 15 (58%) | 0.812 |
| Antihypertensive drugs | | | |
| Thiazides | 13 (45%) | 14 (54%) | 0.511 |
| Beta-blockers | 11 (38%) | 15 (58%) | 0.216 |
| ACE-inhibitors | 10 (35%) | 14 (54%) | 0.304 |
| AT-1 receptor antagonists | 10 (35%) | 14 (54%) | 0.231 |
| Calcium channel blockers | 8 (28%) | 5 (19%) | 0.412 |

ACE = angiotensin converting enzyme; AT = angiotensin; BMI = body mass index; DBP = diastolic blood pressure; GLP-1 = glucagon-like peptide-1; $HbA_{1c}$ = glycosylated haemoglobin; SBP = systolic blood pressure

### Effect of $D_3$ on $HbA_{1c}$ and HOMA-IR

In both groups and without change in the antihyperglycaemic medication regimen, $HbA_{1c}$ increased nonsignificantly when compared to baseline (table 2). However, $HbA_{1c}$ increased significantly less in patients treated with $D_3$ than in the placebo group (mean ± standard deviation +2.9% ± 1.5% vs +6.9% ± 2.1%, p = 0.041, table 2, fig. 2). There was also a significant treatment effect on HOMA-IR (table 2 and fig. 2): whereas HOMA-IR decreased by -12.8% ± 5.6% in the $D_3$ group, it increased by +10% ± 5.4% in the placebo group (p = 0.032). There was no significant difference in the serum levels of high-sensitivity C-reactive protein (hsCRP) as a marker of systemic inflammation (table 2) for both the intra- and intergroup comparisons. We found no statistically significant interaction of $D_3$ treatment effect on insulin treatment.

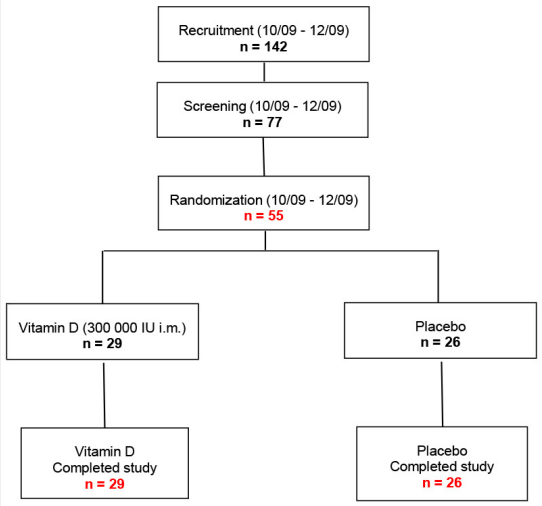


**Figure 1**

Study flowchart

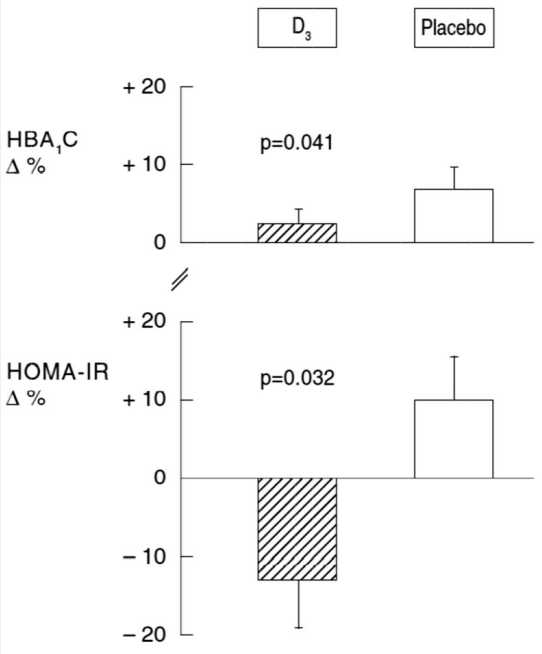


**Figure 2**

Effect of $D_3$ supplementation on the percent changes in $HbA_{1c}$ and HOMA-IR.

$HbA_{1c}$ = glycosylated haemoglobin; HOMA-IR = homeostasis model assessment insulin resistance

### Effect of $D_3$ on calcium/phosphate metabolism and on calci-/phosphotropic hormones

Administration of $D_3$ significantly suppressed intact PTH (table 3), had no effect on plasma ionised calcium and phosphate concentrations (table 4), but significantly increased calciuria in intra- and intergroup comparisons at 3 and 6 months (table 5). In the placebo group, intact PTH was suppressed significantly at 6 months without significant changes in plasma and urinary calcium and phosphate concentrations and 24-hour excretion rates. Serum $1,25(OH)_2$-$D_3$ increased significantly in response to $D_3$, as did fibroblast growth factor 23 (FGF-23), an osteocyte/osteoblast-derived phosphaturic hormone, when compared with baseline values in the $D_3$, but not in the placebo group (table 3). $D_3$ supplementation significantly increased serum 25(OH)-$D_3$ levels in comparison with baseline and in comparison with placebo (table 3). Eleven of the 29 subjects in the $D_3$ group needed a second injection of 150,000 IU $D_3$ after 3 months. The placebo group also exhibited a significant intragroup increase in the serum 25(OH)-$D_3$ concentration from 28 to 62 nmol/l, probably owing to increased sun exposure in the second part of the study. The serum 25(OH)-$D_3$ concentrations correlated positively and significantly with the later termination of the study in the spring/summer months (data not shown).

### Effects of $D_3$ on 24-hour albumin excretion rates and activity of the renin/aldosterone axis

Twenty-four-hour urinary albumin excretion decreased in the $D_3$ group from 200 ± 41 mg to 126 ± 39 mg, p = 0.021, table 5). There was no significant change in plasma active renin and aldosterone concentrations and in the 24-hour excretion rates of the tetrahydro metabolite of aldosterone (table 6).

### Effects of $D_3$ on 24-hour ambulatory blood pressure

Twenty-four-hour ambulatory systolic and diastolic blood pressures decreased significantly within both groups with no significant intergroup difference (table 7).

### Adverse effects

One patient in the placebo group developed a small abscess at the injection site (after the 3-month injection), which healed without antibiotics or surgical intervention. No other side effects were reported.

## Discussion

This study examined a population of slightly $D_3$ deficient (defined as <50 nmol/l [24]), metabolically stable, long-standing (>10 years) T2DM patients with adequate baseline blood pressure and acceptable glycaemic control ($HbA_{1c}$ 7.1% ± 1.0%, table 1). The main findings were: first, $HbA_{1c}$ showed a differential course during treatment with $D_3$, with a significantly smaller increase in the treatment group compared with placebo. Second, markers of in-

sulin resistance were significantly reduced in individuals treated with $D_3$ compared with placebo.

HOMA-IR has been shown to correlate closely with analysis of insulin sensitivity by the euglycaemic insulin clamp method [25]. Based on this calculation, $D_3$ administration ameliorated insulin resistance and significantly limited the rise in $HbA_{1c}$ as compared to placebo during this 6-month intervention trial. The amelioration of insulin resistance could theoretically be indirect via the reported anti-inflammatory effects of $D_3$ [26], but this thesis was not supported by changes in hsCRP levels. However, both groups exhibited normal baseline hsCRP values, suggesting that systemic inflammatory activity was low and rendering demonstration of a putative inhibitory effect more difficult. Other studies examining the effects of $D_3$ in patients at risk for diabetes or normal subjects have failed to demonstrate a significant effect of the intervention on insulin sensitivity [9–11]. Thus, the effect of $D_3$ may be limited to establish T2DM and may depend on the degree of insulin resistance.

**Table 2:** Effect of vitamin D ($D_3$) supplementation on $HBA_{1c}$, HOMA-IR, and hsCRP (mean ± standard deviation, 95% confidence interval).

| **Diabetes parameters and hsCRP** (fasting) | **$D_3$ (n = 29)** | | | | **Placebo (n = 26)** | | | | **Treatment difference** |
|---|---|---|---|---|---|---|---|---|---|
| | Baseline | 3 months | 6 months | Change (%) | Baseline | 3 months | 6 months | Change (%) | p-value |
| Proinsulin (pmol/l) | 31.0 ± 29.4 (19.8 to 42.2) | 33.4 ± 25.6 (23.7 to 43.1) | 32.6 ± 18.9 (25.4 to 39.8) | +5.1 ± 3.9 (+3.6 to 6.6) | 49.4 ± 34.8 (35.3 to 63.5) | 55.5 ± 31.9 (42.6 to 68.4) | 63.8 ± 34.2 (50.0 to 77.6) | +14.1 ± 6.7 (+11.4 to 16.8) | 0.342 |
| C-peptide (nmol/l) | 1.2 ± 0.8 (0.9 to 1.5) | 1.1 ± 0.7 (0.8 to 1.4) | 1.2 ± 0.9 (0.9 to 1.5) | 0.0 ± 2.3 (–0.9 to 0.9) | 0.9 ± 0.8 (0.6 to 1.2) | 1.2 ± 0.9 (0.6 to 1.2) | 1.1 ± 0.7 (0.8 to 1.4) | +22.0 ± 14 (+16.3 to 27.7) | 0.045 |
| Insulin (mU/l) | 12.4 ± 9.1 (8.9 to 15.9) | 12.5 ± 8.4 (9.3 to 15.7) | 11.1 ± 7.6 (8.2 to 14.0) | –11.7 ± 9.6 (–15.4 to 8.1) | 11.4 ± 8.9 (7.8 to 15.0) | 12.3 ± 7.4 (9.3 to 15.3) | 11.4 ± 7.9 (8.2 to 14.6) | 0.0 ± 4.1 (–1.7 to 1.7) | 0.341 |
| Glucose (mmol/l) | 7.8 ± 2.3 (6.9 to 8.7) | 7.4 ± 2.4 (6.5 to 8.3) | 8.1 ± 2.5 (7.1 to 9.1) | +3.7 ± 4.1 (+2.1 to 5.3) | 7.9 ± 2.3 (7.0 to 8.8) | 7.7 ± 2.5 (6.7 to 8.7) | 8.5 ± 2.6 (7.5 to 9.6) | +7.6 ± 4.7 (+5.7 to 9.5) | 0.422 |
| $HBA_{1c}$ (%) | 7.0 ± 1.1 (6.6 to 7.4) | 7.0 ± 1.0 (6.6 to 7.4) | 7.2 ± 1.1 (6.8 to 7.6) | +2.9 ± 1.5 (+2.3 to 3.5) | 7.2 ± 0.9 (6.8 to 7.6) | 7.4 ± 1.0 (7.0 to 7.8) | 7.7 ± 0.9 (7.3 to 8.1) | +6.9 ± 2.1 (+6.1 to 7.7) | 0.041 |
| HOMA-IR | 4.4 ± 2.4 (3.5 to 5.3) | 4.1 ± 2.1 (3.3 to 4.9) | 3.9 ± 2.2 (3.1 to 4.7) | –12.8 ± 5.6 (–14.9 to 10.7) | 4.0 ± 2.3 (3.1 to 4.9) | 4.3 ± 2.1 (3.5 to 5.2) | 4.4 ± 2.5 (3.5 to 5.3) | +10 ± 5.4 (+7.8 to12.2) | 0.032 |
| hsCRP (mg/l) | 1.2 ± 3.1 (0.02 to 2.4) | 1.4 ± 2.0 (0.6 to 2.2) | 1.5 ± 2.0 (0.7 to 2.3) | +25.1±13.2 (+20.1 to 30.1) | 1.5 ± 2.7 (0.4 to 2.6) | 1.8 ± 5.4 (–0.4 to 4.0) | 1.7 ± 2.0 (0.9 to 2.5) | +13.3 ± 10.1 (+9.2 to 17.4) | 0.612 |

$HBA_{1c}$ = glycosylated haemoglobin; HOMA-IR = homeostasis model assessment insulin resistance; hsCRP = high-sensitivity C-reactive protein

**Table 3:** Effect of vitamin D ($D_3$) supplementation on calci-/phosphotropic hormones (mean ± standard deviation, 95% confidence interval).

| **Calci-/phosphotropic hormones** | **$D_3$ (n = 29)** | | | | **Placebo (n = 26)** | | | | **Treatment difference** |
|---|---|---|---|---|---|---|---|---|---|
| | Baseline | 3 months | 6 months | Change (%) | Baseline | 3 months | 6 months | Change (%) | p-value |
| 25(OH)-$D_3$ (nmol/l) | 36.0 ± 18.1 (29.1 to 42.9) | 78.9 ±17.5*“ (72.2 to 85.6) | 84.9 ±16.0* (78.8 to 91.0) | +136 ± 17 (+131 to 144) | 28.1 ± 17.4 (21.1 to 35.1) | 39.9 ± 18.9 (32.3 to 47.5) | 61.7 ± 16.0* (55.2 to 68.2) | +119 ± 21 (+111 to 128) | 0.011 |
| 1,25(OH)-$D_3$ (nmol/l) | 50 ± 7 (47.3 to 52.7) | n.d. | 57 ± 8* # (54.0 to 60.1) | +14 ± 4 (+13 to 16) | 50 ± 9 (46.4 to 53.6) | n.d. | 50 ± 8 (46.8 to 53.2) | 0 ± 5 (–2 to –2) | 0.009 |
| Intact PTH (pmol/l) | 5.6 ± 2.5 (4.7 to 6.6) | 4.7 ± 2.1* (3.9 to 5.5) | 4.0 ± 2.2* (3.2 to 4.8) | –29 ± 13 (–34 to –24) | 5.5 ± 2.3 (4.6 to 6.4) | 5.2 ± 1.9 (4.4 to 6.0) | 3.9 ± 1.5 (3.3 to 4.5) | –30 ± 14 (–36 to –24) | 0.127 |
| FGF-23 (pg/ml) | 38.5 ± 19.3 (31.2 to 45.9) | 44.4 ± 18.0* (37.6 to 51.3) | 48.8 ± 18.2* (41.9 to 55.7) | +27 ± 11 (+23 to 31) | 40.0 ± 20.9 (31.6 to 48.4) | 36.5 ± 18.2 (29.1 to 43.9) | 45.2 ± 19.8 (37.2 to 53.2) | +13 ± 11 (9 to 17) | 0.022 |

FGF-23 = fibroblast growth factor 23; n.d. = not done; PTH = parathyroid hormone

* p <0.05 for intragroup comparison (with own group baseline); # p <0.05 for intergroup comparison (between the groups)

**Table 4**: Effect of vitamin D ($D_3$) supplementation on plasma electrolytes, urea and creatinine (mean ± standard deviation, 95% confidence interval).

| **Parameter** | **$D_3$ (n = 29)** | | | **Placebo (n = 26)** | | |
|---|---|---|---|---|---|---|
| | Baseline | 3 months | 6 months | Baseline | 3 months | 6 months |
| $Na^+$ (mmol/l) | 139 ± 2 (138 to 140) | 138 ± 2 (137 to 139) | 138 ± 3 (137 to 139) | 139 ± 3 (138 to 140) | 138 ± 2 (137 to 139) | 139 ± 3 (138 to 140) |
| $K^+$ (mmol/l) | 3.8 ± 0.4 (3.7 to 4.0) | 3.8 ± 0.5 (3.6 to 4.0) | 3.8 ± 0.4 (3.7 to 4.0) | 3.8 ± 0.3 (3.7 to 3.9) | 3.8 ± 0.5 (3.6 to 4.0) | 3.8 ± 0.4 (3.6 to 4.0) |
| $Cl^-$ (mmol/l) | 103 ± 3 (102 to 104) | 103 ± 2 (102 to 104) | 103 ± 3 (102 to 104) | 104 ± 4 (102 to 106) | 104 ± 2 (103 to 105) | 104 ± 3 (103 to 105) |
| Ion. $Ca^{++}$ (mmol/l) | 1.21 ± 0.05 (1.19 to 1.23) | 1.24 ± 0.04 (1.23 to 1.26) | 1.22 ± 0.04 (1.21 to 1.24) | 1.25 ± 0.05 (1.23 to 1.27) | 1.23 ± 0.05 (1.21 to 1.25) | 1.18 ± 0.05 (1.16 to 1.20) |
| $PO_4$ (mmol/l) | 1.08 ± 0.12 (1.03 to 1.13) | 1.11 ± 0.14 (1.06 to 1.16) | 1.14 ± 0.15 (1.08 to 1.20) | 1.14 ± 0.13 (1.09 to 1.19) | 1.07 ± 0.14 (1.01 to 1.13) | 1.14 ± 0.12 (1.09 to 1.19) |
| Urea (mmol/l) | 6.8 ± 2.5 (5.9 to 7.8) | 6.9 ± 2.1 (6.1 to 7.7) | 7.5 ± 2.6 (6.5 to 8.5) | 6.8 ± 2.8 (5.7 to 7.9) | 7.0 ± 2.5 (6.0 to 8.0) | 6.4 ± 2.7 (5.3 to 7.5) |
| Creatinine (µmol/l) | 72.3 ± 34.6 (59.5 to 85.5) | 75.4 ± 38.9 (60.6 to 90.0) | 78.2 ± 35.6 (64.7 to 91.8) | 68.8 ± 33.8 (55.1 to 82.5) | 69.2 ± 31.9 (56.3 to 82.1) | 72.6 ± 30.9 (60.1 to 85.1) |

It had been planned to enrol all patients between October and November to limit the contribution of skin synthesis of $D_3$ (the recruited subjects all live ~47° N latitude). However, as a result of patient factors (holidays, professional engagements, etc.) enrolment could be completed only at the end of December. The last subjects completed the protocol in July 2010, thereby natural sun exposure increased $D_3$ in both groups. The effect of $D_3$ administration may have been mitigated by the fact that the placebo group exhibited a "spontaneous" increase in 25(OH)-$D_3$ levels most probably owing to increased sun exposure in the spring and early summer. A small but significant reduction of C-peptide levels was noted in the $D_3$ arm relative to placebo. This finding is consistent with the observed tendency to a relative reduction of fasting glucose levels in the $D_3$ arm and may thus reflect a secondary consequence of improved insulin sensitivity.

Previously, $1,25(OH)_2$-$D_3$ has been shown to inhibit renin gene transcription and vitamin D receptor knockout mice demonstrate hypertension [27, 28]. However, this study in T2DM with well-controlled blood pressure did not show evidence for a detectable inhibitory effect of $D_3$ on the activity of the renin/aldosterone system on the basis of the analysis of plasma renin, plasma aldosterone and 24-hour urinary excretion rates of tetrahydro-aldosterone. Also, there was no intergroup treatment difference in the 24-hour ambulatory systolic and diastolic blood pressure measurements.

The observation of a significant decrease in urinary albumin excretion in the $D_3$ group is of interest in view of the association of low $D_3$ status with albuminuria [29] and is confirmatory evidence for the possible retarding effect of $D_3$ agonists on progression of glomerular injury [30].

Our study cannot conclusively answer the question as to whether the observed effects of $D_3$ administration are due to changes in 25(OH)-$D_3$ or $1,25(OH)_2$-$D_3$, although the increase in $1,25(OH)_2$-$D_3$ was limited to the intervention group. The increase in $1,25(OH)_2$-$D_3$ and the decrease in intact PTH are responsible – at least in part – for the significant increase in FGF-23. However, the role of higher circulating levels of 25(OH)-$D_3$ also requires consideration as osteoblasts exposed to 25(OH)-$D_3$ have been shown to produce $1,25(OH)_2$-$D_3$ locally in a paracrine/autocrine fashion and, thereby, to increase the synthesis of FGF-23 [31].

The $D_3$-induced rise in FGF-23 in this study might be viewed adversely since injection of pharmacological amounts of murine FGF-23 into myocardium induced left ventricular hypertrophy in mice [32], and elevated FGF-23 levels have been reported to be independently associated with total mortality in a prospective patient cohort with in-

**Table 5:** Effect of vitamin D ($D_3$) supplementation on 24-hour urinary electrolytes and albumin excretion rates (mean ± standard deviation, 95% confidence interval).

| Parameter | $D_3$ (n = 29) | | | Placebo (n = 26) | | |
|---|---|---|---|---|---|---|
| | Baseline | 3 months | 6 months | Baseline | 3 months | 6 months |
| $Na^+$ (mmol/24h) | 206 ± 34 (193 to 219) | 170 ± 39 (155 to 185) | 191 ± 31 (179 to 203) | 177 ± 36 (163 to 192) | 172 ± 33 (159 to 185) | 184 ± 35 (170 to 198) |
| $K^+$ (mmol/24h) | 73 ± 14 (68 to 78) | 76 ± 17 (70 to 83) | 96 ± 20 (88 to 104) | 63 ± 17 (56 to 70) | 68 ± 19 (60 to 76) | 75 ± 20 (67 to 83) |
| $Cl^-$ (mmol/24h) | 199 ± 29 (188 to 210) | 179 ± 27 (169 to 189) | 189 ± 31 (177 to 201) | 175 ± 29 (163 to 187) | 173 ± 32 (160 to 186) | 187 ± 27 (176 to 198) |
| $Ca^{++}$ (mmol/24h) | 3.8 ± 1.1 (3.4 to 4.2) | 5.1 ± 1.3*# (4.6 to 5.6) | 5.5 ± 1.0*# (5.1 to 5.9) | 3.3 ± 0.9 (2.9 to 3.7) | 3.0 ± 1.2 (2.5 to 3.5) | 4.3 ±1.3 (3.8 to 4.8) |
| $PO_4$ (mmol/24h) | 28 ± 5 (26 to 30) | 31 ± 6 (29 to 33) | 29 ± 5 (27 to 31) | 27 ± 6 (25 to 29) | 27 ± 7 (24 to 30) | 27 ± 5 (25 to 29) |
| Albumin (mg/24h) | 200 ± 41# (184 to 216) | 143 ± 37# (129 to 157) | 126 ± 39*# (111 to 141) | 84 ± 21 (76 to 93) | 66 ± 27 (55 to 77) | 55 ± 24 (45 to 65) |

* p <0.05 for intragroup comparison, # p <0.05 for intergroup comparison (between the groups)

**Table 6:** Effect of vitamin D ($D_3$) supplementation on renin/aldosterone (mean ± standard deviation, 95% confidence interval).

| Renin/aldosterone | $D_3$ (n = 29) | | | | Placebo (n = 26) | | | | Treatment difference |
|---|---|---|---|---|---|---|---|---|---|
| | Baseline | 3 months | 6 months | Change (%) | Baseline | 3 months | 6 months | Change (%) | p-value |
| Active plasma renin (mU/l) | 12.8 ± 3.1 (11.6 to 14.0) | 13.8 ± 2.6 (12.8 to 14.8) | 14.0 ± 3.9 (12.6 to 15.5) | +9.4 ± 5.4 (+7.3 to 11.5) | 13.1 ± 4.1 (11.4 to 14.8) | 12.4 ± 3.4 (11.0 to 13.8) | 13.6 ± 3.8 (12.1 to 15.1) | +3.8 ± 4.7 (+1.9 to 5.7) | 0.451 |
| Plasma aldosterone (nmol/l) | 0.48 ± 0.19 (0.41 to 0.55) | 0.44 ± 0.17 (0.38 to 0.51) | 0.46 ± 0.16 (0.40 to 0.52) | –4.2 ± 4.2 (–5.8 to –2.6) | 0.45 ± 0.15 (0.39 to 0.51) | 0.44 ± 0.14 (0.38 to 0.50) | 0.41 ± 0.17 (0.34 to 0.48) | –9.9 ± 6.9 (–12.7 to –7.1) | 0.721 |
| Tetrahydro-aldosterone (µg/24h) | 55 ± 10 (51 to 59) | 61 ± 11 (57 to 65) | 53 ± 9 (50 to 56) | –3.7 ± 7.6 (–6.6 to –0.8) | 52 ± 11 (48 to 56) | 47 ± 10 (43 to 51) | 44 ± 11 (40 to 48) | –15.4 ± 11.2 (–20 to –11) | 0.537 |

**Table 7:** Effect of vitamin D (D3) supplementation on 24 hour ambulatory blood pressure (mean ± standard deviation, 95% confidence interval).

| Parameter | $D_3$ (n = 29) | | | Placebo (n = 26) | | |
|---|---|---|---|---|---|---|
| | Baseline | 3 months | 6 months | Baseline | 3 months | 6 months |
| Mean systolic BP (mm Hg) | 131 ± 3 (130 to 132) | 129 ± 3* (128 to 130) | 127 ± 3* (126 to 128) | 135 ± 3 (134 to 136) | 133 ± 3 (132 to 134) | 128 ± 2* (127 to 129) |
| Mean diastolic BP (mm Hg) | 83 ± 2 (82 to 84) | 76 ± 3* (75 to 77) | 80 ± 2* (79 to 81) | 82 ± 3 (81 to 83) | 83 ± 3 (82 to 84) | 80 ± 3* (79 to 81) |

BP = blood pressure
* p <0.05 for the intragroup comparison (with own baseline), intergroup comparisons yielded no significant difference

cident end-stage renal disease [33, 34]. The role of FGF-23 in the incidence of coronary heart disease (CHD) in the general population is unclear. However, there is substantial reason to consider that incident CHD is not dependent on FGF-23 levels in the general population. In a prospective, nested, case-control cohort study, from the 51,529-subject Health Professionals Follow-up Study, within the subset with no history of CHD (mean serum creatinine 1.0 mg/dl) no association was found between baseline FGF-23 levels and subsequent nonfatal myocardial infarction and fatal CHD events [35]. Nevertheless, other epidemiological data have shown that FGF-23 concentration is a risk factor for increased all-cause and cardiovascular mortality in Swedish community dwelling adults [36].

Interestingly, it has been demonstrated that insulin-resistant T2DM patients exhibit an impaired FGF-23 and PTH response to an acute phosphate load, sufficient to result in a supernormal hyperphosphataemic response [37]. Higher postprandial serum phosphate in T2DM might account, at least in part, for the systemic vascular calcification observed in this disorder and its status as a cardiovascular risk factor, as a function of duration of diabetes [38], and thus it is possible that higher FGF-23 levels might mitigate diabetic vascular disease, at least in subjects without chronic kidney disease. Since FGF-23 is produced in osteoblasts and osteocytes, and osteocyte density is reduced in experimental diabetes [39], it has been suggested that T2DM may be a state of relative FGF-23 hyporesponsiveness [37]. In fact, osteoblast-specific deletion of the insulin receptor in mice results in a phenotype of systemic insulin resistance and obesity that is mediated in part by osteoblastic endocrine dysfunction characterised by diminished secretion of under-carboxylated osteocalcin [40]. The findings that $D_3$ induced increases in FGF-23 levels in T2DM, as previously reported in non-diabetics [41], and that $D_3$ can improve the course of $HBA_{1c}$ and insulin sensitivity of peripheral tissues (HOMA-IR), raises the possibility that $D_3$ therapy in T2DM, in addition to improving insulin sensitivity for glucose homeostasis in muscle cells and hepatocytes, might also result in increased FGF-23 levels via a similar insulin-sensitising action in osteocytes/osteoblasts.

The strengths of the present study are the placebo-controlled, prospective study design and the fact that no potentially confounding concomitant medication changes were made during the observation period. The chief limitation of this study is the relatively small number of participants and single centre location. In addition, the "spontaneous" rise in 25(OH)-$D_3$ levels in the control population may have narrowed the differences and we cannot assume that the effects are dependent on different $D_3$ doses. Also, we cannot exclude an additional effect of clandestine use of $D_3$ in the placebo group as a result of the subjects' interest in the study hypothesis.

Our results encourage the design and conduct of studies that further explore the roles of $D_3$ and $D_3$ analogues on glycaemic control in T2DM patients. Future studies should – among many other points – establish the dose-response characteristics, examine the best analogue of $D_3$ with regard to the benefit/harm ratio and evaluate the effects in larger study populations and over longer time periods. In view of our study results, the effects of $D_3$ on beta-cell function and insulin secretion merit special attention. In addition, the relevance of increased FGF-23 on cardiovascular morbidity should be evaluated in diabetes.

In summary and conclusion, $D_3$ improved insulin sensitivity (based on HOMA-IR) and affected the course of $HbA_{1c}$ positively compared to placebo in patients with T2DM, but did not weaken, and was well tolerated.

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**Authors' contribution:** Sigrid Jehle examined and followed the patients, calculated/analysed the data, co-interpreted them and co-wrote the manuscript. Alessia Lardi examined and followed the patients and calculated/analysed the data. Barbara Felix referred the patients for screening for this study. Henry N. Hulter designed the protocol with the corresponding author (RK), co-analysed and co-interpreted the data and co-wrote the manuscript. Christoph Stettler analysed the data and advised in interpreting them and made contributions to contents of the manuscript. Reto Krapf designed the protocol, supervised the study and data acquisition, analysed and interpreted the data and co-wrote the manuscript.

***Correspondence:*** *Sigrid Jehle, MD, Department of Medicine, Kantonsspital Bruderholz, University of Basel, CH-4101 Bruderholz, Switzerland, sianjehle[at]bluewin.ch*

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## Figures (large format)

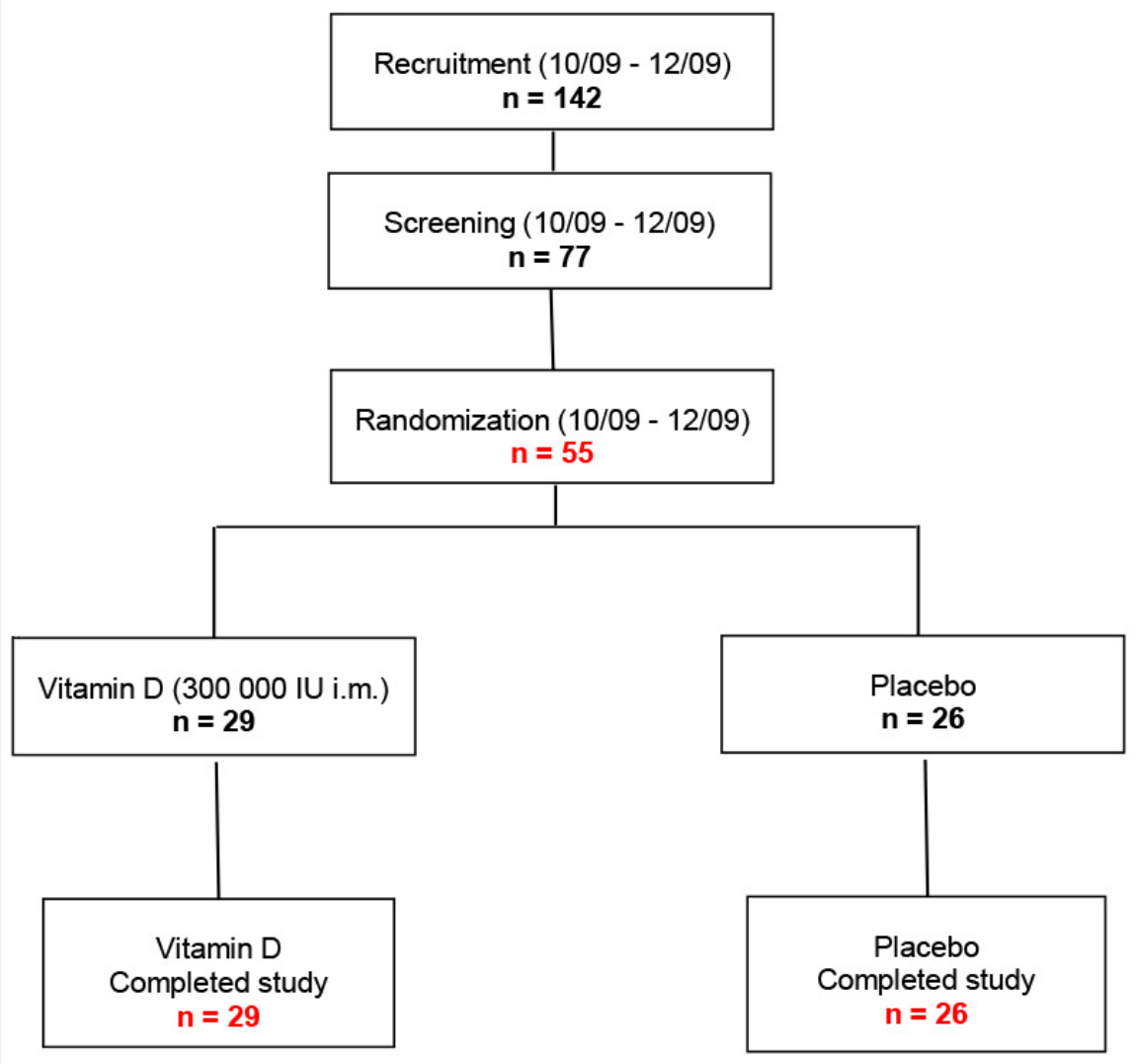


**Figure 1**

Study flowchart

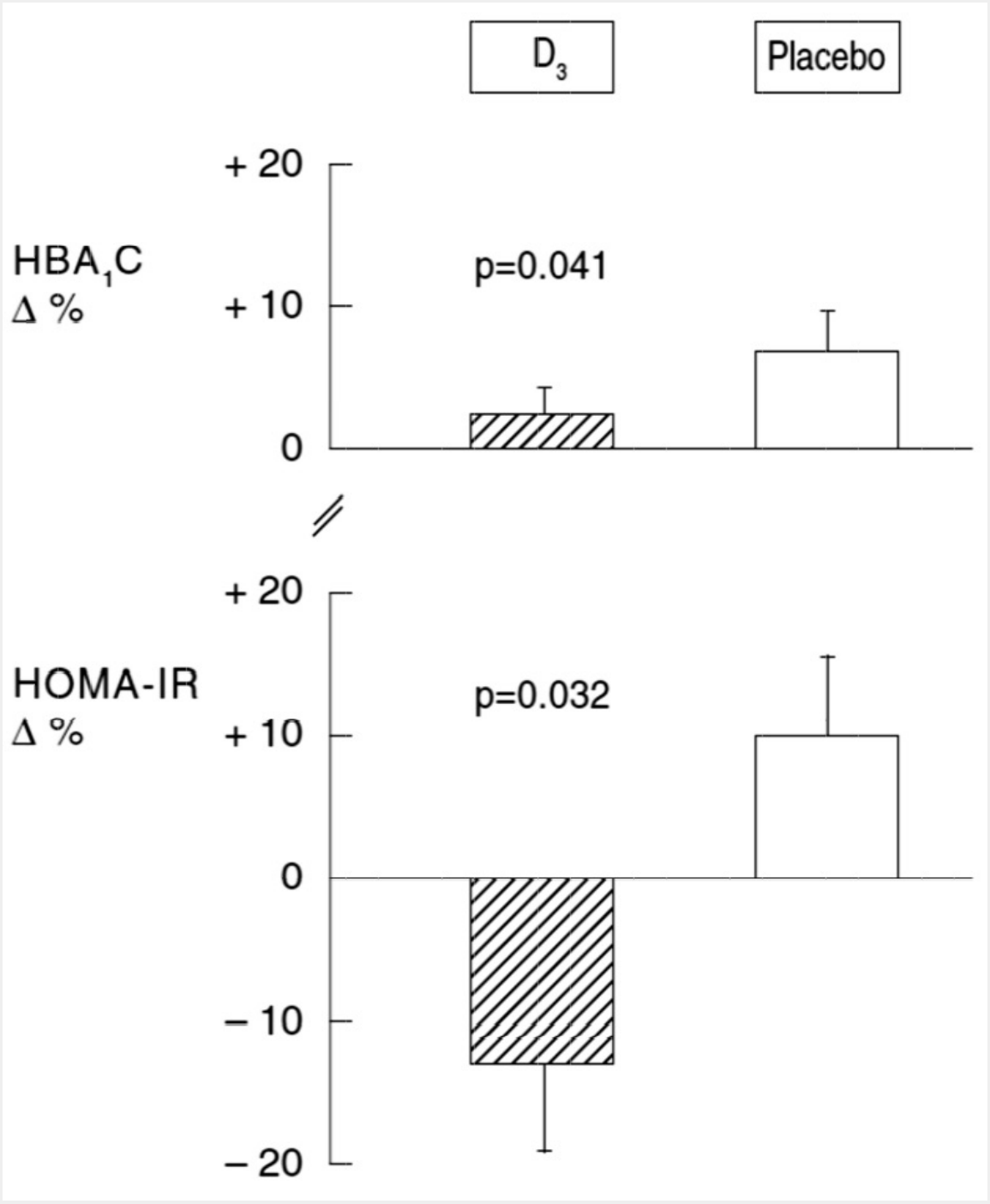


**Figure 2**

Effect of $D_3$ supplementation on the percent changes in $HbA_{1c}$ and HOMA-IR.
$HbA_{1c}$ = glycosylated haemoglobin; HOMA-IR = homeostasis model assessment insulin resistance