Original Communication

Vitamin D3 supplementation improves glycemic control in type 2 diabetic patients: Results from an Italian clinical trial

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Abstract: *Background*: to evaluate the effects of Vitamin D3 on glyco-metabolic control in type 2 diabetic patients with Vitamin D deficiency. *Methods*: one hundred and seventeen patients were randomized to placebo and 122 patients to Vitamin D3. We evaluated anthropometric parameters, glyco-metabolic control, and parathormone (PTH) value at baseline, after 3, and 6 months. *Results*: a significant reduction of fasting, and post-prandial glucose was recorded in Vitamin D3 group after 6 months. A significant HbA_{1c} decrease was observed in Vitamin D3 (from 7.6% or 60 mmol/mol to 7.1% or 54 mmol) at 6 months compared to baseline, and to placebo ($p < 0.05$ for both). At the end of the study period, we noticed a change in the amount in doses of oral or subcutaneous hypoglycemic agents and insulin, respectively. The use of metformin, acarbose, and pioglitazone was significantly lower ($p = 0.037$, $p = 0.048$, and $p = 0.042$, respectively) than at the beginning of the study in the Vitamin D3 therapy group. The units of Lispro, Aspart, and Glargine insulin were lower in the Vitamin D3 group at the end of the study ($p = 0.031$, $p = 0.037$, and $p = 0.035$, respectively) than in the placebo group. *Conclusions*: in type 2 diabetic patients with Vitamin D deficiency, the restoration of value in the Vitamin D standard has led not only to an improvement in the glyco-metabolic compensation, but also to a reduced posology of some oral hypoglycemic agents and some types of insulin used.

Keywords: Vitamin D deficiency, type 2 diabetes mellitus, supplementation

Introduction

Diabetes is increasing exponentially and is appearing more and more precociously, despite the awareness campaigns that are being carried out all over the world [1]. Currently the pharmacological control of diabetes is made a little simpler by the multiple drugs available, even if the optimal control is not always achieved [2].

In 2016 more than 3 million 200 thousand people in Italy reported to suffer from diabetes, 16.5% among people aged 65 and over. The prevalence of self-reported diabetes has almost doubled in the last thirty years [3]. At the economic level, 8% of the total health budget in Italy is invested for diabetes [3–5] or its complications.

Type 2 diabetes is associated with a lack of Vitamin D [6], as proven by low values of Vitamin D identified in diabetic patients [7]. Vitamin D responsive elements in the promoter region of the human insulin gene have been identified [8]. Vitamin D is involved not only in the production and secretion of insulin, but also in the pathways of insulin signaling in insulin-sensitive tissues [9]. Vitamin D deficiency is associated with insulin resistance [10], which is the pathophysiological basis of type 2 diabetes mellitus [11]. A glycosylated hemoglobin (HbA_{1c}) < 7.0% is an index of good glyco-metabolic control, and a negative correlation between the value of HbA_{1c} and the level of Vitamin D has been demonstrated [12].

The administration of Vitamin D may protect from the onset of diabetes mellitus, especially due to the action of Vitamin D on some organs involved in the pathophysiology of diabetes mellitus such as liver, muscle, adipose tissue and on beta cell itself [13, 14]. However, in literature there is no clear evidence of which dose of Vitamin D should be used so that the therapy is as effective as possible and whether this

therapy can help in the management of the complex therapy of the diabetic patient or whether such therapy with Vitamin D could eventually reduce the hypoglycemic therapy doses [15].

The aim of our study was to identify type 2 diabetic patients with variable Vitamin D deficiency, and to administer vitamin D at different doses according to the level of deficiency and to evaluate the glyco-metabolic control after 6 months of therapy. Furthermore, we also checked whether the amount of hypoglycemic agents patients took at the end of the study changed compared to the beginning.

Materials and methods

Study design

This 6-months, double-blind, randomized, placebo-controlled, clinical trial was conducted at the Centre of Diabetes and Metabolic Diseases, Department of Internal Medicine and Therapeutics, University of Pavia, PAVIA, Italy.

Institutional review board approved the study protocol. The trial was conducted in accordance with the 1994 Declaration of Helsinki [16], and its amendments and the Code of Good Clinical Practice. All patients provided written informed consent to participate in this study after a full explanation of the study.

Patients

We enrolled 239 Caucasian type 2 diabetic patients aged > 18 of either sex according to the ESC (European Society of Cardiology) and EASD (European Association for the Study of Diabetes) Guidelines criteria [17] and with Vitamin D deficiency (25 (OH) Vitamin D < 30 ng/ml) [18]. Patients were excluded if they had a history of ketoacidosis or had unstable or rapidly progressive diabetic retinopathy, nephropathy, or neuropathy; impaired hepatic function (defined as plasma aminotransferase and/or gamma-glutamyltransferase level three times higher than the upper limit of normal [ULN] for age and sex), impaired renal function (defined as serum creatinine level higher than the ULN for age and sex), or severe anemia. We also excluded patients with serious cardiovascular disease (CVD) (e.g., New York Heart Association class I-IV congestive heart failure or a history of myocardial infarction or stroke) or cerebrovascular conditions within 6 months before study enrolment. Women who were pregnant or breastfeeding or of childbearing potential and not taking adequate contraceptive precautions were also excluded. None of the patients included in our study used Vitamin D supplements or medication known to affect the serum Vitamin D concentration.

Suitable patients, identified from review of case notes and/or computerized clinic registers, were contacted by the investigators in person or by telephone.

Diet and physical activity

At baseline, all patients were already following an adequate diet. The controlled-energy diet (~600 kcal daily deficit) was based on NCEP-ATP III recommendations [19], that contained 50% of calories from carbohydrates, 30% from fat (< 7% saturated, up to 10% polyunsaturated, and up to 20% monounsaturated), and 20% from proteins, with a maximum cholesterol content of 300 mg/d, and 35 g/d of fiber. A dietitian and/or specialist physician gave standard diet advice. Individuals were also encouraged to maintain their usual physical activity.

Treatments

Investigators randomized patients to take placebo or Vitamin D (cholecalciferol [Vitamin D3]) for 6 months, in a randomized, double-blind, placebo-controlled design (Figure 1). Patients self-administered cholecalciferol (one Vitamin D3 vial after the lunch on a piece of bread) following the schedule below, according to the level of Vitamin D deficiency:

- 25 (OH) Vitamin D < 20 ng/ml:
  - Attack dose: 50,000 IU/weekly for 12 weeks.
  - Maintenance: 25,000 IU/every 2 weeks for 12 weeks.
- 25 (OH) Vitamin D 20–30 ng/ml:
  - Attack dose: 25,000 IU/weekly for 12 weeks.
  - Maintenance: 25,000 IU/every 2 weeks for 12 weeks.

Both placebo and Vitamin D3 were identical, coded vials to ensure the blind status of the study. Randomization was done using a drawing of envelopes containing randomization codes prepared by a statistician. Investigators assessed medication compliance by counting the number of vials returned at the time of specified clinic visits. Throughout the study, we instructed patients to take their first dose of new medication on the day after the study medication delivery. At the same time, Investigators retrieved all unused medication for inventory. All medications were provided free of charge.

Assessments

Before starting the study, all patients underwent an initial screening assessment that included a medical history,

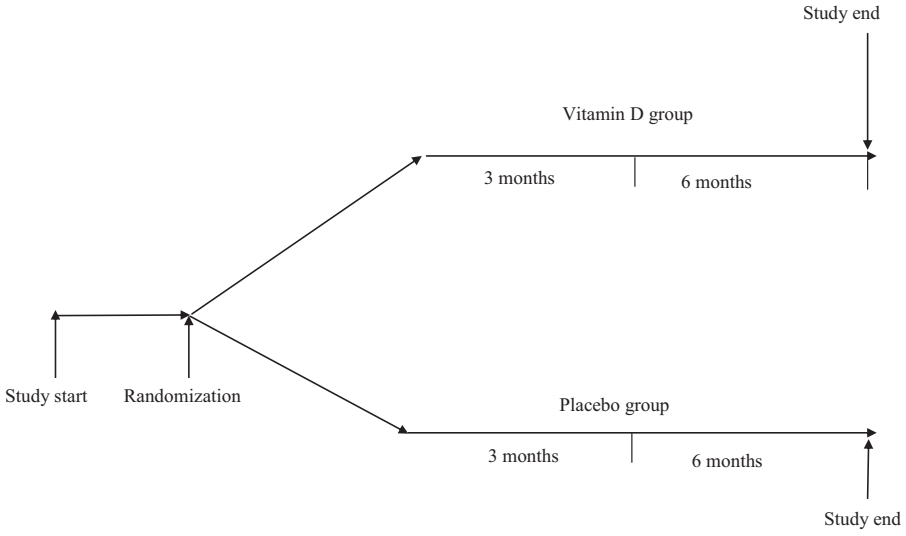

Figure 1. Study design

physical examination, vital signs (blood pressure and heart rate), a 12-lead electrocardiogram, measurements of height and body weight, calculation of body mass index (BMI), assessment of fasting plasma glucose (FPG), post-prandial glucose (PPG), HbA_{1c}, fasting plasma insulin (FPI), HOMA index, lipid profile, calcium, and parathormone (PTH).

All parameters were assessed at baseline, after 3 and 6 months since the study start. Moreover, at baseline, and after 3 and 6 months, patients underwent a blood sample to assess the dosage of 25 (OH) Vitamin D.

All plasmatic variables were determined after a 12-hour overnight fast, with the exception of PPG, which was determined two hours after lunch. A research nurse for all patients drew venous blood samples between 8:00 am and 9:00 am. We used plasma obtained by addition of Na2-EDTA, 1 mg/mL, and centrifuged at 3000 g for 15 minutes at 4 °C. Immediately after centrifugation, the plasma samples were frozen and stored at −80 °C for ≤3 months. Laboratory technicians drew blood samples and the biologist responsible for the laboratory performed the assays. A central laboratory performed all measurements.

Body mass index was calculated by the investigators as weight in kilograms divided by the square of height in meters.

Plasma glucose was assayed using a glucose-oxidase method (GOD/PAP, Roche Diagnostics, Mannheim, Germany) with intra- and interassay coefficients of variation (CsV) < 2% [20].

Glycated hemoglobin level was measured by a high performance liquid chromatography (HPLC) method (DIAMAT, Bio-Rad, USA; normal values 4.2–6.2%), with intra- and interassay coefficients of variation (CsV) of < 2% [21].

Plasma insulin was assayed with Phadiaseph insulin radio immuno assay (RIA) (Pharmacia, Uppsala, Sweden) by using a second antibody to separate the free and antibody-bound 125 I-insulin (intra- and interassay CsV 4.6 and 7.3%, respectively) [22].

The HOMA index was calculated as the product of basal glucose (mmol/l) and insulin levels (μU/ml) divided by 22.5 [23, 24].

Total cholesterol and Tg levels were determined using fully enzymatic techniques [25, 26] on a clinical chemistry analyzer (HITACHI 737; Hitachi, Tokyo, Japan); intra- and interassay CsV were 1.0 and 2.1 for TC measurement, and 0.9 and 2.4 for Tg measurement, respectively. High density lipoprotein-cholesterol level was measured after precipitation of plasma apo B-containing lipoproteins with phosphotungstic acid [27] intra- and interassay CsV were 1.0 and 1.9, respectively; LDL-C level was calculated by the Friedewald formula [28].

Serum intact PTH levels was measured by electro chemiluminescence immunoassay (Cobas 8000, Roche Inc., CA, USA) [29].

Serum 25 (OH) Vitamin D was dosed using Gamma-B 25-hydroxyvitamin D RIA (IDS, Boldon, UK). The CV for interassay analyses is 7.9% [30].

Safety measurements

Treatment tolerability was assessed at each study visit using an accurate interview of patients by the investigators, and comparisons of clinical and laboratory values with baseline levels. Safety monitoring included physical examination, vital sign assessment, weight, electrocardiogram, adverse events, and laboratory tests. All adverse events were recorded.

Statistical analysis

Investigators conducted an intention-to-treat (ITT) analysis in patients who had received ≥ 1 dose of study medication and had a subsequent efficacy observation. We included patients in the tolerability analysis if they had received ≥ 1 dose of trial medication after randomization and had undergone a subsequent tolerability observation. The statistical significance of the independent effects of treatments on the other variables was determined using ANCOVA. Continuous variables were tested using one-way repeated measures ANOVA to assess overall differences within groups. A 1-sample *t* test was used to compare values obtained before and after treatment administration; 2-sample *t* tests were used for between-group comparisons. Normal distribution of data was tested with the Shapiro-Wilk test. Statistical analysis of data was performed using the Statistical Package for Social Sciences software version 11.0 (SPSS Inc., Chicago, Illinois, USA). Data are presented as mean (SD) [31]. For all statistical analyses, $p < 0.05$ was considered statistically significant.

Results

Study sample

A total of 239 patients completed this trial. Of these, 122 were randomized to Vitamin D3, and 117 to placebo. There were 7 patients who did not complete the study and the reasons for premature withdrawal included non-compliance to treatment or lost to follow-up. The characteristics of the patient population at the study entry are shown in Tables 1 and 2.

Anthropometric parameters

No variations of BMI was recorded with neither treatments (Table 1 and 2).

Glyco-metabolic parameters

No FPG, PPG, FPI, and HbA_{1c} variation was obtained in both groups after 3 months. A significant reduction of FPG, and PPG, was recorded in Vitamin D3 group after 6 months ($p < 0.05$ compared to baseline), respectively. A significant HbA_{1c} decrease was observed in Vitamin D3 at 6 months compared to baseline ($p < 0.05$), and compared to placebo group ($p < 0.05$), respectively. No FPI variation was obtained in both groups after 6 months. Regarding insulin resistance, there was a decrease of HOMA index in Vitamin D3 group at 3 months ($p < 0.05$), and at 6 months ($p < 0.01$), compared to baseline, respectively. A significant difference was determined in Vitamin D3 group compared to placebo at 6 months ($p < 0.05$) (Table 2).

No variation of lipid profile was recorded in neither group.

Parathormone and calcium

A significant reduction of PTH was recorded in Vitamin D3 group at 3 months ($p < 0.01$), and at 6 months ($p < 0.001$) compared to baseline, respectively, and this difference was significant at 3 months ($p < 0.05$) and at 6 months ($p < 0.01$) compared to placebo group (Table 2).

A significant increase of calcium was recorded in Vitamin D3 group at 3, and 6 months, compared to baseline, and to placebo group ($p < 0.05$ for both) (Table 2).

25 (OH) Vitamin D3 level

After 3 months of Vitamin D3 supplementation, there was a significant increase of 25 (OH) Vitamin D3 level ($p < 0.01$) in the group with 25 (OH) Vitamin D3 < 20 ng/ml, and a significant increase of this level ($p < 0.01$) in the group with 25 (OH) Vitamin D3 between 20 and 30 ng/ml. Further significant increase was determined in the group with 25 (OH) Vitamin D3 < 20 ng/ml ($p < 0.001$) and in the group with 25 (OH) Vitamin D3 between 20 and 30 ng/ml ($p < 0.001$) at 6 months, and this difference was significant versus placebo ($p < 0.01$) (Table 2).

Hypoglycemic treatment

At the end of the study period, we noticed a change in the amount in mg or units of oral or subcutaneous hypoglycemic agents and insulin, respectively (Table 3). The use of metformin, acarbose, and pioglitazone was significantly lower ($p = 0.037$, $p = 0.048$, and $p = 0.042$, respectively) than at the beginning of the study in the Vitamin D3 therapy group. The units of Lispro, Aspart, and Glargine insulin were lower in the Vitamin D3 group at the end of the study ($p = 0.031$, $p = 0.037$, and $p = 0.035$, respectively) than in the placebo group (Table 3).

Correlations

There was an inverse correlations between FPG and Vitamin D3 level ($r = -0.43$; $p = 0.039$), between PPG ad Vitamin D3 level ($r = -0.38$; $p = 0.044$), and between HOMA-IR and Vitamin D3 level ($r = -0.32$; $p = 0.021$).

Table 1. Data at Baseline, and after 3 and 6 months of Placebo treatment

	Placebo			
	Baseline	3 months	6 months	*p* value 6 months vs Baseline
n (M/F)	117 (57/60)	115 (57/58)	113 (56/57)	–
Age (years)	54.7 ± 9.6	–	–	
Smokers (M/F)	18/15	18/15	17/15	
Diabetes duration (years)	2.7 ± 0.8	–	–	–
Macrovascular complications (n)	43	43	40	0.087
Microvascular complications (n)	35	35	35	0.99
Alcohol use (yes/no) (n)	60	60	59	0.096
Physical activity (yes/no) (n)	67	66	66	0.098
Dairy intake (yes/no) (n)	54	54	53	0.097
Fish intake (yes/no) (n)	68	66	65	0.097
Exposure to sun (yes/no) (n)	74	74	72	0.096
BMI (kg/m^2)	27.2 ± 1.1	27.1 ± 1.0	26.9 ± 0.9	0.096
HbA_{1c} (%)	7.7 ± 0.7	7.7 ± 0.7	7.5 ± 0.5	0.083
FPG (mg(dl)	142.4 ± 16.3	139.5 ± 15.7	137.8 ± 13.4	0.085
PPG (mg/dl)	167.2 ± 25.2	164.7 ± 23.7	160.4 ± 21.1	0.078
FPI (mU/ml)	14.8 ± 7.9	14.6 ± 7.7	14.5 ± 7.6	0.084
HOMA index	5.2 ± 3.6	5.0 ± 3.3	4.9 ± 3.0	0.088
SBP (mmHg)	132.5 ± 5.6	134.2 ± 5.8	132.1 ± 5.2	0.084
DBP (mmHg)	80.1 ± 3.5	79.5 ± 3.2	79.2 ± 3.1	0.092
TC (mg/dl)	174.1 ± 17.2	168.4 ± 15.1	164.2 ± 13.4	0.086
LDL-C (mg/dl)	110.1 ± 10.2	102.0 ± 9.1	100.4 ± 8.8	0.079
HDL-C (mg/dl)	40.1 ± 5.1	41.4 ± 6.0	40.8 ± 5.2	0.093
Tg (mg/dl)	119.4 ± 36.1	125.2 ± 41.0	118.0 ± 35.7	0.087
Creatinine (mg/dl)	0.89 ± 0.3	0.87 ± 0.2	0.89 ± 0.3	0.092
Calcium (mg/dl)	8.5 ± 0.5	8.6 ± 0.7	8.7 ± 0.8	0.087
25 (OH) Vitamin D (ng/ml)	18.6 ± 4.7	18.9 ± 4.9	19.6 ± 5.2	0.059
<20 ng/ml (%)	12.5 ± 5.3 (43.5)	12.7 ± 5.5 (40.2)	13.0 ± 5.7 (38.6)	0.072
20–30 ng/ml (%)	24.7 ± 4.1 (56.5)	25.1 ± 4.4 (59.8)	26.2 ± 4.8 (61.4)	0.061
PTH (pmol/l)	9.2 ± 3.2	9.1 ± 3.0	8.8 ± 2.7	0.065

M: males; F: females; BMI: body mass index; HbA_{1c}: glycated hemoglobin; FPG: fasting plasma glucose; PPG: post-prandial plasma glucose; FPI: fasting plasma insulin; HOMA index: homeostatic model assessment of insulin resistance; SBP: systolic blood pressure; DBP: diastolic blood pressure; TC: total cholesterol; LDL-C: low density lipoprotein-cholesterol; HDL-C: high density lipoprotein-cholesterol; Tg: triglycerides; 25 (OH) Vitamin D: 25-hydroxy vitamin D; PTH: parathormone.

Safety and treatment acceptance

Considering a score among 1 and 10, where 1 is the worst, and 10 is the best, no differences were recorded between groups regarding acceptance of treatment that was well tolerated.

Discussion

In the current study, we reported that a dietary supplementation with Vitamin D3 not only improved the glyco-metabolic compensation in Vitamin D3 deficient patients, but also reduced the consumption of some oral hypoglycemic agents and some types of insulins. Among the oral hypoglycemic agents, we noticed a reduction of the dose of hypoglycemic agents with an insulin-sensitizing action. This could be due to the fact that Vitamin D3 supplementation led to an improvement of insulin sensitivity, demonstrated not only by improvement of glyco-metabolic compensation, but also by HOMA index reduction (−1.7, −27.9%). The reduction of anti-diabetic drugs dose is probably due more to the improvements of insulin-sensitivity that to weight decrease given that BMI did not change during the study. Rolf J et al. [32] conducted a study of thirty-six subjects with type 2 diabetes, treated with metformin and bed-time insulin, with cholecalciferol (40,000 IU per week) supplementation compared to placebo for 6 months. Fasting plasma glucose (−3.6 mg/dl, −2.0%), FPI (−42.0 μg/ml, −76.6%), and HbA_{1c} (−0.2%, −2.5%)

Table 2. Data at Baseline, and after 3 and 6 months of Vitamin D3 treatment

	Vitamin D3				
	Baseline	3 months	6 months	*p* value 6 months vs Baseline	*p* value among groups
n (M/F)	122 (62/60)	121 (61/60)	119 (60/59)	–	–
Age (years)	53.8 ± 9.2	–	–		
Smokers (M/F)	19/16	19/16	19/15		
Diabetes duration (years)	2.5 ± 0.6	–	–	–	–
Macrovascular complications (n)	45	45	42	0.087	
Microvascular complications (n)	38	37	37	0.99	
Alcohol use (yes/no) (n)	64	64	63	0.096	
Physical activity (yes/no) (n)	63	63	62	0.098	
Dairy intake (yes/no) (n)	52	52	51	0.097	
Fish intake (yes/no) (n)	70	70	69	0.097	
Exposure to sun (yes/no) (n)	73	73	72	0.096	
BMI (kg/m^2)	27.6 ± 1.4	27.4 ± 1.3	27.3 ± 1.2	0.086	0.079
HbA_{1c} (%)	7.6 ± 0.6	7.3 ± 0.4	7.1 ± 0.3*°	0.045	0.046
FPG (mg(dl)	140.3 ± 14.8	136.3 ± 12.6	130.4 ± 10.2*	0.042	0.067
PPG (mg/dl)	163.8 ± 22.5	158.1 ± 19.7	151.2 ± 15.8*	0.048	0.059
FPI (mU/ml)	14.1 ± 7.4	14.0 ± 7.2	13.8 ± 7.1	0.094	0.088
HOMA index	6.1 ± 2.1	4.7 ± 3.3*	4.4 ± 3.1*°	0.0091	0.041
SBP (mmHg)	131.7 ± 5.2	132.5 ± 5.5	133.5 ± 5.8	0.091	0.088
DBP (mmHg)	80.2 ± 3.6	80.5 ± 3.8	79.1 ± 2.9	0.089	0.091
TC (mg/dl)	169.5 ± 16.7	168.1 ± 16.1	165.8 ± 15.9	0.088	0.086
LDL-C (mg/dl)	105.4 ± 10.0	102.8 ± 9.5	101.9 ± 9.1	0.075	0.089
HDL-C (mg/dl)	41.1 ± 5.1	41.2 ± 5.2	41.5 ± 5.4	0.091	0.095
Tg (mg/dl)	115.1 ± 35.3	120.4 ± 37.2	111.6 ± 34.6	0.083	0.087
Creatinine (mg/dl)	0.85 ± 0.2	0.86 ± 0.3	0.86 ± 0.3	0.098	0.096
Calcium (mg/dl)	8.8 ± 0.7	9.5 ± 0.8*°	9.9 ± 0.9*°	0.046	0.048
25 (OH) Vitamin D (ng/ml)	18.2 ± 4.4	26.1 ± 6.7*	31.3 ± 4.6*°	0.00076	0.0014
<20 ng/ml (%)	12.1 ± 5.0 (46.1)	19.4 ± 7.8* (38.4)	25.8 ± 4.1*° (0)	0.00062	0.0018
20–30 ng/ml (%)	24.2 ± 3.8 (53.9)	32.7 ± 5.6* (61.6)	36.9 ± 5.2*° (0)	0.00069	0.0028
PTH (pmol/l)	9.3 ± 3.4	7.5 ± 1.3*°	6.3 ± 0.9*°	0.00081	0.0087

*p statistically significant vs Baseline; °p statistically significant vs Placebo. M: males; F: Females; BMI: body mass index; HbA_{1c}: glycated hemoglobin; FPG: fasting plasma glucose; PPG: post-prandial plasma glucose; FPI: fasting plasma insulin; HOMA index: homeostatic model assessment of insulin resistance; SBP: systolic blood pressure; DBP: diastolic blood pressure; TC: total cholesterol; LDL-C: low density lipoprotein-cholesterol; HDL-C: high density lipoprotein-cholesterol; Tg: triglycerides; 25 (OH) Vitamin D: 25-hydroxy vitamin D; PTH: parathormone.

levels were not significantly different from baseline values. In our study, instead, we noted a statistically significant reduction in FPG (−10.0 mg/dl, −7.1%), and HbA_{1c} (−0.5%, −6.6%) compared to baseline; furthermore, the HbA_{1c} value was significantly lower than in the placebo group (−0.2%, −2.6%). Ryu OH et al. [33] found different data with respect to Rolf J et al. and compared to ours. They have seen a slight increase in FPG (+ 0.9, +0.7%) and HbA_{1c} (+ 0.1, + 1.4%), respectively, always after 6 months of treatment with 1,000 IU daily of Vitamin D3. Instead, the PTH data (−0.63 pmol/l, −21.1%) is in agreement with ours (−3.0 pmol/l, −32.3%) being significantly decreased compared to baseline, even if our PTH data is also significantly lower compared to placebo group (−0.4 pmol/l, −4.3%). In addition, the decrease in PTH was already apparent after 3 months of Vitamin D3 therapy (−1.8 pmol/l, −19.6%). Ryu et al. used a lower dose of Vitamin D3 on their patients, this could explain the differences between Ryu data and ours.

Ryu OH et al. [34] also conducted a study doubling the dosage of Vitamin D3 per day (cholecalciferol 2,000 IU/day). They did not notice metabolic changes at the end of the 6-month study, but instead saw an increase in 25 (OH) Vitamin D dosage. They did not start from different cut-offs, as we did, thus standardizing the dose to be given of Vitamin D3. The average reached at the end of the study (22.3 ± 9.7 ng/ml; +10 ng/ml, +81.3%) does not seem satisfactory, as not all of them reached the value of 20 ng/ml and none exceeded the value of 30 ng/ml. Instead, the treatment we proposed was dosed according to baseline Vitamin D3 value, allowing all our patients to reach the goal.

Already after 3 months, patients with 25 (OH) Vitamin D < 20 ng/ml reported a significant increase (+7.3 ng/ml,

Table 3. Type of hypoglycemic agents and dose of each single molecule at baseline and after 6 months of placebo or Vitamin D3 treatment

	Placebo			Vitamin D3			
Hypoglycemic agents	Baseline	6 months	*p* value vs Baseline	Baseline	6 months	*p* value vs Baseline	*p* value among groups
Sulphonylureas							
Glimepiride (mg)	3 ± 1	3 ± 1	0.097	3 ± 1	3 ± 1	0.092	0.93
Gliclazide (mg)	60 ± 30	90 ± 30	0.051	60 ± 30	60 ± 30	0.098	0.077
Biguanide							
Metformin (mg)	2000 ± 500	2000 ± 500	0.097	2250 ± 500	1700 ± 850	0.042	0.037
Alfa-glucosidase inhibitors							
Acarbose (mg)	150 ± 50	200 ± 50	0.048	150 ± 50	100 ± 50	0.039	0.048
Glinides							
Repaglinide (mg)	1.0 ± 0.5	1.0 ± 0.5	0.093	1.5 ± 0.5	1.5 ± 0.5	0.092	0.78
Thiazolidinediones							
Pioglitazone (mg)	30 ± 15	30 ± 15	0.096	30 ± 15	15 ± 7.5	0.047	0.042
DPP-4 inhibitors							
Sitagliptin (mg)	75 ± 25	50 ± 25	0.068	75 ± 25	50 ± 25	0.053	0.65
Vildagliptin (mg)	50 ± 25	50 ± 25	0.094	50 ± 25	50 ± 25	0.094	0.72
Linagliptin (mg)	2.5 ± 2.5	2.5 ± 2.5	0.092	2.5 ± 2.5	2.5 ± 2.5	0.092	0.83
GLP-1 receptor agonists							
Exenatide (mg)	2.0 ± 0.0	2.0 ± 0.0	0.088	2.0 ± 0.0	2.0 ± 0.0	0.095	0.46
Liraglutide (mg)	1.2 ± 0.6	1.2 ± 0.6	0.090	1.2 ± 0.6	0.6 ± 0.6	0.043	0.074
Dulaglutide (mg)	0.75 ± 0.75	0.75 ± 0.75	0.095	1.5 ± 0.0	0.75 ± 0.75	0.041	0.082
SGTL-2 inhibitors							
Dapagliflozin (mg)	7.5 ± 2.5	7.5 ± 2.5	0.085	7.5 ± 2.5	5.0 ± 2.5	0.049	0.078
Empagliflozin (mg)	17.5 ± 7.5	25.0 ± 0.0	0.048	15.0 ± 5.0	10.0 ± 0.0	0.041	0.080
Insulins							
Lispro (IU)	26.4 ± 8.2	23.8 ± 6.2	0.074	26.7 ± 8.3	18.6 ± 5.1	0.044	0.031
Aspart (IU)	28.7 ± 6.3	26.3 ± 5.7	0.086	30.4 ± 10.8	18.2 ± 4.5	0.041	0.037
Detemir (IU)	21.2 ± 8.6	19.2 ± 6.8	0.067	18.7 ± 7.1	20.6 ± 7.2	0.055	0.072
Glargine (IU)	18.3 ± 6.5	16.5 ± 4.2	0.071	20.9 ± 8.3	12.3 ± 2.8	0.038	0.035
Degludec (IU)	16.1 ± 4.4	20.5 ± 8.1	0.058	16.8 ± 4.6	16.2 ± 4.2	0.087	0.082

Data are expressed as mean ± standard deviation (SD); Molecule dose are expressed as mg or international unit (IU). DPP-4: Dipeptidyl Peptidase-4; GLP-1: Glucagon-Like Peptide-1; SGLT-2: Sodium-glucose cotrasporter-2.

+ 60.3%) from baseline, continuing their increase to 6 months (+13.7 ng/ml, +113.2%), while those who had a value of 25 (OH) Vitamin D 20–30 ng/ml at 3 months had a significant increase (+8.5 ng/ml, +26.0%), confirmed then at 6 months (+12.7 ng/ml, +34.4%).

At the end of the study, both values of 25 (OH) Vitamin D in the two groups with different cut-off values were significantly higher than in the placebo group demonstrating a slight nonsignificant increase (+0.5 ng/ml, +4.0%, and +1.5 ng/ml, +6.1%, respectively). With a different concept of administration of Vitamin D3, Maggi S et al. [35] saw that a dose of 300,000 IU of Vitamin D3 in a single oral bolus did not significantly change FPG after 6 months of observation.

A similar sequential treatment scheme with Vitamin D3, although with different dosages (6,000 IU of cholecalciferol for 3 months, followed by 3,000 IU for a further 3 months) was proposed by Sadiya A et al. [36]. The patients considered were type 2 diabetic, obese. No change was observed from baseline to placebo in metabolic parameters such as FPG and HbA_{1c}. With this scheme, they obtained at the end of the study a value of 25 (OH) Vitamin D of 24.8 ng/ml (+13.6 ng/ml, +121.4%). Krul-Poel YHM, et al. [37] used a dosage of Vitamin D3 of 50,000 IU per month, for 6 months. At the end of the study, they agree with Sadiya A, et al., in that, despite an increase of 25 (OH) Vitamin D up to 40.5 ng/ml (+16.4 ng/ml, +68.3%), they did not obtain significant modifications of FPG, FPI, HbA_{1c} and HOMA index. The mechanism by which Vitamin D3 can improve carbohydrate metabolism is currently quite clear, as its receptors are present in many organs that are involved in inducing insulin resistance. First of all, the Vitamin D receptor (VDR) is a 50- to 60-kDa molecule with strong affinity for the active 1a, 25-dihydroxycholecalciferol form; it is a member of the steroid and thyroid receptors family, which act as nuclear transcription factors, regulating gene expression in a ligand-dependent manner [38]. The VDR is present

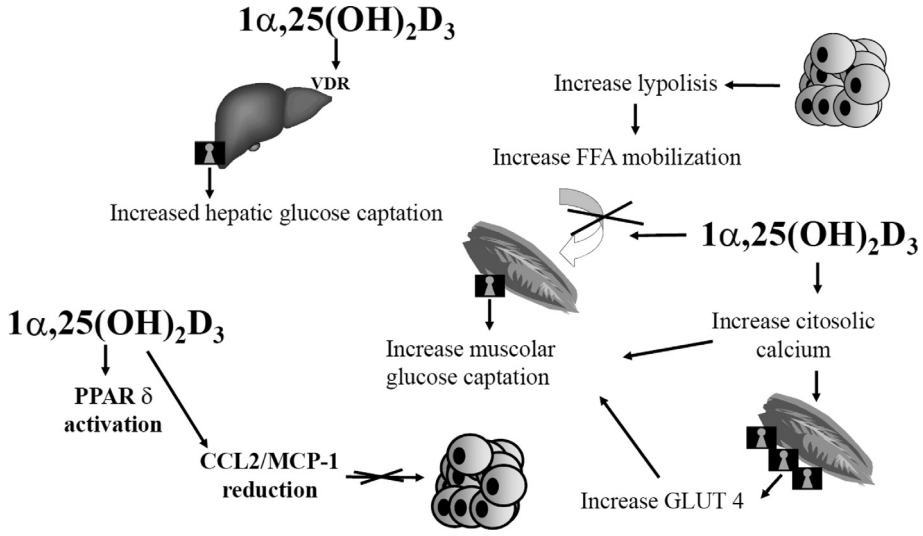

Figure 2. Pathogenic mechanisms linking vitamin D deficiency and insulin-resistance on β-cells. PPAR δ: peroxisome proliferator-activated receptor; GLUT 4: glucose transporter type 4; FFA: free fatty acids; VDR: vitamin D receptor; CCL2: chemokine (C-C motif) ligand 2; MCP-1: monocyte chemoattractant protein 1.

in diverse cell types. Pancreatic islet cells have both VDR and Vitamin D-dependent calcium-binding proteins. Insulin secretion is dependent on changes in intracellular calcium concentration, and 1α, 25-dihydroxycholecalciferol regulates β-cell calcium flux. The VDR is also highly expressed in adipocytes. Activation modulates intracellular calcium concentrations and can effect changes in lipogenesis and lipolysis [39]. It is therefore plausible that Vitamin D3 may be involved in β-cell secretory activity and in modulating the tissue response to insulin. It is not yet clear whether muscle cells expressed VDR or if Vitamin D3 effects on muscle insulin sensitivity, if any, may be indirect [40]. Vitamin D3 deficiency inhibits insulin secretion [41, 42] and modulates lipolysis [43] (Figure 2). Hypovitaminosis D is a risk factor for glucose intolerance. Vitamin D3 concentration is lower in patients with type 2 diabetes mellitus [42] compared to the nondiabetic population. Moreover, 25 (OH) Vitamin D concentration is lower in subjects at risk than those are not at risk for the development of diabetes mellitus and is associated with impaired insulin release [44]. A range of evidence in animal studies indicates that Vitamin D plays an essential role in physiologic pancreatic insulin secretion, since the latter is impaired in Vitamin D-deficient situations and is gradually restored after Vitamin D repletion [45, 46]. It seems that supplementation with Vitamin D improves insulin release and glucose clearance in vivo, independently of calcium and phosphorus concentrations, as well as other dietary factors [47]. Vitamin D not only enhances β-cell biosynthetic activity, but also accelerates transformation of pro-insulin to insulin [48]. Finally, the improvement of insulin sensitivity by 60% noted with Vitamin D supplementation is superior to that noted after treatment with troglitazone or metformin, which improved sensitivity by 54 and 13%, respectively [49].

Our study certainly has limitations: the number of patients is still small in order to draw definitive conclusions, but it can be the starting point for a further and more numerous study. Moreover, the use of the HOMA index is also a limitation; the gold standard method for assessing insulin sensitivity should be clamp, even if this technique is more expensive and complicated.

Conclusions

In type 2 diabetic patients with Vitamin D deficiency, the restoration of value to the Vitamin D standard has led not only to an improvement in the glyco-metabolic compensation, but also to a lower use of some oral hypoglycemic agents and some types of insulin.

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Conflict of interest
The authors declare that there are no conflicts of interest.

Authors contribution
Design and conduction of the study: Giuseppe Derosa, Pamela Maffioli. Data collection: all authors. Data interpretation and manuscript writing: Giuseppe Derosa, Pamela Maffioli. All authors read and approved the final version of the manuscript.

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