


Effect of Vitamin D Supplementation on Obesity-Induced Insulin Resistance: A Double-Blind, Randomized, Placebo-Controlled Trial

Chiara M.A. Cefalo^{1*}, Caterina Conte^{1,2*}, Gian Pio Sorice¹, Simona Moffa¹, Vinsin A. Sun¹, Francesca Cinti¹, Enrica Salomone^{1,3}, Giovanna Muscogiuri^{1,4}, Alex A.G. Brocchi¹, Alfredo Pontecorvi¹, Teresa Mezza¹ , and Andrea Giaccari¹

Objective: The aim was to investigate whether vitamin D supplementation, combined with a hypocaloric diet, could have an independent effect on insulin sensitivity in subjects with both overweight and hypovitaminosis D. Changes from baseline in anthropometric parameters, body composition, glucose tolerance, and insulin secretion were considered as secondary outcomes.

Methods: Eighteen volunteers who were nondiabetic and vitamin D deficient and had BMI > 25 kg/m² were randomized (1:1) in a double-blind manner to a hypocaloric diet + either oral cholecalciferol at 25,000 IU/wk or placebo for 3 months. Hyperinsulinemic-euglycemic clamp to measure insulin sensitivity was performed at baseline and after intervention.

Results: Body weight in both groups decreased significantly (−7.5% in the vitamin D group and −10% in the placebo group; $P < 0.05$ for both), with no between-group differences. Serum 25-hydroxyvitamin D levels in the vitamin D group increased considerably (from 36.7 ± 13.2 nmol/L to 74.8 ± 18.7 nmol/L; $P < 0.001$). Insulin sensitivity in the vitamin D group improved (from 4.6 ± 2.0 to 6.9 ± 3.3 mg·kg^{−1}·min^{−1}; $P < 0.001$), whereas no changes were observed in the placebo group (from 4.9 ± 1.1 to 5.1 ± 0.3 mg·kg^{−1}·min^{−1}; $P = 0.84$).

Conclusions: Cholecalciferol supplementation, combined with a weight loss program, significantly improves insulin sensitivity in healthy subjects with obesity and might represent a personalized approach for insulin-resistant subjects with obesity.

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Introduction

Serum 25-hydroxyvitamin D (25[OH]D) concentration has been reported to be inversely associated with impaired glucose regulation, insulin resistance, β -cell dysfunction, and risk of metabolic syndrome (1,2). Although vitamin D insufficiency, defined as 25(OH)D

levels under 75 nmol/L, is common in subjects with obesity (3), there is still controversy as to the mechanisms underlying vitamin D deficiency in obesity. Enhanced adipose tissue uptake (4), altered vitamin D metabolism in adipose tissue (5), sunlight underexposure, and low consumption of dairy products have all been postulated as possible causes. Moreover, it is still unclear whether the concurrence

¹ Center for Endocrine and Metabolic Diseases, Gemelli University Polyclinic Foundation, Catholic University of the Sacred Heart, Rome, Italy. Correspondence: Teresa Mezza (teresa.mezza@gmail.com) ² Internal Medicine and Transplant Unit, San Raffaele Hospital, Milan, Italy ³ Center for Diabetes and Metabolic Diseases, Association of Italian Knights of the Sovereign Military Order of Malta, Rome, Italy ⁴ Division of Endocrinology, Department of Clinical Medicine and Surgery, Federico II University, Naples, Italy.

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*Chiara M.A. Cefalo and Caterina Conte contributed equally to this work.

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of vitamin D deficiency and insulin resistance in obesity is a causality or merely an independent finding.

Several cross-sectional clinical studies have associated low vitamin D status with insulin resistance in adults (6) and children (7). Higher basal levels of 25(OH)D have been found to predict better β -cell function and lower glycemia in subjects at risk for type 2 diabetes mellitus (T2DM) (8). Vitamin D may have favorable effects on insulin sensitivity through a series of mechanisms: it increases transcriptional activation and expression of the insulin receptor gene, facilitating both basal and insulin-stimulated glucose oxidation and thus improving insulin sensitivity (9); enhances insulin action and signal transduction by regulating extracellular calcium (10); and modulates cytokine-mediated β -cell apoptosis, an important factor in the development and progression of T2DM (11).

In a previous study (12), we reported a direct correlation between 25(OH)D concentration and insulin sensitivity, evaluated by hyperinsulinemic-euglycemic clamp, suggesting that vitamin D deficiency could worsen obesity-related insulin resistance. Since then, further studies on the effects of vitamin D supplementation on insulin resistance have produced inconsistent data, with results from trials showing either no effect (13) or improvements in insulin action (14) in a wide range of patient populations. The inconsistency of the findings is most probably due to differences in the methods used to assess insulin resistance, as indirect indexes of insulin resistance derived from fasting insulin and glucose mostly reflect hepatic insulin sensitivity, whereas post-oral glucose tolerance tests (OGTTs) do not account for all the variables influencing the results, including insulin secretion.

Our aim was therefore to evaluate whether vitamin D supplementation could ameliorate insulin sensitivity in patients at a high risk for diabetes; we designed a randomized, double-blind, placebo-controlled trial in which subjects with obesity were supplemented with vitamin D or placebo on top of a hypocaloric diet to clarify the relationship between hypovitaminosis D and glucose metabolism, with a particular focus on insulin resistance, as evaluated by hyperinsulinemic-euglycemic clamp.

Methods

Study protocol

This was a double-blind, randomized, placebo-controlled trial (ClinicalTrials.gov registration: NCT02020694). The primary outcome measure was a change from baseline in insulin sensitivity after an intervention of 3 months.

Secondary outcomes included a change from baseline in glucose tolerance and insulin secretion and/or a change from baseline in body composition, anthropometric parameters, and phosphocalcic metabolism.

The study protocol was approved by the Ethics Committee of the Catholic University of the Sacred Heart. All subjects provided written informed consent.

Subjects

Participants were recruited from patients attending the outpatient clinic of the Center for Endocrine and Metabolic Diseases of our hospital, for follow-up of thyroid nodules or overweight or obesity. Male and female subjects aged 18 to 70 years with BMI ≥ 30 kg/m² and

vitamin D deficiency (serum 25[OH]D < 75 nmol/L), who wished to be included in a weight loss program were enrolled. The exclusion criteria are discussed in the Supporting Information Methods.

Intervention

Subjects were blindly randomized (1:1), in a parallel manner, to a standardized hypocaloric diet + either oral cholecalciferol at 25,000 IU/wk or placebo once a week for 3 months. Cholecalciferol was provided by Abiogen (Dibase cod.A11CC05, 25,000 IU/2.5 mL oral solution), whereas the placebo was manufactured by our hospital pharmacy and was similar to the study medication in color, smell, and taste. The study medications were dispensed in identical boxes, sequentially numbered as per the random allocation list (by the pharmacist who was not involved in data collection or analysis and who kept the list linking the randomization code to the participants' identity and to the delivered box number). Randomization was carried out in blocks of six by gender and month of recruitment to ensure gender balance and minimize seasonal changes in vitamin D levels. The principal investigator, the co-investigators, and the health care providers had no access to these code breaks and were all blinded to treatment allocation. Compliance to treatment was assessed by interview and inspection of vitamin D/placebo bottles supplied to participants and returned at each visit.

In addition, all subjects received dietary counseling from the same nutritionist, who calculated dietary energy composition by subtracting 500 kcal from the usual individual energy intake, evaluated using the diet history method and a 3-day recall questionnaire. Diet compliance was evaluated through changes in anthropometric parameters via food frequency questionnaires during a monthly nutritional visit.

Assessments

Data on demographics, coexisting illnesses, family history of diabetes, and medications used were collected at the first visit. Anthropometric values (body weight, height, and waist and hip circumferences), a hormonal assessment (parathyroid hormone, 25[OH]D, thyroid function), measurement of electrolytes (calcium, phosphorus), a serum lipid profile (triglycerides, total cholesterol, high-density lipoprotein and low-density lipoprotein cholesterol), and a comprehensive metabolic assessment (OGTT, hyperinsulinemic-euglycemic clamp) were performed at baseline and after 3 months of intervention. A detailed description of metabolic assessments has been added in the Supporting Information Methods.

Body composition

Whole body fat mass, whole body lean mass, and trunk fat mass were assessed by dual x-ray absorptiometry (Delphi-W densitometer; Hologic, Marlborough, Massachusetts) at the beginning and at the end of the intervention.

Statistical analyses

From previous data on a similar cohort of patients with obesity studied at our Center (13) showing a difference in the mean glucose infusion rate between the upper and the lower part of serum 25(OH)D (25[OH]D ≥ 75 and ≤ 50 respectively) of 2.0 ± 1.5 mg·kg⁻¹·min⁻¹, we determined that a sample size of 18 subjects for each arm was necessary to detect a difference of 1 m/kg per minute in insulin-mediated glucose uptake on the basis of a type I error of 0.05 and a type II error of 0.20 (power of 80%).

TABLE 1 Clinical and biochemical characteristics of all subjects at baseline

	Placebo group (n = 9)	Vitamin D group (n = 9)	P
Age (y)	35.3 ± 11.0	45.5 ± 11.1	0.06
Gender (F/M)	6/3	8/1	
Body weight (kg)	111 ± 12.2	100 ± 15.8	0.22
BMI (kg/m ²)	38.7 ± 9.75	36.6 ± 5.14	0.50
WHR	0.94 ± 0.03	0.86 ± 0.08	0.13
25(OH)D (nmol/L)	34.7 ± 21.1	36.7 ± 13.2	0.80
Serum calcium (mmol/L)	2.32 ± 0.09	2.31 ± 0.09	0.90
PTH (ng/L)	46.5 ± 1.41	47.8 ± 19.5	0.90
Fasting glucose (mmol/L)	4.78 ± 0.22	5.08 ± 0.55	0.18
Fasting insulin (μU/mL)	16.8 ± 1.69	13.7 ± 8.59	0.49
Total cholesterol (mmol/L)	4.57 ± 0.66	5.12 ± 0.49	0.22
HDL cholesterol (mmol/L)	1.21 ± 0.27	1.47 ± 0.42	0.22
LDL cholesterol (mmol/L)	2.69 ± 0.22	3.16 ± 0.43	0.22
Triglycerides (mmol/L)	1.43 ± 0.36	1.19 ± 0.60	0.55
AUC glycemia 0'-120'(mmol/L·min·10 ⁴)	85.9 ± 7.70	82.5 ± 19.6	0.62
AUC insulin 0'-120' (μU/mL·min·10 ⁶)	12.3 ± 8.77	9.77 ± 7.90	0.52
Glucose uptake (mg·kg ⁻¹ ·min ⁻¹)	4.93 ± 1.13	4.62 ± 2.08	0.75
Total fat (%)	38.8 ± 7.42	39.7 ± 7.06	0.81
Trunk lean mass (kg)	32.3 ± 1.04	28.3 ± 5.23	0.20
Trunk fat mass (kg)	23.9 ± 1.10	21.1 ± 7.99	0.46

Data are expressed as mean ± SD. P value is for interaction between the two groups. 25(OH)D, 25-hydroxyvitamin D; AUC, area under the curve; F, female; HDL: high-density lipoprotein; LDL: low-density lipoprotein; M, male; PTH, parathyroid hormone; WHR, waist-hip ratio.

Statistical analyses were performed with SPSS (version 20; SPSS, Chicago, Illinois), and statistical significance was set at $P < 0.05$. Normality was assessed by using the Shapiro-Wilk test. Results are reported as mean ± SD for normally distributed data sets or median for nonnormally distributed variables. Differences in baseline and follow-up characteristics between the two groups were tested by using independent t tests for normally distributed continuous variables, Mann-Whitney U tests for skewed variables, and Pearson's χ^2 tests for categorical variables.

Results

Of a total of 45 subjects screened from October 2013 to March 2014, only 42 were considered eligible for the study. During the first visit, two subjects were excluded because of high serum levels of vitamin D and another was excluded because of desire to attempt pregnancy. Six subjects were diagnosed with diabetes after the baseline OGTT and were excluded from the study before randomization. The remaining 36 subjects were randomly assigned to either the vitamin D group or the placebo group. A total of 18 subjects (9 participants for both placebo and vitamin D group) were included in the analysis. At follow-up, three participants refused to sign the informed consent, while an additional eight subjects were lost because of lack of compliance in attending visits or taking medication, and other seven subjects were excluded because of noncompliance with the dietary regimen. A flow-chart (Supporting Information Figure S1) shows the study progression.

More females than males were included. Baseline characteristics were comparable for both groups (Table 1). In particular, serum levels of vitamin D were similar for both groups (vitamin D: 36.7 ± 13.2 nmol/L, placebo: 34.7 ± 21.2 nmol/L; $P = 0.80$) as was insulin sensitivity, expressed as milligrams of glucose consumed per minute per kilogram of body weight (glucose uptake) during hyperinsulinemic-euglycemic clamp (vitamin D: 4.6 ± 2.1, placebo: 4.9 ± 1.1 mg·kg⁻¹·min⁻¹; $P = 0.75$).

After 3 months of intervention with hypocaloric diet + cholecalciferol or placebo, body weight decreased significantly in both groups (vitamin D: -7.5%, placebo: -10% reduction in body weight; $P = 0.0007$ and $P = 0.001$); while no other between-group differences were observed ($P = 0.68$) (Figure 1A).

Plasma 25(OH)D levels increased from 36.7 ± 13.2 to 74.8 ± 18.7 nmol/L in the vitamin D group ($P < 0.001$) and from 34.7 ± 21.1 to 41.7 ± 7.7 nmol/L in the placebo group ($P = 0.41$) (Figure 1B).

Total trunk fat mass, evaluated by dual x-ray absorptiometry, decreased significantly only in the vitamin D group after treatment (vitamin D group decreased from 21.1 ± 7.9 to 17.9 ± 7.8 kg, $P = 0.003$; placebo group from 23.9 ± 1.1 to 21.5 ± 5.9 kg, $P = 0.32$), as reported in Table 2. After 3 months of treatment, a substantial improvement in insulin sensitivity was observed in the vitamin D group (from 4.6 ± 2.1 to 6.9 ± 3.3 mg·kg⁻¹·min⁻¹; $P < 0.001$),

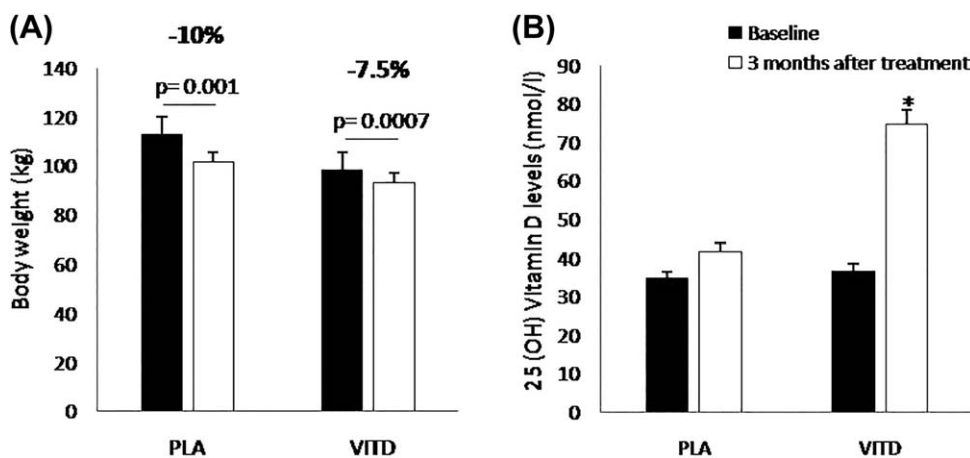


Figure 1 (A) Body weight and (B) 25-hydroxyvitamin D (25[OH]D) levels at baseline (black bars) and after 3 months of treatment (white bars); * $P < 0.001$.

whereas the change from baseline in glucose uptake during the clamp procedure was not significant in the placebo group (from 4.9 ± 1.0 to $5.1 \pm 0.3 \text{ mg}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$; $P = 0.84$) (Figure 2). Seventy-five percent of subjects in the vitamin D group achieved a 20% improvement in insulin sensitivity, versus 33.3% in the placebo group. Nevertheless, the improvement in insulin sensitivity achieved in the supplemented group was not significant compared with the placebo group (P for interaction = 0.16).

Changes from baseline in glucose and insulin areas under the curve were nonsignificant and plasma glucose and insulin levels did not differ at any OGTT time point (Figure 3).

Indirect indexes of β -cell function (i.e., homeostatic model assessment 2 B, insulinogenic index, disposition index) remained consistent in both groups. A clinically irrelevant but statistically significant increment in serum calcium levels was observed in patients treated with vitamin D (from 2.31 ± 0.09 to $2.39 \pm 0.08 \text{ mmol/L}$; $P = 0.01$), while parathyroid hormone levels and lipid profiles remained unchanged in both groups (Table 2).

We noticed that only 60% of subjects in the vitamin D group reached the target serum 25(OH)D concentration of 75 nmol/L after treatment. To confirm the relationship between the improvement in insulin sensitivity and vitamin D supplementation, we performed a further subanalysis on the group treated with Vitamin D between subjects who reached a 25(OH)D concentration of 75 nmol/L and those who did not. At follow-up, both subgroups showed a significant increase in vitamin D levels (Supporting Information Figure S2A), but only subjects with vitamin D higher than 75 nmol/L reached the cutoff of normal serum levels of vitamin D.

Although the subclassification limits the power of analysis, which doesn't reach statistical significance, both groups showed an increase in insulin sensitivity following vitamin D supplementation (25 [OH]D > 75 nmol/L, $P = 0.10$; 25[OH]D < 75 nmol/L,

$P = 0.18$). Further, no difference was observed between groups at follow-up ($P = 0.73$) (Supporting Information Figure S2B).

Discussion

This randomized controlled trial compared the effects of a hypocaloric diet combined with 3 months of vitamin D supplementation (25,000 IU weekly) or placebo on insulin sensitivity, measured using a hyperinsulinemic-euglycemic clamp, in 18 adults with both obesity and vitamin D deficiency. The study revealed that cholecalciferol supplementation combined with weight loss is associated with a significant improvement in insulin sensitivity in vitamin D deficient

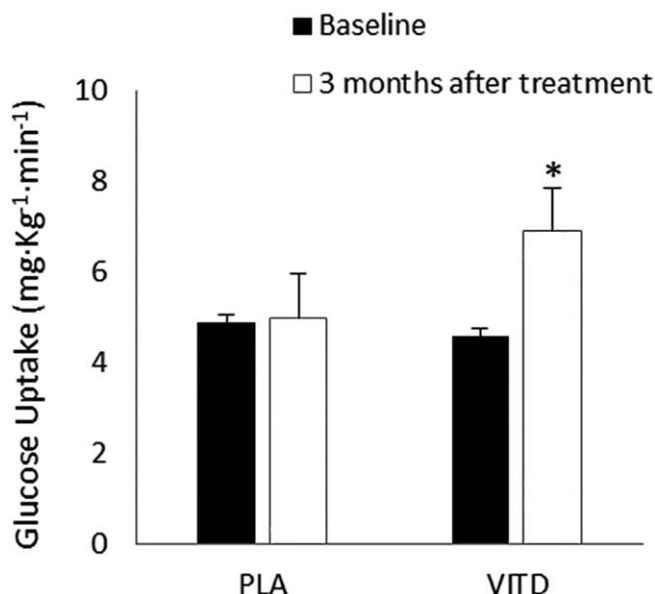


Figure 2 Insulin sensitivity (glucose uptake) at baseline (black bars) and after 3 months of treatment (white bars) in the placebo (PLA) and vitamin D (VITD) groups; * $P = 0.02$.

TABLE 2 Biochemical parameters, β -cell function (HOMA2-B, insulinogenic index, disposition index), and body composition at baseline and after 3 months of treatment in both groups

	Placebo group (n = 9)			Vitamin D group (n = 9)		
	Baseline	Follow-up	P	Baseline	Follow-up	P
25(OH)D (nmol/L)	34.7 ± 21.1	41.7 ± 7.7	0.41	36.7 ± 13.2	74.8 ± 18.7	0.001
Serum calcium (mmol/L)	2.32 ± 0.09	2.32 ± 0.10	0.85	2.31 ± 0.09	2.39 ± 0.08	0.01
PTH (ng/L)	46.5 ± 1.41	40.9 ± 13.6	0.44	47.8 ± 19.5	47.6 ± 20.1	0.97
HOMA2-B	299 ± 107	287 ± 105	0.29	182 ± 84.2	190 ± 107	0.67
Insulinogenic index	1.30 ± 0.36	1.07 ± 0.33	0.51	2.99 ± 5.40	1.07 ± 0.62	0.32
Disposition index	3.38 ± 0.56	3.89 ± 0.04	0.51	4.89 ± 56.0	4.91 ± 5.84	0.65
Total trunk mass (kg)	56.9 ± 9.50	57.2 ± 10.4	0.35	50.0 ± 11.3	37.4 ± 20.5	0.05
Trunk fat mass (kg)	23.9 ± 11.0	21.5 ± 5.88	0.32	21.1 ± 7.98	17.9 ± 78.3	0.003

Data are expressed as mean ± SD. P value is for interaction between baseline and follow-up in each group. 25(OH)D, 25-hydroxyvitamin D; HOMA2-B, homeostatic model assessment 2 B; PTH, parathyroid hormone.

subjects with obesity. However, we failed to demonstrate an unequivocal effect of cholecalciferol supplementation on insulin sensitivity, as the improvements observed in the vitamin D group were not significant versus placebo.

A systematic meta-analysis found that vitamin D supplementation had a bland effect on the reduction of fasting glucose and improvement of insulin resistance in T2DM or impaired glucose tolerance, but no effect on these variables was noted in patients with obesity

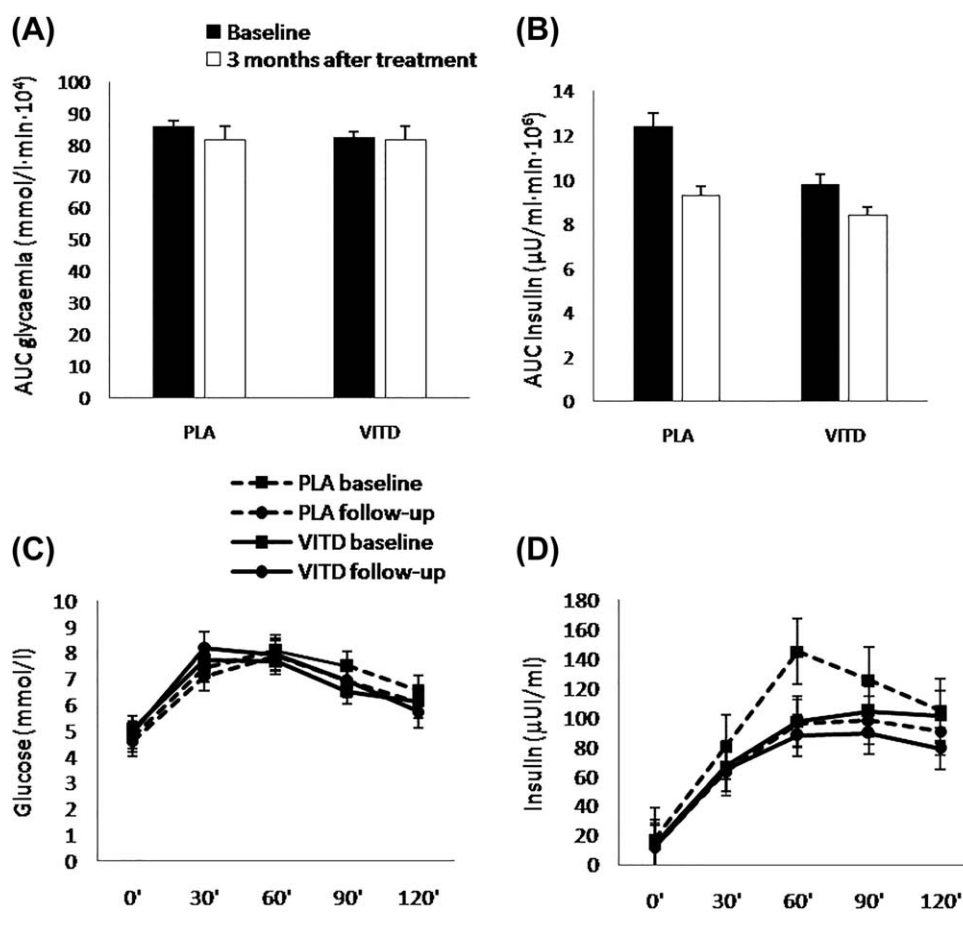


Figure 3 Areas under the curve (AUC) for (A) glycemia and (B) insulin and (C) glucose and (D) insulin curves during oral glucose tolerance tests at baseline and after 3 months of treatment in both groups.

or with normal glucose tolerance (15). In a recently published randomized controlled trial, Gulseth et al. (16) also observed no changes in insulin sensitivity or insulin secretion in T2DM patients after vitamin D3 supplementation. Similarly, Kampmann et al. (17) found no differences in insulin sensitivity, glycemic control, inflammatory parameters, or blood pressure in patients with established T2DM after 12 weeks of vitamin D supplementation, but they suggested a positive effect on insulin secretion and C-peptide levels in the vitamin D-treated group.

However, previous reports have suggested that vitamin D can improve insulin sensitivity both in adolescents and in adults. To date, very few studies have used the gold standard technique to measure insulin sensitivity (i.e., euglycemic-hyperinsulinemic clamp) in order to assess the effect of vitamin D supplementation in subjects with overweight or obesity, and it is therefore difficult to compare these results with data from other studies using surrogate indexes. In a small group of healthy male volunteers ($n = 18$), insulin sensitivity, measured using the clamp procedure, was similar after either calcitriol or placebo treatment, but these subjects had normal body weight and vitamin D levels (18). The findings of a recent randomized controlled trial (19) conducted on subjects with overweight or obesity and with no history of diabetes and vitamin D deficiency contrast with our findings, showing no difference in insulin sensitivity measured with the use of a hyperinsulinemic-euglycemic clamp and insulin secretion measured with the use of an intravenous glucose tolerance test after vitamin D supplementation compared to placebo, despite a significant increase in 25(OH)D concentrations in the vitamin D group. However, in our study, subjects showed a reduction in body weight following a hypocaloric diet, suggesting that supplementation with vitamin D may have a greater effect than lifestyle intervention alone in improving insulin sensitivity in vitamin D deficient subjects with obesity.

In our study, the improvement in insulin resistance following vitamin D supplementation could be related to the important reduction in trunk fat mass observed only in the vitamin D group after intervention. Despite controversial findings on how vitamin D supplementation acts on body weight or fat mass composition, increases in visceral fat mass or trunk fat mass were correlated with pathological obesity and features of metabolic syndrome in this population (20). A well-designed randomized controlled trial combining weight loss intervention with placebo or vitamin D supplementation in 218 women with overweight or obesity revealed that a 12-month daily supplementation with 2,000 IU cholecalciferol was associated with greater reduction in BMI and waist circumference only in those subjects whose 25(OH)D levels rose to 80 nmol/L or higher, suggesting that the beneficial effects of supplementation only kick in at elevated plasma 25(OH)D levels (21). Other studies have reported that calcium and/or vitamin D supplementation contributes to a beneficial reduction in visceral adiposity (22) and have described the mechanism with which such supplements influence the expression of genes correlated with adipogenesis and fatty acid oxidation (23). *In vitro* studies have also shown that the differentiation of preadipocytes into mature adipocytes is halted by 1,25-dihydroxyvitamin D₃ (24). Some of these vitamin D effects could be explained by the expression and activation of different variants of vitamin D receptors in adipose tissue (25).

An additional factor to consider is the increase in serum calcium levels reported in subjects treated with vitamin D; indeed, a significant inverse correlation between high calcium levels and insulin resistance has been reported in the literature. This relationship is

probably explained by the interaction between serum calcium levels and cellular function such as the regulation of glucose uptake in muscle cells, modulating the insulin receptor and insulin sensitivity of these cells (26).

We observed a modest, nonsignificant increase in 25(OH)D levels in the placebo group and no significant improvement in insulin sensitivity despite a clinically relevant 10% weight loss. Serum 25(OH)D concentrations have been shown to increase following weight loss (27), although in a large, controlled, retrospective study (28), this increase did not contribute to improvements in insulin sensitivity and β -cell function. Furthermore, the improvement in insulin sensitivity in the vitamin D-supplemented group was not significant versus placebo. Only 60% of subjects in the vitamin D group reached the target serum 25(OH)D concentration of 75 nmol/L (30 ng/dL), identified as the optimal value for vitamin D homeostasis in epidemiological studies (29). However, when we divided the subjects treated with vitamin D in two subgroups, on the basis of serum 25(OH)D concentration reached after supplementation, we found no significant difference in insulin sensitivity, glucose tolerance, or insulin secretion between groups, while both groups showed an increase in insulin sensitivity following vitamin D supplementation, suggesting that the effect of vitamin D supplementation on insulin sensitivity might be independent of the achievement of the cutoff level of Vitamin D.

Furthermore, we found no improvements in β -cell function due to vitamin D supplementation, at least as assessed by the disposition index. Similarly, glucose and insulin areas under the curve during 120-min OGTT did not apparently improve after treatment; this result is only in apparent contrast with the increase in insulin sensitivity following vitamin D supplementation. An explanation may be that the sample size is probably too small to catch possible changes in glucose tolerance; therefore, an improvement in insulin secretion cannot be excluded. A limited number of interventional studies have examined the effect of vitamin D supplementation on measures of β -cell function (30); these studies have, however, yielded inconsistent results. In a randomized clinical trial, Mitri et al. showed that 16 weeks of cholecalciferol (2,000 IU daily) supplementation improved β -cell function in adults at high risk of diabetes (31).

The strengths of our study are the rigorous assessment of both insulin sensitivity and insulin secretion and the use of gold standard methodology for measuring insulin sensitivity; moreover, the study is well designed, and the participants are well characterized in terms of body composition and anthropometric measures, despite the small sample size, which could lead to insufficient power in detecting differences in study outcomes. Indeed, the main limitation of our study lies in the number of dropouts due to difficulty in following the hypocaloric diet. Despite this limitation, the estimated effect size was higher than anticipated and statistical significance in the primary outcomes changes was detected, even with the reduced final sample analyzed at the end of the study.

In conclusion, our data suggest that vitamin D supplementation, combined with dietary lifestyle intervention, favorably affects insulin sensitivity and body composition, at least in vitamin D deficient subjects with obesity whose serum levels of vitamin D significantly increase after treatment. Our data lay the grounds for future, larger studies aimed at confirming the effect of vitamin D supplementation

on peripheral and hepatic insulin resistance, with the long-run objective to investigate personalized strategies for obesity. **O**

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