

# Relationship between 25-Hydroxyvitamin D and Newly Diagnosed Type 2 Diabetes Mellitus in Postmenopausal Women with Osteoporosis

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## Key Words

Osteoporosis · 25-Hydroxyvitamin D · Type 2 diabetes mellitus

## Abstract

**Objective:** The aim of the study was to determine a correlation between the level of 25-hydroxyvitamin D (25-OHD) and the incidence of diabetes. **Subjects and Methods:** In this prospective observational study, 97 (out of an initial 100) Caucasian women with osteoporosis (OS) were monitored for 2 years for the incidence of diabetes. Logistic regression analysis was used to establish an association with and prognostic value of vitamin D for the onset of type 2 diabetes mellitus, as well as insulin resistance, body mass index (BMI), total cholesterol and triglyceride levels in the development of diabetes. The serum level of 25-OHD was measured using immunochemiluminescence in March and April 2011. **Results:** Of the 97 patients (mean age  $51.64 \pm 5.86$  years, range 36.0–73.0), 21 (21.65%) were diagnosed with diabetes during the observational period. The study showed that the 22 patients with low levels of vitamin D were more susceptible to diabetes (odds ratio = 0.958). The cut-off value of vitamin D using a receiver operating characteristic curve was 62.36 nmol/l with a sensitivity of 39.5% and a specificity of 90.5%. With an increase in BMI and triglyceride levels, women were, respec-

tively, 1,591 and 2,821 times more likely to get diabetes than those without an increase. **Conclusion:** This study showed that the patients with postmenopausal OS and hypovitaminosis D, besides a high BMI, elevated triglyceride levels and insulin resistance, had an increased risk of developing type 2 diabetes.

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## Introduction

According to the International Diabetes Federation, the estimated prevalence of diabetes mellitus (DM) in adults worldwide is 8.3% [1]. The majority of patients (85–95%) suffer from type 2 diabetes mellitus (T2DM) which is a metabolic disorder characterized by hyperglycemia and caused by insulin resistance (IR) and relative insulin deficiency, either or both of which may be present at the time diabetes is diagnosed [1]. Although therapies for T2DM and its complications have improved over the last few decades, the increasing burden of T2DM highlights the need for innovative approaches for the management and prevention of the disease [2].

Vitamin D is a steroid prohormone synthesized in the skin following UV exposure or else acquired by supplemental or dietary intake [3]. Originally, it was described

as regulator of calcium homeostasis, but has since been shown to play a much greater, comprehensive role including protection against immune dysfunction, cancer, cardiovascular conditions, hypertension, metabolic syndrome (MS) and diabetes [3]. Vitamin D status may influence the risk of developing metabolic diseases such as T2DM, MS and IR [4, 5]. Its insufficiency is common among postmenopausal women and it is associated with osteoporosis (OS) [5]. Since 1980, there has been a growing interest regarding its relation to T2DM when Norman et al. [6] identified expression of the vitamin D receptor (VDR) in rat pancreatic cells and demonstrated that a deficiency of vitamin D inhibits the production of insulin. A number of studies have shown that polymorphisms in the VDR gene are implicated in susceptibility to T2DM [7, 8]. However, even nowadays, evidence from randomized and placebo-controlled clinical trials considering the relationship between the level of 25-hydroxyvitamin D (25-OHD) and T2DM is limited [8]. This is particularly true with regard to the underlying mechanism of the associations among serum 25-OHD, glucose homeostasis and insulin resistance which, consequently, leads to the development of T2DM [9, 10].

The objective of this study was to investigate if there is an association between the serum level of 25-OHD and the development of T2DM in postmenopausal women with OS and to determine if 25-OHD has any predictive potential in the onset of T2DM.

## Subjects and Methods

The study included 100 postmenopausal Caucasian women diagnosed with OS and hypovitaminosis D (serum level <75 nmol/l), who were followed for 2 years. This cut-off level was used because for years, it had been shown that a 25-OHD concentration <50 nmol/l or 20 ng/ml is an indication of vitamin D deficiency, whereas a 25-OHD concentration of 51–74 nmol/l or 21–29 ng/ml is considered to indicate insufficiency; concentrations >30 ng/ml are considered to be sufficient [11]. All the studied patients signed a patient consent form according to the Declaration of Helsinki. The inclusion criteria were: age ≤80 years; primary, type 1 OS was defined according to World Health Organization (WHO) criteria [12] as bone mineral density T-score of the spine (L1–L4) and the hip of 2.5 standard deviations (SD) or more below the mean peak bone mass measured on postmenopausal women with a Hologic Discovery A device; not previously treated with bisphosphonates, vitamin D, calcium or any other drug affecting bone metabolism. The exclusion criteria were: underlying conditions in a patient's medical history that could cause secondary OS; a history of intake of glucocorticosteroids, antiepileptics or anticoagulants; previously diagnosed glucose intolerance or T2DM; alcohol abuse; secondary hyperparathyroidism; infectious diseases and any other comorbidity, especially liver and kidney disease.

If the Hologic Discovery A device showed a positive diagnosis for OS, then the following laboratory tests were performed: fasting insulin, blood glucose, serum level of 25-OHD (by immunochemiluminescence, COBAST e-211, Roche), total cholesterol (total-C) and triglyceride (TG) levels. The laboratory tests were performed in March and April because in this period after winter, the lowest 25-OHD levels were expected. Weight and height were recorded in order to determine body mass index (BMI). BMI and the homeostasis model assessment of insulin resistance (HOMA-IR) were calculated in order to determine the level of nutritional status and to quantify IR and beta cell function [ $HOMA-IR = (\text{glucose} \times \text{insulin})/22.5$ ]. The HOMA-IR value of  $\geq 2$  was used to identify patients with IR [13].

A once-monthly oral dose of ibandronate 150 mg, followed by supplementation with calcium (1,000 mg) and 25-OHD (800 IU) was administered to all patients with strict instructions for correct use (intake at least 1 h before breakfast, strictly with a glass of still water, followed by 1 h of walking).

The 97 patients who participated were tested for T2DM over a 2-year period. The diagnosis of T2DM was made according to the 2012 International Diabetes Global Guideline: fasting plasma glucose  $\geq 7.0$  mmol/l (126 mg/dl) or 75 g oral glucose tolerance test with fasting plasma glucose  $\geq 7.0$  mmol/l (126 mg/dl) and/or 2-hour plasma glucose  $\geq 11.1$  mmol/l (200 mg/dl) [14].

Statistical evaluation of the results was performed with the SPSS version 12 for Windows software package. A standard statistical protocol was used for descriptive statistics, followed by a Mann-Whitney U test for establishing differences in age and menopausal age between the 2 groups. To determine the individual contribution of various factors in the emergence of T2DM, taking into consideration the fact that T2DM was given as a binary variable, the logistic regression analysis was used. The receiver operating characteristic (ROC) curve was used to assess the predictive potential of the variables for T2DM development.  $p < 0.05$  was considered significant, while  $p < 0.01$  was deemed as highly significant. The ROC curve provided an opportunity to find the cut-off score with the optimal ratio between sensitivity and specificity (or to maximize between true-positive and true-negative proportions). The y-axis represented sensitivity with values ranging from 0.0 to 1.0 (since the proportions are considered). The x-axis represented  $1 - \text{specificity}$  or the proportion of false-positive values. The area under the curve (AUC) was considered to be the accuracy of the test.

## Results

During the 2-year period, 3 patients withdrew from the study: 1 was diagnosed with breast cancer, another voluntarily decided to not participate after the first check-up and the third died from respiratory failure. Of the 97 patients (mean age  $51.64 \pm 5.86$  years, range 36.0–73.0) at the end of 2 years, 21 (21.65%) developed T2DM after they had been diagnosed with OS while the remaining 76 (78.35%) were negative for T2DM. No statistical significance was found between the groups considering their age ( $p = 0.846$ ) and menopausal age ( $p = 0.823$ ). Both

**Table 1.** Examined parameters for both groups (mean  $\pm$  SD)

	25-OHD, nmol/l	Age, years	Menopausal age, years	BMI	Total-C, mmol/l	TG, mmol/l	HOMA-IR
Group I – T2DM-positive	44.36 $\pm$ 18.99	61.38 $\pm$ 6.03	47.28 $\pm$ 5.59	29.71 $\pm$ 3.49	7.18 $\pm$ 1.14	2.98 $\pm$ 1.11	2.69 $\pm$ 0.29
Group II – T2DM-negative	57.75 $\pm$ 27.59	61.21 $\pm$ 9.14	47.46 $\pm$ 6.28	25.03 $\pm$ 3.04	6.21 $\pm$ 1.17	1.89 $\pm$ 0.67	1.56 $\pm$ 0.44

groups of patients, the one with T2DM and the other without T2DM were homogeneous according to age and menopause age; this permitted the analysis of correlations between variables.

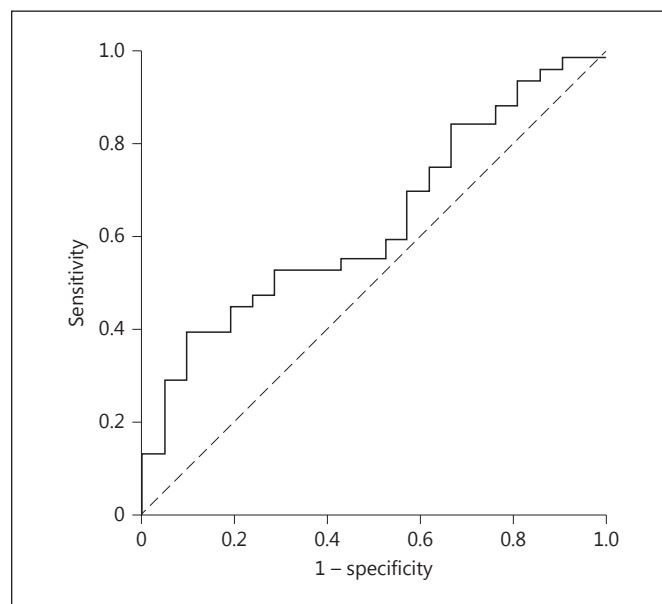
The results of descriptive statistics for both groups are given in table 1. The comorbidities were as follows: all the patients (100%) had hypovitaminosis D, 19 (19.69%) were obese, 84 (86.6%) had elevated total-C levels, 71 (73.2%) had elevated TG levels and 37 (38.14%) had IR.

In the logistic regression analysis, the occurrence (presence) of T2DM was the criterion or dependent variable (dependent/response variable) and the predictors or independent variables (independent/explanatory variables) were: 25-OHD, BMI, total-C and TG. HOMA-IR was highly correlated with the presence of T2DM (a nearly collinear relationship [1]), with this predictor being analyzed independently of other predictors.

Increased amounts of 25-OHD significantly reduced the probability of T2DM occurrence ( $p < 0.05$ ). With the increase in BMI, the probability of diabetes also increases; this finding was also statistically significant ( $p < 0.01$ ). Total-C levels were not significantly associated with the occurrence of diabetes ( $p > 0.05$ ). By increasing the TG parameter, the probability of an incidence of T2DM significantly increased ( $p < 0.05$ ).

The odds ratio (OR) displays chances for each of the predictor variables. For 25-OHD, OR is  $< 1$  (OR = 0.958), indicating that the chances of someone having T2DM were less if his/her 25-OHD serum level was higher. For BMI, women with a higher BMI were 1.591 times more likely to have T2DM. For cholesterol, the chances were equal (OR = 0.983, i.e. close to 1), which means that cholesterol was not recognized as a risk factor for T2DM. Finally, people with higher TG levels were 2.821 times more likely to have T2DM.

The level of 25-OHD correlated negatively with T2DM, which means that the dependent variable (T2DM absence variable) is considered on the ROC chart (fig. 1). Therefore, the values of 25-OHD above the cut-off score indicate a lower risk of T2DM, and those below the cut-off score represent a greater risk of T2DM. The cut-off score

**Fig. 1.** ROC curve for 25-OHD and T2DM.

was 62.36 nmol/l with sensitivity of 39.5% and specificity of 90.5%. The AUC was 0.633 [SE = 0.064, which is on the border of statistical significance ( $p = 0.063$ , therefore  $p > 0.05$ )].

Finally, the correlation coefficients between HOMA-IR and 25-OHD for both groups were very low ( $-0.135$  for the first group and  $0.128$  for the second), since no significant difference existed between them ( $p > 0.05$ ).

## Discussion

Besides IR, a high BMI and elevated TG levels, all well-known positive predictors for developing T2DM, patients with lower 25-OHD levels also had a significantly greater chance of being diagnosed with T2DM ( $p = 0.0256$ ) with an OR of 0.958. Thus, in the case of 25-OHD for the obtained OR of 0.958, the chances for some-

one to have T2DM are less if his/her 25-OHD serum level is higher, meaning that the presented OR is clinically significant.

Given explanations for this relationship are based on identified VDR on beta pancreatic cells, increased IR, reduced insulin secretion and increased autoimmune or inflammatory damage to pancreatic islets [10, 15]. Our 25-OHD findings support the results of earlier clinical and animal studies linking hypovitaminosis D, obesity, IR and T2DM [8, 10].

Our study has some strengths and limitations. Its prospective feature constitutes an element of methodological strength with a certain predictive value to the research findings and also provides insight for further surveys. Limitations include the small sample size and the relatively short follow-up period. Considering the observational design, the study did not elaborate on possible underlying mechanisms or genetic variants in the vitamin D pathways.

The ROC curve shows a cut-off value of 25-OHD, below which postmenopausal women with OS have a greater chance of developing T2DM. With a sensitivity of 39.5% and a specificity of 90.5%, this value is 62.36 nmol/l. From this finding, we can infer that a low serum 25-OHD level is a better positive predictor of T2DM (specificity of 90.5%) than a higher value is a negative predictor for the same disease (sensitivity of 39.5%). This cut-off value is on the edge of statistical significance ( $p = 0.063$ ), probably due to the small sample.

A prospective study carried out by Pittas et al. [16] included 83,779 women who were taking vitamin D and calcium. It concluded that a combined daily intake of  $>1.200$  mg calcium and  $>800$  IU vitamin D was associated with a 33% lower risk of T2DM over 20 years. Our study, on the other hand, followed postmenopausal women taking calcium 1,000 mg and 800 IU of 25-OHD as daily supplementation over 2 years, but we still registered a lower probability of T2DM in those with higher serum levels of vitamin D.

Pittas et al. [17] later published a meta-analysis which concluded that combined supplementation of vitamin D and calcium may play a role in the prevention of T2DM [17]. However, the evidence from current studies is insufficient to be able recommend vitamin D supplementation for the prevention of T2DM [18]. Further prospective studies are needed to establish whether achieving a sufficient 25-OHD level would indeed reduce the risk of DM, the severity of the disease or any of its complications.

Apart from the relation of a low serum level of 25-OHD to greater bone turnover, bone loss and OS, it is in

an inverse association with BMI [4, 19, 20]. It has been calculated in multivariate analysis that a decrease of 0.74 nmol/l in 25-OHD causes an increase in BMI [21]. Considering that vitamin D is a fat-soluble vitamin, excess fat makes it less available for use in the body [3]. Wortsman et al. [22] concluded that obesity-associated vitamin D insufficiency is likely due to the decreased bioavailability of vitamin D from cutaneous and dietary sources because of its deposition in body fat compartments. With a statistically significant difference of  $p = 0.0014$ , we proved that patients with a higher BMI have a higher probability of developing T2DM.

Obesity was found in 19.69% of our participants, but there were also numerous other risk factors for T2DM: an elevated total-C in 86.6%, elevated TG levels in 73.2% and IR in 38.14%. Total-C levels were not significantly connected with T2DM while patients with elevated TG levels had 2.821 times more chance of developing T2DM.

Considering the fact that elevated TG and IR levels are components of MS, we have confirmed the correlation between these parameters and T2DM. IR, one of the major contributors to the pathophysiology of T2DM, assessed by HOMA-IR, was in an almost collinear relationship with the incidence of T2DM as expected. The literature contains more than 40 studies that have produced inverse correlations of vitamin D status (serum 25-OHD) with a risk for MS or with the incidence or severity of its components [8].

## Conclusion

This study showed that hypovitaminosis D in postmenopausal women was a positive predictor for T2DM occurrence. Reliable evidence from carefully designed intervention studies, particularly those based on healthy populations, is needed to confirm observational findings.

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