Study of vitamin D level in type 2 diabetic patients before and after treatment with pioglitazone

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Received 04 January 2015
Accepted 23 April 2015

Egyptian Journal of Obesity, Diabetes and Endocrinology
2015, 1:43–48

Objective
The aim of this study was to evaluate vitamin D level in type 2 diabetic patients before and after treatment with pioglitazone and assess any possible relationship with type 2 diabetic patients who are pioglitazone naive.

Participants and Methods
The study included 50 female participants; of them, 20 were healthy female participants who served as controls and 30 were pioglitazone-naive diabetic patients. All individuals were subjected to history taking and clinical examination, including fasting blood sugar, 2 h postprandial, glycosylated hemoglobin (HbA1c), lipid profile test (total cholesterol, HDL, LDL, triglycerides), kidney function tests (serum creatinine and calculated glomerular filtration rate), and evaluation of serum calcium, phosphorus, and alkaline phosphatase and serum 25-hydroxy vitamin D (by enzyme linked immunosorbant assay) before (basal) and after 3 months of treatment with pioglitazone.

Results
There was an nonsignificant elevation of vitamin D in group 2b (diabetic patients after using pioglitazone for 3 months), in comparison with vitamin D level in group 2a (diabetic patients before using pioglitazone) ($P = 0.117$). Vitamin D levels were found to be inversely associated with HbA1c levels in type 2 diabetic patients ($P = 0.000$ linear regression analysis); it was also found to be inversely associated with fasting and 2 h postprandial blood sugar levels ($P < 0.000$).

Conclusion
Vitamin D could impact glycemic control in terms of the inverse relation of vitamin D with HbA1c%, and at the same time poor glycemic control could impact vitamin D status in uncontrolled diabetic patients. Thiazolidinediones do not have significant effect on vitamin D level in female diabetic patients.

Keywords:
diabetes mellitus, pioglitazone, vitamin D

Egyptian Journal of Obesity, Diabetes and Endocrinology 1:43–48

2356-8062

Introduction

Studies have shown decreased bone formation and an increased risk for fractures in diabetic patients on thiazolidinediones (TZDs). Changes in bone strength from glycation of collagen and negative calcium balance from calcium loss in the urine due to hyperglycemia may also be seen. The TZDs affect bone turnover by increasing the formation of adipocytes instead of the bone-forming osteoblasts from the common mesenchymal stem cell. Vitamin D also suppresses the antigen-presenting capacity of macrophages, modulates the development of CD4 lymphocytes, and inhibits the production of interferon c and interleukin 2 among other cytokines. These cytokines are known to activate macrophages and cytotoxic T cells, which in turn can lead to the destruction of the pancreatic islets seen in type 1 diabetes mellitus (T1DM) [1]. Through its anti-inflammatory effects, vitamin D may reduce the cytokine-induced islet cell death. By modulation of the immune and inflammatory process, vitamin D may also decrease insulin resistance and increase insulin secretion in T2DM, two characteristic defects in this condition [2]. The relationship between vitamin D and B-cell functions may be reciprocal in nature. Insulin secretion is a process dependent on changes in intracellular calcium concentration. The effects of vitamin D on B cells may be by its regulation of extracellular calcium and calcium flux through the B cell [3], or through calcium-independent pathways [4]. Vitamin D insufficiency promotes the development of osteoporosis through the association with parathyroid hormone (PTH). The increase in PTH enhances osteoclast activation; this stimulates an increase in preosteoclast conversion to osteoclasts [5].

Subsequent observational studies reported increased fracture risk after exposure to TZD, in men and in the axial skeleton. A 1.5- to two-fold increase in fracture risk, if attributable to changes in bone mineral density (BMD), would be predicted to be associated with a decrement in BMD approaching 1 SD (10%) in size [6].

 DOI: 10.4103/2356-8062.159994

Vitamin D insufficiency contributes to osteoporosis by decreasing intestinal calcium absorption [7]. Treatment of vitamin D deficiency has been shown to improve BMD [8]. An analysis by the Third National Health and Nutrition Examination Survey (NHANES III) demonstrated a positive correlation between circulating 25-hydroxy vitamin D (25OHD) levels and BMD [9].

The explanation for the increased risk for fractures observed in both randomized trials and observational studies of TZDs remains unclear [10].

In a study by Andrew and colleagues, they reported the results of a 1-year randomized controlled trial of the effects of pioglitazone on BMD and biochemical markers of bone turnover in people with T2DM or impaired glucose tolerance (IGT).

They found that 30 mg/day of pioglitazone did not increase bone loss at the lumbar spine over 12 months in a population of men and women with T2DM. Pioglitazone marginally increased bone loss at the proximal femur, but had no effect on BMD at skeletal sites enriched for cortical bone, the proximal forearm, and whole body.

The link between hypovitaminosis D3 and metabolic disorders, including obesity, metabolic syndrome, T2DM, and CVD requires further investigation, particularly for those most at risk of these combined conditions [11].

Participants and methods

Study design

This is a cross-sectional study, which was conducted on 50 individuals selected from the outpatient clinic of internal medicine and endocrinology of Ain Shams University hospitals from December 2012 to April 2013. They were divided into the following groups: Group 1:20 healthy female subjects as control, Group 2:30 female pioglitazone naïve diabetic patients who were subdivided into 2a and 2b (a before and b after start of pioglitazone), (diagnosed by history, clinical examination, and investigations).

Exclusion criteria

Patients with T1DM, postmenposal women, obese patients (BMI >30%), those with other causes of vitamin D deficiency, and patients on pioglitazone therapy were excluded from the study.

All participants were subjected to the following:

(1) Clinical assessment: Full history taking was carried out. BMI was calculated using the Quetelet formula (weight in kilograms divided by the square of height in meters). Clinical examination including waist circumference and blood pressure was carried out.
(2) Laboratory investigation: Biochemical assays included determination of fasting blood glucose, postprandial plasma glucose, glycated hemoglobin (HbA1c), lipid profile (LDL, total cholesterol, HDL, triglycerides), blood urea, and creatinine using automated standard laboratory techniques. Participants were instructed to fast for 12–14 h, and 10 ml of venous blood was collected by means of venipuncture. Serum was separated by centrifugation and divided into two samples. The first sample was used for measurement of fasting blood sugar, and the second sample was frozen at −20°C until assayed in the laboratory of Clinical Pathology Department, Ain shams University, Faculty of Medicine. Serum lipid profile (total cholesterol, HDL, LDL, and triglycerides) was determined by means of enzymatic hydrolysis, and oxidation of 25-OH vitamin D was measured using enzyme linked immunosorbant assay. Participants were instructed to eat after venipuncture, and another 3 ml of venous blood was collected 2 h after for measuring 2 h postprandial blood sugar. To avoid seasonal variations, all 25(OH)D samples were collected during the spring months for individuals in all groups.

Normal range: 25-OH vitamin D: 25–125 nmol/l, 1 ng/ml = 2.5 nmol/l, 1 nmol/l = 0.4 ng/ml.

Statistical analysis

All data were analyzed using SPSS software (version 11; SPSS Inc., Chicago, Illinois, USA). Baseline characteristics were presented as mean ± SD for the continuous variables and as frequency and percentage for the discrete ones. Comparisons between groups were made using analysis of variance. Correlation between variables was examined using Pearson's correlation coefficient. Multiple linear regression analysis was used to determine the independent predictors of serum vitamin D levels. A *P* value less than 0.05 was considered statistically significant.

Results

The studied participants were divided into two groups: group 1 included 20 healthy female participants who served as controls and group 2 included 30 female pioglitazone-naive diabetic patients.

Group 1 (control) included 20 (40%) female participants. Their mean age was 36.09 ± 7.86 years, mean weight was 80.36 ± 14.98 kg, mean height was 1.71 ± 0.10 m, mean BMI was 27.17 ± 2.94 kg/m^2, mean fasting blood sugar (FBS) was 87.00 ± 6.71 mg/dl, mean 2 h post prandial blood sugar (PPBS) was 125.6 ± 9.99 mg/dl, mean HbA1c was 5.14 ± 0.30%, and mean vitamin D level was 63.18 ± 21.34 nmol/l (Fig. 1). Group 2a included diabetic patients before the initiation of pioglitazone therapy. It included 30 (60%) diabetic female patients with T2DM. Patients' mean age was 40.33 ± 4.99 years, mean weight was 78.37 ± 7.72 kg, mean height was 1.66 ± 0.06 m, mean BMI was 28.43 ± 1.75 kg/m^2 (Fig. 1), mean FBS was 211.13 ± 74.38 mg/dl, mean 2 h PPBS was 268.57 ± 80.44 mg/dl, mean HbA1c was 9.97 ± 2.00%, and mean vitamin D level was 17.87 ± 10.68 nmol/l (Fig. 2). After 3 months of initiating pioglitazone therapy, patients' mean weight was 79.4 ± 7.93 kg, BMI was 28.78 ± 1.933 kg/m^2 (Figs. 2, 3), mean FBS was 143.8 ± 44.596 mg/dl, mean 2 h PPBS was 191.2 ± 54.941 mg/dl, mean HbA1c was 8.27 ± 1.229%, and mean vitamin D level was 20.57 ± 10.553 nmol/l (Tables 1 and 2).

Comparing group 2a (diabetic patients before using pioglitazone) with group 2b (after using pioglitazone)

There were highly statistically significant decrease in FBS, PPBS, and HbA1c levels, highly statistically significant increase in patients' weight and BMI, and nonstatistically significant increase in vitamin D level in diabetic patients after using pioglitazone ($P > 0.005$) Table 3.

Correlating vitamin D level and the studied parameters in all 50 patients

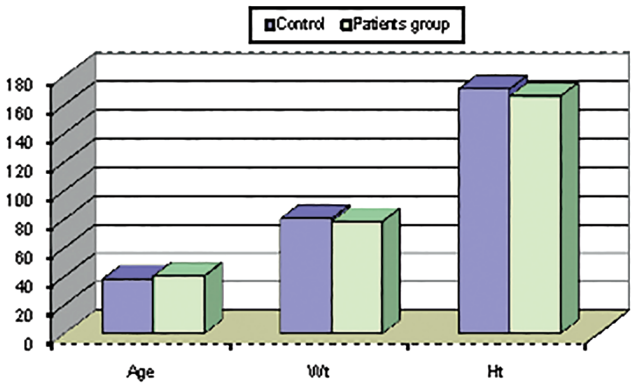
The correlation between vitamin D and other quantitative variables (age, weight, height, BMI, FBS, PPBS, and HbA1c) showed that there was an indirect significant correlation between age and vitamin D level ($P = 0.010$; $r = -0.352$), direct nonsignificant correlation between weight and vitamin D level ($P = 0.358$; $r = 0.130$), indirect nonsignificant correlation between BMI and vitamin D level ($P = 0.206$; $r = -0.178$), an indirect highly significant correlation between FBS and vitamin D level ($P = 0.000$; $r = -0.686$), also an indirect highly significant correlation between PPBS and vitamin D level ($P = 0.000$; $r = -0.621$), and there is an indirect highly significant correlation between HbA1c and vitamin D level ($P = 0.000$; $r = -0.744$) Table 4.

Comparison between control group and patients group regarding demographic data is shown in Fig. 1, Comparison between control group (group 1) and patients group (group 2b) after 3 month of initiating Pioglitazone therapy is shown in Fig. 2, comparison between group 2a and 2b in all studied parameters. Is shown in Fig. 3.

Discussion

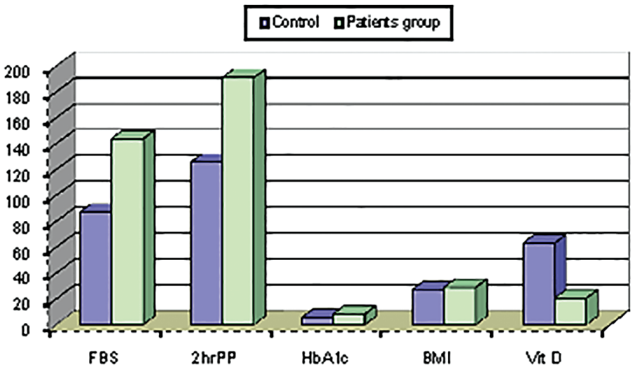
DM is one of the most common noncommunicable diseases worldwide. It is the fourth or fifth leading cause of death in most high-income countries, and there is substantial evidence that it is epidemic in many economically developing and newly industrialized

Figure 1



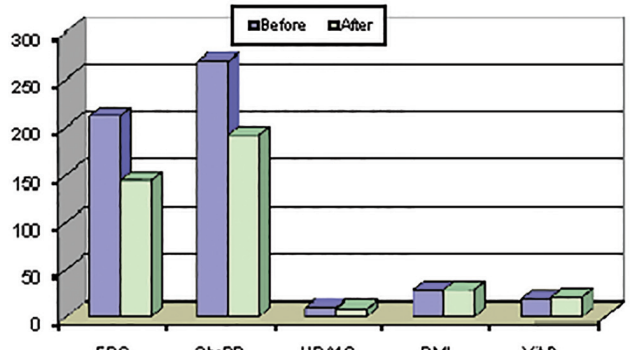
Comparison between control group and patients group regarding demographic data

Figure 2



Comparison between control group (group 1) and patients group (group 2b) after 3 month of initiating Pioglitazone therapy

Figure 3



Comparison between group 2a and 2b in all studied parameters.

countries. Diabetes is undoubtedly one of the most challenging health problems in the 21st century [12].

Vitamin D, the sunshine vitamin, has received a lot of attention recently as a result of a meteoric rise in the number of publications showing that vitamin D plays a crucial role in a plethora of physiological functions and associating vitamin D deficiency with many acute and chronic illnesses [13].

Vitamin D deficiency have been related to the development of autoimmune diseases, such as multiple sclerosis [14,15] and rheumatoid arthritis [16], and vitamin D deficiency appears to be related to the development of DM type 2 and metabolic syndrome [17–19].

Our present study was conducted to determine vitamin D level in type 2 diabetic patients before and after treatment with pioglitazone and to determine the effect of pioglitazone on vitamin D levels.

Vitamin D level was significantly decreased among diabetic patients when compared with healthy controls, with a median of 17.88 nmol/l in diabetic patients before initiating pioglitazone therapy, a median of 20.57 nmol/l after 3 months of pioglitazone therapy, and a median of 63.18 nmol/l in the control group ($P < 0.000$), showing that diabetic patients were in the deficient range and controls were in the insufficient range.

These results are in agreement with those reported by Kostoglou-Athanassiou *et al*. (2013), who found that 25(OH)D3 levels were lower in type 2 diabetic patients than in the control group, with 25(OH)D3 levels being 19.26 ± 0.94 and 25.48 ± 1.02 ng/ml in the patient and control groups, respectively [27].

Our result showed a nonsignificant elevation of vitamin D level in diabetic patients after using pioglitazone for 3 months (group 2b) compared with vitamin D level in diabetic patients before initiating the pioglitazone therapy (group 2a) (20.57 vs. 17.87 ng/dl, respectively; $P = 0.117$).

Interestingly, an inverse relationship was found between vitamin D levels and glycosylated hemoglobin in the

Table 1 Demographic data of type 2 diabetic patients and controls

Demographic data	Control		Patient group		Independent *t*-test	
	Mean	SD	Mean	SD	*t*	*P* value
Age (years)	38.09	7.86	40.33	4.99	1.318	0.192
Weight (kg)	80.36	14.98	78.37	7.72	0.627	0.534
Height (cm)	171	13.0	166	8.0	1.794	0.078
BMI (kg/m^2)	27 017	2.94	28.43	1.75	–1.943	0.058

Table 2 Laboratory data in type 2 diabetic patients and controls

Laboratory data	Group 1		Group 2a		Group 2b	
	Mean	SD	Mean	SD	Mean	SD
FBS (mg/dl)	87.00	6.71	211.13	74.38	143.3	44.6
2 h postprandial (mg/dl)	125.69	9.99	268.5	80.44	191.2	54.94
HbA1c (%)	5.14	0.30	9.97	2.00	8.27	1.23
Vitamin D (nmol)	63.18	21.34	17.88	10.68	20.57	10.55

HbA1c, glycosylated hemoglobin.

Table 3 Comparison between patients as regards the studied parameters before and after initiating pioglitazone therapy

Studied patameters	Before		After		Paired *t*-test	
	Mean	SD	Mean	SD	*t*/*z**	*P* value
FBS (mg/dl)	211.13	74.38	143.80	44.60	5.839	0.000
2 h postprandial (mg/dl)	268.57	80.44	191.20	54.94	5.895	0.000
HbA1c (%)	9.97	2.00	8.27	1.23	5.819	0.000
BMI (kg/m^2)	28.43	1.75	28.78	1.93	–3.604	0.001
Vitamin D (nmol)	17.87	10.68	20.57	10.55	–1.593*	0.117

HbA1c, glycosylated hemoglobin; *Significant correlation.

Table 4 Correlations of vitamin D in patients and controls in each group individually and with all participants

All studied parameters	Group 1 (control)		Group 2a (diabetic patients before pioglitazones)		Group 2b (diabetic patients after pioglitazones)		All participants	
	R	*P* value	*R*	*P* value	*R*	*P* value	*R*	*P* value
Age (years)	–0.180	0.424	–0.12	0.529	–0.203	0.281	–0.352*	0.010
Weight (kg)	0.338	0.124	–0.065	0.733	0.156	0.410	0.130	0.358
Height (cm)	0.292	0.188	0.016	0.933	0.129	0.497	0.280*	0.045
p-FBS (mg/dl)	0.259	0.245	–0.166	0.381	0.097	0.611	–0.686**	0.000
HbA1c (%)	–0.177	0.430	–0.125	0.51	0.039	0.838	–0.744**	0.000
BMI (kg/m^2)	0.275	0.215	0.044	0.819	0.284	0.128	–0.178	0.206
2 h postprandial (mg/dl)	0.420	0.052	0.055	0.771	0.200	0.289	–0.621**	0.000

HbA1c, glycosylated hemoglobin; *Significant correlation; **Highly significant correlation.

entire studied population, when type 2 diabetic patients and controls were analyzed together. It appears that vitamin D may be related to glucose control in diabetic patients. In addition, statistically significant 25(OH) D_3 deficiency and insufficiency was found more in type 2 diabetic patients than in the controls.

Kostoglou-Athanassiou *et al*. (2013) reported that lower 25(OH)D_3 levels were observed in a cohort of type 2 diabetic patients compared with the control group, and an inverse relationship was observed between glycosylated hemoglobin levels and 25(OH) D_3 levels in the patient group, implying that 25(OH) D_3 levels may affect glucose control in DM type 2.

In a cross-sectional analysis of a general population sample in eastern Finland, an inverse association was observed between 25(OH)D3 levels and fasting insulin, fasting glucose, and 2 h glucose tolerance test glucose results, implying that low serum 25(OH) D3 may be associated with impaired glucose metabolism [20].

Similar to our results, Somchodok Chakreeyarat *et al*. (2011) showed that the levels of 25(OH)D in TZD postmenopausal female users were higher (35.3 ± 1.5 vs. 25.9 ± 1.2 ng/dl; $P < 0.001$). The prevalence of vitamin D deficiency was 75.5% in participants not on TZD compared with 34.6% in those on TZD [26].

Although a difference in sun exposure could account for the difference in vitamin D status, the degree of difference is not likely to be enough to be attributable to the higher vitamin D status in the TZD group. Both groups of participants were active, ambulatory, and without other major complications that could lead to limited sun exposure.

It is also likely that TZD may affect dermal vitamin D metabolism. Vitamin D synthesis takes place in the dermal/epidermal junction. It is of note that besides being present in the liver, dermal fibroblasts also express 25-hydroxylase enzymes [21] and produce 25(OH)D upon UVB exposure [22].

In contrast, by taking paired stored baseline and 12-month serum samples from 1605 participants (689 women, 916 men) in ADOPT, a recent study showed that CTX-1 (C-terminal telopeptide for type 1 collagen), a marker for osteoclast activity and bone resorption, was increased by 6.1% in rosiglitazone-treated women and that bone alkaline phosphatase, a marker for osteoblast activity and bone formation, was decreased in patients treated with rosiglitazone [23,24].

In the current study, a stepwise logistic regression model, including TZD, BMI, fasting, postprandial blood sugar level, and age, showed that vitamin D level had no interaction with age, BMI, and blood sugar levels. HbA1c was a significant predictor; it was found that there was an inverse association between 25(OH) D levels and HBA1c ($t = -3.833$; $P = 0.000$). Moreover, the patient group after pioglitazone therapy had better glycemic control reflected as significant decrease in HbA1c%, which resulted in a nonsignificant elevation of vitamin D level in the treatment group.

Similar to the current study results, Christine D *et al*. (2011) and Anne-Thea McGill *et al*. (2008) showed an increasing concentration of HbA1c associated with decreasing 25(OH)D3 concentration, and this association was independent of BMI and age [28,29].

However, these results are not in line with the results of Lee *et al*. (2012), who did not find any association between the serum 25(OH)D level and HbA1C in Korean T2DM patients [30].

An alternative and potentially interesting explanation could be that a poor chronic glycemic control directly affects vitamin D metabolism. The first hydroxylation process takes place in the liver and forms 25-hydroxyvitamin D3 (25(OH)D3), whereas the second hydroxylation step, which produces the final active metabolite, occurs predominantly in the kidney. These reactions are brought about by 25-hydroxylase in the liver and by 1a-hydroxylase in the kidney, and they belong to the cytochrome P450-dependent steroid hydroxylases. Two enzymes in the liver, one in microsomal fraction, and the other in mitochondria catalyzes the 25-hydroxylation of vitamin D. However, no direct evidence of a possible effect of hyperglycemia on 25-hydroxylase has been provided yet, an issue that only specifically designed studies can address [25].

Therefore, a hypothesis that state, there is increase in 25-hydroxylationof vitamin D synthesized from the skin in TZD users maybe the underlying basis of the higher vitamin D status observed with TZD use.

Eventually, in the current study, pioglitazone did not seem to alter vitamin D level, thus is not the cause of the increased fracture risk in diabetic patients.

Conclusion

Vitamin D could impact glycemic control in terms of the inverse relation of vitamin D with HbA1c%, and at the same time poor glycemic control could impact vitamin D status in uncontrolled diabetics. TZDs do not have significant effect on vitamin D level in female

diabetic patients. Therefore, screening for vitamin D insufficiency is essential for all patients with diabetes. Further studies are needed to clarify the association between vitamin D level and the use of TZDs in diabetic patients.

Acknowledgements

Conflicts of interest
None declared.

References

1 Riachy R, Vandewalle B, Belaich S, Kerr-Conte J, Gmyr V, Zerimech1 F, *et al*. Beneficial effect of 1,25 dihydroxyvitamin D3 on cytokine-treated human pancreatic islets. J Endocrinol 2001; 2001:161–168.

2 Palomar X, *et al*. Role of vitamin D in the pathogenesis of type 2 diabetes mellitus. Diabetes Obes Metab 2008; 10:185–197.

3 Ismail A, Namala R. Impaired glucose tolerance in vitamin D deficiency can be corrected by calcium. J Nutr Biochem 2000; 11:170–175.

4 Vertergaard P. Bone metabolism in type 2 diabetes and role of thiazolidinediones. Curr Opin Endocrinol Diabetes obese 2006; 16:125–131.

5 Holick MF. High prevalence of vitamin D inadequacy and implications for health. Mayo Clin Proc 2006; 81:353–373.

6 Douglas IJ, Evans SJ, Pocock S, Smeeth L. The risk of fractures associated with thiazolidinediones: a self-controlled case-series study. PLoS Med 2009; 6:e1000154.

7 Heaney RP. Vitamin D depletion and effective calcium absorption. J Bone Miner Res 2003; 5:13–42.

8 Harwood RH, Sahota O, Gaynor K, *et al*. A randomized, controlled comparison of different calcium and vitamin D supplementation regimens in elderly women after hip fracture: The Nottingham Neck of Femur (NONOF) Study. Age Ageing 2004; 33:45–51.

9 Bischoff-Ferrari HA, Dietrich T, Orav EJ, *et al*. Positive association between 25-hydroxy vitamin D levels and bone mineral density: a population-based study of younger and older adults. Am J Med 2004; 16:634–639.

10 Andrew G, Mark B, Sheryl F, Anne H, Greg G. The skeletal effects of pioglitazone in type 2 diabetes or impaired glucose tolerance: a randomized controlled trial. Eur J Endocrinol 2014; 170:255–262.

11 Vieth R, Bischoff FH, Boucher BJ, Dawson HB, *et al*. The urgent need to recommend and intake of vitamin D is effective. Am J Clin Nutr 2007; 85:649–650.

12 Whiting DR, Guariguata L, Weil C, *et al*.: IDF diabetes atlas: global estimates of the prevalence of diabetes for 2011 and 2030. Diabetes Res Clin Pract 2011; 94:311–321.

13 Wacker M, Holick MF. Vitamin D — effects on skeletal and extraskeletal health and supplementation. Nutrients 2013; 5:111–148.

14 Ho S, Alappat L, Awad A. Vitamin D and multiple sclerosis. Crit Rev Food Sci Nutr 2012; 52:980–987.

15 Weinstock-Guttman B, Mehta B, Ramanathan M, Karmon Y, Henson L, Halper J, *et al*. Vitamin D and multiple sclerosis. Neurologist 2012; 18:179–183.

16 Haga H, Schmedes A, Naderi Y, Moreno A, Peen E. Severe deficiency of 25-hydroxyvitamin D(3) (25-OH-D (3)) is associated with high disease activity of rheumatoid arthritis. Clin Rheumatol 2013; 32:629–633.

17 Pittas A, Nelson J, Mitri J, Hillmann W, Garganta C, Nathan D, *et al*. Plasma 25-hydroxyvitamin D and progression to diabetes in patients at risk for diabetes: an ancillary analysis in the Diabetes Prevention Program. Diabetes Care 2012; 35:565–573.

18 Lim S, Kim M, Choi S, Shin C, Park K, Jang H, *et al*. Association of vitamin D deficiency with 'incidence of type 2 diabetes in high-risk Asian subjects'. Am J Clin Nutr 2013; 97:524–530.

19 Kayaniyil S, Harris S, Retnakaran R, Vieth R, Knight J, Gerstein Hm, Perkins BA, Zinman B, *et al*. Prospective association of 25(OH)D with metabolic syndrome. Clin Endocrinol 2014; 80:502–507.

20 Hurskainen A, Virtanen J, Tuomainen T, Nurmi T, Voutilainen S. Association of serum 25-hydroxyvitamin D with type 2 diabetes and markers of insulin resistance in a general older population in Finland. Diabetes Metab Res Rev 2012; 28:418–423.

21 Ellfolk M, Norlin M, Gyllensten K, Wikvall K. Regulation of human vitamin D(3) 25-hydroxylases in dermal fibroblasts and prostate cancer LNCaP cells. Mol Pharmacol 2009; 75:1392–1399.

22 Vantieghem K, De Haes P, Bouillon R, Segaert S *et al*. HYPERLINK "http://www.ncbi.nlm.nih.gov/pubmed/16598763"UVB-induced 1, 25 (OH) 2D3 production and vitamin D activity in intestinal CaCo-2 cells and in THP-1 macrophages pretreated with a sterol Delta7-reductase inhibitor. J Photochem Photobiol 2006; 99:229–240.

23 Zinman B, Haffner SM, Herman WH, *et al*. Effect of rosiglitazone, metformin, and glyburide on bone biomarkers in patients with type 2 diabetes. J Clin Endocrinol Metab 2010; 95:134–142.

24 Zinman B, Harris SB, Neuman J, Gerstein HC, Retnakaran RR, Raboud J, *et al*. Low-dose combination therapy with rosiglitazone and metformin to prevent type 2 diabetes mellitus (CANOE trial): a double-blind randomized controlled study. Lancet 2010; 376:103–111.

25 Zoppini G, Galletti A, Targher G, Brangani C, Pichiri I, Negri C, *et al*. Glycated haemoglobin is inversely related to serum vitamin D levels in type 2 diabetic patients. PLoS ONE 2013; 8:e82733.

26 Somchodok Chakreeyarat, Sunee Saetung, La-or Chailurkit *et al*. Elevated vitamin D status in postmenopausal women on thiazolidinediones for type 2 diabetes Endocr 2011; 39:278–282.

27 Kostoglou-Athanassiou, Panagiotis Athanassiou, Anastasios Gkountouvas, Philippos Kaldrymides. Vitamin D and glycemic control in diabetes mellitus type 2. Therapeutic Advances in Endocrinology and Metabolism 2013; 4:122–128.

28 Christine Dalgård, Maria Skaalum Petersen, *et al*. Vitamin D Status in Relation to Glucose Metabolism and Type 2 Diabetes in Septuagenarians. Diabetes Care 2011; 34:30–33.

29 Anne-Thea McGil, Joanna M Stewart, Fiona E Lithander, *et al*. Relationships of low serum vitamin D3 with anthropometry and markers of the metabolic syndrome and diabetes in overweight and obesity. N Engl J Med 20083:266–281.

30 Lee SA, Yu JR, Lee J-G, Seong GM, Ko SJ, *et al*. Serum vitamin D status and its relationship to metabolic parameters in patients with type 2 diabetes mellitus. Chonnam Med J 2012; 48:108–115.