

Effect of serum 25-hydroxyvitamin D₃ on insulin resistance and β -cell function in newly diagnosed type 2 diabetes patients

Yan Yang†, Xuejun Zhang†, Mingjing Bao, Limei Liu, Yang Xian, Jichuan Wu, Pengqiu Li*

Department of Endocrinology, Sichuan Academy of Medical Sciences & Sichuan Provincial People's Hospital, Chengdu, Sichuan, China

Keywords

25-Hydroxyvitamin D₃, Insulin resistance, Newly diagnosed type 2 diabetes

*Correspondence

Pengqiu Li

Tel.: +86-189-81838299

Fax: +86-028-87773291

E-mail address: pengqiu19@163.com

J Diabetes Investig 2016; 7: 226–232

doi: 10.1111/jdi.12381

ABSTRACT

Aims/Introduction: To evaluate serum 25-hydroxyvitamin D₃ (25(OH)D₃) in newly diagnosed type 2 diabetes patients and to explore the associations of 25(OH)D₃ with insulin resistance and β -cell function.

Materials and Methods: A total of 97 newly diagnosed type 2 diabetes patients and 69 healthy controls were recruited. Serum 25(OH)D₃ was determined using high-pressure liquid chromatography. Insulin resistance was measured using a homeostasis model assessment of insulin resistance (HOMA-IR). β -Cell function was determined using the HOMA β -cell function index (HOMA- β), early-phase insulin secretion index (Δ I30/ Δ G30) and area under the insulin curve (AUCins). Correlation analysis was carried out using Pearson's correlation and multiple stepwise regression analysis.

Results: Serum 25(OH)D₃ was much lower in patients with newly diagnosed type 2 diabetes ($t = -13.00$, $P < 0.01$), and the prevalence of hypovitaminosis 25(OH)D₃ was 62.9% (61/97) in diabetic patients. Among the diabetic patients, patients with hypovitaminosis 25(OH)D₃ showed higher glycosylated hemoglobin and AUCglu ($P < 0.01$) as well as lower HOMA- β , Δ I30/ Δ G30 and AUCins. Serum 25(OH)D₃ was independently positively correlated with Δ I30/ Δ G30 and AUCins ($P < 0.05$), but was not significantly correlated with either HOMA-IR or HOMA- β . Only triglycerides, glycosylated hemoglobin and Δ I30/ Δ G30 emerged as independent factors associated with serum 25(OH)D₃ in both diabetes patients and the health control group.

Conclusions: The present results further showed a low serum 25(OH)D₃ concentration in patients with newly diagnosed type 2 diabetes. 25(OH)D₃ deficiency is associated with disturbances in glucose metabolism and lipid metabolism. Serum 25(OH)D₃ is not correlated with basal insulin resistance or β -cell function, but is significantly positively correlated with glucose-stimulated insulin secretion and β -cell function.

INTRODUCTION

Vitamin D deficiency or insufficiency is a risk factor in the development of diabetes¹. Among many non-calcemic functions, vitamin D acts as a necessary cofactor for insulin secretion². The main circulating form of vitamin D, 25-hydroxyvitamin D₃ (25(OH)D₃), is considered to be the best indicator

of vitamin D status³. The 25(OH)D₃ concentration was lower in individuals with type 2 diabetes and impaired glucose tolerance than in those with normal glucose tolerance⁴. Abnormal glucose tolerance has been reported to adversely affect insulin sensitivity and β -cell function⁵, and the loss of β -cell function and insulin sensitivity is known to contribute to the development of diabetes⁶. Therefore, vitamin D, measured as 25(OH)D₃, might play a significant role in the pathogenesis of type 2 diabetes⁷.

†Yan Yang and Xuejun Zhang contributed equally to this work.

Received 8 October 2014; revised 17 May 2015; accepted 1 June 2015

The mechanisms whereby low 25(OH)D₃ concentrations increase the risk of type 2 diabetes are still not well understood; the association of low serum 25(OH)D₃ concentrations with type 2 diabetes might be mediated through glucose homeostasis effects. In particular, a direct effect of vitamin D on glucose homeostasis is insulin resistance and/or β -cell dysfunction, the main pathophysiological disorders underlying type 2 diabetes⁸. Several studies have suggested that low vitamin D status contributed to insulin resistance⁹ and was associated with markers of impaired glucose metabolism, such as glycosylated hemoglobin (HbA1c)¹⁰. Vitamin D could play a role in the pathogenesis of type 2 diabetes by affecting insulin resistance, β -cell function, or both¹¹; there is a direct role for 25(OH)D, the major circulating metabolite precursor of the hormonally active form (25(OH)D₃), in pancreatic β -cell function and insulin sensitivity¹². Cross-sectional studies have reported that 25(OH)D is associated with insulin resistance and β -cell function in healthy, glucose-tolerant subjects¹¹, and in subjects who are at risk for type 2 diabetes⁹. However, the effect of serum 25(OH)D₃ level on insulin resistance and β -cell function has not been well documented in diabetes patients.

The homeostasis model assessment (HOMA) of β -cell function (HOMA- β) and insulin resistance (HOMA-IR) from basal plasma glucose and insulin concentrations is a widely used clinical and epidemiologic tool¹³ that, however, only shows what is occurring with glucose homeostasis in the fasting state¹⁴. Vitamin D is well known to play a role in the development and progression of type 2 diabetes, and a high vitamin D status provides protection against type 2 diabetes¹⁵. However, the exact mechanisms through which vitamin D affects diabetes development and progression are not yet fully understood, and the interaction of 25(OH)D₃ with insulin resistance and β -cell function has not been explored in newly diagnosed type 2 diabetes patients. Therefore, to further elucidate the role of vitamin D, measured as 25(OH)D₃, on the potential mechanisms of type 2 diabetes development, the present study was designed to examine the correlation of 25(OH)D₃ with insulin resistance and β -cell function in a cohort of Chinese patients with newly diagnosed type 2 diabetes, with the combined use of HOMA and early-phase insulin secretion index (Δ I30/ Δ G30), as well as area under the insulin curve.

MATERIALS AND METHODS

Patients

We prospectively evaluated 97 consecutive patients admitted to the Sichuan Academy of Medical Sciences & Sichuan Provincial People's Hospital between April and July 2008. The patients included 57 men and 40 women, aged 33–70 years (median age 52 \pm 10 years), who were newly diagnosed with type 2 diabetes mellitus according to the World Health Organization criteria from 1999¹⁶. All patients underwent careful clinical examination to exclude the presence of acute diabetic complications, cancer or any other complications that could influence the calcium phosphorus metabolism. Patients who

reported stress (trauma, surgery or mental stimulation) within the past 3 months, who had a severe infection, chronic renal disease or alcohol use, or who were taking any antidiabetic medications or other medicine (vitamin, mineral supplements or glucocorticoids) that could influence vitamin D metabolism were also excluded from the present study. The 69 sex- and age-matched healthy controls (40 men, 29 women; age 33–70 years, mean 50 \pm 11 years) were recruited into this study undergoing oral glucose tolerance test and physical examination during the same period. Smoking, chronic alcoholism, past or present drug intake, abnormal blood glucose level (>140 mg/dL) and any medical conditions that could influence the calcium phosphorus metabolism constituted the exclusion criteria. The study was approved by the institutional review board for human subjects of Sichuan Academy of Medical Sciences, Sichuan Provincial People's Hospital. Written informed consent was obtained from each participant before participating in the study.

Variable Assessment

Data on baseline characteristics, including height, body mass, waist-to-hip ratio, systolic pressure and diastolic pressure, were measured after overnight fasting at the time of enrolment. Fasting venous blood samples were collected to measure the concentration of HbA1c, blood glucose, triglycerides, total cholesterol, low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), insulin and serum 25(OH)D₃. HbA1c values were measured with the Bio-Rad glycosylated hemoglobin column assay (Bio-Rad, Hercules, CA, USA). Blood glucose was measured by a glucose oxidase method using a UV-Vis spectrophotometer (UV751GD; Shanghai Analytical Instrument Co., Shanghai, China). Insulin concentrations were measured by electrochemiluminescence immunoassay (Roche Diagnostics, Indianapolis, IN, USA). Total cholesterol, HDL-C, LDL-C and triglyceride levels were determined from the blood specimen collected after an overnight fast, and measured on the Olympus AU 5400/AU2700 autoanalyzer (Olympus Co., Tokyo, Japan). The 25(OH)D₃ serum levels were measured by high-pressure liquid chromatography (Shimadzu Corporation, Kyoto, Japan) using an Aichrombond-AQ C18 column (250 \times 4 mm, 5 μ m; Agilent, Los Angeles, CA, USA), as previously described¹⁷. The standard 25(OH)D₃ was purchased from Dr Ehrenstorfer (Augsburg, Germany). The calibration curve was linear over the 0.5–120-ng/mL range ($r = 0.9993$). The extraction recoveries of 25(OH)D₃ ranged from 81.7 to 87.9%, and the inter- and intraday relative standard deviations were <7.7 and <9.9%, respectively. Serum levels of blood glucose and insulin were also determined at 0.5 and 2 h after the oral administration of 75 g glucose.

Assessment of Insulin Resistance and β -Cell Function

The β -cell function was estimated by HOMA- β , early-phase insulin secretion index (Δ I30/ Δ G30) and area under the insulin

curve (AUCins); insulin resistance was estimated using HOMA-IR. HOMA- β and HOMA-IR were calculated using the following formulas: β -cell function (%) = $20 \times \text{insulin} / (\text{glucose} - 3.5)$; and insulin resistance = $\text{fasting serum insulin} (\mu\text{U/mL}) \times \text{fasting plasma glucose} (\text{mmol/L}) / 22.5$, respectively¹³. The total glucose response and insulin secretion were measured by the areas under the glucose concentration curve (AUCglu) and AUCins, and AUC was calculated by the trapezoidal method. The early-phase insulin secretion index was calculated according to the ratio of insulin change to glucose change during the first 30 min after a meal ($\Delta\text{I30}/\Delta\text{G30}$). Deficiency of 25(OH)D₃ was defined as a 25(OH)D₃ concentration <37.5 nmol/L¹⁸.

Statistical Analysis

All statistical analyses were carried out with SPSS, version 13.0 (SPSS Inc., Chicago, IL, USA). Data were expressed as the mean value \pm standard deviation. Measurement data with a normal distribution between the groups were compared using an independent sample *t*-test or one-way analysis of variance (ANOVA) as appropriate. Enumeration data with a normal distribution between groups were compared with a χ^2 -test. Data with a non-normal distribution were analyzed after a logarithmic transformation or square root transformation. The correlation between 25(OH)D₃ and HOMA- β , $\Delta\text{I30}/\Delta\text{G30}$ or AUCins was calculated using Pearson's correlation analysis and multiple stepwise regression analysis. A *P*-value of <0.05 was considered statistically significant.

RESULTS

Serum 25(OH)D₃ Levels Between Diabetic and Control Group

In total, 166 participants were enrolled as previously described in the inclusion criteria of the present study, and there was no significant difference in age and sex distribution between the diabetic and control groups (*P* > 0.05). The serum 25(OH)D₃ levels were significantly lower in patients with newly diagnosed diabetes (36 ± 19 nmol/L) compared with those of the healthy control group (80 ± 26 nmol/L; *t* = -13.00, *P* < 0.01). The prevalence of 25(OH)D₃ deficiency was 62.89% (61/97) in the diabetic group, which was significantly higher than that of the control group, in which no one was identified as being deficient of 25(OH)D₃ ($\chi^2 = 68.60$, *P* < 0.01).

Comparison of Clinical Data Among the Different 25(OH)D₃ Level Groups

According to the distribution of 25(OH)D₃ levels, patients in the diabetic group were further divided into two groups; that is, the 25(OH)D₃ deficiency group and the non-deficiency group, with a cut-off value of 37.5 nmol/L⁷. Comparisons of the clinical data among the patients with or without hypovitaminosis 25(OH)D₃ and healthy controls are listed in Table 1. No significant difference in age and sex distributions, body mass index (BMI), systolic blood pressure, diastolic blood pressure, Ca²⁺ or serum level of phosphorus was

found among the three groups. There were several statistically significant differences between the patients with and without hypovitaminosis 25(OH)D₃. The values of HbA1c, fasting blood glucose, 0.5-h blood glucose, 2-h blood glucose, AUCglu and creatinine clearance rate were significantly higher in the hypovitaminosis 25(OH)D₃ group, whereas fasting insulin, 0.5- and 2-h insulin, AUCins, HOMA- β , $\Delta\text{I30}/\Delta\text{G30}$, HOMA-IR and serum creatinine were comparatively lower (*P* < 0.05) compared with patients without hypovitaminosis 25(OH)D₃. However, after adjustment for age, sex, BMI, waist-to-hip ratio, triglycerides, total cholesterol, LDL-C and HDL-C, differences in AUCglu, AUCins, HOMA- β , $\Delta\text{I30}/\Delta\text{G30}$ and HOMA-IR were found to be statistically insignificant between the groups (*F* = 1.65, *P* > 0.05). No significant difference in BMI, waist-to-hip ratio, systolic pressure, diastolic pressure, triglycerides, total cholesterol, LDL-C or HDL-C was observed between the groups. The serum 25(OH)D₃ level in patients without hypovitaminosis 25(OH)D₃ was comparatively lower than that of the healthy control group (*t* = -5.60, *P* < 0.01).

Comparison of 25(OH)D₃ Levels Among Patients With Different HbA1c Levels

According to the distribution of the HbA1c levels, 97 diabetic patients were divided into three groups: (i) HbA1c levels <8%; (ii) HbA1c levels 8–10%; and (iii) HbA1c levels $\geq 10\%$. The serum 25(OH)D₃ levels in patients with HbA1c levels <8% (44 ± 22 nmol/L) were significantly higher than those of patients with higher HbA1c levels (HbA1c levels 8–10% and HbA1c levels $\geq 10\%$ for 28 ± 7 nmol/L and 30 ± 10 nmol/L, respectively; *F* = 16.67, *P* < 0.01). The distributions of age and sex were found to be statistically insignificant (*P* > 0.05).

Correlation Analysis of Serum 25(OH)D₃ Levels and Clinical Features

The effects of 25(OH)D₃ concentration on relevant clinical features of diabetes patients were investigated (Table 2). Univariate analysis showed negative correlations between the serum 25(OH)D₃ level and concentrations of triglycerides, total cholesterol, LDL-C, and HbA1c. However, after adjustment for age, sex, BMI, waist-to-hip ratio, systolic and diastolic blood pressure, triglycerides, total cholesterol, LDL-C, HDL-C and HbA1c, multiple stepwise regression analysis showed that only triglycerides (*r* = -0.15, *t* = -2.94, *P* < 0.05) and HbA1c (*r* = -0.58, *t* = -3.98, *P* < 0.05) were independently negatively correlated with serum 25(OH)D₃ concentration.

Correlation Analysis of Serum 25(OH)D₃ Levels and Insulin Resistance

With HOMA-IR as a dependent variable, other factors, including serum 25(OH)D₃ levels, age, sex, BMI, waist-to-hip ratio, systolic and diastolic blood pressure, triglycerides, total chole-

Table 1 | Comparison of clinical characteristics among individuals in the different groups

	Control group	Diabetic group		χ ² /F-value	P-value
		Hypovitaminosis 25(OH)D ₃	Non-hypovitaminosis 25(OH)D ₃		
n (case)	69	61	36		
Sex (M/F)	40/29	41/20	16/20	4.84	>0.05
Age (years)	50 ± 11	52 ± 10	53 ± 10	1.23	>0.05
BMI (kg/m ²)	24 ± 3	25 ± 4	25 ± 4	2.16	>0.05
WHR	0.90 ± 0.06	0.96 ± 0.07*	0.94 ± 0.05*	8.54	<0.01
SBP (mmHg)	125 ± 15	128 ± 16	126 ± 15	0.65	>0.05
DBP (mmHg)	74 ± 12	80 ± 11	77 ± 10	3.39	<0.05
TC (mmol/L)	4.7 ± 1.0	5.5 ± 1.3*	5.2 ± 0.7*	9.76	<0.01
TG (mmol/L)	1.3 ± 0.8	3.6 ± 3.5*&	2.4 ± 1.6*	26.41	<0.01
LDL-C (mmol/L)	2.9 ± 0.8	3.5 ± 1.0*	3.2 ± 0.7*	9.62	<0.01
HDL-C (mmol/L)	1.3 ± 0.3	1.1 ± 0.3*&	1.2 ± 0.3	9.62	<0.01
Ca ²⁺ (mmol/L)	2.47 ± 0.11	2.43 ± 0.11	2.44 ± 0.11	1.61	>0.05
25(OH)D (nmol/L)	80 ± 26	26 ± 6*&	52 ± 22*&	127.66	<0.01
HbA1c (%)	5.2 ± 0.4	10.1 ± 3.0*&	7.7 ± 2.6*	120.32	<0.01
FBG (mmol/L)	5.1 ± 0.5	10.2 ± 3.2*&	7.9 ± 2.9*	120.78	<0.01
0.5-h BG (mmol/L)	8.7 ± 1.7	16.0 ± 3.8*&	13.2 ± 3.5*	113.51	<0.01
2-h BG (mmol/L)	6.6 ± 0.8	20.9 ± 5.0*&	16.2 ± 5.0*	470.17	<0.01
FI (mU/L)	7 ± 5	6 ± 4&	11 ± 7*	12.32	<0.01
0.5-h Insulin (mU/L)	52 ± 33	16 ± 12*&	50 ± 40	46.35	<0.01
2-h Insulin (mU/L)	38 ± 22	22 ± 17*&	96 ± 79*	42.10	<0.01
AUCglu (h(mU/L)	14.6 ± 2.0	31.5 ± 7.3*&	25.2 ± 6.8*	195.69	<0.01
AUCins (h(mU/L)	74 ± 40	30 ± 21*&	104 ± 80*	42.80	<0.01
ΔI ₃₀ /ΔG ₃₀	14.5 ± 9.9	1.9 ± 1.9*&	8.3 ± 7.7*	103.81	<0.01
HOMA-β	110 ± 156	21 ± 16*&	75 ± 64*	50.61	<0.01
HOMA-IR	1.5 ± 1.1	2.6 ± 1.8*&	3.5 ± 2.1*	23.52	<0.01
P (mmol/L)	1.31 ± 0.1	1.33 ± 0.1	1.32 ± 0.1	2.26	>0.05
Crea (μmol/L)	88.4 ± 10.3	80.1 ± 16.8*	80.9 ± 15.6*	6.47	<0.01
Ccr (mL/min)	74.2 ± 20.7	89.9 ± 31.2*&	76.7 ± 19.3	5.58	<0.01

ΔI₃₀/ΔG₃₀, early-phase insulin secretion index; AUCglu, area under the glucose curve; AUCins, area under the insulin curve; BG, blood glucose; BMI, body mass index; Ccr, creatinine clearance rate; Crea, serum creatinine; DBP, diastolic blood pressure; F, female; FBG, fasting blood glucose; FI, fasting insulin; HbA1c, glycosylated hemoglobin; HDL-C, high-density lipoprotein cholesterol; HOMA-β, homeostasis model assessment of β-cell function; HOMA-IR, homeostasis model assessment of insulin resistance; LDL-C, low-density lipoprotein cholesterol; M, male; SBP, systolic blood pressure; TC, total cholesterol; TG, triglyceride; WHR, waist-to-hip ratio. *P < 0.05 vs control group; &P < 0.05 vs non-hypovitaminosis 25-hydroxyvitamin D₃ (25(OH)D₃) group.

terol, LDL-C, HDL-C, and HbA1c as independent variables, multiple stepwise regression analysis showed that there was no significant difference between the HOMA-IR and serum 25(OH)D₃ levels.

Correlation Analysis of Serum 25(OH)D₃ Levels and β-Cell Function

Multiple stepwise regression analysis included the HOMA-β, ΔI₃₀/ΔG₃₀ and AUCins individually as dependent variables, and the serum 25(OH)D₃ levels, age, sex, BMI, waist-to-hip ratio, systolic and diastolic blood pressure, triglyceride, total cholesterol, LDL-C, HDL-C, HbA1c, and HOMA-IR as independent variables. The results showed that HOMA-β was independently associated with HbA1c and HOMA-IR (P < 0.01; Table 3), whereas serum 25(OH)D₃ level had no independent effect on HOMA-β. Both ΔI₃₀/ΔG₃₀ and AUCins were associ-

ated with sex, HbA1c, serum 25(OH)D₃ levels, and HOMA-IR (P < 0.05), respectively. Serum 25(OH)D₃ level was independently positively correlated with ΔI₃₀/ΔG₃₀ and AUCins (Table 3).

Correlation Analysis of Serum 25(OH)D₃ Level and Clinical Features, Insulin Resistance, and β-Cell Function Between Diabetic and Control Groups

Univariate analysis showed a significant correlation between serum 25(OH)D₃ level and waist-to-hip ratio, triglycerides, total cholesterol, LDL-C, HDL-C, HbA1c, HOMA-IR, HOMA-β, ΔI₃₀/ΔG₃₀, AUCins, serum creatinine, and creatinine clearance rate (P < 0.01 or P < 0.05; Table 4). However, by multiple stepwise regression analysis, only triglycerides (r = -0.19, t = -3.57, P < 0.01), HbA1c (r = -0.52, t = -3.12, P < 0.01) and ΔI₃₀/ΔG₃₀ (r = 0.16, t = 3.92, P < 0.01) emerged as independent

Table 2 | Correlation analysis of the effect of 25-hydroxyvitamin D₃ on the clinical characteristics of diabetes patients

	Univariate analysis	
	<i>r</i>	<i>P</i>
Systolic blood pressure	0.07	>0.05
Diastolic blood pressure	0.02	>0.05
BMI	0.20	>0.05
Waist-to-hip ratio	-0.10	>0.05
Lipid profile		
Total cholesterol	-0.21	<0.05
Triglyceride	-0.31	<0.01
LDL-C	-0.29	<0.05
HbA1c	-0.49	<0.01

Covariates considered were sex, age, body mass index (BMI), waist-to-hip ratio, systolic and diastolic blood pressure, glycosylated hemoglobin (HbA1c), blood glucose, triglyceride, total cholesterol, low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol.

Table 3 | Multivariate analysis of the effect of 25-hydroxyvitamin D₃ on β-cell function

Dependent variable and covariate	<i>r</i>	<i>t</i>	<i>P</i>
HOMA-β			
HbA1c	-2.04	-8.85	<0.01
HOMA-IR	0.68	6.93	<0.01
ΔI ₃₀ /ΔG ₃₀			
Sex	0.19	2.04	<0.05
HbA1c	-2.23	-5.63	<0.01
25(OH)D ₃	0.71	2.21	<0.05
HOMA-IR	0.35	2.32	<0.05
AUCins			
Sex	0.99	2.23	<0.05
HbA1c	-8.58	-4.57	<0.01
25(OH)D ₃	7.07	4.67	<0.01
HOMA-IR	3.96	5.62	<0.01

ΔI₃₀/ΔG₃₀, early-phase insulin secretion index; 25(OH)D₃, 25-hydroxyvitamin D₃; AUCins, area under the insulin curve; HbA1c, glycosylated hemoglobin; HOMA-β, homeostasis model assessment of β-cell function; HOMA-IR, homeostasis model assessment of insulin resistance.

factors associated with serum 25(OH)D₃ level in diabetic patients and the healthy control group.

DISCUSSION

The current data showed that serum 25(OH)D₃ level was significantly lower in patients with newly diagnosed type 2 diabetes compared with that of the healthy control group, and the prevalence of hypovitaminosis 25(OH)D₃ was 62.9% in the diabetic patients, corroborating the results of the previous studies, which showed that the baseline 25(OH)D₃ concentration was lower in diabetes patients and was inversely associated with the incidence of type 2 diabetes^{19,20}. The vitamin D status of <50 nmol/L has been previously reported to double the risk of newly diagnosed

Table 4 | Correlation of 25-hydroxyvitamin D₃ with clinical characteristics, insulin resistance and β-cell function of participants

	Univariate analysis	
	<i>r</i>	<i>P</i>
Waist-to-hip ratio	-0.29	<0.01
Total cholesterol	-0.31	<0.01
Triglyceride	-0.52	<0.01
LDL-C	-0.32	<0.01
HDL-C	0.35	<0.01
HbA1c	-0.74	<0.01
HOMA-IR	-0.22	<0.01
HOMA-β	0.63	<0.01
ΔI ₃₀ /ΔG ₃₀	0.73	<0.01
AUCins	0.52	<0.01
Crea	0.25	<0.01
Ccr	-0.20	<0.05

ΔI₃₀/ΔG₃₀, early-phase insulin secretion index; AUCins, area under the insulin curve; Ccr, creatinine clearance rate; Crea, serum creatinine; HOMA-β, homeostasis model assessment of β-cell function; HOMA-IR, homeostasis model assessment of insulin resistance. Covariates considered were gender, age, body mass index, waist-to-hip ratio, systolic and diastolic blood pressure, glycosylated hemoglobin (HbA1c), blood glucose, triglyceride, total cholesterol, low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C).

type 2 diabetes after adjustment for clinical covariables²¹. In further support of this association, we observed, in newly diagnosed type 2 diabetes patients with decreasing 25(OH)D₃ concentration, increasing concentrations of HbA1c, blood glucose and AUCglu, with decreasing insulin levels, AUCins, HOMA-β, ΔI₃₀/ΔG₃₀ and HOMA-IR. These differences were statistically insignificant after adjustment for clinical parameters, including age, sex, BMI, waist-to-hip ratio, triglycerides, total cholesterol, LDL-C and HDL-C ($F = 1.65$, $P > 0.05$). However, hypovitaminosis D was positively associated with insulin resistance and negatively correlated with β-cell dysfunction in type 2 diabetes¹¹. HbA1c, a global measure of glucose homeostasis, has also been reported to inversely associate with vitamin D status, as measured by serum 25(OH)D levels^{10,21,22}. Elevated HbA1c levels might be considered for screening people with vitamin D insufficiency¹⁰. Abnormalities could be a result of changes in insulin secretion and sensitivity²¹, and potential mechanisms contributing to vitamin D regulation of glucose homeostasis and insulin sensitivity include enhanced insulin secretion and synthesis²³, and reduced inflammatory processes that reduce the functional capacity of pancreatic β-cells expressing the vitamin D receptor and the enzyme 1-α hydroxylase²⁴.

HOMA is a widely used clinical and epidemiological tool for assessing β-cell function and insulin resistance from basal (fasting) glucose and insulin concentrations²⁵; when used appropriately, it can closely mirror the glucose clamp technique in the assessment of insulin sensitivity²⁶. Therefore, HOMA was used to assess insulin resistance and β-cell function. However,

because HOMA was reported to only show what is occurring with glucose homeostasis in the fasting state¹⁴, AUC_{glu}, Δ I30/ Δ G30 and AUC_{ins} were also used in the present study.

Ample evidence has suggested that vitamin D activity is essential for pancreatic β -cell function, and a higher baseline vitamin D level independently predicted better β -cell function in subjects who were at risk for type 2 diabetes^{27–29}. Insulin resistance has also been reported to associate with vitamin D insufficiency³⁰, and data on both associations in a single study have been reported. The concentration of 25(OH)D was found to be independently associated with both insulin sensitivity and β -cell function among individuals at risk of type 2 diabetes^{9,11}, though another study showed that serum 25(OH)D was not associated with insulin resistance or β -cell function in the Canadian Cree³¹. Such controversy might be a result of the variability of the method used or ethnic differences. In the present study, our multiple stepwise regression analysis was carried out to investigate the association of 25(OH)D₃ levels with the IR and β -cell function. Our results showed no independent effect of serum 25(OH)D₃ level on the insulin resistance index (HOMA-IR) derived in the basal state, suggesting that serum 25(OH)D₃ might not associate with basal or hepatic insulin sensitivity in newly diagnosed type 2 diabetes patients. β -Cell function was determined using HOMA- β , Δ I30/ Δ G30 and AUC_{ins}. The results showed that there was no significant correlation between serum 25(OH)D₃ and HOMA- β , a marker of basal insulin secretion of pancreatic β -cells, and the latter was found to be independently associated with HbA1c and HOMA-IR, corroborating the previous results that insulin sensitivity and secretion are mutually related, and that HOMA- β alone might not accurately assess β -cell function³². Previous studies have shown that vitamin D affects β -cells by increasing the insulin response to glucose stimulation and does not affect basal insulin secretion³³. HOMA- β can be considered one aspect of β -cell function, with the ability to increase basal insulin secretion to meet the elevation of basal glycemia³⁴. Therefore, a correlation analysis of serum 25(OH)D₃ concentration and Δ I30/ Δ G30 as well as AUC_{ins} was carried out. The results showed that serum 25(OH)D₃ concentration was significantly positively correlated with Δ I30/ Δ G30 and AUC_{ins}; serum 25(OH)D₃ was also shown to correlate with sex, HbA1c and HOMA-IR. These results showed that the serum 25(OH)D₃ concentration was less correlated with the basal insulin resistance and basal β -cell function, and was significantly correlated with glucose-stimulated insulin secretion.

Abnormal fat accumulation in the liver, muscle and pancreatic islets plays a crucial role in the development of both β -cell dysfunction and insulin resistance³⁵. Our findings showed that triacylglycerol emerged as an independent factor that is negatively associated with serum 25(OH)D₃ level. A previous study by Liu *et al.*³⁶ reported that vitamin D, as measured by plasma 25(OH)D, was inversely associated with plasma triacylglycerol in non-diabetic adults after adjusting for age and sex, but these associations were no longer significant after further adjustment for BMI, waist circumference and current smoking status. Further study is,

therefore, still required to clarify the correlation of serum 25(OH)D₃ level and triglycerides, and the potential mechanism of their effects on β -cell dysfunction and insulin resistance.

The present study also had some limitations that should be considered when interpreting the results. Most importantly, it was a cross-sectional study; some parameters and confounding factors, including different, non-standardized meals taken by our patients and the degree of proteinuria or preferable urinary albumin excretion, were not considered in the present study. Thus, we can only show the existence of a correlation, but not a causal nexus between hypovitaminosis D and glucose tolerance in type 2 diabetes. In addition, the present study was restricted to one site and took place in a relatively limited number of patients, indicating that interpretation of the results needs to be done with caution. A large cohort of prospective studies should be further carried out to establish the link between hypovitaminosis D and development of type 2 diabetes in newly diagnosed type 2 diabetes patients.

In conclusion, the present findings support earlier results that showed a low serum 25(OH)D₃ concentration in patients with newly diagnosed type 2 diabetes. Vitamin D deficiency is associated with disturbances in glucose and lipid metabolism. Serum 25(OH)D₃ concentration is not correlated with basal insulin resistance or basal β -cell function, but is significantly positively correlated with glucose-stimulated insulin secretion and β -cell function.

ACKNOWLEDGMENTS

We express our great thanks to Lei Zhang, Xianjun Zhu, Yi Yang, Xu Cao and Hui Zhou from Sichuan Province People's Hospital for their help in this work.

DISCLOSURE

The authors declare no conflict of interest.

REFERENCES

1. Soheilykhah S, Mojibian M, Rashidi M, *et al.* Maternal vitamin D status in gestational diabetes mellitus. *Nutr Clin Pract* 2010; 25: 524–527.
2. Holick M. Noncalcemic actions of 1, 25-dihydroxyvitamin D₃ and clinical applications. *Bone* 1995; 17: S107–S111.
3. Ng K, Meyerhardt JA, Wu K, *et al.* Circulating 25-hydroxyvitamin D levels and survival in patients with colorectal cancer. *J Clin Oncol* 2008; 26: 2984–2991.
4. Targher G, Bertolini L, Padovani R, *et al.* Serum 25-hydroxyvitamin D₃ concentrations and carotid artery intima-media thickness among type 2 diabetic patients. *Clin Endocrinol (Oxf)* 2006; 65: 593–597.
5. Rossetti L, Giaccari A, DeFronzo RA. Glucose toxicity. *Diabetes Care* 1990; 13: 610–630.
6. López S, Bermúdez B, Pacheco YM, *et al.* Distinctive postprandial modulation of β cell function and insulin sensitivity by dietary fats: monounsaturated compared with saturated fatty acids. *Am J Clin Nutr* 2008; 88: 638–644.

7. Ozfirat Z, Chowdhury TA. Vitamin D deficiency and type 2 diabetes. *Postgrad Med J* 2011; 1011: 18–25.
8. Alvarez JA, Ashraf A. Role of vitamin D in insulin secretion and insulin sensitivity for glucose homeostasis. *Int J Endocrinol* 2010; 2010: 351385.
9. Kayaniyl S, Vieth R, Retnakaran R, *et al.* Association of vitamin D with insulin resistance and β -cell dysfunction in subjects at risk for type 2 diabetes. *Diabetes Care* 2010; 33: 1379–1381.
10. Kositsawat J, Freeman VL, Gerber BS, *et al.* Association of A1C levels with vitamin D status in US adults data from the National Health and Nutrition Examination Survey. *Diabetes Care* 2010; 33: 1236–1238.
11. Chiu KC, Chu A, Go VLW, *et al.* Hypovitaminosis D is associated with insulin resistance and β cell dysfunction. *Am J Clin Nutr* 2004; 79: 820–825.
12. Pittas AG, Lau J, Hu FB, *et al.* The role of vitamin D and calcium in type 2 diabetes. A systematic review and meta-analysis. *J Clin Endocrinol Metab* 2007; 92: 2017–2029.
13. Matthews D, Hosker J, Rudenski A, *et al.* Homeostasis model assessment: insulin resistance and β -cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985; 28: 412–419.
14. Wallace T, Matthews D. The assessment of insulin resistance in man. *Diabet Med* 2002; 19: 527–534.
15. Knekt P, Laaksonen M, Mattila C, *et al.* Serum vitamin D and subsequent occurrence of type 2 diabetes. *Epidemiology* 2008; 19: 666–671.
16. Alberti KGMM, Zimmet PF. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus. Provisional report of a WHO consultation. *Diabetic Med* 1998; 15: 539–553.
17. Long E, Qing Q, Yang Y. Determination of 25-hydroxy vitamin D3 in human serum by RP-HPLC. *West China J Pharm Sci* 2010; 2: 026 (Chinese).
18. Mosekilde L. Vitamin D and the elderly. *Clin Endocrinol (Oxf)* 2005; 62: 265–281.
19. Cigolini M, Iagulli MP, Miconi V, *et al.* Serum 25-hydroxyvitamin D3 concentrations and prevalence of cardiovascular disease among type 2 diabetic patients. *Diabetes Care* 2006; 29: 722–724.
20. Scragg R, Holdaway I, Singh V, *et al.* Serum 25-hydroxyvitamin D3 levels decreased in impaired glucose tolerance and diabetes mellitus. *Diabetes Res Clin Pract* 1995; 27: 181–188.
21. Dalgård C, Petersen MS, Weihe P, *et al.* Vitamin D status in relation to glucose metabolism and type 2 diabetes in septuagenarians. *Diabetes Care* 2011; 34: 1284–1288.
22. Lau SL, Gunton JE, Athayde NP, *et al.* Serum 25-hydroxyvitamin D and glycated haemoglobin levels in women with gestational diabetes mellitus. *Med J Aust* 2011; 194: 334–337.
23. Palomer X, González-Clemente J, Blanco-Vaca F, *et al.* Role of vitamin D in the pathogenesis of type 2 diabetes mellitus. *Diabetes Obes Metab* 2008; 10: 185–197.
24. Pittas AG, Dawson-Hughes B. Vitamin D and diabetes. *J Steroid Biochem Mol Biol* 2010; 121: 425–429.
25. Wallace TM, Levy JC, Matthews DR. Use and abuse of HOMA modeling. *Diabetes Care* 2004; 27: 1487–1495.
26. Bonora E, Targher G, Alberiche M, *et al.* Homeostasis model assessment closely mirrors the glucose clamp technique in the assessment of insulin sensitivity: studies in subjects with various degrees of glucose tolerance and insulin sensitivity. *Diabetes Care* 2000; 23: 57–63.
27. Boursolon P, Faure-Dussert A, Billaudel B. The de novo synthesis of numerous proteins is decreased during vitamin D3 deficiency and is gradually restored by 1, 25-dihydroxyvitamin D3 repletion in the islets of langerhans of rats. *J Endocrinol* 1999; 162: 101–109.
28. Karnchanasorn R, Ou H-Y, Chiu KC. Plasma 25-hydroxyvitamin D levels are favorably associated with β -cell function. *Pancreas* 2012; 41: 863–868.
29. Kayaniyl S, Retnakaran R, Harris SB, *et al.* Prospective associations of vitamin D with β -cell function and glycemia The PROspective Metabolism and ISlet cell Evaluation (PROMISE) Cohort Study. *Diabetes* 2011; 60: 2947–2953.
30. Forouhi NG, Luan J, Cooper A, *et al.* Baseline serum 25-hydroxy vitamin D is predictive of future glycemic status and insulin resistance the medical research council ely prospective study 1990–2000. *Diabetes* 2008; 57: 2619–2625.
31. Del Gobbo LC, Song Y, Dannenbaum DA, *et al.* Serum 25-hydroxyvitamin D is not associated with insulin resistance or beta cell function in Canadian Cree. *J Nutr* 2011; 141: 290–295.
32. Ahrén B, Pacini G. Importance of quantifying insulin secretion in relation to insulin sensitivity to accurately assess beta cell function in clinical studies. *Eur J Endocrinol* 2004; 150: 97–104.
33. Boursolon P, Billaudel B, Faure-Dussert A. Influence of vitamin D3 deficiency and 1, 25 dihydroxyvitamin D3 on de novo insulin biosynthesis in the islets of the rat endocrine pancreas. *J Endocrinol* 1999; 160: 87–95.
34. Li C-L, Tsai S-T, Chou P. Relative role of insulin resistance and β -cell dysfunction in the progression to type 2 diabetes – the Kinmen Study. *Diabetes Res Clin Pract* 2003; 59: 225–232.
35. Boden G, Shulman G. Free fatty acids in obesity and type 2 diabetes: defining their role in the development of insulin resistance and β -cell dysfunction. *Eur J Clin Invest* 2002; 32: 14–23.
36. Liu E, Meigs JB, Pittas AG, *et al.* Plasma 25-hydroxy-vitamin D is associated with markers of the insulin resistant phenotype in nondiabetic adults. *J Nutr* 2009; 139: 329–334.