Vitamin D Deficiency in Relation to the Risk of Metabolic Syndrome in Middle-Aged and Elderly Patients with Type 2 Diabetes Mellitus

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(Received February 29, 2016)

Summary Vitamin D deficiency is highly prevalent all over the world and dietary intakes of vitamin D are very low in China. In this study we aimed to determine whether vitamin D deficiency is associated with increased risk of metabolic syndrome (MetS) among Chinese type 2 diabetes mellitus (T2DM) patients aged over 50 y. Serum 25-hydroxyvitamin D [25(OH)D] concentrations were measured in a cross-sectional sample of 270 T2DM patients aged over 50 y from Zhejiang. Data on demographic characteristics, anthropometry and other variables were collected. The mean of serum 25(OH)D was 22.93 ng/mL, and percentages of vitamin D deficiency and insufficiency were 43.71% and 39.63%, respectively. Serum 25(OH)D concentrations were significantly lower in subjects with MetS than in those without MetS (21.74 vs 24.96 ng/mL, \(p < 0.001\)), and the prevalence of MetS significantly increased according to tertiles of serum 25(OH)D concentrations. After adjusting for multivariate factors, the adverse effect of lower serum 25(OH)D concentrations was significant (OR: 3.26, 95% CI: 1.03–7.34; \(p = 0.044\)) in the group with BMI \(\geq 24\) kg/m² while the change in OR of MetS for each 10 ng/mL decrease in the serum 25(OH)D concentrations was 2.03 (95% CI: 1.10–3.79). These results suggest that serum 25(OH)D deficiency may be a risk factor of MetS among Chinese type 2 diabetic patients, especially in the T2DM with BMI \(\geq 24\) kg/m². The challenge is determining the mechanisms of vitamin D action for recommendation of vitamin D supplementation that reduces the risks of MetS and progression to T2DM.

Key Words vitamin D, deficiency, metabolic syndrome, type 2 diabetes mellitus

Vitamin D deficiency is now an increasingly recognized worldwide health concern (1) related to its non-classical roles, such as diabetes, heart disease, autoimmune diseases and certain types of cancers. Low serum 25-hydroxyvitamin D [25(OH)D] concentrations are also deemed to be related to the high incidence of type 2 diabetes mellitus (T2DM) (2) because of existing vitamin D receptors on pancreatic \(\beta\) cells and the other insulin-sensitive tissue such as skeletal muscle tissue. Outcomes indicated that fasting plasma glucose (FPG) and the insulin sensitive index improved significantly in a study of vitamin D supplementation for patients with T2DM (3). The elderly are at high risk for vitamin D deficiency worldwide. And it is worth noting that the prevalence of T2DM is 11.6% with 50.1% of pre-diabetes (IGT) among adults over 18 y in China according to the latest international clinical diagnostic criteria (HbA1c \(\geq 6.5\%\)). The burden from T2DM complications and mortality is higher in worldwide.

The metabolic syndrome (MetS) is commonly characterized by dyslipidemia, dysglycemia, abdominal obesity and hypertension (4). MetS is associated with an increased risk of T2DM and cardiovascular complications, and is a complex of interrelated risk factors for diabetes (5). We know that obesity is also one of the risk factors for vitamin D deficiency, because the excess fat absorbs and holds onto the vitamin D so that it cannot be used for bone building or cellular health. The elderly are at high risk for vitamin D deficiency worldwide due to reduced ability of active vitamin D synthesis. Moreover, relationships among serum 25(OH)D and dyslipidemia, abdominal obesity, and hypertension have been explored in different regions and various kinds of people (6–8), but data to support those relationships are inconsistent with only a sparse sample from Asian people with T2DM. In addition to these traditional risk factors, some blood biomarkers such as apolipoprotein (Apo) A1 and ApoB (9), C-reactive protein (CRP), fibrinogen, and homocysteine (10–12), and vitamin D status (13–15)
have been proposed to be involved in its pathogenesis. Previous studies have indicated that low concentrations of serum 25(OH)D may be associated with an increased risk of MetS (8, 16, 17), but whether this association exists among T2DM patients remains unclear. Thus, the aim of our study was to determine whether vitamin D deficiency is associated with increased risk of MetS among Chinese patients with T2DM aged over 50 y.

MATERIALS AND METHODS

Survey and sample. Data collection took place in Lishui (latitude: 28°N), a city of Zhejiang Province, southeast China. A combination of stratified and random sampling methods was adopted and 283 participants aged 50 y and older with a previous diagnosis of T2DM according to the diabetes diagnostic criteria set by the WHO in 1998 (18) were recruited from the Center of Disease Control and Prevention (CDC). People were excluded in the case of a predicted survival of less than 3 y, prior history of diabetic ketosis or ketoacidosis, hypertonic coma, renal calculi, corticosteroid use, caffeine or calcium use, or other serious complications of diabetes.

The study was approved by the Ethics Committee of Soochow University (ESCU-20160001), and it was in compliance with the declaration of Helsinki with all subjects providing written informed consent before participating in the study.

Data collection. In a community interview, a pre-designed questionnaire was administered by trained research assistants to collect information regarding demographic characteristics, lifestyle risk factors (including smoking, alcohol drinking and recreational physical activity) as well as disease history and medication intake. After that, all participants were invited to attend a clinical examination after an overnight fast. We measured the height, weight, waist and hip circumference, and blood pressure of each subject at the baseline visit without hat and shoes.

Assessment of biomarkers. Peripheral venous blood specimens were collected in separation gel coagulation promoting tubes from all participants according to a pre-protocol. They were instructed to fast for 12 h until the early morning before blood collection, take all regular medications except for diabetes medication, take no aspirin or nonsteroidal anti-inflammatory drugs for 48 h before the visit except for those medications taken regularly. Circulating levels of 25(OH)D were measured to be the most reliable measure of overall vitamin D status (19). Serum 25(OH)D levels were measured with an electrochemical luminescence system, COBAS e601 (Roche Diagnostics GmbH, Mannheim, Germany). Vitamin D nutritional status was assessed as “sufficiency” (>20 ng/mL), “insufficiency” <20 ng/mL, or “deficiency” <<20 ng/mL) (20). FPG was measured in serum by using the hexokinase method on an automatic biochemical analyzer called COBAS c702 (Roche Diagnostics GmbH). The total triglycerides (TG), total cholesterol (TC), high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C) concentrations were measured enzymatically on the same machine. Plasma high-sensitive C-reactive protein (hs-CRP), and glycosylated hemoglobin (HbA1c) were measured by the immunoturbidimetric method (Ultrasensitive CRP kit; Roche Diagnostics Gmbh). Insulin was determined by the electrochemical luminescence system with COBAS e601.

The homeostatic model assessment of insulin resistance (HOMA-IR) was computed by using the formula:

$$\text{HOMA-IR} = \frac{\text{fasting glucose (mmol/L)} 	imes \text{fasting insulin (mU/L)}}{22.5}$$

The homeostatic model assessment of β cell function (HOMA-β) was calculated as follows (21, 22):

$$\text{HOMA-β} = \frac{[20 \times \text{fasting plasma insulin (mU/L)}]}{[\text{fasting plasma glucose (mmol/L)} - 3.5]}$$

All of the intra- and interassay coefficients of variation were <13%.

Definition of metabolic syndrome. MetS was defined using the updated guidelines proposed for Chinese by the International Diabetes Federation and the American Heart Association/National Heart, Lung, and Blood Institute (5) as presentation of three or more of the following components: 1) waist circumference ≥85 cm for men or ≥80 cm for women; 2) TG ≥1.7 mmol/L; 3) HDL cholesterol <1.0 mmol/L for men or <1.3 mmol/L for women; 4) systolic blood pressure (SBP) ≥130 mmHg or diastolic blood pressure (DBP) ≥85 mmHg or current use of antihypertensive medications; and 5) fasting glucose ≥5.6 mmol/L or previously diagnosed T2DM or use of oral hypoglycemic agents or insulin.

Statistical analysis. According to the tertile range of serum 25(OH)D concentrations, subjects were assigned to the “Tertile 1 (≥25 ng/mL),” “Tertile 2 (19–25 ng/mL)” and “Tertile 3 (0–19 ng/mL)” group, respectively. We described the demographic characteristics and risk factors with mean and standard deviation (SD) for continuous variables, and for the non-normal distribution data with median and quartiles, as well as for the classification variables with rate. 25(OH)D, FPG, TG, HDL-C, TG/HDL-C, insulin, HOMA-β and HOMA-IR were log-transformed before analysis in the case of skewed distribution. In addition, we performed logistic regression analysis to generate the odds ratios (ORs) and 95% confidence intervals (95% CI) of the prevalence of MetS across tertiles of serum 25(OH)D concentrations in the sample of patients with T2DM for which model 1 was unadjusted. In order to further adjust the confounding factors, we then adjusted for age, gender, residential region (urban/rural) and calcium supplement (yes/no) (model 2). After that, we adjusted for insulin, HOMA-IR and inflammatory factor (hs-CRP) based on model 2 (model 3). Further adjustments were made for the BMI (model 4). In stratified analyses, we assessed the potential effect modification by BMI (<or ≥24 kg/m²). Each corresponding change in ORs and 95% CI of MetS for each 10 ng/mL decrease in the serum 25(OH)D concentrations was calculated. Furthermore, to model the shape of the dose-response relation between serum 25(OH)D and MetS, the association was also evaluated with the use of nonparametric restricted cubic splines.
with 3 knots defined at the 10th, 50th, and 90th percentiles of the distribution of 25(OH)D. A p value of less than 0.05 indicated statistical significance. All the statistical analyses were performed with SAS software (version 9.2; SAS Institute, Cary, NC).

RESULTS

In our study including 270 subjects with T2DM, the percentages of vitamin D deficiency and insufficiency reached 43.71% and 39.63%, respectively, while the percentage of vitamin D sufficiency was only 16.66%. The characteristics of subjects are summarized in Table 1. There was no significant difference among three groups for age, region or calcium supplementation, except for gender. However, compared with Tertile 1 groups, the indicators for metabolic profiles (such as waist circumference, hip circumference, BMI, SBP, and TG) and insulin resistance (insulin and HOMA-IR) were significantly higher, and the higher degree of vitamin D deficiency showed a higher trend of related-indicators. Inversely, the level of HDL-C was lower (p<0.05).

Serum 25(OH)D concentrations were significantly lower in subjects with MetS than in those without MetS (21.74 vs 24.96 ng/mL, p=0.001) (Table 2). As shown in Table 3, the prevalence of MetS was inversely associated with the serum level of 25(OH)D. The median of 25(OH)D in the Tertile 1, 2 and 3 group was 30, 21 and 15 ng/mL, respectively, while the prevalence of MetS was 52.08%, 65.59% and 72.84%, respectively. Compared with the Tertile 1 group, the ORs of MetS in the Tertile 2 and 3 group progressively increased to 1.75 (95% CI: 1.17–2.62), 2.47 (95% CI: 1.31–4.66), respectively (Table 3, model 3, p=0.004). In model 2 adjusting for age, gender, residential region and calcium supplement, the ORs of MetS in the Tertile 2 and 3 group progressively increased to 1.75 (95% CI: 0.98–3.15) and 2.47 (95% CI: 1.31–4.64), respectively. And the OR of MetS for each 10 ng/mL decrease in the serum 25(OH)D concentrations was 1.65 (95% CI: 1.20–2.27) (Table 3, model 1, trend). Further, the increased risk of MetS was not altered in controls for insulin, HOMA-IR or hscRP (Table 3, model 3, trend). After adjusting for BMI based on model 3, we found it became 1.43 (95% CI: 0.74–2.77) and 1.85 (95% CI: 0.88–3.91), respectively (Table 3, model 4, trend). Interestingly, we modeled the association between 25(OH)D level and risk
of MetS using restricted cubic splines. As shown in Fig. 1, the lower degree of 25(OH)D level showed a higher risk of MetS, especially for the adjusted OR of MetS, which reached 2.12 (95% CI: 1.02–4.41) in 14 ng/mL of 25(OH)D levels (p, 0.05).

The association between 25(OH)D level and risk of MetS was appreciably modified by BMI (Table 3). So we divided the subjects into two groups according to the BMI and found a significant inverse association between 25(OH)D level and the ORs of MetS in T2DM with a BMI of $24 \text{kg/m}^2$ while the change in OR of MetS for each 10 ng/mL decrease in the serum 25(OH)D concentrations was 2.03 (95% CI: 1.10–3.79) (Table 3, ptrend = 0.044). There was statistically significant interaction between 25(OH)D level and BMI on risk of MetS (test for interaction: p < 0.001).

**DISCUSSION**

Our study has found that serum 25(OH)D concentration was lower in middle-aged and elderly patients with type 2 diabetes, and the serum level of 25(OH)D was inversely associated with the risk of MetS, especially in those with a BMI $\geq 24 \text{kg/m}^2$. Another study found an association between serum 25(OH)D level and the risk of MetS in individuals aged 30–60 y (23). Our research found the same results in T2DM patients. These results suggested that vitamin D deficiency may be a risk factor for MetS among patients with T2DM over 50 y, which indicates vitamin D might play a role in the prevention and control of diabetes complications.

The relationship between T2DM and vitamin D deficiency has been extensively reported (2, 24). Vitamin D supplementation has been shown to improve, and even prevent, type 2 diabetes mellitus in both human (3) and animal models (25). MetS is a complex of interrelated risk factors in the progression of T2DM, and whether it is associated with vitamin D status has been a widespread concern.

Findings from a general population have also shown an association between serum 25(OH)D level and the risk of MetS. For example, a prospective study carried out in Denmark brought in 4,330 individuals aged 30–60 y, with a 5-y follow-up, and revealed that serum 25(OH)D levels were inversely associated with incidence rates of MetS and hypercholesterolemia, and the ORs per 10 nmol/L higher baseline vitamin D level were 0.95 (p < 0.05) and 0.94 (p = 0.01) for the development of the MetS and hypercholesterolemia, respectively (23). Recently, a study in middle-aged and older Korean adults also reported that serum 25(OH)D concentrations were

**Table 2. Baseline characteristics according to metabolic syndrome (MetS) in patients with type 2 diabetes mellitus (T2DM) (n=270).**

<table>
<thead>
<tr>
<th>Metabolic syndrome</th>
<th>Yes (170)</th>
<th>No (100)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>25(OH)D (ng/mL)</td>
<td>21.74 ± 7.43</td>
<td>24.96 ± 8.76</td>
<td>0.001</td>
</tr>
<tr>
<td>General information</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (y)</td>
<td>66.90 ± 8.41</td>
<td>63.97 ± 9.58</td>
<td>0.009</td>
</tr>
<tr>
<td>Gender (male)</td>
<td>45 (26.47)</td>
<td>33 (33.00)</td>
<td>0.253</td>
</tr>
<tr>
<td>Region (urban)</td>
<td>69 (40.59)</td>
<td>46 (46.00)</td>
<td>0.385</td>
</tr>
<tr>
<td>Ca supplement (yes)</td>
<td>31 (18.34)</td>
<td>14 (14.00)</td>
<td>0.356</td>
</tr>
<tr>
<td>Pulse (time/30 s)</td>
<td>38.61 ± 6.10</td>
<td>38.77 ± 7.17</td>
<td>0.847</td>
</tr>
<tr>
<td>Metabolic profiles</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>88.40 ± 8.42</td>
<td>79.97 ± 8.90</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.78 ± 3.33</td>
<td>22.92 ± 4.02</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Waist-hip ratio (cm/cm)</td>
<td>0.91 ± 0.05</td>
<td>0.87 ± 0.06</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>144.10 ± 15.09</td>
<td>130.20 ± 14.14</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>82.44 ± 8.52</td>
<td>76.82 ± 7.87</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fasting plasma glucose (mmol/L)</td>
<td>7.48 (6.39, 9.26)</td>
<td>7.50 (6.50, 9.33)</td>
<td>0.828</td>
</tr>
<tr>
<td>Glycated hemoglobin (%)</td>
<td>7.20 (6.50, 8.00)</td>
<td>6.90 (6.20, 7.70)</td>
<td>0.010</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.85 (1.31, 2.40)</td>
<td>1.06 (0.87, 1.37)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.17 (1.00, 1.35)</td>
<td>1.56 (1.37, 1.76)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>2.97 (2.47, 3.67)</td>
<td>2.90 (2.33, 3.50)</td>
<td>0.117</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>5.27 (4.53, 6.01)</td>
<td>4.96 (4.37, 5.64)</td>
<td>0.055</td>
</tr>
<tr>
<td>Triglycerides : HDL-cholesterol ratio</td>
<td>1.61 (1.03, 2.28)</td>
<td>0.68 (0.50, 0.97)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Insulin resistance</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin (mIU/L)</td>
<td>10.05 (6.80, 14.28)</td>
<td>6.36 (4.31, 14.85)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>3.59 (2.25, 5.14)</td>
<td>2.51 (1.37, 3.49)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HOMA-β</td>
<td>24.21 (13.81, 35.33)</td>
<td>11.55 (7.96, 21.55)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>High-sensitive C-reactive protein (mg/L)</td>
<td>1.62 (1.01, 3.36)</td>
<td>1.21 (0.55, 2.63)</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Data are mean ± SD, n (%), or median (interquartile range). HOMA-IR, homeostatic model assessment of insulin resistance; HOMA-β, homeostatic model assessment of β cell function.
Vitamin D Deficiency in Relation to Metabolic Syndrome

Vitamin D deficiency was inversely associated with the risk of MetS after adjusting for confounding factors ($p_{trend}=0.0163$) (26). In contrast, a previous investigation in Korean adults aged $\geq 19$ y showed that there was no association between serum 25(OH)D and MetS (27). However, there was no research focus on the relationship between vitamin D and MetS among elderly patients with T2DM. These relationships may be affected by the extraordinarily high levels or the differences in the age of participants and sample collection period (over four seasons). Thus, randomized controlled trials of vitamin D supplementation in older adults are warranted to determine whether this association is causal and reversible.

Several mechanisms may explain how vitamin D affects the risk of MetS. Firstly, the serum TG may be affected by vitamin D through an increase of intestinal calcium absorption; it may ultimately reduce hepatic TG formation and/or secretion via an effect on hepatocellular calcium (28, 29). In addition, vitamin D could modulate lipid levels via suppression of PTH secretion, as elevated PTH concentrations reportedly reduce lipolysis (30). Secondly, the blood pressure may be influenced by vitamin D through regulation of the renin–angiotensin system, an important regulator of blood pressure (31, 32). Last, experimental studies have suggested that vitamin D may exert its beneficial effects by stimulating the expression of an insulin receptor to improve insulin responsiveness for glucose transport or by controlling calcium influx, which is essential for the insulin-mediated intracellular process in insulin-responsive tissues (33).

There are several limitations to this study. Firstly, it was impossible to prove a causal relationship due to the nature of the cross-sectional design. Secondly, the small sample size may affect the stability of the results. Thirdly, 4.6% of the recruited individuals did not participate in the study because they were away from home or refused to respond, which may have caused selection bias. Despite these limitations, to the best of our knowledge, this is the first study to investigate the association of serum 25(OH)D status with MetS in Chinese middle-aged and elderly patients with T2DM.

In summary, our findings suggested that serum 25(OH)D concentrations were inversely associated with the risk of MetS among the middle-aged and elderly.

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### Table 3. Multivariable-adjusted odds ratios (ORs) and 95% CI of metabolic syndrome (MetS) across tertiles of serum 25-hydroxyvitamin D [25(OH)D] concentrations in a sample of patients with type 2 diabetes mellitus (T2DM) ($n=270$).

<table>
<thead>
<tr>
<th>Serum 25(OH)D</th>
<th>p for linear trend</th>
<th>Change in ORs and 95% CI of metabolic syndrome for each 10 ng/mL decrease in the serum 25(OH)D concentrations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tertile 1 ($\geq 25$ ng/mL)</td>
<td>52.08</td>
<td>0.004</td>
</tr>
<tr>
<td>Tertile 2 (19–25 ng/mL)</td>
<td>65.59</td>
<td>0.004</td>
</tr>
<tr>
<td>Tertile 3 (&lt;19 ng/mL)</td>
<td>72.84</td>
<td>0.022</td>
</tr>
</tbody>
</table>

$^1$ ORs and 95% CI were derived from logistic regression models. Model 1: unadjusted. Model 2: adjusted for age, gender, residential region (urban/rural) and calcium supplement (yes/no). Model 3: further adjusted for insulin, HOMA-IR and inflammatory factors (hs-CRP) based on model 2. Model 4: further adjusted for BMI based on model 3.
with type 2 diabetes, indicating that vitamin D deficiency may increase the risk of MetS in diabetes. However, the results should be interpreted with caution because of the aforementioned limitations. Additional well-designed randomized controlled trials are needed to determine the effect of supplemental vitamin D on the risk of MetS.

Acknowledgments

G.-T. Pan and J.-F. Guo contributed equally to this work.

This research was supported by National Natural Science Foundation of China (Grant ref: 81372981, 81372979), Jiangsu Natural Science Foundation (Grant ref: BK2012619), the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD) and Nutrition Research Foundation of Chinese Nutrition Institute—the Specialized Research Foundation for the DSM (Grant ref: 2013-030). We are grateful to the participants in the study and Dian Diagnostics Ltd in Hangzhou, China for their support and assistance. The authors also thank all the doctors and nurses in this study. All the authors declare that they have critically reviewed the manuscript and approved the final version submitted for publication.

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