Type 2 diabetes mellitus (T2DM) has become one of the most common chronic diseases worldwide. By 2013, more than 300 million people had been affected by T2DM (1). In China, there are more than 100 million patients with T2DM and the prevalence of this disease has substantially increased in past decades (2, 3). Because T2DM leads to severe complications such as coronary heart disease, stroke, kidney failure and blindness, numerous studies have been carried out to focus on its pathogenesis.

A growing body of evidence has suggested the involvement of vitamin D in the decline of β-cell function and insulin resistance, which are considered as the major pathological processes of T2DM. An early study has suggested the role of dietary vitamin D in maintaining normal insulin secretion (4). In humans, serum vitamin D levels have been demonstrated to be positively associated with both β-cell function and negatively associated with insulin resistance (5–9). Moreover, vitamin D has been proven to protect against inflammation-induced impairment of β cells (10) and suppress the apoptosis of pancreatic β cells (11). Additionally, vitamin D attenuates insulin resistance through its influences on glucose uptake in skeletal muscle, immunity and inflammatory reaction (12).

Although vitamin D is related to the development of insulin resistance and the impairment of β-cell function, current clinical evidence has not supported the definitive role of vitamin D in the pathogenesis of T2DM (13). Furthermore, relationships between vitamin D and insulin resistance as well as β-cell function vary among different racial and ethnic groups (14). To explore the role of vitamin D in the onset of T2DM in the Chinese Han population, we therefore investigated the relationship between serum 25-hydroxyvitamin D3 (25(OH)D) and insulin resistance as well as β-cell function in patients with newly diagnosed T2DM.

MATERIALS AND METHODS

In this study, 264 patients including 140 males and 124 females were recruited from March 2014 to
November 2017. All participants with an average age of
50.04±9.43 y were in-patients with newly diagnosed
T2DM, fulfilling the criteria of World Health Organiza-
tion 1999, at the Department of Endocrinology of
Anhui Provincial Hospital. The ethnicity of all partici-
pants was Han. To minimize the sunlight exposure dis-
turbance on vitamin D, all blood samples were collected
in either spring (from March to May) or autumn (from
September to November). Participants were excluded
from this study based on the following criteria: (1) indi-
viduals who had acute complications of T2DM, pancre-
atic disease, liver disease, renal disease, cancer or exces-
sive fasting blood glucose; (2) individuals who had taken
vitamin D, calcium, lipid lowering drugs, oral anti-
diabetic agents or insulin therapy; (3) individuals diag-
nosed with type 1 diabetes mellitus, secondary diabetes
mellitus or gestational diabetes mellitus. This study was
approved by the Ethic Committee of Anhui Provincial
Hospital. The ethnicity of all participants before their
consent was obtained from all participants before their
participation 1999, at the Department of Endocrinology of
T2DM, fulfilling the criteria of World Health Organi-
Data were examined for normal distribution by the
change in insulin to the change in glucose from 0 to
where G and I are the average levels of plasma glucose
and serum insulin of MMTT.

The homeostatic model assessment for insulin secre-
tion (HOMA-β) was used to assess β-cell function. The
formula for HOMA-β was as follows: 20×Flns (mIU/L)/
(FPG (mmol/L)−3.5). The early insulin response to a
mixed-nutrient load was calculated as the ratio of the
change in insulin to the change in glucose from 0 to
30 min (ΔI0−30/ΔG0−30). Insulin resistance was assessed by
both homeostasis model assessment for insulin resis-
tance (HOMA-IR), which was calculated as Flns (mIU/L)×FPG (mmol/L)/22.5, and Matsuda insulin
sensitivity index (Matsuda ISI), which was calculated
according to the formula: 10,000/√(G0×I0)×(G×I), where G and I are the average levels of plasma glucose
data presented as mean ± standard deviation (SD). Skewed distributed
data presented as median with interquartile range were
natural logarithm transformed to normality for fur-
eral analysis. All participants were divided into three

Table 1. Comparison of anthropometric and biochemical characteristics of three 25(OHD) tertiles.

<table>
<thead>
<tr>
<th>Group</th>
<th>T1 (n=88)</th>
<th>T2 (n=88)</th>
<th>T3 (n=88)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>25(OHD) (ng/mL)</td>
<td>7.65±1.96</td>
<td>13.53±1.58</td>
<td>19.24±3.06</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Male/female (n)</td>
<td>41/47</td>
<td>48/40</td>
<td>51/37</td>
<td>0.301*</td>
</tr>
<tr>
<td>Age (y)</td>
<td>51.42±10.42</td>
<td>48.91±8.83</td>
<td>50.09±9.06</td>
<td>0.538</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.24±3.47</td>
<td>25.15±3.65</td>
<td>24.22±4.29</td>
<td>0.198</td>
</tr>
<tr>
<td>Scr (μmol/L)</td>
<td>59.02±11.14</td>
<td>63.57±17.10</td>
<td>63.99±15.71</td>
<td>0.058</td>
</tr>
<tr>
<td>Ca (mmol/L)</td>
<td>2.28±0.15</td>
<td>2.27±0.14</td>
<td>2.37±0.80</td>
<td>0.879</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>3.20±1.25</td>
<td>2.91±1.15</td>
<td>3.20±1.91</td>
<td>0.056</td>
</tr>
<tr>
<td>TC (mmol/L)</td>
<td>4.75±1.71</td>
<td>4.49±1.95</td>
<td>4.65±2.28</td>
<td>0.548</td>
</tr>
<tr>
<td>FPG (mmol/L)</td>
<td>8.96±2.21</td>
<td>9.27±2.83</td>
<td>9.87±3.07</td>
<td>0.678</td>
</tr>
<tr>
<td>2hPG (mmol/L)</td>
<td>19.56±12.36</td>
<td>18.49±5.43</td>
<td>18.81±5.76</td>
<td>0.691</td>
</tr>
<tr>
<td>Flns (mIU/L)</td>
<td>2.70 (1.85, 4.63)</td>
<td>2.17 (1.69, 4.47)</td>
<td>2.37 (1.78, 3.68)</td>
<td>0.085</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>10.05±2.21</td>
<td>9.81±2.33</td>
<td>9.92±2.20</td>
<td>0.742</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.24 (0.65, 1.84)</td>
<td>1.04 (0.67, 1.58)</td>
<td>0.87 (0.67, 1.59)</td>
<td>0.005</td>
</tr>
<tr>
<td>HOMA-β</td>
<td>11.74 (6.83, 18.64)</td>
<td>9.69 (6.02, 19.56)</td>
<td>11.00 (6.40, 16.57)</td>
<td>0.245</td>
</tr>
<tr>
<td>ΔI0−30/ΔG0−30</td>
<td>0.49 (0.23, 1.19)</td>
<td>0.62 (0.22, 1.38)</td>
<td>0.59 (0.21, 1.21)</td>
<td>0.167</td>
</tr>
<tr>
<td>ISI</td>
<td>199.84 (107.91, 284.62)</td>
<td>210.49 (113.71, 312.03)</td>
<td>220.93 (144.34, 338.82)</td>
<td>0.009</td>
</tr>
</tbody>
</table>

Normally distributed data are presented as mean±SD, and skewed data are presented as median with interquartile range for ease of interpretation. Skewed data were log transformed to normality before further statistical analysis. Statistical difference among groups was determined by one-way analysis of variance. *Statistical difference was determined by the X²-test.
Association of Vitamin D with Insulin Resistance in Male Diabetic Patients

The average serum 25(OH)D level in this study was 13.47 ± 5.28 ng/mL. The anthropometrical and biochemical characteristics of total patients are shown in Table 1 according to tertiles of serum 25(OH)D levels. Among the three groups, HOMA-IR and Matsuda ISI had significant differences (HOMA-IR, p = 0.005; Matsuda ISI, p = 0.009). Nevertheless, the other parameters had no significant differences (p > 0.05). Although there was no statistical difference in FIns, a decreased trend was indicated with an increase in 25(OH)D level.

Table 1. Correlation between serum 25(OH)D and various indices of glucose metabolism.

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th></th>
<th></th>
<th></th>
<th>Male</th>
<th></th>
<th></th>
<th></th>
<th>Female</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>p</td>
<td>r</td>
<td>p</td>
<td>r</td>
<td>p</td>
<td>r</td>
<td>p</td>
<td>r</td>
<td>p</td>
<td>p</td>
</tr>
<tr>
<td>FPG</td>
<td>-0.002</td>
<td>0.975</td>
<td>-0.101</td>
<td>0.231</td>
<td>-0.141</td>
<td>0.120</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2hPG</td>
<td>-0.039</td>
<td>0.533</td>
<td>-0.001</td>
<td>0.994</td>
<td>-0.031</td>
<td>0.735</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ln FIns</td>
<td>-0.049</td>
<td>0.431</td>
<td>-0.209</td>
<td>0.012</td>
<td>0.061</td>
<td>0.503</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HbA1c</td>
<td>-0.081</td>
<td>0.181</td>
<td>-0.124</td>
<td>0.142</td>
<td>-0.024</td>
<td>0.792</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ln HOMA-β</td>
<td>0.008</td>
<td>0.897</td>
<td>-0.054</td>
<td>0.593</td>
<td>-0.055</td>
<td>0.550</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ln HOMA-IR</td>
<td>-0.042</td>
<td>0.500</td>
<td>-0.273</td>
<td>0.001</td>
<td>0.156</td>
<td>0.086</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ln Δh0–30/ΔG0–30</td>
<td>0.019</td>
<td>0.764</td>
<td>-0.051</td>
<td>0.546</td>
<td>-0.056</td>
<td>0.543</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ln ISI</td>
<td>0.046</td>
<td>0.453</td>
<td>0.219</td>
<td>0.009</td>
<td>-0.166</td>
<td>0.068</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Skewed data were log transformed to normality before further analysis.

Table 2. Linear regression analysis of the association of serum 25(OH)D with indices of HOMA-IR in males.

<table>
<thead>
<tr>
<th></th>
<th>Model 1</th>
<th></th>
<th>Model 2</th>
<th></th>
<th>Model 3</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β (95% CI)</td>
<td>p</td>
<td>β (95% CI)</td>
<td>p</td>
<td>β (95% CI)</td>
<td>p</td>
</tr>
<tr>
<td>ln FIns</td>
<td>-0.209</td>
<td>0.012</td>
<td>-0.273</td>
<td>0.001</td>
<td>-0.241</td>
<td>0.003</td>
</tr>
<tr>
<td>ln HOMA-IR</td>
<td>-0.331</td>
<td>&lt;0.001</td>
<td>-0.276</td>
<td>0.001</td>
<td>-0.244</td>
<td>0.002</td>
</tr>
<tr>
<td>ln ISI</td>
<td>0.219</td>
<td>0.009</td>
<td>0.219</td>
<td>0.009</td>
<td>0.186</td>
<td>0.025</td>
</tr>
</tbody>
</table>

Model 1: adjusted for BMI; Model 2: additionally adjusted for FPG and 2hPG; Model 3: additionally adjusted for TG, TC and Ca.

RESULTS

The average serum 25(OH)D level in this study was 13.47 ± 5.28 ng/mL. The anthropometrical and biochemical characteristics of total patients are shown in Table 1 according to tertiles of serum 25(OH)D levels. Among the three groups, HOMA-IR and Matsuda ISI had significant differences (HOMA-IR, p = 0.005; Matsuda ISI, p = 0.009). Nevertheless, the other parameters had no significant differences (p > 0.05). Although there was no statistical difference in FIns, a decreased trend was indicated with an increase in 25(OH)D level.

The correlations of 25(OH)D with indices of glucose metabolism were assessed in the total population, male population and female population, respectively (Table 2). The 25(OH)D levels were higher in the male population than female population (male: 14.43 ± 5.15 ng/mL, female: 12.34 ± 5.21 ng/mL; p = 0.002). No significant difference in age between the male population and female population was shown (male: 49.56 ± 10.91 y, female: 51.33 ± 10.24 y; p = 0.096). For the male population, serum 25(OH)D was negatively correlated with FIns (r = -0.209, p = 0.012) and HOMA-IR (r = -0.273, p = 0.001), and positively correlated with Matsuda ISI (r = 0.219, p = 0.009). For the total population and female population, no significant correlations were observed (p > 0.05 for all).

To further evaluate whether the serum 25(OH)D was the independent predictor of insulin resistance and insulin sensitivity, multiple stepwise regression analyses were performed in the male population. As shown in Table 3, serum 25(OH)D was an independent predictor for both HOMA-IR and Matsuda ISI after adjustment for confounding factors (p < 0.05 for both).

DISCUSSION

Over the last decades, a number of clinical studies have described the involvement of vitamin D in the onset...
of T2DM in both Eastern and Western populations. However, the definitive association between vitamin D and T2DM has also been doubted (16, 17). Additionally, some Mendelian randomization studies and randomized placebo-controlled trials have challenged the causal association between vitamin D and T2DM (18–21). The development of insulin resistance and β-cell dysfunction are accepted as the hallmarks of the pathogenesis of T2DM. Emerging evidence has demonstrated the involvement of vitamin D in these pathophysiological processes. In this study, no significant association was found between serum 25(OH)D levels and HOMA-β, the index of basal insulin secretion, in patients with newly diagnosed T2DM. Nor did we find a significant association of serum 25(OH)D with ΔI0–30/ΔG0–30, the index of early insulin response to stimulus. Taken together, this study provides no evidence for the role of vitamin D in the decline of β-cell function during the onset of T2DM. Nevertheless, the significant associations of vitamin D with Fins, HOMA-IR and Matsuda ISI suggest the presumable role of vitamin D in the development of insulin resistance and the decrease in insulin sensitivity during the pathogenesis of T2DM.

Several reports have elucidated the possible mechanisms underlying the role of vitamin D in the development of insulin resistance and insulin sensitivity. Vitamin D has been shown to affect the insulin response to glucose stimulation directly by binding of the active form of vitamin D, 1,25-(OH)2-D, to the vitamin D receptors on β cells (22, 23). Furthermore, 1,25-(OH)2-D activates transcription of the human insulin receptor gene, stimulates the expression of insulin receptor, and enhances insulin-mediated glucose transport (24). Vitamin D may reduce insulin resistance by inhibiting the expression of peroxisome proliferator-activated receptor-γ and the differentiation from preadipocytes to mature adipocytes (25). Inflammation is one of the important pathological mechanisms of insulin resistance. Vitamin D could regulate the immunological reaction of macrophages and monocytes, reduce the concentration of interleukin-6 (IL-6), interleukin-1β and tumor necrosis factor-α (TNF-α), and thereby relieve inflammatory reaction (26, 27). Consistently, low vitamin D status is associated with high levels of inflammatory factors such as IL-6, TNF-α, and C-reactive protein (28–30). Remarkably, recent clinical trials have indicated that supplementation of vitamin D may reduce the risk of diabetes mellitus by attenuating the development of insulin resistance rather than by lowering insulin secretion, supporting the association of vitamin D with insulin resistance in the pathogenesis of T2DM (31).

Additionally, the association of low vitamin D with increased insulin resistance and decreased insulin sensitivity could partly be attributed to its action on insulin secretion in pancreatic β-cells via Ca2+ entry. Vitamin D is essential for calcium homeostasis and insulin secretion is dependent on calcium oscillations (32). Early study shows that the active form of vitamin D, 1,25(OH)2-vitamin D, stimulates insulin secretion in isolated rat pancreas (33). Subsequent studies show 1,25(OH)2-vitamin D-evoked Ca2+ oscillations in pancreatic β-cells regulate insulin secretion either by enhancing Ca2+ entry in the pancreatic β cell and/or Ca2+ mobilization from intracellular organelles (34). Moreover, 1,25(OH)2-vitamin D facilitates the insulin response to glucose via its regulatory effects on the calcium pool of β-cells intracellularly and extracellularly (35, 36). A report by Beaulieu et al. further shows that glucose-stimulated insulin secretion is reduced in vitamin D-deficient rats in the presence of hypocalcemia (37).

Another interesting finding in this study is the sex disparity in the relationship between vitamin D and the indices of glucose metabolism in the Chinese Han population. Significant associations of serum 25(OH)D with indices of glucose metabolism were observed in males, whereas such associations were lacking in females. Few studies have focused on the sex disparity in the relationship between vitamin D and insulin sensitivity and β-cell dysfunction up to now. Gao et al. has reported that the vitamin D level is significantly associated with female β-cell function, whereas in the male population, these associations are null (38). For the Western population, vitamin D deficiency was found to be associated with decreased insulin production in males with T2DM (9). This sex disparity may result from the differential effects on glycemic status and risk of type 2 diabetes by endogenous sex hormones (39). Low testosterone has been reported to be associated with greater risks of insulin resistance (40). Recent studies have indicated that serum 25(OH)D level is positively associated with testosterone level in males (41). A study by Wang et al. in Chinese males has drawn a similar conclusion (42). Our findings confirmed this conclusion (Supplemental Online Material, Table S1). Other factors that affect glucose metabolism and the risk for incidence of T2DM, such as physical activity, smoking, body composition and eating habits may also partly explain this sex disparity. Nevertheless, further studies are required to address this issue.

Some limitations of this study should be noted. Firstly, this study is cross-sectional, and the causal relationship between vitamin D and indices of glucose metabolism could not be proven. Secondly, lifestyles such as outdoor exercise, smoking, alcohol consumption and a diet containing high levels of saturated fat and sugar should be considered in future study. Thirdly, parathyroid hormone, which has been shown to influence insulin resistance, was not included in this study due to a lack of information on the participants’ serum parathyroid hormone levels (43). Finally, the sample size of this study was limited. Prospective studies with a large sample size are required to investigate the role of vitamin D in the onset of T2DM.

In conclusion, this study shows the association of vitamin D with the indices of glucose metabolism in Chinese Han patients with newly diagnosed T2DM. Notably, such associations are sex-specific. The positive association with Matsuda ISI and negative association with HOMA-IR suggest the possible role of vitamin D in the development of insulin resistance during the pathogenesis of T2DM in the male Chinese Han population.
Association of Vitamin D with Insulin Resistance in Male Diabetic Patients

Acknowledgements
This study was supported by Integrated Technology Application Research in Public Welfare of Anhui Province (Grant number 17040804012). National Key Research and Development Program of China (Grant number 2016YFB1000905) and National Natural Science Foundation of China (Grant number 81100558). We are grateful to all participants in this study. We also thank all doctors and nurses at the Department of Endocrinology of Anhui Provincial Hospital for their efforts in collecting the clinical data.

Supporting Information
Supplemental Online Material is available on J-STAGE.

REFERENCES

Supporting Information
Supplemental Online Material is available on J-STAGE.


