Hypovitaminosis D in Type 2 Diabetes Mellitus: Association with Microvascular Complications and Type of Treatment

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Abstract. The prevalence of hypovitaminosis D has been recently reevaluated, and diabetes is considered as a risk factor for osteoporosis. We studied the association of the prevalence of hypovitaminosis D with the clinical features of diabetes. We conducted the observational study in 581 Japanese patients with type 2 diabetes mellitus and 51 normal subjects, and analyzed the relationship between serum 25-hydroxyvitamin D (25-OHD) concentration and the clinical features associated with type 2 diabetes. Mean serum 25-OHD concentration in type 2 diabetes patients was 17.0 ± 7.1 ng/ml (Mean ± SD) in winter, and was not statistically different from normal population (17.5 ± 3.6 ng/ml). The prevalence of hypovitaminosis D (<20 ng/ml) was 70.6%. Serum concentrations of 25-OHD were associated with HbA1c (P = 0.013), age (P = 0.070) and serum albumin (P<0.001), but were not related to BMI or the duration of diabetes. The levels of 25-OHD were significantly lower in the population with apparent microvascular complications, although serum creatinine levels were below 2.0 mg/dl. Serum 25-OHD concentrations in the group treated with insulin (15.4 ± 6.5 ng/ml) was lower than those in the patients treated with diet alone (20.8 ± 7.6 ng/ml) and with oral hypoglycemic agents (17.3 ± 7.0 ng/ml). Furthermore, the highest incidence of osteoporotic fracture and/or back deformity was observed in insulin-treated patients with hypovitaminosis D. In conclusion, these results suggest that microvascular complications and insulin treatment in type 2 diabetes patients are associated with the co-existence of hypovitaminosis D, and that hypovitaminosis D in insulin-treated patients is possibly related to the risk of osteoporotic fracture.

Key words: Hypovitaminosis D, Diabetes mellitus, Insulin

VITAMIN D stores are derived from either dietary intake or cutaneous synthesis following ultraviolet irradiation [1, 2]. Vitamin D from either source undergoes 25-hydroxylation in the liver to form 25-hydroxyvitamin D (25-OHD). Serum 25-OHD concentrations are thought to accurately reflect vitamin D stores, because its half-life is approximately 3 weeks [1, 2]. Vitamin D deficiency causes secondary hyperparathyroidism, which can lead to osteomalacia, irreversible bone loss and increased risk of fracture [2–4]. The existence of diabetes mellitus is now considered to be a risk factor to induce bone loss and osteoporotic fracture. In type 1 diabetes, it is apparent that osteopenia occurs especially in the young-onset type 1 diabetes, and that the demineralization process seems to be related to fasting blood glucose concentrations and HbA1c [5–7]. Moreover, it has also been suggested that hypovitaminosis D may be a significant risk factor for glucose intolerance [8]. High prevalence of hypovitaminosis D in type 2 diabetes patients has been reported [9, 10], whereas Ishida et al. [11] could not find any difference in such patients from the control, suggesting that more precise analysis of the relationship between the 25-OHD level and the clinical manifestations of type 2 diabetes is needed.

To address these issues, we analyzed laboratory data from a cross-section of diabetes clinic outpatients who
were evaluated for serum 25-OHD concentrations as part of their routine follow-up visits and compared the data with those in normal population. The aims were to assess the prevalence of hypovitaminosis D in type 2 diabetes mellitus, and to determine whether any association exists between serum 25-OHD concentration and diabetic clinical features.

Materials and Methods

Subjects

Subjects were outpatients who were treated for type 2 diabetes mellitus, as presented in Table 1. Data were collected from patients who visited our hospital, agreed to participate in this study and whose biochemical parameters such as plasma glucose, HbA1c, serum creatinine, and urinary protein excretion were checked as a part of a routine follow-up for diabetes. The hospital’s ethics review committee approved this descriptive study, and informed consent was obtained from each patient. In total, the study included 581 adult type 2 diabetes patients who were not suffering from parathyroid or calcium-related diseases based on biochemical measures and clinical assessment. The patients with apparent chronic renal failure (serum creatinine level >2.0 mg/dl), history of surgical operation of upper gastrointestinal tract, liver diseases, the therapy with glucocorticoid or vitamin D, or supplementation of Ca or vitamin D, were excluded from this study. Institutionalized or hospitalized patients and patients with severe visual disturbance were also excluded from the study. As a control population, 51 randomly selected age- and gender-matched subjects, who visited the hospital for their routine medical check-up, agreed to participate in this study, and were checked for their biochemical parameters and serum 25-OHD levels. In Japan, the company or local district government covers the cost of routine medical check-ups, so there is little bias in the population before random selection. The control subjects were investigated with a protocol similar to that used for the diabetic patient group except for the past or present history of diabetes. No other exclusion criteria other than unwillingness to participate in the study, was applied in the selection of the control group. The incidence of osteoporotic fracture, the height shortness and the progress of round back during 2 years after the measurement of serum 25-OHD were determined by interview or mailed questionnaire in 513 patients with type 2 diabetes who were 50 years of age or more. Among them, 447 of these patients were agreed to answer the questionnaire, 2 of them were dead and the other 64 did not reply.

Assessment of clinical features and serum 25-OHD assays

Age, BMI, the duration of diabetes, overt paresthesia, and the type of treatment were checked when the patients visited our hospital in March. Ophthalmologists with reliable experiences examined them for diabetic retinopathy. Overt proteinuria was assessed by spot urinalysis. Serum 25-OHD levels was measured with direct radioimmunoassay (DiaSorin, Inc., Stillwater, MN). Although it is yet uncertain what concentration of serum 25-OHD level is optimal, it is so far suggested that the lower limit of serum 25-OHD level should be somewhere between 15 and 36 ng/ml [12–16]. We here categorized hypovitaminosis D as a serum concentration of 25-OHD below 20 ng/ml, which is necessary for avoiding the latent secondary hyperparathyroidism that might induce osteoporosis in the elderly. Routine chemical analysis was performed by a Hitachi 7600 automatic analyzer (Hitachi High Technologies, Tokyo, Japan).

Data analysis

The associations with categorical variables and continuous 25-OHD concentrations were examined using analysis of variance (Scheffé’s test) or unpaired Student’s t-test. The relationship between serum 25-OHD concentration as dependent variable and age, BMI, duration of diabetes, HbA1c and serum albumin as in-

<table>
<thead>
<tr>
<th>Variables</th>
<th>Diabetes Patients (N = 581)</th>
<th>Control subjects (N = 51)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>61.6 ± 11.5</td>
<td>58.2 ± 10.6</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>317/264</td>
<td>25/26</td>
</tr>
<tr>
<td>Body Mass Index (kg/m²)</td>
<td>24.0 ± 3.9</td>
<td>22.0 ± 2.8</td>
</tr>
<tr>
<td>Duration of diabetes</td>
<td>11.8 ± 8.6</td>
<td>None</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>7.7 ± 1.4*</td>
<td>4.8 ± 0.6</td>
</tr>
<tr>
<td>Serum 25-OHD (ng/ml)</td>
<td>17.0 ± 7.1</td>
<td>17.5 ± 3.6</td>
</tr>
</tbody>
</table>

*P<0.05 vs. control subjects, ANOVA, Scheffe’s test.
dependent variables were examined using simple regression analysis. The relationship between serum 25-OHD as dependent variable and type of treatment (coded as: 0, without medicine; 1, oral hypoglycemic agent; 2, insulin therapy) as independent variables were examined using multiple regression analysis with all independent variables (age, BMI, duration of diabetes, HbA1c, intact PTH, number of complications and type of treatment) entered into the models. As for microvascular complications, we examined the number of complications as an independent variable for multiple regression analysis, because the progress of diabetic microvascular complications was considered to be associated with each other. In addition, we counted the number of the patients with retinopathy as the sum of the number of the patients with simple retinopathy and proliferative retinopathy. As for the assessment of the osteoporotic fractures, we counted the number of the fracture patients as the sum of the number of apparent clinical fractures, the progress of the height reduction and the round back for \( \chi^2 \)-test. We set the undetectable value (<5 ng/ml) of serum 25-OHD as 2.5 ng/ml in this study. All analyses were performed with the use of statistical software StatView version 5.0 (SAS Inc., Cary, NC).

### Results

The clinical characteristics of the patients are shown in Table 1. The study population of type 2 diabetes patients was aged 22–88 years (mean ± SD 61.6 ± 11.5). Mean BMI was 24.0 ± 3.9 kg/m² (range 13.9–40.6). The duration of diabetes after the diagnosis was between 1 and 53 years (11.8 ± 8.6 years), and mean HbA1c level was 7.7 ± 1.4%. Among these patients, 61.2% of the subjects were treated with oral anti-diabetic drugs such as sulfonylurea and \( \alpha \)-glucosidase inhibitor, while 30.0% were treated with insulin injection. The patients treated without any anti-diabetic drug or insulin was 8.8%. As for microvascular complications, the clinical features of diabetes in the study population are shown in Table 2.

The mean concentration of serum 25-OHD was 17.0 ± 7.1 ng/ml (range, <5 to 47 ng/ml), which was not statistically different from that in age- and gender-matched non-diabetic population (Table 1). The prevalence of hypovitaminosis D (<20 ng/ml) was 70.6% (Fig. 1). As in previous studies on normal population [3, 12, 17], serum intact PTH levels were negatively correlated with serum 25-OHD concentrations in our populations (Fig. 2).

| Table 2. Microvascular complications of diabetes mellitus and serum 25-hydroxyvitamin D (25-OHD) concentration |
|-------------------------------------------------|------------------|
| Variables                                      | Serum 25-OHD (ng/ml) |
| All patients (N = 581)                         | 17.0 ± 7.1        |
| Microvascular complications (%)                |                   |
| Retinopathy                                    |                   |
| None                                           | 60.3 17.6 ± 6.6   |
| Simple                                         | 18.5 16.5 ± 6.4   |
| Proliferate                                    | 21.0 15.1 ± 8.0*  |
| Nephropathy                                    |                   |
| None                                           | 68.0 17.2 ± 7.1   |
| Overt proteinuria                              | 32.0 16.3 ± 6.8   |
| Neuropathy                                     |                   |
| None                                           | 71.7 17.2 ± 7.0   |
| Overt paresthesia                             | 28.3 16.0 ± 7.0   |
| Number of complications                        |                   |
| None                                           | 37.9 17.4 ± 7.0   |
| 1                                              | 28.4 17.0 ± 6.0   |
| 2                                              | 15.3 15.3 ± 6.8*  |
| 3                                              | 18.4 12.7 ± 7.7*  |

\*P<0.05 vs. without microvascular complication. ANOVA, Scheffe’s test.

![Fig. 1. Frequency distribution of serum 25-hydroxyvitamin D (25-OHD) concentrations in type 2 diabetic patients (■) and in control subjects (□). The mean ± SD value was 17.0 ± 7.1 ng/ml and 17.5 ± 3.6 ng/ml, respectively. The patients were categorized according to their serum 25-OHD concentrations in increments of 5 ng/ml.](image-url)
Serum concentrations of men (18.6 ± 7.1 ng/ml) were significantly higher than those in women (15.1 ± 6.0 ng/ml) (Student’s *t*-test, *P* < 0.001). Simple regression statistical analysis showed that serum 25-OHD concentrations decreased according to the increase of HbA1c, while serum albumin concentrations were positively associated with serum 25-OHD concentrations (Table 3). Neither age of the subjects, BMI nor duration of diabetes predicted serum 25-OHD concentrations in type 2 diabetes patients (Table 3). The existence of diabetic proliferative retinopathy was significantly associated with the decrease in serum 25-OHD concentrations (Table 2). There was no significant association between serum concentrations of 25-OHD and proteinuria (with serum creatinine <2.0 mg/dl) (*P* = 0.153) or overt paresthesia (diabetic neuropathy) (*P* = 0.055). However, serum 25-OHD concentrations decreased according to the number of diabetic microvascular complications (Table 2). As for the treatment, the population treated without medicine had higher concentrations of serum 25-OHD than the patients with oral anti-diabetic drugs and those with daily insulin injection (Fig. 3). In addition, serum concentration of 25-OHD in the insulin-treated population (15.4 ± 6.5 ng/ml) was significantly lower than those in the patients treated with diet alone (20.8 ± 7.6 ng/ml) and with oral hypoglycemic agents (17.3 ± 7.0 mg/ml). Furthermore, multiple regression analysis showed that the number of complications and type of treatment were associated with serum concentrations of 25-OHD (Table 4). The assessment of the incidence of osteoporotic fractures (apparent osteoporotic bone fracture, shortening of height and/or progress of round back) in the population with type 2 diabetes aged over 50 years old revealed that the patients with osteoporotic bone deformity or fracture had lower serum 25-OHD concentration (16.5 ± 6.4 ng/ml) than those without them (17.9 ± 7.2 ng/ml) (unpaired Student’s *t*-test, *P* = 0.04). Although the number of microvascular com-

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**Table 3.** Simple regression statistics with dependent variables of serum 25-hydroxyvitamin D concentration

<table>
<thead>
<tr>
<th>Variables (n = 581)</th>
<th>Coefficient</th>
<th><em>P</em></th>
<th>Intercept</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>0.046</td>
<td>0.070</td>
<td>14.2</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>-0.099</td>
<td>0.222</td>
<td>19.3</td>
</tr>
<tr>
<td>Duration of diabetes (year)</td>
<td>-0.029</td>
<td>0.393</td>
<td>17.3</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>-0.512</td>
<td>0.013</td>
<td>20.8</td>
</tr>
<tr>
<td>Serum albumin (mg/dl)</td>
<td>4.461</td>
<td>&lt;0.001</td>
<td>-2.77</td>
</tr>
</tbody>
</table>

**Table 4.** Multiple regression statistics with dependent variables of serum 25-hydroxyvitamin D concentration

<table>
<thead>
<tr>
<th>Variables (n = 581)</th>
<th>Coefficient</th>
<th><em>P</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.049</td>
<td>0.110</td>
</tr>
<tr>
<td>BMI</td>
<td>-0.070</td>
<td>0.426</td>
</tr>
<tr>
<td>Duration of diabetes</td>
<td>0.003</td>
<td>0.943</td>
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<tr>
<td>HbA1c</td>
<td>-0.293</td>
<td>0.239</td>
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<tr>
<td>Number of complications</td>
<td>-0.669</td>
<td>0.027</td>
</tr>
<tr>
<td>Type of treatment</td>
<td>-2.112</td>
<td>0.002</td>
</tr>
<tr>
<td>Intact PTH</td>
<td>-0.054</td>
<td>0.010</td>
</tr>
<tr>
<td>Intercept</td>
<td>23.11</td>
<td></td>
</tr>
</tbody>
</table>

**Fig. 2.** Relationship between serum 25-hydroxyvitamin D (25-OHD) and the serum intact parathyroid hormone (PTH) concentrations in type 2 diabetic patients.

**Fig. 3.** Relation between serum 25-hydroxyvitamin D (25-OHD) concentrations and the type of treatment for diabetes. Each value represents mean ± SD. Statistical analysis was done with ANOVA, Scheffe’s test.
plications did not significantly predict future fracture, the highest incidence of osteoporotic fracture was observed in the patients treated with daily insulin injection ($\chi^2$-test, $P = 0.01$) (Table 5).

### Discussion

Metabolic disorders including diabetes mellitus are now well known to affect the bone metabolism and may result in osteoporosis. Both the increase in bone turnover markers and the decrease in bone formation have been reported in diabetic populations [9, 18]. It has been reported that impaired vitamin D metabolism exists in spontaneously diabetic GK rats [19], and that chronic insulin-deficiency results in a decrease in 1-\(\alpha\) hydroxylase activity and an increase in 24-hydroxylase activity in chronic streptozotocin-induced diabetic rat [20]. The prevalence of hypovitaminosis D in the diabetic population is still controversial [9–11], and recent findings suggest that the lower limit of normal value of serum 25-OHD concentrations should be reevaluated [12–16]. In this study, we found that there was a positive association between age and serum concentration of 25-OHD also in the type 2 diabetes population, though not statistically significant. Serum 25-OHD is known to bind to proteins such as D-binding protein and albumin, and could be considered as one of the nutritional markers. We found a positive association between serum 25-OHD and serum albumin concentrations, suggesting that it is possible that the decrease of protein synthesis affects the concentration of serum 25-OHD in addition to hypovitaminosis D, both of which will deteriorate bone metabolism. We here found that BMI was not associated with serum 25-OHD concentrations in the type 2 diabetes population. It has been reported that obese subjects (BMI $\geq$ 30) had lower serum 25-OHD concentrations and higher iPTH concentrations than did age-matched non-obese control subjects (BMI $\leq$ 25) [24]. In addition, serum 25-OHD levels were negatively associated with body fat content in a study of the general population in the United States [25]. These findings suggest the decreased bioavailability of vitamin D3 due to its deposition in body fat component in obese population. In Japanese non-obese population, we have previously reported that not body fat content but BMI was positively associated with the increase of serum concentration of 25-OHD, suggesting the increase of the amount of non-fat tissue components such as skeletal muscles is related to the increase of 25-OHD [17]. As changes in body weight and body fat distribution are known to be closely related to insulin resistance and the occurrence of type 2 diabetes [26], these results suggest that the imbalance of body composition, for example the increase of visceral fat, in diabetes patients may contribute to the relationship between serum 25-OHD concentration and BMI. Further investigations are needed to clarify full relationship.

The demineralization process especially involves

<table>
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<tr>
<th>Table 5. Relation between serum 25-hydroxyvitamin D (25-OHD) concentrations and the type of treatment for diabetes with or without osteoporotic bone deformity or fracture in the aged patients with diabetes mellitus.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type of treatment</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>Diet</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Oral</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Insulin</td>
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</table>
trabecular bone and seems to be related to the control of blood glucose concentration in insulin-dependent type 1 diabetes mellitus [5–7]. In the present study, we showed that the increase in the HbA1c was negatively associated with serum 25-OHD concentrations in the subjects with type 2 diabetes mellitus, while the duration of diabetes was not associated with serum 25-OHD levels. These results suggest that the control of blood glucose itself affects vitamin D metabolism regardless of the duration of the disease in type 2 diabetes mellitus. The progress of diabetic microangiopathy is known to be closely related to the control of blood glucose and the duration of diabetes. To explore whether the progress of microvascular complications are associated with the existence of hypovitaminosis D, we next analyzed the prevalence of diabetic microvascular complications and their association with serum concentration of 25-OHD. We here showed that the subjects with proliferative diabetic retinopathy had lower serum 25-OHD concentrations than those without retinopathy and those with simple diabetic retinopathy. As for nephropathy, massive proteinuria (more than 3 g per day) has been reported to be associated with the decrease of serum 25-OHD concentrations [27]. However, we could not find significant association with hypovitaminosis D and proteinuria in this study. One of the reasons why proteinuria was not a predictor of hypovitaminosis D might be that the patients with serum creatinine concentrations higher than 2 mg/dl were excluded from this study, so patients with massive proteinuria might have been excluded in part from this study. The concentrations of 25-OHD in the patients with overt paresthesia seemed to be lower than in those without paresthesia, although the difference was not statistically significant (P = 0.055, unpaired Student’s t-test). In addition, the number of complications was significantly associated with serum 25-OHD levels. Furthermore, multiple regression analysis showed that the number of complications was associated with decreased 25-OHD concentrations in type 2 diabetes patients, suggesting that the progress of diabetic microangiopathy is associated with the decrease of serum concentration of 25-OHD.

It has been reported that decreased bone mineral content was found in type 1 diabetes patients and insulin-treated type 2 diabetes patients, whereas both increased and decreased bone mass were found in type 2 diabetes patients treated without insulin [18, 28, 29]. In the present study, we showed that serum concentrations of 25-OHD in insulin-treated type 2 diabetes patients were significantly lower than those in type 2 diabetes patients treated with oral hypoglycemic agents and those in patients without medicine. Although HbA1c in insulin-treated patients were higher than those in oral hypoglycemic agents-treated subjects and in patients without medicine (data not shown), multiple regression analysis showed that the type of treatment was a dependent predictor of hypovitaminosis D in type 2 diabetes mellitus. This finding further suggests that hypovitaminosis D is associated with severe glucose intolerance and insulin-dependency in diabetic subjects. Experimentally, vitamin D is required for normal insulin secretion and glucose tolerance [30], and hypovitaminosis D may be a significant risk factor for glucose intolerance in diabetic patients [8]. It has recently been reported that positive correlation of serum 25-OHD concentration with insulin sensitivity and a negative effect of hypovitaminosis D on β cell function were found in normal population, and that subjects with hypovitaminosis D had a greater prevalence of components of metabolic syndrome than did subjects without hypovitaminosis D [31]. In addition, it has also been reported that a higher intake of vitamin D and Ca intake was associated with the low risk of type 2 diabetes [32]. From this point of view, it is also possible that impaired vitamin D metabolism in type 2 diabetes mellitus adversely affects the glucose intolerance in these patients. Furthermore, we here showed that the incidence of osteoporotic fractures and back deformity in the population with type 2 diabetes aged over 50 years old was associated with the decrease of serum 25-OHD concentration, and that the highest incidence of osteoporotic fracture occurred in the insulin-treated patients, suggesting the adverse effect of hypovitaminosis D on bone metabolism in diabetic patients.

In conclusion, our findings suggest that multiple microvascular complications and insulin treatment in type 2 diabetes patients are associated with co-existence of hypovitaminosis D and that hypovitaminosis D in insulin-treated patients is possibly related to the risk of osteoporotic fracture.

Acknowledgements

We are indebted to Dr. Yasuhiro Uchida (Chugai Pharmaceutical Co., Ltd) for his scientific suggestions. We also give special thanks to Ms. Natsuko Takekawa.
for her excellent secretarial work. Dr. Shigeo Imamura, Dr. Keiko Yamamoto-Hotta, Dr. Kentaro Fujiwara, Dr. Masaki Makino, Dr. Takehiko Mokuno, Dr. Yoshikuni Sawai, who are the staff physicians at the Outpatient Diabetes Clinic, Department of Internal Medicine, Fujita Health University Hospital, are acknowledged for their cooperation during the patient recruitment phase.

References


