Concentrations of Water-Soluble Vitamins in Blood and Urinary Excretion in Patients with Diabetes Mellitus

Hiromi Iwakawa¹, Yasuyuki Nakamura¹, Tomiho Fuku², Tsutomu Fukuwatari³, Satoshi Ugi⁴, Hiroshi Maegawa⁴, Yukio Doi⁵, and Katsumi Shibata⁶

¹Department of Food Science and Human Nutrition, Faculty of Agriculture, Ryukoku University, Otsu, Shiga Prefecture, Japan. ²Department of Nutrition, Faculty of Health and Nutrition, Shubun University, Ichinomiya, Aichi, Japan. ³Department of Nutrition, School of Human Cultures, The University of Shiga Prefecture, Hikone, Shiga, Japan. ⁴Department of Medicine, Shiga University of Medical Science, Otsu, Shiga, Japan.

ABSTRACT: We examined the concentrations of water-soluble vitamins in blood and urinary excretion of 22 patients with type 2 diabetes mellitus (type 2DM) and 20 healthy control participants. Macronutrient and vitamin intakes of type 2DM subjects were measured using a weighed food record method. Control participants consumed a semipurified diet for eight days. Multiple linear regression models were used to determine whether significant differences existed in vitamin concentrations in blood independent of age, sex, and other confounding factors. Concentrations of vitamins B2, B6, C, niacin, and folate in blood were significantly lower in type 2DM subjects than in controls, independent of confounding factors. Renal clearances of vitamins B6, C, niacin, and folate were significantly higher in type 2DM subjects than in controls. In conclusion, concentrations of vitamins B2, B6, C, niacin, and folate in blood were significantly lower in type 2DM subjects than in controls, independent of confounding factors; based on the evidence of increased urinary clearance of these vitamins, the lower levels were likely due to impaired reabsorption processes.

KEYWORDS: water-soluble vitamins, blood vitamin concentration, urine vitamin excretions, type 2 diabetes mellitus, human, Japanese.

Introduction

Type 2 diabetes mellitus (DM) was previously considered to be a disease of Western countries but has now become a worldwide issue. The International Diabetes Federation reported that DM affects at least 285 million people globally, and that the number is expected to reach 438 million by the year 2030, with two-thirds of all DM cases occurring in low- to middle-income countries.¹ Asia accounts for 60% of the world’s diabetic population. Asia has undergone rapid economic development, urbanization, and transitions in nutritional status in recent decades,² which have led to a marked increase in the prevalence of DM within a relatively short time.

The percentage of patients with type 2 DM who die as a direct consequence of the disease, eg because of diabetic ketoacidosis, is small; mortality of patients with type 2 DM has mainly been attributed to its associated complications: microvascular diseases such as diabetic nephropathy or macrovascular diseases such as myocardial infarction and stroke.³,⁴ Increasing evidence has shown the importance of prediabetes and family history of DM in the increasing cardiovascual (CV) risk profile of the general population.⁵ In spite of the availability of extensive treatments for hyperglycemia, the risk of CV disease in patients with type 2 DM has not been sufficiently reduced.⁶,⁷ These findings have prompted the search for other metabolic and nutritional factors that may influence the development of vascular complications in patients with type 2 DM. Some studies have shown that the concentrations of several vitamins in blood were low in patients with type 2 DM,⁸,⁹ and supplement therapy with vitamin B₁ or E has been found to be effective in preventing CV complications in patients with type 2 DM.¹⁰,¹¹ Previous studies have focused on the concentration of a specific vitamin in patients with type 2 DM; the concentrations of water-soluble vitamins in blood and urinary excretion have not yet been comprehensively examined in patients with type 2 DM. Therefore, the aim of the present study was to comprehensively compare the concentrations of water-soluble vitamins in blood and urinary excretion in patients with type 2 DM and healthy control participants.

Methods

Participants.

Patients with type 2 DM. Our study included patients with type 2 DM with significant comorbidities (renal insufficiency,
those receiving insulin therapy, those with thyroid dysfunction, or those receiving warfarin). We invited 30 patients with type 2 DM, of whom 8 patients were excluded from the study because of missing data (dietary records, 24-hour urine, or data on blood chemistries). Thus, 22 patients (13 men and 9 women) with a mean age of 58.9 years (range 36–79 years) with type 2 DM who visited the outpatient clinic of Shiga University of Medical Science Hospital were enrolled in this study. All patients received full explanations of the study and gave informed consent. This part of the study was performed from August to December 2010. The protocol of this study was approved by the institutional review board of the Shiga University of Medical Science (No 22–42, 2010), and the research was performed in accordance with the Declaration of Helsinki.

Control participants. Healthy Japanese college students, 10 males and 10 females with a mean age of 20.7 years (range 19–23 years), served as the control group for the study. Prior to the experiment, they had physical checkups, and their hemotological and blood biochemical analyses showed normal values. All participants received full explanations of the study and gave informed consent. This study was reviewed and approved by the ethical committee of the Incorporated Administrative Agency of Health and Nutrition. All participants were housed in the same facility for eight days. The daily schedule was partly restricted: the lights were turned off at 22:00 in order to promote sleep and the participants got up at 06:00. The precise experimental design is published elsewhere.14 This part of the study was carried out from March 1 to March 8, 2002, for females, and from August 27 to September 3, 2002, for males.

Blood and urine sample collection. Patients with type 2 DM. Fasting blood was collected into ethylenediaminetetraacetic acid tubes. Whole blood and plasma samples were stored at −20°C for later analysis. Twenty-four-hour urine samples before the day of the blood examination were collected at home. Urine samples were stored in ice. After the volumes of the urine samples had been measured, the collected urine samples were immediately treated as described in the “Analyses of blood and urine vitamins” section in order to avoid the destruction of water-soluble vitamins and then stored at −20°C for later analysis.

Control participants. The control participants’ 24-hour urine samples were collected from the second urinary excretion on day 7 to the first one on day 8. After the volumes of the urine samples had been measured, the collected urine samples were immediately treated as described in the “Analyses of blood and urine vitamins” section to avoid destruction of water-soluble vitamins and then stored at −20°C until needed. The blood was taken from a cubital vein at 08:30 on day 8 before breakfast, treated immediately to avoid destruction of water-soluble vitamins, and then stored at −20°C until needed.

Dietary assessment. Patients with type 2 DM. Food intake was recorded by each patient using a weighed food record method with supplemental use of photography at home. Instructions on weighing and taking photographs were given by a registered dietitian. The validation of this method has been reported elsewhere.15 The daily intakes of macro- and micronutrients by each patient were calculated using software (Excel Eiyokun version 4.5, Kenosha, Inc.) based on the fifth revised and enlarged edition of the Standard Tables for Food Composition in Japan.

Control participants. The breakfast time was 08:00–09:00, lunch 12:30–13:10, and dinner 18:30–19:00. Subjects consumed a semipurified diet based on Japanese Dietary Reference Intakes.16 The composition and amount of the semipurified diet are published elsewhere.14

Chemicals. Thiamine hydrochloride, riboflavin, pyridoxine hydrochloride, pyridoxal phosphate monohydrate, cyanocobalamin, nicotinamide, folate, and L (+)-ascorbic acid were purchased from Wako Pure Chemical Industries. 4-Pyridoxic acid (4-PIC) was synthesized by ICN Pharmaceuticals and obtained from Wako Pure Chemical Industries. N4-Methyl nicotinamide (MNA) chloride was purchased from Tokyo Chemical Industries. N4-Methyl-2-pyridone-5-carboxamide (2-Py) and N4-methyl-4-pyr- idone-3-carboxamide (4-Py) were synthesized employing the methods of Pullman and Colowick17 and Shibata et al,18 respectively. All other chemicals used were of the highest purity available from commercial sources.

Analyses of blood and urine vitamins. The concentrations of total vitamin B12 in whole blood and urine were measured by the high-performance liquid chromatography (HPLC)-postlabeled fluorescence method of Kimura et al.19 The concentration of total vitamin B12 in whole blood was determined by the HPLC–lumiflavin method of Ohkawa et al,20 with slight modifications. The concentration of vitamin B12 in urinary excretion was analyzed according to the method of Ohkawa et al.21 Pyridoxal phosphate (a coenzyme of vitamin B6) in plasma was determined using the HPLC method.22 4-PIC, a catabolite of vitamin B6, was measured in urine by the HPLC method.23 Concentrations of vitamin B12 plasma and urine were assayed by the microbiological method with Lactobacillus delbrueckii subsp. lactis ATCC 7870.24 The total nicotinamide content in whole blood was measured by the method of Shibata et al.25 The quantities of Nam, 2-Py, and 4-Py in urine were measured simultaneously by the HPLC method of Shibata et al.26 The content of MNA was measured by the method of Shibata.27 Plasma and urinary folates were determined by the microbiassay method using Lactobacillus casei ATCC 2733.28 Plasma and urine contents of reduced and oxidized ascorbic acid and 2,3-diketogluconic acid were determined by the HPLC method.29

Vitamin clearances. Vitamin clearances in milliliters per minute were calculated from concentrations of vitamins in 24-hour urinary excretion and blood.
Concentrations of water-soluble vitamins in blood and urinary excretion

Table 1. Participant characteristics.

| VARIABLE                        | MEAN ± SD | RANGE   | P    |
|---------------------------------|-----------|---------|------|
| Patients with type 2 DM (N = 22) |           |         |      |
| Age (y)                         | 58.9 ± 10.6 | 36–79  |      |
| Men (%)                         | 59.1      |         |      |
| BMI (kg/m²)                     | 25.6 ± 4.6 | 18.1–34.9 |      |
| Duration of type 2 DM (y)       | 9.2 ± 6.9  | 1–29   |      |
| Use of statins (%)              | 36.4      |         |      |
| Use of metformin (%)            | 27.3      |         |      |
| HbA1c (%)                       | 7.1 ± 1.3  | 5.8–10.6 |      |
| FBG (mg/dL)                     | 146 ± 44   | 73–263 |      |
| Creatinine (mg/dL)              | 0.84 ± 0.22 | 0.55–1.57 |      |
| eGFR (mL/min)                   | 67.1      | 33.7–120.8 |      |
| Control participants for (N = 20) |         |         |      |
| Age (y)                         | 20.7 ± 0.9  | 19–23  | <0.001 |
| Men (%)                         | 50.0      |         |      |
| BMI (kg/m²)                     | 21.4 ± 1.7  | 17.2–24.7 | <0.001 |

Notes: Characteristics of participants by group are shown. Values are shown as the mean ± SD or % (for men), and their range. The prevalence of men in the two groups $P$-values $<0.05$ were considered statistically significant.

Abbreviations: DM, diabetes mellitus; BMI, body mass index; FBG, fasting blood glucose; HbA1c, glycated hemoglobin; eGFR, estimated glomerular filtration rate.

Results

Descriptive statistics. Characteristics of patients with type 2 DM and control participants are shown in Table 1. Mean age was higher in patients than in controls. Mean BMI was larger in patients than in controls. No significant difference was observed in the percentage of men between the groups. The duration of type 2 DM, use of statins, use of metformin, mean glycated hemoglobin (HbA1c), fasting blood glucose, creatinine, and eGFR in patients are also shown in Table 1.

Macronutrient and vitamin intakes by patients with type 2 DM and controls are shown in Table 2. The recommended dietary allowance (RDA) values of the vitamins, as stated in 2010 by the Ministry of Health, are also shown in Table 2.55 The mean values of vitamins $B_6$, $B_12$, $B_9$, and $C$ were approximately the same as the RDA values. Almost half of the patients with type 2 DM had intakes of these vitamins below the RDA values. Some patients’ intakes of vitamins $B_12$ or folate were below the RDA values. Compared to controls, intakes in patients with type 2 DM of protein, fat, vitamin $B_9$, niacin, folate, and NaCl were significantly higher; those of carbohydrates, vitamin $B_6$, and vitamin $B_9$ were significantly lower. Total energy intake and intakes of vitamin $B_1$ and vitamin $C$ were not different between the two groups.

Urinary vitamin excretion in patients with type 2 DM and controls. Urinary vitamin excretion in patients with type 2 DM and controls is shown in Table 3. Mean urinary excretion of vitamin $B_12$ in patients with type 2 DM was significantly lower, and those of vitamin $C$ and of total niacin catabolites were significantly higher, compared to control participants. The other values were not different between the two groups.

Concentrations of vitamins in blood in patients with type 2 DM and controls. The concentrations of vitamins in blood in patients with type 2 DM and controls are shown in Table 4. Without adjustments (Model 0), the concentrations of vitamins $B_6$ and $B_{12}$ in blood were higher, while those of the other vitamins were lower in patients with type 2 DM than in controls. With an adjustment for age (Model 1), the differences in the concentrations of vitamin $B_1$ and $B_{12}$ in blood between the two groups became nonsignificant. With further adjustments for age, sex, BMI, eGFR, urinary excretions of...
vitamin, and dietary intake of each vitamin (Model 2), the concentrations of vitamin B<sub>12</sub>, pyridoxal phosphate (a coenzyme of vitamin B<sub>6</sub>), vitamin C, niacin, and folate in blood remained significantly lower in patients with type 2 DM than in controls. Finally, with the addition of total energy intake to Model 2, the differences in the concentrations of vitamin C in blood between the two groups became non-significant. The concentrations of vitamin B<sub>12</sub>, pyridoxal phosphate (a coenzyme of vitamin B<sub>6</sub>), niacin, and folate in blood remained significantly lower in patients with type 2 DM than in controls. Since concentrations of folate in blood were significantly lower in patients with type 2 DM than in controls, a Model 3 analysis replacing an indicator for group (metformin use = 1, 0 otherwise) in patients with type 2 DM was performed. However, no significant difference was observed in concentrations of folate in blood between patients who were on metformin and those who were not ($P = 0.183$).

Vitamin clearances in patients with type 2 DM and controls. Vitamin clearances in patients with type 2 DM and controls are shown in Table 5. Mean clearance of vitamin B<sub>12</sub> in patients with type 2 DM was significantly lower than that in controls. Mean clearances of vitamin B<sub>6</sub>, niacin, folate, and vitamin C in patients with type 2 DM were significantly higher than those in controls.

### Discussion

The main results of the present study were that among watersoluble vitamins, concentrations of vitamin B<sub>12</sub>, B<sub>6</sub>, niacin, and folate in blood were significantly lower in patients with type 2 DM than in controls, independent of age, BMI, dietary intake, eGFR, and other confounding factors. Despite reduced concentrations of these vitamins in blood, renal clearances of vitamin B<sub>12</sub>, niacin, and folate were significantly higher in patients with DM than in controls.

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**Table 2. Macronutrient and vitamin intakes by patients with type 2 DM and control participants.**

| VARIABLE                        | PATIENTS WITH TYPE 2 DM | CONTROLS | P         | VITAMIN RDA |
|---------------------------------|-------------------------|----------|-----------|-------------|
|                                | MEAN ± SD               | RANGE    | MEAN ± SD |             |
| Total energy (kcal/d)           | 1900 ± 368              | 1082–2467| 2050 ± 255| 0.137       |
| Protein (% kcal)                | 16.6 ± 1.7              | 48.3–95.1| 12.3 ± 0.1| <0.001      |
| Fat (% kcal)                    | 26.0 ± 4.0              | 17.6–33.7| 19.8 ± 0.2| <0.001      |
| Carbohydrate (% kcal)           | 54.8 ± 4.3              | 47.1–60.9| 66.1 ± 1.2| <0.001      |
| Vitamin B<sub>1</sub> (mg/1000 kcal/d) | 0.52 ± 0.09            | 0.33–0.68| 0.54 ± 0.02| 0.312       |
| Vitamin B<sub>2</sub> (mg/1000 kcal/d) | 0.71 ± 0.15            | 0.51–1.22| 0.85 ± 0.01| <0.001      |
| Vitamin B<sub>6</sub> (mg/g protein/d) | 0.022 ± 0.06          | 0.004–0.038| 0.031 ± 0.003| <0.001      |
| Vitamin B<sub>12</sub> (µg/1000 kcal/d) | 5.1 ± 2.2               | 1.1–8.6 | 2.4 ± 0.6 | <0.001      |
| Vitamin C (mg/1000 kcal/d)      | 50.7 ± 18.2             | 28.0–91.1| 49.5 ± 6.2| 0.783       |
| Niacin (mgNE/1000 kcal/d)       | 11.7 ± 6.1              | 7.2–24.1 | 5.6 ± 0.5 | <0.001      |
| Folate (µg/1000 kcal/d)         | 194.8 ± 68.0            | 120.0–409.4| 99.0 ± 12.4| <0.001      |
| NaCl (g/d)                      | 10.4 ± 2.3              | 4.0–13.6 | 3.1 ± 1.0 | <0.001      |

**Notes:** Values shown are the mean ± SD, their range, $P$-values by Student’s t-test comparing mean values between the two groups, and vitamin RDA (recommended dietary allowance). Vitamin RDA values for Japanese adults were taken from reference 31. Niacin intake was expressed as niacin equivalents (NE) where 1 mg niacin equivalent is equal to 1 mg niacin or 60 mg tryptophan. Here, it was shown as mgNE/1000 kcal/d.

**Abbreviation:** DM, diabetes mellitus.

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**Table 3. Urinary vitamin excretion in patients with type 2 DM and controls.**

| VARIABLE                        | TYPE 2 DM | RANGE      | CONTROL | RANGE     | P     |
|---------------------------------|-----------|------------|----------|-----------|-------|
| B<sub>1</sub> (nmol/d)          | 449 ± 327 | 119–1259   | 483.5 ± 176.0 | 199–790 | 0.706 |
| B<sub>6</sub> (nmol/d)          | 1098 ± 2232 | 56–10724 | 571 ± 257 | 155–1208 | 0.283 |
| 4-PIC<sup>1</sup> (µmol/d)      | 16.7 ± 55.2 | 2.2–263.3 | 3.0 ± 0.6 | 2.1–4.42 | 0.259 |
| B<sub>12</sub> (pmol/d)         | 80.3 ± 42.5 | 13.6–73.5 | 119.0 ± 47.8 | 68.6–252.0 | 0.008 |
| C (µmol/d)                      | 406.7 ± 398.3 | 4.4–1757 | 144.1 ± 49.6 | 86.9–257 | 0.006 |
| Sum of niacin catabolites<sup>2</sup> (µmol/d) | 42.2 ± 17.0 | 13.6–73.5 | 28.7 ± 9.4 | 13.6–51.0 | 0.003 |
| Folate (nmol/d)                 | 24.9 ± 29.5 | 2.7–145.7 | 21.1 ± 3.1 | 14.7–29.0 | 0.571 |

**Notes:** <sup>1</sup>A catabolite of vitamin B<sub>6</sub>. <sup>2</sup>Sum of MNA, 2-Py, and 4-Py, which are the major catabolites of niacin. Urinary vitamin excretions in patients with type 2 DM and controls are shown as the mean ± SD with their ranges. $P$-values are by Student’s t-test comparing mean values between the two groups. B<sub>1</sub> to C denote vitamin B<sub>12</sub> to vitamin C.

**Abbreviation:** DM, diabetes mellitus.
Table 4. Concentrations of vitamins in blood in patients with DM and controls.

| VARIABLE                        | MEAN ± SD       | RANGE           | DIFFERENCE | P     | R²   |
|---------------------------------|-----------------|-----------------|------------|-------|------|
| B_{12} (pmol/mL)                |                 |                 |            |       |      |
| DM                             | 100.7 ± 24.2    | 57.2–156.3      |            |       |      |
| Control                        | 86.1 ± 18.7     | 33.8–109.1      |            |       |      |
| Model 0                        | 14.6            | 0.036           | 0.11       |       |      |
| Model 1                        | 35.2            | 0.059           | 0.14       |       |      |
| Model 2                        | 5.0             | 0.84            | 0.33       |       |      |
| Model 3                        | −10.0           | 0.69            | 0.41       |       |      |
| B_{12} (pmol/mL)               |                 |                 |            |       |      |
| DM                             | 184.1 ± 55.0    | 118.3–338.9     |            |       |      |
| Control                        | 214.7 ± 22.8    | 175–258         |            |       |      |
| Model 0                        | −30.5           | 0.027           | 0.12       |       |      |
| Model 1                        | −99.6           | 0.006           | 0.21       |       |      |
| Model 2                        | −123.6          | 0.003           | 0.49       |       |      |
| Model 3                        | −127.9          | 0.006           | 0.49       |       |      |
| Pyridoxal phosphate (a coenzyme of vitamin B_{12}) (pmol/mL) | | | | | |
| DM                             | 58.2 ± 30.5     | 10.5–118.3      |            |       |      |
| Control                        | 10.5–118.3      | 52.7–113.3      |            |       |      |
| Model 0                        | −20.4           | 0.01            | 0.16       |       |      |
| Model 1                        | −67.6           | 0.002           | 0.28       |       |      |
| Model 2                        | −60.3           | 0.014           | 0.58       |       |      |
| Model 3                        | −66.8           | 0.016           | 0.58       |       |      |
| B_{12} * (pmol/mL)             |                 |                 |            |       |      |
| DM                             | 1.26 (0.86, 1.33)| 0.61–3.30      |            |       |      |
| Control                        | 0.50 (0.34, 0.67)| 0.26–0.92      |            |       |      |
| logB_{12}                      |                 |                 |            |       |      |
| DM                             | 0.11 ± 0.46     | −1.38           |            |       |      |
| Control                        | −0.77 ± 0.41    | −1.27           |            |       |      |
| Model 0                        | 0.88            | <0.001          | 0.51       |       |      |
| Model 1                        | −0.11           | 0.755           | 0.61       |       |      |
| Model 2                        | 0.16            | 0.701           | 0.71       |       |      |
| Model 3                        | 0.35            | 0.398           | 0.74       |       |      |
| C (nmol/mL)                    |                 |                 |            |       |      |
| DM                             | 31.5 ± 11.6     | 6.0–58.0        |            |       |      |
| Control                        | 64.5 ± 12.5     | 47–100          |            |       |      |
| Model 0                        | −32.9           | <0.001          | 0.66       |       |      |
| Model 1                        | −33.1           | 0.003           | 0.66       |       |      |
| Model 2                        | −37.3           | 0.009           | 0.69       |       |      |
| Model 3                        | −27.3           | 0.103           | 0.70       |       |      |
| Niacin (nmol/mL)               |                 |                 |            |       |      |
| DM                             | 33.1 ± 14.8     | 0–78.2          |            |       |      |
| Control                        | 60.5 ± 5.6      | 52.8–75.4       |            |       |      |
| Model 0                        | −27.4           | <0.001          | 0.60       |       |      |
| Model 1                        | −55.7           | <0.001          | 0.70       |       |      |
| Model 2                        | −63.0           | <0.001          | 0.73       |       |      |
| Model 3                        | −62.6           | <0.001          | 0.76       |       |      |
Table 4. (Continued)

| VARIABLE | MEAN ± SD | RANGE | DIFFERENCE | P   | R²  |
|----------|-----------|-------|------------|-----|-----|
| Folate (pmol/mL) |          |       |            |     |     |
| DM       | 11.6 ± 5.1 | 3.7–24.0 | −11.9       | <0.001 | 0.39 |
| Controls | 23.5 ± 9.6  | 10.7–51.6  | −24.3       | <0.001 | 0.48 |
| Model 0  |           |         | −24.6       | <0.001 | 0.62 |
| Model 1  |           |         | −24.9       | <0.001 | 0.62 |
| Model 2  |           |         |             |       |     |
| Model 3  |           |         |             |       |     |

Notes: Concentrations of vitamin in blood in patients with type 2 DM and controls are shown as the mean ± SD with their ranges. *Vitamin B₆ concentrations were shown as median (25th, 75th percentile). R², a goodness-of-fit measure. Coefficients for multiple linear regression models were used to examine the differences in concentration of vitamin in blood between the type 2 DM and control groups. Because the distribution of concentration of vitamin B₆ in blood was positively skewed, a logarithmic transformation was used to normalize the distribution. B₁ to C denotes vitamin B₁ to vitamin C. Variables included (P-values by linear regression analyses) are as follows: Model 0, none (crude difference in patients and controls, patient—control). Model 1, age; Model 2, Model 1 variables + sex, BMI, eGFR, urinary excretion, and dietary intake of each vitamin. Model 3, Model 2 variables + total dietary energy intake.

The concentrations of several vitamins in blood in patients with DM have been reported to be lower than normal.²⁻³⁰ The lower concentrations of some vitamins in blood were attributed to enhanced renal clearances of these vitamins, possibly due to impaired reabsorption processes in patients with DM. Thornalley et al.¹⁸ reported that the renal clearance of vitamin B₁ was 16-fold higher in patients with type 2 DM, and concentrations of vitamin B₁ in plasma correlated inversely with the renal clearance of vitamin B₁. Larkin et al.¹⁶ suggested that glucose-induced decreased expression of thiamine transporters in the tubular epithelium might mediate renal mishandling of thiamine in diabetes. However, Fukui et al.¹⁷ did not note any significant differences in the renal clearance of vitamin B₁ between patients with type 2 DM and normal controls. Our results in the present study showed that the concentrations as well as renal clearances of vitamin B₁ in blood in patients with type 2 DM were not significantly different from those in controls. However, mechanisms similar to those postulated by Thornalley et al.¹⁸ may be affecting reabsorption of other water-soluble vitamins in DM. Shibata reported that renal clearances of vitamin E and most water-soluble vitamins were higher in streptozotocin-induced diabetic rats than in controls, despite no higher concentrations of these vitamins in blood.³⁸ Although the concentration of vitamin C in blood in patients with type 2 DM became statistically nonsignificant compared to controls after an addition of total dietary energy intake in the final model, the possibility of over adjustment in analysis cannot be ruled out.

In addition to a higher clearance of niacin in patients with type 2 DM in the present study, a lower concentration of niacin in blood was also found; it is noteworthy that a previous study found the conversion rate of tryptophan to niacin markedly lower in diabetic rats induced by streptozotocin than in control rats.³⁹

Reductions in the concentrations of several vitamins in blood can be related to an increased prevalence of CV complications in patients with type 2 DM. Previous clinical studies and trials examined the benefits of vitamin supplemental therapy in DM for prevention of CV and/or other complications. The combination of vitamin B₁ with B₂, but not alone, has been shown to decrease DNA glycation in leukocytes of DM patients.⁴⁰ A six-month supplementation trial with a combination of vitamins B₆, B₁₂, and folate showed a decrease in retinal edema and an increase in light sensitivity in diabetic patients with nonproliferative retinopathy.⁴¹ Concentrations of vitamin C in plasma have been inversely correlated with HbA₁c and fasting and postprandial blood glucose and oxidative stress, but not to lipid profiles.⁴²,⁴³ Diabetes has also been associated with periodontal disease, and vitamin C supplementation together with dental treatment has been shown to improve chronic periodontitis in newly diagnosed type 2 diabetic patients.⁴⁴ A three-month supplementation of vitamins C and E decreased hypertension and blood glucose while increasing superoxide dismutase and glutathione levels.⁴⁵ Vittone et al examined the effects of niacin plus simvastatin on the progression of coronary stenosis in patients with metabolic syndrome as a subgroup analysis of the HDL-Atherosclerosis Treatment Study and found that the treatment with niacin plus simvastatin reduced changes

Table 5. Vitamin clearances (mL/min) in DM and control.

| VARIABLE | DM  | CONTROL | P   |
|----------|-----|---------|-----|
| B₁ clearance | 3.31 ± 2.63 | 4.04 ± 1.55 | 0.336 |
| B₂ clearance | 3.48 ± 5.49 | 1.87 ± 0.86 | 0.189 |
| B₃₂₆ clearance | 69.3 ± 45.2 | 27.5 ± 7.0 | <0.001 |
| B₁₂ clearance | 0.056 ± 0.045 | 0.179 ± 0.065 | <0.001 |
| Niacin clearance | 0.95 ± 0.45 | 0.33 ± 0.11 | <0.001 |
| Folate clearance | 1.63 ± 1.96 | 0.70 ± 0.23 | 0.038 |
| C clearance | 10.20 ± 11.52 | 1.60 ± 0.59 | 0.002 |

Notes: Vitamin clearances (mL/min) in patients with type 2 DM and controls are shown as mean ± SD. P-values are by Student’s t-Test comparing mean values between the two.
in the mean proximal percent stenosis more than a placebo in participants with metabolic syndrome and in a more insulin-resistant group of participants. They also found that overall primary clinical events were 60% lower with niacin plus simvastatin than with a placebo.46 Smulder et al47 reported that homocysteine concentrations in type 2 DM were increased, even with modest deterioration of renal function or when the vitamin status was in the low to low-normal range, eg 20 pmol/mL for folate; higher than the mean concentration of folate in blood in patients with type 2 DM in the present study. They also showed that fasting homocysteine correlated with macrovascular diseases. Folate supplementation has been shown to lower homocysteine concentrations and may also improve endothelial function in patients with coronary artery disease.48 Sudchada et al49 performed a meta-analysis on the effects of folate supplementation in patients with type 2 DM. They screened 4 studies with 183 patients. Folate supplementation exerted significant effects on homocysteine levels. Although its effects on HbA1c levels were not significant, folate supplementation led to slightly better glycemic control than with a placebo.49

A slight reduction in the intakes of these vitamins and increases in their renal clearances in patients with type 2 DM in the present study may have caused significantly lower concentrations of vitamin B₆, B₁₂, niacin, and folate in blood in patients with type 2 DM than in controls independent of confounding factors. These reductions in the concentrations of some vitamins in blood in patients with type 2 DM may be of clinical relevance, such as the future development of macrovascular diseases.

The strengths of the present study were: (1) the comprehensive measurement of blood, urine, and dietary intakes of water-soluble vitamins in patients with type 2 DM and healthy controls; and (2) the standardized collection of samples and high-quality laboratory measurements. The study was limited by its cross-sectional design; its results must be interpreted cautiously with regard to cause–effect relationships. The age ranges of patients and control participants differed and may have affected the results of statistical analyses. However, R², a goodness-of-fit measure, for most of the concentrations of vitamins in blood markedly increased from Model 0 to Model 3, implying the appropriateness of the statistical methods. We did not collect data on urinary creatinine or NaCl in patients with type 2 DM or controls or blood creatinine in controls. We had to use eGFR in patients and 100 mL/min in controls. The number of patients and controls (22 patients and 20 controls) may appear somewhat small. However, by observing appropriate behavior of R² in the analyses and by viewing previous studies in this field, we think the number of participants was sufficient.

In conclusion, concentrations of vitamin B₆, B₁₂, niacin, and folate in blood were significantly lower in patients with type 2 DM than in controls, independent of age and other confounding factors. These reductions in the concentrations of these vitamins in blood in patients with type 2 DM may be of clinical relevance in areas such as the future development of macrovascular diseases. Thus, changes in the management of patients with type 2 DM, including dietary adjustments and/or vitamin supplementation, may be warranted.

Author Contributions
Conceived and designed the experiments: HI, ToF, and KS. Analyzed the data: HI, YN, and TsF. Wrote the first draft of the manuscript: HI and YN. Contributed to the writing of the manuscript: HM, YD, and KS. Agreed with manuscript results and conclusions: HI, YN, ToF, TsF, SU, HM, YD, and KS. Jointly developed the structure and arguments for the paper: HI, YN, YD, and KS. Made critical revisions and approved final version: ToF, TsF, SU, and HM. All the authors reviewed and approved the final manuscript.

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