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

Hiromi Iwakawa[1], Yasuyuki Nakamura[1], Tomiho Fukui[2], Tsutomu Fukuwatari[3], Satoshi Ugi[4], Hiroshi Maegawa[4], Yukio Doi[1] and Katsumi Shibata[3]

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

CITATION: Iwakawa et al. Concentrations of Water-Soluble Vitamins in Blood and Urinary Excretion in Patients with Diabetes Mellitus. *Nutrition and Metabolic Insights* 2016:9 85–92 doi:10.4137/NMI.S40595.

TYPE: Original Research

RECEIVED: July 22, 2016. **RESUBMITTED:** September 29, 2016. **ACCEPTED FOR PUBLICATION:** October 2, 2016.

ACADEMIC EDITOR: Joseph Zhou, Editor in Chief

PEER REVIEW: Four peer reviewers contributed to the peer review report. Reviewers' reports totaled 665 words, excluding any confidential comments to the academic editor.

FUNDING: This investigation was supported by The University of Shiga Prefecture Grant for Special Research. The authors confirm that the funder had no influence over the study design, content of the article, or selection of this journal.

COMPETING INTERESTS: Authors disclose no potential conflicts of interest.

COPYRIGHT: © the authors, publisher and licensee Libertas Academica Limited. This is an open-access article distributed under the terms of the Creative Commons CC-BY-NC 3.0 License.

CORRESPONDENCE: iwakawa@agr.ryukoku.ac.jp

Paper subject to independent expert single-blind peer review. All editorial decisions made by independent academic editor. Upon submission manuscript was subject to anti-plagiarism scanning. Prior to publication all authors have given signed confirmation of agreement to article publication and compliance with all applicable ethical and legal requirements, including the accuracy of author and contributor information, disclosure of competing interests and funding sources, compliance with ethical requirements relating to human and animal study participants, and compliance with any copyright requirements of third parties. This journal is a member of the Committee on Publication Ethics (COPE).

Published by Libertas Academica. Learn more about this journal.

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 cardiovascular (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 B_1 or E has been found to be effective in preventing CV complications in patients with type 2 DM.[10–13] 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 hematological 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 dietician. 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. N^1-Methylnicotinamide (MNA) chloride was purchased from Tokyo Chemical Industries. N^1-Methyl-2-pyridone-5-carboxamide (2-Py) and N^1-methyl-4-pyridone-3-carboxamide (4-Py) were synthesized employing the methods of Pullman and Colowick[17] 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 B_1 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 B_2 in whole blood was determined by the HPLC–lumiflavin method of Ohkawa et al,[20] with slight modifications. The concentration of vitamin B_2 in urinary excretion was analyzed according to the method of Ohkawa et al.[21] Pyridoxal phosphate (a coenzyme of vitamin B_6) in plasma was determined using the HPLC method.[22] 4-PIC, a catabolite of vitamin B_6, was measured in urine by the HPLC method.[23] Concentrations of vitamin B_{12} in 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 microbioassay 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.

Estimated glomerular filtration rate. Body surface area (BSA) was calculated using the following formula:

$$BSA = 0.007184 \times \text{body weight (kg)}^{0.425} \times \text{height (cm)}^{0.725}.$$[30]

Estimated glomerular filtration rate (eGFR) in patients with type 2 DM was calculated using the following equations:

$$eGFR\ (mL/min) = 194 \times (BSA/1.73) \times Cr^{-1.094} \times age^{-0.287} \quad \text{(for men)}$$

$$eGFR\ (mL/min) = 194 \times (BSA/1.73) \times Cr^{-1.094} \times age^{-0.287} \times 0.739 \quad \text{(for women)}$$[31]

Because concentrations of creatinine in serum in control participants were not available, eGFR was estimated at 100 mL/min.

Statistical analysis. SAS version 9.4 for Windows (SAS Institute) was used. The chi-squared test was used to compare dichotomous variables between patients with type 2 DM and controls. Student's *t*-test was used to determine whether the mean values of the two groups were statistically different from each other. Coefficients for multiple linear regression models were used to examine the differences in concentrations of vitamins in blood between the type 2 DM and control groups.[32,33] Because the distribution of concentration of vitamin B_{12} in blood was positively skewed, a logarithmic transformation was used to normalize the distribution. Model 0 = crude difference in *P*-values of patients and controls (patient—control) by linear regression analyses. Model 1 = age-adjusted difference. Model 2 included Model 2 variables + sex (male = 1, female = 0), body mass index (BMI), eGFR, urinary excretion, and dietary intake of each vitamin. Finally, Model 3 included Model 2 variables + total dietary energy intake. Since metformin, a widely used oral antidiabetic drug, is known to decrease concentrations of vitamin B_{12} and folate in blood,[34] the Model 3 analysis as described above, but replacing an indicator for group (0, metformin non use = 1 otherwise) in patients with type 2 DM, was planned in case the concentrations of vitamin B_{12} and/or folate in blood were lower in patients than in controls. *P*-values <0.05 were considered statistically significant.

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.[35] The mean values of vitamins B_1, B_2, B_6, 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_{12}, niacin, folate, and NaCl were significantly higher; those of carbohydrates, vitamin B_2, and vitamin B_6 were significantly lower. Total energy intake and intakes of vitamin B_1 and vitamin C were not different between the two groups.

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/m^2)	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/m^2)	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.

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_1 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

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_1 (mg/1000 kcal/d)	0.52 ± 0.09	0.33–0.68	0.54 ± 0.02	0.312	0.54
Vitamin B_2 (mg/1000 kcal/d)	0.71 ± 0.15	0.51–1.22	0.85 ± 0.01	<0.001	0.60
Vitamin B_6 (mg/g protein/d)	0.022 ± 0.06	0.014–0.038	0.031 ± 0.003	<0.001	0.023
Vitamin B_{12} (µg/1000 kcal/d)	5.1 ± 2.2	1.1–8.6	2.4 ± 0.6	<0.001	1.2
Vitamin C (mg/1000 kcal/d)	50.7 ± 18.2	28.0–91.1	49.5 ± 6.2	0.783	48.8
Niacin (mgNE/1000 kcal/d)	11.7 ± 6.1	7.2–24.1	5.6 ± 0.5	<0.001	5.8
Folate (µg/1000 kcal/d)	194.8 ± 68.0	120.0–409.4	99.0 ± 12.4	<0.001	117
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 type 2 DM and control 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.

vitamin, and dietary intake of each vitamin (Model 2), the concentrations of vitamin B_2, pyridoxal phosphate (a coenzyme of vitamin B_6), 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_2, pyridoxal phosphate (a coenzyme of vitamin B_6), 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_{12} in patients with type 2 DM was significantly lower than that in controls. Mean clearances of vitamin B_6, 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 water-soluble vitamins, concentrations of vitamin B_2, B_6, 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_6, niacin, and folate were significantly higher in patients with DM than in controls.

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

VARIABLE	TYPE 2 DM	RANGE	CONTROL	RANGE	*P*
B_1 (nmol/d)	449 ± 327	119–1259	483.5 ± 176.0	199–790	0.706
B_2 (nmol/d)	1098 ± 2232	56–10724	571 ± 257	155–1208	0.283
4-PIC[1] (µmol/d)	16.7 ± 55.2	2.2–263.3	3.0 ± 0.6	2.1–4.42	0.259
B_{12} (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[2] (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: [1]A catabolite of vitamin B_6. [2]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_1 to C denote vitamin B_1 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*	R^2
B_1 (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_2 (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_6) (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

(*Continued*)

Table 4. (*Continued*)

VARIABLE	MEAN ± SD	RANGE	DIFFERENCE	*P*	R^2
Folate (pmol/mL)					
DM	11.6 ± 5.1	3.7–24.0			
Controls	23.5 ± 9.6	10.7–51.6			
Model 0			−11.9	<0.001	0.39
Model 1			−24.3	<0.001	0.46
Model 2			−24.6	<0.001	0.62
Model 3			−24.9	<0.001	0.62

Notes: Concentrations of vitamin in blood in patients with type 2 DM and controls are shown as the mean ± SD with their ranges. *Vitamin B_{12} concentrations were shown as median (25th, 75th percentile). R^2, 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 B_{12} 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.[8,9] 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[8] reported that the renal clearance of vitamin B_1 was 16-fold higher in patients with type 2 DM, and concentrations of vitamin B_1 in plasma correlated inversely with the renal clearance of vitamin B_1. Larkin et al[36] 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[37] did not note any significant differences in the renal clearance of vitamin B_1 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 B_1 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[8] 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.

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

VARIABLE	DM	CONTROL	*P*
B_1 clearance	3.31 ± 2.63	4.04 ± 1.55	0.336
B_2 clearance	3.48 ± 5.49	1.87 ± 0.86	0.189
B_6 clearance	69.3 ± 45.2	27.5 ± 7.0	<0.001
B_{12} 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 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 B_1 with B_6, 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 B_6, B_{12}, 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

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 al[47] 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 al[49] 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 B_2, B_6, 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, R^2, 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 R^2 in the analyses and by viewing previous studies in this field, we think the number of participants was sufficient.

In conclusion, concentrations of vitamin B_2, B_6, 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.

REFERENCES

1. IDF Diabetes Atlas 2013. Epidemiology and Morbidity. In: International Diabetes Federation. Available from http://www.idf.org/.
2. Chan JC, Malik V, Jia W, et al. Diabetes in Asia: epidemiology, risk factors, and pathophysiology. *JAMA*. 2009;301:2129–2140.
3. Donnelly R, Emslie-Smith AM, Gardner ID, Morris AD. ABC of arterial and venous disease: vascular complications of diabetes. *BMJ*. 2000;320:1062–1066.
4. American Diabetes Association. Standards of medical care in diabetes. *Diabetes Care*. 2007;30:S4–S41.
5. Ciccone MM, Scicchitano P, Cameli M, et al. Endothelial function in prediabetes, diabetes and diabetic cardiomyopathy: a review. *J Diabetes Metab*. 2014;5:4.
6. The Diabetes Control and Complications Trial Research Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *New Engl J Med*. 1993;327:977–986.
7. Stratton IM, Adler AI, Neil HA, et al. Association of glycaemic with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study. *BMJ*. 2002;321:405–412.
8. Thornalley PJ, Babaei-Jadidi R, Al Ali H, et al. High prevalence of low plasma thiamine concentration in diabetes linked to a marker of vascular disease. *Diabetologia*. 2007;50:2164–2170.
9. Ahn HJ, Min KW, Cho YO. Assessment of vitamin B6 status in Korean patients with newly diagnosed type 2 diabetes. *Nutr Res Pract*. 2011;5:34–39.
10. Rabbani N, Alam SS, Riaz S, et al. High-dose thiamine therapy for patients with type 2 diabetes and microalbuminuria: a randomised, double-blind placebo-controlled pilot study. *Diabetologia*. 2009;52:208–212.
11. Blum S, Vardi M, Brown J, et al. Vitamin E reduces cardiovascular disease in individuals with diabetes mellitus and haptoglobin 2–2 genotype. *Pharmacogenomics*. 2010;11:675–684.
12. Asleh R, Blum S, Kalet-Litman S, et al. Correction of HDL dysfunction in individuals with diabetes and the haptoglobin 2–2 genotype. *Diabetes*. 2008;57:2794–2800.
13. Suksomboon N, Poolsup N, Sinprasert S. Effects of vitamin E supplementation on glycaemic control in type 2 diabetes: systematic review of randomized controlled trials. *J Clin Pharm Ther*. 2011;6:53–63.
14. Shibata K, Fukuwatari T, Ohta M, et al. Values of water-soluble vitamins in blood and urine of Japanese young men and women consuming a semi-purified diet based on the Japanese Dietary Reference Intakes. *J Nutr Sci Vitaminol (Tokyo)*. 2005;51:319–328.
15. Wang DH, Kogashiwa M, Ohta S, Kira S. Validity and reliability of a dietary assessment method: the application of a digital camera with a mobile phone card attachment. *J Nutr Sci Vitaminol*. 2002;48:498–504.
16. Ministry of Health, Labor and Welfare. *Recommended Dietary Allowances in the Japanese Population*. 6th ed. Tokyo: Dietary Reference Intakes; 1999:112–114.
17. Pullman ME, Colowick SP. Preparation of 2- and 6-pyridones of N1-methylnicotinamide. *J Biol Chem*. 1954;206:121–127.
18. Shibata K, Kawada T, Iwai K. Simultaneous micro-determination of nicotinamide and its major metabolites, N1-methyl-2-pyridone-5-carboxamide and N1-methyl-4-pyridone-3-carboxamide, by high-performance liquid chromatography. *J Chromatogr*. 1988;424:23–28.

19. Kimura M, Fujita T, Itokawa Y. Liquid-chromatographic determination of the total thiamin content of blood. *Clin Chem*. 1982;28:29–31.
20. Ohkawa H, Ohishi N, Yagi K. A simple method for micro-determination of flavin in human serum and whole blood by high-performance liquid chromatography. *Biochem Int*. 1982;4:187–194.
21. Ohkawa H, Ohishi N, Yagi K. New metabolites of riboflavin appear in human urine. *J Biol Chem*. 1983;258:5623–5628.
22. Rybak ME, Pfeiffer CM. Clinical analysis of vitamin B6: determination of pyridoxal 5′-phosphate and 4-pyridoxic acid in human serum by reversed-phase high-performance liquid chromatography with chlorite postcolumn derivatization. *Anal Biochem*. 2004;33:336–344.
23. Gregpry JF III, Kirk JR. Determination of urinary 4-pyridovic acid using high performance liquid chromatography. *Am J Clin Nutr*. 1979;32:879–883.
24. Watanabe F, Abe K, Katsura H, et al. Biological activity of hydroxo-vitamin B12 degradation product formed during microwave heating. *J Agric Food Chem*. 1998; 46:5177–5180.
25. Shibata K, Kawada T, Iwai K. High-performance liquid chromatographic determination of nicotinamide in rat tissue samples and blood after extraction with diethyl ether. *J Chromatogr*. 1987;422:257–262.
26. Hiratsuka C, Fukuwatari T, Shibata K. Fate of dietary tryptophan in young Japanese women. *Int J Tryptophan Res*. 2012;5:33–47.
27. Shibata K. Ultramicro-determination of N1-methylnicotinamide in urine by high performance liquid chromatography. *Vitamins*. 1987;61:599–604. (in Japanese).
28. Aiso K, Tamura T. Trienzyme treatment for food folate analysis: optimal pH and incubation time for alpha-amylase and protease treatment. *J Nutr Sci Vitaminol (Tokyo)*. 1998;44:361–370.
29. Kishida K, Nishimoto Y, Kojo S. Specific determination of ascorbic acid with chemical derivatization and high-performance liquid chromatography. *Anal Chem*. 1992;64:1505–1507.
30. DuBois D, DuBois EF. A formula to estimate the approximate surface area if height and weight be known. *Arch Intern Medicine*. 1916;17:863–871.
31. Matsuo S, Imai E, Horio M, et al. Revised equations for estimated GFR from serum creatinine in Japan. *Am J Kidney Dis*. 2009;53:982–992.
32. Zhao L, Stamler J, Yan LL, et al. Blood pressure differences between northern and southern Chinese: role of dietary factors: the International Study on Macronutrients and Blood Pressure. *Hypertension*. 2004;43:1332–1337.
33. Nakamura Y, Ueshima H, Okuda N, et al. Relation of dietary and other lifestyle traits to difference in serum adiponectin concentration of Japanese in Japan and Hawaii: the INTERLIPID Study. *Am J Clin Nutr*. 2008;88:424–430.
34. Sahin M, Tutuncu NB, Ertugrul D, Tanaci N, Guvener ND. Effects of metformin or rosiglitazone on serum concentrations of homocysteine, folate, and vitamin B12 in patients with type 2 diabetes mellitus. *J Diabetes Complications*. 2007; 21:118–123.
35. Dietary Reference Intakes for Japanese 2010. Available at; http://www.mhlw.go.jp/shingi/2009/05/s0529–4.html
36. Larkin JR, Zhang F, Godfrey L, et al. Glucose-induced down regulation of thiamine transporters in the kidney proximal tubular epithelium produces thiamine insufficiency in diabetes. *PLoS One*. 2012;7:e53175.
37. Fukui T, Imai E, Fukuwatari T, et al. Vitamin intakes and their urinary excretion in diabetes mellitus. *J Metabol Clin Nutr*. 2010;13:123–131. (in Japanese).
38. Shibata K. Kinetics of vitamins in streptozotocin induced diabetic rats (in Japanese). 2009. Available at: http://www.shc.usp.ac.jp/shibata/H20-II-10.pdf
39. Shibata K, Ishikawa A, Kondo T. Effects of dietary pyrazinamide on the metabolism of tryptophan to niacin in streptozotocin-diabetic rats. *Biosci Biotechnol Biochem*. 1997;61:1679–1683.
40. Polizzi FC, Andican G, Çetin E, Civelek S, Yumuk V, Burçak G. Increased DNA-glycation in type 2 diabetic patients: the effect of thiamine and pyridoxine therapy. *Exp Clin Endocrinol Diabetes*. 2012;120:329–334.
41. Smolek MK, Notaroberto NF, Jaramillo AG, Pradillo LR. Intervention with vitamins in patients with nonproliferative diabetic retinopathy: a pilot study. *Clin Ophthalmol*. 2013;7:1451–1458.
42. Carter P, Gray LJ, Talbot D, Morris DH, Khunti K, Davies MJ. Fruit and vegetable intake and the association with glucose parameters: a cross-sectional analysis of the Let's Prevent Diabetes Study. *Eur J Clin Nutr*. 2013;67:12–17.
43. Mazloom Z, Hejazi N, Dabbaghmanesh MH, Tabatabaei HR, Ahmadi A, Ansar H. Effect of vitamin C supplementation on postprandial oxidative stress and lipid profile in type 2 diabetic patients. *Pak J Biol Sci*. 2011;14:900–904.
44. Gokhale NH, Acharya AB, Patil VS, Trivedi DJ, Thakur SL. A short-term evaluation of the relationship between plasma ascorbic acid levels and periodontal disease in systemically healthy and type 2 diabetes mellitus subjects. *J Diet Suppl*. 2013;10:93–104.
45. Rafighi Z, Shiva A, Arab S, Mohd Yousof R. Association of dietary vitamin C and E intake and antioxidant enzymes in type 2 diabetes mellitus patients. *Glob J Health Sci*. 2013;5:183–187.
46. Vittone F, Chait A, Morse JS, Fish B, Brown BG, Zhao XQ. Niacin plus simvastatin reduces coronary stenosis progression among patients with metabolic syndrome despite a modest increase in insulin resistance: a subgroup analysis of the HDL-atherosclerosis treatment study (HATS). *J Clin Lipidol*. 2007;1:203–210.
47. Smulders YM, Rakic M, Slaats EH, et al. Fasting and post-methionine homocysteine levels in NIDDM. Determinants and correlations with retinopathy, albuminuria, and cardiovascular disease. *Diabetes Care*. 1999;22:125–132.
48. Thambyrajah J, Landray MJ, Jones HJ, McGlynn FJ, Wheeler DC, Townend JN. A randomized double-blind placebo controlled trial of the effect of homocysteine-lowering therapy with folic acid on endothelial function in patients with coronary artery disease. *J Am Coll Cardiol*. 2001;37:1858–1863.
49. Sudchada P, Saokaew S, Sridetch S, Incampa S, Jaiyen S, Khaithong W. Effect of folic acid supplementation on plasma total homocysteine levels and glycemic control in patients with type 2 diabetes: a systematic review and meta-analysis. *Diabetes Res Clin Pract*. 2012;98:151–158.