Research Article

Daily intake of thiamine correlates with the circulating level of endothelial progenitor cells and the endothelial function in patients with type II diabetes

Ching-Yuen Wong[1], Jianati Qiuwaxi[1], Hua Chen[1], Sheung-Wai Li[2], Hiu-Ting Chan[1], Sidney Tam[3], Xiao-Ou Shu[4], Chu-Pak Lau[1], Yok-Lam Kwong[5] and Hung-Fat Tse[1,6]

[1] Cardiology Division, Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong, China
[2] Department of Medicine, Tung Wah Hospital, Queen Mary Hospital, Hong Kong, China
[3] Department of Clinical Biochemistry Unit, Queen Mary Hospital, Hong Kong, China
[4] Department of Medicine, Vanderbilt Epidemiology Center and Vanderbilt-Ingram Cancer Center, Vanderbilt University School of Medicine, Nashville, USA
[5] Division of Hematology, Department of Medicine, the University of Hong Kong, Queen Mary Hospital, Hong Kong, China
[6] Research Center of Heart, Brain, Hormone and Healthy Aging, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong, China

Our objective was to determine the relationships between levels of different dietary nutrients intake with circulating endothelial progenitor cells (EPC) and vascular endothelial function in type II diabetic patients. We studied the daily dietary nutrients intake, the numbers of circulating $CD34^+/KDR^+$ EPC and $CD133^+/KDR^+$ EPC and brachial artery flow-mediated dilation (FMD) in 88 diabetic patients without prior cardiovascular diseases and 91 sex- and age-matched controls. Compared with controls, diabetic patients had lower $CD133^+/KDR^+$ EPC count (48.3 ± 5.2 *vs*. 84.6 ± 7.6/μL, $p < 0.001$), $CD34^+/KDR^+$ EPC count (311 ± 41 *vs*. 412 ± 36/μL, $p = 0.045$), and FMD ($2.54 \pm 0.37\%$ *vs*. $5.46 \pm 0.47\%$, $p < 0.001$). After adjusted for age, sex, smoking history, body weight, hemoglobin A1c level, total calorie intake, other dietary vitamin intake, use of antihypertensives, and lipid lowering agents, a higher intake of thiamine was significantly associated with a higher level of circulating $CD34^+/KDR^+$ EPC ($\beta = 0.49$, $p = 0.028$) and $CD133^+/KDR^+$ EPC ($\beta = 0.45$, $p = 0.037$) in diabetic patients, but not in controls. Furthermore, an increased intake of thiamine from 1st to 4th quartile in diabetic patients independently predicted an absolute increase in FMD by 1.29% ($p = 0.026$, relative increase = 63.5%). This study demonstrated that daily thiamine intake was positively correlated with the circulating number of EPCs and FMD in patients with type II diabetes, independent of other dietary nutrients intake.

Keywords: Diabetes / Endothelial function / Endothelial progenitor cells / Thiamine

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1 Introduction

Experimental studies suggested that circulating endothelial progenitor cells (EPC) play an important role in postnatal vasculogenesis and re-endothelialization during physiological and pathological conditions [1, 2]. Furthermore, clinical studies demonstrated that the number of circulating EPC correlates with vascular endothelial function in subjects with cardiovascular risk factors [3]. Although the mechanism remains unclear, recent clinical studies have demonstrated that the function and number of circulating EPC are reduced in patients with diabetes [4, 5], and might contribute to the occurrence of macro- and microvascular complications [6]. Currently, there is no information regarding the relationships between the levels of different dietary nutrients intake with the circulating level of EPC and vascu-

Correspondence: Professor Hung-Fat Tse, Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong, China
E-mail: hftse@hkucc.hku.hk
Fax: +852-2818-6304

Abbreviations: EPC, endothelial progenitor cells; **FMD,** flow-mediated dilation

lar endothelial function in patients with type II diabetes. Therefore, the aim of this study was to investigate the relationships between the level of different dietary nutrients intake and the circulating level of EPC in patients with type II diabetes and healthy controls.

2 Materials and methods

2.1 Patients and settings

The study population consisted of consecutive patients with type II diabetes and without a history of acute cardiovascular events recruited from our general outpatient clinic. All patients were diagnosed to have type II diabetes mellitus as defined by WHO criteria [7], and had received treatment with a hypoglycemic drug (oral antidiabetic agents or insulin). Exclusion criteria included poorly control diabetes with hemoglobin A1c (HbA1c) =11%, pregnancy, history of heart failure, myocardial infarction, unstable angina, stroke, renal failure (serum creatinine >1.2 mg/dL), cancer, chronic alcohol abuse, hypo- or hypertension (resting blood pressure <90/50 or >180/110 mmHg), use of any vitamin supplementations, therapy with more than three antihypertensives, and severe diabetes complications (proliferative diabetic retinopathy, macroalbuminuria, painful diabetic peripheral neuropathy requiring morphine derivatives, and diabetic foot syndrome).

The control group consisted of age- and sex-matched healthy subjects recruited from a local health exhibition. All control subjects had normal fasting blood glucose level of <7 mmol/L. Written informed consent was obtained from all subjects. The study was approved by the local Institutional Review Board, and was conducted according to the principles outlined in the Declaration of Helsinki.

2.2 Study protocol

Baseline clinical characteristics including body height, weight, waist and hip circumferences, systolic and diastolic blood pressures were measured in all study subjects. Fasting venous blood sample was obtained from each study subject to measure serum HbA1c, triglyceride, total cholesterol, high- and low-density lipoprotein cholesterol levels and the number of circulating EPC. Data on medications in patients with diabetes were ascertained from the patient's medical record.

2.3 Food frequency questionnaire

The dietary intake of major food groups, and macro- and micronutrients were assessed by a validated food frequency questionnaire for Chinese in all study subjects as described previously [8]. In brief, all subjects were asked how often they ate a specific type or group of food, and how many liangs (37.8 g) they ate of the particular food(s) *per* unit of time (day, week, month, or year) in the 5 years before the reference date. The nutritional conversion into estimated intake of dietary nutrients was performed by custom-made computer software used in the Shanghai Women's Health Study [8]. The intake of micronutrients assessed included carotene, retinal, total vitamin A, vitamin B1 (thiamine), vitamin B2, niacin, vitamin C, vitamin E α, vitamin E β and γ, vitamin E δ, total vitamin E, potassium, sodium, magnesium, calcium, iron, manganese, zinc, copper, phosphorus, selenium, isoflavone, PUFAs, and MUFAs.

2.4 Flow cytometry

Fluorescence-activated cell analysis was performed to determine the number of EPCs as described previously [9]. Briefly, 100 μL of peripheral blood was incubated with a phycoerythrin-conjugated mAb against human KDR (Sigma, St. Louis, MO, USA), followed by a FITC-conjugated CD34 or CD133 antibody (Beckman Coulter, Fullerton, CA, USA). FITC-labeled antihuman CD45 antibody was used for differential gating during flow analysis. FITC labeled IgG1a (Beckman Coulter) and phycoerythrin-labeled IgG2b (Becton Dickinson, Franklin Lakes, NJ, USA) served as the isotypic control for color compensation. Analysis was performed with an automated fluorescence-activated cell counter (Elite, Beckman Coulter) in which 1 000 000 events were counted. The absolute number of cells expressing $CD34^+/KDR^+$ or $CD133^+/KDR^+$ *per* 1 000 000 events in the lymphocyte gate was calculated.

2.5 Vascular endothelial function

Vascular endothelial function was measured as flow-mediated dilation (FMD) of the brachial artery [10, 11]. Subjects were required to fast, to stop all the medications, to avoid smoking, alcohol drinking and exercises for 8 h prior to the measurements. All measurements were performed in a quiet room with the subject supine. Ultrasound scans were performed with a high-resolution ultrasonographic scanner (Agilent Sonos 5500; Philips, Andover, MA, USA) equipped with a 7.5 MHz linear-array transducer. The left brachial artery was scanned over a longitudinal section 3 – 5 cm above the antecubital fossa. Both the patient's arm and the ultrasound probe were secured in position with a stereotactic clamp. A pneumatic tourniquet is held inflated around the forearm at a pressure of 50 mmHg above the systolic blood pressure of the subject for 5 min. The arterial diameter was measured both at rest and after reactive hyperemia 1 min after rapid deflation of the pneumatic tourniquet. Four measurements of the arterial diameter from an end-diastolic frame (identified by the electrocardiographic R wave) were averaged to yield the brachial artery diameter during respective experimental stages. FMD values were calculated as the percentage change of the brachial artery diameters following reactive hyperemia compared with the baseline.

2.6 Statistical analysis

Continuous variables were presented as mean ± 1 standard error. The normality of variables was tested by the Shapiro–Wilk test with appropriate transformations for those with significant results, and the data were back-transformed for presentation. Categorical data were presented as frequencies and percentages. Statistical comparisons were performed with Student's *t*-test or Chi-squared test, as appropriate. Stepwise forward multivariate linear regression models were employed to assess the standardized coefficients (β) indicating the relationships between the dietary intake of nutrients and the circulating number of EPCs with the adjustment of other confounders. In order to have at least 80% power to detect a β of 0.5 with a 5% maximum false positive error rate, we would need a minimum of 88 patients from each group. Calculations were performed with use of the SPSS software (version 14.0). A p value of <0.05 was considered statistically significant.

3 Results

3.1 Study population

The study population consisted of 88 patients with type II diabetes and 91 sex- and age-matched controls. Their baseline clinical characteristics are summarized in Table 1. Patients with type II diabetes had significantly higher BMI, waist-to-hip ratio, systolic and diastolic blood pressures and HbA1c levels, and lower serum HDL cholesterol level compared with controls (Table 1, all p <0.05). There were nonetheless no significant differences between the two groups in the prevalence of smoking, and the serum triglyceride, total and low-density lipoprotein cholesterol levels (Table 1, all p >0.05).

3.2 Circulating EPC levels and FMD

Compared with controls, patients with type II diabetes had significantly lower number of circulating $CD133^+/KDR^+$ EPC (48.3 ± 5.2 *vs*. 84.6 ± 7.6/μL, p <0.001) and $CD34^+/KDR^+$ EPC (311 ± 41 *vs*. 412 ± 36/μL, p = 0.045). Furthermore, FMD was also significantly lower in patients with type II diabetes compared with controls (2.54 ± 0.37% *vs*. 5.46 ± 0.47%, p <0.001).

3.3 Dietary intake of nutrients

The dietary intake of various types of macro- and micronutrients are shown in Table 2. After adjusted for body weight, the total daily caloric, carbohydrate, protein, fat and fruit intake were significantly lower in diabetic patients compared with controls (Table 2, all p <0.05). Furthermore, the body weight adjusted daily intake of vitamin B1 (thiamine), B2, niacin, C, E, potassium, magnesium, iron, manganese, zinc, copper, phosphate, isoflavone, and PUFAs were also significantly lower in diabetic patients compared with controls (Table 2, all p <0.05).

Table 1. Baseline clinical characteristics

	Type II diabetes patients (n = 88)	Controls (n = 91)	p value
Age (years)	60.2 ± 1.1	58.9 ± 1.2	0.41
Sex (male%)	50.0	40.7	0.21
Ever smoker (%)	18.7	25.0	0.31
Body weight (kg)	65.0 ± 1.2	60.8 ± 1.1	0.011
BMI (kg/m^2)	25.6 ± 0.4	24.2 ± 0.4	0.008
Waist-to-hip ratio (%)	92.6 ± 0.7	88.0 ± 0.8	<0.0001
Systolic blood pressure (mmHg)	142 ± 3	126 ± 2	<0.0001
Diastolic blood pressure (mmHg)	79 ± 1	75 ± 1	0.027
Hypertension (%)	61.4	13.2	<0.0001
Hyperlipidemia (%)	54.2	15.4	<0.0001
Total cholesterol level (mmol/L)	4.92 ± 0.09	5.09 ± 0.09	0.19
LDL-C level (mmol/L)	2.91 ± 0.09	3.05 ± 0.07	0.23
HDL-C level (mmol/L)	1.36 ± 0.04	1.52 ± 0.04	0.004
Triglyceride level (mmol/L)	1.44 ± 0.09	1.29 ± 0.08	0.21
HbA1c level (%)	7.64 ± 0.13	5.89 ± 0.38	<0.0001
Diabetes duration	9.3 ± 0.8	–	
Retinopathy (%)	22.4	–	
Nephropathy (%)	12.0	–	
Peripheral neuropathy (%)	24.1	–	
Treatment			
Antihypertensives (%)	69.3	15.4	<0.0001
Lipid lowering agents (%)	26.1	2.2	<0.0001
Oral hypoglycemic agents (%)	84.1	–	
Insulin (%)	13.6	–	

HDL-C, HDL cholesterol; LDL-C, low density lipoprotein cholesterol.

3.4 Relationships between circulating EPC level and dietary intake of nutrients

As shown in Tables 3 and 4, multiple linear regression analysis demonstrated that after adjusted for age, sex, smoking history, body weight, HbA1c level, total calorie intake, other vitamins intake, use of antihypertensives and lipid lowering agents, a higher intake of thiamine was significantly associated with a higher level of circulating $CD34^+/KDR^+$ EPC (β = 0.49, p = 0.028) and $CD133^+/KDR^+$ EPC (β = 0.45, p = 0.037) in diabetic patients, but not in controls (β = –0.34, p = 0.17 and β = 0.021, p = 0.95, respectively).

As meats, fruits, and vegetables are major sources of micronutrients, including thiamine, further multiple linear regression analysis were performed and demonstrated no relationship between intake of meats, vegetables or fruits with the level of circulating $CD34^+/KDR^+$ EPC (meat: β = 0.28, p = 0.16; vegetables: β = –0.38, p = 0.19; fruits: β = 0.057, p = 0.78) and $CD133^+/KDR^+$ EPC (meat: β = 0.33, p = 0.079; vegetables: β = –0.38, p = 0.26; fruits: β = 0.084, p = 0.62) in diabetic patients. Similarly, there

Table 2. Body weight-adjusted dietary intake of macro- and micronutrients in the diabetic patients and the normal controls

Daily intake	Type II diabetes patients ($n = 96$)	Controls ($n = 96$)	p value
Total calories (kcal)	1884 ± 63	2188 ± 94	0.003
Carbohydrate (g)	338 ± 12	395 ± 18	0.006
Protein (g)	71.7 ± 2.4	80.8 ± 3.4	0.006
Fat (g)	27.4 ± 1.4	31.6 ± 2.0	0.035
Daily meats intake (g)	64.9 ± 5.4	63.9 ± 6.8	0.581
Daily fruits intake (g)	58.5 ± 5.1	72.5 ± 5.4	0.015
Daily vegetables intake (g)	132 ± 11	149 ± 11	0.185
Carotene (mg)	1.6 ± 0.1	1.8 ± 0.1	0.124
Retinal (mg)	0.12 ± 0.01	0.14 ± 0.01	0.114
Total vitamin A (mg)	0.37 ± 0.02	0.43 ± 0.02	0.053
Vitamin B1 (thiamine) (mg)	0.97 ± 0.04	1.06 ± 0.05	0.047
Vitamin B2 (mg)	0.74 ± 0.03	0.84 ± 0.04	0.022
Niacin (mg)	16.4 ± 0.6	18.1 ± 0.8	0.023
Vitamin C (mg)	41.8 ± 3.0	47.7 ± 2.8	0.077
Vitamin E a (mg)	1.24 ± 0.05	1.28 ± 0.05	0.330
Vitamin E b and g (mg)	1.40 ± 0.09	2.31 ± 0.29	0.002
Vitamin E d (mg)	1.59 ± 0.09	2.31 ± 0.29	0.016
Total vitamin E (mg)	8.0 ± 0.3	10.3 ± 0.7	0.001
Potassium (mg)	1394 ± 46	1609 ± 66	0.001
Sodium (mg)	368 ± 19	425 ± 29	0.071
Magnesium (mg)	259 ± 8	301 ± 12	0.001
Calcium (mg)	337 ± 17	420 ± 34	0.042
Iron (mg)	17.8 ± 0.6	21.1 ± 1.0	0.001
Manganese (mg)	6.26 ± 0.21	7.27 ± 0.32	0.004
Zinc (mg)	11.1 ± 0.4	12.8 ± 0.5	0.003
Copper (mg)	1.90 ± 0.07	2.22 ± 0.09	0.001
Phosphate (mg)	979 ± 30	1122 ± 48	0.004
Selenium (μg)	51.4 ± 2.5	54.1 ± 27	0.141
Isoflavone (mg)	9.0 ± 0.9	17.4 ± 2.9	0.004
PUFAs (g)	4.87 ± 0.27	6.15 ± 0.42	0.004
MUFAs (g)	10.5 ± 0.6	11.8 ± 0.9	0.084

were no relationship between intake of meats, vegetables or fruits with the level of circulating $CD34^+/KDR^+$ EPC (meat: $\beta = \beta 0.073$, $p = 0.68$; vegetables: $\beta = -0.038$, $p = 0.88$; fruits: $\beta = -0.25$, $p = 0.13$) and $CD133^+/KDR^+$ EPC (meat: $\beta = -0.11$, $p = 0.65$; vegetables: $\beta = -0.27$, $p = 0.31$; fruits: $\beta = -0.27$, $p = 0.14$) for controls.

3.5 Relationships between FMD and dietary intake of thiamine

Figure 1 shows the relationships between FMD and increasing quartiles of thiamine intake in patients with type II diabetes and in controls, respectively. After adjusted for age, sex, smoking history, history of hypertension and hyperlipidemia, total calorie intake and other vitamins intake, an increased intake of thiamine from the first to the fourth quartile predicted an absolute increase in FMD by 1.29% ($p = 0.026$, relative increase = 63.5%), but not in controls ($p = 0.448$). Furthermore, multiple linear regression analysis demonstrated again no relationship between intake of meats ($\beta = 0.15$, $p = 0.41$), vegetables ($\beta = 0.24$, $p = 0.38$), or fruits ($\beta = 0.065$, $p = 0.73$) with FMD in diabetic patients.

Table 3. Multiple linear regression model showing the relationships between different clinical variables and circulating levels of $CD34^+/KDR^+$ and $CD133^+/KDR^+$ EPC in type II diabetic patients

	$CD34^+/KDR^+$		$CD133^+/KDR^+$	
Variables	β	p value	β	p value
Age	−0.10	0.42	−0.017	0.89
Female	−0.23	0.14	−0.008	0.96
Current smoker	0.034	0.81	0.062	0.63
Body weight	−0.17	0.22	−0.035	0.79
Use of antihypertensives	0.085	0.52	0.051	0.69
Use of lipid lowering agents	−0.053	0.64	0.28	0.017
HbA1c level	−0.12	0.33	−0.028	0.81
Total caloric intake	0.13	0.60	−0.28	0.20
Carotene	−0.16	0.65	0.38	0.22
Retinal	0.12	0.50	0.26	0.096
Vitamin B2	−0.18	0.43	−0.25	0.20
Niacin	−0.59	0.072	0.13	0.68
Vitamin C	0.51	0.18	−0.29	0.41
Vitamin E a	0.029	0.85	−0.13	0.39
Vitamin E b and g	0.16	0.54	−0.054	0.87
Vitamin E d	−0.005	0.98	−0.16	0.60
Daily thiamine intake	0.49	0.028	0.45	0.037

Table 4. Multiple linear regression model showing the relationships between different clinical variables and circulating levels of $CD34^+/KDR^+$ and $CD133^+/KDR^+$ EPC in normal controls

	$CD34^+/KDR^+$		$CD133^+/KDR^+$	
Variables	β	p value	β	p value
Age	0.14	0.33	−0.16	0.29
Female	0.17	0.23	0.19	0.21
Current smoker	0.089	0.44	0.12	0.34
Body weight	0.16	0.28	0.21	0.17
Use of antihypertensives	0.081	0.52	0.045	0.74
Use of lipid lowering agents	0.072	0.53	−0.074	0.53
HbA1c level	−0.15	0.27	−0.076	0.60
Total caloric intake	0.31	0.18	0.43	0.098
Carotene	−0.30	0.24	0.073	0.76
Retinal	−0.17	0.35	−0.15	0.42
Vitamin B2	0.49	0.11	0.078	0.77
Niacin	−0.48	0.15	−0.41	0.30
Vitamin C	0.21	0.47	−0.043	0.87
Vitamin E a	0.20	0.30	0.089	0.66
Vitamin E b and g	−0.36	0.12	0.11	0.72
Vitamin E d	0.055	0.84	−0.20	0.57
Daily thiamine intake	−0.34	0.17	0.021	0.95

4 Discussion

4.1 General

The results of the present study confirm the previous findings that the number of circulating EPCs, as determined by using either $CD34^+/KDR^+$ or $CD133^+/KDR^+$ as surface markers was reduced, and the vascular endothelial function measured by FMD was impaired in patients with type II diabetes. Our findings extended this observation and demon-

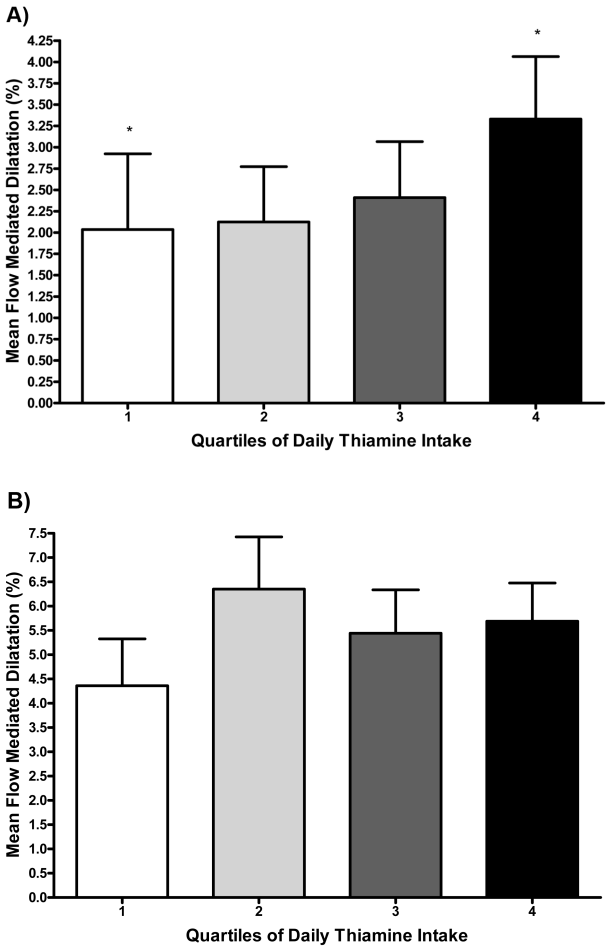

Figure 1. Relationship between FMD and different quartiles of daily thiamine intake in patients with type II diabetes (A) and in controls (B).

strated that the number of circulating EPCs is positively correlated with the daily intake of thiamine in patients with type II diabetes. In addition, multivariate analysis revealed that daily thiamine intake is the only independent factor positively impacting on the circulating number of EPCs, after adjusting for other confounding factors, such as mediations and other vitamins intake. There was nevertheless no relationship between the number of circulating EPCs and daily thiamine intake in control subjects. Furthermore, an increased intake of thiamine from the first to the fourth quartile predicted an increase in FMD in diabetic patients, but not in controls. These findings suggest that thiamine might have a protective effect against the glucotoxicity on circulating EPC and preserve vascular endothelial function in patients with type II diabetes.

In patient with diabetes, hyperglycemia causes vascular damage by overproduction of superoxides *via* the mitochondrial electron transport chain [12, 13]. These superoxides can partially inhibit the glyceraldehyde phosphate dehydrogenase, and thus divert the upstream metabolites from glycolysis to the diacylglycerol pathway, the hexosamine pathway and the polyol pathway with the formation of advanced glycation end products, which subsequently mediate the diabetic vascular complications [14]. These processes can be alleviated by the activation of transketolase that diverts the upstream metabolites from glycolysis to the nonoxidative branch of the pentose phosphate pathway through its cofactor thiamine [14].

Thiamine and its lipid-soluble analog- benfotiamine have been shown to reduce diabetic vascular complications and/or improve vascular endothelial function in clinical [15–17] and experimental studies [14, 18–21] by blocking several pathways of hyperglycemic damage as described above. Furthermore, recent experimental studies have shown that thiamine and their derivative can protect against the glucotoxicity on circulating EPC in patients with type II diabetes [22, 23]. Benfotiamine counteracts the glucotoxicity on EPC differentiation *via* the Akt/FoxO signaling pathway [22]. The phosphatidylinositol 3-kinase/Akt pathway is known to direct cellular processes like differentiation and stress resistance *via* the forkhead family of transcription factors (FoxO1/3a/4), which have been shown to play a role in angiogenesis and vasculogenesis [24, 25]. Benfotiamine has also shown to dose-dependently stimulate proliferation of human EPCs, and to inhibit their apoptosis under hyperglycemic conditions through the protein kinase B/Akt signaling pathway [23]. In addition, benfotiamine supplementation ameliorates the reduction in the number of circulating EPCs in diabetic mice [23].

Previous experimental studies have demonstrated the beneficial effects of thiamine on the functions of endothelial cell [14, 24]. Furthermore, thiamine supplement improved the endothelium-dependent vasodilatation in patients with impaired glucose tolerance or type II diabetes [25]. Our results are consistent with these findings, and provide further indirect evidence to suggest that thiamine might improve vascular endothelial function *via* increasing circulating EPC in patients with type II diabetes.

The present study is, to the best of our knowledge, the first to report that relationship between the dietary thiamine intake with the circulating EPC level and vascular endothelial function in patients with type II diabetes. In view of the emerging evidence to suggest the potential role of circulating EPC in maintenance of vascular endothelium integrity and function, and postnatal neovascularization [1–3], thiamine or its derivative supplementation might be a potential novel therapeutic approach to increase the number of circulating EPC, and improve vascular function in patients with type II diabetes. In this study, reduced intake of various macro- and micronutrients was observed in patients with type II diabetes compared with controls which was likely related to the dietary restriction in diabetic patients to achieve proper glycemic control. Mild thiamine deficiency as measured by serum thiamine level has been reported in patients with diabetes [26, 27]. In normal subjects, the

amount of thiamine intake positively correlated with the serum level of thiamine [28]. On the other hand, recent studies [29] have demonstrated that the lower serum concentration of thiamine in diabetic patients was due to increased renal clearance of thiamine. As a result, the amount of thiamine intake is also not accurately reflected by measuring the urinary or serum concentration of thiamine. Nevertheless, the relatively lower intake of thiamine in diabetic patients than the controls as observed in this study might lead to further reduce in the serum concentration of thiamine in diabetic patients and contribute to the decreased in circulating EPC and endothelial dysfunction. However, this hypothesis needs to be confirmed by future prospective study with the use of thiamine supplement in diabetic patients.

4.2 Limitations

Our study has several limitations. First, this study was designed to explore the relationships between the circulating level of EPC and FMD with the level of different dietary nutrients intake, but not specific with thiamine in type II diabetic patients. As a result, the serum and urinary concentration of thiamine and other nutrients were not determined in this study. Second, the FFQ used in this study has not been specifically validated for the assessment of thiamine intake. Furthermore, misclassification and overestimation of dietary intake is unavoidable with the use of FFQ but would most likely be nondifferential. Therefore, the inclusion of a control group in this study should provide us information on the relative amount of different nutrients intake in diabetic patients as compared with nondiabetic subjects. Third, the relationship between thiamine with the circulating EPC and FMD might be confounded by the other micronutrients, such as folic acid, vitamin B6 and B12 together with thiamine. However, the FFQ used in this study does not capture the intake of those micronutrients [8]. Nevertheless, further analysis on the intake of meats, fruits, and vegetables which are rich in those vitamins and thiamine have failed to show any relationship between their intake with the level of circulating EPC or FMD in diabetic patients. Final, previous studies have shown that FFQ tended to overestimate the intake level of total vegetables and total fruits, and the differences were explained mainly by over-reporting seasonal vegetables and fruits consumption [8]. As the intakes of micronutrients were confounded by the potential overestimation of fruits and vegetables, we have performed further analysis and demonstrated no relationship between them with the level of circulating EPC or FMD in diabetic patients. Furthermore, the study subjects were recruited evenly throughout the whole year which should minimize the over-reporting of seasonal vegetables and fruits consumption.

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The authors have declared no conflict of interest.

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