An investigation of emotion dynamics in major depressive disorder patients and healthy persons using sparse longitudinal networks

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Vitamin B-6 deficiency is common and associated with poor long-term outcome in renal transplant recipients[1,2]

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ABSTRACT

Background: Previous studies have reported low circulating concentrations of pyridoxal-5-phospate (PLP) in renal transplant recipients (RTRs). It is unknown whether this is because of low intake or altered handling, and it is also unknown whether variation in circulating concentrations of PLP influences long-term outcome.

Objective: We compared vitamin B-6 intake and circulating PLP concentrations of RTRs with those of healthy controls and investigated long-term clinical implications of vitamin B-6 deficiency in stable outpatient RTRs.

Design: In a longitudinal cohort of 687 stable RTRs (57% male; mean ± SD age: 53 ± 13 y) with a median (IQR) follow-up of 5.3 y (4.8–6.1 y) and 357 healthy controls (47% male; age 54 ± 11 y), baseline vitamin B-6 was measured as plasma PLP by high-performance liquid chromatography (HPLC). Vitamin B-6 deficiency was defined as PLP <20 nmol/L, and insufficiency as PLP 20–30 nmol/L. Dietary intake was assessed by validated food-frequency questionnaires.

Results: At inclusion [5.3 y (1.8–12.1 y) after transplantation], the mean vitamin B-6 intakes in RTRs and healthy controls were 1.77 ± 0.49 and 1.85 ± 0.56 mg/d, respectively ($P = 0.23$). In these groups, the median plasma PLP concentrations were 29 nmol/L (17–50 nmol/L) and 41 nmol/L (29–60 nmol/L), respectively ($P < 0.001$). Accordingly, deficiency was present in 30% of RTRs compared with 11% of healthy controls. PLP concentrations were inversely associated with glucose homeostasis variables and inflammation variables (all $P < 0.01$). During follow-up, 149 (21%) RTRs died and 82 (12%) developed graft failure. In RTRs, vitamin B-6 deficiency was associated with considerably higher mortality risk (HR 2.14; 95% CI: 1.48, 3.08) than a sufficient vitamin B-6 status, independent of potential confounders. No associations were observed for graft failure ($P = 0.18$).

Conclusions: Vitamin B-6 deficiency is common in RTRs and does not seem to be a consequence of inadequate intake. In addition, this deficient state is clinically relevant and independently associated with an increased risk of mortality in RTRs. The cohort on which the study was based [TransplantLines Food and Nutrition Biobank and Cohort Study (TxL-FN)] was registered at clinicaltrials.gov as NCT02811835. *Am J Clin Nutr* 2017;105:1344–50.

Keywords: vitamin B-6 deficiency, pyridoxal 5′-phosphate, kidney transplantation, diabetes mellitus, inflammation, long-term mortality, long-term graft failure

INTRODUCTION

The preferred treatment of most patients with end-stage renal disease is renal transplantation (Rtx)[9], offering improved prognosis and quality of life at lower costs than dialysis treatment (1, 2). Although short-term prognosis after transplantation has improved over the past decades, long-term success has been disappointing, because stable renal transplant recipients (RTRs) remain at increased risk of mortality, predominantly cardiovascular, compared with the general population (3).

In the search for modifiable factors to improve RTR long-term prognosis, vitamin B-6 might be an interesting target, because previous reports have repeatedly shown that the principal form of vitamin B-6, pyridoxal-5′-phosphate (PLP), is lower in RTRs than in healthy controls (4, 5).

Unfortunately, however, it is not known whether the prevalent vitamin B-6–deficient state in RTRs is caused by inadequate vitamin B-6 intake or altered handling and whether vitamin B-6 deficiency has clinical consequences in this susceptible population. Hence, we aimed to compare circulating PLP concentrations and vitamin B-6 intake in RTRs with those in healthy controls and to investigate the long-term clinical implications of vitamin B-6 deficiency in stable outpatient RTRs.

METHODS

Study population

This prospective cohort study was based on a previously described, well-characterized set of 707 RTRs (6, 7). For the participant flowchart, see **Supplemental Figure 1**. Briefly, this cohort included RTRs (aged ≥18 y) who visited the outpatient clinic of the University Medical Center Groningen, Groningen, Netherlands, between November 2008 and June 2011 and who had a graft that had been functioning for ≥1 y with no history of alcohol and/or drug addiction. We excluded subjects with missing biomaterial (i.e., 11 cases) and subjects using vitamin B-6 supplementation (i.e., 9 cases) from the statistical analyses, which resulted in 687 subjects eligible for analyses. As a control group reflecting the general population, we included 357 healthy kidney donors, none of which had to be excluded because of missing biomaterial or use of vitamin B-6 supplementation. The study protocol was approved by the University Medical Center Groningen institutional review board (METc 2008/186) and adhered to the Declarations of Helsinki and Istanbul.

Data collection and measurements

Information on dietary intake was obtained from a validated semiquantitative food-frequency questionnaire (FFQ), which was developed at Wageningen University to assess nutrient intake (8, 9). Because not all participants completed or returned the FFQ, 191 healthy controls and 627 RTRs had data available on dietary intake derived from the FFQ, whereas all 357 healthy controls and 687 RTRs had plasma PLP concentrations available. The FFQ inquired about intake of 177 food items during the last month, taking seasonal variations into account. For each item, the frequency was recorded in times per day, week, or month. The number of servings was expressed in natural units (e.g., slice of bread or apple) or household measures (e.g., cup or spoon). The questionnaire was self-administered and filled out at home. All FFQs were checked for completeness by a trained researcher, and inconsistent answers were verified with the patients. Validation of the FFQ in RTRs was assessed as previously reported (10). Dietary data were converted into daily nutrient intake by using the Dutch Food Composition Table of 2006 (11). As a cutoff value for sufficient vitamin B-6 intake, the generally accepted recommended daily intake of 1.3 mg/d was used (12). The FFQ did not include information on vitamin supplementation. Use of vitamin supplementation by healthy controls and RTRs was recorded separately, together with recording of medication, with the use of patients' medical records. The variable use of proliferation inhibitors included azathioprine and mycophenolate mofetil. Use of drugs that might affect plasma PLP concentration, including hydralazine (13), penicillin, dopamine, benzodiazepines, antituberculosis drugs, antiepileptic drugs, and theophylline (14), was recorded in both healthy controls and RTRs.

Participants were asked to collect a 24-h urine sample on the day before visiting the outpatient clinic. Urine was collected under oil, and chlorhexidine was added as an antiseptic agent. Urinary albumin was quantified by using nephelometry (Dade Behring Diagnostic), and total urinary protein concentration was determined by means of the Biuret reaction (MEGA AU 510; Merck Diagnostica). Proteinuria was defined as urinary protein excretion ≥0.5 g/24 h.

On completion of the 24-h urine collection, fasting blood samples were obtained the following morning, and venous blood samples were analyzed immediately thereafter. Plasma vitamin B-6 was measured as PLP by means of a validated HPLC method (Waters Alliance) with fluorescence detection (FP-2020; Jasco Inc.) (15). Other laboratory measurements, including glucose homeostasis variables, inflammation variables, lipids, and other liver enzymes, were performed with automated and validated spectrophotometric routine methods (Roche Diagnostics). The glomerular filtration rate was estimated by applying the most recent Chronic Kidney Disease Epidemiology Collaboration equation, which included both serum creatinine and cystatin C (16). Diabetes mellitus was diagnosed according to American Diabetes Association criteria as fasting plasma glucose concentration of ≥7 mmol/L or use of antidiabetic medication (17). Vitamin B-6 sufficiency, insufficiency, and deficiency were defined as plasma PLP >30, 20–30, and <20 nmol/L, respectively (18).

Clinical endpoints

The primary endpoints of this study were all-cause and cardiovascular mortality and death-censored transplant failure. Cardiovascular mortality was defined as death due to cerebrovascular disease, ischemic heart disease, heart failure, or sudden cardiac death according to the International Classification of Diseases, ninth revision, codes 410–447, and graft failure was defined as the necessity to return to dialysis or retransplantation. The continuous surveillance system of the outpatient program ensures up-to-date information on patient status and cause of graft failure. The cause of graft failure was obtained from patient records and was reviewed by a blinded nephrologist. Endpoints were recorded until the end of May 2013. There was no loss due to follow-up for the primary endpoints.

Statistical analyses

Data analyses were performed by using SPSS 22.0 for Windows (SPSS Inc.), STATA version 13.1, and GraphPad Prism version 5.01 for Windows (GraphPad Software).

Data are presented as means ± SDs for normally distributed data, as medians (IQRs) for nonnormally distributed data, and as number (percentage) for nominal data. A 2-sided $P < 0.05$ was considered to indicate statistical significance.

Differences between RTRs and healthy controls in vitamin B-6 were tested by using independent-samples t tests and Mann-Whitney U tests. Univariable linear regression analyses were used to investigate cross-sectional associations of log-transformed plasma PLP with baseline variables (P-trend). Determinants of plasma PLP were identified in a multivariable regression model, in which exposure variables were included according to baseline associations and previous literature (19). These exposure variables included age; sex; BMI (kg/m^2); smoking status; alcohol intake; energy intake; vegetarianism; use of antihypertensives; time spent on dialysis; time since Rtx; intakes of protein, fruit, and vegetables; potassium excretion; vitamin B-6 intake; and use of antidiabetic drugs, statins, calcineurin inhibitor (either cyclosporine or tacrolimus), proliferation inhibitors, or prednisolone. Associates included all other variables that were associated with plasma PLP at baseline but for which information regarding causality was missing. These factors were adjusted for the determinants of

plasma PLP that were identified in the multivariable regression model.

Several subjects had missing values for ≥1 baseline variable [i.e., age, sex, time since Rtx, potassium excretion, high-sensitivity C-reactive protein (hs-CRP), smoking status (6.2%), fruit intake (8.6%), and alcohol intake (9.9%)]. Because excluding subjects with missing values could result in biased prospective results, multiple imputation (fully conditional specification) was used to obtain 5 imputed datasets (20, 21). Rubin's rules were followed to obtain pooled estimates of the regression coefficients and their standard errors across the imputed datasets (22).

The prospective associations of log-transformed plasma PLP with long-term outcomes were first assessed by using Kaplan-Meier curves accompanied by log-rank tests. Secondly, Cox proportional hazard regression analyses were performed, in which adjustments were made for potential confounders, including age, sex, smoking, BMI, time since Rtx, diabetes, alcohol intake, fruit intake, potassium excretion, and hs-CRP. For illustration purposes and to enable more objective comparisons, log-transformed plasma PLP concentrations were standardized to z scores and analyzed as such. In the longitudinal analyses, plasma PLP was entered as a continuous and categorical variable. Cox regression models were built in a stepwise fashion to avoid overfitting and to keep the number of predictors in proportion to the number of events (23). Proportionality of hazards for co-variates was investigated by inspecting the Schoenfeld residuals.

RESULTS

Baseline characteristics and determinants of plasma PLP

The mean age of RTRs was 53 ± 13 y, and 58% were male compared with 54 ± 11 y and 47%, respectively, for controls. Intake of vitamin B-6 was similar in both groups, being 1.77 ± 0.49 mg/d in RTRs and 1.85 ± 0.56 mg/d in controls ($P = 0.23$) (**Figure 1**), and so were the proportions of individuals with lower-than-recommended daily intake, i.e., 15% in RTRs and 13% in controls. However, median plasma PLP concentrations were significantly lower in RTRs than in controls, 29 nmol/L (17–50 nmol/L) compared with 41 nmol/L (29–60 nmol/L) ($P < 0.001$) (Figure 1). Vitamin B-6 insufficiency and deficiency were identified in 22% and 30% of RTRs compared with 18% and 11% of the controls, respectively. None of the controls received treatment regimens that included drugs that have been suggested to affect plasma PLP concentrations, i.e., hydralazine, penicillin, dopamine, benzodiazepines, antituberculosis drugs, antiepileptic drugs, or theophylline. Of the RTRs, none used penicillin, dopamine, antituberculosis drugs, or theophylline, whereas 1 (0.1%), 21 (3%), and 8 (1%) used hydralazine, benzodiazepines, and antiepileptic drugs, respectively.

Baseline characteristics for the overall RTR cohort and according to categories of vitamin B-6 status are shown in **Table 1**. Cross-sectional analyses revealed that plasma PLP was positively associated with alcohol intake, time since Rtx, intake of vegetable protein, fruit intake, potassium excretion, vitamin B-6 intake, and HDL cholesterol and inversely associated with BMI, glucose homeostasis variables, inflammation variables, triglycerides, and proteinuria (all $P < 0.05$).

In a multivariable regression model, age ($\beta = -0.09$, $P = 0.03$), use of antidiabetic drugs ($\beta = -0.09$, $P = 0.02$), time since Rtx
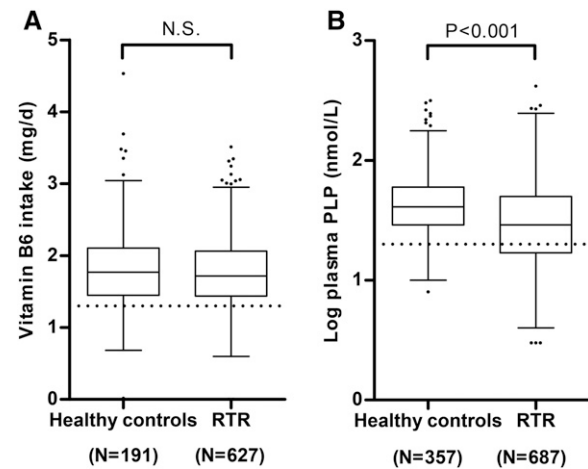
FIGURE 1 Box plots of (A) vitamin B-6 intake and (B) plasma PLP concentrations in healthy controls and RTRs. Boxes indicate IQRs, whereas the extended whiskers indicate observations ≤1.5 IQRs. Black dots represent outliers, i.e., observations outside of the 1.5-IQR range. The dotted horizontal lines show the recommended daily vitamin B-6 intake (1.3 mg/d) and plasma PLP concentration (20 nmol/L) used as cutoffs for vitamin B-6 deficiency. Differences in vitamin B-6 intake and plasma PLP concentrations were tested by an independent-samples t test and Mann-Whitney U test, respectively. PLP, pyridoxal 5′-phosphate; RTR, renal transplant recipient.

($\beta = 0.18$, $P < 0.001$), fruit intake ($\beta = 0.11$, $P = 0.01$), alcohol intake ($\beta = 0.09$, $P = 0.02$), and potassium excretion ($\beta = 0.17$, $P < 0.001$) remained as independent determinants. Adjustment for these independent determinants markedly weakened the association of diabetes ($\beta = -0.04$, $P = 0.47$), serum glucose ($\beta = -0.08$, $P = 0.09$), glycated hemoglobin ($\beta = -0.05$, $P = 0.33$), triglycerides ($\beta = -0.09$, $P = 0.02$), and proteinuria ($\beta = -0.08$, $P = 0.04$) with plasma PLP but left all other baseline associations, including the inflammation variables, materially unchanged.

Vitamin B-6 and mortality

In prospective analyses, with an extended median follow-up of 5.3 y (4.8–6.1 y), 146 of 687 (21%) RTRs died, in 58 (8%) of whom death was due to a cardiovascular cause. Kaplan-Meier analyses revealed a gradual increase in all-cause and cardiovascular mortality across groups with worse vitamin B-6 status (**Figure 2**, log-rank $P < 0.001$ and $P = 0.01$, respectively). In univariable Cox regression analysis, plasma PLP as a continuous variable was associated with all-cause mortality (**Table 2**, model 1). This association remained consistently present independent of adjustment for potential confounders, such as age, sex (model 2), smoking, BMI, time since Rtx, (model 3), diabetes (model 4), alcohol intake, fruit intake, potassium excretion (model 5), and hs-CRP (model 6). When analyzed according to vitamin B-6 status, vitamin B-6–deficient RTRs were at increased risk of all-cause mortality, also independent of potential confounders. Furthermore, analyses with cardiovascular mortality as an endpoint revealed similar point estimates, again without being affected by adjustments for potential confounders (Table 2).

Vitamin B-6 and graft failure

During follow-up, 82 of 687 (12%) RTRs experienced graft failure, mainly due to chronic transplant dysfunction. In the Kaplan-Meier analyses, no associations between plasma PLP

TABLE 1
Baseline characteristics of RTRs, stratified according to vitamin B-6 status[1]

		Vitamin B-6 status				
	Total cohort (N = 687)	Sufficient (n = 326)	Insufficient (n = 153)	Deficient (n = 208)	Standardized β	P-trend
Plasma PLP, nmol/L	29 (17–50)	51 (39–71)	24 (22–27)	14 (10–16)		
Demographics						
Age, y	53 ± 13	53 ± 13	54 ± 12	53 ± 12	−0.04	0.28
Male, n (%)	395 (58)	181 (56)	93 (61)	121 (58)	0.07	0.07
BMI, kg/m²	26.1 (23.3–29.4)	26.0 (32.0–29.0)	25.1 (23.5–28.7)	26.8 (23.4–30.9)	−0.10	0.008
Smoker,[2] n (%)						
Never	266 (42)	138 (44)	56 (39)	72 (39)	−0.12	0.05
Past	291 (43)	141 (45)	69 (48)	81 (44)	0.10	0.13
Current	84 (12)	32 (10)	20 (13)	32 (15)		
Alcohol intake, g/d	3.0 (0.0–11.6)	3.6 (0.2–13.8)	3.1 (0.0–9.8)	1.0 (0.0–6.8)	0.18	<0.001
Energy intake, kcal/d	2169 ± 649	2182 ± 661	2210 ± 606	2102 ± 643	0.02	0.54
Vegetarian, n (%)	13 (2)	6 (2)	2 (1)	5 (2)	0.01	0.75
SBP, mm Hg	136 ± 17	135 ± 17	138 ± 18	136 ± 18	−0.02	0.68
DBP, mm Hg	82 ± 11	82 ± 11	84 ± 11	83 ± 18	−0.03	0.48
Time spent on dialysis, mo	24 (10–47)	21 (9–48)	26 (11–58)	27 (14–46)	−0.07	0.10
Time since Rtx, y	5.3 (1.8–12.1)	6.4 (2.7–14.3)	5.6 (1.6–11.0)	4.4 (1.1–9.0)	0.19	<0.001
Dietary intake[3]						
Total protein, g/d	82 ± 21	82 ± 11	83 ± 20	81 ± 22	0.003	0.95
Animal protein, g/d	52 ± 16	51 ± 16	53 ± 16	52 ± 17	−0.04	0.37
Vegetable protein, g/d	31 ± 10	32 ± 10	30 ± 8	29 ± 10	0.08	0.05
Fruit, g/d	123 (62–232)	140 (70–244)	123 (76–232)	99 (41–182)	0.16	<0.001
Vegetables, g/d	91 (52–122)	91 (57–134)	91 (60–119)	80 (44–113)	0.07	0.08
Potassium excretion, mmol/24 h	72.7 ± 24.0	76.8 ± 24.9	71.7 ± 20.8	66.9 ± 23.4	0.17	<0.001
Vitamin B-6 intake, mg/d	1.77 ± 0.49	1.82 ± 0.51	1.78 ± 0.45	1.68 ± 0.46	0.10	0.02
Glucose homeostasis						
Diabetes, n (%)	165 (24)	69 (21)	30 (20)	66 (32)	−0.11	0.003
Glucose, mmol/L	5.3 (4.8–6.0)	5.2 (4.7–5.8)	5.2 (4.7–6.0)	5.6 (4.9–6.6)	−0.10	0.01
HbA1c, %	6.0 ± 0.8	5.9 ± 0.7	6.0 ± 0.8	6.1 ± 1.0	−0.11	0.004
Inflammation						
hs-CRP, mg/L	1.6 (0.7–4.6)	1.3 (0.6–3.2)	1.6 (0.7–4.2)	2.8 (0.9–7.2)	−0.21	<0.001
Leukocytes, 10⁹/L	7.7 (7.5–7.9)	7.6 (7.3–7.9)	7.7 (7.2–8.1)	8.0 (7.6–8.3)	−0.10	0.009
Lipids						
Total cholesterol, mmol/L	5.1 ± 1.1	5.2 ± 1.1	5.1 ± 1.2	5.1 ± 1.1	0.06	0.11
HDL cholesterol, mmol/L	1.4 ± 0.5	1.5 ± 0.5	1.4 ± 0.4	1.3 ± 0.4	0.26	<0.001
LDL cholesterol, mmol/L	2.9 (2.3–3.5)	2.9 (2.3–3.6)	2.9 (2.4–3.6)	2.8 (2.3–3.4)	0.05	0.22
Triglycerides, mmol/L	1.7 (1.3–2.3)	1.6 (1.2–2.2)	1.7 (1.3–2.2)	1.9 (1.3–2.8)	−0.16	<0.001
Kidney function						
Serum creatinine, μmol/L	124 (99–160)	120 (99–158)	131 (102–171)	127 (99–157)	−0.04	0.35
Cystatin C, mg/L	1.7 (1.3–2.2)	1.6 (1.3–2.5)	1.8 (1.4–2.3)	1.7 (1.3–2.5)	−0.10	0.006
eGFR, mL · min⁻¹ · 1.73 m⁻²	45 ± 19	44 ± 19	44 ± 19	46 ± 19	0.06	0.10
Proteinuria, n (%)	157 (23)	69 (21)	28 (18)	60 (29)	−0.08	0.03
Nonimmunosuppressive drugs, n (%)						
Hydralazine	1 (0.1)	0 (0)	1 (1)	0 (0)	−0.004	0.91
Benzodiazepine	21 (3)	12 (4)	4 (3)	5 (2)	0.07	0.08
Antiepileptic	8 (1)	3 (1)	1 (1)	4 (1)	0.04	0.36
Antihypertensive	606 (88)	280 (86)	144 (94)	182 (88)	−0.04	0.30
Antidiabetic	105 (15)	44 (14)	19 (12)	42 (20)	−0.10	0.009
Statin	362 (53)	170 (52)	82 (54)	110 (53)	−0.01	0.83
Immunosuppressive drugs, n (%)						
CNI						
Cyclosporine	271 (39)	119 (37)	61 (40)	91 (44)	−0.08	0.05
Tacrolimus	123 (18)	58 (18)	26 (17)	39 (19)	−0.02	0.65
Proliferation inhibitor	575 (84)	268 (91)	117 (77)	190 (82)	−0.08	0.05
Prednisolone	687 (99)	326 (100)	153 (98)	205 (99)	−0.03	0.41

[1] Values are means ± SDs or medians (IQRs) unless otherwise indicated. Plasma PLP was log-transformed for linear regression analyses. CNI, calcineurin inhibitor; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; HbA1c, glycated hemoglobin; hs-CRP, high-sensitivity C-reactive protein; PLP, pyridoxal-5′-phosphate; RTR, renal transplant recipient; Rtx, renal transplantation; SBP, systolic blood pressure.

[2] Variables consisting of >2 groups were recoded into dummy variables before being entered in a linear regression model simultaneously.

[3] Dietary intake was assessed by using a validated food-frequency questionnaire.
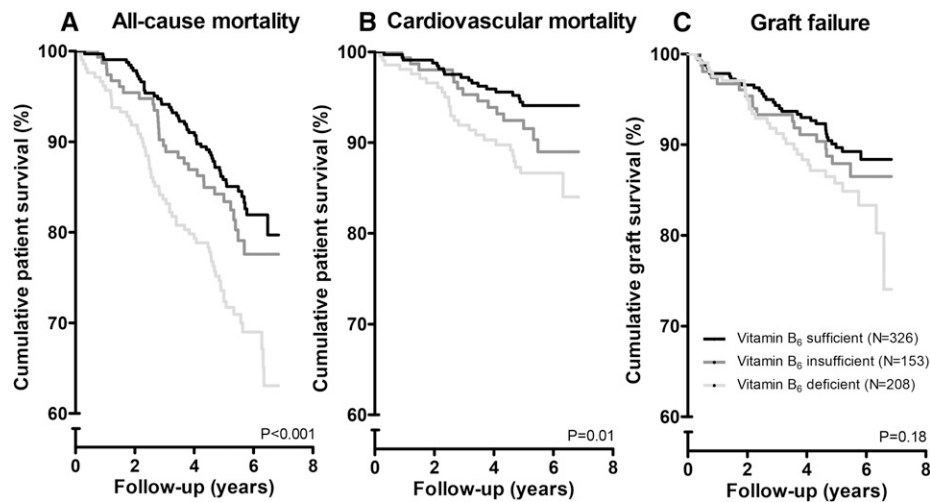
FIGURE 2 Kaplan Meier curves with log-rank tests for (A) all-cause mortality, (B) cardiovascular mortality, and (C) graft failure according to vitamin B-6 status.

concentrations and graft failure were observed (Figure 2, log-rank $P = 0.18$).

DISCUSSION

To the best of our knowledge, this study is the first to compare both vitamin B-6 intake and plasma PLP concentrations between RTRs and healthy individuals. We found a higher prevalence of vitamin B-6 deficiency in RTRs and an indication that this might be the consequence of altered vitamin B-6 handling. Importantly, this vitamin B-6–deficient state, compared with the vitamin B-6–sufficient state, is independently associated with an increased risk of cardiovascular mortality in RTRs.

The vitamin B-6 intake of RTRs in the present study meets the recommended daily intake (12) and complies with data from a previous study in RTRs, which revealed insufficient intake in ∼12% of RTRs (24). The adequate vitamin B-6 intake in RTRs, as well as the overall absence of overt vitamin B-6 deficiency in general populations of developed countries, is explained by the fact that many common foods, such as various meats and vegetables, are high in vitamin B-6 content and thus readily contribute to sufficient intake (19). We found a poor-to-absent association of intake of animal protein and vegetables with circulating PLP concentrations. We can only speculate on a reason for this fact. One possibility is that in RTRs vitamin B-6 coming from food is diverted from the circulation toward sites of

TABLE 2

Cox regression analyses for the association of vitamin B-6 status with all-cause and cardiovascular mortality in RTRs, with subsequent adjustment for potential confounders[1]

| | | Vitamin B-6 status | | | | | |
| | Sufficient (n = 326) | Insufficient (n = 153) | | Deficient (n = 208) | | Continuous (N = 687) | |
Model[2]	Reference HR	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P
All-cause mortality							
1	1.00	1.25 (0.80, 1.96)	0.32	2.14 (1.48, 3.08)	<0.001	0.70 (0.59, 0.82)	<0.001
2	1.00	1.20 (0.77, 1.88)	0.42	2.15 (1.49, 3.09)	<0.001	0.71 (0.60, 0.84)	<0.001
3	1.00	1.17 (0.75, 1.84)	0.49	2.44 (1.66, 3.59)	<0.001	0.67 (0.56, 0.80)	<0.001
4	1.00	1.15 (0.73, 1.80)	0.55	2.40 (1.63, 3.53)	<0.001	0.68 (0.57, 0.81)	<0.001
5	1.00	1.02 (0.65, 1.61)	0.93	2.01 (1.34, 3.01)	0.001	0.74 (0.61, 0.89)	0.001
6	1.00	1.12 (0.71, 1.76)	0.63	2.25 (1.51, 3.37)	<0.001	0.69 (0.57, 0.83)	<0.001
Cardiovascular mortality							
1	1.00	1.71 (0.85, 3.44)	0.13	2.56 (1.40, 4.67)	0.002	0.67 (0.51, 0.87)	0.003
2	1.00	1.63 (0.81, 3.28)	0.17	2.52 (1.38, 4.62)	0.003	0.67 (0.53, 0.89)	0.005
3	1.00	1.75 (0.87, 3.52)	0.11	2.51 (1.33, 4.74)	0.005	0.68 (0.51, 0.91)	0.01
4	1.00	1.72 (0.86, 3.45)	0.13	2.39 (1.26, 4.51)	0.007	0.70 (0.53, 0.93)	0.02
5	1.00	1.52 (0.74, 3.12)	0.25	2.17 (1.12, 4.20)	0.02	0.73 (0.54, 0.99)	0.04
6	1.00	1.65 (0.82, 3.32)	0.16	2.16 (1.12, 4.17)	0.02	0.73 (0.54, 0.99)	0.04

[1] Plasma PLP was log-transformed and standardized to z scores for continuous analyses. One z-score unit corresponds to 0.336 nmol/L log plasma PLP and 2.17 nmol/L plasma PLP. hs-CRP, high-sensitivity C-reactive protein; PLP, pyridoxal-5′-phosphate; RTR, renal transplant recipient.

[2] Model 1, crude model; model 2, adjusted for age and sex; model 3, adjusted as for model 2 and for smoking, BMI, and time since renal transplantation; model 4, adjusted as for model 3 and for diabetes; model 5, adjusted as for model 3 and for alcohol intake, fruit intake, and potassium excretion; model 6, adjusted as for model 3 and for hs-CRP.

ongoing chronic low-grade inflammation (25). Our observation that vitamin B-6 intake in RTRs is similar to that in controls and yet plasma PLP concentrations are lower suggests that the poor vitamin B-6 status in RTRs is the consequence of altered vitamin B-6 handling rather than inadequate intake. These alterations in handling could include decreased absorption from the small intestine, impaired subsequent transport to the liver, aberrant metabolism to active or inactive isoforms, or increased excretion of the vitamin B-6 catabolite in urine (26). However, plasma PLP concentrations were reported to be essentially unaffected by renal function (27), which we corroborated by finding no association between baseline plasma PLP and serum creatinine or estimated glomerular filtration rate. Interestingly, Lacour et al. (4) previously suggested that the deficit in plasma PLP in RTRs could originate from the period on dialysis and that it might be maintained by the immunosuppressive medication used by RTRs; however, they acknowledge that data for such a drug-induced effect were lacking (5). To our knowledge, the potential in vivo effects of prednisolone on vitamin B-6 isoforms have been investigated in one intervention study in experimental animals (28). It was found that long-term prednisolone treatment increased circulating plasma concentrations of PLP, pyridoxal, and pyridoxic acid in rats and mice. However, it is not known whether these findings, which seem consistent with a beneficial effect of prednisolone on vitamin B-6 levels, are also present in humans. Moreover, the possible presence of such an effect cannot explain the fact that we found low rather than high circulating concentrations of PLP in RTRs compared with those in healthy controls, despite similar intake.

One other factor that has been proposed to affect vitamin B-6 handling is diabetes (29–32). Our study extends these reports by showing that antidiabetic drugs, as an indicator of diabetes, independently determine circulating plasma PLP concentrations.

In addition to diabetes, inflammation has been suggested to adversely affect handling of vitamin B-6 through various mechanisms, including increased mobilization of PLP from circulation to sites of inflammation for use by PLP-dependent enzymes that mediate the inflammatory response (25, 33). Our cross-sectional data agree with these reports because both inflammation markers, i.e., hs-CRP and leukocyte count, were associated with plasma PLP concentrations, independent of determinants, and call for mechanistic studies to further unravel the underlying molecular mechanisms. In this regard, it would be useful to distinguish between low status and altered distribution of vitamin B-6 by evaluating alkaline phosphatase, serum albumin, inorganic phosphate, and functional indexes of vitamin B-6, including erythrocyte transaminase activities, plasma kynurenines, and one-carbon metabolites (19). Previous studies on blood PLP and mortality were conducted in populations with different types of pathophysiology and have shown varying results (34–36). The most recent report suggested that the relation between plasma PLP and mortality in patients with coronary artery disease could be secondary to inflammation (36). However, in the present study, adjustment for inflammation had no material effect on the association between plasma PLP and both all-cause and cardiovascular mortality.

Some limitations of this study need to be addressed. First, we did not have information on other B-6 isoforms, such as pyridoxal and pyridoxic acid, and therefore could not estimate vitamin B-6 catabolism. It would be interesting if future studies would explore vitamin B-6 handling by assessing the complete vitamin B-6 profile in this population. Second, although the observational nature of this study enables speculation regarding potential causal mechanisms underlying associations of plasma PLP with diabetes, inflammation, and mortality, it unfortunately precludes conclusions on causality. For evidence regarding causality, intervention studies are essential. Third, one should realize that this study, as with most epidemiologic studies, uses a single baseline measurement for studying the association of variables with outcomes, which in theory could affect the strength and relevance of such associations. However, the intraclass correlation coefficient, an indicator of within-person reproducibility over years, of plasma PLP is excellent, thus allowing for one-exposure assessment of vitamin B-6 status (37). Moreover, if intra-individual variability of plasma PLP over time would be taken into account by including data on repeated measurements, associations that already exist for single measurements of PLP would strengthen, because intra-individual variation would be accounted for. The higher the intra-individual day-to-day variation, thus the lower the intraclass correlation coefficient, the greater one would expect the benefit of inclusion of repeated measurements for finding prospective associations (38, 39). Finally, the FFQ is not well suited for obtaining estimates of precise amounts of vitamin B-6 eaten. The reason is that an FFQ by nature cannot include all food items, but only those that are commonly used in a population. The fact that not every food item is included is also the reason that the energy intake estimated from an FFQ is typically lower than from 24-h recalls or from food records. One can therefore not use data from FFQs for the estimation of precise amounts of intake, but rather for epidemiologic research wherein subjects are ranked and compared, like we do in this study.

Strengths of this study include the large cohort size of this specific population consisting of well-characterized, stable RTRs, in which no cases were lost to follow-up. Also, the availability of appropriate healthy controls positively contributed to the reliability of our data. Moreover, extensive information on metabolic parameters, as well as dietary intake, allowed adjustment for potential confounders.

To conclude, we have shown that vitamin B-6 deficiency is common in RTRs and that it might be the consequence of altered vitamin B-6 handling. Importantly, vitamin B-6 deficiency is independently associated with an increased risk of mortality in RTRs. Because the observational nature of our study precludes conclusions on cause-effect relations, randomized controlled clinical trials are required to determine whether correction of vitamin B-6 status with vitamin B supplements would in fact improve long-term outcome in RTRs with a low vitamin B-6 status. Nevertheless, it would seem prudent to endorse a diet based on foods rich in this vitamin, in particular fruits and legumes, in RTRs with a low vitamin B-6 status.

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