Plasma vitamin B-6 forms and their relation to transsulfuration metabolites in a large, population-based study[1–3]

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ABSTRACT

Background: Vitamin B-6 exists in different forms; one of those forms, pyridoxal 5′-phosphate (PLP), serves a cofactor in many enzyme reactions, including the transsulfuration pathway, in which homocysteine is converted to cystathionine and then to cysteine. Data on the relations between indexes of vitamin B-6 status and transsulfuration metabolites in plasma are sparse and conflicting.

Objective: We investigated the distribution and associations of various vitamin B-6 species in plasma and their relation to plasma concentrations of transsulfuration metabolites.

Design: Nonfasting blood samples from 10 601 healthy subjects with a mean age of 56.4 y were analyzed for all known vitamin B-6 vitamers, folate, cobalamin, riboflavin, total homocysteine, cystathionine, total cysteine, methionine, and creatinine. All subjects were genotyped for the methylenetetrahydrofolate reductase (*MTHFR*) 677C→T polymorphism.

Results: Plasma concentrations of the main vitamin B-6 vitamers—PLP, pyridoxal, and 4-pyridoxic acid—were strongly correlated. Among the vitamin B-6 vitamers, PLP showed the strongest and most consistent inverse relation to total homocysteine and cystathionine, but the dose response was different for the 2 metabolites. The PLP–total homocysteine relation was significant only in the lowest quartile of the vitamin B-6 distribution and was strongest in subjects with the *MTHFR* 677TT genotype, whereas cystathionine showed a graded response throughout the range of vitamin B-6 vitamer concentrations, and the effect was not modified by the *MTHFR* 677C→T genotype.

Conclusion: This large population-based study provided precise estimates of the relation between plasma concentrations of vitamin B-6 forms and transsulfuration metabolites as modified by the *MTHFR* 677C→T genotype. *Am J Clin Nutr* 2007;86:131–8.

KEY WORDS Vitamin B-6, homocysteine, cystathionine, cysteine, methylenetetrahydrofolate reductase, transsulfuration

INTRODUCTION

Vitamin B-6 is a versatile enzyme cofactor that is involved in ≈100 enzymatic reactions (1). Vitamin B-6 exists in 7 forms: pyridoxine, pyridoxine 5′-phosphate (PNP), pyridoxal, pyridoxal 5′-phosphate (PLP), pyridoxamine, pyridoxamine 5′-phosphate (PMP), and the catabolite 4-pyridoxic acid (PA). Pyridoxal and PLP are the major vitamin B-6 forms obtained from animal food products, whereas pyridoxine, pyridoxamine, PNP, and PMP are the main forms obtained from plants (1). Pyridoxine is also the form given as vitamin B-6 supplement. Vitamin B-6 is absorbed in the jejunum and metabolized in the liver (2), which releases PLP (3) with pyridoxal and PA (2) into the circulation. The major catabolic pathway in humans is the hydrolysis of the metabolically active form PLP to pyridoxal, which is followed by oxidation to PA (4).

Orally supplemented pyridoxine is absorbed quickly, which results in a plasma pyridoxine peak that disappears in a few hours (2, 5, 6), strong increases in plasma pyridoxal (2, 5, 7, 8) and PA (2, 5, 7) that normalize in several hours, and an increase in plasma PLP that lasts >24 h (2, 5, 7–9). PLP, pyridoxal, and PA are the major vitamin B-6 forms in plasma (10–12), where most PLP (3), and some pyridoxal—but no PA or pyridoxine—are protein-bound (13). Free plasma pyridoxal but not protein-bound PLP can cross cell membranes (3, 14, 15). Once inside the cell, pyridoxal may be converted to PLP, which is the metabolically active form (1). Plasma PLP is the most commonly used vitamin B-6 index (14, 16, 17). However, pyridoxal (14, 18, 19) and the combinations PLP plus pyridoxal (14, 20) and PLP plus PA (21–23) have also been suggested as useful markers of vitamin B-6 status.

PLP serves as cofactor in both steps in the transsulfuration pathway, in which cystathionine β-synthase and cystathionine γ-lyase convert homocysteine to cystathionine and then to cysteine (24). An inverse relation between plasma PLP and total homocysteine (tHcy) in nonfasting (25) and fasting (23, 26) subjects has been reported by some authors, but most found no such relation (27–35). Similarly, some studies reported a tHcy-lowering effect of pyridoxine supplementation (36, 37), but most investigators found no such effect in fasting (29, 33, 38–45) or nonfasting (46) subjects. An inverse relation between plasma cystathionine and PLP during fasting was reported (35), and both

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fasting (32) and nonfasting (46) plasma cystathionine concentrations were reduced by pyridoxine supplementation. Studies of the relation of plasma PLP (23, 35, 47) and pyridoxine supplementation (32, 43) to total cysteine (tCys) has been negative.

The enzyme methylenetetrahydrofolate reductase (MTHFR; EC 1.5.1.20) catalyzes the irreversible conversion of 5,10–methylenetetrahydrofolate to 5-methyltetrahydrofolate, which serves as methyl donor in the remethylation of homocysteine to methionine. Homozygosity for the common MTHFR 677C→T polymorphism is associated with higher plasma tHcy concentrations (48) and stronger inverse relations of plasma tHcy with folate, vitamin B-12, riboflavin (48), and possibly also with PLP (49).

This report focuses on the concentrations and interrelations of all forms of vitamin B-6 and on the relations of the major vitamin B-6 forms with plasma tHcy and its transsulfuration metabolites cystathionine and cysteine. We also investigated the possible effect modification by the *MTHFR* 677C→T genotype. The study was carried out in a large cohort of healthy Norwegian subjects.

SUBJECTS AND METHODS

Subjects and recruitment

The present study includes 10 601 healthy subjects from the Norwegian Colorectal Cancer Prevention cohort (50) who were randomly selected from the population registries of Oslo and Telemark counties in Norway from 1999 through 2001. All of the participants (men and women) were 50–64 y old.

Written informed consent was obtained from all participants. The Regional Ethics Committee and The Data Inspectorate approved the study protocol.

Blood collection and biochemical analyses

Blood was drawn from nonfasting subjects during normal working hours at 3 study centers: Ullevål Hospital in Oslo, Telemark Hospital in Skien, and Rjukan Hospital in Rjukan (all: Norway). The blood was centrifuged at 1100 × *g* for 10 min at 23 °C, and serum and plasma (which had been drawn into tubes containing EDTA) were separated and kept at −80 °C until they were analyzed.

Plasma tHcy, tCys (51), vitamin B-6 and riboflavin (12), serum folate (52), cobalamin (53) concentrations were measured, and *MTHFR* 677C→T genotypes (54) were determined according to published methods. Cystathionine was analyzed by including it and a deuterated internal standard (d4-cystathionine) in an existing liquid chromatography–tandem mass spectrometry assay (12). Ion pairs were 222.9/133.9 for cystathionine and 226.9/137.9 for d4-cystathionine. Creatinine and total methionine (sum of methionine and methionine sulfoxide) were analyzed by including them and their deuterated internal standards (d3-creatinine and d4-methionine) in a liquid chromatography–tandem mass spectrometry assay (55) by using the ion pairs 114/44.2, 150.2/104, 166.1/73.9, 117/47.2, and 154/108, respectively.

Statistical analysis

Concentrations are given as means and medians (5th, 95th percentiles). Concentration means, age, and sex across genotypes were compared by linear regression after adjustment (where appropriate) for age, sex, and study center. Relations between the vitamin B-6 vitamers PLP, pyridoxal, and PA; other B vitamins; and metabolites were investigated by using partial Spearman correlation after adjustment for age, sex, and study center. The relations between PLP, pyridoxal, and PA were also presented as scatterplots with lowess regression curves (56) with the smoother span and delta both set at 0.01.

The relations between the metabolites and various forms of vitamin B-6 were assessed in multiple linear regression models. Separate regression models were constructed for each of the major vitamin B-6 forms. Age was included as a continuous variable. Categorical variables indicating study center enrollment were used. Vitamin B-6 forms, creatinine, folate, cobalamin, riboflavin, and methionine were included as indicator variables, with one variable used for each concentration quartile. The regression coefficients estimated the difference in mean tHcy between the chosen reference category and the other categories. Mean metabolite concentrations across quartiles of PLP, pyridoxal, or PA were also tested for linear trend. We investigated the possible interaction between the *MTHFR* 677C→T genotype and a vitamin B-6 vitamer by including product terms between genotype and the vitamer concentration in multiple linear regression models in which the transsulfuration metabolites served as the dependent variable; all primary variables were retained in the model. Tests were 2-tailed, and $P < 0.05$ was considered significant.

Statistical analyses were performed by using SPSS software (version 11.0; SPSS, Chicago, IL), except for the lowess regression, which was computed by using R (57).

RESULTS

Population characteristics

The study population ($n = 10\,601$, 49.2% male) was predominantly (>98%) white and had a mean age of 56.4 y (**Table 1**). *MTHFR* 677C→T genotype frequencies were 51.4%, 40.6%, and 8.0% for the *CC*, *CT*, and *TT* genotypes, respectively, and neither sex nor age varied between the genotypes (Table 1).

Vitamin B-6 vitamers

PLP, pyridoxal, and PA were present in all plasma samples. The concentrations and distribution of these vitamers are summarized in Table 1 and **Figure 1**. Median (5th, 95th percentiles) concentrations were 43.7 (16–139), 9.5 (5–39), and 20.3 (10–100) nmol/L for PLP, pyridoxal, and PA, respectively. Only PLP was related to the MTHFR 677C→T polymorphism, and its lowest concentrations (as were those of folate) were found in subjects with the *TT* genotype (Table 1). The concentration of PLP ranged from 4 to 1100 nmol/L, whereas pyridoxal and PA had a wider concentration range of 1 to ≈5000 nmol/L. All 3 species showed a skewed distribution with a long tail in the upper region, and the distributions became essentially symmetric after log transformation (Figure 1).

Pyridoxine and pyridoxamine were detected in 1.9% and 0.85% of the samples; their maximum concentrations were 2970 and 465 nmol/L, respectively. PMP and PNP were rarely detected in plasma; if they were present, their concentrations were always close to the lower limit of quantification of the assay (ie, 0.2 nmol/L for PNP and 4 nmol/L for PMP).

The concentrations of the main species—PLP, pyridoxal, and PA—were strongly related (Figure 1). The plots of PLP versus pyridoxal or PA showed the steepest increase at higher PLP concentrations, whereas pyridoxal and PA had a linear relation

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TABLE 1
Characteristics of the study population[1]

	All subjects (n = 10 601)			*MTHFR* 677C→T genotype									
				CC (n = 5452)			*CT* (n = 4299)			*TT* (n = 850)			
	Mean	Median	Percentiles[3]	Mean	Median	Percentiles[3]	Mean	Median	Percentiles[3]	Mean	Median	Percentiles[3]	P[2]
Total homocysteine (μmol/L)	10.8	10.2	(6.8, 16.4)	10.4	9.9	(6.7, 15.3)	10.9	10.4	(6.8, 16.4)	13.3	11.2	(7.0, 27.0)	< 0.001
Cystathionine (μmol/L)	0.237	0.190	(0.091, 0.525)	0.238	0.190	(0.092, 0.523)	0.236	0.189	(0.090, 0.526)	0.235	0.193	(0.092, 0.527)	0.708
Total cysteine (μmol/L)	285.3	283.7	(237.1, 338.2)	285.3	283.5	(237.1, 339.1)	285.7	284.2	(237.4, 337.3)	282.0	281.2	(233.6, 334.4)	0.013
PLP (nmol/L)	62.7	48.0	(18.7, 152.4)	62.7	47.9	(19.1, 153.3)	63.8	49.0	(18.8, 153.2)	58.2	44.4	(15.6, 143.1)	0.002
PL (nmol/L)	21.5	10.0	(5.2, 41.1)	19.9	9.9	(5.2, 40.6)	23.9	10.2	(5.3, 41.8)	19.6	9.6	(4.9, 41.5)	0.276
PA (nmol/L)	42.0	20.4	(10.3, 110.0)	40.0	20.3	(10.4, 109.0)	44.7	20.5	(10.2, 110.0)	40.3	20.5	(10.0, 109.0)	0.270
Folate (nmol/L)	17.1	13.7	(6.6, 39.4)	17.8	14.5	(7.3, 40.3)	17.0	13.4	(6.6, 39.0)	13.4	10.5	(5.0, 32.1)	< 0.001
Cobalamin (pmol/L)	332.0	307.2	(172.0, 535.9)	333.4	307.7	(174.6, 541.6)	330.5	307.7	(170.4, 532.4)	323.9	299.7	(157.4, 516.0)	0.341
Riboflavin (nmol/L)	18.1	10.5	(4.1, 55.9)	18.5	10.4	(4.1, 57.6)	17.7	10.4	(4.1, 54.8)	17.0	10.7	(3.9, 46.5)	0.178
Total methionine (μmol/L)	23.5	22.5	(16.2, 34.9)	23.5	22.4	(16.1, 34.8)	23.6	22.5	(16.2, 35.3)	23.4	22.4	(16.4, 33.7)	0.186
Creatinine (μmol/L)	69.9	68.9	(50.6, 92.2)	70.0	68.9	(50.4, 91.9)	70.3	69.5	(51.2, 92.9)	67.6	66.3	(49.9, 88.9)	< 0.001
Age (y)	56.4	55	(51, 63)	56.4	55	(51, 63)	56.4	55	(51, 63)	56.3	55	(51, 63)	0.283
Male (%)	49.2			48.2			50.2			50.7			0.068

[1] All concentrations are in plasma, except folate, which is in serum. MTHFR, methylenetetrahydrofolate reductase; PLP, pyridoxal 5′-phosphate; PL, pyridoxal; PA, pyridoxic acid.
[2] Means across genotypes were modelled by linear regression and adjusted (where appropriate) for age, sex, and study center.
[3] 5th, 95th percentiles.

throughout the range of concentrations. All correlations were highly significant ($P < 0.001$) but were somewhat stronger between PLP and pyridoxal (Spearman $r = 0.80$) and between pyridoxal and PA ($r = 0.79$) than between PLP and PA ($r = 0.67$) (**Table 2**).

The vitamin B-6 vitamers showed moderate correlations with folate and riboflavin ($r = 0.35–0.45$) and a weaker correlation with cobalamin ($r = 0.14–0.18$). Methionine and creatinine were more strongly associated with PLP and PA, respectively, than with the other vitamin B-6 vitamers (Table 2).

Homocysteine

The median (5th, 95th percentiles) tHcy concentration for all subjects combined was 10.2 (6.8–16.4) μmol/L, and the concentration increased with the number of *MTHFR* 677T alleles ($P <$ 0.001; Table 1). Plasma tHcy was negatively related to folate, cobalamin, and riboflavin and positively related to creatinine (Table 2).

The association of plasma tHcy with either PLP, pyridoxal, or PA was assessed by using multiple regression analyses after

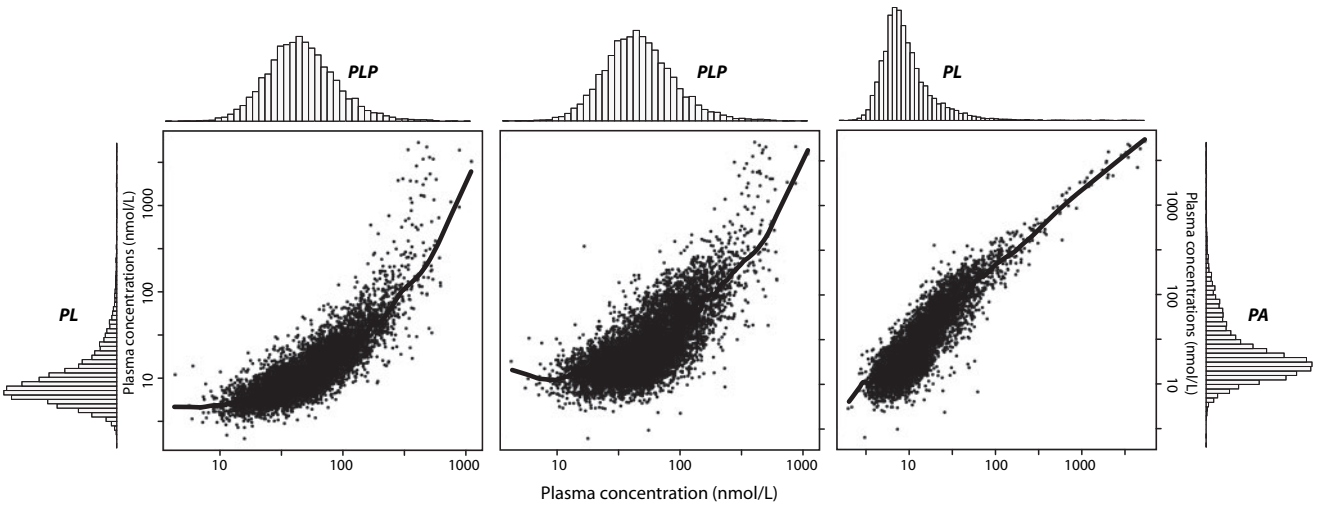

FIGURE 1. Distribution of and correlation between pyridoxal 5′-phosphate (PLP), pyridoxal (PL), and pyridoxic acid (PA) in human plasma. The main panels show scatter plots overlaid with lowess regression curves. Distribution of the separate vitamin B-6 vitamers is presented as histograms projected on the x and y axes. Note that both axes use log scale.

TABLE 2
Partial Spearman correlation coefficients[1]

	tHcy	Cystathionine	tCys	PLP	PL	PA
Cystathionine	0.15	—	—	—	—	—
tCys	0.37	0.04	—	—	—	—
PLP	−0.23	−0.11	0.07	—	—	—
PL	−0.20	−0.16	0.10	0.80	—	—
PA	−0.21	−0.10	0.10	0.67	0.79	—
Folate	−0.44	−0.20	0.13	0.39	0.40	0.40
Cobalamin	−0.24	−0.02[2]	0.08	0.18	0.14	0.18
Riboflavin	−0.18	0.003[3]	0.09	0.35	0.39	0.45
Methionine	−0.07	0.33	−0.02[4]	0.16	0.11	0.07
Creatinine	0.20	0.19	0.16	0.07	0.08	0.19

[1] Adjusted for age, sex, and study center. $n = 10\,601$. tHcy, total homocysteine; tCys, total cysteine; PLP, pyridoxal 5′-phosphate; PL, pyridoxal; PA, pyridoxic acid. All correlations were significant if not indicated otherwise, $P < 0.001$.
[2] $P = 0.09$.
[3] $P = 0.7$.
[4] $P = 0.016$.

adjustment for other B vitamins, creatinine, study center, age, and sex (**Table 3**). Plasma tHcy increased with decreasing concentrations of PLP only in the lowest PLP quartile. Furthermore, the tHcy differences across quartiles were investigated separately in the *MTHFR* 677C→T genotypes and was most pronounced (2.18 μmol/L) in the *TT* genotype ($P < 0.001$ for interaction between PLP and *MTHFR*). Plasma tHcy was similarly related to pyridoxal and PA, but the associations were in general weaker than those with PLP (Table 3).

Cystathionine and cysteine

The median (5th, 95th percentiles) concentrations for all subjects combined were 0.190 (0.091–0.525) μmol/L for cystathionine and 283.7 (237–338.2) μmol/L for tCys; the concentration of cystathionine did not vary with *MTHFR* 677C→T genotype, whereas tCys was lowest in the *TT* group (Table 1). Plasma cystathionine (but not tCys) was higher in men than in women [$\bar{x}$ (5th, 95th percentiles) concentrations: 0.257 (0.099–0.580) and 0.218 (0.086–0.471) μmol/L, respectively] ($P < 0.001$). Cystathionine was inversely related to folate, but not to cobalamin or riboflavin, and was positively related to creatinine and methionine (Table 2).

We investigated the relations of cystathionine to the vitamin B-6 vitamers by using a multiple regression model similar to that described for tHcy but with additional adjustment for methionine. Plasma cystathionine increased (P for trend ≤ 0.007) with

TABLE 3
Difference in plasma total homocysteine across quartiles (Q) of vitamin B-6 vitamers and *MTHFR* 677C→T genotypes[1]

		Genotype				
Determinant	Upper cutoff	All genotypes ($n = 10\,576$)	*CC* ($n = 5452$)	*CT* ($n = 4299$)	*TT* ($n = 850$)	P^2
	nmol/L		*nmol/L*			
PLP						
Q1	32.6	0.73 (0.52, 0.95)	0.57 (0.35, 0.79)	0.54 (0.21, 0.87)	2.18 (0.64, 3.72)	< 0.001
Q2	48.0	0.18 (−0.02, 0.38)	0.25 (0.05, 0.46)	0.10 (−0.20, 0.40)	−0.39 (−1.79, 1.01)	
Q3	73.1	0.09 (−0.10, 0.28)	0.17 (−0.03, 0.36)	0.09 (−0.19, 0.37)	−0.46 (−1.81, 0.89)	
P^3		< 0.001	< 0.001	0.003	0.004	
PL						
Q1	7.5	0.22 (0.01, 0.43)	0.06 (−0.16, 0.28)	0.01 (−0.32, 0.33)	2.03 (0.60, 3.46)	< 0.001
Q2	10.0	−0.14 (−0.34, 0.06)	−0.09 (−0.30, 0.12)	−0.10 (−0.41, 0.20)	−0.46 (−1.85, 0.93)	
Q3	14.7	0.00 (−0.19, 0.20)	0.09 (−0.12, 0.29)	0.00 (−0.29, 0.29)	0.10 (−1.24, 1.44)	
P^3		0.11	1.0	0.89	0.01	
PA						
Q1	15.2	0.34 (0.13, 0.56)	0.29 (0.07, 0.50)	0.34 (0.014, 0.66)	1.41 (−0.07, 2.89)	< 0.001
Q2	20.4	0.03 (−0.18, 0.23)	0.11 (−0.10, 0.32)	−0.01 (−0.32, 0.30)	0.03 (−1.41, 1.47)	
Q3	31.7	0.04 (−0.16, 0.23)	0.05 (−0.16, 0.25)	0.01 (−0.29, 0.31)	0.23 (−1.10, 1.55)	
P^3		0.002	0.007	0.05	0.07	

[1] Comparison of mean values (and 95% CIs) between the highest (referent) quartile (Q4) and each of the other quartiles. MTHFR, methylenetetrahydrofolate reductase; PLP, pyridoxal 5′-phosphate; PL, pyridoxal; PA, pyridoxic acid. Data were obtained by multiple regression with total homocysteine as the dependent variable. The models were adjusted for age, sex, study center, and concentrations of folate, cobalamin, riboflavin, and creatinine.
[2] P for interaction between *MTHFR* 677C→T genotype and vitamin B-6 vitamer.
[3] P for trend across quartiles of vitamin B-6 vitamers.

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TABLE 4
Difference in plasma cystathionine across quartiles (Q) of vitamin B-6 vitamers and *MTHFR* 677C→T genotypes[1]

		Genotype				
Determinant	Upper cutoff	All genotypes (n = 10 576)	*CC* (n = 5452)	*CT* (n = 4299)	*TT* (n = 850)	P^2
	nmol/L		*nmol/L*			
PLP						
Q1	32.6	47.1 (34.8, 59.3)	41.9 (24.5, 59.2)	52.5 (33.0, 71.9)	40.8 (0.2, 81.4)	0.67
Q2	48.0	33.2 (21.9, 44.4)	37.7 (21.6, 53.8)	30.5 (13.0, 48.1)	17.0 (−19.7, 53.6)	
Q3	73.1	25.5 (14.9, 36.2)	20.1 (4.9, 35.4)	28.4 (11.9, 44.8)	36.5 (1.2, 71.8)	
P^3		< 0.001				
PL						
Q1	7.5	55.9 (44.0, 67.9)	58.1 (41.1, 75.0)	56.4 (37.3, 75.5)	19.9 (−17.4, 57.2)	0.40
Q2	10.0	39.9 (28.5, 51.2)	45.3 (29.1, 61.5)	38.3 (20.4, 56.2)	5.1 (−31.2, 41.4)	
Q3	14.7	32.9 (22.0, 43.8)	32.6 (16.9, 48.3)	33.2 (16.2, 50.1)	22.1 (−12.9, 57.1)	
P^3		< 0.001				
PA						
Q1	15.2	32.5 (20.5, 44.6)	37.4 (20.2, 54.5)	29.8 (10.7, 48.8)	−0.3 (−38.7, 38.0)	0.24
Q2	20.4	31.6 (20.1, 43.1)	39.8 (23.2, 56.3)	24.7 (6.7, 42.8)	0.8 (−36.5, 38.1)	
Q3	31.7	22.4 (11.3, 33.5)	22.2 (6.3, 38.1)	26.0 (8.7, 43.4)	−1.5 (−35.9, 32.8)	
P^3		< 0.001				

[1] Comparison of mean values (and 95% CIs) between the highest (referent) quartile (Q4) and each of the other quartiles. MTHFR, methylenetetrahydrofolate reductase; PLP, pyridoxal 5′-phosphate; PL, pyridoxal; PA, pyridoxic acid. Data were obtained by multiple regression with cystathionine as the dependent variable. The models were adjusted for age, sex, study center, and concentrations of folate, cobalamin, riboflavin, creatinine and methionine.

[2] P for interaction between *MTHFR* 677C→T genotype and vitamin B-6 vitamer were obtained by including product terms between genotype and the vitamer concentration in the multiple linear regression models.

[3] P for trend across quartiles of vitamin B-6 vitamers.

decreasing concentration of PLP, pyridoxal, or PA when investigated in the entire study population (**Table 4**). There was no significant vitamin B-6 vitamer × genotype interaction (P for interaction > 0.24). Notably, the dose-response relation was different from that observed with tHcy, in that cystathionine concentration decreased throughout the concentration range of PLP, pyridoxal, or PA.

We also investigated the possible relations of tCys to PLP by using multiple regression after adjustment for other B vitamins, creatinine, study center, age, and sex. No such relation was observed (data not shown; P for trend = 0.59).

DISCUSSION

We measured the concentrations of various vitamin B-6 species in human plasma and assessed their relation to the metabolites involved in transsulfuration—homocysteine, cystathionine, and cysteine—in a large population of healthy adults. We found strong correlations between the 3 major vitamin B-6 vitamers—PLP, pyridoxal, and PA—all of which showed a relation to other B vitamins, in particular folate and riboflavin. Vitamin B-6 vitamers, especially PLP, were inversely related to tHcy and cystathionine but not to tCys.

Vitamin B-6

We detected PLP, pyridoxal, and PA in all of the plasma samples, and these vitamer concentrations were strongly correlated. Pyridoxine and pyridoxamine were found in 1.9% and 0.9% of the samples, respectively. The very high PLP (10, 11), pyridoxal (10, 11, 58), and PA (10, 11, 58) concentrations observed in some samples are most likely caused by the recent intake of high doses of vitamin B-6, although we do not have vitamin supplementation data to verify that possibility. Nonfasting populations are expected to show a greater variation in vitamin B-6 concentrations than are fasting populations, because higher vitamin B-6 concentrations may be attained after a recent meal containing vitamin B-6 and also after the ingestion of a vitamin supplement, which sometimes accompanies a meal. The large variation in PLP, pyridoxal, PA, and pyridoxine at high total vitamin B-6 concentrations could be explained by variable vitamin B-6 intakes and the incomplete conversion of pyridoxine to other forms after recent supplementation because the conversion of pyridoxine to other vitamin B-6 forms takes a few hours (5, 6, 58). The presence of pyridoxamine in some samples was always accompanied by very high PLP, pyridoxal, and PA concentrations and sometimes also by high pyridoxine concentrations, which suggests that it is related to a recent intake of a supplement containing pyridoxine. The faster and stronger increases in plasma concentrations of pyridoxal and PA than in those of PLP that are induced by recent vitamin B-6 supplementation (2, 5, 7) may explain the increased strength of PLP-pyridoxal and PLP-PA to relations with increasing PLP concentrations (Figure 1).

The 3 main vitamin B-6 species showed a moderate correlation with the concentrations of other B vitamins, in particular folate and riboflavin. This correlation is probably due to overlapping dietary sources of these 3 B vitamins, including fruit and vegetables (59). The weak association with cobalamin is probably explained by the fact that cobalamin is mainly derived from food items other than fruit and vegetables—primarily, animal products (59).

Of the vitamin B-6 vitamers, PA showed the strongest relation to creatinine. This finding is in agreement with published data showing that PA is sensitive to renal function (23) and that it accumulates during renal failure (60).

The associations of vitamin B-6 vitamers with other B vitamins and renal function indicate that these factors are potential confounders in investigations of the relation of vitamin B-6 status and clinical outcomes or metabolite concentrations. It has been suggested that the ratio of PA to pyridoxal can distinguish between increases in PA concentrations that are due to increased dietary intake and those that are due to renal impairment (60).

Homocysteine

The influence of vitamin B-6 on tHcy is moderate in this study and is present only at vitamer concentrations in the lowest quartile, which agrees with findings of a previous study (25). We also found that this relation was strongest for PLP and pyridoxal in the *TT* group. The genotype effects may explain why most authors report no PLP-tHcy relation (27–35). This also agrees with the fact that most studies report no effect of vitamin B-6 supplementation on fasting plasma tHcy concentrations (29, 33, 38–45).

PLP serves as the cofactor of cystathionine β-synthase (24), which could partly explain the inverse relation between vitamin B-6 and plasma tHcy. Vitamin B-6 nutrition may also affect homocysteine status by influencing the folate-metabolizing enzyme serine hydroxymethyltransferase (61).

Cystathionine and cysteine

All 3 major vitamin B-6 forms—in particular, PLP and pyridoxal—were inversely related to cystathionine concentrations. This suggests that cystathionine degradation catalyzed by cystathionine γ-lyase is the rate-limiting step in transsulfuration. Cystathionine was found to be inversely related to the concentration of the major B-6 vitamer forms throughout their concentration ranges, which is consistent with the linear relation of PLP concentrations to cystathionine γ-lyase activity in the liver of rats (62). Thus, the dose response differed from that observed for tHcy, which increased only at low vitamin B-6 vitamer concentrations. Such differences between the relations of vitamin B-6 status to homocysteine and to cystathionine are in accordance with the greater sensitivity of cystathionine γ-lyase enzyme than of cystathionine β-synthase to vitamin B-6 status (35, 62–64).

Plasma concentrations of cystathionine increase (65) and those of PLP decrease (66, 67) in the hours after the consumption of proteins, and recent protein intake may therefore enhance the inverse relation of vitamin B-6 to cystathionine. Conversely, vitamin B-6 intake, either from food or vitamin supplement, may counteract this short-term effect.

In accordance with published reports, we observed no relation between PLP and the concentration of tCys (23, 35, 47). However, this observation allows no inference about the role of transsulfuration in cysteine homeostasis, partly because tCys is mainly protein-bound in plasma and undergoes complex displacement and disulfide exchange reactions with homocysteine (68). Furthermore, cysteine is a component of dietary protein and is obtained from food.

MTHFR 677C→T polymorphism

We observed that plasma tHcy increased and folate decreased according to the number of *MTHFR* 677T alleles. A folate × *MTHFR* 677C→T genotype interaction as a determinant of plasma tHcy has been shown in numerous studies (48, 69). The association of PLP (70), pyridoxal, and PA with tHcy is modified by the *MTHFR* 677C→T genotype. Thus, vitamin B-6 shares this effect modification with other nonfolate B vitamins involved in homocysteine metabolism, including riboflavin (69) and cobalamin (71). A likely explanation is that impaired 5-methyltetrahydrofolate formation and homocysteine remethylation in the *TT* genotype direct homocysteine to the transsulfuration pathway.

Of the vitamin B-6 vitamers, only PLP had its lowest concentrations in subjects with the *TT* genotype, and this difference between genotypes was modest compared with that found for folate in these subjects. One may speculate whether a lower PLP concentration reflects a greater flux through the transsulfuration pathway in subjects with the *TT* genotype. Likewise, it has been speculated that greater metabolic activity decreases the concentrations of cofactors involved, including vitamin B-6 (72).

Neither the cystathionine concentration nor the relation of vitamin B-6 to cystathionine was modified by the *MTHFR* 677C→T genotype. This finding agrees with the fact that MTHFR and related folate species are not involved in cystathionine metabolism (24).

Conclusions

In this study, we showed that plasma concentrations of the main vitamin B-6 vitamers were strongly correlated but also had a moderate association with other B vitamins, in particular folate and riboflavin, that was due to overlapping dietary sources of these vitamins. The population size was large enough to provide precise estimates of the metabolic effects of differences in vitamin B-6 status on the plasma concentrations of tHcy and cystathionine. These associations were in accordance with experimental data on the role of PLP as a cofactor for cystathionine β-synthase and cystathionine γ-lyase. PLP and pyridoxal had the strongest association with these transsulfuration metabolites, which may reflect the role of PLP as cofactor and the ability of pyridoxal to cross cell membranes (3, 14, 15). The inverse relation between PLP and tHcy was strongest and the PLP concentration was lowest in subjects with the *MTHFR* 677TT genotype, possibly because of impaired homocysteine remethylation and increased flux through the transsulfuration pathway. Thus, the present study shows that the transsulfuration metabolites in humans reflect the role of vitamin B-6 as a cofactor for cystathionine β-synthase and cystathionine γ-lyase.

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