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Effect of vitamin B-6 nutrition and diabetes on vitamin B-6 metabolism

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Vitamin B-6 content, some B-6 dependent enzymes, and alkaline phosphatase were examined in rat tissues subjected to different nutritional conditions and treated with or without streptozotocin. There was almost no B-6 in the plasma from the nutritionally deficient rat. The ratio of pyridoxal phosphate to pyridoxal in the plasma was decreased by injection of streptozotocin and was inversely correlated to an increase in alkaline phosphatase activity. Vitamin B-6 content in the liver of the nutritionally deficient rats was approximately 50% of that of the controls and this decrease correlated to aspartate aminotransferase activity in this tissue. Vitamin B-6 content in the skeletal muscle from the nutritionally deficient rats decreased to less than 20% of the control level and this decreased level correlated to the glycogen phosphorylase activity in this tissue. © Elsevier Science Inc. 1997
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Introduction

Vitamin B-6 is found in biological tissues and fluids as pyridoxine (PN), pyridoxal (PL), pyridoxamine (PM), and their phosphorylated derivatives. Phosphate esters of dietary vitamin B-6 are hydrolyzed before absorption and are transformed into Pyridoxal phosphate (PLP) in the intestinal wall. PLP is then again dephosphorylated and appears in the form of PL in the blood stream.[1] In plasma, B-6 vitamers occur mainly as PL and PLP, and this PLP arises primarily from liver, probably in association with the secretion of albumin.[2] PLP in plasma is incorporated into various organs and tissues after dephosphorylation, and is again transformed into an active form such as PLP or pyridoxamine phosphate (PMP).

In the course of the study on the glycation reaction of aspartate aminotransferase (AST) [EC2.6.1.1][3,4] we found a decrease in the ratio of PLP/PL in plasma from rats treated with streptozotocin (stz), which seemed to be related to the levels of alkaline phosphatase (ALP) activity in the plasma.

In this study we report the relationship between vitamin B-6 levels and alkaline phosphatase (ALP) in plasma, and also the distribution of the B-6 vitamers in various tissues of rats subjected to diet with different vitamin B-6 content and treated with or without stz.

Methods and materials

Animals

Twenty-four male weanling rats of the Wistar strain were divided into four groups. Groups 1 and 2 were fed a vitamin B-6 free diet and groups 3 and 4 were fed a complete diet ad libitum for 4 weeks. The diet contained 20% casein with or without 2.9 mg/kg pyridoxine (Harper composition, Oriental Yeast, Tokyo, Japan). The complete composition of the diet has been described previously.[5] After 1 week, groups 2 and 4 were injected with intraperitoneal stz (80 mg/kg stz) in citrate buffer, pH 4.5 (0.5 mL of 16 mg/mL per 100 g body weight) and groups 1 and 3 were administered vehicle and served as controls.

Chemicals

All reagents used were of the highest grade available and were purchased from Wako Pure Chemicals (Osaka, Japan), Boehringer (Mannheim, Germany), Sigma Chemicals Co. (St. Louis, MO, US), or Oriental Yeast Co. (Osama, Japan). All dietary materials were purchased from Oriental Yeast Co.

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Preparation

Rats were decapitated and blood was collected into heparinized tubes. A portion of the blood was used to analyze glucose and hemoglobin, and the remaining blood was centrifuged at 2,000 rpm for 10 min. The resultant precipitate was washed with equal volumes of saline twice and suspended in 2 volumes of water. The suspension was then subjected to sonic oscillation and the supernatant, centrifuged at 10,000 × g for 20 min, was used to measure hemoglobin, glycohemoglobin, and AST activity. The plasma fraction was used for the determinations of ALP and B-6 vitamers. Plasma (0.5 mL) was diluted to 1 mL with water, 27 μl of 9N-HClO was added, mixed for 1 min using a vortex, centrifuged at 10,000 × g for 5 min, and the resultant supernatant was used for analysis of vitamin B-6. Rat tissues were immediately removed, divided into two or three pieces depending on the analysis and, except for a piece of liver tissue for the measurement of AST, all tissue samples were frozen in liquid nitrogen. Pieces of liver and muscle tissue were each homogenized with 9 volumes of 1N-HClO and the supernatants were used to analyze vitamin B-6 contents. Another piece of liver was homogenized with 9 volumes of 0.25 M sucrose-5 mM phosphate-0.1 mM EDTA (pH 7.4) and the supernatant, centrifuged at 40,000 × g for 60 min, was used to analyze AST activity. Similarly, another piece of muscle was homogenized with 9 volumes of 0.1 M PIPES [piperazine-N-N'-bis(2-ethanesulfonic acid)] buffer, pH 6.0, containing 0.5 mM EDTA, 57 mM 2-mercaptoethanol, and 0.1 M sodium fluoride. The homogenate was centrifuged at 10,000 × g for 20 min and the resulting supernatant was used to analyze phosphorylase activity.

Methods

B-6 vitamers were determined using an HPLC system according to the method originally described by Edwards et al.[6] and modified by Yagi et al.[7] as previously described.[8] The mobile phase consisted of 0.075 M sodium phosphate-0.075 M sodium perchloride buffer, pH 3.38, containing 8.5 mL acetonitril and 0.5 mL triethanolamine per liter. To enhance the fluorescence of PLP, sodium bisulphite (1 g/L) in 0.25 M sodium phosphate adjusted to pH 11.7 was used as the post-column reagent. A Shimazu RF-535 fluorescence monitor was used for detecting the fluorescence of vitamin B-6. The excitation wavelength was 325 nM and the emission wavelength was 420 nM. Glycogen phosphorylase was determined in the direction of glycogen formation as previously described.[9] The assay solution contained 1% glycogen, 20 mM glucose-1-phosphate in PIPES buffer. Assays were performed at 30°C in the presence of 1 mM AMP. Inorganic phosphate released during the reaction was assayed by the method of Fiske-Subbarow.[10] AST activity was determined by a modification of the method of Karmen.[11] The reaction mixture contained 0.1 mL of 0.5 M aspartate, 0.1 mL of 0.2 M 2-oxo-glutarate, 0.3 mg of NADH, and 5 U of malate dehydrogenase in 3 mL. The reaction products were measured with a spectrophotometer (at 340 nM; Hitachi U-3000). ALP, glucose, and glycohemoglobin were determined using Wako Kits: Alkaliphospha B-test Wako (ALP), Glucose B-test Wako (glucose), and Glycohemoglobin B-test Wako (glycohemoglobin).

Statistical analysis

Analysis of variance was performed to determine whether there were significant ($P < 0.05$) differences among the groups. The Student Newman Keuls multiple comparison test was used to determine which means were significantly different. All data are presented as the mean of each group.

Results

Effects of vitamin B-6 deficiency on stz injection and blood glucose, hemoglobin, and plasma vitamin B-6

Blood glucose and HbA1c were determined and the results obtained are shown in *Table 1*. The blood glucose level was lower in vitamin B-6 deficient rats (group 1) than in control rats (group 3). These levels were elevated by stz injection in both groups 2 and 4. HbA1c levels correlated to the blood glucose of each group.

Effects of vitamin B-6 deficiency and stz injection on the B-6 contents in rat tissues

Vitamin B-6 contents were measured in rat tissues, i.e., plasma, liver, and gastrocnemius muscle (*Table 2*). In the plasma, PLP was the main form in the control rats (group 3), although both PLP and PL were detected. The PLP/PL ratio changed from 1.76 to 0.41 after injection of stz (group 4). On the other hand, total B-6 vitamer contents (PLP + PL) increased to approximately 150% of that of controls. In the plasma of rats fed a vitamin B-6 deficient diet, low B-6 levels were detected and data obtained are shown in parentheses, because these levels are below the detection limit (groups 1 and 2). In the liver, PMP and PLP were the main form of B-6 compounds detected and the contents were elevated by stz injection (groups 3 and 4). B-6 vitamer content in the nutritionally deficient groups (groups 1 and 2) was approximately 50% of that of the controls (group 3) and no effect of stz was observed. In the muscle, PLP was the main B-6 compound; however, some PMP was also detected. The B-6 vitamer in rats fed the deficient diet was less than 20% of the controls and the contents in both vitamin B-6 deficient and control groups were not affected by stz injection.

Table 1 Effect of Vitamin B-6 deficiency and stz on blood glucose and hemoglobin

Group	1	2	3	4	Q0.05sx
Vitamin B-6	—	—	+	+	
stz (80 mg/kg)	—	+	—	+	
Blood glucose (mg/dl)	118.4 ± 4.97[a]	445 ± 64.0[b]	145 ± 6.32[a]	662 ± 165[b]	212
HbA1c	3.19 ± 0.16[a]	10.56 ± 1.64[b]	3.29 ± 0.20[a]	13.32 ± 1.34[b]	4.27
Hb(g/dl)	14.5 ± 0.9[a]	16.1 ± 1.8[a]	15.0 ± 0.7[a]	16.1 ± 2.0[a]	2.9

Within a row, values with different superscripts are significantly different ($P < 0.05$). Values are means ± SD.

Table 2 Effects of Vitamin B-6 and stz on vitamin B-6 contents

Group	1	2	3	4	Q0.05sx
Plasma (nmol/L)					
PLP	(15 ± 6)	(34 ± 33)	625 ± 70[a]	415 ± 7[b]	210
PL	(2 ± 2)	(6 ± 4)	355 ± 80[a]	1,002 ± 190[b]	280
Total	(17 ± 7)	(40 ± 36)	980 ± 117[a]	1,417 ± 250[b]	378
% of control*			100	144.6	
PLP/PL	(7.5)	(5.7)	1.76	0.41	
Liver (nmol/g)					
PMP	16.22 ± 1.71[b]	16.11 ± 3.87[b]	23.69 ± 2.07[a]	42.39 ± 2.52[c]	5.64
PLP	12.24 ± 0.96[b]	9.98 ± 1.59[b]	32.42 ± 1.59[a]	41.85 ± 2.68[c]	3.82
Total	28.47 ± 1.87[b]	26.09 ± 5.47[b]	56.11 ± 2.29[a]	84.24 ± 0.83[c]	6.47
% of control*	50.7	46.5	100	150.1	
Muscle (nmol/g)					
PMP	1.35 ± 0.20[b]	1.62 ± 0.69[b]	6.91 ± 0.75[a]	6.53 ± 0.39[a]	1.27
PLP	8.09 ± 0.88[b]	10.66 ± 3.49[b]	44.10 ± 2.97[a]	45.43 ± 6.50[a]	7.54
Total	9.44 ± 1.01[b]	12.28 ± 4.17[b]	51.01 ± 3.37[a]	51.90 ± 6.94[a]	8.36
% of control*	18.5	24.1	100	101.9	

Within a row, values with different superscripts are significantly different ($p < 0.05$).
*: Group 3.
Values are means ± SD.

Effects of vitamin B-6 deficiency and stz injection on enzyme activity

ALP activity in the plasma was elevated by stz injection in both vitamin B-6 deficient and control rats as shown in *Table 3*. This elevation of ALP reflected the difference of the ratios of PLP/PL in the plasma from rats treated with and without stz. AST activity in the presence or absence of PLP was determined in erythrocytes, liver, and muscle (*Table 3*). In erythrocytes, the activity was greatly decreased in vitamin B-6-deficient rats and slightly increased by the addition of PLP. Hepatic AST activity was induced by stz injection in both vitamin B-6-deficient and control rats. In the skeletal muscle, AST activity was elevated by the addition of PLP in rats under all conditions (groups 1 to 4). Phosphorylase activity, which is considered to be the most important PLP enzyme in muscle, was measured in the presence of AMP. Unlike AST activity in the liver and muscle, phosphorylase activity from rats fed a vitamin B-6-deficient diet was less than 15% of that from the control rats.

Discussion

In this study we investigated metabolism of B-6 vitamers in rats under different vitamin B-6 dietary conditions, treated with or without stz. When B-6 vitamers are ingested orally at a physiological level, they are known to appear in the portal vein in the form of PL, regardless of the form ingested.[1] In fact, B-6 vitamers in the plasma consist of PL

Table 3 Effects of vitamin B-6 and stz on enzyme activity

Group	1	2	3	4	Q0.05sx
AP (μ kat/L)	2.2 ± 0.7[a]	5.1 ± 2.1[a]	2.9 ± 0.2[a]	11.8 ± 4.4[b]	5.23
AST					
Erythrocyte (nkat/gHb)					
−PLP	112 ± 20[b]	102 ± 23[b]	1,030 ± 163[a]	878 ± 200[a]	285
+PLP	258 ± 48[b]	260 ± 45[b]	1,100 ± 216[a]	867 ± 77[a]	293
EAST	2.32 ± 0.27[b]	2.61 ± 0.59[b]	1.08 ± 0.13[a]	1.02 ± 0.19[a]	1.03
Liver (nkat/g tissue)					
−PLP	101 ± 38[a]	330 ± 233[a]	252 ± 103[a]	1,247 ± 333[b]	415
+PLP	131 ± 68[a]	923 ± 483[b]	285 ± 75[a]	1,263 ± 183[c]	678
+PLP/−PLP	1.31 ± 0.39[a]	3.53 ± 1.49[b]	1.24 ± 0.40[a]	1.05 ± 0.16[a]	2.1
Muscle (nkat/g tissue)					
−PLP	29 ± 6[b]	56 ± 22[bc]	107 ± 40[ac]	108 ± 43[a]	51.3
+PLP	357 ± 47[b]	363 ± 47[b]	485 ± 83[a]	533 ± 72[a]	108
+PLP/−PLP	12.7 ± 2.9[b]	7.5 ± 1.9[ab]	5.9 ± 4.4[a]	6.1 ± 3.2[a]	6.6
GP(nkat/g tissue)					
−PLP	242 ± 21[b]	215 ± 28[b]	1,609 ± 157[a]	1,614 ± 272[a]	358
+PLP	239 ± 16[b]	220 ± 25[b]	1,575 ± 25[a]	1,558 ± 177[a]	273
+PLP/−PLP	0.99 ± 0.10[a]	1.12 ± 0.13[a]	0.98 ± 0.06[a]	0.97 ± 0.07[a]	1.0

Within a row, values with different superscripts are significantly different ($p < 0.05$). Values are means ± SD.

absorbed through the intestinal wall and PLP, which is secreted from liver in a form bound to albumin. We found a decrease in the ratio of PLP/PL in the plasma in rats treated with stz. On the other hand, ALP activity in the plasma was elevated in the rats treated with stz. Recently, elevated plasma levels of PLP in cases of hypophosphatasia and also in zinc-deficient rats have been reported.[12–14] The elevated PLP levels in the plasma result in vitamin B-6 deficiency because PLP cannot cross plasma membranes, but first must be dephosphorylated to PL before it can enter tissues. ALP is known to non-specifically react with many phosphorous compounds, including PLP.[15] As PLP in the plasma must be dephosphorylated into PL by means of ALP, we attempted to determine if a relationship existed between the ratio of PLP/PL and ALP in the plasma from rats treated with and without stz. As shown in *Figure 1*, a high inverse correlation coefficient ($r = -0.85$) was obtained between PLP/PL and ALP activity. B-6 vitamer levels in the liver from the deficient rats decreased to approximately half of that of the controls and stz injection had no effect, whereas in the rats given a vitamin B-6 containing diet the B-6 levels were increased by stz injection. Although hepatic AST activity in the vitamin B-6 deficient rats (group 1) decreased to approximately half of that of controls (group 3), the activity without PLP from the deficient rats treated with stz (group 2) was maintained at a rather high level compared with that from the group 3, suggesting that PLP may be used for AST preferentially, rather than the other B-6 enzymes. In diabetes, it is well known that an increase in AST activity in the liver is due to increased enzyme synthesis. In both the controls (group 2) and the vitamin B-6-deficient rat (group 4), stz injection induced hepatic AST activity because in both groups AST activity was similar when PLP was added. There are some reports on an alteration of vitamin B-6 metabolism in type I diabetes.[16–18] Rogers et al.[16] reported a reduction of plasma PLP and an increase in cytosolic AST activities in diabetic rats. Their observation is consistent with our data; however, they did not determine plasma PL. Ellis et al.[17] reported an association of a deficiency of vitamin B-6 with diabetes by monitoring the erythrocyte AST activity. AST, which has an important role for the gluconeogenic pathway, may have some relation to insulin deficient diabetes through vitamin B-6 metabolism. Vitamin B-6 content in the muscle from the deficient rats was approximately 20% of that of controls and the decreased levels were lower than that found in the liver.

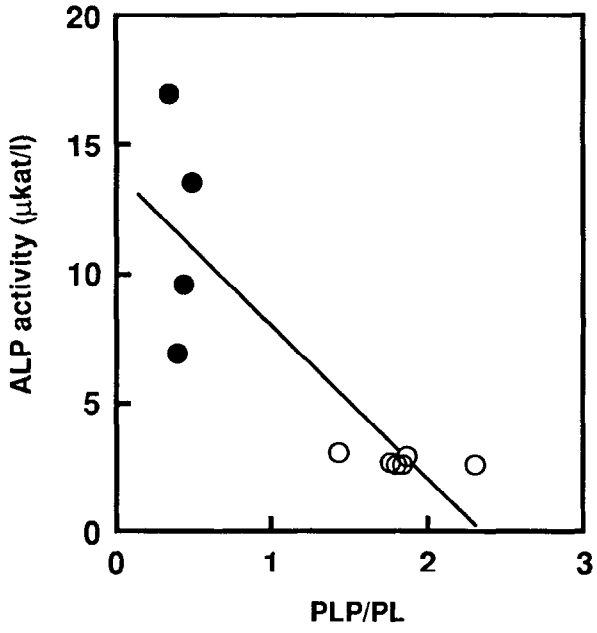

Figure 1 Relationship between ALP and PLP/PL in the plasma from rats treated with or without stz ($n = 10$, $r = 0.85$, $P < 0.001$). Data from rats treated with (closed circles) and without (open circles) stz. Experimental conditions are described in the text.

Phosphorylase activity in vitamin B-6-deficient rats decreased in parallel with the decrease of the B-6 content. On the contrary, the decrease in AST activity in the muscle was to much lesser extent compared with phosphorylase activity, suggesting that PLP may be preferentially used for AST molecule, rather than for the phosphorylase molecule also found in this tissue. On the other hand, AST activity in muscle was elevated when determined in the presence of PLP, even in control rats. The reason for such high amounts of free apo-enzyme unbound to PLP in the rats with normal vitamin B-6 nutritional status is unclear.

From the results obtained, we conclude that in diabetic conditions PL is preferentially incorporated into the liver and utilized there, and in the vitamin B-6-deficient conditions, PL is also preferentially incorporated into the liver and then in other tissues, such as muscle, until PLP or PL in the plasma is exhausted. Also, PL incorporated into the tissues may be preferentially used by AST in the liver and muscle, especially under a limited nutritional status of vitamin B-6.

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