Effect of aging on the bioavailability of vitamin B-6 in rats

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An age-related decline in vitamin B-6 status has been demonstrated in humans and rodents. In this study, the bioavailability of vitamin B-6 was evaluated in 3- and 20-month-old, male Fischer 344 rats by administration of a single, oral dose of [^{3}H]pyridoxine. In addition, plasma pyridoxal 5'-phosphate concentration and alkaline phosphatase and erythrocyte aspartate aminotransferase activity were measured. Plasma pyridoxal phosphate concentration was 75% lower in the aged rats compared with the 3-month-old rats. Plasma alkaline phosphatase activity was reduced and erythrocyte aspartate aminotransferase activity increased in the aged rats. There was no difference in the stimulation of erythrocyte aspartate aminotransferase activity with pyridoxal phosphate. Approximately 99% of the radioisotope was absorbed by rats from both age groups. No differences were observed in the tissue distribution of [^{3}H]vitamin B-6 between the two age groups or the retention of radioactivity by the various tissues examined. The urinary excretion of total ^{3}H, as well as [^{3}H]4-pyridoxic acid, was not different between the two age groups. Reduced plasma pyridoxal phosphate concentration with aging does not appear to be the result of decreased intestinal absorption of vitamin B-6, increased plasma alkaline phosphatase activity, or altered metabolic utilization of vitamin B-6. Based on this information, plasma pyridoxal phosphate concentration may not be an adequate assessment parameter for vitamin B-6 status as affected by aging.

Keywords: aging; vitamin B-6; bioavailability

Introduction

Several studies have demonstrated a decline in vitamin B-6 status with aging in humans, as indicated by plasma pyridoxal 5'-phosphate (PLP) concentration.[1-3] An age-related decline in plasma PLP concentration has also been observed for rats.[4,5] However, erythrocyte aspartate aminotransferase (EAST) activity was increased with aging.[4] Reduced vitamin B-6 status in aged rats was shown not to be a result of reduced intake of vitamin B-6.[4,5] Bode et al.[4] reported alterations in the vitamin B-6 content of several tissues with aging, and this finding was suggested to be a result of changes in protein metabolism.[4] Glycogen phosphorylase activity, however, was not altered with aging in the muscle, liver, or brain.[5] Recently, Bode et al.[6] observed no age-related differences in the biokinetics of vitamin B-6 in rats. However, differences were observed in the tissue levels of [^{14}C]B-6 vitamers after oral administration.

The cause for reduced plasma PLP concentration with aging is not clearly understood. Some suggestions for an age-related reduction in plasma PLP concentration have been made, including reduced intestinal absorption, alterations in plasma alkaline phosphatase (PALP) activity, and alterations in erythrocyte vitamin B-6 metabolism.[7] Plasma PLP has been reported to reflect tissue PLP levels, and therefore is used as a primary assessment parameter for vitamin B-6 status.[8,9] Nutrient status can be influenced by the bioavailability (intestinal absorption and metabolic utilization) of the nutrient, as well as the extent of urinary excretion of the nutrient. In this study, [^{3}H]pyridoxine was orally administered to 3- and 20-month-old Fischer 344 rats for direct assessment of vitamin B-6 bioavailability. Results of this study will identify possible age-related changes in vitamin B-6 bioavailability. Furthermore, this study will investigate the adequacy of plasma PLP as a parameter for vitamin B-6 status as affected by aging.

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Materials and methods

Reagents

All vitamin B-6 standards, 1-octane sulfonic acid, aspartate aminotransferase, alkaline phosphatase, bovine hemoglobin standard, and hemoglobin assay kits were purchased from Sigma Chemical Co. (St. Louis, MO USA). [^{3}H]Pyridoxine hydrochloride (3.75 Ci/mmol) was purchased from Amersham Inc. (Arlington Heights, IL USA). The distribution of tritium was methyl, 61.9%; 5-methylene, 2.0%; 4-methylene, 21.9%; C-6, 11.9%. Water was purified for chromatographic use with a Nanopure II system (Barnstead Co., Newton, MA USA). 2-Propanol, formic acid, ethyl ether, and phosphoric acid were purchased from Baxter Scientific Co. (McGraw Park, IL USA). Scintillation cocktail (Ecolite) was purchased from ICN Biomedicals (Irvine, CA USA).

Animals and diets

Male Fischer 344 rats, 3- and 20-months old, were purchased from Harlan Sprague-Dawley, Inc. (Indianapolis, IN USA). They weighed approximately 300 g and 450 g, respectively. All rats were housed individually in stainless-steel metabolism cages and were fed, ad libitum, a semi-purified diet (ICN Biochemicals, Costa Mesa, CA USA), which contained sucrose, 62.7%; casein, 18%; cottonseed oil, 10%; cellulose, 5%; mineral mix (ICN Salt Mixture No. 2), 4%; and vitamin mix (ICN Vitamin Diet Fortification Mixture). The diet contained 7 mg pyridoxine HCl/kg diet. After 8 days on the semi-purified diet, all rats were orally administered [^{3}H]pyridoxine HCl (1 μCi/kg body weight). A 48-hour fecal collection and 24-hour urine samples were collected prior to euthanasia. Rats were euthanized 2 or 5 days after oral administration of the isotope. Blood and tissue samples were collected following decapitation.

Sample preparation

Plasma was obtained after centrifugation of heparinized blood. An equal volume of trichloroacetic acid (14%) was added to the plasma followed by centrifugation. The supernatant was extracted with an equal volume of ethyl ether for partial removal of the trichloroacetic acid and lipids. Urine samples were deproteinated by ultrafiltration with micropartition tubes and YMT membrane filters (Amicon, Danvers, MA USA). For measurement of radioactivity, fecal and tissue samples were homogenized in water and total homogenate volumes were recorded. For high performance liquid chromatography (HPLC) analysis, 1 g of liver was homogenized in an equal volume of deionized water. An equal volume of trichloroacetic acid (14%) was added to the homogenate for protein precipitation. The supernatant was extracted twice with an equal volume of ethyl ether. Hemolysates of red blood cells were prepared by lysis of red blood cells in water, followed by centrifugation.

HPLC equipment and method

Chromatographic analyses were conducted with a Rainin HP Drive module (Rainin Instrument Co., Woburn, MA USA), sample injection valve (Rheodyne, model 7125), a fluorescence detector (Model LS 40, Perkin Elmer, Norwalk, CT USA), a Dynamax HPLC Method Manager Integration Software Program (Rainin Instrument Co., Woburn, MA USA) and MacIntosh SE computer (Apple Computer, Inc., Cupertino, CA USA).

Plasma PLP was measured by use of one mobile phase containing 8 mmol/L octane sulfonic acid and 0.033 mmol/L potassium phosphate, pH 2.2, and employing an Ultrasphere C_{18}-IP column (Beckman).[10] The wavelengths for fluorometric detection were 295 nm for excitation and 405 nm for emission.

Hepatic B-6 vitamer analyses were conducted by HPLC using a gradient elution method[10] consisting of 2 mobile phases and an Ultrasphere C_{18}-IP column (Beckman). Mobile phase A consisted of 8 mmol/L octane sulfonic acid and 0.033 mmol/L phosphoric acid, pH 2.2. Mobile phase B consisted of 17% isopropanol and 0.033 mmol/L phosphoric acid, pH 2.2.

Measurement of radioactivity

Fecal and tissue homogenates, urine 4-pyridoxic acid (4-PA), and liver HPLC fractions were measured for radioactivity in scintillation fluid employing a liquid scintillation spectrophotometer (Beckman LS 1800). A quench curve for tritium was used to correct for quenching. Radioactivity of samples was expressed as percent of oral dose or percentage of distribution of total radioactivity.

Spectrophotometric analysis

EAST and PALP activity was determined by measuring the change in absorbance at 340 nm and 405 nm, respectively. Exogenous PLP was added to the erythrocyte hemolysates to give a final concentration of 0.08 mmol/L to assess the EAST activity coefficient.[11] Hemoglobin (Hbg) levels of red blood cell hemolysates were determined spectrophotometrically at 540 nm.[12]

Statistical analysis

Mean values were analyzed by one-way or two-way analysis of variance.[13]

Results

Food intake and plasma PLP concentrations

There was no significant difference in food intake between the 3- and 20-month-old rats *(Table 1)*. Vitamin B-6 status, as determined by plasma PLP concentration, was 75% lower in the aged rats compared with the 3-month-old rats. There was a 31% increase in EAST activity in the 20-month-old rats compared with the 3-month-old rats. There was no difference in the EAST activity coefficient. PALP activity was significantly lower in the aged rats.

Table 1 Food intake, plasma pyridoxal phosphate, and erythrocyte aminotransferase and alkaline phosphatase activity for 3- and 20-month-old rats*†

Age	3 Months	20 Months
Food intake (g/day)	16 ± 8	14 ± 6
Plasma PLP (nmol/L)	295 ± 78	80 ± 38[a]
EAST (U/g Hbg)	14.9 ± 2.1	21.6 ± 5.9[a]
EAST coefficient	1.6 ± 0.1	1.5 ± 0.2
Alkaline phosphatase (U)	251 ± 41	154 ± 29[a]

*Values represent the mean ± SD of seven rats per age group.
†Values within each row followed by a different superscript were significantly different ($P < 0.05$).

Radioactivity in feces and tissues.

Approximately 99% of the oral dose was absorbed, as indicated by 1% or less of the radioactivity in the 48-hour post-administration fecal samples for both age groups *(Table 2)*. The level of radioactivity excreted in the feces was slightly but significantly lower in the aged rats. No significant differences were observed in the level of radioactivity for the liver, muscle, kidney, brain, or plasma between the two age groups *(Table 2)*. The level of radioactivity in the muscle remained constant with time for both age groups. Losses in radioactivity with time were observed for kidney, liver, and plasma and were similar for both age groups. No age-related differences were observed in the distribution of radioactivity among the B-6 vitamers in the liver *(Table 3)*.

Urinary excretion of [^{3}H]vitamin B-6

For both age groups, approximately 8% of the oral dose was excreted in the urine within 1 day after administration of the isotope *(Table 4)*. By day 5, almost 20% of the oral dose was excreted by the rats, regardless of age. There was no significant difference in the rate of excretion of vitamin B-6 with respect to age. The percent of radioactivity in the urine that was excreted as [^{3}H]4-PA was not significantly different between the two age groups.

Discussion

Factors that can influence nutrient status include dietary intake, bioavailability of the nutrient, and physiological status. The primary parameter used for the assessment of vitamin B-6 status is plasma PLP concentration.[14] A decline in plasma PLP concentration has been reported for rats aged 3 weeks to 25 months.[5,7] To assess the effect of aging on vitamin B-6 status and bioavailability, 3- and 20-month-old rats were fed a purified diet 8 days prior to administration of a single oral dose of [^{3}H]PN and euthanized 2 or 5 days after administration.

In agreement with the findings of Cochary et al.,[5,7] we demonstrated a significant decrease in plasma PLP concentration between the 3- and 20-month-old Fischer 344 rats. Possible mechanisms suggested for the decreased plasma PLP concentration with aging include decreased intestinal absorption, increased PALP activity, and altered erythrocyte vitamin B-6 metabolism.[7]

Similar to our findings, PLP-dependent EAST activity was reported to increase with age, without a difference in the EAST activity coefficient.[4] The lack of difference in hemoglobin levels with aging[4] would infer that there is a greater level of enzyme in the erythrocyte, resulting in greater EAST activity. Thus, there may be a greater concentration of PLP in the erythrocyte of the aged rat without a difference in the portion of EAST bound to PLP. The sequestering of PLP by erythrocytes has been proposed as a contributing factor for the decrease in plasma PLP and increase in erythrocyte PLP observed in pregnant mice.[15] Aging does not influence the blood hematocrit in rats.[4]

Table 2 [^{3}H]Vitamin B-6 in rat excreta and tissues*†‡

Age	Percentage of oral dose (%)	
	3 Months	20 Months
Feces		
Day 2	1.1 ± 0.2	0.7 ± 0.2
Liver		
Day 2	8.2 ± 0.9[a]	8.1 ± 1.1[a]
Day 5	4.4 ± 1.4[b]	4.3 ± 1.4[b]
Muscle		
Day 2	2.8 ± 1.0[a]	2.6 ± 0.3[a]
Day 5	2.6 ± 0.3[a]	2.6 ± 0.3[a]
Kidney		
Day 2	0.25 ± 0.07[a]	0.30 ± 0.05[a]
Day 5	0.14 ± 0.04[b]	0.16 ± 0.01[b]
Brain		
Day 2	0.33 ± 0.11[a]	0.29 ± 0.11[a]
Day 5	0.42 ± 0.20[b]	0.42 ± 0.13[b]
Plasma		
Day 2	1.37 ± 0.12[a]	1.27 ± 0.17[a]
Day 5	0.99 ± 0.53[b]	0.78 ± 0.09[b]

*Values represent means ± SD of three to seven rats per group.
†Values within each column followed by a different superscript were significantly different ($P < 0.05$) with respect to time.
‡Values were not significantly different ($P > 0.05$) with respect to age.

Table 3 Percent distribution of [^{3}H]vitamin B-6 in liver*†

B-6 Vitamins	Percent (%)	
	3-Month-old-rats	20-Month-old-rats
PLP	24.3 ± 7.5	27.0 ± 3.0
4-PA	20.0 ± 10.5	21.0 ± 9.6
PMP	20.0 ± 3.0	24.7 ± 3.2
PL	5.7 ± 4.1	3.0 ± 3.5
PN	3.0 ± 2.0	2.3 ± 2.3
PM	24.3 ± 8.4	22.7 ± 9.3

*Values represent means ± SD of three livers per age group.
†Values were not significantly different ($P > 0.05$) between the two age groups.

Table 4 Urinary excretion of [^{3}H]Vitamin B-6*†

Age	Percentage of oral dose (%)	
	3 Months	20 Months
Day 1	8.8 ± 2.6	8.1 ± 1.8
Day 2	4.9 ± 1.0	7.1 ± 0.6
Day 3	2.4 ± 0.8	2.7 ± 0.4
Day 4	1.2 ± 0.2	1.6 ± 0.2
Day 5	0.6 ± 0.06	0.8 ± 0.4
Percent of urinary radioactivity excreted as [^{3}H] 4-PA		
Day 1	12.3 ± 5.4	10.4 ± 3.8

*Values represent means ± SD of three rats per age group.
†Values were not significantly different ($P > 0.05$) with respect to age.

Reduced erythrocyte phosphatase activity has been suggested as a possible cause for "trapping" of PLP because dephosphorylation to pyridoxal is necessary for exiting the erythrocyte.[16] Thus, sequestration of PLP by erythrocytes may contribute to low plasma PLP concentrations observed with aging.

Increased PALP activity has been proposed as an explanation for decreased plasma PLP levels.[6,17,18] Increased PALP activity has been reported in pregnant women with a corresponding decrease in PLP and increase in pyridoxal (PL) concentration in the plasma.[16] No relationship between plasma PLP levels and PALP activity was observed in 7- and 19-month-old Wistar rats.[4] In the present study, PALP activity was greater for the 3-month-old rats compared with the aged rats. In agreement with these findings, no correlation in serum alkaline phosphatase activity (SALP) and age in humans was observed.[19] Furthermore, SALP activity was reported to be lower with aging in men.[20] Therefore, it can be concluded that reduced plasma PLP concentration in the aged rat is not a result of increased PALP activity.

The use of radiolabeled pyridoxine has facilitated the study of vitamin B-6 bioavailability in rats by direct assessment of the intestinal absorption, metabolism, and urinary excretion of the vitamin. Such bioavailability studies can provide much-needed information concerning causes for marginal vitamin B-6 status. The intestinal absorption of [^{3}H]PN was approximately 99% for both 3- and 20-month-old rats, which is in agreement with the observation that PN is well absorbed by a passive, nonsaturable process.[21] Thus, a reduction in plasma PLP concentration in the aged rat is not a result of decreased intestinal absorption of vitamin B-6.

Age-related differences in the metabolism of vitamin B-6 have been reported.[5,22,23] It has been suggested that there is reduced turnover of PLP in tissues as a result of altered protein metabolism, metabolic rate, and physical activity with aging.[5] In this study, the level of radioactivity in various tissues was measured 2 and 5 days after administration of [^{3}H]PN to compare uptake and turnover of vitamin B-6 between the two age groups. Two and 5 days after administration of the isotope, no differences were observed in the level of radioactivity in the liver, muscle, kidney, or brain between the two age groups. Similar to our findings, Bode et al.[6] reported no age-related differences in the distribution of ^{14}C label in the kidneys, brain, and muscle of rats 1 day after a single, oral dose of [^{14}C]PN. The level of ^{14}C label in the liver was slightly but significantly reduced 1 day after oral administration. However, this difference was not observed after 47 days.[6] Although plasma PLP was significantly reduced in the aged rats, there was no difference in plasma radioactivity between the two age groups. Such a discrepancy may be due to plasma radioactivity not reflecting steady-state concentrations. Bode et al.,[6] however, reported no age-related difference in the distribution of ^{14}C label in blood of rats 47 days after oral administration of [^{14}C]PN.

There was no difference in the distribution of radioactivity among the hepatic B-6 vitamers. The liver is the major organ involved with PLP-dependent protein metabolism. Therefore, differences in protein utilization can influence the retention of vitamin B-6 in the liver and the requirement for the vitamin.[24] Results from this study would suggest that possible age-related differences in protein utilization do not affect vitamin B-6 turnover or the interconversion of B-6 vitamers in the liver.

The liver is the major source of plasma PLP, thus a reduction in liver PLP could infer reduced synthesis. An age-related decline in hepatic PLP concentration was reported. However, PL kinase and PLP hydrolase activity were not associated with this decline.[4] The lack of difference in the distribution of [^{3}H]PLP in the liver between the two age groups would suggest that reduced plasma PLP concentration with aging is not a result of impaired hepatic PLP biosynthesis.

PLP and pyridoxamine phosphate (PMP) concentration were reported to be decreased in muscle and kidney and increased in the brain with aging.[4] Approximately 90% of PLP in muscle is bound to glycogen phosphorylase; however, no age-related differences in the activity of this enzyme were observed in the muscle, nor in the liver and brain.[5] Muscle has been shown to represent a slower pool of vitamin B-6 when the organism is not in a deficient state.[5] In our study, no difference was observed in the level of radioactivity present in muscle. In addition, the level of radioactivity remained constant with time. Similar to our findings, Bode et al.[6] demonstrated little change in muscle radioactivity up to 12 days after oral administration of [^{14}C]PN.

The urinary excretion data indicated that differences in plasma PLP concentration between the two age groups were not a result of increased excretion of vitamin B-6 with aging. Although Bode et al.[6] reported a slight but significant increase in urinary excretion of ^{14}C with aging, such a difference could not account for the marked reduction in plasma PLP concentration. 4-PA is the irreversible product of vitamin B-6 metabolism and is formed primarily in the liver. The lack of difference in the urinary excretion of 4-PA between the two age groups further supports the observation that hepatic vitamin B-6 metabolism is not altered with aging.

Results from this study demonstrate that the intestinal absorption, hepatic metabolism, and urinary excretion of vitamin B-6 are not associated with reduced plasma PLP concentration observed with aging. These results are in general agreement with recent findings reported by Bode et al.[6] In addition, altered PALP activity does not appear to be associated with reduced plasma PLP concentration in the aged rats. Plasma PLP concentration may not be an adequate measure of tissue vitamin B-6 levels, and therefore not an adequate assessment parameter for vitamin B-6 status as affected by aging. Studies are currently being conducted to evaluate the effect of aging on erythrocyte PLP concentration and the mechanism for increased EAST activity.

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