

Hip fracture patients may be vitamin B₆ deficient

Controlled study of serum pyridoxal-5'-phosphate

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Deficiency of vitamin B₆ in rats may result in defective bone formation, possibly due to decreased activity of the enzyme ornithine decarboxylase which requires pyridoxal-5'-phosphate (PLP) as a co-factor and is responsible for production of intracellular putrescine, a metabolic regulator. We studied 3 groups of patients (62 fit ambulant out-patients, 21 elective arthroplasty patients, and 20 hip fracture

patients) and assayed their PLP status by high performance liquid chromatography. The reference range derived from the out-patients was 13–106 nmol/L. 3 of the arthroplasty group and 10 of the fracture group had serum PLP concentrations less than 13 nmol/L ($P < 0.01$). We conclude that PLP may be an etiologic factor in hip fracture by virtue of its role in the activity of a key regulatory protein.

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Pyridoxal-5'-phosphate (PLP) is the major B₆ vitamin present in human serum, and serum concentrations of this correlate well with tests of the biological function of the vitamin such as the tryptophan load test (Hamfelt 1967a) and PLP saturability of erythrocyte AST activity (Hamfelt 1967b). The serum concentration is also relatively constant within an individual so it can reliably be used as an assessment of vitamin B₆ status (Baysul et al. 1966, Hamfelt and Tuverno 1972, Lumeng and Li 1974, Brown et al. 1975).

PLP participates as a co-enzyme in many important metabolic processes due to its ability to form Schiff bases with amino acids. One such enzyme is ornithine decarboxylase (ODC, EC 4.1.1.17) which is the rate-limiting enzyme in the biosynthesis of polyamines (Bachrach 1984, Russell 1981). Inhibition of this enzyme reduces intracellular putrescine and spermidine concentrations (Relyea and Rando 1975, Mamony et al. 1976) which results in an up to 80 per cent decrease in the synthesis of DNA (Kay and Pegg 1973, Poso and Janne 1976) with cessation of cell proliferation (Fillingame et al. 1975, Mamony et al. 1976). Similarly, deficiency of PLP results in decreased activity of ODC with consequent decreased cellular proliferation (Raina and Janne 1975, Eloranta et al. 1976, Pegg 1977).

A further function of putrescine in osteoblasts is to regulate activity of the enzyme glucose-6-phosphate dehydrogenase (Dodds et al. 1986a) which is responsible for the reduction of NAD(P) to NAD(P)H. This is significant because NAD(P)H is required for the

vitamin K cycle concerned with γ -carboxylation of osteocalcin (Price 1985, Hauschka 1986, Lian and Gundberg 1988). This process initiates a conformational change in the protein which allows binding of hydroxyapatite and its subsequent accumulation in bone matrix (Price 1985, Hauschka 1986, Lian and Gundberg 1986).

Deficiency of PLP is known to adversely affect bone growth in rats resulting in fewer, shorter, more irregular trabeculae in tibial metaphyses (Rodda 1975) and also in retarded maturation of callus and delayed union in metatarsal fractures (Dodds et al. 1985, 1986b). It is possible that chronic PLP deficiency may result in a long-term diminution in cell proliferation contributing to a generalized weakening of bone structure which may predispose the individual to fractures. We examined the PLP status of patients admitted with femoral fractures to determine if their PLP status was deficient relative to other patients admitted for orthopedic procedures.

Patients and methods

Three groups of patients were chosen: a reference population of 62 fit ambulant individuals attending for out-patient phlebotomy; 21 patients admitted for elective arthroplasty; and 20 patients admitted for hip fracture due to minimal trauma (Table 1).

Table 1. Demographic details and type of fracture in the elective and fracture groups. PLP (nmol/L)

Fracture Group				Arthroplasty Group			
Sex	Age	Fracture	PLP	Sex	Age	PLP	
F	63	C	11	M	61	24	
F	72	T	21	M	63	39	
F	73	T	9	F	69	8	
F	78	C	13	F	69	16	
F	79	C	12	F	70	38	
F	79	C	16	F	70	23	
F	79	T	11	F	71	91	
F	80	T	13	M	71	47	
F	82	T	4	F	71	8	
F	83	C	31	M	73	45	
F	85	T	27	F	75	28	
F	85	T	12	M	76	29	
F	87	U	14	F	77	10	
F	87	U	9	F	78	57	
F	89	T	47	F	79	21	
F	89	U	8	F	80	72	
F	89	T	41	F	80	66	
F	90	U	21	M	80	20	
F	92	C	57	F	82	51	
F	94	C	27	F	83	26	
				F	83	48	

C cervical, T trochanteric, U unspecified

Samples were collected from the orthopedic patients within 24 hours of admission and before any surgery. Serum was stored at -80°C until analysis which was performed in a single batch to minimize between batch variations. PLP was assayed by high performance liquid chromatography (HPLC; Reynolds and Brain 1992).

Reference range. The reference population had an age range of 23–82 (mean 54, *SD* 15) years. To determine whether an age- and sex-matched reference range was required, results were separated into 4 groups by sex and age (<60 and >60 years old). These groups comprised: 16 men <60; 12 men >60; 20 women <60; and 14 women >60. The results from each group were compared with the two-sided Smirnov test (Sprent 1989, Conover 1980) and in no case could the null hypothesis that all groups were equal be rejected. Age dependence was tested by Spearman rank correlation of age against PLP concentration (Conover) with a similar result (P 0.3). Sex-dependence was also tested by the two-tailed Wilcoxon-Mann-Whitney test (Sprent) (P 0.3) and by the Kruskal-Wallis test (P 0.7). Thus a single reference range could be determined. Since the distribution of PLP results was shown to be non-Gaussian by the values of skewness and kurtosis (Sachs) and by the Kolgomorov-Smirnov "goodness-of-fit" test (Conover 1980),

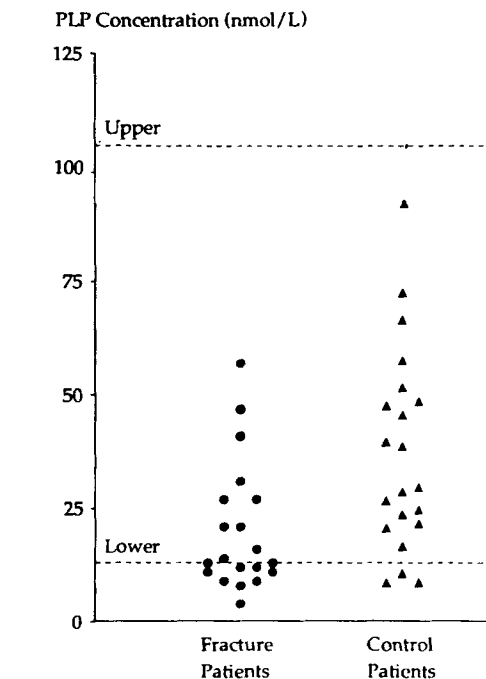


Figure 1. PLP concentrations and upper and lower limit of reference range in fracture and control patients.

the reference range was determined as the 2.5–97.5 interpercentile range after probability plotting of LOG10 transformed data (Barnett 1979) and was 13–106 nmol/L.

Results

The PLP status of fracture patients appeared to differ from that of the elective patients (Figure 1). The age distribution of the 2 groups was slightly different (fracture group age range 63–94 (mean 83, *SD* 7.6) years; elective group age range 61–83 (mean 74, *SD* 6.3) years, as proven by the Wilcoxon-Mann-Whitney test (P 0.0003) and the Kruskal-Wallis test (P 0.0006). However, the PLP concentration was not related to age when tested by Spearman rank correlation (P 0.6).

Due to the age distribution differences, an age-sex matched data set could not be created, but since the PLP concentration did not appear to be related to the patient's age, the difference in age distribution between fracture and elective groups was considered irrelevant. Thus, the differences in PLP concentration were tested by the Wilcoxon-Mann-Whitney test (P 0.006) and the Kruskal-Wallis test (P 0.01). In both

cases the null hypothesis was rejected and it was therefore concluded that there was a difference in serum PLP concentrations between fracture and elective surgery groups.

Discussion

The reference range we determined is similar to others (Coburn and Mahuren 1983, Shephard et al. 1987, Edwards et al. 1989, Millart and Lamiabie 1989), and 18 of 21 of the elective surgery patients fell within this range, whilst 10 of the 20 fracture patients fell below the lower limit of the reference range. However, it does not prove that low PLP is a factor in the etiology of femoral fractures. There are a number of explanations which need to be considered.

PLP concentration may actually be age-dependent, and the assay method used in this study may be unable to detect this. If so, then the concentrations measured may be normal for the age group who suffer hip fracture: other investigators, in a study of 617 men (Rose et al. 1976) found a small age-related decrease in PLP of 3.6 nmol/L/decade. In our study the mean age of the fracture group was 83 years and of the elective group, 74 years with median PLP concentrations of 14 nmol/L and 29 nmol/L, respectively. It is therefore unlikely that age had any significance since there was only a difference of 9 years on average whilst there was a PLP difference of 15 nmol/L.

Serum PLP concentrations were depressed in the fracture group but are not an etiologic factor for fractures. It may be some other factor, which may or may not be related to PLP, which is associated with fractures: low serum PLP may be due to dietary factors or to a poorer level of general health in the fracture patients. It may be these other factors and not PLP deficiency, which are etiologically important in falls and hence fractures.

In conclusion therefore, we have shown that patients with hip fracture have lower circulating serum PLP concentrations than patients admitted for elective joint surgery. This deficiency does not appear to be accounted for by the slightly greater age of the fracture patients and may therefore be an etiologic factor. However, we have not checked the alternative explanations, e.g., that serum PLP concentrations fall rapidly after a fracture in response to the injury, that low serum PLP is directly related to fractures, or that other dietary factors, such as calcium, alcohol or vitamin D intake, are important. Nor have we been able to measure serum osteocalcin concentrations to assess whether there is a difference between the control and fracture groups.

References

- Bachrach U. Physiological aspects of ornithine decarboxylase. *Cell Biochem Funct* 1984; 2 (1): 6–10.
- Barnett R. Clinical laboratory statistics. Little Brown & Co, Boston, 2nd Ed 1979.
- Baysul A, Johnson B A, Linkswiler H. Vitamin B₆ depletion in man: blood vitamin B₆, plasma pyridoxal-5'-phosphate, serum cholesterol, serum transaminases and urinary vitamin B₆ and 4 pyridoxic acid. *J Nutr* 1966; 89: 19–23.
- Brown R R, Rose D P, Leklem J E, Linkswiler H, Anand R. Urinary-4 pyridoxic acid, plasma pyridoxal phosphate, and erythrocyte aminotransferase levels in oral contraceptive users receiving controlled intakes of vitamin B₆. *Am J Clin Nutr* 1975; 28 (1): 10–9.
- Coburn S P, Mahuren J D. A versatile cation exchange procedure of measuring the seven major forms of vitamin B₆ in biological samples. *Anal Biochem* 1983; 129 (2): 310–7.
- Conover W. Practical non-parametric statistics. John Wiley & Sons, New York 1980.
- Dodds R A, Catterall A, Bitensky L, Chayen J. Osteolytic retardation of early stages of fracture healing induced by vitamin B₆ deficiency. *Clin Sci (Suppl)* 1985; 85: 21.
- Dodds R A, Dunham J, Bitensky L, Chayen J. Putrescine may be a natural stimulator of glucose 6 phosphate dehydrogenase. *FEBS Lett* 1986a; 201 (1): 105–8.
- Dodds R A, Catterall A, Bitensky L, Chayen J. Abnormalities in fracture healing induced by vitamin B₆ deficiency in rats. *Bone* 1986b; 7 (6): 489–95.
- Edwards P, Liu P K, Rose G A. A simple liquid chromatographic method for measuring vitamin B₆ compounds in plasma. *Clin Chem* 1989; 35 (2): 241–5.
- Eloranta T O, Kajander E O, Raina A M. A new method for the assay of tissue. S adenosylhomocysteine and S adenosylmethionine. Effect of pyridoxine deficiency on the metabolism of S adenosylhomocysteine, S adenosylmethionine and polyamines in rat liver. *Biochem J* 1976; 160 (2): 287–94.
- Fillingame R H, Jorstad C M, Morris D R. Increased cellular levels of spermidine or spermine are required for optimal DNA synthesis in lymphocytes activated by concanavalin A. *Proc Natl Acad Sci U S A* 1975; 72 (10): 4042–5.
- Hamfelt A. Enzymatic determination of pyridoxal phosphate in plasma by decarboxylation of L tyrosine 14 C (U) and a comparison with the tryptophan load test. *Scand J Clin Lab Invest* 1967a; 20 (1): 1–10.
- Hamfelt A. Pyridoxal phosphate concentration and aminotransferase activity in human blood cells. *Clin Chim Acta* 1967b; 16 (1): 19–28.
- Hamfelt A, Tuvemo T. Pyridoxal phosphate and folic acid concentration in blood and erythrocyte aspartate aminotransferase activity during pregnancy. *Clin Chim Acta* 1972; 41: 287–98.
- Hauschka P V. Osteocalcin: the vitamin K-dependent Ca²⁺-binding protein of bone matrix. *Haemostasis* 1986; 16 (3–4): 258–72.
- Kay J, Pegg A. Effect of inhibition of spermidine formation on protein and nucleic acid synthesis during lymphocyte activation. *FEBS Lett* 1973; 29: 301–4.
- Lian J, Gundberg C. Osteocalcin: the vitamin K dependent Ca²⁺ binding protein of bone matrix. *Haemostasis* 1986; 16: 258–72.

- Lumeng L, Li T K. Vitamin B₆ metabolism in chronic alcohol abuse. Pyridoxal phosphate levels in plasma and the effects of acetaldehyde on pyridoxal phosphate synthesis and degradation in human erythrocytes. *J Clin Invest* 1974; 53 (3): 693-704.
- Mamony P S, Bohlen P, McCann P P, Bey P, Schuber F, Tardif C. Alpha methyl ornithine, a potent competitive inhibitor of ornithine decarboxylase, blocks proliferation of rat hepatoma cells in culture. *Proc Natl Acad Sci U S A* 1976; 73 (5): 1626-30.
- Millart H, Lamiabie D. Determination of pyridoxal-5' phosphate in human serum by reversed phase high performance liquid chromatography combined with spectrofluorimetric detection of 4 pyridoxic acid-5' phosphate as a derivative. *Analyst* 1989; 114 (10): 1225-8.
- Pegg A E. Role of pyridoxal phosphate in mammalian polyamine biosynthesis. Lack of requirement for mammalian S adenosylmethionine decarboxylase activity. *Biochem J* 1977; 166 (1): 81-8.
- Poso H, Janne J. Inhibition of polyamine accumulation and deoxyribonucleic acid synthesis in regenerating rat liver. *Biochem J* 1976; 158 (2): 485-8.
- Price P A. Vitamin K-dependent formation of bone Gla protein (osteocalcin) and its function. *Vitam Horm* 1985; 42: 65-108.
- Raina A, Janne J. Physiology of the natural polyamines putrescine, spermidine and spermine. *Med Biol* 1975; 53 (3): 121-47.
- Relyea N, Rando R R. Potent inhibition of ornithine decarboxylase by beta, gamma unsaturated substrate analogs. *Biochem Biophys Res Commun* 1975; 67 (1): 392-8.
- Reynolds T, Brain A. A simple internally standardised isocratic HPLC assay for vitamin B₆ in human serum. *J Liquid Chromatogr* 1992; 15: 897-914.
- Rodda R A. Bone growth changes in pyridoxine deficient rats. *J Pathol* 1975; 117 (3): 131-37.
- Rose C S, Gyorgy P, Butler M, Andres R, Norris A H, Shock N W, Tobin J, Brin M, Spiegel H. Age differences in vitamin B₆ status of 617 men. *Am J Clin Nutr* 1976; 29 (8): 847-53.
- Russell D H. Ornithine decarboxylase: a key regulatory protein. *Med Biol* 1981; 59 (5-6): 286-95.
- Sachs L. Applied statistics: a handbook of techniques (translated by Reynarowycz Z). Springer Verlag, New York, 2nd ed. 1984.
- Shephard G S, Louw M E, Labadarios D. Analysis of vitamin B₆ vitamers in plasma by cation exchange high performance liquid chromatography. *J Chromatogr* 1987; 416 (1): 138-43.
- Sprent P. Applied non-parametric statistical methods. Chapman & Hall, London 1989.