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Diane Catharine Darcy for the degree of Master of Science in Foods and Nutrition presented on August 12, 1983

Title: The Response of Elderly and Young Women to an Oral Dose of Pyridoxine (Vitamin B-6).

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The metabolism of vitamin B-6 was studied in 5 elderly females ages 66 to 87 years and in 5 young women ages 22 to 30 years in a 14 day experiment. Blood was drawn on day 1. This was followed by a pyridoxine (PN) supplementation period where the subjects ingested daily 2 mg of PN.HCl (days 2-6). The subjects recorded their dietary intake and collected their 24-hour urine specimens on days 10-12. On day 14, each subject received an oral PN loading dose of 0.1 mg PN.HCl per kg lean body mass. Blood was drawn before, and at 1/2, 1, 3, and 5 hours post-dose and urine was collected 0-5 and 5-24 hours post-dose. In blood collected on day 1 plasma pyridoxal 5'phosphate levels, in nM, were: 53.1±44.6 for the elderly women, and 68.7±43.1 for the young subjects. Dietary analyses revealed that the self-selected diets of the elderly contained 0.023±0.005 mg vitamin B-6 per g of protein and of the young 0.019±0.004. Urinary excretion

of vitamin B-6 and 4-pyridoxic acid was not different at this time. Compared to the response of the young subjects to the oral PN loading dose, the elderly absorbed the PN dose with equal efficiency. Plasma pyridoxal 5'phosphate levels were lower in the elderly group in response to the PN dose, than the young suggesting metabolic differences in vitamin B-6 metabolism between the two groups.

The Response of Elderly and Young Women To an Oral Dose of Pyridoxine (Vitamin B-6)

by

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The Response of Elderly and Young Women to an Oral Dose of Pyridoxine (Vitamin B-6).

I. INTRODUCTION

Dramatic declines in plasma pyridoxal 5' phosphate (PLP) levels have been observed in older adults (Hamfelt, 1964; Walsh, 1966; Lumeng and Li, 1974; and Rose et al., 1976), which suggest marginal vitamin B-6 status in this group. Increased stimulation of erythrocyte transaminase activity by PLP added in vitro (Jacobs et al., 1968) and abnormal tryptophan metabolism (Ranke et al., 1960) also suggest compromised vitamin B-6 status in the elderly. However, according to the currently available guidelines by Leklem and Reynolds (1981), adequate vitamin B-6 status assessment has not been done in the elderly. It is, therefore, unknown whether or not vitamin B-6 absorption and metabolism in the elderly are different from that in younger adults. Current dietary recommendations for vitamin B-6 intake (NAS-NRC, 1980) are the same for all adults of the same sex, regardless of age. Additional studies on vitamin B-6 metabolism in the elderly adults are needed to better equip nutritionists in advising this population on dietary habits.

Decreased dietary vitamin B-6 intake by elderly

adults has been reported (Vir and Love, 1977, & 1979; Garry et al., 1982; Jacobs et al., 1968). Decreased plasma PLP levels, and altered functional tests of vitamin B-6 status (i.e. transaminase activity and the tryptophan load test) may represent a normal phenomonen of ageing, a dietary vitamin B-6 deficiency, or changed metabolism of this vitamin. Altered metabolism of vitamin B-6 could have serious implications for the well-being of elderly adults because vitamin B-6 as the coenzyme PLP has a crucial role in protein metabolism. PLP is a coenzyme for over 60 enzymes which participate in amino acid metabolism, the formation of hemoglobin, neurotransmitters, and polyamines; PLP has a role in the immune response.

The aim of the research reported in this thesis was to identify possible causes for the apparent decline in vitamin B-6 status with age. We hypothesized decreased vitamin B-6 intake, decreased vitamin B-6 absorption, or changed vitamin B-6 metabolism as possible mechanisms of the observed status decline with age. Accordingly, we compared in elderly and young women, dietary vitamin B-6 intake, plasma and urinary concentrations of vitamin B-6 compounds, and response to an oral dose of pyridoxine (PN), a crystalline form of vitamin B-6. By measuring vitamin B-6 compounds in timed blood and urine specimens obtained after the PN dose, we observed any age

differences in the absorption and metabolism of PN.

II. REVIEW OF LITERATURE

Vitamin B-6 is a nutrient required by humans and a wide variety of other animal species (Gyorgy, 1968). The term vitamin B-6 refers to a collection of six vitamers: three non-phosphorylated vitamers pyridoxal (PL), pyridoxamine (PM), pyridoxine (PN); and their respective phosphorylated forms pyridoxal 5'phosphate (PLP), pyridoxamine 5'phosphate (PMP), and pyridoxine 5'phosphate (PNP). The non-phosphorylated forms as well as PNP, can be converted to PLP and PMP, the active coenzymatic forms of vitamin B-6. The B-6 vitamer structures and their major interconversions in mammals are presented in figure 1. Non-phosphorylated vitamers are phosphorylated by pyridoxal kinase. Both PNP and PMP are converted to PLP by the enzyme PNP oxidase, although PNP appears to be the preferred substrate of the enzyme. Comparing PNP and PMP as substrates for this oxidase in rabbit liver, Choi et al.(1983) found a seven fold increase in turnover time with the PNP substrate. The vitamers PMP and PLP are also interconvertable by transamination. A variety of phosphatases, primarily membrane-bound alkaline phosphatase, convert PNP, PLP, and PMP to their non-phosphorylated forms. PL is converted to 4-pyridoxic acid (4-PA) mainly through aldehyde dehydrogenase, but also by the enzyme aldehyde

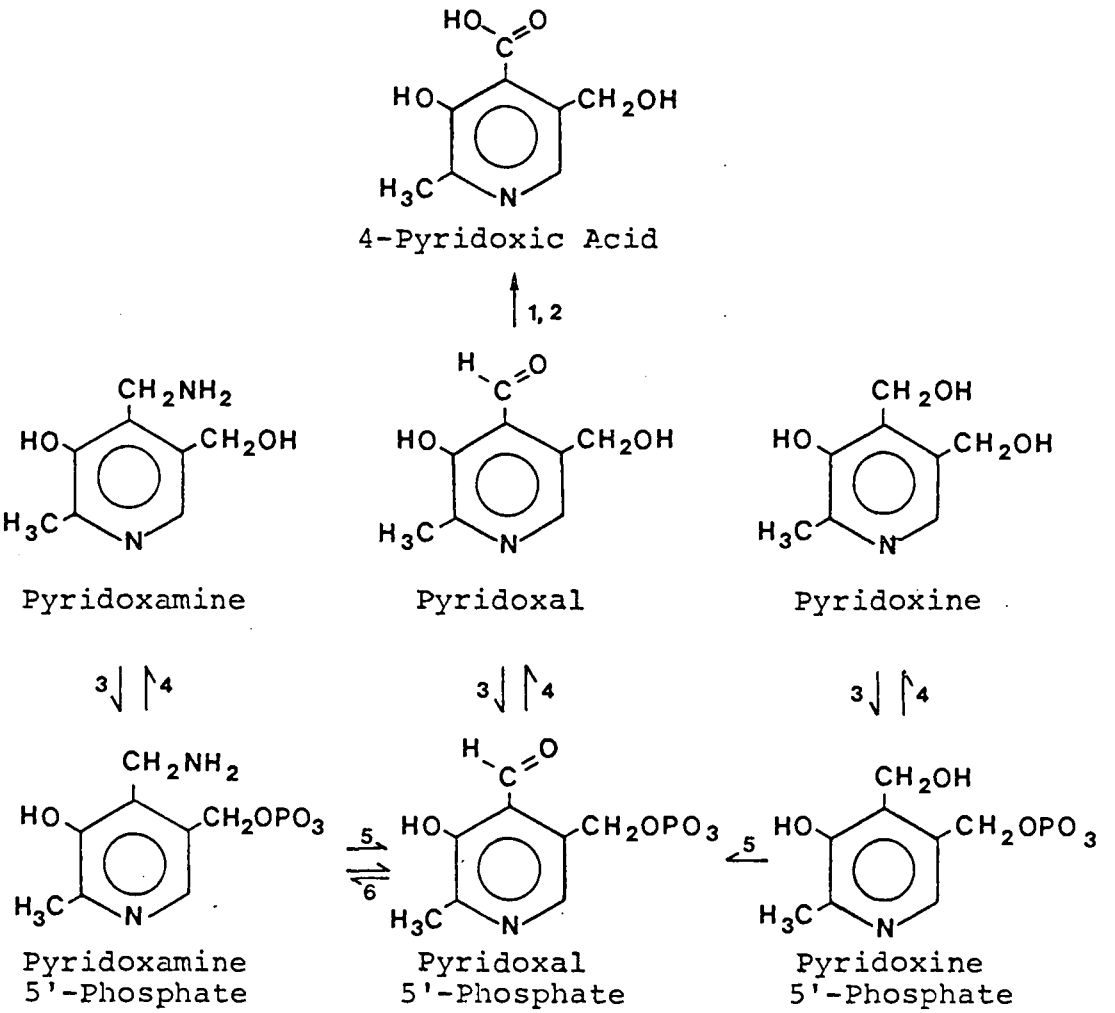

Figure 1. Mammalian B-6 vitamer interconversions.
Enzymes involved in these reactions are:

1. aldehyde dehydrogenase
2. aldehyde oxidase
3. pyridoxine kinase
4. alkaline phospatase
5. PNP oxidase
6. transaminases

oxidase (Stanulovic, 1980). Four-pyridoxic acid represents a metabolic end point and is not further metabolized (Reddy, Reynolds, and Price, 1958).

Vitamin B-6 Function

The importance of vitamin B-6 as a nutrient is evident in the wide variety of functions it performs. PLP functions include coenzymatic roles in over 60 enzyme systems, a structural role in glycogen phosphorylase, and a secondary role in fat metabolism.

Most notably, vitamin B-6 is involved in amino acid metabolism as a conenzyme for transaminases, decarboxylases, racemases, dehydratases, and desulfatases (Lehninger, 1975; Sauberlich & Canham, 1980). Examples of reactions involving these PLP-dependant enzymes are listed in table 1. As a coenzyme in amino acid metabolism PLP functions in Schiff base formation. The carbonyl group of PLP and the primary amino groups of amino acids form the Schiff base. The reaction site on the amino acid may be the alpha, beta, or gamma carbon following the electron drawing or destabilizing influence of the aldimino group formed by the carbonylamine (Dakshinamurti, 1982).

Examples of the physiologic significance of vitamin B-6 roles in amino acid metabolism are also listed in table 1. PLP functions in the synthesis or formation of

TABLE 1 Examples of Vitamin B-6 Function in Amino Acid Metabolism

PLP Dependant Enzyme		Examples	Physiologic Role
Transaminase	a	glutamate $\rightleftharpoons$ α-keto glutarate	gluconeogenesis
	a	alanine $\rightleftharpoons$ pyruvate	non-essential amino acid synthesis
	b	γ-aminobutynic acid (GABA) $\longrightarrow$ succinic semialdehyde	catabolism of neurotransmitter
Decarboxylase	a	histidine $\longrightarrow$ histamine	formation of hormone
	a	5-OH tryptophan $\longrightarrow$ serotonin	formation of neurotransmitters
	a	DOPA $\longrightarrow$ Dopamine	
	b	glutamate $\longrightarrow$ GABA	
Dehydratases	a	Serine $\longrightarrow$ pyruvate	gluconeogenesis
Desulfatases	a	cysteine $\longrightarrow$ pyruvate	gluconeogenesis
Hydrolases	a	cystathionine $\longrightarrow$ cysteine + homoserine	non-essential amino acid synthesis
	a	3-OH kynurenine $\longrightarrow$ 3-OH anthranilic acid	niacin formation from tryptophan
	b	ornithine $\longrightarrow$ putrescine	spermine synthesis

a) Lehninger, 1976
b) Dakshinamurti, 1982

niacin , non-essential amino acids, porphyrins, polyamines, neurotransmitters, and substrates for gluconeogenesis. In addition, PLP is a coenzyme for the degradation of the neurotransmitter, gamma-aminobuteryic acid (Dakshinamurti, 1982; Lehninger,1976).

PLP has a role in carbohydrate metabolism through the enzyme glycogen phosphorylase. Using NMR spectroscopy, Withers et al.(1981) have demonstrated conformational changes in the enzyme due to ionic interactions of the phosphate group of PLP with the active site in glycogen phosphorylase. This structural role of PLP is unique and separate from its coenzymatic roles.

In the past, vitamin B-6 has been reported to affect various endocrine activities including gonadotrophic, adrenocorticotrophic, thyroid stimulating, and growth hormones (Sauberlich, 1968). Current understanding of the hypothalmal-pituitary axis explains the association of vitamin B-6 and these hormones by the role of PLP in neurotransmitter metabolism (Dakshinamurti, 1982). The pituitary, adrenal and thyroid output of the above hormones are regulated by hypothalmal hormones or releasing factors, which in turn are regulated by dopamine and serotonin. Synthesis of these two neurotransmitters is vitamin B-6 dependant (Frohman, 1980; Krieger, 1980).

Vitamin B-6 may, however, be related to hormone regulation more directly. Litwack (1979) reported that PLP competitively binds to the deoxyribonucleic acid site of the activated glucocorticoid complex. PLP may then be capable of inhibiting or decreasing glucocorticoid induced protein synthesis in the liver. By this mechanism intracellular PLP levels can modulate glucocorticoid activity.

Vitamin B-6 reportedly has a role in lipid metabolism, although this is thought to be a secondary one acting through hormonal mediation (Sauberlich, 1968). Vitamin B-6 has a role in nerve myelination. The synthesis of sphingosine may involve a PLP dependant enzyme (Dakshinamurti, 1982). Since rapid myelination occurs within the first two years of human life, this vitamin B-6 action is most critical during this time period. Myelination continues, however, at a slow rate until about 20 years of age (Dakshinamurti, 1982).

Deficiency

The clinical symptoms of human vitamin B-6 deficiency are inconsistant and varied among individuals. In a classic experiment, Snyderman et al.(1953) reported symptoms of VB deficiency in two human infants. One infant developed convulsive seizures when maintained on a vitamin B-6 deficient diet for 76 days. Seizures were

alleviated by acute vitamin B-6 administration. The other deficient infant reported by Snyderman developed hypochromic microcytic anemia which was nonresponsive to iron, but was reversed by vitamin B-6 supplementation. No explaination was given for this variety in the infants' response to the vitamin B-6 deficiency. The most frequently reported clinical symptoms of vitamin B-6 deficiency are irritability, cheilosis, convulsions (in infants), seborrheic dermatitis, and hypochromic microcytic anemia (Sauberlich, 1981).

Clinical symptoms are often expressed only after severe deficiency of the nutrient. Biochemical indicators of vitamin B-6 deficiency, therefore, are useful and are likely to be the most sensitive in detecting early vitamin B-6 deficiency (Sauberlich, 1981). Appropriate biochemical tests for deficiency of this vitamin include the tryptophan load test (TLT), urinary 4-PA excretion, plasma PLP levels, and erythrocyte transaminase activities and in vitro stimulation by PLP (Sauberlich, 1981). For the TLT a vitamin B-6 deficiency is suggested by an increase in urinary excretion of metabolites in the tryptophan to niacin pathway following a dose of tryptophan (Ranke et al., 1960). Consistently low urinary 4-PA excretion may be indicative of vitamin B-6 deficiency (Shultz and Leklem, 1981). It is important when interpreting urinary

4-PA results to be aware of other factors which may influence 4-PA excretion. For example, high-protein diets cause a decrease in 4-PA excretion. Miller and Leklem (1978) have reported low 4-PA excretion in vitamin B-6 sufficient humans on a high protein diet. Low plasma PLP levels reflect low vitamin B-6 intake and a possible compromised status of the vitamin (Li & Lumeng, 1981). Shultz and Leklem (1981) have provided tentative guidelines for the evaluation of marginal vitamin B-6 status using urinary 4-PA and plasma PLP concentrations. Erythrocyte transaminase activity may be normal or high in vitamin B-6 sufficient states. Therefore stimulation by PLP added in vitro, provides a better indicator of vitamin B-6 deficiency. A frequently reported parameter is the transaminase index, a ratio of transaminase-stimulated activity (with added PLP) to unstimulated activity (without added PLP) (Sauberlich et al., 1972).

Food Sources

Vitamin B-6 is ubiquitously distributed in relatively small amounts throughout the food supply. PN is the form primarily found in plant food sources, whereas PL and PM are in animal sources (Orr, 1969). In general, foods high in protein (e.g. meats, legumes, nuts) are also good sources of vitamin B-6. Exceptions

to this generality are avocado and banana which are high in the vitamin but low in protein, and eggs and dairy products which are good sources of protein but are not rich in vitamin B-6. Whole grain breads and cereals are richer sources of the vitamin than refined breads and cereals.

Absorption

Brin (1978) recently reviewed information which shows that vitamin B-6 is absorbed in the small intestine. The linear relationships between vitamin B-6 intake and excretion indicate diffusion as the mechanism of absorption. Other studies, using two different techniques, have expanded the understanding of the mechanism of vitamin B-6 absorption. Using an everted intestinal sac technique, Middleton (1977) demonstrated that PN uptake was linear with PN concentration, and that anoxia had no effect on mucosal uptake of this vitamer. These results suggest diffusion, because PN uptake shows no saturation kinetics and is independent of apparent energy requirements. Hamm, Mehansho, and Henderson (1979) and Mehansho, Hamm, and Henderson (1979) similarly suggested diffusion of other B-6 vitamers in vascularly perfused small intestine. Neither PM, PL or their phosphorylated forms showed saturation kinetics. At physiologic levels, the phosphorylated B-6 vitamers, PLP

and PMP, were absorbed after phosphate removal by alkaline phosphatase. Dephosphorylation was rapid, and absorptive rates of these vitamers were similar to PL and PM. However the phosphorylated forms of vitamin B-6 were absorbed into the mucosa when they were present in high concentrations (Mehansho et al., 1979).

Bioavailability

Human bioavailability studies suggest that dietary vitamin B-6 is incompletely absorbed and or metabolized from food sources. Tarr, Tamura, and Stokstad (1981) estimated the vitamin B-6 bioavailability from a semi-purified diet as compared to a typical American diet. An average of 71% bioavailability was estimated from the American diet, by comparing differences in plasma PLP levels in this group to subjects receiving the semi-purified diet. Urinary vitamin B-6 levels suggested a 79% bioavailability of vitamin B-6 when compared to the same standard.

Employing a triple-lumen perfusion tube to measure vitamin B-6 bioavailability, Nelson, Lane, and Cerda (1976) found decreased absorption of this vitamin from orange juice as compared to a synthetic source of vitamin B-6 (a solution of PN.HCl with glucose, salts, folacin, and ascorbic acid). Differences were attributed to binding of vitamin B-6 by some compound in the orange

juice which was less than 3000 daltons.

Distribution and Transport

The major metabolic pathways of a PN dose are schematically represented in figure 2. Once absorbed from the digestive tract, vitamin B-6 is assimilated by the liver. From the liver, B-6 vitamers and metabolites are released into the plasma which transport the compounds throughout the body. Other tissues, in turn, release vitamers and metabolites back into the plasma. Thus, the plasma concentration of B-6 vitamers and metabolites are the net result of the flux of vitamin B-6 compounds into and from the plasma.

The vitamin B-6 levels in any given tissue of the body (e.g., liver, blood cells, muscle, brain) are dependent on equilibria between protein bound and free vitamers and cell membrane permeabilities. While it appears that phosphorylated B-6 vitamers may be transported across cell membranes, the most physiologically significant transport forms are the nonphosphorylated vitamers (Lumeng and Li, 1980). Protein binding may keep a vitamer in the extra- or intra-cellular compartments. For example, that proportion of plasma PL bound to albumin will remain in the plasma (Lumeng and Li, 1980).

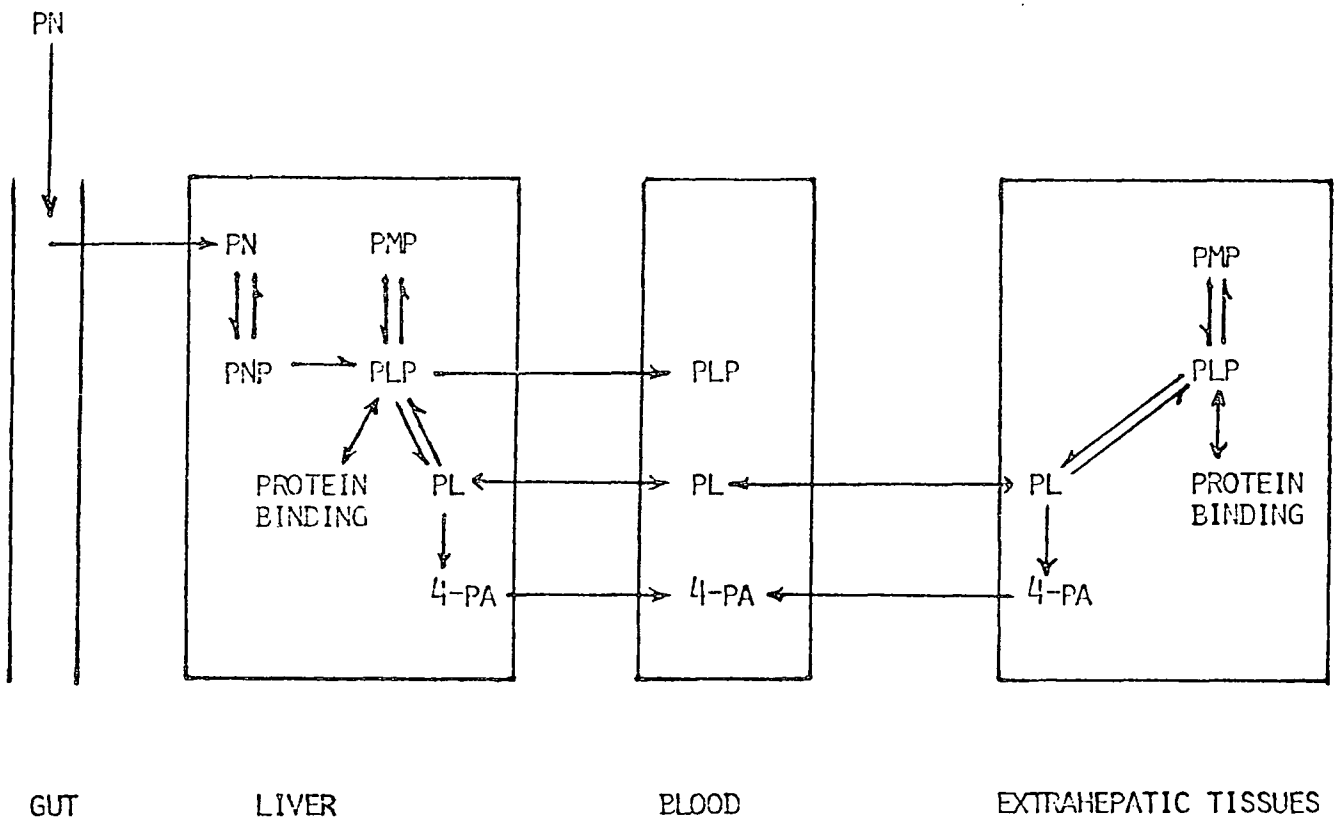

Figure 2. Major metabolic pathways of a PN dose.

Regulation

PLP and PMP are the main mammalian intracellular B-6 vitamers, with very small amounts of the non-phosphorylated forms present. In vivo studies indicate that intracellular PLP levels are tightly regulated; however, the concentration ratios of PMP to PLP vary widely from tissue to tissue. Rat liver contains approximately equal amounts of each coenzymatic form, whereas skeletal muscle contains approximately five times more PLP than PMP. Rat brain has the opposite trend, with PMP predominating (Lumeng and Li, 1980). Since the aldehyde group of PLP is very reactive with protein, the homeostatic need to regulate intracellular PLP levels is easily understood. For example, at high intracellular levels free PLP can inactivate aldolase by intracellular binding. Aldolase does not require PLP as a coenzyme (Shapiro, Enser, and Horecker, 1968).

PLP, PL, and PN are the major B-6 vitamers in human plasma. Plasma PLP levels are about 3.5 times higher than PN or PL, and the two non-phosphorylated forms exist in approximately equal amounts. Low levels of PM and PMP are also present in plasma (Li and Lumeng, 1981).

Tissue Metabolism

The mammalian liver appears to possess all of the major enzymes for the B-6 vitamer interconversions that

are listed in figure 1 (Lumeng, Lui, and Li, 1980). Li, Lumeng, and Veitch (1974) have suggested that hepatic PLP levels are primarily regulated by catabolic rather than synthetic enzymes, with unbound PLP subject to the action of phosphatases. According to this theory, the balance of hepatic PLP levels is maintained through protein binding and PLP catabolism.

More than 50% of the PLP in rat liver is bound (Li et al., 1974). In this tissue, PLP binds to a variety of proteins including glycogen phosphorylase, transaminases, and other unidentified proteins with molecular weights of 200,000 and 100,000 daltons (Bosron et al., 1978). Employing unphysiologically high levels of PLP, McCormick and Merrill (1980) observed that PNP oxidase is inhibited by its end product, PLP, and suggested that intracellular PLP levels are controlled by PLP production via the PNP oxidase.

Lumeng, Lui, and Li (1980) incubated isolated rat hepatocytes with radiolabeled PN and found that newly synthesized PLP was not readily miscible with exogenous protein-bound hepatic pools of PLP. Therefore, excess unbound hepatic PLP is either released to the plasma, or converted to PL or 4-PA before release.

Hepatic vitamin B-6 metabolism and plasma vitamin B-6 levels are intimately related. The liver appears to be the only source of plasma PLP as demonstrated by

Lumeng, Brashear, and Li (1974) in canine organ ablation studies. Of the organs tested, only excision of the liver gave no increase in plasma PLP levels in response to an intravenous dose of PN or PL. Conversely, the source of plasma PMP is unknown, but is probably not from the liver (Lumeng, Lui, and Li, 1980). Plasma non-phosphorylated B-6 vitamers and 4-PA appear to origonate from a variety of sources. Tissues containing aldehyde dehydrogenase, the main enzyme responsible for 4-PA synthesis in mammals, include liver, red cells, intestine, muscle, brain, kidney, heart, and lung (Stanulovic, 1980). It is unknown if all these tissues contribute to 4-PA levels in the plasma. Both liver and red cells are reported to release PL and PN into the plasma (Lumeng, Lui, and Li, 1980; Anderson, 1980). Studies by Lumeng et al. (1974) have demonstrated that in the human, almost all of plasma PLP is bound to albumin. The capacity of albumin to bind this vitamer far exceeds the physiologic levels of PLP in the plasma. Lumeng et al. (1974) suggest the protein bound PLP is in equilibrium with some small amount of free PLP, and it is this free PLP which can be hydrolyzed to PL before entering cells.

Plasma PL may also bind to albumin, although it appears that under normal conditions more than half remains free. Plasma PL may represent the transport form

of the vitamin because of its unbound status and cell membrane permeability. Plasma PN is unbound and may readily diffuse into cells (Anderson, 1980).

Mammalian red blood cells (RBC) also contain all of the major enzymes for B-6 vitamer interconversions shown in figure 1 (Shane, 1978). RBC have twice the PL levels of plasma (Anderson, 1980), but PLP levels are approximately equal between the two compartments (Shane, 1978). Intracellular levels of PLP in RBC may be regulated by hemoglobin (Hb) binding (Lumeng and Li, 1980). Anderson (1980) has reported PLP binding to the diphosphoglycerate site on Hb. Both compounds lower the oxygen affinity of the pigment. She suggests that RBC are very important in the overall metabolism of vitamin B-6 and may serve as a reservoir for PL to maintain constant plasma levels. Fonda and Harker (1980) incubated labeled PN with human RBC. After 60 minutes, about 80% of the label had been incorporated into the RBC as PLP which was associated with Hb.

PLP has a structural role in glycogen phosphorylase (GP) and most of the PLP in muscle is bound to this protein (Black, Guirard, Snell, 1977). Although GP is only 5% of the soluble protein in skeletal muscle, this tissue may act as a reservoir for PLP since muscle constitutes about 40% of the total body weight (Lumeng and Li, 1980). High levels of vitamin B-6 intake have

been shown to increase GP levels in rat muscle (Black et al., 1977), but dietary deprivation of vitamin B-6 does not deplete GP levels. However, GP levels decrease with deficient energy intake, indicating that if muscle does act as a PLP reservoir, it does so only in energy restriction (Black, Guirard, Snell, 1978).

Little research has been done regarding vitamin B-6 metabolism in the brain. The rat brain appears to have a slow uptake of the vitamin . When rats were given a single intra-peritoneal injection of radiolabeled PN, brain radioactivity peaked at 7 days post-injection, while the liver radioactivity peaked at 4 hours. Total radioactivity in the brain was less than 10% of that in the liver (Segalman and Brown, 1981).

Excretion

Both 4-PA and non-phosphorylated B-6 vitamers are excreted in urine. Four-pyridoxic acid is the major metabolite of the vitamin, and urinary excretion increases with increasing intake of vitamin B-6 as a supplement or in food. A ratio of 4-PA excretion to vitamin B-6 intake is reported in humans to be anywhere from 20% (Johansson et al., 1966) to 50% (Reddy, Reynolds, and Price, 1958). Higher values have been reported in other studies (Wozenski et al., 1980; Shultz and Leklem, 1981).

There is indication that urinary 4-PA levels also vary with dietary protein intake. Miller and Leklem (1978) have reported decreased excretion of 4-PA in humans fed a high-protein diet. The suggested explanation was that increased protein consumption increases the need for vitamin B-6 as a coenzyme involved in amino acid metabolism; hence there was decreased degradation of the vitamin and excretion as 4-PA. While the research of Itoh and Okada (1973) does not exactly duplicate these findings, decreased 4-PA excretion per g nitrogen intake was reported in rats consuming high protein levels.

Vitamin B-6 is also excreted in small amounts in the urine as PL, PM, and PN with almost negligible amounts of phosphorylated forms (Brin, 1978). Recent dietary intake of vitamin B-6 is reflected by urinary vitamin B-6 levels (Sauberlich, 1981).

PN Loading Doses and Supplementation in Humans

Vitamin B-6 loading doses are usually given as PN (Li, 1978; Johansson et al., 1966; Mitchell et al., 1976) in uniform amounts regardless of body size. Within an hour of an oral or intravenous dose of PN, plasma PLP and total vitamin B-6 (which includes both phosphorylated and nonphosphorylated forms) increase (Mitchell et al., 1976); Wozenski, Leklem, and Miller, 1980). Lumeng, Lui,

and Li (1980) found that PLP and PL were the only vitamers to increase significantly after a PN dose. Urinary excretion of 4-PA and vitamin B-6 also increase in response to a vitamin B-6 dose (Wozenski et al., 1980; Johansson et al., 1966)

Wozenski et al. (1980) administered equimolar doses of PL, PN, and PM to men. Vitamin B-6 compounds determined in timed blood and urine collections were used as indicators of absorption and metabolism. Plasma total vitamin B-6 levels increased rapidly, peaking at 1/2 or 1 hour after the PN or PL dose, and at 1 hour after PM. The greatest magnitude of response was with the PL dose. The most rapid and largest apparent peak of plasma PLP was observed with the PN supplement. As expected, urinary 4-PA excretion was most rapid and of greatest magnitude following PL administration.

Physiological Effects of Age

Physiological changes are apparent with aging, but these changes are individual and somewhat independant of chronologic age. Implications of these changes on the human nutritional status of vitamin B-6 have not been determined. The elderly represent a diverse group often without an identified set of standards for nutritional assessment in the laboratory. Biochemical tests can be used for both young and elderly groups, but different normal ranges may exist. For instance, results of glucose tolerance tests change with age (Todhunter, 1980). Likewise, other standards may need to be adapted for correct interpretations of biochemical data in healthy aged individuals.

The most noteworthy physiological change with age is the net cell loss from most major organs: kidney, liver, muscle, and heart (Libow, 1981; Hyams, 1978). This cell loss results in body composition changes leading to a decrease in lean body mass (LBM) despite the maintenance or increase of body weight in adult life. Total body water decreases with age, and water distribution also changes. Extracellular fluid (ECF) volume remains constant with age, but intracellular fluid (ICF) volume decreases (Lindeman, 1981). Consequently, as a percent of total body water ECF increases, while ICF decreases.

Organs from older individuals may also function

differently than those from younger ones. For example, there are apparent differences in liver metabolism in the elderly. Drugs which are metabolized by the liver microsomal system show increased half-lives in older adults, suggesting diminished hepatic capabilities (Sherman and Libow, 1981). Plasma albumin levels are depressed in the elderly, which may suggest a decrease in liver synthesis of this protein. Note that at the present time it is unknown if the apparent metabolic differences associated with ageing are due to a decrease in the number of cells or decreased cellular metabolism (Watkin, 1980).

It is thought that glomerular filtration rate (GFR) decreases with age because of the decline in the number of functional glomeruli and reduced renal blood flow. Decreased GFR may also increase the half-life of drugs which are excreted unchanged in urine (Sherman and Libow, 1981).

Gastrointestinal morphology changes with age. Shorter villi in the elderly results in decreased absorptive area (Webster and Leeming, 1975). Gastric acid secretion declines as does gut motility (Libow, 1981). Xylose absorption tests are commonly performed to determine absorptive efficiencies in individuals. In elderly subjects these tests are difficult to interpret. Typically, these tests involve an oral dose of xylose

followed by timed urine collections. Recovery of xylose in the urine represents the absorptive efficiency of the intestinal tract. Reviewing this subject, Mayershon (1982) suggested that timed urine xylose measurements are dependant on renal xylose excretion. Since GRF changes with age, these results are unsuitable for cross-sectional age comparisons. When existing data for oral and intravenous xylose tests were reintrepreted as a ratio by Mayerson, all adult age groups showed remarkably constant ratios up to 65 years of age. This suggests absorptive differences occur only after that age. Ratios drop by about 15% for existing data on 70-year-olds.

Other studies suggest no age-related impairment of carbohydrate, fat, or protein absorption. Southgate and Durnin (1970) in a balance study, compared intake and excretion in different age groups. No age or sex differences in apparent digestability of protein, fat, or available carbohydrate was observed. Cheng et al. (1978) observed no significant difference in protein utilization when comparing nitrogen balance in young and elderly groups on varying dietary protein intake.

Age and Vitamin B-6

Three vitamin B-6 biochemical indicators have been used to investigate vitamin B-6 status in the elderly. These are plasma PLP, tryptophan load test (TLT), and

transaminase activity and stimulation. A summary of these results are listed in table 2.

Hamfelt (1964), Walsh (1966), Lumeng and Li (1974), and Rose et al. (1976) have observed declines in plasma PLP levels with age. In cross-sectional studies, Hamfelt, Walsh, and Lumeng and Li have reported plasma PLP levels at age 65 are approximately half the levels of their 20-year-old subjects. Rose et al. estimated that plasma PLP levels decline at a rate of 0.09 ng per mL plasma per year. No explanations were given for the observed age related declines.

Ranke et al. (1960) discovered abnormally high xanthurenic acid (XA) excretion in elderly subjects given the TLT. The levels of XA excreted by the elderly were twice that of the younger subjects, which is suggestive of a vitamin B-6 deficiency in the elderly group. Excretion of XA by the elderly was normalized after 3 weeks of a daily 15 mg supplement of vitamin B-6.

Plasma, serum, and erythrocyte transaminases have been determined in elderly groups. These include serum glutamic oxaloacetic transaminase (SGOT), plasma GOT (PGOT), erythrocyte GOT (EGOT), and erythrocyte glutamic pyruvic transaminase (EGPT). The most sensitive test for vitamin B-6 deficiency is the EGPT. The GOT apparently has a higher affinity for PLP and is less likely to lose this coenzyme in early deficiency (Baysal, Johnson and

TABLE 2 Summary of Reported Differences in Vitamin B-6 Status of Unsupplemented Elderly & Young Adults

References	Subj.[a] no	Subj. Sex[b]	Young & Elderly Ages[c] yr	TLT[d] mg XA	Plasma PLP ng/mL	Erythrocyte Transaminase Activity GPT[e]	GOT[f]	Simulated GPT[g]
Ranke et al., 1960	44	NG	X̄25	16.5				
			X̄76	32.6				
Hamfelt, 1964	59	NG	20-29		11.3			
			760		3.4			
Walsh, 1966	24	NG	20		11.0			
			65		3.5			
Jacobs, et al., 1968	64	F	20			200		425
			65			125		325
	70	M	20			250		475
			65			200		400
Lumeng & Li, 1974	94	M	20		14.0			
			65		8.0			
Rose et al. 1976	627	M	18-29				25.5	
			60-69				25.0	

a) Total number of subjects from each reference.
b) F = Female subjects, M = Male subjects, NG = sex of subjects not reported.
c) Selected ages for reporting elderly and young subject levels for various Vitamin B-6 parameters.
d) Tryptophan load test. Subjects received 15g of tryptophan, reported as mg xanthurenic acid (XA) excreted.
e) Glutamic pyruvic transaminase (GPT) activity reported in moles pyruvate/10^{12} rbc/hr.
f) Glutamic oxaloacetic transaminase (GOT) activity reported in international units.
g) GPT activity in the presence of excess PLP, reported in moles pyruvate/10^{12}rbc/hr.

Linkswiler, 1966), and serum transaminases are variable and more indicative of disease states than vitamin B-6 status (Sauberlich, 1981). Jacobs, Cavill, and Hughes (1968) observed significant decreases in EGPT levels in both sexes. Both Jacobs et al. and Rose et al. (1976) found decreased EGOT levels with age, but differences between the young and elderly groups were not statistically significant. Stimulation of transaminase activity by the in vitro addition of PLP is a measure of apoenzyme. As mentioned before, the index of transaminase activity (a ratio of PLP-stimulated to unstimulated activities) gives a good measure of vitamin B-6 status. Jacobs et al. (1968) observed a decline in stimulation of EGPT with age, indicating a reduction of this apoenzyme with age. Stimuation of EGOT showed no statistically significant age correlation (Jacobs et al., 1968; Rose et al., 1976).

No direct interpretation of any of these data can be made with regard to vitamin B-6 status. In a recent conference on vitamin B-6 methods and status assessment, Leklem and Reynolds (1981) defined status assessment as "properly done by dietary intake with regard to protein and vitamin B-6, and two biochemical measurements". According to this definition, proper vitamin B-6 status assessment has not been done in an older age group. It is also important to note that not all vitamin B-6

indicators may be useful in the assessment of vitamin B-6 status of elderly populations. The TLT, for example, is only useful when a standard normal range has been established for comparison of results (Leklem et al., 1975). This standard does not exist for an elderly group, decreasing the usefulness of the TLT for vitamin B-6 status assessment in this population.

Age and Dietary Vitamin B-6 Intake

Analysis of the dietary intakes by elderly populations almost universally show a lower than recommended energy intake (Monroe, 1980; Stiedman, 1978). Causes for this reported lower energy intake may be varied, including poverty, social isolation, ineffective mastication and impaired appetite. The nutritional implications of this lower energy intake are that the elderly must eat nutrient dense foods to achieve the recommended dietary allowances of vitamins and minerals (Natow & Heslin, 1980).

Dietary intakes by the elderly are most frequently reported to be low in vitamins A, E, D, and the minerals calcium and iron (Yearick et al., 1980; Garry et al., 1982; Bowman and Rosenberg, 1982). Vir and Love (1977, 1979); Garry et al. (1982); and Jacobs et al. (1968) have reporded vitamin B-6 intakes by the elderly. All researchers found significant numbers of the elderly

groups studied had vitamin B-6 intakes below the 1980 RDA. The RDA, however, is based on the assumption that 100 g of protein are consumed per day (NAS-NRC,1980). When vitamin B-6 intake is considered per g protein intake, only Garrys' group failed to achieve the recommended 0.02 mg vitamin B-6 per g protein ratio proposed as the Dietary Standard for Canada, (1975). The groups studied by Jacobs, and Vir and Love consumed diets adequate in vitamin B-6 when protein intake was also considered.

III. MATERIALS AND METHODS

Subjects

Table 3 presents descriptive data on the ten volunteers participating in this project. Five older Caucasian females, who were 66 to 87 years of age (subjects 1-5), were recruited from among retired Foods and Nutrition faculty and staff, a local retirement facility, and a senior citizens' center. Five younger Caucasian females, ages 22 to 30 years (subjects 6-10), were recruited from among the Oregon State University Foods and Nutrition student population. All subjects except number 5 had previous experience in recording diet histories and collecting 24-hour urine samples. Approval of this project was obtained from the Oregon State University Committee on Human Subjects. An informed consent form approved by this committe was signed by the subjects in this study.

The subjects had no current liver or kidney disease, as determined by a questionnaire. The elderly subjects were at least 15 years post-menopausal. None of the young subjects were currently using oral contraceptives. Two of the younger subjects were primiparas, having given birth one year or more prior to the study; none of the other younger subjects reported a term pregnancy. No subject was pregnant during the study. Four of five

TABLE 3 Descriptive Data on Subjects

GROUP	SUBJECT	AGE	HEIGHT	WEIGHT	URINARY CREATININE[a]	LBM[b]	HEMATOCRIT[c] PS	HEMATOCRIT[c] TI	HEMOGOBLIN[c] PS	HEMOGOBLIN[c] TI
	no	yr	cm	kg	g/d	kg	%		g/dL	
Elderly	1	66	161.0	62.6	0.75	29.2	d	40.5	d	14.6
	2	67	163.0	72.1	1.11	39.6	42.5	41.5	14.5	14.9
	3	69	163.5	55.1	0.92	34.2	46.0	46.5	15.5	15.8
	4	74	158.0	73.1	1.14	40.6	46.0	44.5	16.6	16.7
	5	87	157.5	66.7	0.68	27.3	45.0	41.5	16.4	15.2
$\bar{X} \pm$ SD		73[1]	160.6	65.9	0.92[2]	34.2[2]	44.9	42.9	15.8	15.4
		± 9	± 2.8	± 7.4	±0.21	± 6.0	± 1.6	± 2.5	± 1.0	± 0.8
Young	6	30	170.0	62.8	1.19	42.0	45.5	45.5	16.0	17.0
	7	27	165.5	65.5	1.05	38.0	43.5	42.5	15.5	15.0
	8	27	159.0	78.2	1.43	49.1	42.0	41.5	16.2	15.4
	9	24	161.5	58.2	1.13	40.2	39.0	37.5	14.2	13.6
	10	22	174.0	70.8	1.15	40.7	38.0	36.5	13.5	13.6
$\bar{X} \pm$ SD		26[1]	166.0	67.2	1.19[2]	42.0[2]	41.6	40.7	15.1	14.9
		± 3	± 6.1	± 7.6	±0.14	± 4.2	± 3.1	± 3.7	± 1.2	± 1.4

a) Three-day average of creatinine excretion during the basal period.
b) Lean Body Mass (LBM) during the basal period as estimated from a three-day mean of urinary creatinine excretion (Forbes & Bruning, 1976).
c) Presupplement (PS) blood sample was collected before the PN supplementation period, or, one month after this period for subjects 1, 9 and 10. TI blood sample was collected on the experimental day, one hour after the PN loading dose of 0.1 mg PN.HCl/kg LBM.
d) Not Measured.

1 $P < 0.001$ 2 $P < 0.04$ between groups.

young subjects were in the early part of their menstrual cycle (<day 10) on the loading dose day.

Subjects 1, 6, 8, and 10 reported occasional use of vitamin supplements before this investigation. Subject 5 in the elderly group took a daily supplement, and was the only person who reported ingesting a personal supplement the week before the study. All subjects discontinued their personal supplements during the study, taking only the PN supplements and loading dose provided them by the principal investigator.

Experimental Design

The protocol for this two-week study is outlined in table 4. In the morning of day 1, a presupplement blood sample (PS) was drawn from fasting subjects for the determination of plasma PLP and vitamin B-6 as well as hemoglobin and hematocrit. Clinical evaluation of the PS sample gave a general indication of nutritional status and initial levels of plasma vitamin B-6 compounds. For various reasons, the PS blood sample of subjects 1, 9, and 10 were drawn 13 to 14 days after the PN loading dose day. All other aspects of the experimental design were the same for these individuals. This 4 week delay in the PS collection of these subjects positioned the PS and T0 blood sampling periods similarly in relation to the menstural cycle of the young women. Since one older

TABLE 4 Protocol for the Experimental Design

Day No.	Treatment
1	Presupplement (PS) Day. A blood sample was drawn from fasting subjects.
2 3 4 5 6	Supplementation Period. Liquid supplements of PN were ingested daily; 2mg/mL/d PN.HCl (Sigma Chemical Co., St. Louis, Mo.).
7 8 9	No treatment period.
10 11 12	Basal Period. Diet histories were recorded and 24-hour urines were collected.
13	No treatment day.
14	Experimental Day. A loading dose of 0.1 mg PN.HCl/kg. Lean body mass was given to each fasting subject. Blood was drawn before the dose and at 1/2, 1, 3 and 5 hours after the dose. Urine was collected from the ingestion of the dose until 5 hours after the dose and from 5 to 24 hours thereafter.

subject was also delayed in having her PS sample drawn, the same procedure was followed for her. While plasma PLP levels are reportedly unaffected by the menstrual cycle (Shane, 1978), this PS delay was done as a control measure for these individuals in the young group.

In an effort to assure that the vitamin B-6 status of the two groups of subjects would be comparable, each subject ingested 2 mg of PN.HCl daily from day 2 to day 6 (supplementation period). All subjects were given 5 amber-glass bottles, each containing 2 mg of PN.HCl (2mg of PN.HCl, Sigma Chemical Co. St. Louis MO, in 2 mL of solution). These supplements were kept refrigerated until use. The subjects ingested one 2 mg PN.HCl supplement each morning before breakfast, rinsed the bottle twice with water and drank each rinsing. The subjects were reminded by telephone to take their supplements.

The subjects received no treatments during the following 3 days, days 7-9. During this interval increased urinary excretion of vitamin B-6 and 4-PA resulting from PN supplementation should have decreased and stabelized (Wozenski et al.,1980). In the following three-day basal period, days 10-12, the subjects ate their typical self-chosen diets, recorded their dietary intake, and collected 24-hour urine specimens daily. Subjects, who were informed of the importance of accurate

diet histories, were given both written and verbal instructions on recording diets and collecting urine. The function of the basal period was to provide information on possible differences in the diet and urinary excretion of vitamin B-6 compounds between these free-living young and old women on their self-selected diets.

Daily urinary creatinine was determined during the 3-day basal period to estimate the subjects' LBM. LBM was calculated using the mean basal creatinine excretion by the equation of Forbes and Bruning (1976). Since the subjects were following their own schedules for the timing of the 24-hour basal urine collections, an additional no treatment day (day 13) was necessary for the synchronization of timed urine collections on the loading dose day.

The PN dose was administered on day 14. Response to this dose, as measured by changes in the concentrations of vitamin B-6 compounds in timed blood and urine collections provide indirect measures of vitamin B-6 absorption and metabolism (Wozenski et al., 1980). Time-dependent changes in blood and urine levels of vitamin B-6 compounds reflect differences in cellular uptake, interconversions, degradation, and excretion of the vitamin. Accordingly, at 7 AM on day 14, the loading dose day, blood was drawn from the fasting subjects. The

subjects then ingested a PN.HCl dose of 0.1 mg PN.HCl per kg LBM and without delay completely voided their bladders before starting the 0-5 hour post-dose urine collection. Blood samples of 20mL each were drawn at 1/2, 1, 3, and 5 hours after the dose (T1/2, T1, T3, and T5, respectively). Immediately after the 5 hour blood drawing, subjects completely voided their bladders ending the 0-5 hour urine collection and initiating the 5-24 hour collection. A luncheon menu was provided from which subjects selected their noon meal. Height and weight of the subjects were recorded prior to the noon luncheon. The subjects recorded their dietary history on this day; their 5-24 hour urine specimen ended the following morning.

Rationale for Selection of Dose Size

This study was unique in that the loading dose of PN, a water soluble vitamin, was based on the subjects' LBM. Since this experiment was designed to provide indirect measures of metabolism in two different age groups, basic pharmacokinetic principles were employed. Pharmacokinetics, the study of drug absorption, metabolism, and excretion, is monitored in humans by blood and urine drug levels (Burgen and Mitchell, 1978). Similarly in this experiment, absorption, metabolism, and excretion of the PN loading dose were monitored by

measuring vitamin B-6 compounds in timed blood and urine collected after the dose. The rate of drug distribution in the body is size-dependent. Since a dose of a water soluble drug (or vitamin) will distribute among all the water soluble compartments in the body, body size indicates the total volume in which the drug (or vitamin) will distribute, or the volume of distribution. Hence, the drug (or vitamin) would distribute at different rates in large and small individuals, making it difficult to interpret metabolism based on blood levels. When this research was planned, it was anticipated that subject size would vary, therefore a dose based on size was employed to eliminate a distribution variable.

Additionally, dose dependant kinetics may alter blood profiles unpredictably. Dose dependant kinetics result if one or another process involved in absorption, distribution, protein binding, metabolism, or excretion of a drug (or vitamin) is capacity limited or saturable (Kaplan and Jack, 1979). Correct interpretation of an in vivo metabolic response to a dose in two physically different groups would not be possible if dose dependent kinetics were involved in the response. This is especially true if metabolic capacity is limited in only one of the two test groups. In this experiment, metabolic dose response was monitored primarily by time dependent changes in plasma PLP. Since the only known

source of plasma PLP is the liver (Lumeng et al., 1974), the elderly group may have a limited capacity to prdoduce PLP because both liver weight and the number of hepatocytes decrease with age (Libow, 1981). To account for known physical differences between the groups in the present study, the PN loading dose was given as a function of LBM. The assumptions implicit in this method are that liver weight is proportional to LBM and that LBM of the young and elderly subjects could be estimated with equal accuracy by urinary creatinine levels.

To provide approximatly a 3 mg loading dose to subjects, a 0.1 mg PN.HCl per kg LBM dose was chosen. Wozenski et al.(1980) reported that a loading dose of 1 mg PN.HCl is necessary for a measurable response in blood and urine samples, while variability becomes large when a loading dose above 4 mg PN.HCl is administered. The mean dose for the elderly group was 3.5+0.5 mg PN.HCl, and for the young group 4.3+0.4 mg PN.HCl. Table 6 presents the average basal creatinine excretion from which the LBM was calculated according to the Forbes and Bruning method (1975), and the individual PN doses given, as calculated from the 0.1 mg per kg LBM standard. While these PN loading doses were low, they were approximately double the recommended dietary allowance (NAS-NRC, 1980).

Sample Collection

Blood was collected from the antecubital vein into heparinized evacuated tubes. All samples were drawn as 20 mL collections by two registered medical technologists. Samples were centrifuged at 4 C to pack the red blood cells. Plasma was separated from the red cells without delay and stored at -15 C until assay. Hct and Hb were determined in the PS and T1 blood samples before centrifugation.

All urine collections were preserved with toluene. Subjects kept their urine in a dark cool place during each 24 hour period. Completed urine collections were obtained the following morning, measured and mixed, and aliquots were stored at -15 C.

Total Vitamin B-6

Total vitamin B-6 was measured in hydrolyzed urine and plasma according to the microbiological procedure with Saccharomyces uvarum as proposed by Miller and Edwards (1981). Since this yeast responds to all forms of the vitamin in a hydrolyzed sample, growth of this organism measures the concentration of all B-6 vitamers, and is termed total vitamin B-6. In this thesis, total vitamin B-6 is referred to simply as vitamin B-6. Control urine samples had interassay variations between 2 and 10%. Recovery of PL added to plasma before hydrolysis, ranged between 80 and 100%. The coefficient

of variation of the plasma control was 6%. Sample preparation and the vitamin B-6 assay were performed in subdued light to avoid destruction of the vitamers by light.

Pyridoxal 5'Phosphate

Plasma PLP was measured by a modification of the tyrosine decarboxylase method of Chabner and Livingston (1970). In addition to the existing modification in our laboratory by Leklem (personal communication), a procedural change was made in the perchlorate precipitation step. After deproteinization with perchlorate buffer, rather than transfer the entire volume of the supernatent as in the Leklem procedure, 1 mL of the supernatent was transferred to 0.2 mL of cold potassium acetate buffer for perchlorate precipitation. Recovery of PLP added to the plasma was between 86 and 108%. The plasma PLP determination was performed under yellow safelights to avoid the destruction of PLP by light.

Urinary 4-Pyridoxic Acid

Urinary 4-PA was determined fluorometrically by the procedure of Reddy, Reynolds, and Price (1958). Recovery of 4-PA added to a urine sample from each subject ranged from 80 to 97%. The coefficient of variation of 4-PA in

the urine control was 4%.

Creatinine

Urinary creatinine was quantitated by an automated method from the procedure of Pino, Bennotti, and Gardyna (1965).

Hemoglobin and Hematocrit

Hb and Hct were determined by standard procedures.

Lean Body Mass

LBM was determined from the mean daily excretion of creatinine by the equation of Forbes and Bruning (1975):LBM=7.38+0.02908 mg creatinine per day.

Dietary Analysis

Nutritional analysis of the diets were calculated by computer using the Ohio State Data Base (Schaum, Mason, and Sharp, 1973). Vitamin B-6 food values which were missing from the data base were obtained from Orr (1969).

Statistical Analysis of Data

Approximate t test for two means was used for statistical analysis. The elderly group was compared to the young group for all plasma and urinary levels of vitamin B-6 compounds in the presupplement, basal, and

loading dose collections, and for dietary vitamin B-6 intakes as recorded in the diet histories. Due to the large number of t tests performed, the Bonferroni joint estimation assigned a 0.99 confidence coefficient for the level of significance necessary in this study (Snedecor and Cochran, 1980). Confidence coefficients of 0.99 or more will be considered statistically significant.

IV. RESULTS AND DISCUSSION

Descriptive Data on Subjects

Despite age differences, the heights and weights of the elderly and young subjects were similar (Table 3). However, the elderly subjects had a lower LBM, as measured by urinary creatinine. Decrease in LBM with age has been supported by other reports (as reviewed by Lindeman, 1981). The Hb and Hct values for all of the subjects in both groups were within acceptable limits (Sauberlich, Skala, Dowdy, 1974).

Nutrient Intake During the Three-day Basal Period

The National Academy of Sciences' Recommended Dietary Allowance (RDA) set goals for daily nutrient consumption in the United States. Adequate dietary energy and protein levels are based on a variety of conditions including body weight, activity levels, sex, ambient temperature and health (NAS-NRC, 1980). In the dietary assessment of protein and energy intake of subjects in this study, only body weight and sex will be considered. Energy intakes were low in both groups (Table 5). Mean kcal per kg per day intakes during the basal period for the older group were 24±5, and for the younger group 27±8. Neither group consumed even the lower limit of the recommended energy intake proposed by NAS-NRC

TABLE 5

Mean Daily Intake of Energy, Protein and Vitamin B-6 During the 3-Day Basal Period

GROUP	SUBJECT	ENERGY [a]	KCAL / BODY WT.	PROTEIN	PROTEIN / BODY WT.	VITAMIN B-6 [b]	VITAMIN B-6 / PROTEIN
	no	kcal/d	kcal/kg	g/d	g/kg	mg/d	mg/g
Elderly	1	1859	30	59.5	0.95	1.28	0.022
	2	1187	16	58.1	0.81	0.94	0.016
	3	1246	23	51.6	0.94	1.49	0.029
	4	1910	26	77.1	1.05	1.83	0.024
	5	1579	24	66.3	0.99	1.46	0.022
$\bar{X} \pm$ SD		1556 ± 435	24 ± 5	62.0 ± 14.8	0.95 ±0.09	1.40 ± 0.52	0.023 ±0.005
Young	6	2355	38	103.4	1.65	1.65	0.016
	7	1484	23	56.9	0.87	1.54	0.027
	8	2490	32	103.6	1.32	1.79	0.017
	9	1404	24	50.3	0.86	0.93	0.018
	10	1264	18	37.7	0.53	0.64	0.017
$\bar{X} \pm$ SD		1799 ± 590	27 ± 8	70.4 ± 29.9	1.05 ±0.44	1.31 ± 0.54	0.019 ± 0.004

a) For each subject, daily intakes varied < 30% of their 3-day mean intake.

b) For each subject, daily intakes varied < 50% of their 3-day mean intake.

(1980) for their age groups: 25-40 kcal/kg for females 51-75 years old and 29-44 kcal/kg for females 23-50 years. Since weight was not monitored throughout this two week experiment, it is not known if subjects had lost weight during this time. Both young and elderly groups had sufficient protein intakes as the mean dietary levels were above the suggested 0.8 g of mixed quality protein per kg body weight level in both groups (NAS-NRC, 1980).

No subject in either group had a mean vitamin B-6 intake at the 2 mg level of the 1980 RDA for their age and sex groups (Table 5). Three subjects in each group received more than two-thirds of the RDA for vitamin B-6, and one in each group slightly less than half the RDA. Subject 10 in the young group, who was consuming a diet inadequate in protein and kcal, ingested only 1/3 of the RDA for vitamin B-6. The 1980 RDA for vitamin B-6 for females is based on the estimated daily consumption of 100g of protein. For those consuming more or less protein, vitamin B-6 requirements may vary (NAS-NRC, 1980). To account for varying protein intake, the Dietary Standard for Canada (1975) recommends a ratio of 0.020 mg vitamin B-6 per g of protein. Mean dietary ratios of the elderly and young groups met this established value with 0.023 ± 0.005 and 0.019 ± 0.004 mg vitamin B-6 per g protein respectively. However, 4 of 5 young subjects (numbers 6, 8, 9 and 10) and one elderly subject (number

2) failed to consume this recommended amount. Clear group differences in dietary protein choices, which may affect this ratio, were not apparent. According to present dietary guidelines, these results suggest that the older subjects in this study may be at a lower risk of developing a vitamin B-6 deficiency than the younger females.

Vitamin B-6 Status of Subjects

Assessment of vitamin B-6 status is most accurate when two or more biochemical measurements along with dietary intake are examined (Leklem and Reynolds, 1981). In the present study, PS and TO blood levels with basal urinary and dietary data were used to evaluate the subjects' vitamin B-6 status (Table 6). Tentative marginal status guidelines were established for plasma PLP, and urinary 4-PA and vitamin B-6 excretion values for females ingesting 1.25 to 1.50 mg of vitamin B-6 per day by Shultz and Leklem (1981). These guidelines, which do not consider influence of age on these vitamin B-6 parameters, were used to assess the status of the subjects in this project. Two different blood samples, PS and TO, were collected to evaluate the effect of a brief PN supplementation period on vitamin B-6 status. The PS and TO blood samplings were separated in time by a 13 day interval, during which the PN supplement was administered

TABLE 6 Comparison of Plasma and Urinary Vitamin B-6 Compounds to Vitamin B-6 Status Guidelines.[a]

GROUP	SUBJECT	PLASMA PLP[b]		URINARY[c]		PERSONAL[d]
		PS	TO	4-PA	Vitamin B-6	SUPPLEMENTATION
	no	nM		u moles/d		
Elderly	1	21.0	32.2	3.26	0.64	O
	2	24.3	26.1	6.14	0.79	N
	3	111.6	119.5	10.75	0.86	N
	4	17.7	13.3	5.64	0.59	N
	5	90.8	58.1	4.72	0.96	D
$\bar{X} \pm$ SD		53.1	49.8	6.10	0.77	
		± 44.6	± 42.2	± 2.71	± 0.25	
Young	6	44.7	47.1	6.29	0.75	O
	7	30.6	32.2	4.87	0.75	N
	8	49.8	60.0	6.81	1.02	O
	9	79.7	84.6	5.48	0.96	N
	10	138.9	50.2	4.80	1.13	O
$\bar{X} \pm$ SD		68.7	54.8	5.65	0.92	
		± 43.1	± 19.4	± 1.24	± 0.25	
Marginal Guidelines		32-36		4.6-5.2	0.6-0.7	

a) Marginal Status Guidelines - Marginal levels for females ingesting 1.25 - 1.50 mg of vitamin B-6/day (Shultz & Leklem, 1981).
b) Presupplement (PS) blood sample collected before the PN supplementation period or one month after this period for subjects 1, 9 and 10. TO blood sample was collected before the PN loading dose of 0.1 mg PN.HCl/kg LBM on the experimental day.
c) Presented as a 3-day mean of urine collections during the basal period.
d) Subject responses to a questionnaire indicating No (N), occasional (O) and daily (D) personal multi-vitamin ingestion before the study.

on days 2-6 (Table 4).

Mean plasma PLP values of either group were not suggestive of a vitamin B-6 deficiency in either PS or TO blood samplings (Table 6). However, subjects 1, 2, 4 and 7 had both PS and TO plasma PLP values in the marginal range, <32-36 nM. Supplementation with 2mg of PN.HCl per day for five days had no significant effect on the subjects' mean plasma PLP values (PS vs TO). Despite PN supplementation mean plasma PLP levels declined slightly in the TO sample for both groups (Table 6). The observed decrease with supplementation is the result of bias introduced by a few individuals. With the exception of subjects 4, 5 and 10, PN supplementation slightly increased individual TO PLP levels above the PS concentration as anticipated. Explanations for the reversed trend in subjects 4, 5, and 10 may be varied. Only subject 5 reported personal multi-vitamin supplementation on a regular basis before the study. Presumably, this accounts for the decline in her TO sample. Subject 10 reported occasional multi-vitamin ingestion and it is unknown if her personal supplementation was responsible for the large difference between PS and TO PLP levels. This subject reported gastrointestinal illness on day three of the basal period, two days before the TO sample was drawn, which may account for her decline. Subject 4 did not report

personal vitamin supplementation and her very small TO decline is unexplained.

Basal urine collections provided two indicators of vitamin B-6 status assessment, 4-PA and vitamin B-6 excretion. According to the guidelines by Shultz and Leklem, neither indicator suggests marginal vitamin B-6 status in the means of urinary 4-PA and vitamin B-6 in elderly or young group (Table 6). Average individual basal excretion values, however, suggest that subjects 1, 5, 7 and 10 excreted marginal levels of 4-PA, and subjects 1 and 4 excreted marginal amounts of vitamin B-6.

Subjects with two or more marginal biochemical measures were considered to have marginal vitamin B-6 status. Collated biochemical test results suggest that subjects 1, 4 and 7 had marginal vitamin B-6 status. All three of these subjects had average basal dietary vitamin B-6 intakes within or above the 1.25-1.54 mg per day range used by Shultz and Leklem to establish these marginal guidelines for assessing vitamin B-6 status (Table 6). The dose response of subjects 1, 4 and 7 to the PN loading dose on the experimental day will be considered individually to identify possible atypical responses. It is interesting to note that subjects 5 and 10 who have elevated PLP levels have normal urinary 4-PA and vitamin B-6 levels. No explanation is readily

available.

Loading Dose Responses

Absorption and metabolism of PN by the subjects in this study were monitored by measuring changes in vitamin B-6 compounds in timed blood and urine collections following the oral ingestion of the PN loading dose on the experimental day. An in vivo method such as this one in humans has limitations in that the interpretation of the results is often a process of elimination. For example, changing blood levels of PLP may be indicative of hepatic metabolism, but are not a direct measure of metabolism since no hepatic tissue was sampled. Inferences on hepatic metabolism of vitamin B-6 can be made based on dose response consistencies with various animal metabolic studies. In this thesis, when hypotheses which are possible but do not support the observed response are rejected.

An additional limitation to human experimentation is the finite number of acceptable blood collections that can be made. The timing of the possible blood collections may not coincide with the fluctuations in plasma vitamin B-6 compounds. Where peak plasma levels are important to the interpretation of a metabolic response, actual peak levels may not have been sampled. In recognition of this limitation, the highest levels of

a plasma parameter at any sampling time will be termed measured-peak.

Plasma vitamin B-6 levels correspond to the concentration of all forms of this vitamin. Changes in plasma vitamin B-6 levels following a PN dose reflect increases in PN levels from absorption of the dose and from increases in the other B-6 vitamers. Therefore, absorption efficiencies of the oral PN dose by subjects in this study were monitored by measured-peak plasma vitamin B-6 levels and placement of the measured-peak in the five hour blood sampling period. Changes in the plasma levels of vitamers other than PN, following a PN dose, reflect metabolism of the dose. For example, increases in plasma PLP levels after a PN dose reflect a metabolic interconversion of the dose.

Blood Response

Following the PN dose, group mean plasma vitamin B-6 and PLP levels rose from pre-dose levels (Fig. 3). Measured-peak concentrations of mean plasma vitamin B-6 and PLP occurred in both groups at the T1/2 and T1 blood samplings, respectively.

At all time periods, the mean plasma levels of PLP and vitamin B-6 are higher in the young group than in the elderly group. From the zenith, elderly and young group levels declined for both plasma variables, although

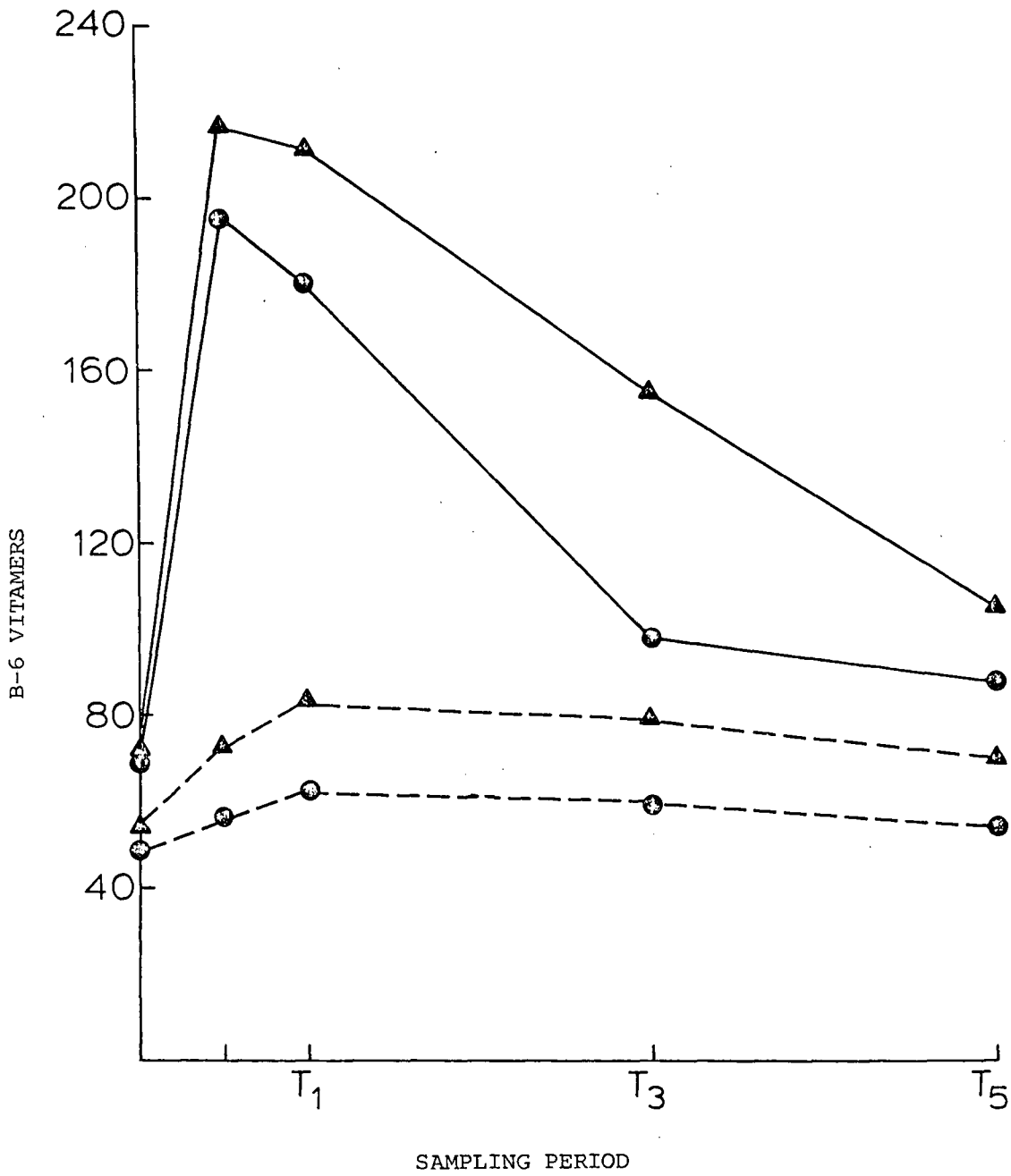

Figure 3. Plasma Vitamin B-6 (——) and PLP (- - -) in elderly (●) and young (▲) females from timed blood collections after a PN loading dose. Blood samples were collected before the dose (T0) and at $\frac{1}{2}$, 1, 3 and 5 hours ($T\frac{1}{2}$, T1, T3 and T5 respectively) after a dose of 0.1 mg PN.HCl per kg lean body mass. Standard deviations for plasma Vitamin B-6 and PLP levels were as much as 50% and 100% respectively from the group means.

values remain elevated in the T5 sampling after the dose. Most notably, the elderly group's vitamin B-6 concentration fell more rapidly from T1 to T3 and stabalized at T3 to T5, while the younger group continues a steady decline from the T1 to T5 collection period.

Metabolic response to a PN loading dose can be best measured by changes in the levels of vitamin B-6 compounds that occur after the administration of the dose. Each individual subjects T0, or predose, blood value was subtracted from values at each successive time period to give the magnitude change in plasma concentration corresponding to the dose response (Fig.4, & Fig. 5). This change was termed baseline. Mean baseline values were lower in the elderly group for each variable at all sampling times (Fig.4 & Fig.5). Baseline vitamin B-6 levels in the elderly group again show a rapid decline from the T1 to T3 samples (Fig. 4A). The measured peak heights and peak arrival times from baseline vitamin B-6 blood samplings, were similar to those reported by Wozenski et al. (1980) for subjects receiving a 4 mg PN.HCL dose. In the present study, small baseline PLP responses were seen in both groups although there was much individual variation. The elderly group PLP levels were consistently much lower than the levels of the young subjects (Fig. 5). The elderly baseline plasma PLP concentrations were less than half

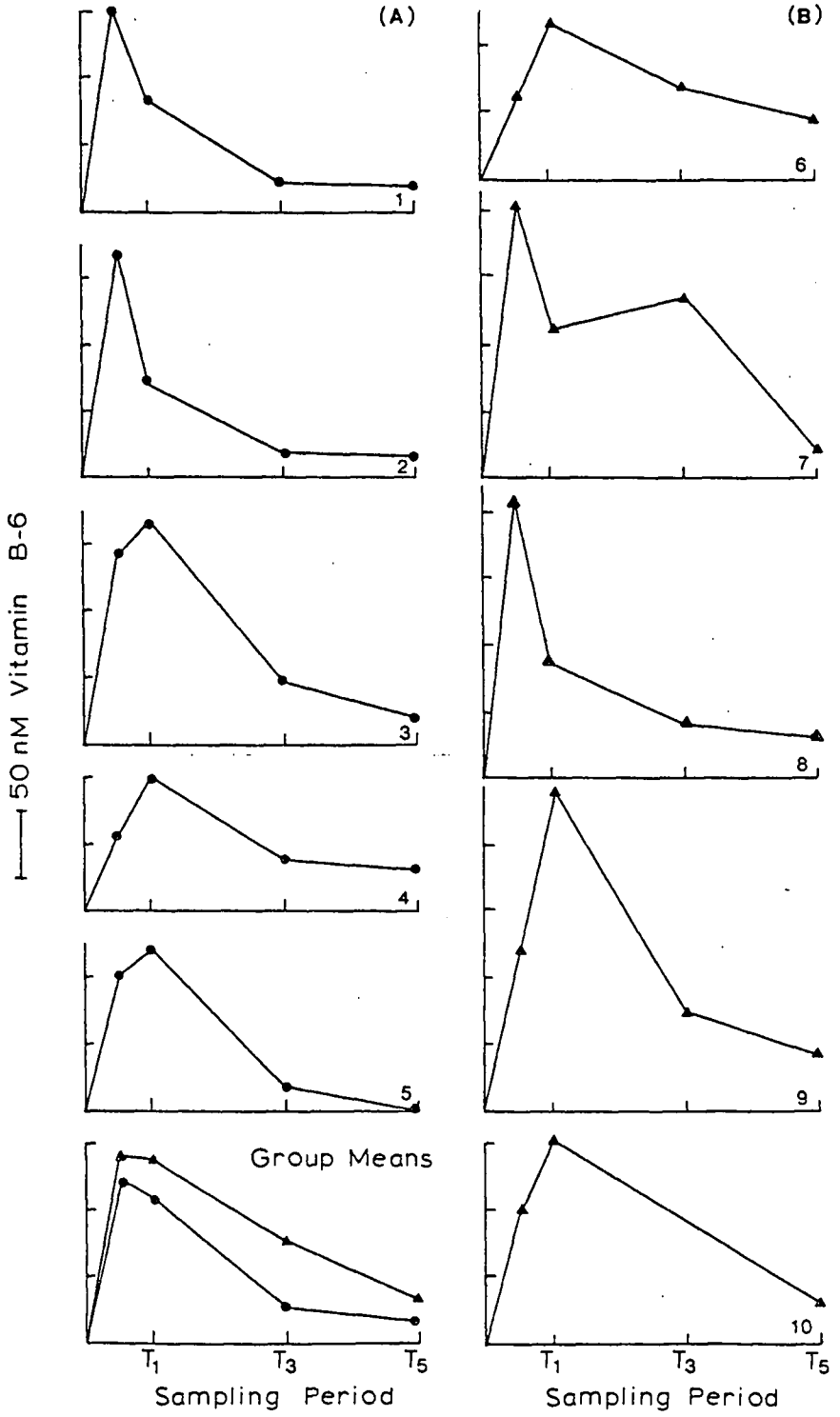

Figure 4. Individual plasma B-6 (——) response of elderly (Part A, ●) and young (Part B, ▲) subjects to a PN dose. Response was measured as baseline plasma Vitamin B-6 levels from timed blood collections after an oral loading dose of 0.1 mg PN.HCl per kg lean body mass. Plasma levels before the dose (TO) were subtracted from the levels in each post-dose plasma sample to give baseline values. The T3 sample was not drawn from subject 10. Mean group responses are also listed.

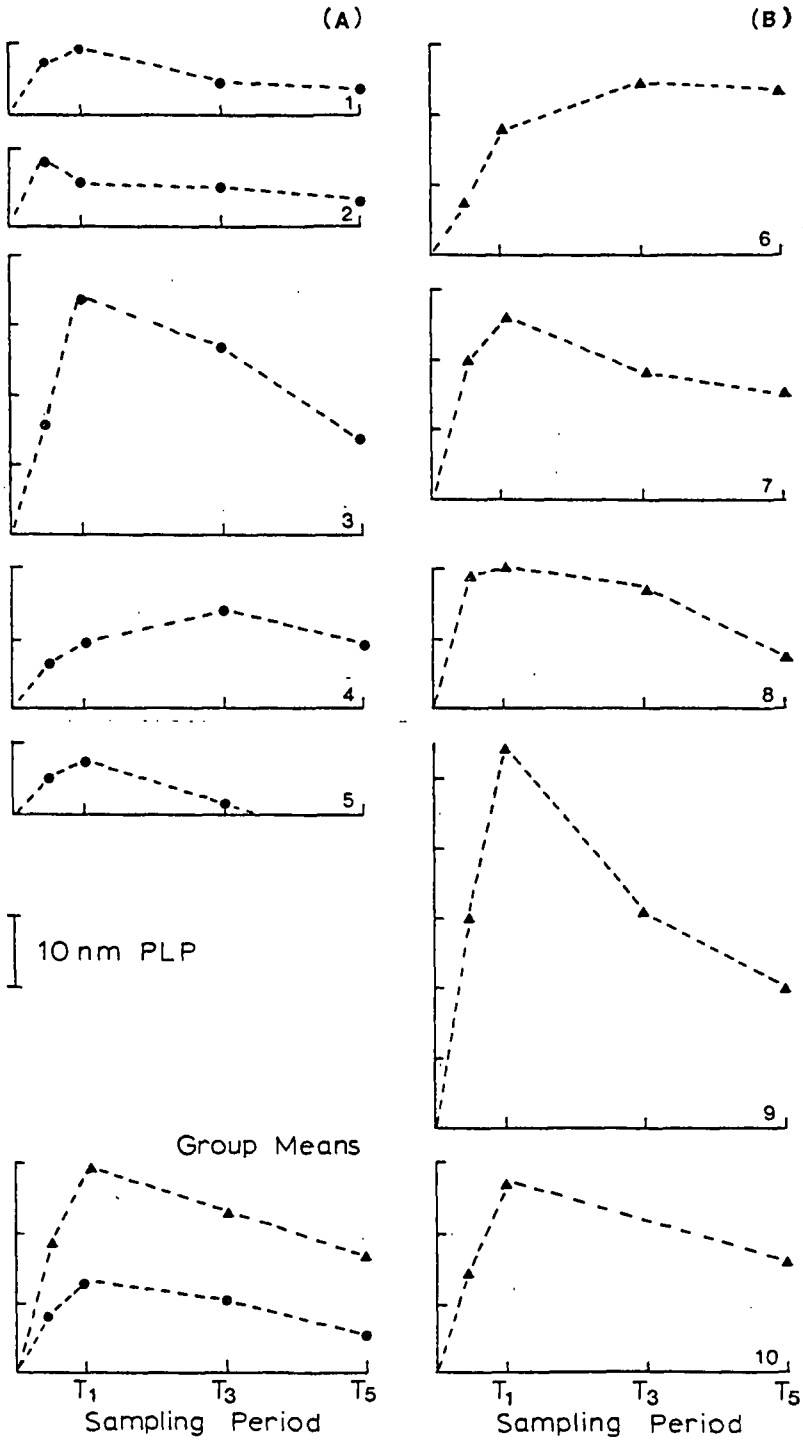

Figure 5. Individual plasma PLP (-----) response of elderly (Part A, ●) and young (Part B, ▲) subjects to a PN dose. Response was measured as baseline plasma PLP levels from timed blood collections after an oral loading dose of 0.1 mg PN.HCl per kg lean body mass. Plasma levels before the dose (TO) were subtracted from the levels in each post-dose plasma sample to give baseline values. The T3 sample was not drawn from subject 10. Mean group responses are also listed.

that of the young group at the T1/2, T1, T3 blood sampelings. At the T5 sample, the elderly plasma PLP levels were one-third of those in the young group. The PLP measured-peak in this study is at the T1 sampling, not at the T1/2 sample as observed by Wozenski et al.(1980). The young female group PLP levels were similar to those reported by Wozenski et al. in young males, but the elderly female PLP levels were lower.

The responses of suspected vitamin B-6 deficient subjects 1, 4 and 7 were included in baseline group means. Baseline plasma vitamin B-6 responses in subjects 1 and 4 were similar to their elderly peers (Fig. 4A). Subject 7 was also similar in response to her young peers, with the exception of levels at T3 (Fig. 4B). No consistent trends existed in the baseline plasma PLP response of these subjects (Fig.5). Since the baseline PN dose responses were neither high nor low when compared to their group peers, the responses of subjects 1, 4 and 7 were considered typical for their groups.

Neither of the two baseline plasma variables were statistically different between groups at any sampling time (Figs. 4 & 5, Table 7). When small sample sizes are used to compare two populations, the chance sampling from either end of a Gaussian distribution curve would result in large standard deviations from the mean. A large variation was observed in group baseline plasma variables

TABLE 7 Baseline Plasma Vitamin B-6 and PLP Loading Dose Response[a]

GROUP	SUBJECT	VITAMIN B-6 HOURS AFTER DOSE				PLP HOURS AFTER DOSE				PN DOSE[b] HALF-LIFE
		0.5	1.0	3.0	5.0	0.5	1.0	3.0	5.0	
	no		nM				nM			hr
Elderly	1	153.1	87.3	21.1	19.9	7.1	9.2	4.7	3.5	0.85
	2	170.2	72.5	20.2	17.1	9.6	5.5	5.3	3.5	0.45
	3	143.3	166.1	47.4	23.6	15.3	33.8	26.3	13.1	1.40
	4	59.0	100.9	39.0	31.0	6.3	9.1	14.0	9.5	1.65
	5	103.3	124.9	18.3	4.0	5.2	8.0	1.1	4.1	1.15
$\bar{X} \pm$ SD		125.8	110.3	29.2	19.1[1]	8.7[2]	13.1	10.3	5.1[3]	1.10
		± 44.7	± 36.6	±13.2	± 9.9	±4.0	±11.6	±10.1	± 6.6	±0.47
Young	6	68.0	119.4	69.4	43.2	8.0	18.4	24.7	23.5	2.74
	7	212.4	113.3	136.3	22.4	20.8	26.5	18.0	15.7	3.05
	8	219.6	86.6	43.5	32.3	19.8	21.0	17.8	7.6	0.45
	9	120.7	236.2	73.6	45.0	30.2	54.9	31.4	20.0	1.45
	10	103.7	151.6	c	32.7	14.9	27.1	c	16.7	2.50
$\bar{X} \pm$ SD		144.9	141.4	80.7	35.1[1]	18.7[2]	29.6	23.0	16.7[3]	2.04
		± 67.7	± 57.8	±39.4	± 9.2	± 8.2	±14.6	+ 6.5	± 5.9	±1.07

a) Baseline responses = plasma levels at each time period - the initial plasma levels at TO (before PN dose was administered).

b) Estimated time to clear half of the baseline measured-peak Vitamin B-6 levels from the plasma.

c) Blood sample was not collected.

1. $p < 0.03$ 2. $p < 0.04$ 3. $p < 0.02$

(Table 7). Standard deviations in plasma vitamin B-6 levels were 50% from the mean for several time periods in both groups. For example, the elderly group mean T3 vitamin B-6 level was 29.2+ 13.2 nM. For baseline plasma PLP levels, standard deviations are as much as 100% from the mean in the elderly group and 50% in the younger group mean. Group variation did not increase as a result of the loading dose, because PS and T0 plasma vitamin B-6 and PLP levels had similarly large standard deviations from the mean (Appendix Table 1, & Table 6 respectively). Although statistically significant differences between groups were not observed, interesting trends exist which suggest metabolic response differences in the elderly.

Differences in subject post-dose responses were not due to differences in the level of the PN loading dose. A low non-significant correlation ($r=0.33$) exists for individual dose and response as determined by the measured-peak baseline plasma vitamin B-6 levels (Table 8). For example, subject 8, who received the largest dose (5 mg PN.HCL), did not have the highest measured-peak vitamin B-6 concentration (Fig. 4B & Table 7). Subjects 1 and 5, who both received the smallest dose (3.0mg PN.HCL), did not have the lowest measured-peak response (Fig. 4A & Table 8).

Absorption rates of the PN dose were estimated from the baseline plasma vitamin B-6 values. Measured-peak

TABLE 8 Lean Body Mass (LBM) Estimation, PN Dose Assignments, and Measured-Peak Plasma Vitamin B-6 Values in Response to the PN Dose

GROUP	SUBJECT	URINARY CREATININE[a]	LBM[b]	PN.HCL DOSE[c]		MEASURED - PEAK[d]	MEASURED-PEAK DOSE[e]
	no.	g/d	kg	mg	Mmoles	nM	nM/moles
Elderly	1	0.75	29.2	3.0	14.6	153.1*	10.5
	2	1.11	39.6	4.0	19.4	170.2*	8.8
	3	0.92	34.2	3.5	17.0	166.1**	9.8
	4	1.14	40.6	4.0	19.4	100.9**	5.2
	5	0.68	27.3	3.0	14.6	124.9**	8.6
$\bar{X}$ ± SD		0.92[1]	34.2[1]	3.5[2]	17.0[2]	143.0	8.6
		± 0.21	± 6.0	±0.5	± 2.4	± 29.5	± 2.0
Young	6	1.19	43.2	4.5	21.9	119.4**	5.4
	7	1.05	38.0	4.0	19.4	212.4*	10.9
	8	1.43	49.1	5.0	24.3	219.6*	9.0
	9	1.13	40.2	4.0	19.4	236.2**	12.2
	10	1.15	40.7	4.0	19.4	151.6**	7.8
$\bar{X}$ ± SD		1.19[1]	42.0[1]	4.3[2]	20.9[2]	187.8	9.1
		± 0.14	± 4.2	±0.4	± 2.2	± 49.9	2.6

a) Presented as a 3-day average of the basal period.
b) Lean body mass as estimated from the average creatinine excretion according to the method of Forbes & Bruning, 1976.
c) Dose was administered as 0.1 mg PN.HCl/kg LBM.
d) Highest baseline vitamin B-6 plasma level, occurring 0.5* or 1 hour** after the PN.HCl loading dose on the experimental day.
e) Ratio of measured-peak height to the level of PN loading dose administered on the experimental day.

1. $p < 0.04$ between groups 2. $p < 0.03$ between groups.

height and time of peak arrival of this variable estimates the efficiency with which the oral PN dose was absorbed and transported into the bloodstream. The mean measured-peak concentration of vitamin B-6 occurs at the T1/2 sample in both groups (Fig. 4). Measured-peak height at 125.8nM ± 44.7 in the old and 144.9nM ± 67.7 in the young. Measured-peak height was not very different between groups indicating that efficiency of absorption and delivery of a PN dose, as measured by changes in plasma vitamin B-6 compounds, does not change with increasing age.

Clearance of vitamin B-6 from plasma was rapid in the elderly group as evidenced by changes in the T1 to T3 levels (Fig. 4A). Baseline mean plasma vitamin B-6 levels in the elderly group were similar to levels in the young group at T1/2 and T1, but at the T3 sample, levels in the elderly group were approximately 60% below those in the younger group (Fig. 4).

As a measure of plasma vitamin B-6 dose clearance, PN dose half-life was determined (Table 7). This value was calculated as the time for plasma vitamin B-6 levels in each subject to decline to half the value of the baseline measured-peak. The elderly group required 1.1 ± 0.5 hours to clear half the plasma vitamin B-6 dose while the young group required 2.0 ± 1.1 hours. Vitamin B-6 cleared from the blood is transported into body cells and

excreted in the urine.

Baseline plasma PLP levels suggest an age-related trend of decreased phosphorylation of the PN dose in the liver and or decreased transfer of newly synthesized PLP into the blood. While absorption of the PN dose appears to be unimpaired in the elderly group, a two fold response difference in plasma PLP levels existed between the groups suggesting differences in vitamin B-6 metabolism between the young and elderly subjects. Individual variability is greater in the elderly group than in the young group and in particular, subject 3 who has a PLP response notably different from her peers (Fig. 5A & Table 7). When this subject is excluded from the group means, half of the baseline PLP blood samples in the elderly group become statistically different ($P<0.01$) from the young group (T 1/2 $p<0.025$, T1 $P<0.020$, T3 $P<0.005$, T5 $P<0.010$; Appendix Table 2).

Plasma vitamin B-6 is a measure of all vitamins including PLP, which represents the major phosphorylated B-6 vitamer in plasma (Li and Lumeng, 1981). A ratio of plasma PLP to vitamin B-6 was determined to compare the percentage of plasma vitamin B-6 represented as the major phosphorylated form. This ratio was termed percent phosphorylated vitamer (% p-vitamer). In the fasting undosed state, individuals in both groups have from 60 to 80% of the total vitamin B-6 as PLP. This is indicated by

the PS and TO % p-vitamer values (Fig. 6). Although % p-vitamer declined in response to the loading dose, a remarkably constant difference existed between the elderly and young groups. Where differences between group vitamin B-6 levels are small, less than 30nM, the elderly group had about 20% less phosphorylated form present in the plasma, compared to the young group. With the exception of the T3 sample, all blood samplings, on the PS and the experimental days, resulted in less than a 30nM difference in vitamin B-6 concentration between groups. Since plasma PLP levels reflect hepatic metabolism (Lumeng et al., 1974), this constancy suggests an age-related metabolic difference that is unperturbed by a loading dose.

Remarkable group similarities exist in the levels of non-phosphorylated vitamers at all blood sampling periods, again with the exception of T3, on the experimental day (Fig. 6). Non-phosphorylated B-6 vitamers represent the major membrane transport and urinary excretion forms. These vitamers are the plasma vitamin B-6 'exit' forms. Group similarities in levels of these vitamins suggest similar metabolic and excretory opportunities to both young and old tissues.

As a measure of the ability to convert the PN dose to PLP and transport it into the blood, a ratio between baseline plasma PLP and vitamin B-6 levels was also

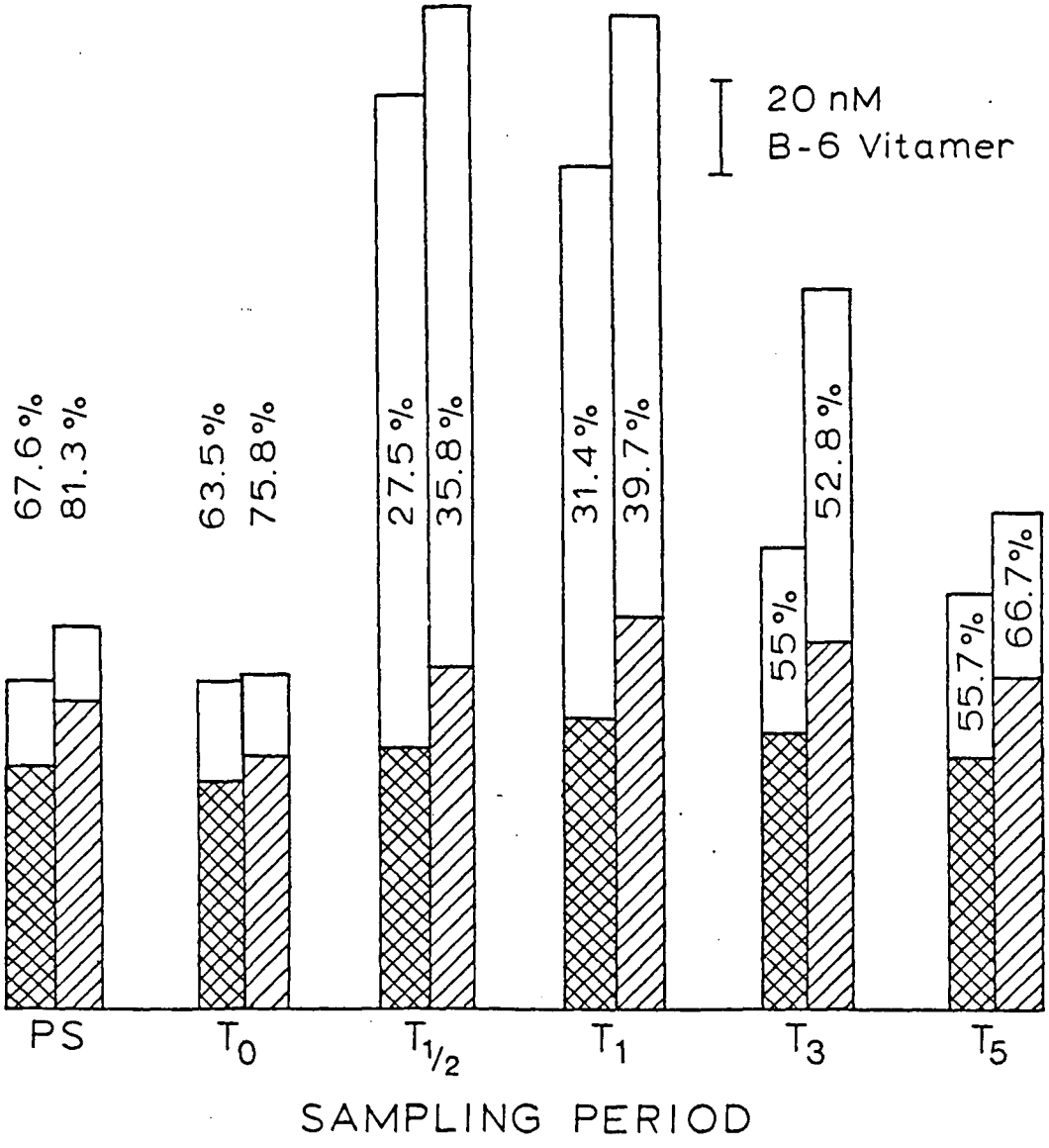

Figure 6. Percent of phosphorylated vitamer (% p-vitamer) in the plasma of elderly (▨) and young (▨) females from the pre-supplement (PS), pre-dose (TO) and timed post-dose ($T\frac{1}{2}$, T1, T3, T5) periods. The % p-vitamer is the fraction of plasma vitamin B-6 (total area under the barograph) represented by PLP (shaded area). Unshaded areas represent non-phosphorylated vitamer levels.

calculated as baseline % p-vitamer (Fig. 7). Baseline %p-vitamer indicated the elderly group phosphorylated about half as much PLP in response to the PN dose at T1/2 and T1 sampling periods. This difference is statistically significant at the 1% level in the T1 sample ($P<0.01$). The baseline % p-vitamer increases with time as the vitamin B-6 levels decline in both groups at the T3 and T5 samples. Since the vitamin B-6 clearance rate was greater between the T1 and T3 samples in the elderly groups, baseline % p-vitamer had a large increase and became similar to the ratio of the young group.

Urine Response

The 24 hour urine collections on the experimental day were divided into two excretion periods, 0 to 5 and 5 to 24 hours post-dose. Mean group excretion was similar for both variables in the 0 to 5 hour collection period (Table 9). Urinary 4-PA excretion was again similar between the two groups in the second collection period. Group differences in the 5 to 24 hour vitamin B-6 excretion were highly significant ($P<0.001$), the elderly group excreting less of the vitamin than the younger group. The higher dietary vitamin B-6 intake by the young subjects may be a factor in the increased urinary vitamin B-6 excretion (Table 9) The total 24 hour urinary excretion of these variables on the experimental day is

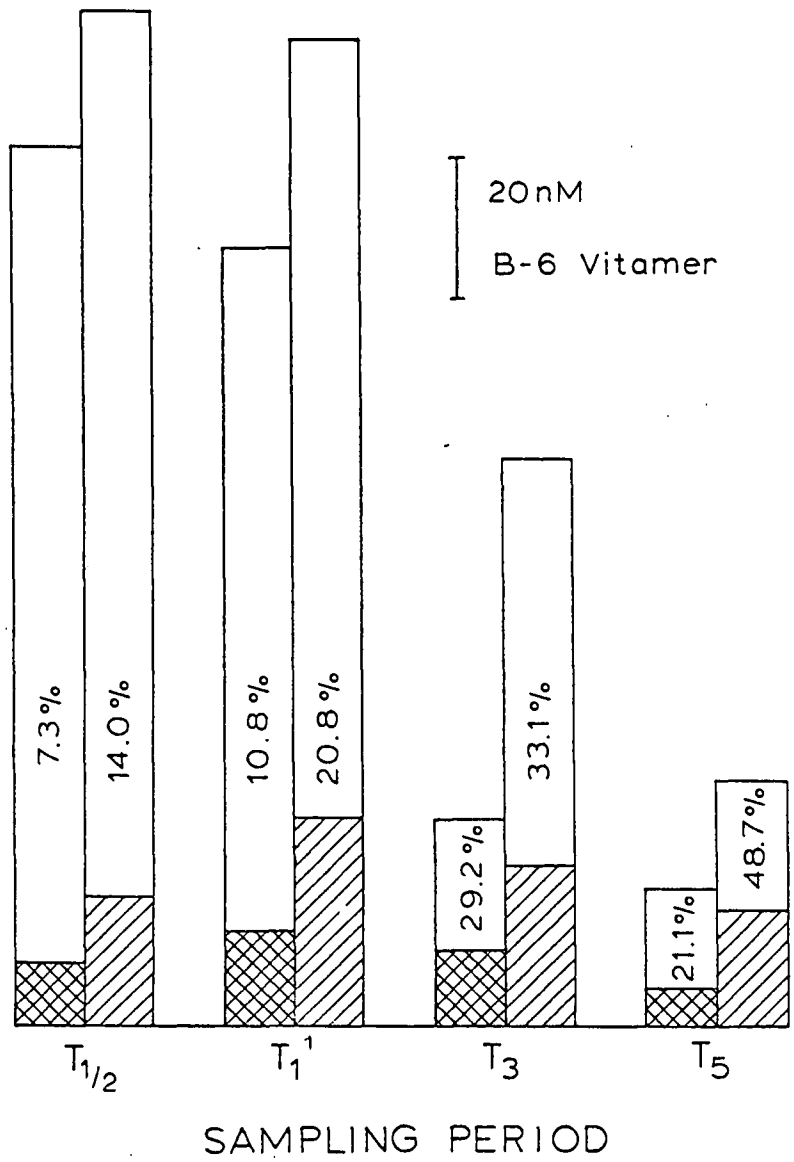

Figure 7. Baseline phosphorylated vitamer in the plasma of elderly (▨) and young (▨) females during response to the PN dose. Pre-dose plasma levels were subtracted from levels in each post-dose plasma sample to give a baseline value. The fraction of PLP (shaded area) in the plasma vitamin B-6 (total area under the barograph) was calculated for each post-dose collection.

1. $p < 0.01$

TABLE 9 Urinary 4-PA and Vitamin B-6 Excretion[a] After a PN Dose

GROUP	SUBJECT	VITAMIN B-6 INTAKE		4-PA HOURS POST-DOSE			VITAMIN B-6 HOURS POST-DOSE		
		Dose	Diet	0-5	5-24	0-24[b]	0-5	5-24	0-24[b]
	no	u moles/period		u moles/period		u moles/d	u moles/period		u moles/d
Elderly	1	14.6	4.0	3.72	4.90	8.62	0.63	0.51	1.13
	2	19.4	3.3	8.39	4.56	12.95	0.65	0.59	1.24
	3	17.0	9.0	5.59	8.79	14.38	0.59	0.68	1.27
	4	19.4	5.6	5.14	7.64	12.78	0.43	0.48	0.91
	5	14.6	5.1	3.16	5.01	8.17	0.46	0.51	0.96
$\bar{X} \pm$ SD		17.0[1]	5.4	5.20	6.18	11.38	0.55	0.56[2]	1.10[3]
		± 2.4	±2.2	±2.04	±1.91	± 2.80	±0.10	±0.08	±0.16
Young	6	21.9	6.1	4.75	9.85	14.60	0.61	0.87	1.48
	7	19.4	9.3	3.31	7.58	10.89	0.40	0.85	1.25
	8	24.3	9.3	6.08	6.96	13.05	0.97	1.16	2.14
	9	19.4	7.1	4.69	3.67	8.36	0.69	1.03	1.72
	10	19.4	3.8	5.61	6.67	12.27	0.62	0.79	1.41
$\bar{X} \pm$ SD		20.9[1]	7.1	4.89	6.95	11.83	0.66	0.94[2]	1.60[3]
		± 2.1	±2.3	±1.06	±2.22	± 2.36	±0.21	±0.15	±0.35

a) The 24 hour post-dose urine collection period consisted of two samples, 0 to 5 hours, and 5 to 24 hours post-dose collections.
b) Sum of the two post-dose urine collections.

1. p 0.03 2. p 0.001 3. p 0.02

increased from average basal levels (Tables 6 and 9). The levels of 4-PA excreted on the loading dose day are increased approximately two fold over the basal period in both groups, whereas vitamin B-6 excretion is increased only about 1.5 times in the elderly and young groups. Similarities in 4-PA excretion in both collection periods on the experimental day, indicate similar 4-PA levels were filtered from the plasma and excreted. This observation suggests comparable metabolic dose response in the young and elderly subjects. A wide variety of tissues have the enzymes to synthesize 4-PA (Stanulovic, 1980). The major metabolic pathway for synthesis of this metabolite from PN is: PN → PNP → PLP → PL → 4-PA (Lumeng and Li, 1980; Segalman and Brown, 1981) (Fig. 2). The major regulatory step in the synthesis of 4-PA is the protein binding of PLP. Newly synthesized PLP that remains unbound is rapidly metabolized to 4-PA (Lumeng et al., 1980). Depending on the tissue of origin, 4-PA may be synthesized through PLP or directly from PL which may be preexisting or the result of the PN dose interconversion (Fig. 2). The major route of metabolite synthesis (i.e. from PLP to PL) is likely to change with time from the first to the second urinary collection period. However, large differences in intracellular binding of PLP would account for a difference in the amount of 4-PA synthesized at any given time. In the

liver, protein bound PLP as synthesized from the PN dose would directly limit the available substrate for 4-PA synthesis. In extra-hepatic tissues there is a possible competition for substrate. If a net loss of intracellular PL to protein bound PLP occurs, less 4-PA will be produced. Presumably, the more saturated the PLP intracellular binding proteins are, the greater will be the synthesis of 4-PA (Fig. 2). In the present study, the group similarities in the urinary 4-PA data suggests that intracellular PLP-binding proteins are similarly saturated in both groups, hence catabolism of the dose to 4-PA and subsequent excretion was comparable between groups.

Group levels of vitamin B-6 excreted in the second urine collection period were statistically different ($P<0.001$). Urinary vitamin B-6 excretion reflects dietary vitamin B-6 intake, and group differences in the 5-24 hour urine collection might be explained on this basis. Moreover, recovery of dietary vitamin B-6 as urine vitamin B-6 compounds again show similarities between groups (Table 10). The 24-hour recovery of dietary vitamin B-6 in the vitamin B-6 levels of both the young and elderly group were not statistically different. This indicates that differences in the 5 to 24 hour urinary vitamin B-6 excretion were due to dietary, not metabolic differences between groups. Recoveries of dietary vitamin

TABLE 10

Recovery of Dietary Vitamin B-6
As Urinary Vitamin B-6 Compounds After a PN Dose

GROUP	SUBJECT	VITAMIN B-6 INTAKE		4-PA RECOVERY[a] HOURS POST-DOSE		VITAMIN B-6 RECOVERY[b] HOURS POST-DOSE	
		Dose	Day	0-5	0-24	0-5	0-24
	no	u moles		%		%	
Elderly	1	14.6	18.6	25.5	46.3	4.3	6.1
	2	19.4	22.8	43.2	56.6	3.3	5.4
	3	17.0	26.0	32.9	55.3	3.5	4.9
	4	19.4	25.0	26.5	51.1	2.2	3.6
	5	14.6	19.7	21.6	41.5	3.2	4.9
$\bar{X} \pm$ SD		17.0[1]	22.4[2]	29.9	50.2	3.3	5.0
		± 2.4	± 3.2	± 8.4	± 6.3	±0.8	± 0.9
Young	6	21.9	28.0	21.7	52.1	2.8	5.3
	7	19.4	28.8	17.1	37.8	2.0	4.3
	8	24.3	33.6	25.0	38.8	4.0	6.4
	9	19.4	26.6	24.2	31.4	3.5	6.5
	10	19.4	23.3	28.9	52.7	3.2	6.0
$\bar{X} \pm$ SD		20.9	28.0[2]	23.4	42.6	3.1	5.7
		± 2.2	± 3.76	± 4.4	± 9.4	±0.8	±0.9

a) 4-PA Recovery = 4-PA excreted/Vitamin B-6 ingested. 0-5 hour recovery represents the 0 to 5 hour 4-PA excretion/PN dose, and the 0-24 hour recovery represents the daily 4-PA excretion/daily Vitamin B-6 ingestion, including the PN dose.

b) Vitamin B-6 Recovery = Vitamin B-6 excreted/Vitamin B-6 ingested. The 0-5 hour recovery represents 0-5 hour excretion/dose; 0-24 hour recovery represents 0-24 hour excretion/daily Vitamin B-6 ingestion.

1. $p < 0.03$ 2. $p < 0.04$

B-6 in the other urine collection periods were also similar between the two groups and suggest no metabolic differences (Table 10).

V. COMMENTS AND CONCLUSIONS

We postulated three explanations for the apparent decline in vitamin B-6 status in elderly people. These were decreased vitamin B-6 intake, decreased absorption, or changed metabolism of vitamin B-6. Results of this study suggest that changes in vitamin B-6 metabolism may account for the previously observed decrease in vitamin B-6 status with age.

Dietary vitamin B-6 intakes by the elderly subjects in this investigation were adequate in relation to protein intake (Dietary Standards for Canada, 1975). This trend was not seen in the young subjects. The individual vitamin B-6 intakes of the elderly subjects were consistantly higher per g of protein than the recommended adequate levels (0.020 mg vitamin B-6/g protein), while the younger subjects were consistantly lower (Table 5). Accordingly, any observed low plasma PLP response to the orally administered PN dose by the elderly group in this study could not be attributed to dietary insufficiencies. Before the 5 day PN supplementation the mean plasma PLP levels in the elderly were only slightly lower than those in the younger group (Table 6). These findings did not duplicate the earlier dramatic observations of Hamfelt (1964); Walsh (1966); Lumeng and Li (1974); and Rose et al.,(1976). An

explanation for these different findings may be differences in the subjects' dietary vitamin B-6 intake. The elderly subjects in this study were educated women who are informed about nutrition. It might be expected that these women would have adequate dietary vitamin B-6 intakes. Hamfelt, Walsh, Lumeng and Li, and Rose et al. did not report dietary vitamin B-6 intake for their subjects.

Trends in subjects' response to the PN dose suggests no age-impairment of PN absorption , because measured-peak vitamin B-6 height and time of peak arrival of vitamin B-6 were similar for the two groups (Fig. 4, & Table 8). While the elderly in this study had adequate vitamin B-6 intake and no apparent impairment of vitamin B-6 absorption, their metabolism of vitamin B-6 as measured by their response to the PN loading dose response was different from that of the young controls.

Six observations from the experimental day that deserve mention in a comprehensive discussion of the vitamin B-6 metabolism of these two groups of women. These observations are: (1) lower baseline plasma PLP levels in the elderly at each sampling time; (2) constancy of group differences in % p-vitamer; (3) decreased baseline % p-vitamer in the elderly at each sampling time; (4) group similarities in 4-PA excretion; (5) increased PN dose clearance from plasma in the

elderly; and (6) group similarities in the levels of non-phosphorylated vitamer. Each observation will be discussed separately. The most important variables in evaluating PN dose metabolism in this study are plasma PLP and urinary 4-PA responses. Both variables reflect metabolic conversions of the PN dose (Fig. 1). In future studies, laboratory methods should be used that measure specific B-6 vitamers in blood and urine of subjects who have received a PN loading dose. Group differences may exist in the major non-phosphorylated forms that are circulated and excreted. For example, Leklem et al.(1980) suggested metabolism of the dietary vitamer PN in subjects who excreted large amounts of PL.

Changes in post dose plasma PLP levels reflect hepatic metabolism of the PN dose and transport of the PLP vitamer into the plasma (Lumeng et al., 1974). Three lines of evidence from the experimental day suggest that reduced hepatic PLP synthesis or impaired PLP transport may exist in the elderly group. The first observation was that at all sampling periods, baseline plasma PLP levels in the elderly were less than half that in the young group. In addition, low initial baseline %p-vitamer levels indicate the elderly subjects phosphorylated about half as much of the PN dose as did the young (Fig. 7). These two observations could indicate an age dependent decline in liver PN kinase activity such

that less PLP is synthesized in response to the PN dose. Alternatively, these observations could be consistent with intracellular trapping of the newly synthesized PLP with unimpaired PLP synthesis. This later proposal is in effect an impaired transport mechanism. Whichever interpretation is correct for the possible mechanisms for these group differences in PLP response, the mechanism is constant (Fig. 6). Group differences in %p-vitamer are remarkably constant and unpreturbed by the PN dose (Observation 3). Reasons for the apparently impaired transport of newly synthesized PLP may range from membrane abnormalities to increased intracellular binding of PLP. If the sole reason for intracellular trapping of PLP is an impairment of hepatic transport in the elderly, one might expect this impairment to be reflected in a delayed time of measured-peak plasma PLP level. Since this event was not observed, a simple transport impairment in the elderly group is not indicated (Fig. 5). If high levels of unbound PLP are trapped intracellularly, one would expect large increases in urinary 4-PA excretion.

The fourth observation obtained from the loading dose experiment was that similarities existed in group 4-PA excretion for both the 0-5 and the 5-24 hour urine collection periods. In addition the % increase in excretion from the basal period was similar between

groups indicating similar conversions of the PN dose to 4-PA. In other words, the intracellular PLP and PL levels available for conversion to 4-PA were similar in both groups. This observation then implies similar saturation of intracellular PLP binding proteins. This suggests that high levels of unbound PLP were not trapped intracellularly in either group. If group similarities in PLP saturation of protein existed, then both the young and the elderly subjects have similar vitamin B-6 status. This is analogous to the transaminase assay which is used to assess vitamin B-6 status by quantitating the saturation of a transaminase enzyme with the coenzyme, PLP (Sauberlich et al., 1972). Likewise, the saturation of PLP tissue pools would indicate vitamin B-6 status is similar in both groups.

Blood levels of vitamin B-6 are the net result of the flux of vitamers into and from the blood. Therefore, increased plasma clearance in the elderly group from the T1 to T3 sampling may be the result of two events: a net movement of vitamers into cells and urinary excretion. Current theory maintains that non-phosphorylated vitamers are the primary membrane transport and excretory forms of vitamin B-6 (Lumeng and Li, 1980). However, concentrations of plasma non-phosphorylated B-6 vitamers were similar in both groups at the T1 sampling (Fig. 6). The increased clearance rate in the elderly group after

the T1 sampling is inconsistant with current theory and the sixth observation of similar plasma levels of non-phosphorylated vitamers in both groups (Fig. 6).

It is unknown if renal excretion contributes substantially to increased clearance rates of vitamin B-6 from the T1 to T3 blood samples in the elderly because only one urine sample was obtained for the entire 5 hour blood collection period. While the group plasma vitamin B-6 levels were very different in the T3 sample, concentrations in the T1 and T5 samples were very similar between groups. If the elderly vitamin B-6 excretion rates were faster between the T1 and T3 blood samplings, this event could not be detected in this 0 to 5 hour urine collection interval. Additional shorter urine collection periods would have been helpful in identifying mechanisms for increased vitamin B-6 clearance by the elderly group during this time period.

If the increased vitamin B-6 clearance rate in the elderly group is the result of non-phosphorylated B-6 vitamer movement into cells, intracellular trapping of the vitamer could explain all the phenomona. If intracellular trapping is the mechanism, then the existance of a high affinity intracellular PLP-binding protein in the elderly group would be consistent with the decreased plasma PLP levels in response to the dose, the constant differences in group %p-vitamer levels and

increased plasma vitamin B-6 clearance by the elderly. The existence of a high affinity intracellular PLP-binding protein might also be consistent with the theory of similar PLP saturation in both groups because binding affinities may enter into the ability to saturate tissue pools.

In conclusion, the observations gathered in this study may indicate decreased PN kinase activity and/or intracellular of PLP in elderly females. The response of young and elderly females to a loading dose of PN was a pilot project. While data interpretation in this study is speculative, new research directions were identified. Future research in vitamin B-6 metabolism and ageing should examine both enzymatic and storage capacities in elderly tissues.

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VII. APPENDIX

Appendix Table 1
Presupplement (PS) and predose (TO) plasma VB levels in elderly and young females.

GROUP	SUBJECT	PLASMA VITAMIN B-6 PS	TO
	no	nM	
ELDERLY			
	1	37.5	45.9
	2	38.9	43.4
	3	126.6	126.7
	4	34.3	48.5
	5	113.8	88.5
	x	70.2	70.6
	+SD	+45.9	+36.4
YOUNG			
	6	51.9	58.0
	7	44.8	53.4
	8	62.3	74.4
	9	92.4	112.9
	10	161.9	61.4
	X	82.7	72.0
	+SD	+47.9	+24.1

Appendix Table 2
Mean baseline PLP response to a PN dose.

GROUP		PLASMA PLP LEVELS HOURS AFTER DOSE T1/2	T1	T3	T5
		nM			
ELDERLY	X	7.0 [1]	8.0 [2]	6.3 [3]	3.1 [4]
	+SD	+1.9	+1.7	+5.5	+5.6
YOUNG	X	18.7	29.6	23.0	16.7
	+SD	+8.2	+14.6	+6.5	+5.9

a) Means for the elderly group are excluding the baseline PLP values of subject 3 (n=4).

1. $p<0.025$. 2. $p<0.020$. 3. $p<0.005$. 4. $p<0.010$.