AN ABSTRACT OF THE THESIS OF

Janet Elizabeth Harris for the degree of Master of Science in Nutrition and Food Management presented on March 16, 1990

Title: Effect of Estrogen Replacement Therapy on Vitamin B-6 Status of Postmenopausal Women

Abstract Approved: _____
Dr. Lorraine T. Miller

This investigation was conducted to determine the effect of estrogen replacement therapy (ERT) on vitamin B-6 status of postmenopausal women. Nineteen postmenopausal women served as subjects. Nine (54.7 ± 4.7 years) were taking ERT (experimental group); ten (56.8 ± 2.3 years) were not (control group). For three consecutive days, subjects recorded their dietary intake and collected their 24-hour urine specimens. On the fourth day, a fasting blood sample was drawn from the subjects. The dietary intake of vitamin B-6, as well as the concentration of total vitamin B-6 in plasma (PB6) and urine (UB6) were measured. PB6 and UB6 were determined by a microbiological method with Saccharomyces uvarum as the assay organism.

The mean age, height, hematocrit and hemoglobin values were similar for the two groups. The experimental group was significantly heavier than the control group ($p<0.05$). The experimental group had a lower mean PB6 than the control group: 47.7 ± 19.7 nmol/L vs. 56.2 ± 20.6 nmol/L. These means were not significantly different ($p=0.05$). PB6 was positively correlated with dietary vitamin B-6 intake ($p=0.0001$) and vitamin B-6 to protein ratio ($p=0.0021$). When the means were adjusted for dietary vitamin B-6 and the vitamin B-6 to protein ratio, the mean PB6 of the experimental group (42.7 nmol/L) was significantly lower than that of the control group (60.6 nmol/L) ($p<0.05$). PB6 was not positively correlated with either age ($r=0.20$) or the vitamin B-6 dietary history score ($r=0.15$).

UB6 was similar for the two groups. UB6 correlated positively with daily dietary intake of vitamin B-6 ($r=0.51$, $p<0.05$) and the ratio of vitamin B-6 to protein ($r=0.47$, $p<0.05$). UB6 was not significantly correlated to urine volume ($r=0.05$).

The mean daily intakes of vitamin B-6 and protein were similar for the two groups. One of the 19 subjects had a vitamin B-6 intake that was less than 67 percent of the RDA. Most subjects' (89%) intake of vitamin B-6 was adequate when the ratio of 0.016 mg of vitamin B-6 per g of protein was used as the standard.

THE EFFECT OF ESTROGEN REPLACEMENT THERAPY ON VITAMIN B-6 STATUS OF POSTMENOPAUSAL WOMEN

by

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INTRODUCTION

Young women using estrogens for contraceptive purposes do not consistently have a lower vitamin B-6 status or a significantly increased requirement for the vitamin (Leklem, 1986). However, there is convincing evidence that vitamin B-6 status is sacrificed in some women using these drugs (Lumeng, Cleary and Li, 1974; Lind, 1980; Hamfelt, Soderhjelm and Mikaelson, 1984; Miller, 1986).

Several indicators of vitamin B-6 status decline with age (Walsh, 1966; Lumeng and Li, 1974; Rose et al., 1976; Lee and Leklem, 1984). In addition, the dietary intake of vitamin B-6 by the elderly, especially women, has frequently been identified to be inadequate (Vir and Love, 1977; Hampton, Chrisley and Driskell, 1977; Chrisley and Driskell, 1979; Garry et al., 1982).

The combined effect of age and estrogen use on the vitamin B-6 status of postmenopausal women is an area of concern. It was proposed that the use of estrogen replacement therapy (ERT) by postmenopausal women would provoke an even sharper decline in their vitamin B-6 status when compared to postmenopausal women who do not use ERT.

The primary aim of this study was to determine vitamin B-6 status in postmenopausal women using ERT by measuring the concentration of total vitamin B-6 in plasma (PB6) and urine (UB6) and the dietary intake of the vitamin. A secondary aim was to assess the dietary patterns that accounted for the observed dietary intake and the relationship between dietary intake and the biochemical indices used in this study to assess vitamin B-6 status.

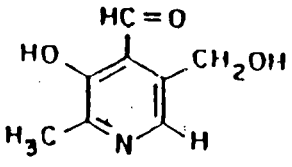
REVIEW OF LITERATURE

VITAMIN B-6

Vitamin B-6 plays an important role in amino acid metabolism and is also involved in the metabolism of carbohydrate, lipids and nucleic acids. Over 100 enzymes have been identified for which pyridoxal 5'-phosphate (PLP), the chief active form of vitamin B-6, is a coenzyme (Sauberlich, 1985). Because several comprehensive reviews on vitamin B-6 are available (National Academy of Sciences, 1978; Tryfiates, 1980; Leklem and Reynolds, 1981, 1988; Reynolds and Leklem, 1985), only a brief review of vitamin B-6 metabolism will be presented here.

Absorption. The six forms of vitamin B-6 are: The three free forms, pyridoxal (PL), pyridoxamine (PM) and pyridoxine (PN); and their respective phosphorylated forms, pyridoxal 5'-phosphate (PLP), pyridoxamine 5'-phosphate (PMP) and pyridoxine 5'-phosphate (PNP). The structures of these B-6 vitamers and the metabolite, 4-pyridoxic acid, (4PA) are shown in Figure 1.

Intestinal absorption of vitamin B-6 is dependent on the amount of the vitamin present in the intestinal lumen and does not require energy (Yamada and Tsjue, 1980). Absorption, which is by passive diffusion, is rapid and occurs predominately in the jejunum, and to a lesser extent

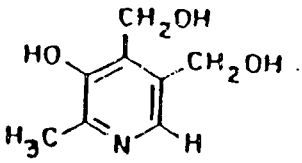


PYRIDOXAL

(PL)

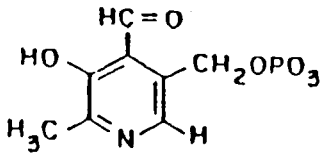
PYRIDOXAMINE

(PM)



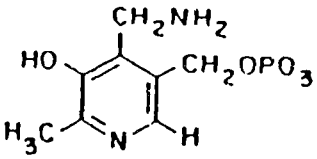
PYRIDOXINE

(PN)



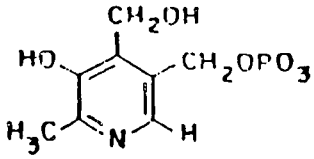
PYRIDOXAL
5'-PHOSPHATE

(PLP)



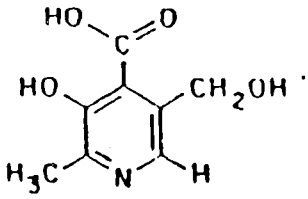
PYRIDOXAMINE
5'-PHOSPHATE

(PMP)



PYRIDOXINE
5'-PHOSPHATE

(PNP)



4-PYRIDOXIC ACID
(4PA)

Fig 1. The B-6 vitamers and 4-pyridoxic acid.

in the ileum (Booth and Brain, 1962). It involves mainly PL, PM and PN since a membrane-bound alkaline phosphatase hydrolyzes most of the phosphylated forms in the intestinal lumen (Ink and Henderson, 1984).

Metabolism, Transport and Storage. The nonphosphorylated forms of vitamin B-6 absorbed by the intestinal tract are initially concentrated in the liver where they are converted to the phosphorylated forms by PL (PM or PN) kinase (EC 2.7.1.35), an enzyme which requires ATP and a divalent cation such as Zn or Mg (Merrill et al., 1984). PNP and PMP can then be converted to PLP by a flavin mononucleotide-dependent oxidase (EC 1.4.3.5). PLP and PMP are interconvertible through transamination by aminotransferases (Lumeng, Li and Lui, 1985). PLP bound to albumin is released into the plasma by the liver, the organ from which plasma PLP is derived (Lumeng, Brashear and Li, 1974). The metabolic interconversions and degradation of the B-6 vitamers are summarized in Figure 2.

Some important differences in vitamin B-6 metabolism have been identified in several tissues in the body. Lumeng and co-workers (1985) reviewed these differences and the important, but different, roles that the liver and erythrocytes play in the inter-organ transport and metabolism of vitamin B-6. In man, PMP (PNP) oxidase activity is found only in the liver and erythrocytes. The liver plays a central role in vitamin B-6 metabolism. It possesses a high PMP (PNP) oxidase activity and releases

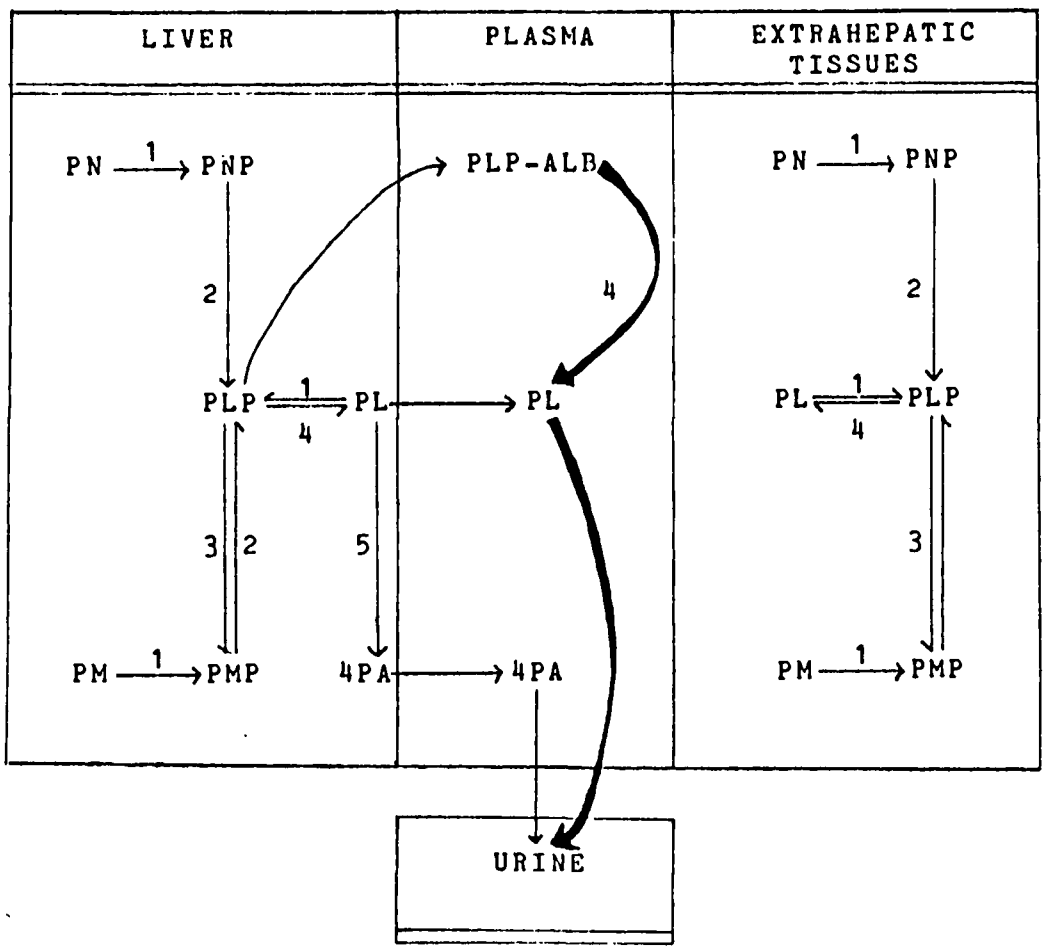


Fig 2. Metabolic pathways of vitamin B-6. Adapted from Lumeng et al., 1984. Boldface arrows represent the major pathways involved in the degradation of plasma PLP. Reactions are catalyzed by: 1- pyridoxal kinase, 2- pyridoxine-P oxidase, 3- aminotransferases, 4- alkaline phosphatase and 5- aldehyde oxidase and aldehyde dehydrogenase.

PLP, PL and 4PA into the plasma. (See Figure 2). Erythrocytes, which have a very low PMP (PNP) oxidase activity, are also an important source of PL for tissues which lack PMP (PNP) oxidase. Most cells have PL kinase activity (Lumeng et al., 1985) and, therefore, can rephosphorylate PL intracellularly. (See Figure 2).

PLP, accounting for more than 50 percent of the plasma vitamin B-6, is the major transport form of vitamin B-6 (Lumeng, Lui and Li, 1980). PLP in plasma and erythrocytes is tightly bound to albumin and hemoglobin, respectively, by means of a Schiff base linkage. These PLP-protein complexes generally cannot cross membranes as efficiently as the nonphosphorylated forms, suggesting a protective mechanism by which PLP can be retained in the cell (Brin, 1978). This interaction also prevents PLP from being transported directly into cells, such as erythrocytes, and slows the rate of degradation by alkaline phosphatase (Lumeng et al., 1974), thus regulating intracellular levels of PLP and PMP.

Lumeng et al. (1984) performed several experiments in dogs to identify the metabolic fate of plasma PLP and examine the role of various organs in plasma PLP clearance. PLP attached to albumin was administered intravenously to dogs that had undergone hepatectomy; nephrectomy; combined removal of the stomach, small intestine and spleen; or a sham operation. There were no significant differences among the four groups of dogs in plasma clearance of PLP,

suggesting that the liver, kidney, spleen and gastrointestinal tract do not degrade plasma PLP. Changes in plasma B-6 vitamers over time (0.5 to 5.0 hours post-dose) indicated that the conversion of PLP to PL is an important in vivo pathway in the degradation of plasma PLP. PL not only accumulated in plasma, but was also excreted in urine. (See Figure 2. Note boldface arrows).

Small quantities of vitamin B-6 are stored in the body. PLP and PMP are the predominant forms of vitamin B-6 in mammalian tissues (Li and Lumeng, 1981). Although the liver is particularly rich in both PLP and PMP, PLP bound to glycogen phosphorylase in muscle represents the major body pool of vitamin B-6 (Black, Guirard and Snell, 1977).

Excretion. Four-pyridoxic acid (4PA), which does not possess biological activity, is the major excretory metabolite of vitamin B-6 metabolism (Brin, 1978). PLP and PMP synthesized in excess of the binding capacity of proteins are dephosphorylated by alkaline phosphatase (Lumeng and Li, 1975). The PL or PM formed is oxidized to 4PA by aldehyde oxidase (FAD-dependent) and aldehyde dehydrogenase (NAD-dependent) (McCormick and Merrill, 1980) in the liver. (See Figure 2). Since urinary 4PA excretion generally reflects dietary intake, it is not a good indicator of body stores (Sauberlich, 1981). Urinary total vitamin B-6 also reflects recent dietary intake of the vitamin (Miller and Edwards, 1981).

Biochemical Function. Vitamin B-6 serves as a coenzyme for a wide variety of metabolic reactions in the body. Its most notable role is in the metabolism of amino acids in which PLP serves as the coenzyme of a number of enzymes including aminotransferases, decarboxylases and dehydratases.

The most common and best known of these are aminotransferases, which are important in the synthesis and catabolism of amino acids. Decarboxylation reactions are employed in the synthesis of several neuroactive amines, such as serotonin, tyramine, histamine, epinephrine and γ-amino butyric acid. Serine, homoserine and threonine are converted to their α-keto acids by dehydratases. PLP is required for desulfhydration of homocysteine and cysteine in the metabolism of methionine. Vitamin B-6 is also involved in the conversion of tryptophan to niacin, the biosynthesis of porphyrins and plays a conformational role in glycogen phosphorlylase.

Nutritional Requirements and Assessment. The current recommended dietary allowance (RDA) for vitamin B-6 is 2.0 mg for adult males, 1.6 mg for adult women, 2.2 mg during pregnancy and 2.6 mg during lactation. Because the requirement for vitamin B-6 is related to protein intake, the 1989 RDA is based on 0.016 mg of vitamin B-6 per g of protein.

There are several methods for assessing the vitamin B-6 status of humans. These include direct measurement of

various forms and metabolites of vitamin B-6 in blood and urine; estimation of erythrocyte aminotransferase activity and its stimulation in vitro by PLP; and measurement of metabolites of tryptophan or methionine excreted in urine following loading doses of either of these two amino acids. Although the concentration of PLP in plasma is considered to be a reliable and sensitive indicator of vitamin B-6 nutritional status, Leklem and Reynolds (1981) recommend using two biochemical measurements combined with an evaluation of dietary intake of vitamin B-6 and protein for proper vitamin B-6 status assessment. This would be particularly important when a drug interaction with vitamin B-6 metabolism is suspected.

Deficiency. Typical deficiency symptoms in humans, summarized by Sauberlich and Canham (1980), include the following: a) personality changes - mental depression, irritability, nervousness, convulsions and confusion; b) skin changes - seborrheic lesions about the eyes, nasolabial folds and mouth; pellagra-like dermatitis; cheilosis; glossitis; and stomatitis; c) other changes - abnormal tryptophan metabolism, weight loss, hypochromic anemia, electroencephalographic abnormalities, impaired immune response and impaired motor function. These deficiency symptoms were observed in several studies in which human subjects were made vitamin B-6 deficient with the use of a vitamin B-6 deficient diet and/or 4-deoxypyridoxine, a pyridoxine antagonist.

ESTROGEN REPLACEMENT THERAPY

Estrogen replacement therapy (ERT) continues to be a controversial topic. Women faced with the decision of whether or not to take estrogens must weigh the benefits of ERT against its risks and undesirable side affects.

Menopause. Many biological functions including those which are under hormonal control become impaired with age (Change and Roth, 1979). Menopause, which occurs at the termination of menstruation, is a form of primary ovarian hypofunction that is experienced by all women who survive to middle life (Kicklighter and Kulkarni, 1984). The median age of onset of menopause is 50 years (Hammond and Maxson, 1982). Surgical menopause results from the surgical removal of the uterus and ovaries, oophorohysterectomy; or surgical removal of the ovaries, oophorectomy. In 1982, women in the postmenopausal age range numbered 40 million in the United States (Hammond and Maxson, 1982).

Menopause provokes a variety of disturbances in some women, but may be asymptomatic in others. The most important factor influencing the development of symptoms during the postmenopausal years is the degree of estrogen depletion and the rate at which estrogen levels decrease (Gambrell, 1982). Hot flashes, the most common symptom, occur in 75 to 85 percent of women who experience natural menopause. Because estrogens protect against parathyroid hormone-mediated bone resorption, loss of estrogen after

natural or surgical menopause is one of the risk factors that contributes to osteoporosis, a serious and extremely costly disease of older women.

Synthetic Estrogens. Estrogen is the most specific and effective drug available for relief of the hot flashes associated with menopause. Of the currently available methods for the prevention and treatment of osteoporosis, ERT has been the most frequently prescribed medication (Chestnut, 1984). Conjugated equine estrogen or its equivalent given cyclically in a dosage of 0.625 mg per day can retard postmenopausal osteoporosis (Fish and Don, 1985).

Conjugated equine estrogens (Premarin, Ayerst Laboratories, New York, N.Y.) are obtained from the urine of pregnant mares. This drug consists of estrone sulfate (48 percent), equilin sulfate (26 percent), 17-dihydroequilin sulfate (15 percent) and other conjugated estrogens in smaller amounts. Based on in vivo data indicating differences produced by different conjugated estrogens in urinary excretion levels of active ingredients, generic conjugated estrogens are not therapeutically equivalent (USPDI, 1990). The use of a progestogen is an important facet of ERT. Ten days of cyclic progestogen is recommended to reduce the risk of endometrial cancer (Gambrell, 1982). ERT is usually administered on a cyclic dosage schedule, such as three weeks with medication and one week without, to approximate

the natural menses and to avoid overstimulation of estrogen-activated tissues (Barnhart, 1986).

At a recent Consensus Development Conference on Osteoporosis sponsored by the National Institute of Health (1984), it was concluded that ERT should be given, if there are no complications, to women whose ovaries are removed before the age of 50 and to women who have had a natural menopause. In addition, it was suggested that the duration of ERT need not be limited. In one study, more than half of the postmenopausal women in one metropolitan area had used estrogens for at least three months (Weinstein, 1980). The prevalence of estrogen use by postmenopausal women has been growing rapidly for a number of years.

VITAMIN B-6 AND ESTROGENS

All classes of steroid hormones act by binding to intracellular receptor proteins (Hughes, 1984). After an initial uptake by the target cell, estrogen interacts with a cytosolic protein receptor. The estrogen-protein complex then migrates to the nucleus where it interacts with specific binding sites on the chromosomes initiating DNA-directed, RNA-mediated new protein synthesis and expression of biological function of the steroid (Rochefort and Wesley, 1984).

The estrogen-vitamin B-6 interaction is complex. PLP has been shown to interact with the estrogen-receptor complex and inhibit its binding to DNA. This interaction leads to inhibition of the action of the estrogen (DiSorbo

and Litwack, 1982). The level of plasma PLP is inversely correlated with the uptake of estrogen into nuclei of target tissues (Holley et al., 1983). Additionally, a number of PLP-dependent enzymes are inducible by estrogens, either directly or indirectly because estrogens cause an increase in the level of plasma glucocorticoids, which, in turn, stimulate enzyme protein synthesis (Rose, 1978). This could lead to increased PLP binding and altered tissue distribution of PLP.

Estrogens, which are commonly used by postmenopausal women to relieve symptoms associated with menopause, have been found to affect several parameters used to measure vitamin B-6 status. The majority of the studies that have investigated the effects of estrogen on vitamin B-6 status and metabolism have involved young women using estrogen-progestogen preparations for contraceptive purposes. The influence of oral contraceptives on vitamin B-6 metabolism was reviewed by Miller (1985).

Tryptophan Metabolism. The concern that oral contraceptives (OC) may have an effect on vitamin B-6 status was aroused in 1966 when Rose demonstrated an increased urinary excretion of tryptophan metabolites by OC users after an oral dose of tryptophan (Rose, 1966). Abnormal tryptophan metabolism was subsequently exposed by several researchers (Price, Thorton and Mueller, 1967; Aly, Donald and Simpson, 1971; Price, Rose and Toseland, 1972; Adams et al., 1973; Miller et al., 1975; Leklem et al.,

1975b; and Donald and Bosse, 1979). Since abnormal tryptophan metabolism is a sensitive indicator of vitamin B-6 status, it was assumed that OC users were vitamin B-6 deficient or had an increased requirement for vitamin B-6. The tryptophan-niacin pathway and the enzymes requiring PLP are presented in Figure 3.

More recent studies by Bender and co-workers (Bender and Wynick, 1981; Bender, Tagoe and Vale, 1982; and Bender, 1983) offer an alternative explanation to the abnormal tryptophan load tests that are observed in women using estrogens. Bender and Wynick (1981) used estrone sulphate to study the interaction between apokynureninase and PLP. Inhibition of kynureninase by estrone sulphate was measured in the presence of a saturating concentration of kynurenine and varying concentrations of PLP, and vice versa. Enzyme kinetic studies indicated uncompetitive inhibition of kynureninase by estrone sulphate with respect to PLP when the enzyme was preincubated with a saturating concentration of PLP. As a result of the inhibition of kynureninase by estrogen, the liver pool of kynurenine would subsequently increase. Normalization, which occurs by pyridoxine administration, is a result of increased kynureninase activity due to activation of the excess apokynureninase in the liver.

Bender, Tagoe and Vale (1982) conducted another study to determine the extent to which kynureninase is inhibited by estrogen in vivo. Administration of estrogen sulphate

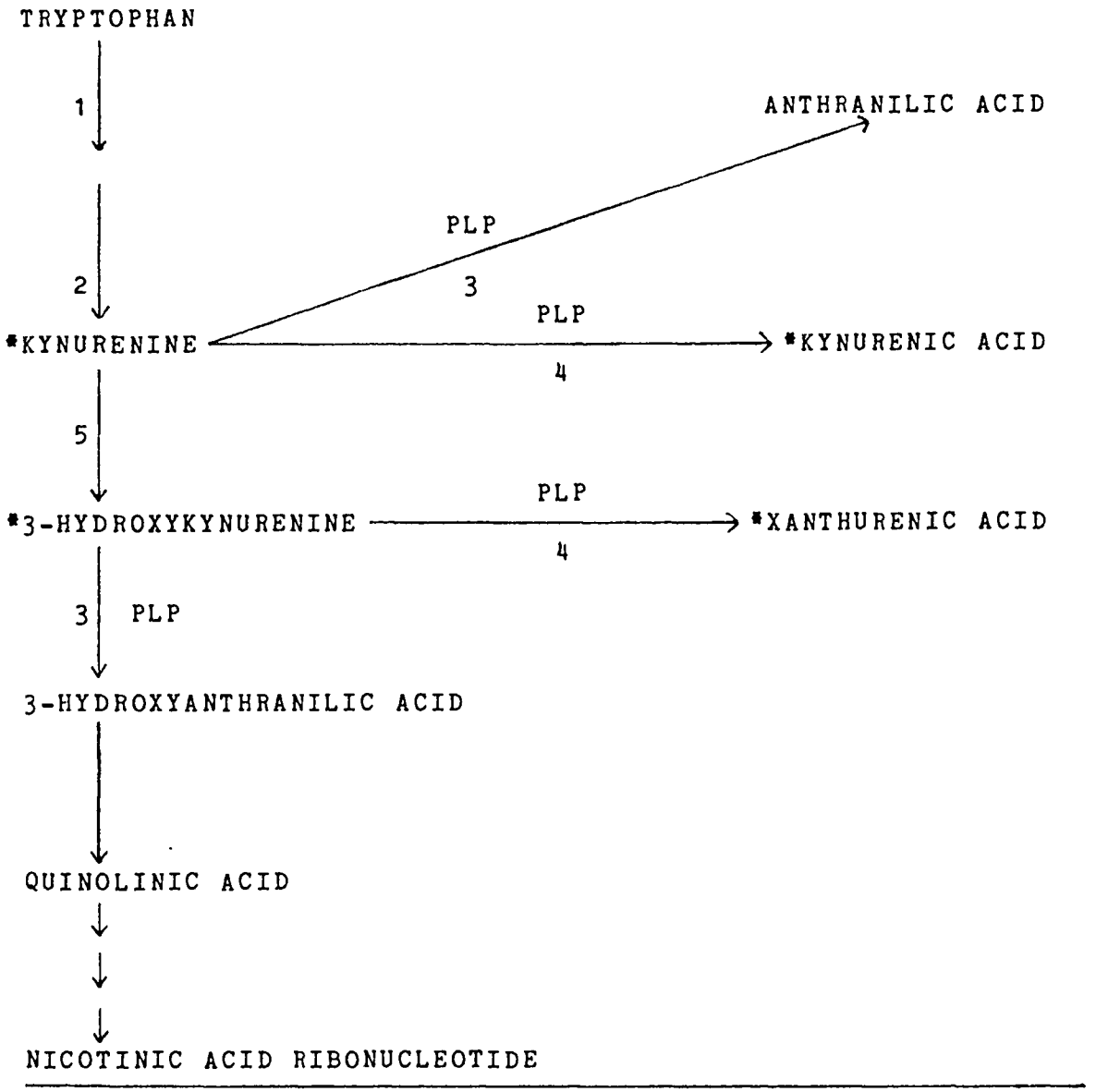


Fig 3. The tryptophan-nicotinic acid ribonucleotide pathway. Adapted from Rose, 1978. (*) indicates the metabolites which are elevated in vitamin B-6 deficiency. (PLP) indicates the reactions which are pyridoxal phosphate dependent. Reactions are catalyzed by: 1- tryptophan oxygenase, 2- formamidase, 3- kynureninase, 4- kynurenine aminotransferase and 5- kynurenine hydroxylase.

to ovariectomized rats caused a reduction in the activity of liver kynureninase and a considerable excess of liver apokynureninase. According to Bender and his associates, estrogens do not significantly affect vitamin B-6 requirements. They proposed that the tryptophan load test is unreliable as an index of vitamin B-6 nutritional status in women receiving estrogens.

Vitamin B-6 in Blood. Measuring plasma PLP levels of women who had used OC for six months or longer, Lumeng et al. (1974) found that the mean PLP concentration of OC users was significantly lower than that of an age-matched control group of non-users, especially in the 25 to 34 year old group. They also followed changes in plasma PLP concentration from two weeks before OC use to six months after. During the first three months Lumeng et al. observed a significant drop in plasma PLP in nine out of ten women. These levels returned toward normal in most of the women by the sixth month of OC use, suggesting that in most women dietary supply and perhaps increased economy of PLP utilization (redistribution of PLP in the body) are sufficient to accommodate the increased requirement for vitamin B-6.

Brown et al. (1975) and Miller et al. (1975) found no significant differences in plasma PLP when comparing OC users to non users. Hamfelt et al. (1984) found that nine percent of 275 women who had used OC for at least five years had low levels of plasma PLP. Determining vitamin

B-6 status in young women using OC, Lind (1980) and Hoagland (1981) found that the mean plasma and red blood cell vitamin B-6, as well as plasma PLP (Miller, 1985) were significantly lower in 26 OC users than in 25 non users.

Aminotransferase Activity and Stimulation. Conflicting data on the effect of oral contraceptives on vitamin B-6 metabolism were generated from studies that measured the activity and/or stimulation by PLP of erythrocyte enzymes (aspartate aminotransferase, EAST and alanine aminotransferase, EALT). These discrepancies may be related to differences in the methods that were used in these investigations.

Aly et al. (1971) found elevated EAST levels but not EALT levels in OC users. Rose et al. (1973) concluded that this enzyme assay is of no help for identifying vitamin B-6 deficiency in women taking OC because EAST activity is elevated by steroid hormones. Adams et al. (1973) found decreased activity of EAST and EALT and Miller et al. (1975) found the activity of EAST was higher in OC users than in non-users, but the stimulation in vitro by PLP was similar for the two groups. Brown et al. (1975) found that OC use produced no significant changes in either EAST or EALT levels.

Urinary 4-Pyridoxic Acid. There are also conflicting data regarding the urinary excretion of 4PA by OC users. Brown et al. (1975), Miller et al. (1974), and Donald and Bosse (1979) found no significant difference in 4PA

excretion when comparing OC users to non-users. However, Adams et al. (1973) found that urinary excretion of 4PA in OC users was lower than that of non-users.

Vitamin B-6 Requirements of Estrogen Users. Bosse and Donald (1979) proposed that an intake of vitamin B-6 between 1.5 and 5.0 mg per day (but closer to 1.5 mg) was adequate to meet the needs of OC users. In this depletion/repletion study, 0.96 mg of vitamin B-6 per day was not adequate to resume predepletion levels of most of the vitamin B-6 parameters that were measured. Brown et al. (1975) proposed that between 0.8 and 2.0 mg of vitamin B-6 per day were needed to maintain normal levels of urinary 4PA, plasma PLP, and erythrocyte aminotransferase activity. Although it does not seem necessary for women who are taking estrogens to supplement their diets with vitamin B-6, it would be beneficial for those women who consume low amounts of vitamin B-6 to improve their intake of this vitamin to assure adequate intake.

PHYSIOLOGICAL EFFECTS OF AGING

Demographic studies reveal that the number and proportion of older people in our society is increasing rapidly (Kohrs, 1982). Improved hygienic standards, improved access to medical care, and positive economic development have been main factors contributing to an increased life span (Werner, 1983). Unfortunately, there are problems associated with this increased percentage of elderly. Although comprising 12 percent of the population,

the elderly account for 29 percent of total personal health care expenditure (Libow, 1981). Older persons have a higher hospitalization rate and are the leading consumers of prescription medications, with their use of medications accounting for 25 to 35 percent of the U.S. drug expenditure (Simonson, 1984). The incidence of chronic disease also increases with age, with greater than 80 percent of older persons experiencing at least one such condition (Simonson, 1984). Since women outlive men by approximately eight years in later life, the elderly population has a greater proportion of women than men (Kohrs, 1982).

The age-related changes that occur in humans are numerous and the aging process varies from one individual to another. There are several age-related physiological changes that could account for abnormal metabolism of vitamin B-6 among older populations. For example, the metabolizing capacity of the liver seems to decrease with age (Hyams, 1978). This could account for less vitamin B-6 being converted to PLP. Plasma alkaline phosphatase levels have been reported to increase with age (McPherson et al., 1978; Kelly et al., 1979), possibly causing a drop in plasma PLP concentration by causing more PLP than normal to be dephosphorylated to PL.

Body Composition. At any age, females generally possess a larger proportion of adipose tissue than do males (Greenblatt et al., 1982). There is an increase in total

body fat with aging and a corresponding decrease in lean body mass (Novak, 1972). This tissue loss is caused by cell death, and as cells die they are replaced by fat and connective tissue (Watkin, 1982). Other changes in body composition that occur are decreased plasma volume, decreased total albumin and decreased total body water (Lamy, 1982; Platt, 1983).

Absorption. Several age-related changes in the function of the gastrointestinal tract have been documented. However, the effect of these changes is still speculative. In some cases, they may have no overall effect because age-related changes may contradict each other.

Gastric emptying time is increased due to a decrease in the activity and motility of the stomach (Lamy, 1982). A general decrease in gastric cell function occurs, which produces an elevation of gastric pH due to reduced acid secretion (Greenblatt et al., 1982). In view of decreased hydrochloric acid secretion, protein digestion is less efficient (Poleman and Capra, 1984). In addition, active transport of selected nutrients is impaired in some elderly (Gelb and Straus, 1983), suggesting that transport activity may, in general, be decreased. There may also be a decreased capacity to absorb some nutrients due to either a decrease in intestinal blood flow or shortened villi.

Metabolism. Changes in hepatic metabolism with age are highly variable and unpredictable. Due to a decrease

in cardiac output of approximately one percent per year, blood flow to the liver decreases with age (Hyams, 1983). Metabolism in the liver may take longer and some substances may have an increased half-life with age (Vestal, 1982). There may also be a decrease in the size of the liver and in liver microsomal enzymes with age (Hyams, 1983). These changes are further complicated by the use of alcohol.

Excretion. Decreased cardiac output also results in decreased blood flow to the kidney (Sourander, 1983), which in turn results in a gradual decrease in kidney function. Throughout life there is also a decrease in the number of functioning nephrons. Glomerular filtration rate is decreased by one-third over a lifetime, so that substances, possibly including nutrients, excreted via the kidney can be expected to have reduced total clearance (Greenblatt et al., 1982).

VITAMIN B-6 AND AGE

A large number of surveys have assessed the dietary intake of elderly persons. These studies have consistently reported widespread inadequate dietary intake, especially of calcium, iron and several water-soluble vitamins. Vitamin B-6 intake has frequently been identified to be low in elderly women (Vir and Love, 1977; Chrisley and Driskell, 1979; Hampton et al., 1977; Gary et al., 1982). Until recently, however, relatively few studies have examined the dietary intake of middle-aged, postmenopausal women (ages 45 to 65 years). A two-year dietary survey of

160 middle-aged women ranging in age from 37 to 66 years (mean age of 53 years) was conducted by Sempos et al. (1984). Approximately 40 percent of the women had usual intakes of vitamin B-6 that were less than 70 percent of the 1980 RDA, and nine percent consumed amounts of vitamin B-6 that were 50 percent below the recommended level.

Inasmuch as the low vitamin B-6 intakes reported in these studies may be due to underreporting of food consumption or to the lack of data on the vitamin B-6 content of many foods, it is helpful to examine biochemical evidence available on the vitamin B-6 and age relationship. Studies on plasma levels of PLP, the biochemically active form of the vitamin, have shown that there is generally a decrease with age (Hamfelt, 1964; Rose et al., 1976; Walsh, 1966; Lumeng and Li, 1974). Activity of erythrocyte aminotransferases has also been shown to be low in the elderly (Ranke et al., 1960; Jacobs, Cavill and Hughes, 1968; Chrisley and Driskell, 1979; Vir and Love, 1977). Supplementing diets of subjects with pyridoxine normalizes levels of both of these vitamin B-6 status indicators (Ranke et al., 1960; Jacobs et al., 1968).

Under conditions of controlled vitamin B-6 intake, Lee and Leklem (1985) demonstrated that healthy, middle-aged, postmenopausal women who are not taking estrogens excrete more urinary 4PA, have lower plasma levels of PLP and total vitamin B-6, and have greater erythrocyte pyridoxine kinase activity than younger women. Tryptophan load tests (before

and after supplementation) in this study suggest that 2.3 mg of vitamin B-6 per day is adequate for older and younger women.

A series of experiments by Fonda and co-workers (Fonda, Eggers and Mehta, 1980; Fonda and Eggers, 1980; Fonda et al., 1980) examined vitamin B-6 metabolism in the liver, blood and brains of young adult (10 to 12 months) and senescent mice (30 to 33 months). The in vivo metabolism of labeled vitamin B-6 in the brains, livers and blood cells of mice of selected ages from 10 to 33 months was studied following an intravenous injection of labelled pyridoxine. Following injection each mouse was killed, blood was collected, and the brains and liver were removed.

Compared to those of the younger mice, the livers and brains of the senescent mice took up significantly more PN (perhaps indicating a deficiency of the vitamin), but converted a smaller percentage of PLP to PMP via transamination (perhaps indicating a decrease in the total amino acid aminotransferase activity) (Fonda et al., 1980c; Fonda et al., 1980b). The livers and brains of the senescent mice also had significantly more PNP, PLP 4PA, and PL. Fonda et al. (1980c) proposed that the increased rate of PLP hydrolysis is due to either greater alkaline phosphatase activity or to less PLP being bound to protein, and that senescent animals need more vitamin B-6 to provide the same amount of PMP as that found in young adult mice. The plasma of the senescent mice was found to contain

higher levels of 4PA and PL than that of the young adult mice, while the blood cells took up and metabolized PN similarly in both age groups (Fonda and Eggers, 1980a).

MATERIALS AND METHODS

SUBJECTS

Nineteen postmenopausal women served as subjects in this study. Nine of the subjects (mean age 54.7 ± 4.7 years), the experimental group, had been taking estrogen replacement therapy with or without progestin for ten months or longer. The other ten (mean age 56.8 ± 2.3 years), the control group, either had never taken estrogen replacement therapy or had discontinued its use for at least six months before this investigation.

As determined by personal interview and a questionnaire (see Appendix), the subjects were in good health, free from any known liver, kidney or metabolic disorder and not taking any drugs, other than estrogen and progestin by the experimental group, known to affect either vitamin B-6 status or the results of the microbiological assay of vitamin B-6. Because alcohol interferes with vitamin B-6 metabolism (Lumeng and Li, 1974), no subject was consuming more than 3 ounces of hard liquor, 12 ounces of wine or 36 ounces of beer daily. Any subject who had been taking nutrient supplements discontinued their use for at least two weeks before participating in this research.

Before the study, all subjects were informed of the purpose of this investigation and were instructed on how to record their dietary intake and collect urine. An informed

consent form approved by the Oregon State University Human Subjects Committee was signed by each subject after she fully understood the purpose of the study and her responsibilities. The informed consent form and approval of this investigation by the Human Subjects Committee are given in the Appendix.

EXPERIMENTAL DESIGN

To determine the influence of estrogen replacement therapy on vitamin B-6 status of postmenopausal women, the dietary intake of vitamin B-6 as well as the concentration of vitamin B-6 in plasma and urine were measured. The subjects kept dietary records and collected complete, 24-hour urine specimens under toluene for three consecutive days. Between 0700 and 0900 hours on the morning of the fourth day, a registered medical technologist withdrew 20 mL of blood from each of the fasting subjects into heparinized, evacuated tubes. After blood was drawn, several body measurements including height, weight, skinfolds (triceps, biceps, subscapular, suprailiac and abdominal), diameters (bideltoid, wrist and elbow) and circumferences (waist, abdomen, upper arm, thigh and buttocks) were taken by co-researcher, Susan Worley. Worley examined these data along with data from the questionnaire in an investigation of factors influencing body composition of postmenopausal women. These results are published elsewhere (Worley, 1987). The subjects also

completed a self-administered questionnaire (see Appendix) which they returned on the fourth day.

METHODS

Immediately after the blood was drawn, it was placed on ice and protected from light. Hematocrit and hemoglobin were determined by the microhematocrit and cyanomethemoglobin methods, respectively (Makarem, 1980). The remaining blood was centrifuged at 3,000 rpm for 20 minutes at 4 degrees C, and two portions of plasma (two mL each) were stored in vials at -15 degrees C until analyzed. After each 24-hour urine collection was thoroughly mixed and volume recorded, two portions of approximately 50 mL each were stored in plastic bottles at -15 degrees C until analyzed.

Total vitamin B-6 in plasma and urine was measured according to the microbiological assay with Saccharomyces uvarum ATCC 9080 as proposed by Miller and Edwards (1981). Since autoclaving with acid converts the phosphorylated forms of vitamin B-6 to their respective free forms (See Figure 1), growth of this organism measures the concentration of all the B-6 vitamers and is termed "total vitamin B-6". A control sample was analyzed with each assay. The plasma control sample had a mean value of 99.0 $\pm$ 0.7 nmol/L (assayed four times) and the urine control sample had a mean value of 87.0 $\pm$ 0.3 umol/24 hours (assayed four times).

The vitamin B-6 and protein content of the three-day

diets were computed using the Ohio State University nutrient database (Schaum et al., 1973) which had been supplemented and revised in 1984. Vitamin B-6 food values which were missing from the database were obtained from Orr (1969). The vitamin B-6 to protein ratio (mg of vitamin B-6 per g of protein per day) was determined from these data. A vitamin B-6 diet history score was calculated from the food frequency tables on pages four and five of the questionnaire. (See Appendix).

STATISTICAL ANALYSIS

Statistical counseling was obtained from the Statistics Department of Oregon State University. Analysis of covariance was used to determine if the mean total plasma vitamin B-6 level of the experimental group was statistically lower than the level of the control group. Estrogen use, years of age, dietary intake of vitamin B-6, vitamin B-6 to protein ratio and dietary vitamin B-6 history score were used as covariates. A two-tailed Student's t test for small sample sizes was used to test the difference between two means and the Pearson product moment coefficient of correlation (r) was used to test the linear relationship of two variables. A p value of less than or equal to 0.05 was considered significant in each case. All data are expressed as mean $\pm$ standard deviation. Statistical concepts and methodologies used in this research are based on the 2nd Edition of "Statistics by Example" (Sincich, 1985).

RESULTS AND DISCUSSION

GENERAL HEALTH AND DEMOGRAPHIC DATA

Descriptive data on the nine subjects using estrogen replacement therapy (experimental) and ten control subjects are presented in Table 1. The subjects' age, height, hematocrit and hemoglobin values were similar for the two groups. The mean weight of the experimental group, 77.8 $\pm$ 13.8 kg, was significantly higher than the mean weight of the control group, 67.7 $\pm$ 8.2 kg ($p<0.05$). The weight of subject #4 (107.7 kg) was much higher than the other subjects in both groups. However, even if this subject's weight is deleted, the mean weight of the experimental group (74.1 $\pm$ 8.6 kg) is still higher than that of the control group.

The subjects were generally healthy. With an exception or two, their hemoglobin and hematocrit values were within the normal range for women (Tietz, 1976). Several subjects had conditions commonly associated with aging, such as hypertension and arthritis. Also, several of the subjects in both groups were taking unprescribed and prescribed medications in addition to the estrogen and progestin used by the experimental group. None of these medications, except estrogen, are known to have an effect on vitamin B-6 metabolism (Bhagavan, 1985). A brief description of the medical history and prescription medications taken by each subject is given in the Appendix.

This information was taken from questions seven and eight of the questionnaire (see Appendix).

Table 1

Descriptive data on experimental and control subjects

Group	Subject	Age	Height	Weight	Hematocrit	Hemoglobin
		y	cm	kg	1	g/L
Experimental	1	55	172.0	57.3	0.42	145
	2	57	159.5	82.0	0.40	151
	3	58	157.5	78.1	0.42	155
	4	53	163.3	107.7	0.42	149
	5	53	165.3	74.5	0.43	150
	6	63	169.5	73.6	0.40	144
	7	46	164.0	85.4	0.35	160
	8	52	171.0	69.1	0.39	150
	9	55	173.5	72.7	0.42	146
		54.7[a]	166.2	77.8[b]	0.41	151
		±4.7	±5.4	±13.8	±0.03	±5
Control	21	58	177.5	82.7	0.42	154
	22	57	170.5	69.5	0.44	146
	23	54	173.0	55.4	0.41	142
	24	58	162.5	62.3	0.42	154
	25	55	160.5	70.0	0.45	166
	26	55	165.0	65.9	0.42	152
	27	57	164.3	78.6	0.42	148
	28	59	168.5	60.9	0.38	138
	29	61	157.5	68.6	0.42	152
	30	54	171.0	62.7	0.42	149
		56.8	167.0	67.7[b]	0.42	151
		±2.3	±6.2	±8.2	±0.02	±9

[a] X ± SD.
[b] Means are statistically different ($p<0.05$).

Eleven of the 19 subjects held university positions as faculty or staff. Only three women were full-time homemakers. For the most part, this was a healthy, noninstitutionalized, educated, motivated and health conscious group of women. All inferences from data obtained from these subjects should be made with this in mind. Poor dietary intake and clinical signs of malnutrition, which are strongly related to income level, are found more frequently among older people who are poor and less educated (Nestle, 1985).

Data on the estrogens and progestins used by the experimental group are given in Table 2. Premarin (Ayerst Laboratories; New York, NY) was the estrogen most commonly taken (seven out of nine subjects). The average length of time on ERT was 66 $\pm$ 42 months, ranging from 10 to 132 months (11 years). Eight of the nine subjects were taking ERT because of hot flashes and/or to prevent or retard osteoporosis. "Hysterectomy" was the other most common reason for taking ERT. The most common daily dosage of estrogen taken by the experimental group was 0.625 mg. Subject #9 was only taking 0.05 mg of estrogen per week or 0.007 mg per day. Because this is an extremely small dose, all data were analyzed with and without data from subject #9. Five of the nine experimental subjects were taking a progestin in addition to estrogen.

Table 2

Estrogens and progestins used by experimental group

Subject	Estrogen[a]			Progestin[b]		
	Name	Amount	Duration	Name	Amount	Duration
		mg/d	mo		mg/d	mo
1	Premarin[c]	0.625	79	Provera[d]	10	7
2	Premarin	0.625	36	Provera	10	36
3	Premarin	0.300	102			
4	Premarin	0.625	15	Medoxypro.[e]	10	15
5	Premarin	0.625	96	Medoxypro.	10	60
6	Premarin	0.625	132			
7	Premarin	0.625	10			
8	Fred Meyer[f]	0.625	84	Medoxypro.	10	6
9	Estrovis[g]	0.007	48			

[a] Administered on a cyclic dosage schedule, such as 3 weeks on and 1 week off.
[b] Administered during the last 5 to 10 days of the estrogen cycle.
[c] Conjugated estrogen; Ayerst Laboratories, New York, N.Y.
[d] Medoxyprogesterone acetate; The Upjohn Company, Kalamazoo, MI.
[e] Medoxyprogesterone acetate; Generic.
[f] Conjugated estrogen; Generic.
[g] Quinestrol; Parke-Davis, Morris Plains, N.J.

PLASMA TOTAL VITAMIN B-6 CONCENTRATION

The major objective of this study was to examine the effect that estrogen use had on PB6 of postmenopausal women. The plasma total vitamin B-6 concentrations (PB6) of the 19 subjects are presented in Table 3. The experimental group had a lower mean PB6 (47.6 $\pm$ 19.7 nmol/L) than the control group (56.5 $\pm$ 20.7 nmol/L). These means are not statistically different due to wide variations among the subjects of each group. There was a fourfold range in PB6 values among the experimental subjects and almost a threefold range among the control subjects. Additionally, control subject #25 had a PB6 of 110.5 nmol/L. Without this subject's PB6 value, the mean PB6 for the control group is 50.4 $\pm$ 8.7 nmol/L and is still higher than that of the experimental group. An explanation for this subject's high PB6 value is given in a later section.

The results of analysis of covariance are presented in Table 4. The only two covariates that had a significant effect on PB6 are dietary vitamin B-6 ($p=0.0001$) and vitamin B-6 to protein ratio ($p=0.0021$). The other two covariates, age and vitamin B-6 dietary history score, did not have an effect on PB6 (respective p values are 0.43 and 0.19). The statistics were repeated with and without subject #9 because of the low level of estrogen taken by this subject. Without subject #9, the same two covariates are significant and the means of PB6 are still

Table 3

Plasma vitamin B-6 of experimental and control subjects

Group	Subject	Plasma vitamin B-6[a]
		nmol/L
Experimental	1	37.0
	2	79.0
	3	68.0
	4	21.0
	5	39.0
	6	41.0
	7	27.9
	8	48.6
	9	67.0
		47.6[b]
		± 19.7
Control	21	50.2
	22	41.4
	23	41.9
	24	44.3
	25	110.5
	26	47.4
	27	61.4
	28	55.0
	29	46.2
	30	66.2
		56.5
		± 20.7

[a] Determined using S. uvarum.
[b] X ± SD.

Table 4

Analysis of covariance for plasma total vitamin B-6: effect of subject's age, dietary vitamin B-6, vitamin B-6 to protein ratio and vitamin B-6 diet history score.

I. Including subject #9

SOV[a]	DF[b]	SS[c]	MS[d]	F[e]	P-value	P<0.05
Age	1	0.6879	0.6879	0.6737	0.4266	
Diet B-6	1	24.3766	29.3766	28.7687	0.0001	*
B-6: Pro	1	14.9501	14.9501	14.6407	0.0021	*
B-6 Hist	1	1.9497	1.9497	1.9094	0.1903	
Estrogen	1	12.2584	12.2584	12.0047	0.0042	*
Error	13	13.2747	1.02113			
Total	18					

TABLE 4 (continued)

II. Without subject #9

SOV[a]	DF[b]	SS[c]	MS[d]	F[e]	P-value	P<0.05
Age	1	0.7178	0.7178	0.6873	0.4233	
Diet B-6	1	29.8708	29.8708	28.6023	0.0002	*
B-6: Pro	1	15.6542	15.6542	14.9894	0.0022	*
B-6 Hist	1	2.1376	2.1376	2.0468	0.1780	
Estrogen	1	11.1705	11.1705	10.6962	0.0067	*
Error	12					
Total	17					

Group	Unadjusted means PB6, nmol/L	Adjusted means[f] PB6, nmol/L
Experimental	47.1 ± 19.7	42.7
Control	56.2 ± 20.6	60.7

[a] Source of variation.
[b] Degrees of freedom.
[c] Sum of squares term.
[d] Mean square (SS-DF).
[e] F-value.
[f] Statistically different ($p<0.05$).

statistically different. Since the same conclusions are reached with or without subject #9 and neither one is seen as being statistically preferable (March, 1986), all results reported in this research include the data from subject #9. There is a significant difference between PB6 values (experimental group < control group) when PB6 values are adjusted for dietary vitamin B-6 and vitamin B-6 to protein ratio. The adjusted means for the experimental and control groups are 42.7 nmol/L and 60.6 nmol/L, respectively.

A minor objective of this study was to examine the effect that age had on PB6 of postmenopausal women by comparing our results to other values reported in the literature for PB6, particularly from assays done in our laboratory with S. uvarum. The 19 women that participated in this study ranged in age from 46 to 63 years, with 15 of the women falling within the range of 55 $\pm$ 3 years. There was no linear relationship ($r=0.20$, $p>0.05$) between age and PB6 in this investigation. This is most likely explained by the relatively narrow range in the subjects' ages and the variability of their dietary vitamin B-6 intakes. This will be discussed in more detail later. Results for PB6 (using S. uvarum) from two previous studies also conducted in the O.S.U. Nutrition and Food Management Department research laboratory are reported in Table 5. The results of Lee and Leklem's (1985) study support the hypothesis of increasing age being inversely related to PB6 when

Table 5

Reported values of plasma vitamin B-6 using microbiological assay with S. uvarum[a]

Reference	Subjects	Age	Plasma B-6	Diet B-6
	no	y	nmol/L	mg/d
Lind, 1980	26 OC users	21 ± 1.9	44.3 ± 15.0	1.4 ± 0.5
	25 control	22 ± 2.3	60.4 ± 25.1	1.6 ± 0.5
Lee & Leklem 1985[b]	5 young women	24.4 ± 3.2	49.4 ± 9.7	initial values in study before controlled diet
	7 middle-aged	55.3 ± 4.0	46.0 ± 13.0	
	"	"	81.4 ± 19.7	2.3 mg/d
	"	"	59.6 ± 8.4	controlled diet
This study	9 ERT users	54.7 ± 4.7	47.7 ± 19.7	1.61 ± 0.54
	10 control	56.8 ± 2.3	56.5 ± 20.7	1.54 ± 0.44

[a] Only studies conducted at O.S.U. are reported here.
[b] None used estrogen.

estrogen is not a factor. The middle-aged postmenopausal women researched by Lee and Leklem had a mean PB6 level of 46.0 ± 13.0 nmol/L. They reported PB6 values for the initial week of the study in which vitamin B-6 intake was the usual, voluntary diet of the subjects and for week two of the study in which vitamin B-6 intake was controlled and constant at 2.3 mg per day. The initial values are used here for comparison because they are probably more comparable to the vitamin B-6 intake of the subjects in the present study. The young women studied by Lee and Leklem had a mean PB6 level of 49.4 ± 9.7 nmol/L and the young women studied by Lind (1980) that were not using estrogen had a mean PB6 level of 60.4 ± 25.1 nmol/L. The mean PB6 level of the middle-aged, postmenopausal women in the present study that were not taking ERT was 56.5 ± 20.7 nmol/L. This is higher than those of the young women in Lee and Leklem's investigation, but lower than the young women in Lind's.

The postmenopausal estrogen users of this study had a higher PB6 than did the young estrogen users of Lind (1980). This may be explained by the different levels of estrogen taken by the two groups. The common daily dose of estrogen taken during menopause is 0.625 mg (equivalent to approximately 0.025 mg ethinyl estradiol), while that taken for contraception is 30 to 35 ug (0.030 to 0.035 mg ethinyl estradiol)(Schneider and Wagner, 1971). Contrary to the hypothesis, the middle-aged postmenopausal women of the

present study that were using ERT and the middle-aged postmenopausal women in the study by Lee and Leklem that were not using ERT had PB6 levels that were similar (47.7 ± 19.7 and 46.0 ± 13.0 nmol/L, respectively. It appears that the data reported in the present study do not make a strong case for supporting the age - PB6 hypothesis. However, it is difficult to compare estrogen users vs. non-users because, besides age being a factor, one must consider the differences in levels of estrogen taken and length of time estrogen was used. (See Table 2.)

Progestin taken in addition to estrogen by the experimental group did not significantly affect PB6. As previously discussed by Miller (1985), only the estrogen component of oral contraceptives, not the progestin, appears to affect vitamin B-6 status.

Cigarette smoking may have affected the PB6 in two of the control subjects: subject #22 (13 to 17 cigarettes per day) and subject #29 (7 to 8 cigarettes per day). The PB6 levels of these subjects (41.0 nmol/L and 46.0 nmol/L, respectively) were below the mean for their group (56.2 ± 20.6 nmol/L). Cigarette smoking has been shown to lower vitamin B-6 levels (Serfontein et al., 1986).

In our laboratory, PB6 is about 70 to 80 percent PLP (Miller and Edwards, 1981). Schultz and Leklem (1980) have suggested that a plasma PLP level of less than 7.8 to 8.8 ng/mL (or 46.7 to 52.7 nmol/L) is indicative of marginal vitamin B-6 status among females. The comparable PB6

marginal level would be approximately 9.75 to 12.57 ng/mL (or 58.35 to 75.22 nmol/L). Thirteen subjects in the present study (6 experimental and 7 control) had PB6 values less than 58.35 nmol/L.

While clinical signs of vitamin B-6 deficiency have not been reported in postmenopausal women (whether using ERT or not using ERT), the implications of such low PB6 values is of concern. For example, Talbot, Miller and Kerkvleit (1987) recently demonstrated that improving vitamin B-6 nutriture (in this case by supplementation) in elderly persons resulted in improved immunocompetence as measured by lymphocyte function tests.

URINARY TOTAL VITAMIN B-6 EXCRETION

The subjects' urinary total vitamin B-6 (UB6), determined in acid-hydrolyzed urine, is shown in Table 6. The mean UB6 was similar for the experimental and control groups: 1.01 $\pm$ 0.36 umol/d and 1.03 $\pm$ 0.33 umol/d, respectively. As previously reported (Donald and Bosse, 1979; Aly et al., 1971), estrogen use does not appear to affect UB6. Several subjects had a wide variation of UB6 from day to day. Coefficients of variability for the individual 3-day means of the 19 subjects ranged from 2.3 percent to 59.0 percent (Table 6). In general, urine collections were carefully collected (Worley, 1986). Different dietary intakes of vitamin B-6 by the subjects are more likely to be the cause of variation in UB6. (See section on UB6 and diet.)

Table 6

Urinary excretion of vitamin B-6 of experimental and control subjects

Group	Subject	Day 1	Day 2	Day 3	X ± SD	Coefficient of variability
		----------umol/24 hrs----------				
Experimental	1	0.90	2.10	1.53	1.51 ± 0.60	39.7
	2	0.93	0.95	0.98	0.95 ± 0.03	3.2
	3	1.39	0.88	0.79	1.02 ± 0.32	31.4
	4	0.57	0.56	0.53	0.55 ± 0.02	3.6
	5	0.97	0.63	0.51	0.70 ± 0.24	34.3
	6	1.63	1.00	0.92	1.18 ± 0.39	33.1
	7	0.59	0.69	0.89	0.72 ± 0.15	20.8
	8	0.86	0.88	0.85	0.86 ± 0.02	2.3
	9	1.30	1.60	1.83	1.58 ± 0.27	17.1
					1.01 ± 0.36[a,b]	
Control	21	0.81	0.90	0.76	0.82 ± 0.07	8.5
	22	1.10	0.81	0.73	0.88 ± 0.19	21.6
	23	1.10	0.85	0.81	0.92 ± 0.16	17.4
	24	0.86	0.83	0.82	0.84 ± 0.02	2.4
	25	2.56	1.57	1.61	1.91 ± 0.56	29.3
	26	1.77	0.71	0.68	1.05 ± 0.62	59.0
	27	0.79	1.60	0.75	1.05 ± 0.48	45.7
	28	0.78	0.73	1.51	1.01 ± 0.44	43.6
	29	0.83	0.70	0.69	0.74 ± 0.08	10.8
	30	0.89	1.32	1.09	1.10 ± 0.22	20.0
					1.03 ± 0.33[a,b]	

[a] X ± SD.
[b] Means are not statistically different.

An age-related decrease in UB6 has been reported (Schultz and Leklem, 1981). In the investigation by Lee and Leklem (1985) in which the subjects were administered a controlled diet, middle-aged women excreted only about 80% as much total UB6 as compared to the younger subjects. The differences in UB6 in young and middle-aged women cannot be explained by differences in vitamin B-6 dietary intake. UB6 did not correlate with mean urine volume in the present study (data not given).

DIETARY VITAMIN B-6 AND PROTEIN

Mean vitamin B-6 intake data gathered from the subjects' three-day dietary records and from question 24 of the self-administered questionnaire are presented in Table 7. The mean daily vitamin B-6 intake of the experimental group was 1.61 $\pm$ 0.54 mg, ranging from 0.75 mg to 2.62 mg. For the control group, the daily vitamin B-6 intake ranged from 1.13 mg to 2.55 mg and had a mean value of 1.54 $\pm$ 0.44 mg. The difference between groups was not statistically significant. Hoaglund (1981) also found no difference in daily vitamin B-6 intake between users and non-users of oral contraceptive agents.

Fifty-eight percent of the subjects (11 out of 19) had mean daily vitamin B-6 intakes that were less than 100 percent of the RDA (NRC, 1989). One subject had a mean vitamin B-6 intake that was less than 67 percent of the RDA. In a two-year study of middle-aged women, Sempos et

Table 7

Vitamin B-6 intake of subjects of experimental and control subjects

Group	Subject	B-6 Diet History Score[a]	Dietary Intake[b,c] B-6	Protein	B-6:Pro	% RDA[d] B-6	Protein	B-6:Pro
			mg/d	g/d	mg/g	%	%	%
Experimental	1	2.7	1.53	66.0	0.023	95.6	144	144
	2	3.6	2.04	53.4	0.038	127.5	81	238
	3	2.9	1.28	103.5	0.012	80.0	166	75
	4	3.4	0.75	59.8	0.013	46.9	70	81
	5	3.3	1.18	43.4	0.027	73.8	73	169
	6	3.5	1.62	69.8	0.023	101.3	119	144
	7	3.6	1.57	68.0	0.023	98.1	100	144
	8	3.3	1.88	73.8	0.025	117.5	133	156
	9	3.5	2.62	83.8	0.031	163.8	144	194
		3.3	1.61	69.1	0.024	100.5	114	149
		± 0.3	± 0.54	± 17.4	± 0.008	± 33.7	± 35	± 51
Control	21	3.5	1.19	59.5	0.020	74.4	90	125
	22	3.1	1.56	81.3	0.019	97.5	146	119
	23	2.7	1.21	61.5	0.020	75.6	139	125
	24	3.3	1.47	69.4	0.021	91.9	139	131
	25	3.7	2.55	83.9	0.030	159.4	150	188
	26	3.3	1.51	56.1	0.027	94.4	106	169
	27	3.2	1.13	53.4	0.021	70.6	85	131
	28	1.5	1.63	59.3	0.027	101.9	122	169
	29	3.5	1.20	72.6	0.017	75.0	132	106
	30	3.2	1.95	91.8	0.021	121.9	183	131
		3.1	1.54	68.9	0.022	96.3	129	139
		± 0.6	± 0.44	± 13.1	± 0.004	± 27.3	± 30	± 25

[a] Calculation is described in appendix.
[b] From Schaum, Mason and Sharp, 1973.
[c] Values are means of three consecutive days.
[d] Based on 0.8g/kg of body weight.

al. (1984) reported that 40 percent of the 151 women studied had usual vitamin B-6 intakes that were less than 70 percent of the RDA. Garry et al. (1982) examined the dietary intakes of 270 non-institutionalized, healthy men and women who were older than 60 years of age. Eighty-six percent of the 145 women studied were receiving less than 75 percent of the 1980 RDA for vitamin B-6 and 61 percent were receiving less than 50 percent of the 1980 RDA. Vitamin B-6 was one of the nutrients, along with vitamin D, vitamin E and folic acid, judged by Garry et al. (1982) to be inadequate based on the finding that more than one-fourth of the population studied had intakes that were less than 50 percent of the 1980 RDA. Eighty-four percent of the 51 young women studied by Hoaglund (1981) were receiving less than 100 percent of the 1980 RDA and 44 percent were receiving less than 67 percent of the 1980 RDA for vitamin B-6. While poor dietary intake of vitamin B-6 has been reported in elderly women (Garry et al., 1982; Vir and Love, 1977), it appears that vitamin B-6 intake relative to the RDA is poor in all women, whether young, middle-aged or elderly.

Since the requirement for vitamin B-6 is based on protein intake, we examined the protein intake of our subjects. The mean daily protein intake was 69.1 $\pm$ 17.4 g for the experimental group and 68.9 $\pm$ 13.1 g for the control group. Five subjects failed to receive 100 percent of the RDA for protein (based on 0.8 g per kg of body

weight). Fifty-three percent of the subjects (10 out of 19) had a daily protein intake that was greater than 125 percent and 16 percent (three out of 19) had a daily protein intake that was greater than 150 percent of the RDA for protein. The effects of a high protein diet on plasma vitamin B-6 and urinary vitamin B-6 are addressed in following sections.

The mean vitamin B-6 to protein ratios for the experimental and control groups were 0.024 $\pm$ 0.008 and 0.022 $\pm$ 0.004, respectively. These differences, again, were not statistically different. Although the protein intakes were high relative to the RDA, most subjects had adequate vitamin B-6 intake with respect to protein, based on the suggested ratio of 0.016 mg of vitamin B-6 per g of protein (NRC, 1989). Only two of the 19 subjects (both from the experimental group) had a vitamin B-6 to protein ratio less than 0.016. Since the current RDA for vitamin B-6 (1.6 mg for adult women) is based on a diet containing 100 g of protein, a person receiving less than that may not require 1.6 mg of vitamin B-6. Although the protein intakes of the subjects in this study were high relative to the RDA, only one subject had a mean daily protein intake that exceeded 100 g (Subject #3). Generally, foods that are high in protein are also high in vitamin B-6. The B-6 to protein ratio of subject #3 is an exception to this rule because protein intake was high (103.5 g/d), but vitamin B-6 was low (1.28mg). Skim milk powder, which is low in

vitamin B-6, contributed to the high level of protein in this subject's diet. Guthrie and Crocetti (1983) examined USDA Nationwide Food Consumption Survey data on vitamin B-6 intake of women in relation to protein intake and total vitamin B-6. When vitamin B-6 intake was compared to a standard based on 0.02 mg of vitamin B-6 per g of protein, only 2.9 percent of the 6,457 respondents had intakes less than 60 percent of this standard. However, when vitamin B-6 intake was compared to the 1980 RDA (2.0 mg); 35.3 percent of the respondents had intakes that were less than 60 percent of the RDA. In other words, poor intake may be exaggerated when only the 1980 RDA (2.0 mg/d) is used as a reference.

There was a wide range of average intakes of vitamin B-6 and protein among subjects, but the day to day variation of each subject's intake during the three days of the study was relatively minimal. As is evident from Table 8, 11 of the 19 subjects had a three-day range (difference between the highest and the lowest daily vitamin B-6 intake) of vitamin B-6 that was less than 0.5 mg. With respect to protein, 11 of the 19 subjects had a three-day range of protein that was less than 25 g. Most of the subjects that had highly variable vitamin B-6 intakes also had highly variable intakes of protein. The coefficients of variability for vitamin B-6 to protein ratio ranged from 3.0 percent to 60.4 percent, with a mean value of 22.0 $\pm$ 14.0. Hoaglund (1981) also reported daily variation of

Table 8

Ranges of vitamin B-6 and protein intakes during the three day reporting period

Group	Subject	Vitamin B-6 Range[a]	Protein Range[b]
		mg	g
Experimental	1	0.28	6.7
	2	0.41	3.0
	3	0.30	9.7
	4	0.52	24.1
	5	0.46	19.0
	6	0.38	5.0
	7	0.60	33.7
	8	0.44	32.0
	9	0.40	21.1
Control	21	0.35	24.7
	22	0.81	6.9
	23	0.31	11.6
	24	0.85	31.5
	25	1.53	72.5
	26	0.44	9.8
	27	0.67	35.2
	28	0.80	44.8
	29	0.90	32.2
	30	0.25	56.0

[a] Difference between highest daily vitamin B-6 intake and lowest daily vitamin B-6 intake.

[b] Difference between highest daily protein intake and lowest daily protein intake.

vitamin B-6 intake. Seventy percent of the young women studied had a three-day range of vitamin B-6 that exceeded 0.5 mg. While approximately 20 percent of these young women studied by Haoglund had daily ranges of vitamin B-6 that exceeded the mean vitamin B-6 intake of their group, none of the postmenopausal women in this investigation had a daily range greater than the mean for their group. This suggests that middle-aged women have a more consistent vitamin B-6 intake from day-to-day than do younger women.

In Table 9, the percentages of total vitamin B-6 per major food group of the subjects in this study are compared to the percentages of total vitamin B-6 per major food group in the national food supply in 1980 (Sauberlich, 1981). Compared to the latter, the women in this study received less vitamin B-6 in their diets from meat and more from cereals (especially fortified breakfast cereals) and fruits. Perhaps this is due to the current emphasis on a low-fat, high-fiber diet. Results of several studies indicate that the bioavailability of vitamin B-6 from plant sources is lower than that from animal sources (Kabir, Leklem and Miller, 1983; Gregory and Ink, 1985). The middle-aged women studied by Sempos et al. (1984) rarely reported consuming legumes, nuts, dark green vegetables and whole grains. Sempos et al. attributed the low vitamin B-6 intake of their subjects to poor food choices.

Table 9

Comparison of percentages of total dietary vitamin B-6 from major food groups

Food Group	Data From U.S. Food Supply[a]	Data From Present Study[b]	Difference >5%
	%	%	
Meats	40.2	21.4	*
Cereals	10.2	19.3	*
Legumes	5.4	5.4	
Vegetables	22.2	21.8	
Fruits	8.2	16.9	*
Eggs	2.1	1.7	

[a] Adapted from data of Marston and Peterkin (1980) as cited by Sauberlich, 1981.

[b] Determined from three day diet records.

Subject #25 consumed two cups of fortified breakfast cereal (one cup oat cereal, 1/2 cup rice cereal and 1/2 cup shredded wheat) per day. It is interesting to note that this subject not only had the highest vitamin B-6 intake (2.55 mg/d) but also had the highest PB6 value (110.5 nmol/L). This suggests that the bioavailability of vitamin B-6 (PN) added to ready-to-eat cereals is good. These fortified cereals can provide a substantial amount of vitamin B-6. Improving vitamin B-6 intake from dietary sources alone may be better than relying on supplements. Garry et al. (1982) reported that subjects not taking supplementary vitamin B-6 had a median intake of less than 50 percent of the RDA, while subjects taking supplementary vitamin B-6 had a median intake of approximately 275 percent of the RDA, which approaches toxic levels if taken for a long time (Driskell, 1984).

The individual dietary history scores (Table 7) did not correlate with actual vitamin B-6 dietary intake. The mean dietary history scores for the experimental and control groups were 3.3 $\pm$ 0.3 and 3.1 $\pm$ 0.6, respectively. The dietary history scores ranged from 1.5 to 3.7, with only four subjects (two in each group) having scores that were not between 3.1 and 3.7. This made it difficult to make comparisons between individual subjects. The poor correlation between the vitamin B-6 dietary history scores and the calculated dietary intake of vitamin B-6 may have been due to the high degree of recall necessary to estimate

servings per week correctly and confusion in determining serving size. It may have been more beneficial if just the number of servings per week of foods that had a high score would have been compared.

As also found in the study by Worley (1986), the subjects of the present investigation had daily intakes of protein, phosphorus, iron, thiamin, riboflavin, niacin and vitamin C that exceeded 67 percent of the RDA. Four subjects had an average daily intake of vitamin A that was less than 67 percent of the RDA and one subject had an average daily intake of calcium that was less than 67 percent of the RDA.

URINARY VITAMIN B-6 AND DIET

Mean daily urinary total excretion of vitamin B-6 (UB6) was positively correlated with mean dietary intake of vitamin B-6 ($r = 0.51$; $p<0.05$) and the mean daily ratio of vitamin B-6 to protein ($r = 0.47$; $p<0.05$). Urinary vitamin B-6 excretion has been found to decrease with depletion and increase with repletion of vitamin B-6 (Donald and Bosse, 1979) and generally reflects recent dietary intake of the vitamin (Miller and Edwards, 1981). Because the urinary excretion of vitamin B-6 reflects recent dietary intake, the positive correlation found in this study between UB6 and dietary vitamin B-6 suggests that the calculated dietary intakes for vitamin B-6 are good estimates of the subjects' dietary intake of this vitamin.

The level of protein in the diet also has a slight effect on UB6. Miller, Schultz and Leklem, (1985) evaluated UB6 excretion during 15-day periods of three different levels of protein. As the level of protein intake increased (0.5 to 1.0 to 2.0 g protein per kg of body weight), the excretion of UB6 decreased slightly, although not significantly. The concentrations of plasma PLP, PB6 and urinary 4PA were also inversely related to protein intake suggesting that more vitamin B-6 is retained in the body with increased dietary protein intake (Miller et al., 1985).

The percent of dietary vitamin B-6 excreted as UB6 ranged from 6 to 26. (See Table 10). A wide range (3 to

Table 10

Percent of dietary vitamin B-6 excreted in urine of experimental and control subjects

Group	Subject	Day 1			Day 2			Day 3		
		Diet[a]	Urine[b]	% Excreted[c]	Diet	Urine	% Excreted	Diet	Urine	% Excreted
		nmol/d	nmol/d	%	nmol/d	nmol/d	%	nmol/d	nmol/d	%
Experimental	1	8.45	0.90	11	8.75	2.10	24	10.12	1.53	15
	2	10.89	0.93	9	13.33	0.95	7	12.14	0.98	8
	3	7.79	1.39	18	6.60	0.88	12	8.39	0.79	9
	4	3.27	0.57	17	6.37	0.56	9	3.81	0.53	14
	5	7.20	0.97	13	8.27	0.63	8	5.53	0.51	9
	6	8.93	1.63	18	8.87	1.00	11	11.13	0.92	8
	7	10.65	0.59	6	7.08	0.69	10	10.29	0.89	9
	8	12.14	0.86	7	11.90	0.88	7	9.52	0.85	9
	9	14.64	1.30	9	15.17	1.60	11	17.02	1.83	11
Control	21	8.09	0.81	10	7.20	0.90	13	6.01	0.76	13
	22	11.90	1.10	9	7.08	0.81	11	8.93	0.73	8
	23	8.27	1.10	13	6.43	0.85	13	6.84	0.81	12
	24	11.19	0.86	8	8.98	0.83	9	6.13	0.82	13
	25	12.02	2.56	21	21.12	1.57	7	12.38	1.61	13
	26	13.27	1.77	13	9.04	0.71	8	4.70	0.68	14
	27	8.98	0.79	9	6.25	1.60	26	5.00	0.75	15
	28	12.55	0.78	6	7.79	0.73	9	8.69	1.51	17
	29	5.36	0.83	15	5.36	0.70	13	10.71	0.69	6
	30	10.95	0.89	8	12.44	1.32	11	11.36	1.09	10

[a] Dietary vitamin B-6 intake.
[b] Urinary vitamin B-6 excretion.
[c] Percent of dietary vitamin B-6 excreted in urine.

25 percent) of values for percent of dietary vitamin B-6 excreted in urine as vitamin B-6 have been reported (Kabir, 1984).

As mentioned earlier, UB6 is highly correlated with dietary vitamin B-6 intake and thus one would expect a more consistent percent of excretion. These inconsistencies can probably be explained by differences in the levels of protein, fiber and glycosylated vitamin B-6 in the subjects' diets or to inadequacy or error in vitamin B-6 content of food.

Extremely low levels of vitamin B-6 in urine have been shown to correspond to other biochemical signs of vitamin B-6 deficiency (Sauberlich et al., 1972). Several researchers have recommended levels of UB6 to be used as minimal levels which would be reflective of inadequate vitamin B-6 intake. These suggested values include 0.59 nmol/24 hours (Linkswiler, 1967), 0.33 - 0.53 umol/24 hours (Sauberlich et al., 1972), and 0.5 - 0.7 umol/24 hours (Schultz and Leklem, 1981). According to these criteria, the UB6 of subject #4 (days 1, 2 and 3), subject #5 (day 3) and subject #7 (day 1) reflect inadequate vitamin B-6 intake. Indeed the vitamin B-6 intakes of these subjects were below ideal.

PLASMA VITAMIN B-6 AND DIET

PB6 was positively correlated with dietary vitamin B-6 intake ($p = 0.0001$). The relationship between PB6 and dietary vitamin B-6 intake has not been studied

extensively. In the experiment by Lee and Leklem (1985), PB6 increased three- to four-fold with daily supplementation of 8.0 mg of vitamin B-6. Lind (1980), however, found no correlation between PB6 and either vitamin B-6 intake or vitamin B-6 to protein ratio.

PB6 was also positively correlated to the vitamin B-6 to protein ratio ($p = 0.0021$). Guthrie and Crocetti (1983) suggest that using this dietary indicator to predict biochemical status would be more accurate than using vitamin B-6 intake without regard to protein. Both vitamin B-6 intake and vitamin B-6 to protein ratio were good predictors of vitamin B-6 status in this study. The two subjects that had vitamin B-6 to protein ratios less than 0.016 had PB6 values that might be considered marginal (less than 58.35 nmol/L) (Schultz and Leklem, 1980). Of the 12 subjects that had vitamin B-6 intakes less than 1.6 mg/d, 10 had PB6 values that could be considered marginal.

A high protein diet has been shown to affect PB6. In a previous study (Miller et al., 1985) PB6 decreased significantly ($p<0.05$) as protein intake increased. The correlation coefficient for PB6 and protein intake was -0.48 ($p<0.05$). In the present study, no correlation was found between protein intake and PB6. When protein is expressed as a percent of the RDA, the correlation coefficient is 0.16 and when protein is expressed as g per day the correlation coefficient is 0.23. The differences

between the results of this study and Miller et al. are that the latter was a controlled metabolic study in which widely different levels of protein (0.5, 1.0 and 2.0g/kg of body weight) and a constant level of vitamin B-6 were fed.

These results demonstrate an estrogen-related decrease of PB6 in postmenopausal women using ERT. Biochemical changes, such as lowered plasma levels of a vitamin, precede the development of vitamin deficiency. Although overt symptoms are not evident in this marginal state, functional deficits may be present. Two important questions which need to be addressed by continued research of this subject are:

1) Do postmenopausal women who use ERT need a higher daily dietary allowance of vitamin B-6 than other young or adult women?

2) Are postmenopausal women who use ERT at risk for developing clinical symptoms of vitamin B-6 deficiency?

SUMMARY

The primary aim of this study was to determine vitamin B-6 status in postmenopausal women using ERT. The concentration of total vitamin B-6 in plasma (PB6) and urine (UB6) and the dietary intake of the vitamin were measured. Since estrogens used for contraceptive purposes lower plasma PLP (Miller, 1986; Lumeng et al., 1974) and vitamin B-6 declines with age (Hamfelt, 1964; Lumeng and Li, 1974; Lee and Leklem, 1985), it was hypothesized that the subjects taking ERT (experimental group) would have a lower vitamin B-6 status than the control group. The experimental group did have a lower mean PB6 (47.7 $\pm$ 19.7 nmol/L) than the control group (56.2 $\pm$ 20.6 nmol/L), but this difference was not statistically significant. Because of the results of analysis of covariance, the means were adjusted for dietary vitamin B-6 and protein intake. After adjustment, the mean PB6 of the experimental group (42.7 nmol/L) was significantly ($p<0.05$) lower than that of the control group (60.6 nmol/L).

The subjects in this investigation were taking varying amounts of estrogen. The duration of ERT differed widely from subject to subject, as well as whether or not progestogen was taken. It would be interesting to conduct a controlled diet study of a population of postmenopausal women who had a more consistent ERT, for instance, 0.625 mg/day for at least five years.

The combined affect of age and estrogen use on the vitamin B-6 status of postmenopausal women was also an area of concern to the researchers. There was no linear relationship between age and PB6 in this study because the subjects were similar in age and had a wide range of vitamin B-6 intake. When the results of this experiment are compared to others of similar research areas and methods, the age:vitamin B-6 hypothesis is not fully supported.

A secondary aim of this investigation was to assess the dietary patterns that accounted for the observed dietary intake and the relationship between dietary intake and the biochemical indices used (PB6 and UB6). PB6 was correlated with dietary intake of vitamin B-6 ($p=0.0001$) and vitamin B-6 to protein ratio ($p=0.0021$). Likewise, UB6 was correlated with dietary vitamin B-6 intake ($r = 0.51$, $p<0.05$) and vitamin B-6 to protein ratio ($r = 0.47$, $p<0.05$). The mean daily intakes of vitamin B-6 and protein were similar for the two groups. Eighteen of the 19 subjects had vitamin B-6 intakes that were greater than 67 percent of the RDA and most subjects (all but two) had adequate vitamin B-6 intake with respect to protein (vitamin B-6 to protein ratio greater than 0.016 mg/g).

Vitamin B-6 intake has been frequently reported to be low in the elderly (Hampton et al., 1977; Garry et al., 1982). In most studies, vitamin B-6 intake is reported relative to the 1980 RDA of 2.0 mg. However, since the

requirement for vitamin B-6 is related to protein intake, it is also appropriate to evaluate dietary vitamin B-6 adequacy with regard to vitamin B-6 to protein ratio. Only one of the women in this study consumed more than 100 g of protein per day on which the present RDA for vitamin B-6 (1.6 mg) is based.

The women in this study received a large percentage of their vitamin B-6 from cereals, particularly fortified breakfast cereals. Evidence from this investigation suggests that the bioavailability of the vitamin B-6 found in breakfast cereals is high and that fortified breakfast cereals could provide an excellent source of vitamin B-6.

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APPENDICES

Appendix A. Informed Consent

Department of Foods & Nutrition

Oregon State University

INFORMED CONSENT

Vitamin B-6 Status and Body Composition
of Postmenopausal Estrogen Users

The purpose of this research is (a) to determine the effect of estrogen replacement therapy on vitamin B-6 status in postmenopausal women and (b) to determine some of the factors affecting body composition of postmenopausal women. Vitamin B-6 status will be measured by dietary intake of vitamin B-6, plasma vitamin B-6 and urinary excretion of vitamin B-6; body composition will be determined by skinfold and circumference measurements, and urinary excretion of creatinine.

If I agree to participate in this investigation, I will keep an accurate record of foods and beverages I consume for three consecutive days. On these same three days, I agree to collect complete 24-hour urine specimens in the containers provided for me by the investigators. For recording my diet and collecting urine, I will follow the detailed instructions that are provided for me. On the fourth day, I consent to have a registered medical technologist obtain 20 ml (equivalent to 4 teaspoons) of blood from a vein in my forearm between 7 and 9 o'clock in the morning. I will not have eaten or drunk anything except water from 10 o'clock the evening before to the time my blood is drawn the following morning. At the time blood is drawn, I will complete a questionnaire including questions about my age, use of estrogen, use of prescription and non-prescription drugs, nutrient supplements and alcohol, dietary habits and amount of exercise. After breakfast I consent to having skinfold and circumference measurements made at several sites on my body. I also consent to having my height and weight measured. I understand that to participate in this investigation, I should not have taken a vitamin supplement for at least 2 weeks.

I understand that there may be some slight discomfort when the medical technologist draws blood from my arm. There may be a slight bruise at the site of needle entry. There are no risks involved in collecting urine, recording my diet, and having anthropometric measurements made.

I know that I will receive no direct benefits from volunteering to participate in this research.

I have been assured that any information obtained from me in connection with this study will remain confidential.

I understand that I am free to withdraw from this study at any time.

All of my questions regarding this investigation have been answered.

If I have any additional questions later, I will contact Dr. Lorraine Miller, Janet Harris or Susan Worley at 754-3561. I have been given a copy of this form for my records.

Subject	Date
Witness	Date

Appendix B. Instructions for Recording Food

1. Please record each food and beverage you consume on a separate line. Be sure to include all snacks.

2. Record the exact amount: liquids in household measures (cups), fluid ounces or milliliters; vegetables and fruits in cups or inches; beans, grains or pasta in cups dry, or cups cooked; bread in slices, indicating what kind of bread; meats, fish and cheeses in ounces (an average meat portion is 3 oz., a slice of American cheese is about 1 oz.).

3. Please specify if a food is consumed raw. Also indicate if it was prepared from fresh, canned or frozen products.

4. Indicate how the food was prepared, such as fried or boiled.

5. If a food is a mixture (sandwich, soup, casserole) list the ingredients separately in their proportions or amounts.

6. Use brand names whenever possible, or mention comparable brand name products. Specify if it is a "diet" product.

7. For fruits and vegetables indicate if skin was removed.

8. Indicate if milk consumed is whole, skim, 1%, or 2%.

9. Provide any other information you feel might be helpful. Remember: the more accurate your record, the more accurate the analysis will be.

If there are any questions about your diet record, please call Janet Harris at 754-9014, or Susan Worley at 754-9209, or leave message with the Foods and Nutrition Department at 754-3561, from 8:00-12:00 and 1:00-4:30.

Appendix C. Diet Record Sheet

NAME_____ DATE_____ LEAVE A BLANK SPACE BETWEEN EACH MEAL
USE A SEPARATE SHEET FOR EACH DAY

LOCATION time & place	FOOD specify each food or beverage on a separate line	SOURCE canned, dried frozen, fresh	BRAND be specific	PREPARATION fried, baked, raw, etc.	AMOUNT measure in cups, inches, etc.	FOR OFFICE USE AMT code	WT code

Appendix D. Instructions for Collection of Urine

1. Collect all urine in containers provided.

2. Label all containers carefully with your initials and date.

3. On day 1, completely empty bladder immediately after rising. Discard this urine. Thereafter, collect all urine during the next 24 hours in the containers provided. Completely empty bladder at the same time the following morning and label this urine as belonging to that collected on the preceding day (24-hour period). Continue collecting urine in this manner for the following 2 days. It is important that the collection made on rising is done at the same time each day.

4. Urine will be collected starting with breakfast on the day you start your diet recording. Return urine samples between 7:30 a.m. and 11:00 a.m. to room 106, Milam Hall.

5. Store urine in a cool place, protected from light. Do not store urine in your refrigerator.

6. Please be careful not to spill or lose any urine. If this does happen, let us know. The urine collections are a very critical part of this study.

7. Drink approximately the same amount of fluids each day, if possible.

If there are any questions about your urine collection, please call Janet Harris at 754-9014, or Susan Worley at 754-9209, or leave a message with the Foods and Nutrition Department at 754-3561, from 8:00-12:30 and 1:00-4:30.

Appendix E. Questionnaire

NUTRITIONAL SURVEY OF POSTMENOPAUSAL WOMEN

Please answer the following questions as completely and as honestly as possible. If you need more space than that which is provided, please continue your answer on page five or on the back of the page. If you do not wish to answer a question, draw a line through it. If you do not understand a question, please ask for assistance. The accuracy of this study depends upon you. All information is confidential. Thank you for your cooperation!

NAME _____ DATE _____

1. Have you been taking estrogens regularly for the last six months? (Circle one number)
   1 NO
   2 YES
   → 1a. How many months have you been taking estrogens continuously?
   _____MONTHS
   1b. What is the complete brand name of the estrogens you are taking? (Include a label if possible. Be sure to remove your name from label.)
   _____BRAND NAME
   1c. Have you switched estrogen brands any time during the last six months? (Circle one number)
   1 NO
   2 YES
   → 1d. Please list the other estrogen brands you have taken during the past six months and give number of months taken for each.

BRAND NAME	MONTHS TAKEN
_____	_____
_____	_____
_____	_____

   1e. Please indicate whether or not you are taking estrogens for each of the following reasons. (Circle one number for each reason)

	YES, A REASON	NO, NOT A REASON
a. hot flashes.....	1	2
b. to prevent/retard osteoporosis.	1	2
c. other (please specify _____ _____)	1	2

   1f. Since you started using estrogens, has your weight stayed the same, increased, or decreased? (Circle one number)
   1 STAYED THE SAME
   2 INCREASED
   3 DECREASED
   → 1g. By about how many pounds has your weight increased or decreased?
   _____POUNDS

2. Have you been taking progestogens regularly for the last six months? (Circle one number)
   1 NO
   2 YES
   → 2a. How many months have you been taking progestogens continuously?
   _____MONTHS
   2b. What is the complete brand name of the progestogens you are taking? (Include a label if possible. Be sure to remove your name from label.)
   _____BRAND NAME

3. Are you presently on a special diet? (Circle one number)
   1 NO
   2 YES
   → 3a. Briefly describe this diet: _____
   _____

4. Do you smoke cigarettes? (Circle one number)
   1 NO
   2 YES
   → 4a. How many cigarettes do you smoke per day?
   _____CIGARETTES PER DAY

5. Do you ever drink alcoholic beverages? (Circle one number)
   1 NO
   2 YES
   → 5a. About how many servings (equivalent to 1 ounce of liquor, 4 ounces of wine, or 10 ounces of beer) do you consume per week?
   _____SERVINGS PER WEEK

6. Do you drink coffee, tea, or soft drinks containing caffeine? (Circle one number)
   1 NO
   2 YES
   → 6a. How many cups per day?
   _____CUPS PER DAY

7. Do you have a history of medical problems? (Circle one number)
   1 NO
   2 YES
   → 7a. Briefly describe your medical problems: _____
   _____

8. Are you presently using any prescription drugs other than estrogens?
   1 NO
   2 YES
   → 8a. In the table below, please list the prescription drugs you are taking, the dosage, the number of pills you take per day, and the number of months you have been taking them.

PRESCRIPTION NAME	DOSAGE	NUMBER OF PILLS PER DAY	NUMBER OF MONTHS TAKEN

9. Have you been using any supplements such as vitamins or minerals regularly for the last six months? (Circle one number)
   1 NO
   2 YES
   → 9a. In the table below, please list the supplements you are taking, the dosage, the number of pills you take per day, and the number of months you have been taking them.

SUPPLEMENT NAME	DOSAGE	NUMBER OF PILLS PER DAY	NUMBER OF MONTHS TAKEN

10. Circle the word(s) below that you feel best describes your lifestyle.
    1 SEDENTARY
    2 MODERATELY ACTIVE
    3 VERY ACTIVE

11. Do you regularly engage in physical activity? (Circle one number)
    1 NO
    2 YES
    → 11a. In the table below, please describe the type of activity you engage in, the number of minutes per day, and the number of days per week.

TYPE OF ACTIVITY (walking, swimming, etc.)	MINUTES PER DAY	DAYS PER WEEK

12. During which of the following time periods do you eat the most food? (Circle one number)
    1 BEFORE 11 A.M.
    2 BETWEEN 11-2 P.M.
    3 BETWEEN 2-5 P.M.
    4 BETWEEN 5-7 P.M.
    5 AFTER 7 P.M.

13. How many people including yourself live in your home?
    _____NUMBER OF PEOPLE

14. How often do you prepare the meals for yourself or yourself and others you live with? (Circle one number)
    1 ALL OF THE TIME
    2 MOST OF THE TIME
    3 SOME OF THE TIME
    4 SELDOM OR NEVER

15. Please fill out the following table which describes your daily meal pattern. Indicate which meals you usually eat, what time you usually eat them, and where you usually eat them.

	DON'T EAT	YES DO EAT	WHAT TIME EATEN	WHERE EATEN (USUALLY)
Breakfast	1	2 →	_____ →	_____
Lunch	1	2 →	_____ →	_____
Dinner	1	2 →	_____ →	_____
Snacks	1	2 →	_____ →	_____

16. How many hours of television do you watch weekly? (Circle one number)
    1 1 HOUR OR LESS
    2 UP TO 2 HOURS
    3 UP TO 4 HOURS
    4 UP TO 6 HOURS
    5 MORE THAN 6 HOURS

17. How often do you eat meals and/or snacks while watching television? (Circle one number)
    1 NEVER
    2 ONCE IN AWHILE
    3 ALMOST ALWAYS

18. Are you presently working for pay or as a volunteer? (Circle one number)
    1 NO
    2 YES
    → 18a. How many hours per week do you work?
    _____HOURS PER WEEK

19. What was your approximate weight at the following ages?
    a. weight at age 25: _____POUNDS
    b. weight at age 35: _____POUNDS
    c. weight at age 45: _____POUNDS
    d. weight at age 55: _____POUNDS

20. What is your present weight?
    _____POUNDS

21. What is your approximate height (without shoes)?
    _____INCHES

22. Has your height changed since you were age 25? (Circle one number)
    1 NO
    2 YES
    → 22a. What was your approximate height at age 25?
    _____INCHES

23. What is your present age?
    _____YEARS

24. Please put a number indicating approximately how many servings of each of the following you eat per week:

A. FRUITS (one small fruit or 1/2 cup is one serving)

_____Citrus	_____Dried fruit	_____Banana
_____Apples	_____Raisins (1/3 cup)	_____Avocado (1/4 med.)
_____Berries		
_____Melon		
_____Plums		
_____Pears		

Other (please specify_____)

B. VEGETABLES (1 small veg. or 1/2 cup is one serving)

_____Green beans	_____Greens	_____Dried beans
_____Tomatoes	_____Broccoli/cauliflower	_____Lentils
_____Potatoes	_____Sweet potatoes	_____Soybeans
_____Celery	_____Corn	
_____Mushrooms	_____Cabbage	
_____Carrots	_____Onions, mature	

_____Other (please specify_____)

C. BREADS AND CEREALS (1 slice or 1/2 cup is one serving)

_____White bread	_____Whole wheat bread	_____Brewer's yeast (3 Tb)
_____White rice	_____Whole wheat pasta	
_____Saltines/soda crackers (4-6)	_____Brown rice	_____Wheat germ (1/4 cup)
	_____Rye bread	_____Wheat bran (1/2 cup)
_____Cookies	_____Cornbread	_____Soy flour (1/4 cup)
_____Other (please specify_____)		_____Breakfast cereal

D. MEATS (3 ounces is one serving)

_____Shellfish	_____Canned tuna/salmon	_____Organ meats
_____Shrimp	_____Fish	_____Fresh tuna/salmon
_____Eggs (2 = 1 serv.)	_____Red meats	_____Poultry

E. MILK AND MILK PRODUCTS

_____Milk, all kinds (1 fluid cup is one serving)
_____Yogurt (1 cup is one serving)
_____Cheeses, all kinds (1 ounce is one serving)
_____Cottage cheese (1/2 cup is one serving)
_____Other (please specify_____)

F. MISCELLANEOUS

_____Jam/jelly (2 Tb)	_____Peanut butter (1 1/2 Tb)	____Sunflower seeds (1/4 cup)
_____Honey (2 Tb)	_____Almonds (10)	
_____Candy bar (1)	_____Peanuts (10)	____Walnuts (14 halves)
_____Soft drinks (12 oz.)		
_____Cake or pie (1 slice)		____Filberts (1/4 cup)
_____Other (please specify_____)		

25. Please use this space for any additional comments you may have.

THANK YOU ! ! !

Appendix F. Calculation of Vitamin B-6 Dietary History Score

The vitamin B-6 dietary history score was derived from pages 4 and 5 of the questionnaire. Foods were scored according to mg of vitamin B-6 per serving. Foods with less than 0.1 mg of vitamin B-6 per serving (first column) were given two points. The foods in the second column were given four points and contained between 0.1 and 0.3 mg of vitamin B-6 per serving. Eight points were given to foods in the third column. These foods contained greater than 0.3 mg of vitamin B-6 per serving. Any food recorded under "other" was grouped in the appropriate column and scored accordingly. The total score was then divided by the total number of servings per week to obtain an average value whioh was termed vitamin B-6 dietary history score.

Appendix G. Brief description of subject's medical history

Group	Subject	Prescription Drugs Taken[a]	Medical Conditions: Past and Current
Experimental	1	None	Severe anemia leading to complete hysterectomy, eye problems
	2	Lopressor, Moduretic	Hypertension
	3	Synthyroid, Diazapem, Starax	Thyroid removed, migraine headaches, boils, shingles, uticaris, colitis, jaw clenching
	4	None	None
	5	None	None
	6	None	None
	7	None	Hypothyroidism, oophorohysterectomy
	8	None	Hysterectomy, allergies
	9	Lithabid, Descryl, Propine, Vancenase	Duodenal ulcer, hay fever, glaucoma, depression
Control	21	None	None
	22	None	Gallbladder surgery, hysterectomy
	23	None	None
	24	Dyazide, Thyroid pills	Headaches, severe kidney infection when eight years old, calcification of thyroid and breasts, hypertension
	25	Lopressor, Diazyne	Hypertension
	26	None	None
	27	None	Chronic cystitis
	28	None	Mastectomy, surgery for varicose veins
	29	None	None
	30	None	Neck and back problems from a car accident, arthritis

[a] Other than estrogens and progestins taken by experimental group. (See Table 2.)