AN ABSTRACT OF THE THESIS OF

Andrea M. Bolli for the degree of Master of Science in Nutrition and Food Management presented on August 20, 1997.

Title: Vitamin B6 Status Over Time and Its Relation to Symptoms of Carpal Tunnel Syndrome

Abstract approved: _____
James E. Leklem

Research suggests that, in individuals with carpal tunnel syndrome (CTS), low plasma pyridoxal 5'-phosphate (PLP) concentrations are related to an increased incidence and severity of symptoms associated with CTS. This study was designed to determine the relationship between plasma and red blood cell PLP concentrations and the severity and incidence of CTS symptoms. Thirty people with CTS were selected for a 9 month exercise study. Subjects were divided into either vitamin users or non-vitamin users based on supplement use data gathered at the beginning of the study. Blood was drawn at 1, 6 and 9 months. CTS symptoms questionnaires and health questionnaires were also administered at these intervals. The symptoms questionnaires were used to gather data on the frequency and nature of hand and wrist symptoms. Health questionnaires focused on vitamin supplement usage including frequency, amount and length of use. Mean plasma PLP, total plasma vitamin B6 and erythrocyte PLP concentrations were significantly higher in the sixteen vitamin users when compared to the fourteen non-vitamin users ($p<0.001$). While there was variation in plasma PLP and total plasma vitamin B6 over

time, within each group, there were no significant changes in any of the status measures over the nine month period. Mean erythrocyte PLP concentration, in particular, was stable over time. In vitamin users, the intensity of pain, numbness and tingling was significantly higher when compared to non-vitamin users. In both groups, plasma PLP was negatively correlated with pain. This correlation reached statistical significance in vitamin users at month one and nine ($p<0.01$), but not at month six; a statistically significant correlation between these two variables was not found in non-vitamin users at any time point. Pain was also negatively and significantly correlated with plasma total vitamin B6 and erythrocyte PLP in vitamin users. No other symptoms were significantly correlated with the status measures. These results indicate that a higher vitamin B6 status may be related to a decrease in the severity of pain experienced by some individuals with CTS.

Vitamin B6 Status Over Time and Its Relation to Symptoms of Carpal Tunnel Syndrome.

By

Andrea M. Bolli

A THESIS

Submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

Completed August 20, 1997

Commencement June, 1998

Master of Science thesis of Andrea M. Bolli presented on August 20, 1997

Approved:

James Leklem, representing Nutrition Science

Chair of Department of Nutrition and Food Management

Dean of Graduate School

I understand that my thesis will become part of the permanent collection at Oregon State University Libraries. My signature below authorized release of my thesis to the reader upon request.

Andrea M. Bolli, Author

TABLE OF CONTENTS

TABLE OF CONTENTS (cont.)

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDIX FIGURES

LIST OF APPENDIX TABLES

LIST OF APPENDIX TABLES (cont.)

Vitamin B6 Status Over Time and its Relation to Symptoms of Carpal Tunnel Syndrome

INTRODUCTION

Over the years, there has been heightened interest in trying to cure a variety of ailments with non-traditional means. Increasingly, people are looking to vitamins, minerals and herbs, rather than prescription drugs and surgery, to promote health and well being. One such disorder that falls into this category is carpal tunnel syndrome. Carpal tunnel syndrome is a disorder that affects the median nerve of the wrist and causes a variety of neurological problems, including pain and numbness. Traditionally, carpal tunnel syndrome has been treated with steroid injections and surgery. However, because both of these methods have a high failure rate, 50 % and 40% respectively, researchers have been trying to find a more successful alternative (Amadio, 1987).

Vitamin B6, known to be essential for synthesis of neurotransmitters, such as gamma aminobutyric acid and serotonin, associated with analgesia, has been used in the treatment of carpal tunnel syndrome (Dakshinamurti and Paulose, 1985). At present, however, there is no conclusive evidence as to whether supplementation of vitamin B6 can reduce symptoms associated with the syndrome. The possible link between vitamin B6 and carpal tunnel syndrome was proposed in the 1970's. Research suggested that impaired vitamin B6 status, as defined by low transaminase activity, caused carpal tunnel syndrome; intakes near 150 mg vitamin B6/day would cure the disorder (Ellis et al., 1979; Folkers et al., 1978). Later studies did not support this conclusion and instead suggested that vitamin B6 can, in some individuals, reduce the severity and frequency of

ymptoms when given in doses of 100-300 mg/day (Bernstein and Dinesen, 1993; Guzman et al., 1989).

The active form of vitamin B6, pyridoxal 5'-phosphate (PLP), serves as a cofactor to enzymes involved in serotonin and gamma amino butyric acid (GABA) synthesis (Dakshinamurti and Paulose, 1985; Scriver, 1960). Serotonin, a neurotransmitter associated with pain, has been shown to possess an analgesic like effect. GABA is an inhibitory neurotransmitter also associated with a higher pain threshold. Research, in animals, has shown that impaired vitamin B6 status, evidenced by low plasma PLP concentrations, results in reduced synthesis of both serotonin and GABA (Behbehani, 1990; Samanin, 1976). Conversely, pharmacological intakes of vitamin B6 appear to increase the neurotransmitter concentrations in the brain and spinal fluid thereby increasing analgesia (Fu et al., 1990; Bartoszyk and Wild, 1990). As a result, it is possible that individuals with impaired vitamin B6 status may experience more pain when compared to individuals with either adequate or high concentrations of plasma PLP.

Studies trying to identify the relationship between carpal tunnel syndrome and vitamin B6 are complicated by the fact that carpal tunnel syndrome is just that – a syndrome which, by definition, is a group of symptoms and signs of disordered function related to one another by means of some anatomic, physiologic, or biochemical peculiarity. In addition to vitamin B6, research efforts must also consider body weight, activity, lifestyle, diet, alkaline phosphatase activity and supplement use (frequency, amount, length of use). All are factors that either influence vitamin B6 status or are associated with an increased risk of carpal tunnel syndrome.

With the prevalence of carpal tunnel syndrome on the rise, it is important that the effect of vitamin B6 be conclusively determined. In part, it is the intent of this study to investigate the role of vitamin B6 in carpal tunnel syndrome by taking a more comprehensive approach that will include monitoring vitamin B6 status over time, evaluation of nerve function, evaluation of symptomology, detailed data on supplement use, and determination of exercise habits.

HYPOTHESES

Low vitamin B6 status, as reflected by low plasma and erythrocyte PLP is positively correlated to both an increased frequency and severity of symptoms associated with carpal tunnel syndrome in people either afflicted with or susceptible to carpal tunnel syndrome.

Vitamin B6 status will neither improve nor decline over the nine month study period.

OBJECTIVES

The primary objective is to better understand the interrelationship between vitamin B6 status and carpal tunnel syndrome by comparing two groups of people with carpal tunnel syndrome – one taking vitamin B6 supplements and one taking no vitamin B6 supplements. Specific objectives are as follows:

1. To monitor the stability of vitamin B6 status over a nine month period by determining

plasma PLP, total plasma vitamin B6 and erythrocyte PLP concentrations.

2. To determine if a significant correlation exists between carpal tunnel symptoms (e.g. pain, numbness, tingling) and plasma PLP, total plasma vitamin B6 and erythrocyte PLP.
3. To determine if vitamin users have a significantly lower frequency of reported carpal tunnel symptoms when compared to non-vitamin users.

4. To determine if a significant correlation exists between nerve conduction and plasma PLP, total plasma vitamin B6, and erythrocyte PLP.

LITERATURE REVIEW

VITAMIN B6

History

The term vitamin B6 was first used in 1934 by Paul Gyorgy to describe a substance essential for proper development of rats (Gyorgy, 1934). Animal studies over the next four years led to the isolation of the crystalline form of vitamin B6 by several different research groups (Gyorgy, 1938; Kuhn and Wednt, 1938; Lepovsky, 1938). Because this substance was a derivative of pyridine, the compound was named 3-hydroxy-4,5-dihydroxy-2-methyl pyridine (Harris and Folkers, 1939). For simplicity, the name was shortened to pyridoxine (PN). In the early 1940s, microbiological research resulted in the discovery of two additional forms of vitamin B6 - pyridoxal (PL) and pyridoxamine (PM) (Snell et al., 1942). Consequently, vitamin B6 became the generic term used to describe all 3-hydroxy-2-methyl pyridine derivatives. The following decade (1950s) saw the discovery of the active form of the vitamin - pyridoxal 5'-phosphate (PLP). Isolation of pyridoxamine 5'-phosphate (PMP) and pyridoxine 5'-phosphate (PNP) occurred shortly thereafter.

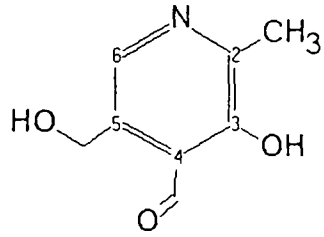
The essentiality of vitamin B6 to humans was first documented in 1939 when Spies and colleagues (1939) reported irritability, weakness, abdominal pain and nervousness in patients consuming a low vitamin B6 diet. Administration of 50 mg vitamin B6 as PN-HCL reversed the symptoms. Convulsive disorders were later documented by Snyderman et al. (1953) in infants consuming vitamin B6 deficient diets. These early studies paved the way for continued vitamin B6 research.

Structure and Chemistry

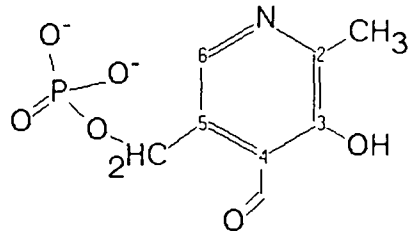
The basic structure of all vitamin B6 compounds consists of a pyridine ring. The specific vitamer formed depends upon the side chain located at position #4 (see Figure 1). For example, PL is the vitamer formed if the side chain is a formyl group. An aminomethyl group in this position forms PM and a hydroxymethyl group PN. All three vitamers also exist in the phosphorylated forms -- pyridoxal 5'-phosphate (PLP), pyridoxamine 5'-phosphate (PMP), and pyridoxine 5'-phosphate (PNP). The phosphorylated compounds, namely PLP, are the active forms of the vitamin found in organs and tissues (Rabinowitz and Snell, 1948). All known reactions of PLP dependent enzymes involve formation of a planar Schiff base (Hughes et al., 1962). Base formation is followed by bond labilization caused by electron withdrawal into the coenzymes pyridine ring. Depending on which bond is labilized, PLP can catalyze transamination, decarboxylation, racemization or numerous side chain reactions (see Table 1)(Leklem, 1993). Two other forms of vitamin B6 have also been identified – 4-pyridoxic acid (4-PA) and 5-O-(B-D-glycopyranosyl). 4-pyridoxic acid is the metabolic end product of vitamin B6 and is formed with the substitution of a carboxyl group at the #4 position. The glucoside is a form of vitamin B6 found in plant products. Glucoside linkage occurs at the #5 position.

The various forms of vitamin B6 are pH sensitive - stable in acid, but destroyed at an alkaline or neutral pH. pH also influences the stability of B6 vitamers in heat and light. For example, vitamin B6 can withstand heat under acidic, but not alkaline conditions. The degree to which vitamin B6 remains active in light also varies with pH.

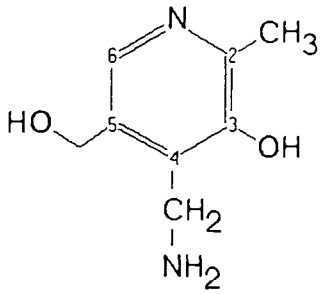
Figure 1. Structure of the B6 vitamers (Adapted from Leklem, 1991)



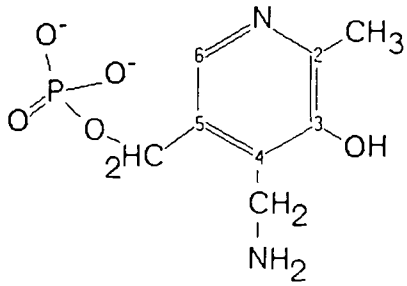
PL



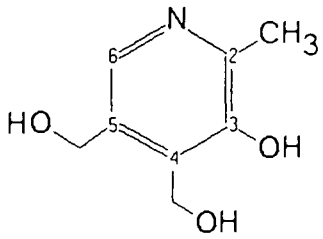
PLP



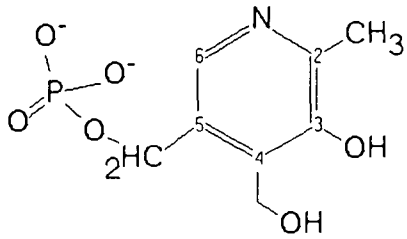
PN



PNP



PM



PMP

Table 1. Enzymatic reactions catalyzed by pryidoxal 5'-phosphate.

Type of Reaction	Reaction or Enzyme
α–carbon reactions	
Transamination	Alanine $\leftrightarrow$ pyruvate + PMP
Racemization	D-amino acid $\leftrightarrow$ L-amino acid
Decarboxylation	5-OH Tryptophan $\rightarrow$ 5-OH tryptamine + CO_2
Oxidative deamination	Histamine $\rightarrow$ imidazole-4-acetaldehyde + NH_4
Loss of the side chain	THF + Serine $\rightarrow$ glycine + N5,10-methyleneTHF
β-carbon reactions	
Replacement (exchange)	Cysteine synthetase
Elimination	Serine and theonine dehydratase
γ-carbon reactions	
Replacement (exchange)	Cystathionine $\rightarrow$ cysteine + homoserine
Elimination	Homocysteine desulfhydrase
Cleavage	Kynurenine $\rightarrow$ anthranilic acid

Adapted from: Leklem, 1991.

Toxicity

Although vitamin B6 is water soluble, it can be toxic when taken in large amounts for extended periods of time. Evidence of vitamin B6 toxicity in animals was reported in 1942 (Antopol and Tarlov, 1942). In 1983, vitamin B6 was shown to cause irreversible nerve damage in humans when taken in pharmacological doses (Schaumburg et al., 1983). Early research on the toxicity of vitamin B6 indicated that chronic intake of 2000-6000 mg/day would result in nerve problems. Further research found that toxicity could occur at a much lower amount -- 200-500 mg/day (Berger and Schaumburg, 1984; Parry and Bredesen, 1985).

Occurrence in Foods

Vitamin B6 is widespread in the food supply and most foods contain varying proportions of the three vitamers (see Table 2). In general, PN and PM are predominant in plant products and PL in animal products. Because of its stability, pyridoxine, as PN-HCl, is the form used in vitamin supplements and fortified foods (Bauernfeind and Miller, 1978). The vitamers usually exist as the phosphorylated compound. A fourth form of vitamin B6 has been identified in plant foods, pyridoxine 5'-glucoside (Gregory, 1988). Ample amounts of vitamin B6 can be obtained by eating a variety of foods. With the exception of some legumes and bananas, meat, fish and poultry are the best sources of vitamin B6. These products contain the highest amount of the vitamin per serving and the majority of the vitamin B6 is considered to be bioavailable (Kabir et al., 1983a; Gregory and Ink, 1987). A 100 gram portion of meat, fish or poultry provides an average of 0.35 mg vitamin B6 (range 0.170-0.683 mg/100g). Dairy products are considered a poor source of the vitamin providing only 0.010-0.110 mg/100g. The vitamin B6 content of plant foods varies considerably ranging from 0.019 mg/100g for peaches to 3.515 mg/100g for rice bran. Some of the best plant sources include potatoes, soybeans, lentils, filberts, brown rice, and bananas. Fortified cereals have also become a significant source of vitamin B6 in the diet providing an average of 1.5mg/100g serving. Based on NHANES II data (Kant and Block, 1990), animal products, alcoholic beverages, cold cereals and potato products represent the major sources of vitamin B6 in the diets of 19-74 year olds. The average vitamin B6 intake for the total population was determined to be 1.48 ± 0.01. On average, males consumed 1.85 ± 0.02 mg vitamin B6 and females 1.14 ± 0.01 mg vitamin B6.

Table 2. Vitamin B6 content of selected foods and percentage of the unphosphorylated vitamers present.

Food	Vitamin B6 (mg/100 mg)	PN (%)	PL (%)	PM (%)
Vegetables				
Beans lima, frozen	0.150	45	30	25
Cabbage, raw	0.160	61	31	8
Carrots, raw	0.150	75	19	6
Peas, green, raw	0.160	47	47	6
Potatoes, raw	0.250	68	18	14
Tomatoes, raw	0.100	38	29	33
Spinach, raw	0.280	36	49	15
Broccoli, raw	0.195	29	65	6
Cauliflower, raw	0.210	16	79	5
Corn, sweet	0.161	6	68	26
Fruits				
Apples, red delicious	0.030	61	31	8
Apricots, raw	0.070	58	20	22
Apricots, dried	0.169	81	11	8
Avocados, raw	0.420	56	29	15
Bananas, raw	0.510	61	10	29
Oranges, raw	0.060	59	26	15
Peaches, canned	0.019	61	30	9
Raisins, seedless	0.240	83	11	6
Grapefruit, raw	0.034	-	-	-
Legumes				
Beans, white, raw	0.560	62	20	18
Beans, lima, canned	0.090	75	15	10
Lentils	0.600	69	13	18
Peanut Butter	0.330	74	9	17
Peas, green, raw	0.160	69	17	14
Soybeans, dry, raw	0.810	44	44	12
Nuts				
Almonds, without skins, shelled	0.100	52	28	20

Table 2, continued

Food	Vitamin B6 (mg/100 g)	PN (%)	PL (%)	PM (%)
Nuts				
Pecans	0.183	71	12	17
Filberts	0.545	29	68	3
Walnuts	0.730	31	65	4
Cereals/Grains				
Barley, pearled	0.224	53	42	6
Rice, brown	0.550	78	12	10
Rice, white, regular	0.170	64	19	17
Rye flour, light	0.090	64	22	14
Wheat, cereal, flakes	0.292	79	11	10
Wheat flour, all purpose, white	0.060	55	24	21
Oatmeal, dry	0.140	12	49	39
Cornmeal, white and yellow		-	-	-
Bread, white	0.040	-	-	-
Bread, whole wheat	0.180	-	-	-
Meat/poultry/fish				
Beef, raw	0.330	16	53	31
Chicken breast	0.683	7	74	19
Pork, ham, canned	0.320	8	8	84
Flounder fillet	0.170	7	71	22
Salmon, canned	0.300	2	9	89
Sardine, Pacific canned, oil	0.280	13	58	29
Tuna, canned	0.425	19	69	12
Halibut	0.430	-	-	-
Milk/eggs/cheese				
Milk, cow, homog.	0.040	3	76	21
Milk, human	0.010	0	50	50
Cheddar	0.080	4	8	88
Egg, whole	0.110	0	85	15

All values taken from Orr, 1969

Recommended Dietary Allowance (RDA)

Although the essentiality of vitamin B6 in human nutrition was becoming known early in the century, the RDA for vitamin B6 has been set in just the last 25 years. Currently, the RDA for males is 2.0 mg/day and for females 1.6 mg/day (National Research Council, 1989). For males and females, these values are approximately 0.2 mg/day and 0.4 mg/day lower, respectively, than those recommended by the 9th edition of the RDA (National Research Council, 1980). The lowering of the RDA is a topic that is currently being debated. The RDA for vitamin B6 covers the needs of approximately 97% of the population by including a margin of safety. The safety factor accounts for differences in bioavailability, metabolism and other conditions that can impact nutrient needs (see Table 3). Protein intake has been proven to influence vitamin B6 requirements. Protein and vitamin B6 status have an inverse relationship. For example, with a high protein intake, more vitamin B6 is needed to metabolize the excess amino acids. As a result, vitamin B6 concentrations in individuals with a high protein diet tend to be lower than individuals consuming less protein (Baker et al., 1969; Miller and Linkswiler, 1967; Kelsay et al., 1968). This relationship reflects the major role of PLP in amino acid metabolism and has resulted in setting the RDA for vitamin B6 in terms of a vitamin B6 to protein ratio. Based on numerous studies, the current vitamin B6:protein ratio that is used in setting the RDA is 0.016 mg B6/g protein (National Research Council, 1989). This is an amount that is established in relation to the upper boundary of acceptable levels of protein intake, approximately 126 grams/day for men and 100 grams/day for women (National Research Council, 1989).

Table 3. Factors affecting an individual's vitamin B6 requirement.

1. **Dietary**

    a. Physical structure of a food
    b. Forms of vitamin B6; those due to processing
    c. Binding of forms of vitamin B6

2. **Defect in delivery to tissues**

    a. Impaired gastrointestinal absorption
    b. Impaired transport -- albumin synthesis, binding, phosphatase activity

3. **Physiological/Biochemical**

    a. Physical activity -- increased loss, gluconeogenesis
    b. Protein -- enzyme induction
    c. Increased catabolism/turnover -- phosphatase activity, illness
    d. Impaired phosphorylation and/or interconversion, competing pathways, nutrient deficiencies, drugs
    e. Pregnancy -- demand of fetus
    f. Growth -- increased cell mass, repair
    g. Excretion rate -- urinary, sweat, menstrual loss
    h. Lactation -- adequate levels in milk
    i. Sex -- differences in metabolism
    j. Age -- differences in metabolism

4. **Genetic**

    a. Apoenzyme defects -- altered binding to apoenzyme
    b. Altered apoenzyme levels -- biochemical individuality

5. **Disease prevention/treatment**

    a. Which? Heart, cancer, diabetes, PMS, kidney, alcohol

Adapted from: Leklem, 1993

Status Measures

The best method with which to assess vitamin B6 status is still a topic of debate. Indices used to determine status can be divided into direct measures and indirect measures. Direct measures quantify a specific form of the vitamin in the blood or urine. Conversely, indirect measures determine the enzyme activity or the concentration of compounds whose metabolism is vitamin B6 dependent. Based on the available literature, Leklem (1990) has compiled a list of values to be used when assessing vitamin B6 status (see Table 4). When assessing status, a minimum of three indices should be used. Currently, three main direct measures are used: plasma PLP, total plasma vitamin B6 and urinary 4-PA. Erythrocyte PLP is sometimes used in conjunction with other status indicators to provide a more comprehensive picture of vitamin B6 nurtiture. Plasma PLP has been the most frequently used indice (Leklem, 1990). The validity of plasma PLP as a status indicator was confirmed by Lumeng et al. in 1978. Their work with rats demonstrated that plasma PLP is reflective of both dietary intake and muscle concentration. Shultz and Leklem (1981) found a similar relationship in humans when they showed that plasma PLP was significantly correlated with dietary vitamin B6 intake. This was further demonstrated by Lee and Leklem (1985) who found that when the dose of vitamin B6 was increased from 2.3 mg/day to 10.3 mg/day, the mean plasma PLP concentration of young women increased from 62.4 ± 20.2 to 210 ± 52 nmol/L. When dietary content of vitamin B6 is altered, plasma PLP concentrations increase or decrease proportionate to dietary intake until concentrations reach a new steady state level within 3-4 weeks (Brown et al., 1975). Because PLP is the predominant form of the vitamer

Table 4. Indices for evaluating vitamin B6 status and suggested values for adequate status in adults.

Indicies	Suggested value for adequate status
Direct	
Blood	
Plasma pyridoxal 5'-phosphate	> 30 nmol/L
Plasma pyridoxal	NV
Plasma total vitamin B6	> 40 nmol/L
Erythrocyte pyridoxal 5'-phosphate	NV
Urine	
4-Pyridoxic acid	> 3.0 μmol/d
Total vitamin B6	> 0.5 μmol/d
Indirect	
Blood	
Erythrocyte alanine transaminase index	< 1.25
Erythrocyte aspartic transaminase index	< 1.80
Urine	
2 g Tryptophan load; xanthurenic acid	< 65 μmol/d
3 g Methionine load; cystathionine	< 350 μmol/d
Diet Intake	
Vitamin B6 intake, weekly average	> 1.2-1.5 mg/d
Vitamin B6: protein ratio	> 0.020
Other	
Electroencephalogram pattern	NV

NV = no value established; limited data available
Adapted from: Leklem, 1990.

present in the plasma, approximately 50-75% of the total vitamin B6 (Lumeng et al., 1980; Vanderslice et al., 1981), fasting plasma PLP values are considered to be a reliable measure of vitamin B6 status.

Several factors can influence plasma PLP concentration which has caused some researchers to question the usefulness or validity of this status measure. For example, a high protein intake has been shown to decrease plasma PLP concentrations (Canham et al., 1969). Miller et al. (1985) found an inverse relationship between protein intake and plasma PLP in subjects given 0.5 to 2.0 g protein/kg/day. The importance of plasma alkaline phosphatase activity to plasma PLP concentration is becoming more recognized. Alkaline phosphatase is a membrane bound enzyme that hydrolyzes circulating PLP allowing uptake of PL into the tissues. In 1980, Anderson (1980) demonstrated an inverse relationship between plasma PLP concentration and plasma alkaline phosphatase activity. Because of this relationship, a high activity of alkaline phosphatase may cause an erroneous diagnosis of deficiency where low PLP is not indicative of low vitamin B6 status. The effect of high alkaline phosphatase activity is evident in patients with cirrhotic liver. Individuals with this condition display low plasma PLP concentrations (Henderson et al., 1986; Bonjour, 1980). Research has found that patients with cirrhosis have the same level of vitamin B6 metabolic enzymes (Merrill et al., 1986). Therefore, the low PLP concentrations are not due to a defect in vitamin B6 metabolizing enzymes, but rather can be partly attributed to the elevated alkaline phosphatase activity in the plasma (Anderson et al., 1980). Because of this interrelationship, if plasma PLP is used as a status measure, it is suggested that alkaline phosphatase activity also be determined

(Wan et al., 1993; Reynolds, 1995). Plasma PLP values reported in the literature range from 27-75 nmol/L for males and 26-93 nmol/L for females (Leklem, 1991). A plasma PLP concentration of >30 nmol/L has been suggested to serve as the concentration considered adequate (Leklem, 1990). Regardless of its shortcomings, plasma PLP is still the most reliable measure of vitamin B6 status.

To provide a more comprehensive picture of status, erythrocyte PLP has been used in conjunction with plasma PLP. Plasma PLP gives an extracellular measure of status whereas erythrocyte PLP gives an "intracellular measure." It must be kept in mind, however, that mature erythrocytes are not a true cell since they contain no nucleus. Because most of the PLP dependent enzymes act intracellulary, it is thought that erythrocyte PLP would be a useful measure of status (Reynolds, 1995). Furthermore, both *in vitro* and *in vivo* studies have found human erythrocytes to be responsive to alterations in PN levels. In vitro, the uptake of PN into the erythrocyte is very rapid. A study by Anderson et al. (1979) found that when increasing amounts of PN were added to whole blood (0.15 to 6 umol/L) 55-40% of the dose, respectively, entered the erythrocyte within 1 minute. A comparable effect has been seen in humans. Anderson, et al. (1971) found that when human subjects were given a 40 mg oral dose of PN, 6% entered the erythrocyte within 20 minutes. Similar findings were found by Reynolds et al. (1995) who observed that oral ingestion of 100 mg PN resulted in a rapid increase in erythrocyte PLP. A peak concentration of 4000 nmol/L was reached in 40 minutes (pre-supplementation erythrocyte PLP concentrations = 100 nmol/L). Concentrations decreased over the next 4 hours. A greater percentage of PN enters the red cell if given intravenously. For example, Anderson et al. (1989) found 23% of a 48.6 umol

intravenous dose of PN entered the erythrocyte within 1 minute. This percentage dropped off as the dose increased – approximately 12.5% of the PN was taken up by the erythrocyte when given at a dose of 118 umol/L. The usefulness of erythrocyte PLP as a status indicator is still unclear. A recent study by Hansen et al. (1996b) looked at changes in vitamin B6 status in nine women fed different intakes of protein. Erythrocyte transaminase activity, urinary 4-PA and plasma PLP were used as status indicators. The study results suggested that the erythrocyte did not respond rapidly to changes in vitamin B6 intake. Therefore, the erythrocyte was not considered to be as good an indicator of status as plasma PLP. Another benefit of erythrocyte PLP is that it may not be influenced by as many factors as is plasma PLP. For example, disease states that cause alterations in plasma PLP via changes in alkaline phosphatase activity may have a less dramatic effect on erythrocyte PLP concentrations when compared to plasma PLP. Such conditions include hypophosphatasia (Coburn and Whyte, 1988), aging (Kant et al., 1988), and pregnancy (Barnard et al., 1987). Alkaline phosphatase is anchored to the outer membrane of the cell. Although the erythrocyte does contain a phosphatase, to date, researchers have not detected alkaline phosphatase activity inside the cell (Coburn and Whyte, 1988). As a result, the intracellular PLP concentration may be independent of the extracellular alkaline phosphatase activity. Until more research is done in this area, no definite conclusions can be drawn. Measurement of erythrocyte PLP is not without problems however. For example, using the radioenzymatic assay, recovery of PLP from erythrocytes is not satisfactory ranging anywhere from 50-150% (Reynolds, 1995). In addition, erythrocytes may not accurately represent other body tissues. Furthermore, hemoglobin tightly binds PLP. As a result, when using the existing methodology,

variants in hemoglobin may affect the calculated PLP concentration and lead to a diagnosis of adequacy (tight binding) or deficiency (weak binding). For example, the alteration in the shape of the hemoglobin molecule that occurs with sickle cell anemia results in a tighter binding of PLP (Kark et al., 1982). As a result, individuals with sickle cell anemia have a higher concentration of PLP (Natta and Reynolds, 1984). Lastly, at present, there are no established values with which to evaluate status based on erythrocyte PLP.

Total plasma vitamin B6 is a third direct measure of vitamin B6 status and a concentration >40 nmol/L is considered adequate. Few studies have been done to definitively establish the relationship of total plasma vitamin B6 to status (Leklem, 1990). As a result, there are no accepted guidelines with regards to normal levels for any segment of the population. In addition, an acid hydrolysis step during the assay results in the conversion of PN-glycoside to free PN. After this point, it is difficult to discriminate between the conjugate and the non-conjugated forms (Reynolds, 1995; Andon, 1989). As a result, the assay could give falsely high values for vitamin B6. However, because the PN-glucoside content of the plasma is minimal, the conversion to free PN would most likely have an insignificant impact on results. In addition, total plasma vitamin B6 is influenced by all the factors that affect plasma PLP. Because of these various shortcomings, total plasma vitamin B6 should not be used as the sole status measure.

The final direct measure is urinary 4-PA. Adequate vitamin B6 status is indicated by excretion of >3.0 μmol/day (Leklem, 1990). Like other status measures, urinary 4-PA levels are influenced by protein intake – the higher the dietary protein, the lower the urinary 4-PA excretion. For example, men who consumed a diet containing 1.6 mg

vitamin B6 and 0.5 g protein/kg excreted approximately 46% of the vitamin B6 ingested as urinary 4-PA. When protein intake was increased to 2.0 g protein/kg, the amount of vitamin B6 excreted as urinary 4-PA dropped to 29% (Miller et al., 1985). The relationship between urinary 4-PA and protein is further supported by Hansen et al. (1996) who looked at the effect of three different intakes of protein on urinary 4-PA excretion in women. As the level of dietary protein increased, urinary excretion of 4-PA decreased. Urinary 4-PA is only a short term indicator of vitamin B6 status reflecting recent intake and not tissue reserves.

Indirect measures used to assess vitamin B6 status include amino acid load tests and determination of erythrocyte transaminase activity. Rather than determining blood concentrations, indirect methods measure whether dietary intake of a specific nutrient is adequate enough to meet metabolic needs. Until tryptophan sales were banned in the United States, the tryptophan load test was one of the most common methods with which to assess status. A tryptophan load test involves ingestion of approximately 2-5 grams of tryptophan followed by the measurement of urinary metabolites. With a vitamin B6 deficiency, there are elevated concentration of urinary 3-hydroxykynurenine, kynurenine and xanthurenic acid (Greenberg et al., 1949). Following a 2 gram tryptophan load, average urinary excretion of xanthurenic acid is 30-40 μmol/day. Concentrations of xanthurenic acid >65 μmol/day suggest vitamin B6 deficiency (Leklem, 1990). Ingestion of 1.25 to 1.50 mg vitamin B6 corrects the abnormal tryptophan metabolism. The methionine load test has also been used to assess vitamin B6 status. Following ingestion of a large dose of methionine urinary excretion of cystathionine is measured. Conditions that can influence methionine metabolism include pregnancy, lactation and myocardial

infarction (Reynolds, 1995; Clarke et al., 1991; Stampfer et al., 1992). Concentrations of cystathionine above 350 μmol/day are indicative of deficiency (Leklem, 1990). A final status measure is determination of erythrocyte alanine (EALT) and aspartic acid (EAST) transaminase activity and percent stimulation. Determination of transaminase activity is considered to be a long term measure of vitamin B6 status. Activity levels prior to incubation and percent stimulation following incubation with PLP are used to diagnosed deficiency. Activity levels less than 1.25 and 1.80 for EALT and EAST, respectively, are considered adequate (Leklem, 1990).

Bioavailability

Vitamin B6 nutriture is greatly influenced by bioavailability - the proportion of dietary B6 vitamers that are absorbed and utilized by the body. The first indication of reduced bioavailability of vitamin B6 in foods came from rat growth studies conducted by Sarma and colleagues (1947). They found that growth rates of rats varied depending upon what type of plant food formed the basis of the diet. Similarly, reduced bioavailability of vitamin B6 was demonstrated in humans by Harding et al. (1959) who found that men receiving canned combat rations (1.9 mg of vitamin B6/day) displayed abnormal tryptophan metabolism indicative of a vitamin B6 deficiency. Continued research found that several factors can influence vitamin B6 bioavailability, most notably the amount of pyridoxine glucoside, fiber content and reaction products formed from either PN or PL during processing.

The bioavailability of vitamin B6 from plant products can be influenced by the content of pyridoxine 5'-glucoside. The glucoside content of plant foods ranges

anywhere from 5-80% of the total vitamin B6 (see Table 5) (Kabir et al., 1983b; Gregory and Ink, 1987; Hardin et al., 1986). The amount of PN-glucoside present in foods is a concern because it has been shown to have less vitamin B6 activity when compared to PL, PM, PN or their respective phosphorylated forms. For example, PN glucoside is approximately 58% bioavailable relative to PN (Gregory, 1991). The reduced bioavailability of plant products was demonstrated by a study that looked at vitamin B6 absorption from orange juice. Nelson et al. (1976) found that vitamin B6 present in orange juice was incompletely absorbed; it was determined that approximately 50% of the vitamin B6 was in the glucoside form. The effect of PN-glucoside was further confirmed by Kabir and colleagues (1983c) who compared the vitamin B6 bioavailability from whole wheat and peanut butter to tuna. Subjects were fed 1.6 mg vitamin B6/day with 50% of this amount coming from whole wheat, peanut butter or tuna. Based on urinary, fecal and blood data and assuming bioavailability of tuna to be 100%, researchers determined bioavailability of wheat bread to be 75% and peanut butter 63%. Linear regression analysis of bioavailability and glucoside content found a significant relationship between the two factors. More recent research supports these findings. Hansen et al. (1996a) compared the effect of a high glucoside diet (1.52 mg/day) to a low glucoside diet (1.44 mg/day) on vitamin B6 status. It was found that the higher the content of glucoside in the diet, the higher the excretion of the unmetabolized glucoside in the urine and the lower the vitamin B6 status of the subjects (determined via erythrocyte and plasma PLP concentrations and erythrocyte transaminase stimulation). A study by Gilbert and Gregory (1992) found that PN-glucoside, in addition to having decreased bioavailability, may also impair utilization of non-glycosylated

Table 5. Vitamin B6 and glycosylated vitamin B6 levels in selected foods.

Food mg/100g	Vitamin B6 mg/100g	Glycosylated Vitamin B6
Vegetables		
Carrots, canned	0.064	0.055
Cauliflower, frozen	0.084	0.069
Broccoli, frozen	0.119	0.078
Spinach, frozen	0.208	0.104
Cabbage, raw	0.140	0.065
Sprouts, alfalfa	0.250	0.150
Potatoes, cooked	0.394	0.165
Potatoes, dried	0.884	0.286
Beets, canned	0.018	0.005
Yams, canned	0.067	0.007
Beans/Legumes		
Soybeans, cooked	0.627	0.357
Beans, navy, cooked	0.381	0.159
Beans, lima, frozen	0.106	0.039
Peas, frozen	0.122	0.018
Peanut Butter	0.302	0.054
Beans, garbanzo	0.653	0.111
Lentils	0.289	0.134
Animal Products		
Beef, ground, cooked	0.263	NV
Tuna, canned	0.316	NV
Chicken breast, raw	0.700	NV
Milk, skim	0.005	NV
Nuts/Seeds		
Walnuts	0.535	0.038
Filberts	0.587	0.026
Cashews, raw	0.351	0.046
Sunflower seeds	0.997	0.355
Almonds	0.086	0.000

Table 5. Continued

Food mg/100g	Vitamin B6 mg/100g	Glycosylated Vitamin B6
Fruits		
Orange juice, frozen concentrate	0.165	0.078
Orange juice, fresh	0.043	0.016
Tomato juice, fresh	0.097	0.045
Blueberries, frozen	0.046	0.019
Banana	0.313	0.010
Banana, dried chips	0.271	0.024
Pineapple, canned	0.079	0.017
Peaches, canned	0.009	0.002
Apricots, dried	0.206	0.015
Raisins, seedless	0.230	0.154
Cereal/Grains		
Wheat bran	0.903	0.326
Shredded wheat, cereal	0.313	0.087
Rice, brown	0.237	0.055
Rice, bran	3.515	0.153
Rice, white	0.076	0.015
Rice cereal, puffed	0.098	0.007
Rice cereal, fortified	3.635	0.382

NV = no value established; limited data available
Taken from: Leklem, 1989 (unpublished)

forms. They looked at the effect of PN-glucoside on the metabolic utilization of PN in rats. Following a dose of either 0, 36 or 72 nmol glucoside and 240 nmol ^{14}C PN, rats were killed and the distribution of vitamin B6 metabolites in the liver and urine were quantitated. Urinary and hepatic PNP and PLP were directly related to the glucoside dose; ^{14}C labeled PL, PN, and PM in the liver and the concentration of urinary 4-PA were inversely proportional to the glucoside dose. It was concluded that the glucoside quantitatively alters metabolism and *in vivo* retention of PN and the glucoside may retard utilization of the nonglycosylated form.

It has also been suggested that fiber may reduce vitamin B6 bioavailibilty. When studying the effects of fiber, complications arise because high fiber foods also contain significant amounts of PN-glucoside. A study by Leklem et al. (1980) estimated the vitamin B6 in wheat bread to be 5-10% less bioavailable than the vitamin B6 found in animal products. The reduced bioavailibilty could not be attributed solely to the fiber content of the wheat bread. Rather, researchers felt it was a combination of the fiber and glucoside. Similar results were found in 1983 by Lindberg et al. (1983) who looked at the effect of cooked wheat bran on the bioavailability of vitamin B6 in 10 men. Subjects were fed a constant diet with bran (1.69 mg B6) or without bran (1.66 mg B6). Plasma PLP, plasma total vitamin B6, urinary 4-PA, urinary B6, and fecal B6 were determined. The bran diet reduced vitamin B6 bioavailability by 17%. This was evidenced by a significant increase in fecal B6 and decreased amounts of urinary 4-PA, plasma PLP and total plasma vitamin B6. As long as vitamin B6 intake was adequate, researchers felt that the decrease in vitamin B6 bioavailability caused by the addition of bran would not pose a serious problem for vitamin B6 nutriture. Processing and storage have been shown to

decrease the content of vitamin B6 either by destroying the vitamin or by changing the form. For example, heating can lead to the formation of a bond between PL and the amino acid lysine. This bond cannot be hydrolyzed by digestive enzymes and therefore the vitamin B6 is 25-30% less biologically available (Ink et al., 1986). If all of the possible influences on vitamin B6 bioavailability are taken into account, the bioavailability of foods that make up the average American diet is estimated to be between 71-79% (Tarr et al., 1981).

Interorgan Metabolism

Based on animal data, absorption of PL, PM and PN occurs in the proximal jejunum via a nonsaturable process (Booth and Brain, 1962; Middleton, 1979; Mehansho et al., 1979; Hamm et al., 1979). Additional research on vitamin B6 absorption suggests that a saturable component may exist (Henderson, 1985). Absorption of the 5'-phosphates is minimal and they are instead converted to the non-phosphorylated form through the action of alkaline phosphatase. Absorption of the non-phosphorylated forms is rapid with approximately 40%, 23%, and 18% of PL, PN, and PM, respectively, being absorbed within 10 minutes (Ink and Henderson, 1984). Following absorption, the vitamers are transported to the liver where they are taken up by passive diffusion followed by metabolic trapping (Mehansho, 1980). Uptake of vitamin B6 into the liver is fairly rapid. For example, Snell and Haskell (1971) demonstrated in rats that, within one hour, 80% of an oral PN-HCl supplement was in the liver.

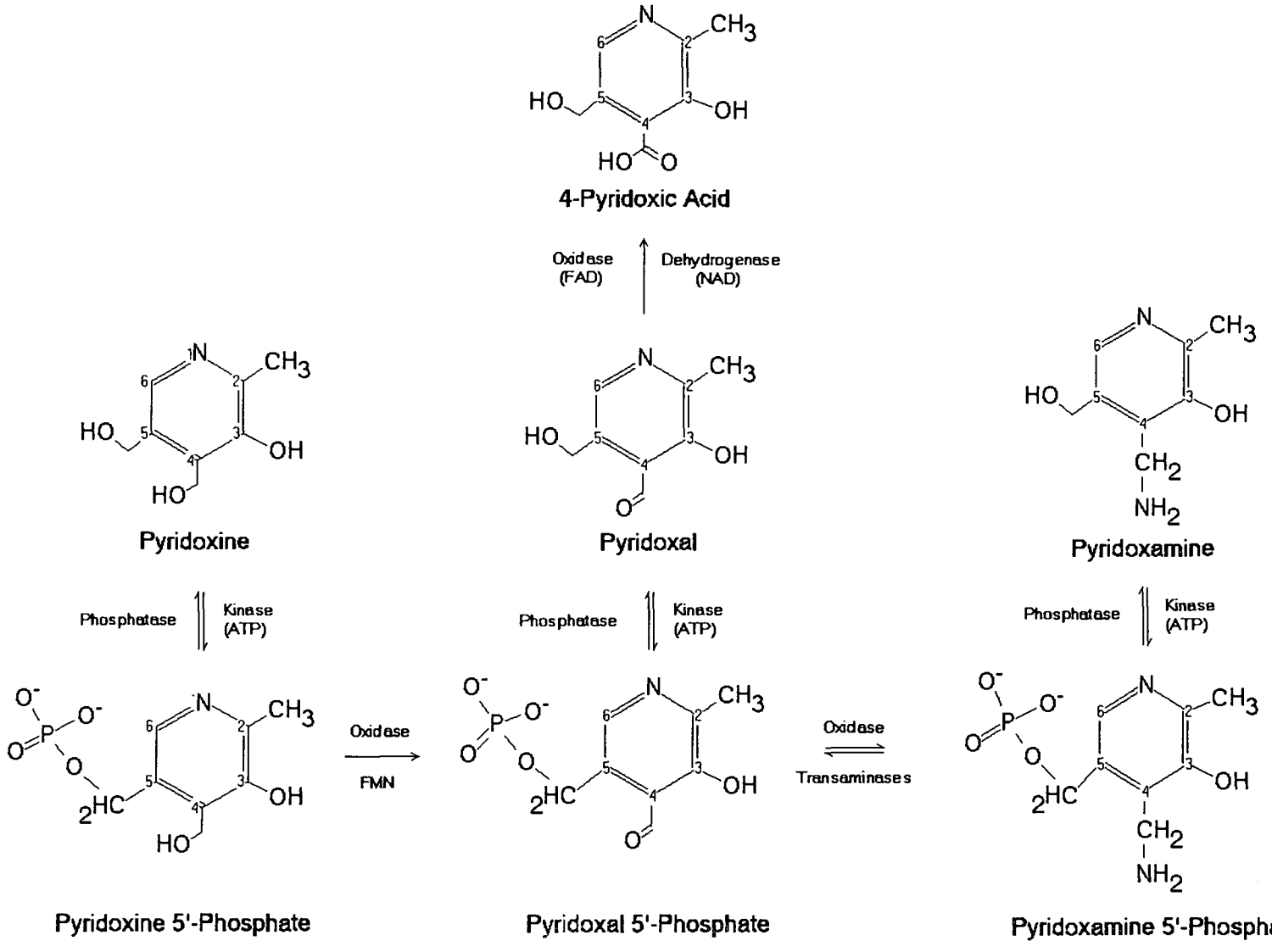
The liver is the primary organ involved in vitamin B6 metabolism with the end result being release of plasma PLP into the bloodstream. Pyridoxal 5'-phosphate synthesis

occurs via the pathway shown in Figure 2. In the liver, pyridoxal kinase catalyzes the conversion of PL, PM and PN to their respective 5'-phosphates, PLP, PMP and PNP (McCormick et al., 1961). This step is a reversible reaction and the phosphorylated compounds can be dephosphorylated by the action of phosphatase. Because of this reversibility the conversion of the free vitamers to the phosphorylated compounds represents a key site of regulation (Li et al., 1974). Plasma pyridoxal 5'-phosphate and PL are the most important forms of vitamin B6 released into the plasma and are converted to PLP via the flavin dependent enzyme, pyridoxine phosphate oxidase (Wada and Snell, 1961; Pogell, 1958; Morisue, 1960). Regulation of the oxidase occurs through product inhibition. As a result, high PLP concentrations will inhibit the enzyme and flux through this pathway will decrease (Merrill, 1978). The major metabolic end product of vitamin B6, 4-PA, is formed from PL via a NAD dependent dehydrogenase or a FAD dependent aldehyde oxidase (Stanulovic, 1976; Kjelgaard, 1951). Formation of urinary 4-PA is the main route for vitamin B6 elimination with approximately 40-60% of an adequate intake being excreted in this form (Wozenski et al., 1980, Leklem, 1988). At high doses of vitamin B6, the amount of vitamin B6 that is excreted as urinary 4-PA decreases as intake increases. For example, with intakes near 100 mg, much of the vitamin B6 is excreted unchanged (Rabinowitz and Snell, 1949). Once PLP is released from the liver, it circulates in the plasma bound to albumin. Albumin acts to protect PLP from hydrolysis as well as deliver it to the tissues (Dempsey and Christensen, 1962). Because the phosphorylated form is unable to cross the plasma membrane, tissue uptake involves cleavage of the phosphate group and subsequent uptake of PL into the cell. The conversion rate of PLP to PL is thought to be dependent on alkaline phosphatase activity,

a deficiency of which has the potential to impair vitamin B6 status (Middleton, 1977; Middleton, 1978). Once in the cell, PL is rephosphorylated to PLP. Pyridoxal 5'-phosphate is the major form of the vitamin found in the plasma, comprising 60-70% of the total (Shultz and Leklem, 1981; Leklem, 1990). Pyridoxal is next highest in concentration followed by PN and PM. Pyridoxamine 5'-phosphate and PNP are almost never detected in the plasma (Lumeng et al., 1985).

Pyridoxine and PL are also taken up by simple diffusion into the erythrocytes Mehansho and Henderson, 1980). Therefore, the erythrocytes are thought to be important in the transport and metabolism of vitamin B6. Through the action of the PN kinase and an FMN dependent oxidase, PN is converted first to PNP, then to PLP and finally to PL (Anderson, 1989). The PL can then be released into the plasma. The initial uptake of PN and PL into the erythrocyte is rapid but then slows as PN and PL are converted to PLP. Only when the concentration of the non-phosphorylated forms is reduced by conversion to the phosphorylated forms can more PN or PL be taken up (Anderson, 1980). In the erythrocyte, PLP is tightly bound to the beta chain of the hemoglobin molecule. The binding of PLP has been shown to lower the oxygen binding affinity of the hemoglobin molecule (Kark et al., 1982, Maeda et al., 1976). Pyridoxal is tightly bound to the alpha chain. In contrast to the effect of PLP, the binding of PL has been shown to increase the oxygen binding affinity of the hemoglobin molecule (Benesch, 1973; Ink et al., 1982). As a result of this tight binding, erythrocyte concentrations of PL have been found to be up to five times greater than the PL concentrations detected in the plasma (Friedrich, 1988). The binding affinity may be a limiting factor of how much erythrocyte PL is

Figure 2. Metabolic interconversion of the B6 vitamers.



available to contribute to PLP synthesis and therefore how much PL is available to the rest of the body (Benesch, 1973).

In addition to the plasma and erythrocyte, the muscle plays an important role in vitamin B6 metabolism. Studies have shown that the majority of the body's reserve of vitamin B6 is located in the muscle - approximately 80% of the total pool of 1 mmol (Coburn et al., 1988). The majority of pyridoxal 5'-phosphate found in the muscle is bound to the enzyme glycogen phosphorylase. The binding of PLP to glycogen phosphorylase also acts as a storage mechanism and protects PLP from hydrolysis (Fonda and Harker, 1982). Pyridoxal is the main source of PLP to this tissue. There is some evidence indicating that the muscle acts as storage for vitamin B6. For example, supplementation of vitamin B6 (160 mg for 6 weeks) has been shown to increase total vitamin B6 in the muscle by 25% (Coburn et al., 1991). However, research indicates that very little of this reserve is available to tissues except through tissue breakdown. For example, research has found that there is considerable release of PLP from the muscle following prolonged food deprivation (Black, 1977; Guirard and Snell, 1978).

Alkaline Phosphatase

Plasma PLP can be influenced by several factors. Although dietary factors such as vitamin B6 and protein intake receive the most attention, several studies have shown that hydrolysis of PLP to PL by alkaline phosphatase (AP) plays a crucial role in the regulation of tissue and blood levels. Circulating plasma PLP is hydrolyzed by AP to PL (Whyte et al., 1988). Pyridoxal is then taken up into the tissues. The activity of AP is important since a high activity can decrease plasma PLP levels. Conversely, low AP

activity is associated with an increase plasma PLP concentration, possibly making less PL available to the tissues and PLP dependent enzymes (Whyte, 1989; Whyte, 1994).

Alkaline phosphatases, discovered in 1911, are glycoproteins that hydrolyze phosphate monoesters. Phosphatases are anchored to the cell membrane lipid bilayers by a phosphatidinositol-glycan moiety (McComb et al., 1979). This moiety is attached to the carboxyl terminus of the protein. Alkaline phosphatases are expressed in virtually all tissues but are individually most abundant in hepatic, skeletal and renal tissue. These liver/bone/kidney isoenzymes are collectively known as tissue nonspecific alkaline phosphatase (TNSAP). Three compounds have been identified as natural substrates for TNSAP - phosphatidylinositol, inorganic phosphate and PLP (McComb et al., 1979; Whyte et al., 1985).

Insight into the physiological function of TNSAP comes from studies of individuals with hypophosphatasia, a heritable metabolic bone disease that is characterized by low levels of TNSAP and elevated levels of plasma PLP (Whyte, 1989; Whyte, 1994). The discovery that hypophosphatasia resulted in increased plasma PLP was made by Whyte et al. in 1985. For example, in 14 patients (age 1.5-45 years) diagnosed with hypophosphatasia, plasma PLP concentrations ranged from 214 to 3839 nmol/L, with an average concentration of 1174 nmol/L. In comparison, the average plasma PLP concentration of the control group was 57 ± 26 nmol/L. Results of numerous studies on hypophosphatasia and vitamin B6, led to the conclusion that the plasma PLP was elevated because of lack of PLP hydrolysis and not from increased synthesis. Further studies have helped to conclusively identify the role of AP in PLP metabolism (McComb, 1979; Whyte, 1989; Harris, 1990; Moss, 1992). Support for the AP-PLP link also comes

from research on individuals with elevated activities of TNSAP. For example, Merrill et al. (1986) found patients with cirrhotic liver to have increased AP activity and decreased plasma PLP. Anderson et al. (1989) demonstrated that plasma PLP is lowest in patients having the highest activities of AP.

Several other factors can influence AP activity, for example, depressed plasma zinc concentrations. Alkaline phosphatase is a zinc metalloenzyme. Therefore, low zinc can cause a decrease in AP activity and result in elevated plasma PLP. A study by Wan et al. (1993) found that rats made zinc deficient during gestation and lactation experienced a 48% decrease in serum AP activity and a 68% increase in plasma PLP. High levels of glucocorticoids have also been shown to reduce AP activity. Nielsen and colleagues (1988) found a 6 % decrease in AP activity in patients taking 40 mg/day of the synthetic glucocorticoid, prednisone. Some evidence also indicates that body weight may influence AP activity. Menahan et al. (1985) compared alkaline phosphatase activity in obese mice and normal controls. The alkaline phosphatase activity of the obese animals was three times higher than that of the lean controls (257 ± 16 versus 80 ± 8 IU). This dramatic difference in alkaline phosphatase activity was attributed to an increased proportion of hepatic alkaline phosphatase isozyme in the plasma of the obese mice compared to the controls. Similar findings have been found in humans. Golik et al., (1991) who looked at the AP activity in 60 obese people (BMI 36.8 ± 2.8 versus 34.2 ± 2.6), found that 28% of the persons had elevated AP activity. Many values were near 119% of the upper limit of normal. A similar situation occurs in diabetes. In a group of hospitalized diabetic patients (n=72, mean body weight: 66 kg), elevated alkaline phosphatase activity was found in 17% of the individuals; 11% of the individuals in a group of untreated diabetic

patients (n=185, mean body weight: 72 kg) were found to have elevated alkaline phosphatase activity. The elevated activity was thought to represent an intrinsic feature of the condition which was unrelated to the treatment, duration, or complications among established diabetics (Goldberg et al., 1977). The possible influence of body weight was not addressed. Lastly, season can influence AP with a higher activity seen during the summer than in the winter (Devgun et al., 1981).

Functions

Vitamin B6, as PLP, is involved in virtually every major metabolic pathway. Pyridoxal 5'-phosphate acts as a cofactor for over one hundred enzymes (Sauberlich, 1985) that participate in carbohydrate, lipid and protein metabolism (see Figure 3). The role in amino acid metabolism is what PLP is most noted for. A brief summary of some of PLP's functions - gluconeogenesis, glycogenolysis, red blood cell function, niacin formation, lipid metabolism, immune function and steroid modulation - follows.

Gluconeogenesis

Gluconeogenesis is the process by which the liver converts non-carbohydrate substances (e.g. lactate, amino acids, glycerol) to glucose. Vitamin B6, as PLP, is essential for both transamination and deamination reactions that occur during gluconeogenesis. For example, the enzyme alanine aminotransferase requires PLP to transaminate pyruvate to alanine. Angel (1980) showed that, in rats, a vitamin B6 deficiency resulted in decreased activity of this aminotransferase. In humans however, evidence indicates that a vitamin B6 deficiency results in an insignificant change in

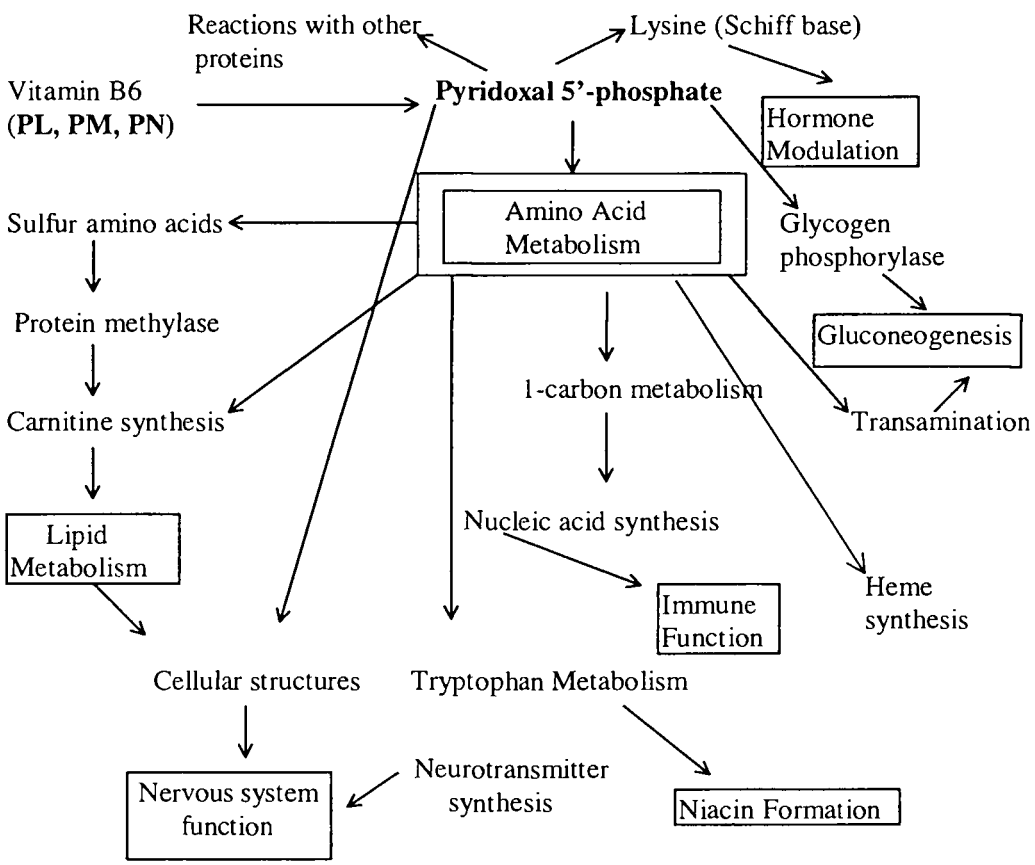
enzyme activity. This was demonstrated by Rose (1975). Women who were fed 0.19 mg B6 for 4 weeks showed no significant change in plasma glucose, a measure indicative of gluconeogenesis. The alanine that is formed in the peripheral tissues is then exported to the liver and serves as a substrate for gluconeogenesis.

Pyridoxal 5'-phosphate is also needed for glycogenolysis, the breakdown of liver and muscle glycogen to provide glucose. Pyridoxal 5'-phosphate serves as a cofactor for glycogen phosphorylase (Cori and Illingsworth, 1952). This enzyme cleaves off and phosphorylates one glucose unit from the glycogen chain. The resulting glucose 1-phosphate is available as energy. The majority of vitamin B6 in the body is associated with this enzyme (Chastiotis et al., 1982).

Erythrocyte function

Vitamin B6 is required for transamination reactions in the red blood cell with PLP serving as a cofactor for erythrocyte aspartate aminotransferase (EAST) and erythrocyte alanine aminotransferase (EALT). Both enzymes catalyze removal of the amino group from an amino acid and subsequent transfer to an alpha keto acid with formation of the corresponding amino acid and alpha keto derivative. For example, EAST transfers the amino group from glutamic acid to oxaloacetate forming alpha ketogluatarate and aspartate. The action of EALT is the same except that the amino group from glutamic acid is transferred to pyruvate forming alanine and alphaketoglutarate. Transamination reactions are an important mechanism for amino acid synthesis or degradation. Furthermore, transamination aids in the transport of amino acids and helps to maintain amino acid homeostasis. In addition to the role it plays in transamination, PLP is needed

Figure 3. Functions of pyridoxal 5'-phosphate.



Adapted from Leklem, 1993

for heme synthesis by serving as a cofactor for delta-aminolevuline acid synthetase (Kikuchi et al., 1978). This enzyme catalyzes the condensation reaction between glycine and succinyl CoA. This reaction is crucial since glycine and succinyl CoA provide all of the carbon and nitrogen used in heme biosynthesis.

Niacin Formation

Pyridoxal 5'-phosphate functions in four different steps as a coenzyme in the pathway that converts tryptophan to niacin. However, there is only one direct PLP requiring step. Kynureninase, the enzyme that catalyzes the conversion of 3-hydroxykynurenine to 3-hydroxyanthranilic acid has an ultimate requirement for PLP and a vitamin B6 deficiency has been proven to result in decreased activity of kynureninase (Brown, 1985). As a result, there is a decrease in the amount of niacin that is formed from tryptophan. This effect was demonstrated by Leklem and colleagues (1975). Researchers fed subjects 0.19 mg vitamin B6/day for four weeks and then measured the excretion of niacin metabolites following a tryptophan load test. Excretion of N'-methylnicotinamide and N'-methyl-2-pyridone-5-carboxamide, the two niacin metabolites of interest, were found to be half of what would have been if subjects were fed adequate amounts of vitamin B6.

Lipid Metabolism

The role of vitamin B6 in lipid metabolism is still being unraveled. Animal studies have yielded mixed results. For example, Greenberg (1964) found monkeys fed vitamin B6 deficient diets developed atherosclerotic lesions and fatty liver. Rat studies,

however, have yielded mixed results. Some researchers have found inadequate vitamin B6 concentrations to elevate triglyceride synthesis (Sabo et al., 1971), some have shown it to decrease synthesis (Angel and Song, 1973) and yet others have shown it to not change triglyceride synthesis when compared to normal controls (Angel, 1975; Desikachar and McHenry, 1954). Other animal studies have found a decreased conversion of linoleic acid to arachadonic acid (Witten and Holman, 1952; Cunnane et al., 1984). Human studies have done little to solidify the vitamin B6-lipid relationship. One study by Mueller and Iacono (1963) found slight, but insignificant changes in fatty acid levels in both the plasma and red blood cell. This finding is supported by recent work done at Oregon State University. Researchers found vitamin B6 deficient females, as defined by plasma PLP and total plasma vitamin B6 concentrations below the recommended level, to show no change in fatty acid profiles or lipid levels when compared to normal controls (Kim et al, 1997).

The major function of vitamin B6 in lipid metabolism may prove to be its role in carnitine synthesis. Carnitine, a compound synthesized from the amino acids methionine and lysine, is required to deliver fatty acyl units to the mitochondria for beta-oxidation. Rats made vitamin B6 deficient had lower levels of carnitine in the plasma, liver, skeletal, muscle, heart and urine. Vitamin B6 repletion returned carnitine levels to normal (Cho and Leklem, 1990). Further research is required to solidify the relationship between vitamin B6 and lipid metabolism.

Immune Function

Evidence indicates that vitamin B6 is important for immune functioning. Animal studies have found that low plasma vitamin B6 concentrations is correlated with both decreased lymphocyte production and decreased antibody response to antigens (van den Berg et al., 1988; Chandra and Puri, 1985). In humans, PLP is needed for DNA synthesis by serving as a cofactor for serine transhydroxymethylase. This enzyme is needed for 1 C transfers which are important for maintenance of the immune system (Axelrod and Traktellis, 1964). Talbott et al. (1987) found an enhanced immune response following administration of 50 mg PN-HCl/day for 2 months. These results were supported by Meydani et al. (1991) who looked at the relationship between vitamin B6 status and the immune system in elderly females. Low vitamin B6 status, as evidenced by reduced plasma PLP concentrations, was associated with decreased *in vitro* indices of cell mediated immunity.

Hormone Modulation

A final area where vitamin B6 appears to be important is in the action of steroid hormones. Pyridoxal 5'-phosphate has been shown, *in vitro*, to extract steroid hormone receptor complexes from tight nuclear binding and inhibit the interactions of hormone receptor complexes with DNA and nucleoproteins (Cidlowski and Thanassi, 1981). These influences indicate that PLP may depress or terminate the action of steroid hormones. Animal studies which have looked at the effect of a vitamin B6 deficiency on hormone action have found that there is an increased and prolonged nuclear accumulation

and retention of steroid hormones (Symes et al., 1988; Bender et al., 1988). In addition, there is an enhanced biological responsiveness and sensitivity of target tissues to steroid hormone action (Bowden et al., 1986; Bender, 1987).

NEUROBIOLGOY OF VITAMIN B6

Overview

Although vitamin B6 was discovered in the mid 1930's, only recently has its importance to human nutrition been acknowledged. Over one-half of the reactions catalyzed by PLP involve transformation of amino acids. As stated previously, PLP is required for transamination, racemization and decarboxylation.

The crucial role of vitamin B6 in the nervous system is evident from the fact that the neurotransmitters, dopamine, norepinephrine, serotonin, and gamma aminobutyric acid (GABA) are synthesized by PLP dependent enzymes. Critical brain metabolites including taurine, sphingolipids and polyamines also require PLP for synthesis (Dakshinamurti et al., 1990). Because of the variation in the affinities of PLP dependent enzymes, vitamin B6 deficiency can result in decreased synthesis in some of the neurotransmitters. The two enzymes most affected by a vitamin B6 deficiency are 5-hydroxytryptophan decarboxylase, involved in serotonin synthesis and glutamic acid decarboxylase involved in GABA (Roberts and Frankel, 1950). Inadequate vitamin B6 intake appears to have little or no effect on dopamine or norepinephrine concentrations in the brain and spinal fluid of rats (Dakshinamurti, 1985).

Some of the earliest evidence indicating the importance of vitamin B6 in the peripheral (PNS) and central nervous system (CNS) came from animal experiments that

identified that a vitamin B6 deficiency in rats would induce seizures. For example, Dakshinamurti and Stephens (1969) induced a dietary deficiency of vitamin B6 in dams during the last week of gestation by feeding rats a diet devoid of vitamin B6. Biochemical assessment showed that the rat pups had a congenital vitamin B6 deficiency. In addition, they exhibited spontaneous convulsions which were characterized by high pitched screams followed by generalized convulsion. Analysis of brain tissue showed depressed levels of both PLP and glutamic acid decarboxylase. A short time later, Stephens et al. (1971) induced a vitamin B6 deficiency in dams during lactation. Uni- and bipolar EEG recordings were taken. Controls displayed slow wave EEG activity. In contrast, vitamin B6 deficient rats had abnormal EEG patterns evidenced by spike activity. In addition, evoked potentials presented abnormalities in latency, wave form and response to repetitive stimuli. Pyridoxal 5'-phosphate and GABA concentrations in the cerebellum were significantly lower than the controls. Vitamin B6 deficiency has the same effects in humans. For example, research by Snyderman (1950) found that infants fed a diet containing inadequate amounts of vitamin B6 displayed abnormal nervous system activity and seizures. Similar problems occurred a few years later when numerous infants were accidentally fed a commercial formula devoid of vitamin B6 (Coursin, 1954). All infants displayed abnormal CNS activity including hyperirritability and convulsive seizures. During this same time period, Hunt et al. (1954) reported data on an infant with seizures that could be controlled only with administration of vitamin B6 supplements. Within minutes of taking the supplement, electroencephalograms dramatically improved. As a result, this condition became as

"pyridoxine dependency." In both the animal and human experiments, administration of PN stopped the seizures and CNS activity returned to normal.

Serotonin

One neurotransmitter vitamin B6 is thought to influence is serotonin. Serotonin is a neurotransmitter secreted by the nuclei in the brain stem. In addition to influencing mood and sleep, several studies suggest that brain and spinal cord serotonin play a crucial role in nociception (Carstens et al., 1981; Willer et al., 1984; Dakshinamurti et al., 1990). Serotonin is formed from the amino acid tryptophan. Tryptophan is first hydroxylated by tryptophan hydroxylase to 5-hydroxytryptophan followed by decarboxylation to 5-hydroxytryptamine, also known as serotonin. Decarboxylation is catalyzed by the PLP dependent enzyme, 5-hydroxydecarboxylase. Therefore, a vitamin B6 deficiency, evidenced by lower plasma PLP concentrations, could impair serotonin synthesis and increase nociception.

The link between serotonin and pain was first demonstrated by Tenen (1967). In his experiments, animals pre-treated with *p*-chlorophenylalanine, a compound that depresses seretonin levels by inhibiting tryptophan hydroxylase, had decreased jump response thresholds to presentations of electric shock. The negative effects of *p*-chlorophenylalanine were reversed with 5-hydroxytryptophan injections. The results of Tenen's study were supported by Fibiger et al. (1972). Following administration of *p*-chlorphenylalanine, rats were given different intensities of shock on opposite sides of the cage. Shock sensitivity and reactivity in a spatial preference task were analyzed. Upon conclusion of the study, rats given p-chlorophenylalanine were found to be more

reactive to electric shock (based on the number of times animals crossed the cage during shocks presentations); the animals showed no difference in their shock detection threshold however (the amount of time the rat spends on either the electrified or non-electrified side of the cage. The reactivity of the animals to electric shock disappeared when given 5-hydroxytryptophan. Similar results have been found in humans when given *p*-chlorophenylalanine for migraine headaches. Subjects complained of skin and muscular algesias and experienced pain when they spoke or made facial expressions. These symptoms ceased within 5-30 days of discontinuation of the drug, the amount of time it takes the body to completely metabolize *p*-chlorophenylalanine.

Support for serotonin's role in pain also comes from studies which have looked at the effect of compounds that block reuptake of serotonin. For example, rats given fluoxetine, a compound used to inhibit serotonin reuptake, and then presented with different intensities of electric shock demonstrated analgesia and increased pain thresholds when compared to controls. Fluoxetine also minimized the hyperalgesia associated with *p*-chlorophenylalanine (Messing, 1975). Similar results were obtained when a group of rats were given quipazine, a seretonin receptor agonist. Rats given the agonist had a higher tolerance for tail compression when compared to normal controls. The algesia was antagonized with methergoline, a serotonin receptor blocker (Samanin, 1990)

Dietary factors, such as vitamin B6 may also influence nociception by influencing seretonin synthesis. Much of the evidence supporting the vitamin B6-pain-seretonin relationship comes from animal data. Only a few studies have looked at the affect of a vitamin B6 deficiency on serotonin concentrations. However, all have found that a low

plasma PLP concentration corresponds with a decrease in 5-hydroxydecarboxylase activity. Consequently, seretonin levels in the brain and spinal fluid are depressed. This relationship was demonstrated by Dakshinamurti et al. (1976). They found that vitamin B6 deficiency drastically reduced serotonin concentrations in the brain of rats. Mean serotonin concentration of the deficient rats was 3.34 ± 0.11 nmole/g compared to a concentration of 7.31 + 0.28 nmol/g for controls. A later study done by Dakshinamurti and Paulose (1983) looked at the differences in brain PLP and serotonin concentrations in two groups of rats – one supplemented with vitamin B6 and one devoid of vitamin B6. Vitamin B6 deficient rats were found to have significantly lower brain concentrations of PLP as well as significantly lower concentrations of serotonin in the cerebral cortex. Pyridoxine administration resulted in a significant increase in brain seretonin and PLP concentration.

The importance of vitamin B6 in pain thresholds is supported by studies showing that PN administration successfully increases serotonin concentrations and increases pain thresholds. This effect is demonstrated by a study conducted by Jurna et al. (1990) who looked at the effect of intraperitoneal vitamin B6 administration (189 mg/kg) on impulse discharges in rats. They found that vitamin B6 decreased nociceptive activity evoked by electrical stimulation in neurons of the rat thalamus in a dose dependent manner. The minimum dose that proved effective was 40 mg/kg. Higher doses produced a more pronounced effect of a longer duration. These researchers hypothesized that PLP may induce or enhance neuronal antinociceptive mechanisms in the central nervous system by upregulating seretonin synthesis and/or other inhibitory neurotransmitters. This hypothesis was supported by a study done by Fu et al. (1990) who pretreated rats with a

subcutaneous injection of 33 mg vitamin B6 for 7 days. Following supplementation, researchers found vitamin B6 pretreatment enhanced afferent inhibition of nociceptive neurons in the spinal dorsal horn to noxious skin heating. The enhanced afferent inhibition was thought to be caused by an increase in the synthesis of inhibitory neurotransmitters, especially serotonin. Similar results were found by Bartoszyk and Wild (1990) who demonstrated that, in rats, vitamin B6 showed antinociceptive activity against pain provoked by heat, pressure and chemicals. This analgesic effect was thought to be caused by upregulation of serotonin synthesis following intraperitoneal vitamin B6 supplementation (166-667 mg B6/kg). The affect of vitamin B6 on brain serotonin concentrations, in rats, was confirmed by Dakshinamurti et al. (1990). They analyzed the effect of vitamin B6 supplementation (33.4 mg PN/day) on brain serotonin concentrations compared to control given a saline injection. After 7 days, rats were killed and brain tissue was analyzed for serotonin concentration. Vitamin B6 administration significantly increased serotonin concentrations: 4.25 ± 0.32 nmol/g tissue for supplemented animals compared to 1.97 ± 0.12 nmol/g tissue for controls.

Results from animal data is reinforced by studies done in humans. Pyridoxine administration has been shown to decrease pain associated with diabetic neuropathy, premenstrual syndrome (PMS) and vertebral pain syndrome. For example, a study by Bernstein and Lobitz (1985) found that 150 mg of PN-HCl/day for 6 months decreased pain in subjects with diabetic neuropathy. The data showed that PN did not cure the neuropathy but rather was able to modify the pain threshold. Supplementation with vitamin B6 during PMS has also been shown to be beneficial for some individuals. As early as 1977, Kerr demonstrated that 40-100 mg PN-HCL reduced headache and

tenderness in 56 of 70 women complaining of PMS. These findings were supported by an open double-blind cross over study which found partial or complete recovery from PMS when women (n=25) took an average dose of 500 mg PN-HCl per day. Similarly, Hallman (1987), after supplementing women with 300 mg PN-HCL/day, found a favorable effect of vitamin B6 on 13 symptoms associated with PMS. Symptoms looked at included headache, tension, and tenderness. Vitamin B6 has also been shown to decrease pain in patients with painful vertebral syndrome, especially if used in conjuction with the pain medication, diclofenea. For example, Bruggemann et al. (1990) looked at the affect of vitamin B6 supplementation (300mg/day for a 2 week maximum) in combination with diclofenae compared to diclofenae alone. Pain frequency and intensity was evaluated via a self-administered questionnaire. Data from the 376 patients indicated that the combination of vitamin B6 and diclofenae was more effective then the medication alone. Similar results were obtained by Destito et al. (1987). Forty individuals, all with painful vertebral syndrome were randomly assigned to receive either vitamin B6 (300 mg/day for 10 days) or gangliosides. Pain scores were generated using the subjects responses to a list of questions. Seventeen of the vitamin supplemented patients improved compared to 15 of the subjects treated with gangliosides. Pain scores significantly decreased over the study period and in 12 of the vitamin supplemented subjects, pain disappeared completely by the end of the study. Individuals suffering from lumboischialgia have also been successfully treated with vitamin B6 (Marcolongo and Fioravanti, 1987). Forty individuals were treated orally with 300 mg vitamin B6/day for 10 days. Pain was evaluated at days 3, 6, and 10 by asking subjects to rate the severity of

their symptoms. At the end of the study, the intensity of all symptoms was found to decrease, especially spontaneous pain, night-time pain and provoked pain.

Based on the above studies, it appears that depressed concentrations of brain and spinal cord serotonergic neurotransmission are associated with an increased sensitivity to noxious stimuli. Conversely, increases in serotonergic neurotransmission are related to analgesia. Of the factors that can influence serotonergic neurotransmission, vitamin B6 appears to play a crucial role.

γ–aminobutyric Acid

A second neurotransmitter influenced by vitamin B6 status is gamma aminobutyric acid (GABA). GABA is the major inhibitory neurotransmitter formed via the decarboxylation of the amino acid glutamate. Glutamate decarboxylase, the enzyme that catalyzes the synthesis of GABA, has an absolute requirement for PLP. Research has shown that low PLP concentrations in the brain and spinal fluid result in decreased glutamate decarboxylase activity (Roberts, 1963; Kurlemann, 1992; Dakshinamurti, 1990). Reduced glutamate decarboxylase activity in turn can influence central nervous system excitability through GABA synthesis. For example, low GABA concentrations can result in hyperexcitability leading to epileptic like convulsions (McCormick, 1961). Like seretonin, GABA appears to have a significant influence in the perception of pain. Several studies support the role of GABA in antinociception. A study by Li et al. (1989) looked at the influence of GABA on peripheral excitatory neurons and peripheral inhibitory neurons in the thalamus of rats. Administration of GABA was found to significantly inhibit electrical discharges of the excitatory neurons and increase the

inhibitory neurons. The results suggest that GABA could either antagonize or partly antagonize the excitatory action of noxious stimuli and produce analgesia. GABA's analgesic affect is further supported by a study in rats that looked at the affect of chronic administration of GABA stimulating drugs (Ignatov and Andreev, 1992). Pain was induced by daily peritoneal electrostimulation for 2 weeks. GABA metabolism was altered as evidenced by increased degradation. After the 2 week period, GABA enhancing drugs were administered for 14 days resulting in normalization of GABA metabolism and increased resistance to pain. Studies which have assessed the effect of GABA receptor antagonists also lend credence to GABA's analgesic effect. Many of these studies have found that antagonists such as bicuculline significantly decrease GABA concentrations and increase base line firing of medullary neurons associated with pain (Behbehani et al., 1990). As a result, nociception is elevated. Conversely, analogues of GABA are able to increase antinocicpetion in rats as measured by the hot plate test and tail compression test. The ability to increase antinocicpetion is especially true of sodium valoprate, a compound that significantly increases GABA concentration. A study by Grafova et al. (1994) found administration of sodium valoprate to substantially reduce manifestations of phantom pain syndrome in humans.

As was the case with serotonin, vitamin B6 is required for GABA synthesis. Most of the evidence linking vitamin B6 to GABA synthesis comes from deficiency studies and analysis of GABA metabolism in individuals with PN dependent seizures. Several researchers have shown a vitamin B6 deficiency to impair GABA synthesis. For example, Roberts et al. (1985) found that the glutamic acid decarboxylase activity was approximately three fold lower in rats who were vitamin B6 deficient compared to rats

being supplemented with vitamin B6. Consequently, GABA concentration was also significantly decreased in the brains of vitamin B6 deficient rats. GABA concentrations increased significantly one hour after injection with PN-HCl. Similarly, Bayoumi and Smith (1972) compared glutamic acid decarboxylase activity between vitamin B6 deficient rats and normal controls. Enzyme activity was 40% lower in deficient animals. However, after intraperitoneal injection of 10 mg PN-HCl, glutamic acid decarboxylase activity increased immediately and within 2 minutes was four times higher than controls.

Further evidence for the relationship between vitamin B6 and GABA comes from studies that have assessed GABA metabolism in individuals with PN dependent seizures. Pyridoxine dependent seizures are a rare autosomal recessive disorder in which there is defective binding of PLP to glutamate decarboxylase, the rate limiting enzyme in GABA metabolism (Mikati et al., 1991; Bankiet et al., 1983; Ebadi and Klangkalya, 1978). Individuals with this disorder have normal vitamin B6 status but abnormally low levels of GABA due to impaired synthesis. For example, GABA concentrations of 13-16 pmol/mL in the cerebrospinal fluid have been reported in these individuals compared to normal values of 54-184 pmol/mL (Kurlemann et al., 1992). GABA concentrations return to normal and seizures cease almost immediately with administration of 10-100mg of PN-HCl daily. Discontinuation of PN-HCl results in recurrence of the seizures and abnormally low GABA concentrations.

CARPAL TUNNEL SYNDROME

Because of the apparent ability of vitamin B6 to influence pain thresholds, it has been suggested that vitamin B6 may be useful in the treatment of carpal tunnel syndrome

(CTS). Carpal tunnel syndrome is a disorder that affects the median nerve of the wrist. Compression of the nerve results from a decrease in the size of the tunnel through which the nerve runs either from trauma or from an increase in the volume of the tunnel contents (e.g edema) (Ditmars and Houin, 1986). The resulting increase in pressure leads to nerve ischemia which in turn leads to localized metabolic dysfunction of the median nerve. Over 200,000 people in the United States develop CTS each year making it the most common peripheral entrapment neuropathy (Byers et al., 1984). This syndrome is believed to be the leading cause of pain, numbness and tingling in the hands. Other common symptoms include hand weakness and decreased dexterity (Ditmars and Houin, 1986).

Many factors are thought to place an individual at risk for CTS. Traditionally, development of CTS has been attributed to jobs that require repetitive hand motions. Other causes include anatomic compression and systemic diseases such as diabetes (Ditmars and Houin, 1986). Recently, some research has indicated that excess body weight promotes the development of CTS. Obese individuals have been shown to be at greater risk for developing CTS when compared to normal weight individuals. For example, a study conducted by Werner et al. (1994) found body mass index (BMI) to be significantly higher in individuals with CTS. When slender individuals (BMI<20) were compared to obese individuals (BMI>29), it was found that obese people were 2.5 times as likely to develop CTS. Sixteen percent of the slender individuals were diagnosed with CTS compared to 39% of the obese subjects. This difference was thought to be caused by excess fluid in the tissues which reduced the diameter of the carpal tunnel. Similar conclusions were drawn by Nathan and colleagues (1992). After tracking 361 industrial

workers between the years 1984 and 1989, researchers found obesity was linked to an increased prevalence of impaired nerve function; sensory conduction of the nerve decreased as body weight increased. Nathan and colleagues also concluded that BMI could be used to predict which individuals would experience changes in the median nerve and be at risk for developing CTS.

Treatment of CTS usually involves splinting, activity modification and steroid injection. However, these methods often have a high failure rate. Current studies indicate that over one-half of all CTS patients ultimately require surgery (Amadio, 1987). A finding that increased plasma levels of vitamin B6 would help alleviate symptoms of CTS, pain for example, would present a significant breakthrough in the treatment of this common condition.

VITAMIN B6 AND CARPAL TUNNEL SYNDROME

Much controversy and uncertainty exists regarding the relationship between vitamin B6 and CTS. A summary of the studies is given in Table 6. The majority of the early work in this area was done by Ellis and colleagues. Consequently their studies have had a large impact on much of the current thinking about vitamin B6 and CTS. As early as 1970, this research group proposed that CTS was caused by a vitamin B6 deficiency. Their data implied that treatment based solely on vitamin B6 therapy was sufficient to provide complete resolution of symptoms in all or nearly all patients. One of their first studies (Ellis et al., 1976) looked at the effect of PN supplementation on individuals having what they termed "a severe clinical status associated with CTS." Vitamin B6 status was assessed using erythrocyte alanine aminotransferase activity (EALT). Ten subjects with

Table 6. Summary of carpal tunnel syndrome studies

Research Group	n	Method	Treatment	Status Measure	CTS Diagnosis	Findings
Ellis et al. 1976	10	Non-random	300 mg B6/day for 4 weeks	EAST	None	People w/CTS have low B6 status; B6 supplementation cures CTS
Ellis et al. 1977	11	Non-random	100 mg B6/day for 6 weeks	EAST	Some type of nerve data	Low vitamin B6 status causes CTS
Folker et al. 1978	1	Cross-over	2 mg/day, 100mg/d, placebo, 100 mg/day (each 11 weeks)	EAST	Clincial exam	Vitamin B6 influences the etiology of CTS
Ellis et al. 1979	22	Cross-over Placebo	100mg B6/day for 10 months	EAST	Clinical exam & nerve data	Causal relationship between vitamin B6 and CTS; B6 reduced CTS symptoms
Ellis et al. 1980	15	Non-random	None	EAST	Clinical exam	Individuals with CTS had low transaminase activity
Ellis et al. 1981	4	Non-random	None	EAST	None	Low transaminase activity is associated with CTS
Ellis et al. 1982	7	Double blind	50 mg B6/day for 12 weeks	EAST	Clincial exam	Those receiving supplements had fewer symptoms than those receiving placebo

Table 6. (cont) Summary of carpal tunnel syndrome studies

Research Group	n	Method	Treatment	Status Measure	CTS Diagnosis	Findings
Ellis et al. 1982	10	Longitudinal8 yr followup	100 mg B6/day for 12 weeks	EAST	Clinical exam	No recurrence of CTS in those who continued to take 100-200 mg B6/day
Byers et al. 1984	33	Non-random	None	EALT	NCS	Peripheral neuropathy rather than CTS was associated with low vitamin B6 status
Smith et al 1984	5	Non-random	100 mg B6/day for 9-26 weeks	PL, PLP EAST	Clinical exam & NCS	No evidence of B6 deficiency with CTS. No improvement in NCS with supplements
Amadio 1985	19	Non-random Retrospective	100mg B6/day for 12 weeks	None	Clinical exam & NCS	Vitamin B6 reduced symptoms in some people (pain, tingling)
Salkheld et al., 1985	61	Non-random	100 mg B6/day for 12 weeks	EAST	NCS	Number of clinical symptoms correlated with vitamin B6 status
Tredici et al. 1985	24	Non-random	150-300 mg B6/day for 6 months	None	NCS	No change in NCS Decrease in frequency of pain with B6
Driskell et al 1985	28	Non-random	100 mg B6/day for 5-58 wks	EALT PLP	NCS?	97% saw an improvement with B6 40% became asymptomatic

Table 6. (cont) Summary of carpal tunnel syndrome studies

Research Group	n	Method	Treatment	Status Measure	CTS Diagnosis	Findings
Fuhr et al. 1989	8	Non-random	None	PLP Not Deproteinized	None	Subjects with CTS had low plasma PLP concentrations
Stransky et al., 1989	10	Random Double blind	150 mg B6/day for 10 weeks	None	NCS	Vitamin B6 had no advantage over other treatments for CTS
Kasdan et al., 1987	494	Non-random From hospital	200 mg B6/day for 6 months	None	Clinical exam	Vitamin B6 supplementation reduced the severity of CTS symptoms in 68% of the subjects
Guzman et al., 1989	12	Non-random	150 mg B6/day for 3 months	EAST	Clinical exam & NCS	A higher transaminase activity was associated with less pain and tingling
Bernstein & Dinesen 1993	16	Non-random	200 mg B6/day for 3 months	None	NCS	No change in NCS following supplementation Vitamin B6 appeared to reduce pain
Frazblau et al,. 1996	125	Non-random Industrial workers	None	PLP EAST	NCS	Vitamin B6 was not correlated with CTS development or CTS symptoms
Kenniston et al,. 1997	441	Non-random Industrial workers	None	PLP	NCS	High plasma PLP concentration was associated with a lower incidence of pain and tingling

low transaminase activity (activation coefficient (AC): 1.27 ± 0.10) were given 300 mg PN-HCl/day for 4 weeks. At the end of the study period, all subjects had adequate vitamin B6 status (AC: 1.10 ± 0.06) and the clinical condition, based on subjective evaluation of 17 different symptoms (e.g. weakness of hand grip, paresthesia of hands, pain in hands) improved. It was concluded that patients with CTS have inadequate vitamin B6 status (measured via EALT) and that both the syndrome and low transaminase activity are relieved by therapy with PN. However, since no nerve conduction studies were conducted, it is difficult make a positive diagnosis of CTS. A very similar study was conducted by Ellis et al. (1977) the following year. Eleven patients with low vitamin B6 status based on EALT (AC: 1.26 ± 0.10) diagnosed with CTS using what appears to be electromyographic data were studied. Following administration of 100 mg PN-HCl/day for 6 weeks, researchers found vitamin B6 status to improve in all patients (AC: 1.01 ± 0.05). Subjects also reported alleviation of symptoms such as pain, stiffness and impaired finger flexion. Based on the evidence, it was concluded that CTS is caused by inadequate vitamin B6 status. These findings were supported by a third study done later that year. After treating 1 CTS/low vitamin B6 status patient with 2 mg PN/day for 11 weeks, 100 mg PN/day for 12 weeks, a placebo for 9 weeks and 100 mg PN/day for another 11 weeks, Folkers and colleagues (1978) concluded that vitamin B6 supplementation eliminated any signs of inadequate vitamin B6 status and any signs of CTS. The activity coefficient of EALT decreased from the initial value of 1.23 to 1.14 with supplementation of 2 mg vitamin B6/day; a decrease to 1.01 was seen when the subject was given 100 mg/day PN-HCl. The activity coefficient during the placebo period was 1.12 and then dropped back down to 1.01 with the reintroduction of 100

mg/day supplements. Supplementation with 2 mg vitamin B6/day increased enzyme activity by 50-70%. Only with supplements of 100 mg/day did the subject become asymptomatic and the transaminase activity return to normal. The conclusions drawn must be questioned for several reasons. First of all, they are based on data gathered from only one patient. In addition, no information was provided on how an initial diagnosis of CTS was made or how it was determined that the patient was cured of CTS. Ellis and colleagues' (1979) next study was a little more scientific in that it used a crossover placebo design. Researchers looked at the effect of 100 mg PN-HCl/day for 10 months on 22 individuals who had low vitamin B6 status (AC: 1.26 $\pm$ 0.06) and had CTS. All individuals receiving the vitamin supplement became asymptomatic and had improved hand function. Self-administered symptoms questionnaires indicated the absence of pain, stiffness and paresthesia following supplementation. However, since the symptoms' scores were compared to control values gathered from other studies, it is hard to know what the severity of the symptoms was prior to supplementation. Electromyography data also indicated subjects had improved nerve conduction. Vitamin B6 status, as measured by EALT, was also adequate (AC:1.01 $\pm$ 0.05). All individuals receiving the placebo had low vitamin B6 status and continued to suffer from CTS. Again, no objective methods were used to make an accurate diagnosis of CTS.

Similar studies continued into the next decade. Vitamin B6 status was determined in fifteen patients with CTS (Ellis et al., 1980). Based on blood values for EALT (AC: 1.38 $\pm$ 0.07), it was concluded that CTS represents a deficiency disease of vitamin B6. Shortly thereafter, blood samples were taken from four patients requesting surgery for

CTS (Ellis et al., 1981). Determination of EALT activity revealed that all subjects had impaired vitamin B6 status as defined by the activation coefficient. In 1982, Ellis decided to see if his conclusions would stand up to a more controlled study. In a double blind study (n=7) subjects (AC: 1.22 $\pm$ 0.09) were treated with 50 mg PN for 12 weeks or with a placebo. Erythrocyte glutamate aminotransferase activity was measured in both groups and changes in CTS symptomology were evaluated by asking subjects to rate their symptoms (e.g. tightness, strength) on a scale of 1 to 5. After analyzing the data, researchers concluded that those receiving PN had normal vitamin B6 status and a decrease in symptoms. Symptoms' scores, which were correlated with the activity level of the transaminase, dropped from a mean of 49 $\pm$ 9 to 11 $\pm$ 6 when receiving the supplement. Those receiving the placebo experienced no changes in CTS symptoms (mean score: 53 $\pm$ 10). A final study done by Ellis and colleagues (1988) looked at the long term effect of vitamin B6 administration on CTS. Patients with "severe crippling" in their hands were treated with 100 mg PN-HCl/day for 12 weeks. At the end of the study period, all individuals were asymptomatic. Patient follow-up over the next 8 years found that their was no recurrence of CTS in individuals who continued vitamin B6 supplementation. After compiling all the data from their studies, Ellis and colleagues concluded that severe vitamin B6 deficiency was a major factor in the development of CTS. They recommended a daily dosage of 100-200 mg PN-HCl per day for at least 90 days to cure CTS. After this time period some individuals may be able to discontinue supplementation. However, most will find recurrence of CTS and will required continued supplementation. Although Ellis and colleagues conducted much research, their findings must be scrutinized since almost all of their studies are flawed by questionable scientific

design. For example, only one study used a double blind placebo control methodology. Moreover, the same sample population was used for more than one study. Furthermore, the research was based on a case history of only one patient. In addition, little information was given on treatment of blood samples. Ellis and colleagues used EALT as the only measure of vitamin B6 status. Based on the activity coefficient, subjects were diagnosed as vitamin B6 deficient. However, an accurate diagnosis of deficiency can be made only if several (i.e three or more) indices are used. In addition, if EALT activity is to be accurate, the erythrocytes must not be frozen but analyzed as soon as possible. There is no mention of how the blood samples were handled. Furthermore, many of the CTS patients studied had no electrodiagnostic (electromyography/nerve conduction velocity) tests. Without these tests, it is not possible to determine if an individual is truly suffering from CTS or peripheral neuropathy. A study by Byers et al.(1984) showed that peripheral neuropathy is often mistaken for CTS. Peripheral neuropathy is characterized by prolonged distal latencies in three or more separate motor or sensory nerves. In addition, there is no marked difference when comparing the median and ulnar nerves in the same hand if the median nerve is involved. In comparison, CTS is defined as prolonged median motor and/or sensory distal latencies; all other nerve conduction values are normal. In this study, researchers categorized subjects with symptoms suggestive of CTS into 4 groups by using standardized electrodiagnostic criteria - CTS, peripheral neuropathy, CTS/peripheral neuropathy and normal. Vitamin B6 status was assessed via EAST (AC: 1.24). Analysis of the results showed that peripheral neuropathy more so than CTS was highly correlated with impaired vitamin B6 status. Their findings suggest that the positive response reported in subjects with CTS taking supplements of vitamin

B6 may actually be related to unrecognized peripheral neuropathy which compounds the symptomology. As one can see, data from such tests is critical if firm conclusions are to be drawn about the benefits of vitamin B6 therapy.

Later studies attempted to verify Ellis' findings by using more rigorously structured methodologies. A study by Fuhr et al. (1989) was the only one to find a statistically significant correlation between low plasma PLP concentrations and the occurrence of CTS. All subjects (n=8) were patients who had surgical decompression 12 months or more prior to the study or were scheduled for CTS surgery. Blood analysis showed the average plasma PLP level in 6 normal control subjects to be approximately 2.5 times higher than that determined in 8 subjects with CTS (22.5 ± 3 nmol/L vs 9.03 ± 3 nmol/L). The findings have to be interpreted cautiously however. The method used by Fuhr et al. to determine plasma PLP was flawed in that it did not result in complete deproteinization of the sample; thus, it is likely that some of the PLP remained bound to albumin. Because of this error in methodology, the reported values were lower than would be expected and the skewed values make it difficult to compare the results to other studies. In addition, because the study failed to make any type of therapeutic intervention, no conclusions can be drawn regarding the possible relationship between vitamin B6 deficiency and the relief of CTS symptoms. One of the most recent studies on the vitamin B6-CTS relationship comes from a study by Franzblau et al. (1996). They looked at the relationship between vitamin B6 and CTS symptoms in 125 randomly selected workers. All subjects underwent electrophysiologic testing to check nerve function and completed a health questionnaire. Blood samples were drawn and analyzed for EAST and plasma PLP. The mean EAST specific activity was 1.04 ± 0.18 and the

mean plasma PLP concentration was 128.0 ± 81.1. Their findings indicated that their was no correlation between vitamin B6 indices and nerve conduction studies or vitamin B6 status and CTS symptoms. The conclusions of this study are compounded by several flaws. For example, there was no indication of vitamin supplement use by subjects, a factor that could influence interpretation. Researchers also neglected to indicate if blood samples were drawn from fasted subjects. Secondly, parametric statistical tests were used on non-normally distributed data which would lead to inaccurate conclusions. In addition, lack of regression analysis made it impossible to determine whether status measures made an independent contribution to predicting any of the outcome variables. Lastly, EAST activity was determined after freezing the blood samples. Freezing decreases transaminase activity and may lead to inaccurate results.

Several studies have looked at both the vitamin B6/CTS correlation as well as the effect of administration of vitamin B6 on the associated symptoms. The results suggest that vitamin B6 can be useful as an adjuvant treatment in patients undergoing surgery. A study by Smith et al. (1984) assessed the effect of 100 mg PN-HCl given for 9-26 weeks to 5 individuals with CTS. All patients had idiopathic CTS for at least 3 months prior to the onset of the study. Clinical examination and blood tests were used to screen patients for CTS that could be secondary to other conditions such as hypothyroidism. Blood samples were drawn and measured for leukocyte and plasma PL and PLP. Erythrocyte aspartate aminotransferase was also determined. The biochemical data indicated that subjects had a normal vitamin B6 status (plasma PLP range: 50.8-264 nmol/L; AC range: 1.11-1.62). In addition, there were no improvements in nerve conduction, a measure used to determine the severity of CTS. Although four of the patients claimed some partial

symptomatic relief, there was no consistent improvement in clinical findings or neurophysiological measurements following pyridoxine treatment. Researchers concluded that vitamin B6 deficiency was not a factor in the development of CTS. Similar results were found by Stransky et al. (1989) in a randomized, double-blind, placebo controlled study. Following ten weeks of vitamin B6 supplementation (150 mg/day), 10 of the 15 patients (including those given the placebo) showed an improvement in symptoms. Researchers concluded that vitamin B6 has no advantage over conservative therapy for CTS. These findings are supported by Amadio (1985) who evaluated the effect of vitamin B6 supplementation on 19 individuals diagnosed with CTS. Diagnosis was based on both a history of the typical pattern of hand numbness, pain and impairment and nerve conduction abnormalities. No biochemical tests were done to assess vitamin B6 status. Patients were classified into three groups based on symptom severity (mild, severe, moderate) and supplements of 100 mg PN-HCl/day were given for a 12 week period. Clinical improvement was defined as progression from a more severe symptoms class to a less severe one and successful treatment was defined as a significant relief of the initial complaints. At the conclusion of the study, two of four patients with mild symptoms reported some symptoms improvement. The 12 patients with moderate symptoms and the 3 patients classified as having severe CTS symptoms did not report any improvement in CTS symptoms with vitamin B6 therapy alone. However, a combination of vitamin B6 and splinting helped some of the patients with moderate and severe symptoms. After evaluating the data, researchers concluded that PN treatment alone was not sufficient to reduce the symptoms of CTS. However, in conjunction with other treatments (e.g. splinting) vitamin B6 did offer some

improvement. A similar conclusion was drawn by Guzman et al. (1989) who looked at the effect of 150 mg PN-HCl/day for 3 months on CTS severity. Twelve patients were identified as having CTS by a combination of clinical and electrophysiological criteria including hand pain, tingling and sensory and motor loss. Distal motor latency was also measured. Blood samples were analyzed for EAST activity. None of the patients were found to have vitamin B6 deficiency (AC 1.47 ± 0.15). The activity coefficient decreased significantly in all patients. In 6 patients, there was clinical and electrophysiological improvement in that they showed a significant decrease in distal motor latency (5.57 ± 1.36 ms compared to the initial value of 6.20 ± 1.72 ms). Distal motor latency is an indicator of CTS severity. Researchers concluded vitamin B6 supplementation could be recommended with other treatments.

Many of the studies that have tried to solidify the vitamin B6-CTS relationship indicate that the more likely role of vitamin B6 is its ability to reduce symptoms - apparently because of its ability to change pain thresholds and possibly reduce edema. A study by Sharma and colleagues (1990) found vitamin B6 deficient rats to have a lower tolerance for pain; administration of vitamin B6 (100 mg/kg bw of PN) produced mild morphine like effects. The animal data is supported by human studies. For example, Keniston and colleagues (1997) looked at the relationship between plasma PLP concentrations and the frequency of CTS symptoms in 441 industrial workers. Researchers found no significant difference in the plasma PLP concentration of the controls when compared to individuals with CTS (45.7 ± 2.1 nmol/L) . They also noted that in males, a high concentration of plasma PLP was associated with less frequent symptoms, especially pain and tingling. The data suggest that the high concentrations of

vitamin B6 may reduce pain by enhancing serotonin synthesis. A similar conclusion was drawn by Schwieger et al.(1990) who evaluated the effect of vitamin B6 supplementation on 53 subjects. Using a randomized double-blind design, it was found that the group treated with 200 mg PN-HCl per day for 6 months experienced less intense pain; the clinical condition of the placebo group deteriorated. Again, this study supports the relationship between vitamin B6 and analgesia. This finding is supported by a study conducted by Salkheld et al.(1985). They found no significant difference in EAST activity and percent stimulation between 61 subjects with CTS and those without (SA: 2.05 ± 0.14). However, administration of vitamin B6 (100 mg PN-HCl per day for 12 weeks) did significantly reduce the number of symptoms from 9.9 ± 2.1 to 2.8 ± 2.5. The number of clinical symptoms was inversely correlated with vitamin B6 status, but was not statistically significant. Researchers felt this was because the subject's biochemical status improved more rapidly than the decline in symptoms. The Salkheld findings have been supported by several other studies. For example in a double blind study, Driskell et al. (1986) assessed the effect of 100 mg PN-HCl for 5 to 58 weeks on 28 individuals with CTS. Presence of CTS was based on symptomology and determination of nerve conduction velocity. Vitamin B6 status before and after supplementation was determined by plasma PLP and EALT. Initially 25% of the subjects had inadequate vitamin B6 status; status of all supplemented subjects were within the normal range at the end of the study. Twenty-seven of the 28 subjects experienced an improvement in their symptoms and 12 of the subjects became asymptomatic. Results of a study by Kasdan and colleagues (1987) supports those found by Driskell. Records of 1075 patients complaining of paresthesia or pain in the median nerve were reviewed. Final diagnosis of

CTS was based on history, clinical examination and in some cases electrical diagnostic studies. Out of the 494 patients who were given 100 mg PN-HCl per day, 68% had satisfactory alleviation of symptoms. A case was considered to have good alleviation of symptoms if the patient no longer had nocturnal symptoms and only occasional finger tingling during the day. Kasdan et al. found that patients who are going to respond to PN treatment do so within 3 months. In more severe cases, some improvement may be seen in the initial 3 months but complete disappearance of nocturnal symptoms and finger tingling will require more time. Similarly, a 4 month supplementation study by Tredici et al. (1985) looked at the effect of either 150 mg PN-HCl/day (n=16) or 300mg PN-HCl/day (n=8) on CTS patients. Subjects were screened for CTS by a combination of clinical and electrical data including distal motor latency. Subjects were asked to numerically rate their symptoms. At the end of the study, supplementation was found to significantly improve both subjective (self-reported symptoms) and objective (electrical data) evaluation of CTS. In patients receiving 150 mg/day vitamin B6 initial mean symptom scores decreased from 23 ± 12 to 12 ± 10 at the end of two months. After four months, scores had dropped to 6 ± 6. In subjects treated with 300 mg vitamin B6/day, symptom scores declined from 27 ± 12 to 12 ± 10. At the end of the study period, average scores were 12 ± 9. Distal motor latency also declined in each group. Subjects receiving 150 mg/day experienced a decrease from 5.3 ± 0.9 msec to 4.4 ± 1.0 between study onset and end. Distal motor latency in individuals receiving 300 mg/day decreased from 6.2 ± 1.8 to 4.6 ± 1.2 msec. In 20 of the patients, recovery was so complete that steroid injection or surgical decompression was not needed. It was concluded that vitamin B6 supplementation should be considered in treatment of CTS as part of the

medication scheme used prior to surgery. Bernstein et al. (1993) also tried to solidify the vitamin B6-CTS relationship by supplementing 16 subjects with 200 mg PN-HCl per day for 3 months. Standard clinical and electrophysiological parameters (e.g. motor nerve latency) were used to monitor CTS. Pain was assessed using the McGill pain questionnaire and the visual analog scale. Following the supplement period, researchers found no change in electrophysiological data. However, the subjects' perception of pain showed a significant decrease, with the visual analog scale score decreasing by 24.8 points. It was concluded that vitamin B6 can be an effective therapeutic modality in the treatment of CTS. The ability of vitamin B6 to, in some individuals, reduce pain associate with CTS suggests that vitamin B6 status does affect GABAergic or serotonergic mechanisms.

To summarize, twenty-one studies have looked at the relationship between vitamin B6 and CTS. Initially, a vitamin B6 deficiency was thought to be the cause of CTS. As research continued, the focus switched to one in which vitamin B6 was believed to decrease the intensity and frequency of CTS symptoms. Eight of the twenty-one studies surveyed were contributed by Ellis and colleagues. Two looked at transaminase activity in subjects with CTS; the other studies analyzed the effect of vitamin B6 supplementation on the presence of CTS. After looking at all of the data this research group came to two conclusions – a vitamin B6 deficiency causes CTS and supplementation of vitamin B6 will cure the syndrome. After carefully reviewing the data, it is obvious that the studies are flawed in several areas. For example, many of the studies provide no methodology information or if they do it is extremely vague. Secondly, only one study had any type of

valid nerve conduction data gathered (Ellis, 1979). In addition, the results and conclusions of several of the studies are based on data from only one individual.

Since the Ellis era, several other research groups have tried to determine the relationship between CTS and vitamin B6. Based on the results, it appears that vitamin B6 may play a role in the perception and frequency of symptoms. Eight studies looked at the effect of vitamin B6 supplementation on CTS symptoms. In each study, some of the subjects reported less intense or less frequent symptoms. None of the studies found any evidence that CTS was related to a vitamin B6 deficiency. However, again, only four of the researchers used standardized nerve conduction data to distinguish between the presence of CTS and the presence of peripheral neuropathy. In the other studies, CTS was diagnosed via clinical exam, via a self-administered symptoms questionnaire or a combination of both. Six studies (twelve if Ellis et al. is included) measured transaminase activity; only four looked at plasma PLP and none included erythrocyte PLP or total plasma vitamin B6 as additional status measures. Moreover, alkaline phosphatase activity was never considered as a possible influence on vitamin B6 status. Lastly, none of the studies reported whether any of the subjects took vitamins prior to the start of the study. Data on vitamin use is necessary to help determine the efficacy of supplement use to help treat CTS. To date, none of the studies have addressed the vitamin B6-CTS relationship over time in a systematic manner. Furthermore, none have collected all of the data necessary to help determine if and how vitamin B6 is related to CTS.

METHODS

OVERVIEW

This research project was part of a collaborative study with Dr. Peter Nathan, a hand surgeon at the Portland Hand Surgery and Rehabilitation Center in Portland, Oregon. For this thesis, the nine month study had four main objectives: 1) to monitor the stability of vitamin B6 status over a nine month period; 2) to determine if a significant correlation exists between carpal tunnel symptoms and vitamin B6 status; 3) to determine if vitamin users have a significantly lower incidence of reported CTS symptoms when compared to non-vitamin users; and, to determine if a significant correlation exists between nerve conduction and vitamin B6 statsus. Vitamin B6 status was evaluated by taking a total of three blood draws. Subsequent analysis involved determination of plasma and erythrocyte pyridoxal 5'-phosphate and total plasma vitamin B6. Alkaline phosphatase activity was also determined. Other pertinent information was gathered via administration of a diet/health questionnaire and a symptoms questionnaire. A staff member from Dr. Nathan's office administered nerve conduction studies.

SUBJECT SELECTION

Carpal tunnel syndrome is frequently associated with repetitive hand motion. However, a study conducted by Nathan et al. (1992) has indicated that a sedentary lifestyle could also be associated with CTS. Because of this apparent relationship, the sample population was drawn from individuals who were not engaged in a regular exercise program prior to the conduction of this study and who were experiencing

symptoms associated with CTS. The study was open to both males and females between 20-50 years of age. Individuals were recruited for the study through poster advertising and electronic mail. Prestudy screening included health assessment by medical history and blood chemistry profiles and diagnosis of CTS via nerve conduction studies and self reported symptoms. Prior to initiation into the study, all subjects had to meet the following criteria: normal health history based on a self-reported questionnaire, normal blood chemistry screen, nerve conduction test indicative of slowing in the median nerve, and presence of some CTS symptoms (e.g. pain). Supplement use data gathered during the prestudy screening was used to divide subjects into vitamin users and non-vitamin users. Vitamin use was defined as use of a vitamin B6 containing supplement 5 days or more each week; non-vitamin use was defined as no supplement use or use of supplements that research has shown will not influence blood concentrations of vitamin B6 (e.g. calcium).

EXERCISE

Subjects enrolled in the study concurrently participated in a supervised exercise program 3 days a week for one-half to one hour each session. For the first month, subjects exercised for 30 continuous minutes at 60% of their maximal heart rate. Thereafter, the length of exercise was increased until subjects could complete 60 minutes of continuous exercise. After one month, subjects also worked toward a target heart rate that was 85% of their maximal heart rate. Heart rate was self monitored and recorded. The maximum training heart rate was based on the maximal treadmill test done at the

study onset. Activities available to subjects included treadmill, ergometer cycling, and stairstepper.

SAMPLING PROCEDURES

Antecubital venous blood was collected after an overnight fast at 3 time points during the study - study onset (month 1), midpoint (month 6) and end (month 9). Approximately 35 mL of blood was drawn into heparinized tubes and kept on ice (30 minute maximum) until centrifugation. Blood was centrifuged at 10,000 g for 15 minutes 4^0C. The plasma was aliquoted and erythrocytes were washed three times with 5 ml of 0.9% saline solution. All samples were stored at -40^{o}C until analysis.

BLOOD ANALYSES

Plasma and erythrocyte PLP, total plasma vitamin B6 and alkaline phosphatase were determined in each sample. Plasma PLP and erythrocyte PLP concentration were determined using the method of Chabner and Livingston (1970). In this procedure, plasma is deproteinized with perchloric acid and erythrocytes are deproteinized with trichloroacetic acid prior to incubation with ^{14}C tyrosine in the presence of tyrosine decarboxylase. The amount of $^{14}CO_2$ given off in the reaction is proportional to the amount of PLP in the sample. Radioactivity was counted in a Beckman LS 5000 TD liquid scintillation counter and counts were compared to a standard curve. Linear regression was used to calculate the concentration. The coefficient of variation (CV) for the plasma PLP assay was 5.5% for a control sample (n=20). Recovery of PLP averaged

92% ± 14%. The CV for the erythrocyte PLP assay was 8.5% for a control sample (n=20). Recovery of PLP from erythrocytes was lower than from plasma, averaging 71% ± 10%.

Total plasma vitamin B6 was determined via the microbiological method of Miller and Edwards (1981). The assay had a CV of 8.4% (n=21). Deproteinized plasma was autoclaved to release the phosphate groups so all the vitamin B6 was in the free form. Samples were then diluted (approximate concentration 1 ng B6/mL solution) and incubated with *S. uvarum*. Following a 22 hour incubation period, the samples were read on the Evlyn colorimter with a 660 nm filter. Percent transmittance and the dilution factor were used to calculate the concentration of total plasma vitamin B6.

Alkaline phosphatase was measured colorimetrically by the method of Roy (1970). Plasma was incubated with thymolpthaline monophosphate for 10 minutes. As the alkaline phosphatase cleaves off the phosphate groups, a blue color results. Samples were then read on the Beckman DU-40 spectrophotometer at 500 nm. The CV for the alkaline phosphatase assay was 1.9% (n=5). All samples were analyzed in duplicate unless otherwise noted.

HEALTH QUESTIONNAIRES AND VITAMIN USE

Self administered health questionnaires were completed at each time point. The initial questionnaire focused on demographic data, prior medical conditions, occupational history, current health status, diet, medication and supplement use. Follow-up questionnaires emphasized diet, medication use (type, frequency, amount), and supplement use. Information gathered about the supplements included the brand of

vitamin, frequency of use, dosage and length of use. At the time the questionnaires were gathered, they were evaluated for completeness; follow-up phone calls or interviews were made in instances where information was incomplete or unclear. A trained technician administered symptoms questionnaires and NCS.

SYMPTOMS QUESTIONNAIRE

At the initial interview, subjects were questioned orally about the presence, frequency and nature of hand and wrist symptoms. Three specific CTS symptoms - numbness, tingling and nocturnal awakening - and three nonspecific symptoms - pain, tightness and clumsiness - were recorded. Each symptom was scored as either absent (0) or present (1). Symptom frequency was rated as never (0), monthly (1), twice/month (2), weekly (3) or daily (4). Total symptom frequency was the sum of the individual symptom frequency.

NERVE CONDUCTION STUDIES

Nerve conduction tests were administered by a trained technician from Dr. Nathan's office. Nerve studies were made on the median nerve, bilateral and included the maximum latency difference (MLD) by the Kimura technique (1979). The MLD was adjusted for a hand temperature of 34^0C. A MLD of $\geq$ 40msec was considered to indicate abnormality. Slowing was rated as absent (0) or present (1) if there was an abnormality in one or both median nerves. Combined MLD equaled the sum of the left MLD value and the right MLD value.

STATISTICS

The data was analyzed using SPSS. Because the data was not normally distributed, plasma PLP, erythrocyte PLP, and total plasma vitamin B6 were log transformed before analysis. Symptom data was also log transformed. Statistical comparison of plasma PLP, erythrocyte PLP, total plasma vitamin B6, alkaline phosphatase, nerve conduction tests and symptoms both within and between groups was performed using repeated measures ANOVA. Simple linear regression was used to determine if a correlation existed between the vitamin B6 status measures and symptoms and nerve conduction tests. Data are expressed as mean $\pm$ SD. P values <0.05 were considered to be statistically significant.

RESULTS

Fifty individuals were selected for the study. However, because of illness, time conflicts and other complications, complete data was available for only thirty subjects . Mean age, height, weight, percent body fat and VO_2 max are given in Table 7. All subjects had hemoglobin concentrations and hematocrit values within the normal range. In each group there was one smoker (~0.5 packs/day) and eight drinkers. The range of alcohol intake was 0.25 to 1.5 drinks of distilled alcohol, wine or beer per day.

Table 7. Subject characteristics.

	Non-vitamin user (n=14)	Vitamin user (n=16)
Age (years)	45.8 ± 8.6	45.2 ± 8.6
Gender	10 female/4 male	13 female/4 male
Body Weight (kg)	88.4 ± 19.3	83.5 ± 20.8
Height (cm)	168.1 ± 8.0	169.4 ± 7.7
Body fat (%)	29.7 ± 5.6	29.5 ± 5.6
Absolute VO_2 (L/min)	2.6 ± 5.7	2.6 ± 6.7

All values are means and SD

Body fat percentages were measured via skinfolds

VO_2 (max) was determined on a treadmill

SUPPLEMENT USE

The distribution and dosage of vitamin B6 supplements is given in Table 8. Sixteen subjects were taking a median dose of 4 mg vitamin B6/day at study onset, 3.5 mg/day at midpoint and 3 mg/day at the conclusion of the study. The average length of vitamin use among subjects was 4 years (range 3 months to 20 years, median 2 years). Eleven subjects took only a multi-vitamin, three took both a multi-vitamin and B-complex, one took pure PLP in combination with a multi-vitamin and one subject used only a B-complex supplement. On average, subjects took vitamins six days per week. Thirteen subjects took one tablet each time they took a supplement, two subjects took two supplements, and one subject took three. Nine subjects took a supplement one to two days before the first blood draw. Four vitamin users took a supplement three to five days prior and three subjects had not taken a supplement for a month. Prior to the second

Table 8. Distribution of vitamin B6 supplements in the sixteen vitamin users.

Milligrams B6	Month 1	Month 6	Month 9
	Number of subjects at dose		
1 - 2	6	4	6
2 - 4	4	5	4
5 - 10	1	2	1
11- 30	1	0	0
50 - 60	1	2	2
60 - 99	0	0	1
100	1	3	2
>100	2	0	0

blood draw, fifteen of the sixteen vitamin users had taken a supplement one day before the blood draw. Nine of the sixteen vitamin users subjects took a supplement one to two days before the last blood draw; seven had taken a supplement three to five days earlier. Five vitamin users changed their dose of vitamin B6 during the study. Of these five individuals, one subject increased the dose received by a supplement and three vitamin users decreased the amount of vitamin B6 taken. One vitamin user decreased the amount of vitamin B6 received from the supplement between month one and six and then increased the dose between month six and nine.

PLASMA PYRIDOXAL 5'-PHOSPHATE

Mean plasma PLP concentrations for the three time points are given in Table 9. The plasma PLP concentrations for vitamin users ranged from 17.6 to 277.0 nmol/L at month one, 52.0 to 302.9 at month six and 20.9 to 271.2 at month nine. For vitamin users, plasma PLP concentration was significantly correlated with the dose of vitamin B6 provided by the supplement. Mean plasma PLP concentrations for vitamin users were approximately three times higher ($p<0.001$) than those of non-vitamin users which ranged from 9.8 to 59.5 nmol/L, 13.3 to 62.4 and 20.0 to 67.5 at months one, six and nine, respectively. Based on the recommended plasma PLP concentration of >30nmol/L, nine of the fourteen non-vitamin had low vitamin B6 status at the study onset (9.8 to 29.1 nmol/L), seven at midpoint (13.3 to 27.6 nmol/L) and five at the conclusion (20.0 to 29.3 nmol/L). Three of the non-vitamin users had inadequate vitamin B6 status at all three timepoints, four at two of the time points and four at one time point. Surprisingly, two subjects in the supplement group had low plasma PLP values at study onset (17.6 and 19.4 nmol/L). These values correspond to a supplement intake of 2 mg vitamin B6/day. One vitamin user, different from the two with inadequate vitamin B6 status at the beginning, was found to have a low plasma PLP value at the end of the study (20.9 nmol/L). This subject was also taking a supplement containing 2 mg of vitamin B6. Within each group, plasma PLP concentrations were not found to significantly change over the nine month period. For vitamin users, the mean plasma PLP concentration increased by 80% between month's one and six and then dropped by 35% between month's six and nine. Overall, the mean 22% increase between month one and nine was

not statistically significant. All sixteen vitamin users experienced at least a 10% change in plasma PLP concentration between month one and six and fifteen subjects between month six and nine

Mean plasma PLP concentrations for non-vitamin users showed less fluctuation than those of vitamin users, increasing 22% between month one and six and another 10% between month six and nine. Again, the overall mean increase of 35% was not statistically significant. The plasma PLP concentration of thirteen of the fourteen non-vitamin users changed by at least 10% between each time point.

TOTAL PLASMA VITAMIN B6

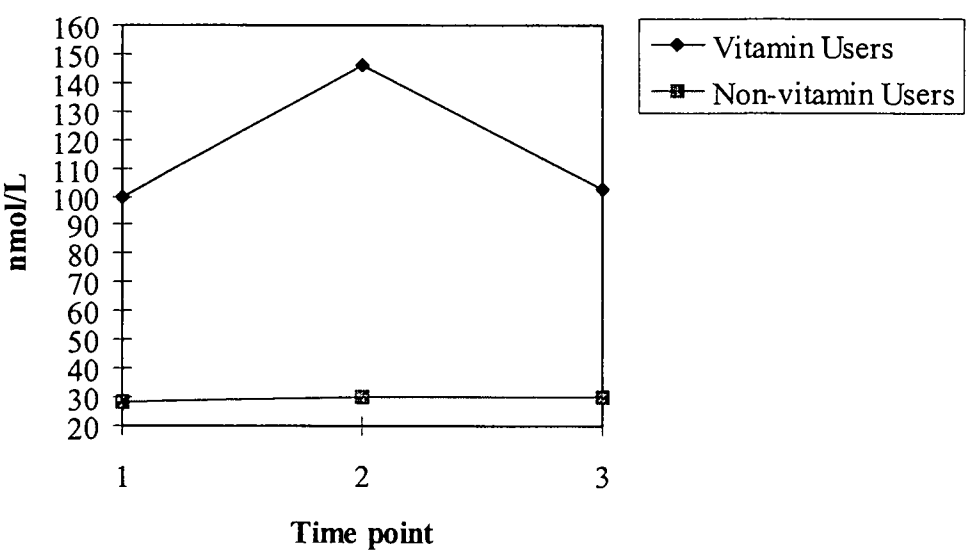
It is evident from the mean total plasma vitamin B6 concentrations shown in Table 10, that the data for total plasma vitamin B6 closely paralleled that seen for plasma PLP. A strong correlation was found between plasma vitamin B6 and the milligrams of vitamin B6 provided by the supplement. Total plasma vitamin B6 concentrations for non-vitamin users were approximately three times lower than vitamin users and showed less variation in the range of values when compared to vitamin users – 17.4 to 87.5 nmol/L, 20.3 to 72.5 nmol/L and 30.8 to 97.5 at month's one, six and nine, respectively. Based on the recommended concentration of >40 nmol/L for total plasma vitamin B6, seven non-vitamin users were below this value at the study onset (17.4 to 36.2) and midpoint (20.3 to 37.9 nmol/L). The number of subjects below the suggested concentration dropped to three by the end of the study (30.8 to 36.6). Low total plasma vitamin B6 concentration was seen continually in two subjects. Four subjects had low

Table 9. Mean plasma PLP concentrations over time.

		Month 1	Month 6	Month 9
			nmol/L	
VU (n=16)	mean	99.5	146.0	102.3
	SD	80.8	81.5	69.7
		[a]	[a]	[a]
NU (n=14)	mean	28.2	30.1	30.3
	SD	14.8	14.5	12.3

(a) means significantly different between groups ($p<0.05$).
VU – vitamin users; NU – non-vitamin users

Figure 4. Mean plasma PLP concentrations over time.



concentrations at two time points and three other non-vitamin users had low concentrations at one time point. Three vitamin users would be considered at risk for vitamin B6 deficiency at month one (32.7 to 39.3 nmol/L). The total plasma vitamin B6 concentration of one of these subjects dropped slightly lower, 38.9 nmol/L, by month nine. Vitamin users had significantly higher ($p<0001$) concentrations of total plasma vitamin B6 with values ranging from 32.4 to 364 nmol/L at month one, 57.6 to 506 nmol/L at month 6 and 38.9 to 547 nmol/L at month 9.

As was the case with plasma PLP concentrations, neither group showed significant changes in total plasma vitamin B6 throughout the nine months. The mean concentrations of total plasma vitamin B6 for vitamin users increased by 65% between month one and six and then dropped 20% by month nine. Although an overall increase of 35% was seen, it was not statistically significant. Fluctuations in total plasma vitamin B6 vitamin B6 concentration were not as great in non-vitamin users. No change in concentration was seen between month one and six; the plasma concentration increased 22% at month nine. This 22% increase was not statistically significant.

PERCENT OF TOTAL PLASMA VITAMIN B6 PRESENT AS PLASMA PLP

The percentage of total plasma vitamin B6 accounted for by plasma PLP was not significantly different between the two groups. For vitamin users, plasma PLP represented an average of 72% of total plasma vitamin B6 at month one, 75% at month six and 65% at month nine. Plasma PLP was a smaller percentage of total plasma vitamin B6 in non-vitamin users – 64%, 70% and 64% at month one, six and nine, respectively.

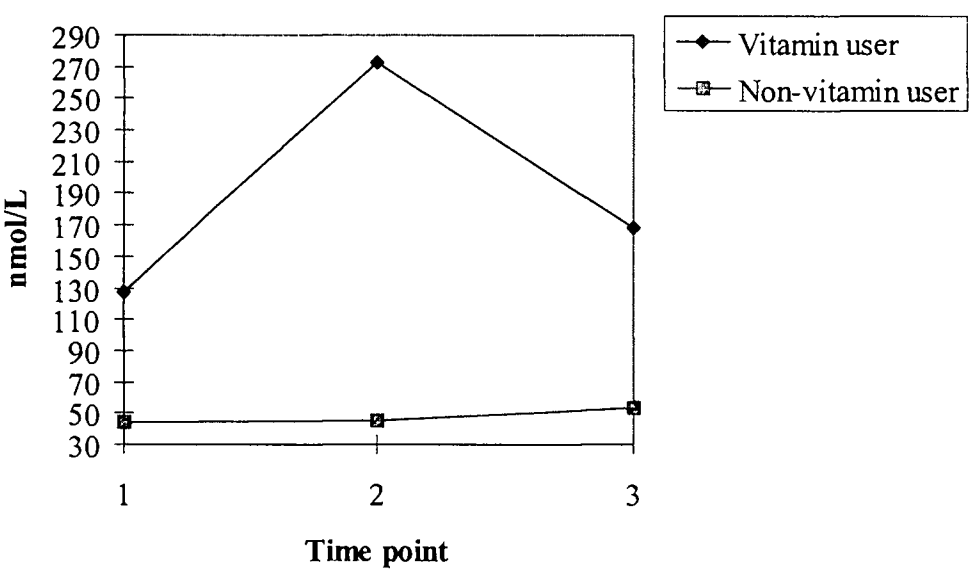
Table 10. Mean total plasma vitamin B6 concentrations over time.

		Month 1	Month 6	Month 9
			nmol/L	
VU (n=16)	mean	127.4	272.6	168.4
	SD	119.0 [a]	343.1 [a]	141.9 [a]
NU (n=14)	mean	44.7	45.7	53.6
	SD	20.1	16.6	17.3

(a) means statistically different between the groups ($p<0.05$).

VU – vitamin user; NU – non-vitamin user.

Figure 5. Mean total plasma vitamin B6 concentration over time.



ERYTHROCYTE PYRIDOXAL 5'-PHOSPHATE

Erythrocyte PLP concentrations for vitamin users ranged from 41.0 to 430 nmol/L at month one, 53.3 to 888 nmol/L at month six and 47.8 to 452 nmol/L at month nine (see Table 11). A significant correlation ($p<0.001$) was found between the amount of vitamin B6 in the supplement and erythrocyte levels of this vitamer. Non-vitamin users had a much narrower range of erythrocyte PLP concentrations: 37.1 to 65.7 nmol/L at month one, 36.3 to 97.0 nmol/L at month six and 40.7 to 102.5 nmol/L at month nine. On average, vitamin users had an erythrocyte PLP concentration two times higher ($p<0.001$) than those for non-vitamin users.

Of all the status measures, erythrocyte PLP showed the most stability and did not significantly change over the nine month period in either group. However, for vitamin users, erythrocyte PLP concentrations changed more than 10% in all subjects at each time point. This resulted in an increase in erythrocyte PLP concentration of 20% between months one and six; the mean concentration returned to prestudy levels by month nine. Twelve of the fourteen non-vitamin users showed a change in PLP concentrations of 10% or more between study onset and midpoint; all fourteen showed at least a 10% change between study midpoint and end. Erythrocyte PLP concentrations for non-vitamin users increased by 11% between month's one and six. There were no further changes in erythrocyte PLP concentrations between month six and month nine. In vitamin users, erythrocyte PLP was significantly correlated with plasma PLP at all three time points ($p<0.001$). In contrast, no correlation between plasma and erythrocyte PLP was found among the non-vitamin users.

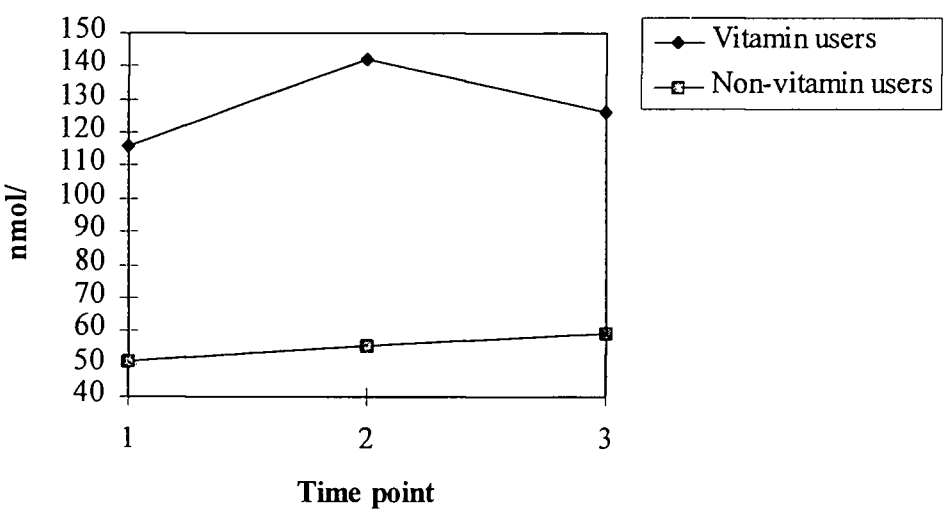
Table 11. Mean erythrocyte PLP concentrations over time.

		Month 1	Month 6	Month 9
			nmol/L	
VU (n=16)	mean	115.7	142.2	125.9
	SD	105.8	209.2	128.2
		[a]	[a]	[a]
NU (n=14)	mean	50.8	55.4	59.3
	SD	9.3	15.7	14.3

All values are means and SD; (a) means significantly different between the groups ($p<0.05$).

VU – vitamin users; NU – non-vitamin user.

Figure 6. Mean erythrocyte PLP concentrations over time.



ALKALINE PHOSPHATASE

Mean alkaline phosphatase activity was significantly higher in non-vitamin users when compared to the mean activity value for vitamin users ($p<0.03$). No subjects had values outside the normal range of 12.5 - 44.4 U/L (Roy, 1970) and the average difference between groups was 10%. Activity levels for vitamin users ranged between 11.6 to 33.7 U/L for month one, 12.0 to 36.2 U/L for month 6 and 13.6 to 37.7 for month nine. Activity levels for non-vitamin users ranged between 19.9 to 38.0, 18.1 to 33.5 and 17.5 to 37.7 for months one, six and nine respectively. Mean alkaline phosphatase activity showed minimal fluctuation and did not change significantly in either group over the study period (see Table 12). In addition, alkaline phosphatase activity was not found to be significantly correlated with plasma PLP concentration in either vitamin or non-vitamin users. Plasma alkaline phosphatase activity was not correlated with body weight in either group.

SYMPTOMS QUESTIONNAIRES

A summary of the frequency of the CTS symptoms is given in Table 13. The frequency of four of the six symptoms associated with CTS – numbness, pain, tightness, and tingling were significantly higher in vitamin users ($p<0.01$) when compared to non-vitamin users. The frequency of nocturnal awakening and clumsiness was not found to significantly differ between the two groups. Over the nine month study period, numbness was the symptom vitamin users experienced most frequently. Eight of the sixteen

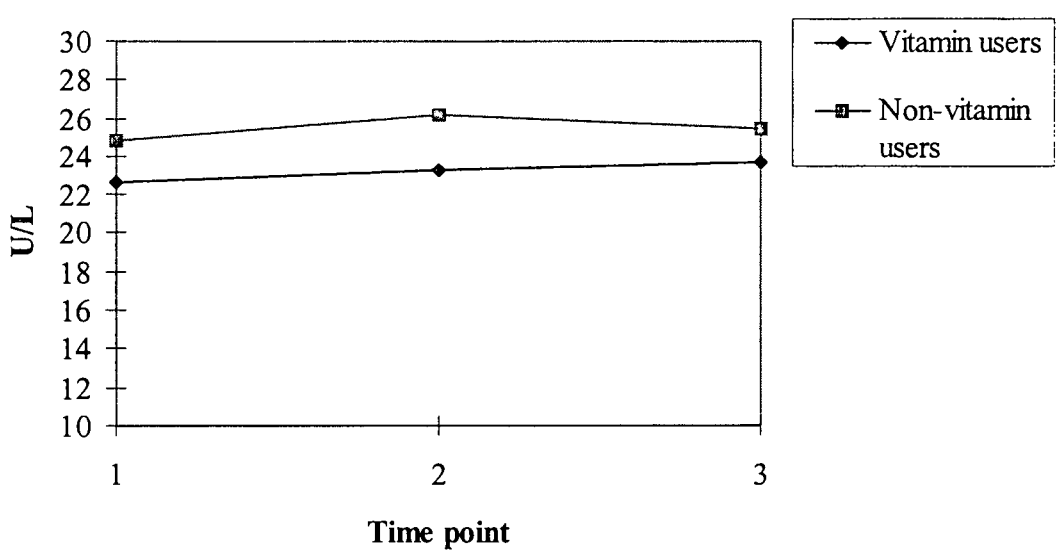
Table 12. Mean plasma alkaline phosphatase activity over time.

		Month 1	Month 6	Month 9
			U/L	
VU (n=16)	mean	22.6	23.3	23.7
	SD	6.2 [a]	6.1 [a]	5.7 [a]
NU (n=14)	mean	24.8	26.2	25.4
	SD	4.7	4.5	5.4

(a) means significantly different between the groups ($p<0.05$).

VU – vitamin user; NU – non-vitamin user.

Figure 7. Mean plasma alkaline phosphatase activity over time.



vitamin users reported daily occurrence and approximately one-third of the vitamin users reported weekly bouts of numbness. Tightness, pain and tingling were the next most common symptoms, with approximately one-half of the vitamin users complaining of daily or weekly frequency of these symptoms at each of the time points. The remaining vitamin users did not appear to be bothered by tightness, pain, or tingling. Seven of the sixteen vitamin users complained that CTS caused them to awake during the night on a daily or weekly basis. Clumsiness was the symptom that troubled vitamin users the least -- approximately 50% of the subjects reported clumsiness to not be a problem. In non-vitamin users, numbness was also the symptom complained about most often, with 50% to 80% of the subjects complaining of numbness on a daily or weekly basis. Tingling and nocturnal awakening were common complaints among non-vitamin users, with one-third reporting the symptoms on a daily or weekly basis. Pain, tightness and clumsiness among the non-vitamin users was reported to occur on a daily or weekly basis in 25% of the subjects for the first two time points. Thirty percent of the subjects reported occurrence of these symptoms at the end of the study.

The intensity of pain, numbness and impaired activity reported by vitamin users was significantly higher ($p<0.01$) when compared to non-vitamin users (see Table 14). For either group, the severity of symptoms did not significantly change over the nine month period. Overall, vitamin users experienced moderate pain several times a day. Pain episodes did not usually last more than 20 minutes. In comparison, non-vitamin users complained of mild pain. The frequency and duration of the pain was approximately the same as that seen in vitamin users. Numbness followed a similar trend as did pain. Vitamin users experienced moderate numbness several times during the day

Table 13. Mean frequency score of carpal tunnel syndrome symptoms.

		Month 1	Month 6	Month 9
Numbness	VU (n=16)	3.1 ± 1.3	2.8 ± 1.6	3.0 ± 1.2
	NU (n=14)	[a] 1.8 ± 1.7	3.1 ± 1.4	[a] 1.6 ± 1.5
Tingling	VU	[a] 2.5 ± 1.4	2.6 ± 1.7	[a] 2.8 ± 1.1
	NU	1.3 ± 1.8	2.7 ± 1.3	1.4 ± 1.6
Awakening	VU	1.8 ± 1.8	1.6 ± 1.8	1.5 ± 1.6
	NU	1.3 ± 1.7	2.1 ± 1.6	.86 ± 1.5
Pain	VU	[a] 1.9 ± 1.8	[a] 2.3 ± 1.8	[a] 1.5 ± 1.7
	NU	[a] .86 ± 1.4	[a] 1.2 ± 1.8	[a] .21 ± .80
Tightness	VU	[a] 2.1 ± 1.8	1.9 ± 2.0	[a] 1.9 ± 1.7
	NU	.92 ± 1.5	1.7 ± 1.8	.36 ± .84
Clumsiness	VU	1.3 ± 1.5	1.6 ± 1.7	1.3 ± 1.6
	NU	.78 ± 1.4	1.4 ± 1.7	.71 ± 1.3

All values are means and standard deviations; (a) means significantly different between the groups.
Frequency scores are based on a five point scale – (0) never, (1) monthly, (2) biweekly, (3) weekly, (4) daily.
VU – vitamin user; NU – non-vitamin user.

Table 14. Mean intensity score of carpal tunnel syndrome symptoms.

		Month 1	Month 6	Month 9
Pain	VU (n=16)	9.7 ± 2.6	[a] 10.2 ± 3.6	[a] 9.8 ± 2.5
	NU (n=14)	8.5 ± 2.8	7.6 ± 3.0	7.6 ± 4.1
Numbness	VU	12.4 ± 3.3	[a] 12.7 ± 9.9	[a] 13.3 ± 3.0
	NU	12.0 ± 3.5	10.5 ± 3.5	10.1 ± 3.7
Activity	VU	[a] 13.4 ± 4.7	[a] 13.6 ± 6.4	11.8 ± 3.3
	NU	10.1 ± 2.0	9.9 ± 1.9	12.8 ± 4.1

All values are means and standard deviations; (a) means significantly different between groups.

or night; non-vitamin users experience mild numbness. Lastly, impaired ability to carry out certain activities (e.g. writing, gripping items, buttoning) was more difficult for vitamin users than it was for non-vitamin users. On average, vitamin users rated their difficulty with these activities as moderate whereas non-vitamin users complained of mild difficulty.

Regression analysis of the symptom data (log transformed) with the three vitamin B6 status indicators (plasma PLP, total plasma vitamin B6 and erythrocyte PLP) found pain to be negatively correlated with plasma PLP in both groups. The statistical significance of this relationship was much stronger in vitamin users. In this group, the correlation between plasma PLP and pain was statistically significant at month one ($r = -0.58$, $p<0.01$) and month nine ($r = -0.34$, $p<0.01$). Although a negative correlation between pain and plasma PLP concentrations of vitamin users was also seen at month six ($r = -0.32$), it was not statistically significant. Plasma PLP concentrations of non-vitamin users were also negatively correlated with pain at each timepoint ($r = -0.26, -0.42, -0.37$ at month one, six and nine, respectively). However, none of the correlations reach statistical significance. In vitamin users, pain was also found to be negatively correlated with both total plasma vitamin B6 ($p<0.003$) and erythrocyte PLP ($p<0.03$). Total plasma vitamin B6 was found to be negatively correlated to numbness ($p<0.05$) in vitamin users. No other correlations were found in non-vitamin users.

Figure 8. Mean pain intensity over time.

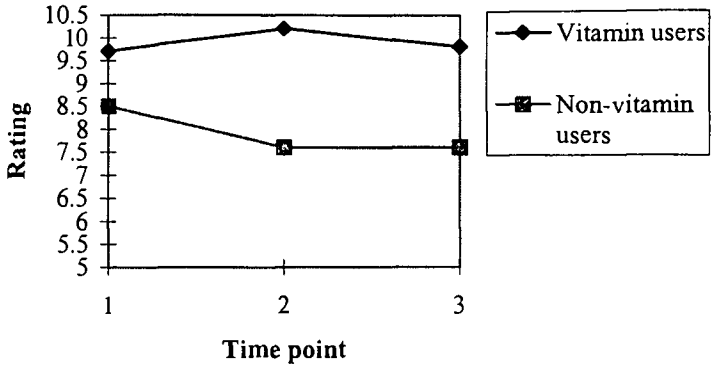


Figure 9. Mean numbness intensity over time.

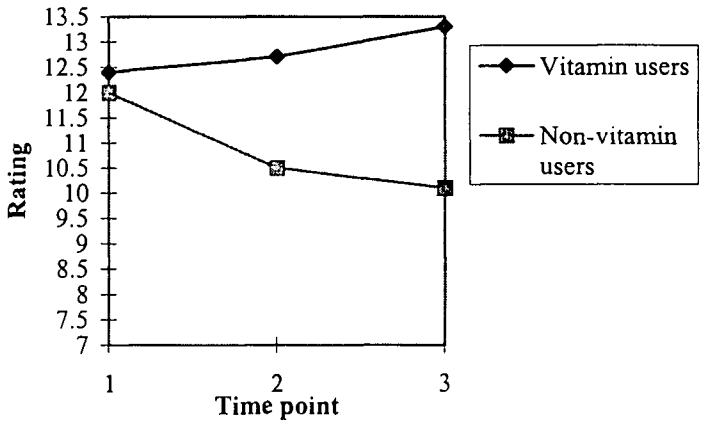
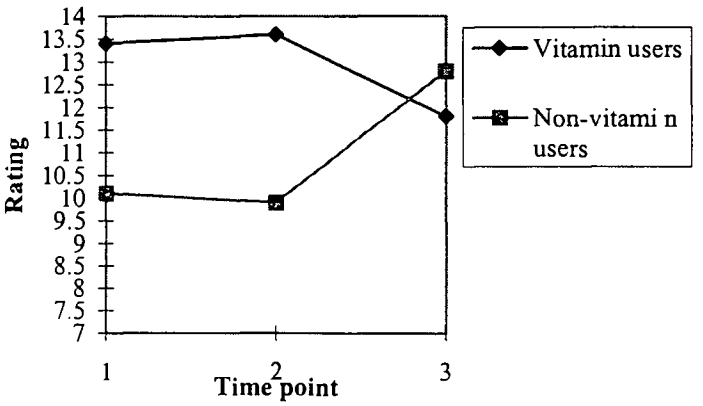


Figure 10. Mean activity impairment over time.



EXERCISE

All 30 subjects participated in the exercise sessions at the study onset. By midpoint only 20 individuals came on a regular basis; this number dropped to 15 by the end of the study. Several of the subjects requested suggestions for home exercise. However, compliance to home exercise was not closely monitored so it is difficult to know how many individuals actually completed the assigned activities. No significant change was seen in body weight in either group; body weight did not significantly differ between vitamin and non-vitamin users. However, both vitamin users and non-vitamin users saw a significant increase in VO_2 max between study onset and study conclusion ($p<0.05$). The mean VO_2 max for vitamin users increased from 2.3 ± 4.7 L/O_2 to 2.5 ± 5.3 L/O_2; the VO_2 max of non-vitamin users increased from 2.5 ± 5.8 L/O_2 to 2.7 ± 6.7 L/O_2. Among the vitamin users, nine subjects had a change in maximal oxygen uptake of ten percent or more, five had a change between zero and four percent and two saw a decline in VO_2 max. A similar trend was seen in non-vitamin users. Nine non-users increase their VO_2 max by at least 10%. Four subjects increased their VO_2 max between zero and four percent; one subject experienced a decrease. Maximal oxygen uptake was not significantly different between the two groups at either time point. Non-vitamin users also saw a significant decrease in percent body fat over the nine months ($p<0.01$). Eleven of the non-vitamin users had a decrease in body fat, one subject had no change in body composition and two subjects experienced an increase in percent body fat. Overall, the mean percent body fat of non-users decreased from 31.6 ± 6.6 to 28.8 ± 5.6 of total body mass. No significant change in body composition was seen in vitamin users.

In vitamin users, body weight was positively and significantly correlated with the intensity of pain and numbness ($p<0.01$) and negatively correlated with the frequency of nocturnal awakening and tightness ($p<0.05$). A negative correlation between body weight and pain intensity ($p<0.009$) and pain frequency ($p<0.05$) was found in non-vitamin users. No significant correlation between body weight and frequency of nocturnal awakening or tightness was seen in non-vitamin users.

NERVE CONDUCTION STUDIES

No correlations were found between the mean maximal latency difference (MLD) in the right hand or the MLD in the left hand and plasma PLP, total plasma vitamin B6, erythrocyte PLP and body weight in either group. Combining theMLD's for both hands did not result in any significant correlations. The maximal latency difference did not significantly change in either group over the nine month period (refer toTable 15). Mean combined MLD's for vitamin users showed a steady increase. Values jumped 17% between month one and six and then another 2% between month six and nine. Values for non-vitamin users also showed a continual increase between all three time points. However, the changes were not as pronounced. A marginal 1% increase was seen between month one and six. Values increased another 4% between month six and nine. Vitamin users had significantly higher MLD values for the left hand when compared to non-vitamin users. No other significant differences in MLD were found between the groups.

Table 15. Mean maximal latency difference.

		Month 1	Month 6	Month 9
VU	right	.53 ± .19	.51 ± .22	.52 ± .25
(n=16)	left	[a] .53 ± .24	[a] .52 ± .18	[a] .50 ± .16
	combined	.82 ± .36	.98 ± .35	1.0 ± .35
NU	right	.52 ± .23	.55 ± .18	.57 ± .21
(n=14)	left	.38 ± .08	.39 ± .08	.39 ± .08
	combined	.90 ± .21	.91 ± .27	.95 ± .27

All values are means and SD;

(a) means significantly different between groups ($p<0.05$).

VU – vitamin users; NU – non-vitamin users

DISCUSSION

Many of the vitamin and mineral concentrations in the blood vary over time. No studies have been done to determine if plasma PLP, total plasma vitamin B6 or erythrocyte PLP follow a similar pattern of fluctuation. Knowledge of these effects could be useful when setting the Recommended Dietary Allowance for the population. This study provided a unique opportunity to determine if changes occur in the plasma concentration of vitamin B6 indices by monitoring two groups of individuals with carpal tunnel syndrome – one group of vitamin users and one group of non-vitamin users. The data indicate that B6 vitamers show little fluctuation over time.

In this study, the time of year did not seem to affect the concentration of the B6 vitamers. No significant change in mean plasma PLP, total plasma vitamin B6 and erythrocyte PLP was seen in either group over the nine month period. The mean plasma PLP concentration of the vitamin users was almost two fold higher at the study midpoint when compared to the prestudy mean concentration. However, since plasma PLP concentrations decreased by the conclusion of the study, the overall change was not significant. The fluctuations seen in plasma PLP concentration are most likely a reflection of two factors: the amount of time that elapsed between supplement intake and the blood draw, and, secondly, the amount of vitamin B6 in the supplement. For example, prior to the first and last blood draw, the majority of vitamin users had taken a supplement three to five days before the draw. Prior to the second blood draw, however, the majority of vitamin users had taken a supplement the day before the blood draw. Because a supplement was taken only one day before the blood draw, plasma PLP concentrations would be higher. If timing of intake is not considered, it could be falsely

concluded that seasonal influences were the cause of the higher plasma PLP concentration seen at study midpoint. In addition to when the supplement was taken, changes in supplement dosage by some subjects also contributed to the variation. No effort was made in this study to influence vitamin B6 intake (either increase or decrease), so subjects were able to change their intake of vitamin B6 throughout the course of the study. For example, during the middle of the study, three subjects, compared to one subject at study onset, were taking 100 mg vitamin B6/day. Because blood levels of the vitamers are directly related to the amount of vitamin B6 in the supplement, the more subjects ingesting a higher amount of vitamin B6, the greater the mean plasma PLP concentrations would be. In a study that looked at the mean plasma PLP concentration in individuals taking a range of supplements of 1.0 to 62 mg vitamin B6/day, Shultz and Leklem (1985) found that a change in supplement intake from 15 to 25 mg/day increased plasma PLP concentration from 160.5 $\pm$ 63.3 nmol/L to 256.8 $\pm$ 162.8 nmol/L. With a 5 mg/day supplement mean plasma PLP concentration was 125 $\pm$ 38 nmol/L. In individuals taking 2.2 mg/day, mean plasma PLP concentration was 74 $\pm$ 25 nmol/L. Since both timing of supplement intake and supplement dosage could explain the variation seen in mean plasma PLP concentrations, it appears as though vitamin B6 shows little variation over time. In comparison to vitamin users, non-vitamin users had a slight but insignificant increase in mean plasma PLP concentration at the last two time points. The increase in mean plasma PLP concentrations of non-vitamin users is reflected by the number of subjects who had plasma PLP concentrations below the suggested 30 nmol/L. At the first time point, nine subjects were below the recommended value. The number dropped to seven at the second time point and five at the third time point.

The concentration of mean total plasma vitamin B6 followed the same pattern as plasma PLP – vitamin users experienced a large increase in total plasma vitamin B6 concentration followed by a modest decrease; non-vitamin users experienced an increase in mean total plasma vitamin B6 concentration only between study midpoint and end. In both groups, the fluctuations in total plasma vitamin B6 concentration were not as dramatic as the changes seen with plasma PLP concentration. It is possible that changes seen in total plasma vitamin B6 concentration are less than those seen in plasma PLP since PLP is only one component of total vitamin B6. For example, although there is no evidence to support this, PL, the other main component of total vitamin B6, may by less susceptible to changes in vitamin B6 intake.

Erythrocyte PLP showed the least fluctuation of all status measures. Mean erythrocyte PLP concentration in vitamin users increased by 20 % between study onset and midpoint but then dropped back down to prestudy concentrations by the conclusion of the study. In non-vitamin users, erythrocyte PLP concentration showed a marginal increase between study onset and midpoint and then plateaued. Erythrocyte PLP, in vitamin users, was found to be significantly correlated with plasma PLP. This correlation, not seen in vitamin users, may be due to a broader range of erythrocyte and plasma PLP values in vitamin users when compared to those of non-vitamin users. As a result of this wide range, the possibility of finding a significant correlation is increased. Furthermore, the correlation between plasma and erythrocyte PLP may be caused by the high intake of PN of vitamin users. It has been shown that PN, the form of vitamin B6 found in supplements, is rapidly taken up by the erythrocyte and then converted to PLP (Anderson et al., 1989). It is unlikely that non-vitamin users, eating a normal mixed diet,

would be able to ingest as much PN as the vitamin users. As a result, non-vitamin users would have a lower concentration of PN entering the erythrocyte and, consequently, not as much PLP synthesis would occur in the red cell.

No study has directly addressed the possibility of seasonal fluctuations in vitamin B6 status. However, the few studies that have looked at the influence of circadian rhythm on vitamin B6 status, have found no significant changes in vitamin B6 concentrations over the 24 hour period. For example, Leinert et al. (1983) looked at changes in the concentration of serum PLP in eight males over a 24 hour period (6 samples/person). Total vitamin B6 intake for the day was 2 mg. Although a large interindividual variation was found (24.3-64.7 nmol/L), intraindividual PLP concentration did not significantly differ. Similar results were found by Mascher et al. (1993) in a group of ten females. Although the results of Leinart et al. and Mascher are only for a 24 hour period, if looked at in conjunction with the results of this study it is fairly safe to hypothesize that B6 vitamers show no significant fluctuations over time. This conclusion is further supported when you look at the other major factors that can influence vitamin B6 concentrations -- diet, alkaline phosphatase activity and storage. It has been shown that blood concentrations of retinol and ascorbic acid vary with the seasons (Winklhofer-Roob et al., 1997). Individuals have significantly higher concentrations of retionol and ascorbic acid during the spring and summer. The increase was attributed to an increased availability of foods during these seasons (i.e fresh fruits and vegetables) that contain vitamin A and vitamin C. In comparison, the major food sources of vitamin B6 in the diet - animal products, potato products and fortified cereals - do not exhibit seasonal variation (Kant and Block, 1990). As a result, it is unlikely that significant seasonal changes in vitamin

B6 status would occur. Another factor that has been shown to influence vitamin B6 concentrations by increasing the conversion of PLP to PL is alkaline phosphatase activity (Whyte et al. 1988). Alkaline phosphatase does exhibit seasonal variation, with a significantly higher activity seen in the summer when compared to the winter (Devgun et al., 1981). Theoretically, the increased activity could influence vitamin B6 status by decreasing plasma PLP concentrations. In the present study, no significant differences were seen in alkaline phosphatase activity between the seasons. The small number of subjects in this study is most likely the cause for the lack of agreement between this study and those that have demonstrated seasonal variation in alkaline phosphatase activity. The ability of vitamin B6 to be stored in the muscle may be another possible reason that no statistically significant difference in vitamin B6 status was seen over time. Vitamin B6 supplementation has been shown to increase the pool of muscle B6. For example, Black et al. (1977) found that the amount of vitamin B6 in rat muscle increased in response to high intakes of the vitamin. The same response was found in humans given 160 mg vitamin B6/day. Researchers found total vitamin B6 in the muscle to increase by 25 % (Coburn et al., 1991). With these "storage" mechanisms in place, changes in plasma concentration of B6 vitamers may be minimized. Lastly, exercise has been shown to have an impact on B6 vitamer concentrations. However, the affects are acute and no studies have found long term changes in vitamin B6 status as a result of exercise. For example, Manore et al. (1987) who looked at the effect of exercise on vitamin B6 metabolism in women found no significant difference in vitamin B6 status indicators between untrained and trained women.

As expected, the mean concentrations of all the B6 vitamers were significantly higher in vitamin users when compared to non-vitamin users at all three time points. Plasma PLP and total plasma vitamin B6 concentrations were three times higher in vitamin users; erythrocyte PLP concentrations were two times higher. The range of values calculated for plasma PLP, total plasma vitamin B6 and erythrocyte PLP for each group agrees with that reported in the literature. Shultz and Leklem (1985) found vitamin users taking a mean dose of 5 mg vitamin B6/day had plasma PLP concentrations between 68.4 nmol/L and 148.1 nmol/L. Plasma PLP concentrations of individuals with an average intake of 40 mg/day ranged from 94.3 nmol/L to 201.4 nmol/L. In comparison, non-vitamin users consuming a normal mixed diet, had a plasma PLP concentration anywhere from 20 nmol/L to 80 nmol/L (Reynolds, 1995). Kretsch et al. (1995) determined the plasma PLP concentration in young women consuming a known amount of vitamin B6. Subjects consuming 1.5 mg vitamin B6/day had an average plasma PLP concentration of 30 nmol/L. Intakes of 1.0 mg/day corresponded to a plasma PLP concentration of 19 nmol/L. Hansen et al. (1996b) found erythrocyte PLP and total plasma vitamin B6 concentrations, in individuals consuming an average of 1.5 mg vitamin B6/day, to range between 40-50 nmol/L and 40-46 nmol/L, respectively. The plasma PLP concentrations given above are for healthy individuals.

When dealing with persons who have CTS, the question has to be asked - does this condition influence their B6 vitamer concentration? Early work in the area of vitamin B6 and CTS suggested that a low vitamin B6 status caused CTS. However, since transaminase activity was the only status measure used and the methodology used was not well described, it is difficult to compare results on vitamin B6 status in subjects with

CTS. However, in the few studies that have used plasma PLP as a status measure in individuals with CTS, mean plasma PLP concentration is above the recommended 30 nmol/L. For example, Driskell et al. (1985) found subjects with CTS to have a mean plasma PLP concentration of 52.6 $\pm$ 8.1 nmol/L. Similarly, Keniston and colleagues (1997) found no significant difference in the plasma PLP concentration of the control group and the group with CTS (38.7$\pm$16.5 nmol/L versus 38.8$\pm$19.1 nmol/L). Franzblau et al. (1996) also found that in a group of individuals with CTS, some taking supplements and some not, the mean plasma PLP concentration was 128.0 $\pm$ 81.1 nmol/L. Because no studies that have looked at vitamin B6 status in individuals with CTS have included total plasma vitamin B6 or erythrocyte PLP concentrations as status measures the results found can not be compared.

As stated previously, mean plasma alkaline phosphatase activity was not found to significantly change over time. However, activity was found to be higher in non-vitamin users when compared to vitamin users. The reason for this finding is uncertain. Theoretically, the higher alkaline phosphatase activity of the non-vitamin users could result in lower plasma PLP concentrations. It is difficult to conclude that plasma PLP concentrations were affected since no activity values were outside the normal range and since there was no significant correlation between alkaline phosphatase activity and plasma PLP concentration. No studies have looked at plasma alkaline phosphatase activity in individuals with CTS so results cannot be confirmed. Body weight could have also influence plasma alkaline phosphatase activity since overweight individuals have been shown to have a higher plasma alkaline phosphatase activity when compared to normal controls (Golik et al., 1991). However, in this study, body weight was not

significantly different between vitamin and non-vitamin users. In addition, plasma alkaline phosphatase activity was not significantly correlated with body weight in either group. Further studies will be required to determine if this is a consistent difference and if so, what is responsible for the difference.

Based on the knowledge of vitamin B6's role in the nervous system and neurotransmitter synthesis, this study also attempted to determine if vitamin B6 status has an influence on the frequency and perception of neurological symptoms associated with CTS. Theoretically, the higher the blood concentration of B6 vitamers, the less frequent and the less intense the symptoms. This idea is partially supported by the data.

The relationship between plasma PLP concentration and CTS symptoms found in this study is similar to that seen in other studies. The data presented in this study and in others (Kasdan et al., 1987; Guzman et al., 1989; Berstein and Dinesen, 1993; Keniston et al., 1997; Amadio, 1985; Salkheld et al., 1985; Tredici et al., 1985; Driskell et al, 1985) suggest that higher vitamin B6 concentrations (namely plasma PLP) are associated with less intense and less frequent symptoms. For example, Keniston et al. (1997) found that in a group of industrial workers (n=441) individuals with a higher plasma PLP concentration had a significantly lower incidence of pain and tingling. A study by Franzblau et al. (1996), which also looked at plasma PLP concentrations in industrial workers and the possible relation to CTS, did not find a significant correlation between CTS symptoms and vitamin B6. However, the plasma PLP values they reported were not normally distributed and should have been logged transformed. Because no transformation was done, the large standard deviation would minimize the likelihood of finding any significant correlations. In addition, the data indicate that some subjects were

taking vitamins. Because of this, the data may have been better presented if subjects were divided into vitamin and non-vitamin users. No other studies have looked at the relationship between vitamin B6 indices and CTS symptoms in free living subjects. However, studies that have looked at the relationship between vitamin B6 status and symptom intensity following supplementation support the results found in this study. For example, Bernstein and Dinesen (1993) found that subjects supplemented with 200 mg vitamin B6/day for three months had a significant decrease in pain intensity. Similar results were found by Driskell et al. (1985) who found that 97% of the individuals (n=28) treated with vitamin B6 experienced an improvement in symptom intensity.

The decreased intensity of pain associated with a higher plasma concentration of vitamin B6 (PLP) suggest that the relationship between vitamin B6 and pain may be related to the role of PLP in the synthesis of serotonin (Carstens et al., 1981; Willer et al., 1984; Dakinshimutri, 1990) and GABA (Roberts, 1963; Bayoumi and Smith, 1972), both neurotransmitters associated with analgesia . The decarboxylase enzymes that catalyze the synthesis of these neurotransmitters have an absolute requirement for PLP (Dakshinamurti, 1990). Numerous studies have shown that a vitamin B6 deficiency results in decreased enzyme activity and therefore reduced concentrations of serotonin and GABA (Roberts and Frankel, 1990). Conversely, both animal and human studies have demonstrated that vitamin B6 supplementation can increase serotonin and GABA synthesis and result in decreased pain. Fu et al. (1990) found that rats pretreated with vitamin B6 (as pyridoxine) for 7 days exhibited an increased tolerance for noxious stimuli. This affect was attributed to an increase in serotonin synthesis caused by the vitamin B6 supplementation. Similarly in humans, vitamin B6 supplementation (300 mg

PN-HCL/day for 10 days) has been shown to reduce pain associated with painful vertebral syndrome (Bruggermann et al., 1990). As in the animal studies, the decreased pain was attributed to an upregulation in serotonin synthesis. The inhibitory neurotransmitter GABA, is also increased under conditions of a high vitamin B6 intake. For example, Roberts et al. (1985) found that glutamic acid decarboxylase activity was three times higher in vitamin B6 supplemented rats when compared to normal controls. In addition, Ignatov and Andreev (1992) demonstrated that chronic administration of GABA stimulating drugs increased rats resistance to pain. Grafova et al. (1994) found administration of sodium valoprate, a compound that significantly increases GABA concentrations to substantially reduce manifestations of phantom pain syndrome in humans.

In addition to plasma PLP, total plasma vitamin B6 and erythrocyte PLP in vitamin users was significantly correlated with a decreased incidence of pain. That this correlation was not found in non-vitamin users may again reflect that a narrow range of values (as seen in non-vitamin users) makes significant correlations less likely when compared to a broader range of values (as seen in vitamin users). Secondly, in vitamin users, plasma PLP comprised a larger percentage of total plasma vitamin B6. Plasma PLP was correlated with pain and plasma PL (defined as the difference between total plasma vitamin B6 and plasma PLP) was not. It is possible that the increased percentage of PLP in vitamin users was enough to make the correlation between total plasma vitamin B6 and pain significant.

None of the vitamin B6 indices were found to significantly correlate with tightness, tingling, clumsiness or nocturnal awakening. The small sample size may have

contributed. However, the lack of a significant correlation may also be due to tightness, tingling, clumsiness and nocturnal awakening being influenced by one of the other numerous neurotransmitters in the body. While vitamin B6 is necessary for synthesis of neurotranmitters other than serotonin and GABA, blood concentrations of vitamin B6 do not necessarily affect synthesis of other neurotransmitters as much. For example, taurine, norepinephrine and dopamine also require vitamin B6 for synthesis. However, the PLP dependent enzymes that catalyze their synthesis have a much higher affinity for PLP. As a result, alterations in vitamin B6 status have little or no influence on the concentration of these neurotransmitters (Dakshinamurti, 1985). The amount of vitamin B6 ingested by subjects in this study may be another factor that contributed to the lack of correlation with other symptoms. No supplementation intervention was done. As a result, the intake of vitamin B6 was much lower when compared to other studies that have found a significant decrease in symptoms other than pain. Subjects in this study were taking a median dose of 4 mg/day compared to the 150-300 mg/day reported in other studies. Kasdan et al. (1987) found that supplementation of 200 mg vitamin B6/day for six months reduced the severity of tingling and nocturnal awakening. Similarly, Amadio (1985) demonstrated that 100 mg vitamin B6/day for 12 weeks could reduce tingling in some individuals. It is possible that increased synthesis of other neurotranmitters that influence tightness or clumsiness are seen only at the higher level of supplementation. A time discrepancy between symptom data collection and the blood draw could have been influential as well. Every effort was made to collect symptom data the same day as the blood draw. However, because of scheduling conflicts, this was not always possible. As a result,

vitamin B6 concentrations and symptom intensity and frequency are not perfectly matched.

A paradoxal relationship was found in this study. Vitamin users, who had the highest concentration of vitamin B6, also had the highest frequency and intensity of symptoms when compared to non-vitamin users. This finding may be the result of "self-help" treatments. The possibility of the use of vitamin B6 supplements to lessen the severity of CTS has been touted by the popular press. It is quite likely that many of the vitamin users in this study have heard of this connection and were using vitamin supplementation as one method to treat CTS. Body weight may be another factor that influenced symptom intensity between the groups. Overweight individuals appear to have more severe symptoms when compared to normal weight subjects. This relationship was demonstrated by Nathan et al. (1992) who found that overweight subjects were more likely to develop CTS and have more intense symptoms. Werner et al. (1994) found a similar relationship. Subjects with CTS had a significantly higher BMI. For example, when slender indivduals (BMI<20) were compared to obese individuals (BMI>29), it was found that the obese subjects were 2.5 times has likely to develop CTS. However, in the present study, body weight was not significantly different between the groups nor was it correlated with symptoms intensity or frequency.

This study provided no evidence that CTS was caused by inadequate B6 vitamer concentrations or that vitamin B6 was able to cure CTS. Although this conclusion is directly opposite to the numerous studies published by Ellis and colleagues (Ellis et al., 1976; Ellis et al., 1977; Ellis et al., 1979; Ellis et al., 1980; Ellis et al., 1981; Ellis et al., 1982), it is supported by the results of several research groups that have shown

individuals with CTS to have normal vitamin B6 levels and that vitamin B6 has no significant relationship with nerve conduction. In the twelve studies (Ellis et al. excluded) that have looked at vitamin B6 status in individuals with CTS all but Fuhr et al. (1989) found that vitamin B6 concentrations in individuals with CTS did not significantly differ from the B6 vitamer concentrations of healthy individuals. As was hypothesized by Ellis et al., the results by Fuhr et al. suggested that individuals with CTS have impaired vitamin B6 status. However, the methods used to determine plasma PLP were incorrect and resulted in abnormally low PLP concentrations. No studies to date have been able to support the theory of Ellis and colleagues that vitamin B6 supplementation will improve nerve conduction in subjects with CTS. For example, Bernstein and Dinesen (1993) found no improvement in nerve conduction following vitamin B6 supplementation. The same conclusion was drawn by Tredici et al. (1985) and Smith et al. (1984). Neither research group found vitamin B6 supplementation to elicit changes in the conduction of the median nerve. Lastly, when standardized electrodiagnostic criteria and erythrocyte aspartate aminotransferase were used to asses the relationship between CTS and vitamin B6 status, Byers et al. (1984) found that peripheral neuropathy rather than CTS was highly correlated with inadequate vitamin B6 status. As stated previously, Ellis and colleagues suggested that a low vitamin B6 status caused CTS. In this study, comparison of blood vitamer concentrations with recommended levels showed that five to nine non-vitamin users had plasma PLP concentrations below the suggested 30 nmol/L over the three time points. However, this finding should not be interpreted to mean that the inadequate vitamin B6 status was the cause of or was caused by CTS. Vitamin B6 is a problem nutrient for many individuals and plasma PLP values below the suggested

concentration are not uncommon in healthy individuals consuming an unsupplemented normal mixed diet.

The finding that CTS cannot necessarily cure CTS makes sense physiologically. Carpal tunnel syndrome is similar to a pinched nerve. The syndrome results when the carpal tunnel becomes smaller due to edema, trauma or other factors. As a result, the nerve becomes compressed and individuals experience a variety of neurological problems including pain and numbness. Vitamin B6 cannot necessarily cure the trauma or increase the size of the carpal tunnel. Rather, it appears to be able to alter the pain signals that are transmitted to the brain by influencing serotonin and GABA synthesis.

Vitamin B6 status did appear to affect the intensity of pain felt by individuals with CTS. However, when you consider that vitamin users, who had the highest concentration of vitamin B6, also had the highest symptoms scores, you have to ask about the efficacy and usefulness of using vitamin B6 to treat CTS. This consideration is also raised when you notice that several of the vitamin users who have been using supplements for many years are still troubled by severe symptoms. This study, as have others, demonstrated that vitamin B6, like all therapies, is only helpful to some individuals. This same conclusion was drawn by Kasdan et al. (1987) who found that vitamin B6 supplementation decreased symptoms in 68% of the subjects and Amadio (1985) who found vitamin B6 supplementation helped approximately 30% of the subjects.

Many other uncontrollable factors could have confounded the results. For example, some subjects may have figured out that the study was designed to determine the relationship between vitamin B6 status and CTS. As a result, some subjects may have altered their diet or supplement usage. In addition, any time you have self-reported data,

there is always room for error. For example, if an individuals symptoms were especially severe the day the questionnaire was completed, the subject may have over generalized by indicating that symptoms were always so intense. Conversely, if symptoms were not present the day the questionnaire was given, the answers may tend to indicated that symptoms are relatively infrequent and fairly mild. Furthermore, some subjects were using pain relievers throughout the course of the study which may have minimized their symptoms when compared to those subject who were not taking any pain medications. Lastly, it must be kept in mind that CTS is a syndrome and because of the nature of a syndrome, it is almost impossible to control all confounding factors.

In conclusion, the B6 vitamer data and the pain data suggest that vitamin B6 may be useful for some individuals in lessening the intensity of symptoms associated with CTS. This is especially true for pain. Although there were no statistically significant effects of B6 vitamer concentration on the frequency of symptoms and on the intensity of certain symptoms, a general trend was observed -- the higher a subject's vitamin B6 concentrations, the less intense and the less frequent the symptoms. The trends may have reached statistical significance had there been a greater number of subjects. No significant correlation was found between vitamin B6 and nerve conduction indicating that individuals with CTS should not rely on vitamin B6 as a method to cure the syndrome.

As a result of this study, there are still several unanswered questions -- should additional research be conducted, and, if so, how can the methodology be better designed to more effectively test the possible role of vitamin B6 on CTS symptomology? One of the limitations in the study of CTS is that research efforts are complicated by the

numerous factors that make up a syndrome. If future studies in the area of vitamin B6 and CTS are to continue, a few modifications to the design are needed. First of all, the vitamin B6 content of the diet must be controlled by feeding pre-prepared meals that contain a known amount of vitamin B6. Secondly, subjects should be divided into two groups, one receiving a placebo and one group receiving 150-300 mg vitamin B6/day for three months. In addition, it is important that every effort is made to collect symptom data the same day as the blood draw. Having subjects keep a daily log of symptom intensity and symptoms frequency may be useful in reducing the amount of over generalizing that occurs.

It is hoped that the findings of this study will not only stimulate additional vitamin B6/CTS research, but that future research will address the relationship between vitamin B6 and neurotransmitters. Concrete knowledge of this relationship would be useful in many conditions. Based on animal data, both vitamin B6 depletion and supplementation can significantly alter neurotransmitter synthesis. Currently, pharmacological doses of vitamin B6 are being used in the treatment of a variety of conditions. However, the clinical significance of vitamin B6 in relationship to CTS and neurotransmitters is not fully established. With the limited data available in this area and with an improvement in the methods used to accurately diagnose an individual with CTS, it is recommended that future studies investigate the relationship between CTS and vitamin B6.

SUMMARY AND CONCLUSIONS

It was hypothesized that vitamin B6 status would not significantly change over time and that high vitamin B6 concentrations would be negatively correlated with CTS symptom intensity and frequency. The objectives of the study were: 1) to monitor the stability of vitamin B6 status over a nine month period by determining plasma PLP, total plasma vitamin B6 and erythrocyte PLP; 2) to determine if a significant correlation exists between carpal tunnel symptoms (e.g. pain, numbness, tingling) and plasma PLP, total plasma vitamin B6, and erythrocyte PLP; 3) to determine if vitamin users have a significantly lower incidence of reported carpal tunnel symptoms when compared to non-vitamin users, and; 4) to determine if a significant correlation exists between nerve conduction and plasma PLP, total plasma vitamin B6 and erythrocyte PLP.

Changes in vitamin B6 status and its relationship to CTS were analyzed in 30 individuals with CTS - 16 vitamin users and 14 non-vitamin users. Blood samples, following an overnight fast, were taken at study onset, midpoint and conclusion. Health questionnaires addressing supplement used and symptoms questionnaires asking about specific types of symptoms and their frequency and intensity were also given at this time. No effort was made to influence vitamin B6 intake. In order to determine if any significant changes occur in vitamin B6 status over time, blood was analyzed for plasma PLP, total plasma vitamin B6 and erythrocyte PLP. The relationship between vitamin B6 status and CTS symptoms was determined by comparing vitamin B6 indices with CTS symptom frequency and intensity. Plasma alkaline phosphatase activity was also determined.

Standard statistical methods were used to analyze the data. These included a paired t-test, analysis of variance (ANOVA) and correlation coefficients (r). Plasma PLP, total plasma vitamin B6 and erythrocyte PLP were log transformed to normalize the data. A p value of <0.05 was considered statistically significant.

Plasma PLP, total plasma vitamin B6, erythrocyte PLP and plasma alkaline phosphatase activity did not differ significantly within groups. Vitamin users had significantly higher concentration of all the vitamin B6 status measures ($p<0.01$) when compared to vitamin users. Non-vitamin users had a significantly higher plasma alkaline phosphatase activity ($p<0.03$). Symptom intensity and frequency was significantly higher in vitamin users when compared to non-vitamin users ($p<0.01$). This somewhat paradoxal finding possibly relates to the knowledge of some of the vitamin users that vitamin B6 has been used in the treatment of CTS. In vitamin users, plasma PLP was significantly and negatively correlated with a decreased intensity of pain at timepoints one ($r = -0.58$, $p<0.01$) and three ($r = -0.34$, $p<0.01$). A negative, but non-significant correlation ($r = -0.32$) was seen been pain and plasma of vitamin users at time point two. In non-vitamin users, pain intensity was also negatively correlated with plasma PLP ($r = -0.26$, -0.42 and -0.37 at time points one, two and three, respectively). However, this correlation did not reach statistical significance. The inverse relationship between plasma PLP and pain possibly reflects the necessity of PLP in the synthesis of serotonin and GABA. Both are neurotransmitters associated with analgesia.

The results suggest that a high intake of vitamin B6 can decrease the intensity of CTS symptoms, namely pain. However, the effect is highly variable among individuals

and does not consistently improve the symptom profile. In addition, vitamin B6 appears to have no ability to improve conduction of the median nerve.

BIBLIOGRAPHY

Abraham GE and Hargrove JT. Effects of vitamin B6 on premenstrual symptomology. Infertility 1980;3:155-65.

Amadio PC. Pyridoxine as an adjunct in the treatment of carpal tunnel syndrome. J Hand Surg 1985;10A:237-41.

Amadio PC. Carpal tunnel syndrome, pyridoxine and the work place. J Hand Surg 1987;12A:875-79.

Anderson BB, Fulford-Jones CE, Child JA, Beard ME, Bateman CJT. Conversion of vitamin B-6 compounds to active forms in the red blood cells. J Clin Invest 1971;50:1901-09.

Anderson BB, O'Brien H, Griffin GE, Mollin DL, Hydrolysis of pyridoxal-5'-phosphate in plasma in conditions with raised alkaline phosphatase. Gut 1980;21:192-94.

Anderson BB, Perry GM, Clements JE, Greany MF. Rapid uptake and clearance of pyridoxine by red blood cells in vivo. Am J Clin Nutr 1989;50:1059-63.

Anderson BB, Perry GM, Modell CB, Child JA, Mollin DL. Abnormal red-cell metabolism of pyridoxine associated with beta-thalassaemia. Br J Haematol 1979;41:497-507.

Andon MB, Reynolds RD, Moser-Veillon PB, Howard MP. Dietary intake of total and glycosylated vitamin B-6 and the vitamin B-6 nutritional status of unsupplemented lactating women and their infants. Am J Clin Nutr 1989;50:1050-58.

Angel JF. Lipogenesis by hepatic and adipose tissues from meal-fed pyridoxine-deprived rats. Nutr Rept Int 1975;11:369-78.

Angel JF. Gluconeogenesis in meal-fed, vitamin B-6 deficient rats. J Nutr 1980;110:262-69.

Angel JF and Song G-W. Lipogenesis in pyridoxine-deficient nibbling and meal-fed rats. Nutr Rept Int 1973;8:393-403.

Axelrod AE and Trakatellis AC. Relationship of pyridoxine to immunological phenomenon. Vitamin Horm 1964;22:591-607.

Baker EM, Canham JE, Nunes WT, Sauberlich HE, McDowell ME. Vitamin B-6 requirement for adult men. Am J Clin Nutr 1964;15:59-66.

Barnard HC, DeKock JJ, Vermaak WJH, Potgieter GM. A new perspective in the assessment of vitamin B-6 nutritional status during pregnancy in humans. J Nutr 1987;117:1303-06.

Bartoszyk GD and Wild A. Antinociceptive effects of pyridoxine, thiamine, and cyanocobalamin in rats. Ann NY Acad Sci 1990;585:473-76.

Bauernfeind JC and Miller ON. Vitamin B-6: nutritional and pharmaceutical usage, stability, bioavailability, antagonist, and safety. In: Human Vitamin B-6 Requirements. Washington DC: National Academy of Science, 1978:78-110.

Beaton JR, Bearre JL , Beaton GH, Caldwel EF, Ozawa G, McHenry EW. Chronological sequence of biochemical defects in vitamin B6-deprived rats. J Biol Chem 1954;207:385-91.

Behbehani MM, Jiang MR, Chandler SD, Ennis M. The effect of GABA and its antagonists on midbrain periaqeductal gray neurons in the rat. Pain 1990;40:195-204.

Bender DA. Oestrogens and vitamin B6, actions and interactions. Wld Rev Nutr 1987;51:140-88.

Bender DA. Vitamin B6 requirements and recommendations. Eur J Clin Nutr 1989;43:289-309.

Bender DA, Bowden JF, Coulson WF, Dewji NR, Sutton J, Symes EK. Vitamin B6 deficiency enhances end-organ sensitivity to steroid hormones. In: Leklem JE and Reynolds RD, eds. Clinical and physiological applications of vitamin B6. New York, NY: Alan R. Liss Inc, 1987:45-9.

Benesch RE, Young S, Suzuki T, Bauer C, Benesch R. Pyridoxal compounds as specific reagents for the alpha and beta N-termini of hemoglobin. Proc Natl Acad Sci 1973;70:2595-99.

Bernstein AL and Dinesen JS. Brief communication: effect of pharmacological doses of vitamin B6 on carpal tunnel syndrome, electroencephalographic results, and pain. J Am Coll Nutr 1993;12:73-6.

Bernstein AL and Lobitz CS. A clinical and electrophysiologic study of the treatment of painful diabetic neuropathies with pyridoxine. In: Leklem JE and Reynolds RD, eds. Clinical and physiological applications of vitamin B6. New York, NY: Alan R Liss Inc, 1985:415-23.

Black AL, Guillard BM, Snell EE. Increased muscle phosphorylase in rats fed high levels of vitamin B-6. J Nutr 1977;107:1962-68.

Bonjour JP. Vitamins and alcoholism. III. Vitamin B6. Int J Vitam Nutr Res 1980;50:215-30.

Booth CC and Brain MC. The absorption of tritium-labeled pyridoxine hydrochloride in the rat. J Physiol 1962;164:282-94.

Bowden JF, Bender DA, Symes EK, Coulson WF. Increase uterine uptake and nuclear retention of [3H]oestradiol through the oestrous cycle and enhanced end-organ sensitivity to oestrogen stimulation in vitamin B6 deficient rats. J Ster Biochem 1986;25:359-65.

Brown RR, Rose DP, Leklem JE, Linswiler H, Amand R. Urinary pyridoxic acid, plasma pyridoxal phosphate and erythrocyte aminotransferase levels in oral contraceptive users receiving controlled intakes of vitamin B6. Am J Clin Nutr 1975;28:10-19.

Bruggemann G., Koehler CO, Koch EMW. The therapy of acute lumbago with diclofenac and B-vitamins; results of a double-blind clinical study. Klin Wochenschr 1990;68:116-20.

Byers CM, DeLisa JA, Frankel DL, Kraft GH. Pyridoxine metabolism in carpal tunnel syndrome with and without peripheral neuropathy. Arch Phys Med Rehabil 1984;65:712-16.

Canham JE, Baker EM, Harding RS, Sauberlich HE, Plough IC. Dietary protein - its relationship to vitamin B6 requirements and function. Ann NY Acad Sci 1969;166:16-29.

Chabner B and Livingston D. A simple enzymatic assay for pyridoxal phosphate. Anal Biochem 1970;34:413-25.

Chandra RK and Puri S. VitaminB-6 modulation of immune responses and infection. In: Reynolds RD and Leklem JE, eds. Vitamin B-6: Its role in health and disease. New York, NY: Alan R. Liss Inc, 1985:163-75.

Chastiotis D, Sahlin K, Hultman E. Regulation of glycogenolysis in human muscle at rest and during exercise. J Appl Physiol: Respirat Environ Exerc Physiol 1982;53:708-15.

Cho Y-O and Leklem JE. In vivo evidence for a vitamin B-6 requirement in carnitine synthesis. J Nutr 1990;120:258-65.

Cidlowski JA and Thansassi JW. Pyridoxal phosphate, a possible cofactor role in steroid hormone action. J Ster Biochem 1981;15:11-16.

Clarke R, Daly L, Robinson K, Naughten E, Cahalane S, Fowler B, Grahm W. Hyperhomocysteinemia: an independent risk factor for vascular disease. N Eng. J Med 1991;3:1149-55.

Coburn SP, Lewis DLN Fink WJ, Mahuren JD, Schaltenbrand WE, Costill DL. Human vitamin B-6 pools estimated through muscle biopsies. Am J Clin Nutr 1988;48:291-4.

Coburn SP and Whyte MP. Role of phosphatases in the regulation of vitamin B-6 metabolism in hypophosphatasia and other disorders. In: Leklem JE and Reynolds RD, eds. Clinical and physiological applications of vitamin B6. New York, NY: Alan R. Liss Inc, 1988:65-79.

Coburn SP, Ziegler FJ, Costill DL, Mahuren D, Fink WJ, Schaltenbrand WE, Pauly TA, Pearson DR, Conn PS, Guilarte TR. Response of vitamin B-6 content of muscle to changes in vitamin B-6 intake in men. Am J Clin Nutr 1991;53:1436-52.

Cori CF and Illingsworth B. The prosthetic group of phosphorylase. Proc Natl Acad Sci USA 1957;43:547-52.

Coursin DB. Convulsive seizures in infants with a pyridoxine-deficient diet. JAMA 1954;154:406-08.

Cunnane SC, Manku MS, Horrobin DF. Accumulation of linoleic and γ-linoleic acids in tissue lipids of pyridoxine-deficient rats. J Nutr 1984;114:1754-61.

Dakshinamurti K, LeBlancq WD, Herchl R, Havlicek V. Nonparallel changes in brain monoamines of pyridoxine-deficient growing rats. Exp Brain Res 1976;26:355-66.

Dakshinamurti K and Paulose CS. Consequences of decreased brain serotonin in the pyridoxine-deficient young rat. Prog Neuropsychopharmacol Biol Psychiatry 1983; 7:743-46.

Dakshinamurti K, Paulose CS and Siow YL. Neurobiology of pyridoxine. In: Leklem JE and Reynolds RD, eds. Vitamin B-6: Its role in health and disease. New York, NY: Alan R. Liss Inc, 1985:99-121.

Dakshinamurti K, Paulose CS, Viswanathan M, Siow YL, Sharma SK. Neurobiology of pyridoxine. Ann NY Acad Sci 1990;585:128-44.

Dakshinamurti K, Sharma SK, Bonke D. Influence of B vitamins on binding properties of serotonin receptors in the CNS of rats. Klin Wochenschr 1990;68:142-145.

Dakshinamurti K and Stephens MC. Pyridoxine deficiency in the neonatal rat. J Neurochem 1969;16:1515-22.

Dempsey WB and Christensen HN. The specific binding of pyridoxal 5'-phosphate to bovine plasma albumin. J Biol Chem 1962;237:111320.

Desikachar HSR and McHenry EW. Some effects of vitamin B6 deficiency on fat metabolism in the rat. Biochem J 1954;56:544-47.

Destito D, Canonico N, Tentori-Montalto T. Analgesic activity of vitamins B1, B6 and B12 in the painful vertebral syndrome. In: Gergershagen HU and Zimmermann M, eds. B-vitamins in pain. Hamburg, Germany: pmi verlag, 1987:30-41.

Devgun MS, Patterson CR, Martin BT. Seasonal changes in the activity of serum alkaline phosphatase. Enzyme 1981;26:301-05.

Dimpfel W, Spuler M, Bonke D. Influence of repeated vitamin B administration on the frequency pattern analyzed from rat brain electrical activity (tele-stero-EEG) Klin Wochenschr 1990;68:136-41.

Driskell JA, Wesley RL, Hess IE. Effectiveness of pyridoxine hydrochloride treatment on carpal tunnel syndrome patients. Nutr Rep Int 1986;34:1031-39.

Ebadi M and Klankgkalya B. On the mechanism of pyridoxal related convulsions as implicated in enhanced transport of GABA. Neuropharmacology 1979;18:301-07.

Ellis JM. Treatment of carpal tunnel syndrome with vitamin B6. South Med J 1987;80:882-84.

Ellis JM, Azuma J, Watanabe T, Folkers K, Lowell JR, Hurst GA, Ahn CH, Shuford EH, Ulrich RF. Survey and new data on treatment with pyridoxine of patients having a clinical syndrome including the carpal tunnel and other defects. Res Commun Pathol Pharmacol 1977;17:165-77.

Ellis JM and Folkers K. Clinical aspects of treatment of carpal tunnel syndrome with vitamin B6. Ann NY Acad Sci 1990;585:302-20.

Ellis JM, Folkers K, Levy M. Shizukuishi S, Lewandowski J, Nishii S, Schubert HA, Ulrich R. Response of vitamin B6 deficiency and the carpal tunnel syndrome to pyridoxine. Proc Natl Acad Sci 1982;79:7494-98.

Ellis JM, Folkers K, Levy M, Takemura K, Shizukuishi S, Ulrich R, Harrison P. Therapy with vitamin B6 with and without surgery for treatment of patients having the idiopathic carpal tunnel syndrome. Res Commun Chem Pathol Pharmacol 1981;33:331-44.

Ellis J, Folkers K, Watanabe T, Kaji M, Saji S, Caldwell JW, Temple CA, Woods FS. Clinical results of a cross-over treatment with pyridoxine and placebo of the carpal tunnel syndrome. Am J Clin Nutr 1979;32:2040-46.

Ellis JM, Kishi T, Azuma J, Folkers K. Vitamin B6 deficiency in patients with a clinical syndrome including the carpal tunnel defect. Biochemical and clinical response to therapy with pyrdoxine. Res Commun Chem Pathol Pharmacol 1976;13:743-57.

Fedde KN and Whyte MP. Alkaline phosphatase (tissue-nonspecific isoenzyme) is a phosphoethanolamine and pyridoxal-5'-phosphate ectophosphatase: normal and hypophosphatasia fibroblast study. Am J Hum Genet 1990;47:767-75.

Fibiger HC, Mertz PH, Campbell BA. The effect of *para*-chlorophenylalanine on aversion thresholds and reactivity to foot shock. Physiol Behav 1972;8:259-63.

Folkers K, Ellis J, Watanabe T, Saji S, Kaji M. Biochemical evidence for a deficiency of vitamin B6 in the carpal tunnel syndrome based on a cross-over clinical study. Proc Natl Acad Sci 1978;75:3410-12.

Fonda ML and Harker CW. Metabolism of pyridoxine and protein binding of the metabolites in human erythrocytes. Am J Clin Nutr 1982;35:1391-99.

Franzblau A, Rock CL, Werner RA, Albers JW, Kelly MP, Johnston EC. The relationship of vitamin B6 status to median nerve function and carpal tunnel syndrome among active industrial workers. JOEM 1996;38:485-91.

Fu QG, Sandkuhler J, Zimmermann M. B-vitamins enhance afferent inhibitory controls of nociceptive neurons in the rat spinal cord. Klin Wochenschr 1990;68:125-28.

Fuhr JE, Farrow A, Nelson HS. Vitamin B6 levels in patients with carpal tunnel syndrome. Arch Surg 1989;124:1329-30.

Gilbert JA and Gregory JF III. Pyridoxine-5'-β-D-glucoside affects the metabolic utilization of pyridoxine in rats. J Nutr 1992;122:1029-1035.

Goldberg DM, Martin J, Knight AH. Elevation of serum alkaline phosphatase activity and related enzymes in diabetes mellitus. Clin Biochem 1977;10:8-11.

Golik A, Rubio A, Weintraub M, Byrne L. Elevated serum liver enzymes in obesity: a dilemma during clinical trials. Int J Obesity 1991;15:797-801.

Grafova VN, Danilova EL, Reshetniak VK. The action of sodium valproate in central pain syndromes. Eksp Klin Farmakol 1994;57:8-11. (abstract only)

Greenberg ID. Arteriosclerotic, dental and hepatic lesions in pyridoxine deficient monkeys. Vitamins and Hormones 1964;22:677-94.

Gregory JF and Ink SL. Identification and quantification of pyridoxine-β-glucoside as a major form of vitamin B-6 in plant-derived foods. J Agr Food Chem 1987;35:76-82.

Guzman FJL, Gonzalez-Buitrago JM, De Arriba F, Mateos F, Moyano JC, Lopez-Alburquerque T. Carpal tunnel syndrome and vitamin B6. Klin Wochenschr 1989;67:38-41.

Gyorgy P. Vitamin B-2 and the pellegra-like dermatitis of rats. Nature 1934;133:448-49.

Gyorgy P. Crystalline vitamin B-6. J Am Chem Soc 1938;60:983-84.

Hamm MW, Mehansho H, Henderson LM. Transport and metabolism of pyridoxamine and pyridoxamine phosphate in the small intestine of the rat. J Nutr 1979;109:1552-9.

Hansen CM, Leklem JE, Miller LT. Vitamin B6 status indicators decrease in women consuming a diet high in pyridoxine glucose. J Nutr 1996a;126:2512-2518.

Hansen CM, Leklem JE, Miller LT. Vitamin B-6 status of women with a constant intake of vitamin B6 changes with three levels of dietary protein. J Nutr 1996b;12:1891-1901.

Harding RS, Plough IS, Friedemann TE. The effect of storage on the vitamin B-6 content of packaged army ration with a note on the human requirement for the vitamin. J Nutr 1959;68:323-31.

Harris H. The human alkaline phosphatases: what we know and what we don't know. Clin Chim Acta 1990;186:133-50.

Harris SA and Folkers K. Synthesis of vitamin B6. J Am Chem Soc 1939;61:1245-47.

Henderson JM, Codner MA, Hollins B, Kutner MH, Merrill AH. The fasting B6 vitamer profile and response to a pyridoxine load in normal and cirrhotic subjects. Hepatology 1986;6:464-71.

Hughes RC, Jenkins WT, Fischer EH. The site of binding of pyridoxal-5'-phosphate to heart glutamic-aspartic transaminase. Proc Natl Acad Sci USA 1962;48:1615-18.

Hunt DA, Stokes J, Wallace W, McCrory WW, Stroud HH. Pyridoxine dependency: report of a case of intractable convulsions in an infant controlled by pyridoxine. Pediatrics 1954;13:140-45.

Ignatov ID, Andreev BV. Role of the GABA system in adaptation to long-term pain stimulation. Patol Fiziol Eksp Ter 1992;4:66-70. (abstract only)

Ink SL and Henderson LM. Vitamin B-6 metabolism. Ann Rev Nutr 1984;4:445-70.

Ink SL, Gregory JF, Sartain DB. Determination of pyridoxine β-glucoside bioavailability using intrinsic and extrinsic labeling in the rat. J Agri Food Chem 1986;34:857-62.

Ink SL, Mehansho H, Henderson LM. The binding of pyridoxal to hemoglobin. J Biol Chem 1982;257:4753-57.

Jurna I, Carlsson KH, Komen W, Bonke D. Acute effects of vitamin B6 and fixed combinations of vitamin B1, B6 and B12 on nociceptive activity evoked in the rat thalamus: dose-response relationships and combinations with morphine and paracetamol. Klin Wochenschr 1990;68:129-35.

Kabir H, Leklem JE, Miller LT. Relationship of the glycosylated vitamin B-6 content of foods of vitamin B-6 bioavailability in humans. Nutr Rept Int 1983a;28:709-16.

Kabir H, Leklem JE, Miller LT. Measurement of glycosylated vitamin B-6 in foods. J Food Sci 1983b;48:1422-25.

Kabir H, Leklem JE, Miller LT. Comparative vitamin B-6 bioavailability from tuna, whole wheat bread and peanut butter in humans. J Nutr 1983c;113:2142-20.

Kant AK and Block G. Dietary vitamin B-6 intake and food sources in the US population: NHANES II, 1976-1980. Am J Clin Nutr 1990;52:707-16.

Kant AK, Moser-Veillon PB, Reynolds RD. Long-term stability of pyridoxal phosphate in frozen human plasma. Fed Proc 1984;43:A486-88.

Kark JA, Bingiovanni R, Hicks CU, Tarassof G, Hannah JS, Yoshida GY. Modification of intracellular hemoglobin with pyridoxal and pyridoxal 5'-phosphate. Blood Cells 1982;8:299-314.

Kelsay J, Miller LT, Linkswiler HM. Effect of protein intake on the excretion of quinolinic acid and niacin metabolites by men during vitamin B6 depletion. J Nutr 1968;94:27-31.

Keniston RC, Nathan PA, Leklem JE, Lockwood RS. Vitamin B6, vitamin C and carpal tunnel syndrome: A cross-sectional study of 441 adults. JOEM 1997; in press.

Kikuchi G, Kumar A, Talmage P. The enzymatic synthesis of gamma-aminolevulinic acid. J Biol Chem 1958;233:1214-19.

Kim, M-S, Leklem JE, Hardin K, Ridlington J, Wander R, Du SH. Effect of vitamin B-6 status on fatty acid and lipid metabolism in women. FASEB J 1997;11;A233.

Kimura J. The carpal tunnel syndrome localization of conduction abnormalities within the distal segment of the median nerve. Brain 1979;102:619-35.

Kretsch MJ, Sauberlich HE, Skala JH, Johnson HL. Vitamin B-6 requirement and status assessment: young women fed a depletion diet followed by a plant- or animal-protein diet with graded amounts of vitamin B-6. Am J Clin Nutr 1995;61:1091-1101.

Kuhn R and Wendt G. Uber das antidermatitische vitamin der hefe. Ber Deut Chem Ges 1938;71B:780-82.

Kurlemann G, Ziegler R, Gruneberg N, Bomelburg T, Ullrich K, Palm DG. Disturbance of GABA metabolism in pyridoxine dependent seizures. Neuropediatrics 1992;23:257-59.

Lee CM and Leklem JE. Difference in vitamin B6 status indicator responses between young and middle-aged women fed constant diets with two levels of vitamin B6. Am J Clin Nutr 1985;42:226-34.

Leinert J, Simon I, Hotzel D. Methoden und deren wertung zur bestimmung des Vitamin B6 versorgungszustandes beim menschen. Int J Vit Nutr Res 1983;53:166-78.

Leklem JE. Vitamin B-6 metabolism and function in humans. In: Leklem JE, Reynolds RD, eds. Clinical and physiological applications of vitamin B-6. New York, NY: Alan R. Liss, Inc, 1988: 3-23.

Leklem, JE. Vitamin B-6: A status report. J Nutr 1990;120:1503-7.

Leklem JE. Vitamin B-6. In: Machlin, LJ, ed. Handbook of vitamins, 2nd Ed. New York, NY: Marcel Dekker, Inc, 1991:341-92.

Leklem JE. Vitamin B-6. In: Machlin LJ, ed. Handbook of Vitamins. New York, NY: Marcel Dekker, Inc, 1993:341-92.

Leklem JE, Brown RR, Rose DP, Linkswiler H, Arend R. Metabolism of tryptophan and niacin in oral contraceptive users receiving controlled intakes of vitamin B-6. Am J Clin Nutr 1975;28:146-56.

Leklem JE, Miller LT, Perera AD, Peffers DE. Bioavailability of vitamin B-6 from wheat bread in humans. J Nutr 1980;110:1819-28.

Lepovsky S. Crystalline factor I. Science 1938;87:169-70.

Li T-K, Lumeng L, Veitch RL. Regulation of pyridoxal 5'-phosphate metabolism in liver. Biochem Biophys Res Comm 1974;61:627-34.

Li YR, Yang YZ, Sun MZ. The influence of GABA injected into cerebral ventricle on electrical activities of pain-excitation neurons and pain-inhibition neurons in nucleus parafascicularis of the thalamus of rats. Chen Tzu Yen Chiu 1989;14:334-338. (abstract)

Lindberg AS, Leklem JE, Miller LT. The effect of wheat bran on the bioavailability of vitamin B6 in young men. J Nutr 1983;113:2578-86.

Lumeng L, Lui A, Li T-K. Plasma content of B-6 vitamers and its relationship to hepatic vitamin B-6 metabolism. J Clin Invest 1980;66:688-95.

Lumeng L, Ryan MP, Li T-K. Validation of the diagnostic value of plasma pyridoxal 5'-phosphate measurements in vitamin B-6 nutrition of the rat. J Nutr 1978;108:545-52.

Maeda N, Takahashi K, Aono K, Shiga T. Effect of pyridoxal 5'-phosphate on the oxygen affinity of human erythrocytes. Br J Haematol 1976;34:501-09.

Manore, MM, Leklem JE, Walter MC. Vitamin B6 metabolism as affected by exercise in trained and untrained women fed diets in carbohydrate and vitamin B6 content. Am J Clin Nutr 1987;46:995-1004.

Mascher H. Determination of total pyridoxal in human plasma following oral administration of vitamin B6 by high-performance liquid chromatography with post-column derivization. J Pharm Sci 1993;82:972-74.

McComb RB, Bowers GN Jr, Posen S. Alkaline phosphatase. New York, NY: Plenium Press, 1979.

McCormick DB . Vitamin B6 and brain function. NY State J Med 1961;62:617-19.

McCormick DB, Gregory M, Snell EE. Pyridoxal phosphokinases.I.Assay, distribution, purification, and properties. J Biol Chem 1961;236:2076-84.

Mehansho H and Henderson JM. Transport and accumulation of pyridoxine and pyridoxal by erythrocytes. J Biol Chem 1980;255:11901-07.

Mehansho H, Buss DD, Hamm MW, Henderson LM. Transport and metabolism of pyridoxine in rat liver. Biochem Biophys Acta 1980;631:112-23.

Mehansho H, Hamm MW, Henderson LM. Transport and metabolism of pyridoxal and pyridoxal phosphate in the small intestine of the rat. J Nutr 1979;109:1542-51.

Menahan LA, Sobocinski KA, Austin BP. Characterization of elevated plasma alkaline phosphatase activity in genetically obese mice. Metabolism 1985;34:272-77.

Merrill AH, Henderson JM, Wang E, Codner MA, Hollins B, Millikan WJ. Activities of the hepatic enzymes of vitamin B6 metabolism for patients with cirrhosis. Am J Clin Nutr 1986;44:461-67.

Messing RB. Phebus L, Fisher L, Lytle LD. Analgesic effects of fluoxetine HCL, a specific uptake inhibitor for serotoninergic neurons. Psychopharm Commun 1975;1:511-21.

Meydani SW, Ribaya-Mercado JD, Russell RM, Sahyoun N, Morrow PD, Gershoff SN. Vitamin B-6 deficiency impairs interleukin 2 production and lymphocyte proliferation in elderly adults. Am J Clin Nutr 1991;53:1275-80.

Middleton HM. Intestinal absorption of pyridoxal 5-phosphate: disappearance from perfused segments of rat jejunum in vivo. J Nutr 1979;109:975-81.

Miller LT and Edwards M. Microbiological assay of vitamin B-6 in blood and urine. In: Leklem JE and Reynolds RD, eds. Methods in vitamin B-6 nutrition. New York, NY: Plenium Press, 1981:45-55.

Miller LT, Leklem JL, Schultz TD. The effect of dietary protein on the metabolism of vitamin B-6 in humans. J Nutr 1985;115;1663-72.

Miller LT and Linkswiler HM. Effect of protein intake on the development of abnormal tryptophan metabolism by men during vitamin B-6 depletion. J Nutr 1967;93:53-9.

Moss DW. Perspectives in alkaline phosphatase research. Clin Chem 1992;38:2486-92.

Mueller JF and Iacono JM. Effect of deoxypryidoxine-induced vitamin B-6 deficiency on polyunsaturated fatty acid metabolism in human beings. Am J Clin Nutr 1963;12:358-67.

Nathan PA, Keniston RC, Myers LD, Meadows KD. Obesity as a risk factor for slowing of sensory conduction of the median nerve in industry. J Occup Med 1992;34:379-383.

National Research Council, Recommended Dietary Allowances 9th ed. Washington DC: National Academy Press, 1980:155-60.

National Research Council, Recommended Dietary Allowances 10th ed. Washington DC: National Academy Press, 1989:142-50.

Natta CL and Reynolds RD. Apparent vitamin B6 deficiency in sickle cell anemia. Am J Clin Nutr 1982;40:235-42.

Nelson EW, Lane HJR, Cerda JJ. Comparative human intestinal bioavailability of vitamin B-6 from a synthetic and natural source. J Nutr 1976;106:1433-37.

Nielsen HK, Thomsen K, Eriksen EF, Charles P, Storm T, Mosekilde L. The effects of high-does glucocorticoid administration on serum bone gamma carboxyglutamic acid-containing protein, serum alkaline phosphatase and vitamin D metabolites in normal subjects. Bone and Mineral 1988;4:105-13.

Orr ML. Pantothenic acid, vitamin B-6 and vitamin B-12 in foods. Home Economics Research Report No. 36, US Dept. of Agriculture, Washington, D.C., 1969.

Paulose CS and Dakshinamurti K. Effect of pyridoxine deficiency in young rats on high affinity serotonin and dopamine receptors. J Neurosci Res 1985;14:263-70.

Poggell B. Enzymatic oxidation of pyridoxamine phosphate to pyridoxal phosphate in rabbit liver. J Biol Chem 1958;232:761-76.

Rabinowitz J and Snell EE. Vitamin B6 group: Urinary excretion of pyridoxal, pyridoxamine, pyridoxine and pyridoxic acid in human subjects. Proc Soc Exp Biol Med 1949;70:235-40.

Reynolds RD. Bioavailability of vitamin B-6 from plant foods. Am J Clin Nutr 1988;48:863-7.

Reynolds RD. Biochemical methods for status assessment. In: Vitamin B6 metabolism in pregnancy, lactation and infancy. Raiten DJ, ed. Boca Raton, FL: CRC Press, 1995:41-59.

Roberts E. Gamma Aminobutyric acid (GABA) - vitamin B6 relationships in the brain. Am J Clin Nutr 1963;12:291-307.

Roberts E, Wein J, Simonsen DG. γ-Aminobutyric acid (γABA), vitamin B6 and neuronal function-a speculative synthesis. Vitamins & Hormones 1964;22:503-59.

Roberts E, Younger F, Frankel S. Influence of dietary pyridoxine on glutamic decarboxylase activity of brain. J Biol Chem 1951;191:277-85.

Rose DP, Leklem JE, Brown RR, Linkswiler HM. Effect of oral contraceptives and vitamin B-6 deficiency on carbohydrate metabolism. Am J Clin Nutr 1975;28:872-78.

Roy AV. Rapid method for determining alkaline phosphatase activity in serum with thymolpthalien monophosphate. Clin Chem 1970;16:431-36.

Sabo DJ, Francesconi RP, Gershoff SN. Effect of vitamin B-6 deficiency on tissue dehydrogenases and fat synthesis in rats. J Nutr 1971;101:29-34.

Salkeld RM and Stotz R. Vitamin B6 deficiency: an etiological factor of the carpal tunnel syndrome. In: Reynolds RD and Leklem JE, eds. Vitamin B6: Its role in health and disease, current topics in health and disease. New York, NY: Alan R Liss Inc, 1985:463-67.

Samanin R, Bernasconi S, Quattrone A. Antinociceptive action of quipazine: relation to central serotonergic receptor stimulation . Psychopharmacologia 1976;46:219-22.

Sarma PS, Snell EE, Elvehjem CA. The bioassay of vitamin B6 in natural materials. J Nutr 1947;33:121-28.

Sauberlich HE. Interaction of Vitamin B6 with other nutrients. In: Reynolds RD and Leklem JE, eds. Vitamin B6: Its role in health and disease, current topics in health and disease. New York, NY: Alan R Liss Inc, 1985:193-217.

Schwieger G, Karl H, Schonhaber E. Relapse prevention of painful vertebral syndromes in follow-up treatment with a combination of vitamins B1, B6 and B12. Ann NY Acad Sci 1990;585:540-42.

Scriver CR. Vitamin B6-dependency and infantile convulsions. Pediatrics 1960;26:62-74.

Sharma SK, Bolster B, Dakshinamurti K. Effects of pyridoxine on nociceptive thalamic unit activity. Ann NY Acad Sci 1990;585:549-53.

Shizukuishi S, Nishii S, Ellis J, Folkers K. The carpal tunnel syndrome as a probable primary deficiency of vitamin B6 rather than a deficiency of a dependency state. Biochem Biophys Res Commun 1980;95:1126-30.

Shultz T and Leklem J. Urinary 4-pyridoxic acid, urinary vitamin B-6 and plasma pyridoxal phosphate as measures of vitamin B-6 status and dietary intake of adults. In: Leklem JE and Reynolds RD, eds. Methods in vitamin B-6 nutrition. New York, NY: Plenium Press, 1981:297-320.

Shultz TD and Leklem JE. Supplementation and vitamin B6 metabolism. In: Reynolds RD and Leklem JE, eds. Vitamin B6: Its role in health and disease. New York, NY: Alan R. Liss, Inc, 1981:295-205.

Sicuteri F, Anselmi B, Del Bianco PL. 5-hydroxytryptamine supersensitivity as a new theory of headache and central pain: a clinical pharmacological approach with *p*-chlorophenylalanine. Psychopharmacolgia 1973; 29:347-56.

Snell EE, Guillard BM, Williams RJ. Occurrence in natural products of a physiologically active metabolite of pyridoxine. J Biol Chem 1942;143:519-30.

Snell EE and Haskell BE. In: Florkin M and Stotz EH, eds. Comprehensive Biochemistry. Elsevier, Amsterdam, 1971;21:47-67.

Snyderman SE, Holt EH Jr., Carreters R, Jacobs KG. Pyridoxine deficiency in the human infant. Am J Clin Nutr 1953;1:200-07.

Spies TD, Bean WB, Ashe WF. A note on the use of vitamin B-6 in human nutrition. JAMA 1939;112:2414-15.

Stampfer MF, Malinow R, Willett WC, Newcomer LM, Upson B, Ullmann T, Tisheler PV, Hennekens CH. A prospective study of plasma homocyst(e)ine and of myocardial infarction in US physicians. JAMA 1992;268:877-81.

Symes EK, Bender DA, Bowden J-F, Coulson WF. Increased target tissue uptake of, and sensitivity to, testosterone in the vitamin B6 deficient rat. J Ster Biochem 1984;20:1089-93.

Talbott MC, Miller LT, Kerkvliet NI. Pyridoxine supplementation: effect on lymphocyte responses in elderly persons. Am J Clin Nutr 1987;46:659-64.

Tarr JB, Tamura T, Stokstad ELR. Availability of vitamin B6 and pantothenate in an average American diet in man. Am J Clin Nutr 1981;34:1328-37.

Tenen SS. The effects of *p*-chlorophenylalanine, a serotonin depletor, on avoidance acquisition, pain sensitivity, and related behaviour in the rat. Psychopharmacologia 1967;10:214-19.

van den Berg H, Mulder J, Spanhaak S, van Dokkum W, Ockhuizen T. The influence of marginal vitamin B-6 status on immunological indices. In: Leklem JE and Reynolds RD, eds. Clinical and physiological applications of vitamin B-6. New York, NY: Alan R. Liss Inc, 1988: 147-55.

Wada H and Snell EE. The enzymatic oxidation of pyridoxine and pyridoxamine phosphates. J Biol Chem 1961;236:2089-95.

Wan DYY, Cerklewski FL, Leklem JL. Increased plasma pyridoxal 5'-phosphate when alkaline phosphatase activity is reduced in moderately zinc-deficient rats. Biol Trace Elem Res 1993;39:203-10.

Werner RA, Albers JW, Franzblau A, Armstrong TJ. The relationship between body mass index and the diagnosis of carpal tunnel syndrome. Musc Nerv 1994;17:632-36.

Whyte MP. Alkaline phosphatase: physiologic role explored in hypophosphatasia. In: Peck WA, ed. Bone and mineral research Vol. 6. Amsterdam: Elsevier Science Publishers, B.V. (Biomedical Division), 1989: 175-218.

Whyte MP, Landt M, Ryan FM, Mulivor RA, Henthorn PS, Fedde KN, Mahuren JD, Coburn SP. Alkaline phosphatase: Placental and tissue-nonspecific isoenzymes hydrolyze phophoethanoloamine, inorganic pyrophosphate, and pyridoxal 5'-phosphate. J Clin Invest 1995;95:1440-45.

Whyte MP, Mahuren JD, Fedde KN, Cole FS, McCabe ERB, Coburn SP. Perinatal hypophosphatasia: tissue levels of vitamin B6 are unremarkable despite markedly increased circulating concentrations of pyridoxal-5'-phosphate (evidence for an ectoenzyme role for tissue nonspecific alkaline phophatase). J Clin Invest 1988;81:1234-39.

Whyte MP, Mahuren JD, Vrabel LA, Coburn SP. Markedly increased circulating pyridoxal-5'-phosphate concentrations in hypophosphatasia: alkaline phosphatase acts in vitamin B6 metabolism. J Clin Invest 1985;76:752-56.

Wild A and Bartoszyk GD. Additive antinociceptive effects of vitamins B1, B6 and B12 in the writhing test and antinociception in the heat coil test. In: Gergershagen HU and Zimmermann M, eds. B-vitamins in pain. Hamburg, Germany: pmi verlag, 1987:9-17.

Winklhofer-Roob BM, van't Hof MA, Shmerling DH. Reference values for plasma concentrations of vitamin E and A and carotenoids in a Swiss population from infancy to adulthood, adjusted for seasonal influences. Clin Chem 1997;43:146-53.

Witten PW and Hollman RT. Polythenoid fatty acid metabolism. VI. Effect of pyridoxine on essential fatty acid conversions. Arc Biochem Biophys 1952;41:266-73.

Wozenski JR, Leklem JE, Miller LT. The metabolism of small doses of vitamin B-6 in man. J Nutr 1980;110:275-85.

APPENDICES

Table A.1. Individual plasma PLP concentrations of vitamin users (nmol/L)

Subject	Month 1	Month 6	Month 9
1	92.4	88.9	160.9
2	63.7	224.6	69.2
3	104.0	112.7	118.2
4	66.6	87.5	46.2
5	62.5	105.1	88.1
6	176.0	263.2	175.3
7	203.0	243.2	271.2
8	39.8	185.8	59.9
9	83.1	54.5	53.3
10	44.0	302.9	44.3
11	17.6	89.6	35.8
12	243.0	203.6	205.2
13	64.4	78.0	86.7
14	277.0	172.4	128.2
15	19.4	71.9	74.1
16	34.9	52.0	20.9

Table A.2. Individual plasma PLP concentrations for non-vitamin users (nmol/L)

Subject	Month 1	Month 6	Month 9
1	43.4	35.3	41.9
2	9.8	15.9	22.3
3	25.9	26.2	30.9
4	17.5	25.4	32.4
5	27.5	41.3	39.6
6	52.1	37.2	21.9
7	29.1	34.8	45.3
8	13.3	13.3	20.0
9	18.7	14.9	25.3
10	59.5	62.4	67.5
11	15.6	22.1	30.1
12	33.6	49.4	35.4
13	18.2	27.6	38.4
14	30.3	42.1	29.3

Table A.3. Individual erythrocyte PLP concentrations of vitamin users (nmol/L)

Subject	Month 1	Month 6	Month 9
1	113.0	81.6	104.7
2	45.5	84.8	49.9
3	84.7	74.9	87.5
4	70.2	64.1	74.2
5	67.3	70.2	57.2
6	147.0	142.8	149.3
7	430.0	447.0	439.5
8	66.8	87.9	57.0
9	64.5	61.5	65.8
10	70.9	888.7	90.1
11	42.2	68.1	62.6
12	250.0	301.3	452.5
13	41.0	53.3	47.8
14	240.0	121.6	101.3
15	55.8	113.6	123.5
16	62.8	59.9	51.7

Table A.4. Individual erythrocyte PLP concentrations for non-vitamin users (nmol/L)

Subject	Month 1	Month 6	Month 9
1	53.1	38.7	45.7
2	41.0	59.3	61.1
3	51.7	50.3	51.5
4	38.0	40.9	40.7
5	51.2	43.8	54.2
6	60.3	51.9	57.1
7	61.1	70.5	69.7
8	59.7	66.7	57.7
9	55.3	57.3	61.5
10	39.5	36.3	57.1
11	37.1	48.9	51.4
12	51.3	59.6	59.4
13	46.7	54.5	60.7
14	65.7	97.0	102.5

Table A.5. Individual total plasma vitamin B6 concentrations for vitamin users (nmol/L)

Subject	Month 1	Month 6	Month 9
1	116.0	119.2	241.2
2	89.5	295.7	101.6
3	140.0	139.6	166.5
4	100.0	103.2	82.2
5	70.5	136.1	123.5
6	26.50	355.9	266.4
7	364.0	506.8	547.3
8	54.5	254.1	92.9
9	117.0	79.0	71.1
10	60.3	1466.2	84.6
11	32.7	99.9	48.2
12	349.0	367.2	432.6
13	85.9	102.7	114.3
14	360.0	180.1	175.4
15	32.4	98.4	108.3
16	39.3	57.6	38.9

Table A.6. Individual total plasma vitamin B6 concentrations for non-vitamin users (nmol/L)

Subject	Month 1	Month 6	Month 9
1	54.5	49.3	65.7
2	17.4	30.3	30.8
3	36.2	46.2	59.9
4	33.3	33.4	45.8
5	40.6	37.9	57.3
6	84.0	41.8	34.0
7	45.4	72.5	72.0
8	26.0	24.8	36.6
9	30.3	20.3	50.3
10	87.5	67.6	97.5
11	44.2	62.7	52.4
12	44.2	62.7	52.4
13	29.0	34.5	53.7
14	50.1	56.4	41.4

Table A.7. Individual plasma alkaline phosphatase activity for vitamin users (U/L)

Subject	Month 1	Month 6	Month 9
1	17.4	16.6	19.4
2	11.6	12.0	13.6
3	21.7	21.9	20.0
4	19.4	20.1	17.8
5	21.2	21.8	21.6
6	22.7	20.0	23.4
7	25.3	24.6	26.1
8	21.5	24.6	26.1
9	20.7	19.5	23.1
10	33.7	22.6	26.4
11	26.7	25.2	22.0
12	18.9	20.8	21.4
13	22.7	22.2	21.2
14	31.8	36.2	37.7
15	32.1	31.8	27.6
16	14.0	32.7	32.0

Table A.8. Individual alkaline phosphatase activity for non-vitamin users (U/L)

Subject	Month 1	Month 6	Month 9
1	22.6	22.1	24.0
2	22.4	18.1	17.5
3	24.6	26.4	29.7
4	27.2	29.8	26.2
5	20.0	19.7	21.9
6	24.2	25.8	23.3
7	30.0	33.5	37.7
8	22.9	28.7	27.8
9	23.0	28.1	25.7
10	22.6	23.3	21.0
11	38.0	30.9	33.8
12	19.9	22.5	22.2
13	22.9	26.5	23.0
14	26.6	30.9	21.2

Table A.9. Individual numbness frequency scores for vitamin users

Subject	Month 1	Month 6	Month 9
1	4	4	4
2	4	3	2
3	4	4	3
4	1	4	2
5	0	0	2
6	3	4	4
7	4	4	4
8	4	0	0
9	4	3	4
10	4	3	4
11	3	4	3
12	3	1	3
13	3	0	4
14	1	4	4
15	3	3	1
16	3	4	3

Table A.10. Individual numbness frequency scores for non-vitamin users

Subject	Month 1	Month 6	Month 9
1	3	4	1
2	4	4	3
3	0	3	3
4	3	3	0
5	3	4	1
6	1	4	4
7	0	3	3
8	0	0	3
9	0	4	2
10	4	0	0
11	0	3	3
12	0	4	0
13	4	0	0
14	3	4	0

Table A.11. Individual tingling frequency scores for vitamin users

Subject	Month 1	Month 6	Month 9
1	4	4	4
2	3	3	2
3	4	4	3
4	1	4	3
5	0	0	2
6	3	4	4
7	4	3	3
8	4	4	0
9	4	3	4
10	1	4	4
11	3	4	3
12	3	1	1
13	0	0	3
14	2	3	3
15	3	0	2
16	2	0	3

Table A.12. Individual tingling frequency scores for non-vitamin users

Subject	Month 1	Month 6	Month 9
1	0	3	0
2	3	3	0
3	0	3	3
4	0	3	0
5	3	4	1
6	1	4	4
7	0	3	3
8	0	0	3
9	0	3	2
10	4	0	0
11	0	3	0
12	0	1	4
13	4	0	0
14	4	4	0

Table A.13. Individual noctural awakening scores for vitamin users

Subject	Month 1	Month 6	Month 9
1	3	4	3
2	4	3	0
3	4	4	3
4	0	0	0
5	0	0	0
6	0	3	3
7	0	0	0
8	0	0	0
9	4	4	4
10	4	3	3
11	1	0	3
12	4	1	3
13	0	0	0
14	0	0	0
15	3	3	2
16	1	0	0

Table A.14. Individual nocturnal awakening scores for non-vitamin users

Subject	Month 1	Month 6	Month 9
1	0	3	0
2	0	3	0
3	0	3	3
4	0	0	0
5	3	3	0
6	4	4	4
7	0	0	0
8	0	0	0
9	0	3	2
10	3	0	0
11	3	3	3
12	0	0	0
13	4	0	0
14	4	4	0

Table A.15. Individual pain frequency scores for vitamin users

Subject	Month 1	Month 6	Month 9
1	3	4	4
2	4	4	2
3	4	4	4
4	1	4	2
5	0	0	0
6	0	0	0
7	0	0	0
8	0	4	4
9	4	3	3
10	4	4	4
11	1	3	0
12	2	0	0
13	0	0	0
14	0	3	1
15	4	3	0
16	3	0	0

Table A.16. Individual pain frequency scores for non-vitamin users

Subject	Month 1	Month 6	Month 9
1	0	0	0
2	3	4	0
3	0	0	3
4	0	0	0
5	3	0	0
6	3	4	0
7	0	0	0
8	0	4	0
9	0	0	0
10	0	0	0
11	3	3	0
12	0	0	0
13	0	0	0
14	0	0	0

Table A.17. Individual tightness frequency scores for vitamin users

Subject	Month 1	Month 6	Month 9
1	4	4	4
2	3	4	3
3	4	4	4
4	0	4	3
5	0	0	0
6	3	0	3
7	1	0	3
8	4	4	4
9	4	0	0
10	4	4	3
11	0	0	1
12	4	3	3
13	0	0	0
14	2	0	0
15	0	4	0
16	1	3	0

Table A.18. Individual tightness frequency scores for non-vitamin users

Subject	Month 1	Month 6	Month 9
1	0	0	0
2	3	4	3
3	0	0	0
4	0	0	0
5	3	4	0
6	4	4	0
7	0	1	0
8	0	3	1
9	0	0	0
10	0	0	0
11	0	3	0
12	0	0	0
13	3	3	1
14	0	0	0

Table A.19. Individual clumsiness frequency scores for vitamin users

Subject	Month 1	Month 6	Month 9
1	3	4	4
2	0	0	0
3	1	4	3
4	0	4	2
5	0	0	0
6	0	0	0
7	3	4	4
8	0	0	0
9	1	0	0
10	3	1	3
11	3	0	0
12	3	0	0
13	0	3	1
14	0	1	0
15	1	2	3
16	3	3	0

Table A.20. Individual clumsiness scores for non-vitamin users

Subject	Month 1	Month 6	Month 9
1	0	0	0
2	3	4	2
3	4	4	4
4	0	0	0
5	0	0	0
6	1	3	0
7	0	1	0
8	0	0	0
9	0	1	3
10	0	0	0
11	0	0	0
12	0	0	0
13	3	3	1
14	0	3	0

Table A.21. Individual pain intensity scores for vitamin users

Subject	Month 1	Month 6	Month 9
1	9	17	18
2	10	9	8
3	11	16	12
4	10	13	10
5	8	8	8
6	5	5	5
7	8	5	6
8	8	10	10
9	14	12	15
10	14	13	12
11	13	11	11
12	8	11	8
13	8	5	5
14	8	9	8
15	12	11	11
16	0	8	10

Table A.22. Individual pain intensity scores for non-vitamin users

Subject	Month 1	Month 6	Month 9
1	7	9	5
2	13	13	9
3	9	9	17
4	5	5	5
5	12	6	5
6	8	11	5
7	9	9	9
8	11	12	14
9	5	5	5
10	9	5	5
11	9	11	12
12	5	5	5
13	12	5	5
14	5	5	5

Table A.23. Individual numbness intensity scores for vitamin users

Subject	Month 1	Month 6	Month 9
1	14	17	18
2	14	13	17
3	11	12	14
4	11	18	13
5	8	9	9
6	12	14	14
7	17	12	13
8	7	9	9
9	16	15	17
10	13	14	13
11	18	15	17
12	10	15	15
13	8	6	9
14	13	11	11
15	14	13	13
16	8	10	11

Table A.24. Individual numbness intensity scores for non-vitamin users

Subject	Month 1	Month 6	Month 9
1	14	12	8
2	15	13	10
3	19	19	21
4	9	9	7
5	15	11	8
6	13	13	12
7	9	10	12
8	9	6	11
9	12	10	11
10	10	6	6
11	10	11	12
12	6	7	8
13	11	7	8
14	16	13	8

Table A.25. Individual activity scores for vitamin users

Subject	Month 1	Month 6	Month 9
1	22	31	14
2	16	12	18
3	8	8	13
4	20	14	19
5	8	8	9
6	10	10	11
7	12	15	11
8	10	11	8
9	13	13	9
10	22	25	9
11	14	19	11
12	12	10	12
13	8	9	9
14	11	9	14
15	17	13	10
16	11	11	11

Table A.26. Individual activity scores for non-vitamin users

Subject	Month 1	Month 6	Month 9
1	12	12	21
2	12	11	14
3	14	14	8
4	10	9	18
5	8	8	13
6	12	12	19
7	10	8	18
8	8	11	11
9	9	8	9
10	8	8	11
11	11	10	11
12	8	10	11
13	11	10	8
14	8	8	18

Table A.27. Individual body weight (kg) for vitamin users

Subject	Month 1	Month 6	Month 9
1	61	63	64
2	66	69	65
3	69	72	74
4	62	63	61
5	73	72	74
6	96	97	96
7	114	103	99
8	113	113	111
9	93	95	97
10	72	73	75
11	87	92	95
12	49	50	49
13	83	84	86
14	73	71	73
15	77	75	82
16	133	133	131

Table A.28. Individual body weight (kg) for non-vitamin users

Subject	Month 1	Month 6	Month 9
1	101	107	106
2	128	124	124
3	107	104	105
4	93	93	89
5	73	72	69
6	55	55	56
7	111	116	111
8	95	95	95
9	83	86	84
10	99	88	85
11	102	96	101
12	71	69	68
13	92	90	92
14	83	83	83

Table A.29. Individual vitamin B6 dose for vitamin users

Subject	Month 1	Month 6	Month 9
1	105	5	60
2	3	3	3
3	3	3	3
4	2	2	2
5	2	2	2
6	52	52	52
7	100	100	100
8	5	5	5
9	10	3	2
10	50	100	2
11	2	2	2
12	52	52	52
13	2	2	2
14	166	100	100
15	2	4	4
16	2.6	2.6	2.6

Table A.30. Individual percent body fat for vitamin users

Subject	Month 1	Month 9
1	16.3	16.9
2	19.2	18.4
3	36.5	30.5
4	19.8	19.2
5	26.4	28.3
6	34.4	36.3
7	36.3	32.2
8	23.3	29.5
9	25.9	20.7
10	18.1	16.3
11	36.2	35.6
12	30.8	29.0
13	34.7	35.1
14	26.5	31.8
15	30.5	30.8

Table A.31. Individual percent body fat for non-vitamin users

Subject	Month 1	Month 9
1	24.8	22.3
2	34.6	31.4
3	39.6	36.1
4	27.3	25.3
5	21.9	21.9
6	35.6	29.9
7	36.5	30.6
8	40.5	35.0
9	25.6	21.2
10	25.1	28.2
11	40.3	38.0
12	30.0	23.4
13	28.5	30.7

Table A.32. Individual absolute VO_2 max for vitamin users (L O_2/min)

Subject	Month 1	Month9
1	2.3	2.6
2	1.6	2.0
3	2.5	2.5
4	2.3	2.5
5	2.3	2.3
6	2.6	2.6
7	1.8	2.0
8	1.7	1.7
9	2.6	3.4
10	3.2	3.6
11	2.9	3.0
12	2.2	2.8
13	2.1	2.3
14	1.7	2.0
15	2.9	2.1

Table A.33. Individual VO_2 max for non-vitamin users (L O_2/min)

Subject	Month 1	Month 9
1	3.7	4.1
2	3.1	2.9
3	1.8	1.8
4	2.8	3.4
5	2.7	2.2
6	1.9	2.3
7	2.3	2.2
8	2.1	2.7
9	7.8	2.2
10	3.0	3.5
11	1.9	2.3
12	2.7	2.8
13	2.6	2.9

B.1. Health/diet history questionnaire -- long form

Dr. Jim Leklem
Dept. Nutrition & Food Management
Oregon State University

Project Name _____

Project Dates _____

Code #: _____ Today's Date: _____

Age: _____ Date of Birth: _____ Place of Birth: _____

Sex: M / F Predominant Place of Residence: _____

Present Employment: _____

Race (circle one): a. American Indian b. Black
c. Caucasian d. Hispanic
e. Chinese f. Japanese
g. Polynesian/Pacific Islander h. Other Asian (specify) _____
i. Other (specify) _____

Marital Status (circle one): a. Single b. Married c. Divorced/Separated d. Widowed

How many people live in your household? _____

Do you have any children? __ Yes __ No If yes, give ages _____

Females : MENSTRUAL and REPRODUCTIVE HISTORY

When did your last menstrual period begin? _____

Do you have regular menstrual periods? __ Yes __ No

How long is your menstrual cycle? _____

Do you have problems with your menstruation? __ Yes __ No

If yes, please explain:

Are you pregnant? __ Yes __ No Breast Feeding? __ Yes __ No

Have you ever been pregnant? __ Yes __ No

If yes, how many times? _____

How many children have you carried? _____

Please check if you have had any of the following complications of pregnancy:

__ 1. hyperemesis gravidarum (morning sickness)
__ 2. pre-eclampsia or eclampsia (toxemia)
__ 3. high blood pressure
__ 4. severe edema (swelling of your legs and feet)
__ 5. numbness and tingling in your hands, wrists, or arms
__ 6. gestational diabetes
__ 7. premature birth(s) (please indicate gestational age of infant(s))

_____

__ 8. kidney or bladder infections
__ 9. premature rupture of membranes
__ 10. small for dates infant (less than 5 lbs or 2500 g at term)

HEIGHT / WEIGHT: Height (ft. & in.) _____ Present weight: _____
Most ever weighed _____ What year _____
Length of time you have maintained current weight _____

DIETARY HISTORY

Dieting: Are you currently on a special diet? __ Yes __ No
If yes, for what purpose? (please check as many as apply):
__ 1. weight loss
__ 2. weight gain
__ 3. control serum lipids
__ 4. diabetes
__ 5. kidney failure
__ 6. ulcers
__ 7. diverticulitis
__ 8. allergies
__ 9. heart trouble
__ 10. high blood pressure
__ 11. pregnancy
__ 12. breast feeding
__ 13. other (please specify):

If you are on a diet, was it prescribed by a doctor, dietitian, or nurse? __ Yes __ No

If you are on a diet, what kind is it? (please check as many as apply):
__ 1. low fat
__ 2. low protein
__ 3. high protein
__ 4. low salt
__ 5. low carbohydrate
__ 6. low sugar
__ 7. low calorie
__ 8. low cholesterol
__ 9. high calorie
__ 10. a bland diet
__ 11. other (please specify):

If you are currently on a diet, for how long have you been on this diet? _____

If dieting, is your dieting associated with any commercial weight loss program?
Yes __ No If yes, please specify what program:

HABITS: A. Smoking:

1) Do you currently smoke? __ Yes __ No

If yes, please check below what you do smoke, and how much per day:

Cigarettes	_____	Packs per day	_____
Cigars	_____	Number per day	_____
Pipe	_____	Pipe Loads per day	_____

At what age did you start smoking? _____

2) If you do not currently smoke, did you ever smoke? __ Yes __ No

If yes, at what age did you start? _____

If yes, when did you quit? _____

Was this the only time you have quit? __ Yes __ No

If you quit, please check below what you did smoke, and how much per day:

Cigarettes	_____	Packs per day	_____
Cigars	_____	Number per day	_____
Pipe	_____	Pipe Loads per day	_____

3) Does anyone else in your household smoke? __ Yes __ No

If yes, please list type and how much per day:

Cigarettes	_____	Packs per day	_____
Cigars	_____	Number per day	_____
Pipe	_____	Pipe Loads per day	_____

B. Alcohol:

1) Do you drink alcoholic beverages? __ Yes __ No

If yes, how many times do you drink per month? _____

If yes, what do you drink and how many drinks do you consume each time you drink?

Beer	_____	Number of drinks at one time	_____
Wine	_____	Number of drinks at one time	_____
Liquor	_____	Number of drinks at one time	_____
Other	_____	Number of drinks at one time	_____

C. Caffeine:

1) Do you drink beverages containing caffeine? __ Yes __ No

If yes, which of the following beverages do you drink, and how much?

Coffee	_____	Number of cups per day	_____
Tea	_____	Number of cups per day	_____
Soda	_____	Number of 12 oz servings per day	_____

2) Do you drink any decaffeinated or caffeine-free beverages? __ Yes __ No

If yes, which of the following beverages do you drink, and how much?

Coffee	_____	Number of cups per day	_____
Tea	_____	Number of cups per day	_____
Soda	_____	Number of 12 oz servings per day	_____

D. Diet Soda Pop and other Sugarless Beverages

1) Do you drink any beverages containing artificial sweetners? __ Yes __ No

If yes, what do you drink and how many drinks (ounces, servings) per day?

Are you a vegetarian? __ Yes __ No If yes, circle the type of vegetarian diet you follow: a. ovo-lacto b. ovo c. lacto d. vegan

Do you take vitamins? (circle one): a. yes, daily b. yes, frequently (3 to 6 times/wk) c. often (once or twice/wk) d. occasionally (less than once/wk) e. never
If yes, what type, how much, and for how long have you taken them?

Type	Amount per day	How long have you taken

Do you take any other nutritional supplements (such as iron, calcium, other minerals, amino acids, fiber, supplement drinks [such as Ensure], etc)? __ Yes __ No

Type	Amount per day	How long have you taken

Please list all foods which you refuse to eat, can not eat, or prefer not to eat:

Please list those foods and beverages that you eat/drink almost every day:

EXERCISE LEVEL: Are you currently involved in a regular exercise program?
__ Yes __ No If yes, describe:

Type of Exercise	# Minutes (continuous)	Distance covered or repititions	# days/wk

Do you monitor your heart rate during exercise? __ Yes __ No
If yes, what heart rate do you try to maintain while exercising? _____

If you do not have a regular fitness program, what types of exercise would you get in a typical week?

MEDICAL HISTORY:

Have you ever had a glucose tolerance test? __ Yes __ No If yes, please explain when, the reason, and the results:

Have you ever had a stress electrocardiogram? __ Yes __ No If yes, please explain when, the reason, and the results:

Have you ever had any health risk screening tests, such as serum cholesterol, blood glucose, or blood pressure? __ Yes __ No If yes, please explain what tests you had, and what were the results and recommendations you received:

MEDICAL HISTORY (Check any condition for which you have been diagnosed and give AGE at diagnosis)

Diagnosis	Age at Diagnosis
__ 1. acquired immunodeficiency syndrome (AIDS)	
__ 2. diabetes	
__ 3. hypoglycemia	
__ 4. hypothyroidism	
__ 5. hyperthyroidism	
__ 6. goiter	
__ 7. osteoporosis	
__ 8. hepatitis	
__ 9. cirrhosis	
__ 10. kidney stones	
__ 11. nephritis	
__ 12. cystitis	
__ 13. high blood pressure	
__ 14. angina	
__ 15. ulcer	
__ 16. pancreatitis	
__ 17. ulcerative colitis	
__ 18. recurring gastritis	
__ 19. allergies/hayfever	
__ 20. hypoadrenalism (Addison's disease)	
__ 21. spastic colon/diverticulitis	
__ 22. carpal tunnel syndrome	
__ 23. rheumatoid arthritis	
__ 24. systemic lupus erythematosus	
__ 25. mental depression requiring regular medication	
__ 26. asthma	
__ 27. insomnia requiring frequent medication	
__ 28. emphysema	
__ 29. heart problems (specify)	
__ 30. cancer (specify type)	
__ 31. chronic infection (specify)	
__ 32. tuberculosis	
__ 33. chronic headache or other pain (specify)	
__ 34. hereditary condition (specify)	
__ 35. premenstrual syndrome	
__ 36. other condition (specify)	

Comments:

Are you currently suffering from any cold, flu, or allergy symptoms? __ Yes __ No
If yes, please specify:

Do any of your first-degree relatives (mother, father, brother, sister, son, daughter) have any of the following conditions? __ Yes __ No If yes, indicate which condition and his/her relationship to you:

__ 1. diabetes
__ 2. heart disease before age 60
__ 3. cancer before age 60
__ 4. high blood pressure before age 60
__ 5. allergies

Have you ever had a nerve conduction/muscle stimulation study? __ Yes __ No
If yes, when, for what reason, and what were the results?

Have you ever had any other special diagnostic tests (such as special X-ray studies or a CAT-scan) __ Yes __ No If yes, please specify:

SURGICAL HISTORY (Please specify any type of surgery you have had and the date and age when it occurred):

Operation	Age or Year

MEDICATION HISTORY (Check any which you take on a regular basis and when and how often): Medication Taking Currently? How often?

__ 1. sleeping tablets
__ 2. aspirin
__ 3. cold medications
__ 4. barbiturates
__ 5. tranquilizers
__ 6. diuretics
__ 7. blood pressure tablets
__ 8. antibiotics
__ 9. thyroid hormones
__ 10. oral contraceptives
__ 11. insulin
__ 12. oral hypoglycemics
__ 13. corticosteroids
__ 14. estrogens (female hormones)
__ 15. isoniazid
__ 16. pain medications
__ 17. muscle relaxants
__ 18. theophylline
__ 19. antiarrhythmatics
__ 20. ulcer medications
__ 21 antacids
__ 22. digoxin
__ 23. antidepressants
__ 24. seizure medications
__ 25. other medications (please specify):

How long did you fast prior to having your blood drawn? __ more than 12 hrs
__ 8-12 hrs __ less than 8 hrs

COMMENTS:

Checked by _____ Date _____

B.2. Health/diet history questionnaire -- short form

Dr. Jim Leklem
Dept. Nutrition & Food Management
Oregon State University

Project Name _____
Project Dates _____
Code # _____

Today's Date _____

DIET

Do you consume fortified cereals (e.g. Special K, Total, Cream of Wheat). If so, what kind and how often.

Please list the type of fruit/fruit juice consumed most often.

Please list any other foods that you eat/drink almost every day.

SUPPLEMENT USE

Do you take vitamins? If so, what type, how often, number per day and for how long have you taken them? (Include both vitamins and other nutritional supplements e.g. Ensure)

Brand	frequency of use	# of tablets/day	length of use	last taken

MEDICATION HISTORY

Please check all medications taken on a regular basis.

Medication	Brand	Dosage	Frequency of use	Last taken
__ aspirin				
__ ibuprofen				
__ acetaminophen				
__ sleeping tablets				
__ cold medications				
__ allergy medications				
__ barbiturates				
__ tranquilzers				
__ diuretics				
__ blood pressure tablets				
__ antibiotics				
__ thyroid hormone				
__ oral contraceptives				
__ corticosteroids				
__ estrogens				
__ isoniazid				
__ muscle relaxants				
__ ulcer medications				
__ antacids				
__ antidepressants				
__ other medications(please specify):				

Have you been sick in the past month? If so, how did you treat the condition?

How do you feel today?

How long did you fast prior to having your blood drawn?

___ more than 12 hours ___ 8-12 hours ___ less than 8 hours

Time of last meal/snack _____

Weight _____

Additional Comments:

B.3. Symptoms questionnaire

Name _____
Date: _____

The following questions refer to your symptoms for a typical twenty-four hour period during the *past two weeks* (circle one answer to each question)

How severe is the hand or wrist pain that you have at night?
1 I do not have hand or wrist pain at night.
2 Mild pain.
3 Moderate pain
4 Severe pain
5 Very severe pain

How often did hand or wrist pain wake you up during a typical night in the past two weeks?
1 Never
2 Once
3 Two or three times
4 Four or five times
5 More than five times

Do you typically have pain in your hand or wrist during the daytime?
1 I never have pain during the day
2 I have mild pain during the day
3 I have moderate pain during the day
4 I have severe pain during the day
5 I have very severe pain during the day

How often do you have hand or wrist pain during the daytime?
1 Never
2 Once or twice a day
3 Three to five times a day
4 More than five times a day
5 The pain is constant

How long, on average, does an episode of pain last during the daytime?
1 I never get pain during the day
2 Less than 10 minutes
3 10 to 60 minutes
4 Greater than 60 minutes
5 The pain is constant throughout the day

Do you have numbness (loss of sensation) in your hand?
1 No
2 I have mild numbness
3 I have moderate numbness
4 I have severe numbness
5 I have very severe numbness

Do you have weakness in your hand or wrist?
1 No weakness
2 Mild weakness
3 Moderate weakness
4 Severe weakness
5 Very severe weakness

Do you have tingling sensations in you hand?
1 No tingling
2 Mild tingling
3 Moderate tingling
4 Severe tingling
5 Very severe tingling

How severe is numbness (loss of sensation) or tingling at night?
1 I have no numbness or tingling at night
2 Mild
3 Moderate
4 Severe
5 Very severe

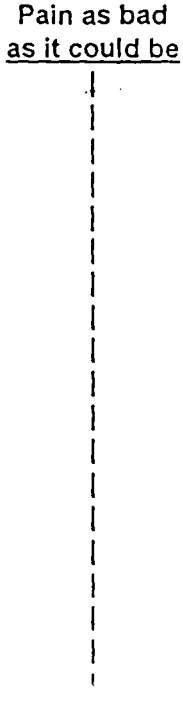
How often did hand numbness or tingling wake you up during a typical night during the past two weeks?
1 Never
2 Once
3 Two or three times
4 Four or five times
5 More than five times

Do you have difficulty with the grasping and use of small objects such as keys or pens?
1 No difficulty
2 Mild difficulty
3 Moderate difficulty
4 Severe difficulty
5 Very severe difficulty

On a typical day during the past two weeks have hand and wrist symptoms caused you to have any difficulty doing the activities listed below? Please circle one number that best describes your ability to do the activity.

Activity	Difficulty				
	No	Mild	Moderate	Severe	Cannot do at all due to Symptoms
Writing	1	2	3	4	5
Buttoning of clothes	1	2	3	4	5
Holding a book while reading	1	2	3	4	5
Gripping of a telephone handle	1	2	3	4	5
Opening of jars	1	2	3	4	5
Household chores	1	2	3	4	5
Carrying of grocery bags	1	2	3	4	5
Bathing and dressing	1	2	3	4	5

Make a mark (-) along the line from the extremes, "No pain at all" and "Pain as bad as it could be," which you think represents your current pain in your hands.



B.4. Informed Consent

Department of Nutrition and Food Management
Oregon State University
in cooperation
with Dr. Peter Nathan
Portland Hand Surgery and Rehabilitation Center

The purpose of this investigation is to evaluate the nutritional status of persons who have had nerve conduction studies performed. This study will relate blood levels of vitamins to nerve conduction information and to symptoms associated with carpal tunnel syndrome.

I have received a thorough explanation of this research and I understand the following:

1. I will undergo a second (midpoint) blood draw. This is in addition to the blood draws taken at the onset and the one to be taken at the conclusion of the study.

2. I will complete a questionnaire (titled Health/Diet History) dealing primarily with my nutrition/diet practices, drug use, and health history since the last blood draw.

3. A registered medical technologist will draw 25 ml (about 2 tablespoons) of blood from a vein in my forearm. This blood will be used to determine vitamins levels in our laboratory.

4. I will have my blood drawn under fasting conditions (i.e. having not eaten or drunk anything except water since 8 p.m. the night before).

I also understand that:

1. I will receive no direct benefits from this testing except information on my vitamin status.

2. I many receive a slight bruise from having my blood drawn. A sterile, disposable needle will be used to draw blood into disposable tubes which have a slight vacuum. For blood drawing, the needle will be inserted in my arm vein only once.

3. Any information obtained from me will be confidential. Individual information will not be shared with any person in the company you are employed. The only persons who have access to information from me are the principal investigators, Dr. Leklem (OSU) and Dr. Nathan (Portland Hand Surgery and Rehabilitation Center). All information will be filed in one of the investigator's office.

4. I am not committed to continue participation in the study. I may discontinue participation at any time without penalty or loss of benefits.

5. Questions about the research or any aspects of the blood drawing aspects of this study should be directed to Dr. Jim Leklem (541-737-0969). Questions related to the study in whole should be directed to Paul Emerick (541-737-6785).

All of my questions have been answered to my satisfaction.

Signed_____ Date_____
Printed Name_____ Phone_____
Present Address_____

Principal Investigator_____
Witness_____