AN ABSTRACT OF THE THESIS OF

Nathan D. Bills for the degree of Doctor of Philosophy in Foods and Nutrition presented on June 11, 1990

Title: IN VIVO AND IN VITRO DETERMINATION OF THE BIOAVAILABILITY OF VITAMIN B-6 FROM PLANT FOODS CONTAINING PYRIDOXINE GLUCOSIDE

Abstract approved: _____
Dr. James E. Leklem

To test the hypothesis that vitamin B-6 bioavailability is inversely correlated with percent vitamin B-6 as pyridoxine glucoside, the bioavailability of vitamin B-6 in ten plant foods was assessed. Nine men, aged 20-33 yrs, were fed a constant diet (3.2 mg of B-6) for 49 days. Incremental 24-hr urinary 4-pyridoxic (4-PA) acid excretion in response to test doses of pyridoxine or test foods fed in addition to the basal diet was used to estimate the vitamin B-6 bioavailability. The percent bioavailability calculated from the urinary 4-PA excretion after the pyridoxine doses was: walnuts, 87; bananas, 60; tomato juice, 43; wheat bran, -46; shredded wheat, -23; spinach, 17; orange juice, 19; broccoli, 62; cauliflower, 56; and carrots, - 1. There was a strong inverse

relationship between percent pyridoxine glucoside and bioavailability for six of the ten foods ($R = -.94$, $p < 0.01$). However, wheat bran and shredded wheat had negative bioavailability while the two crucifers, broccoli and cauliflower, had high bioavailability despite a high percentage of pyridoxine glucoside.

To evaluate different methods of in vitro measurement of bioavailable vitamin B–6 and to determine which method best predicted vitamin B–6 bioavailability measured in the feeding experiment, the ten plant foods were assayed by three different procedures. The first two methods quantified pyridoxine glucoside by a differential growth assay using *S. uvarum* after prior incubation with or without ß-glucosidase. Pyridoxine glucoside content was lower ($p<0.005$) when measured by the first method which used cold buffer extraction than when measured by the second method which used hot water extraction of the foods. In the third method, foods were treated with digestive enzymes (pepsin followed by pancreatin), extracted with methanolic HCl and quantified by *S. uvarum*. Of the three procedures, the best correlation with food vitamin B-6 bioavailability obtained in the human feeding study was with the third method, treatment with digestive enzymes prior to quantification.

IN VIVO AND IN VITRO DETERMINATION OF THE BIOAVAILABILITY OF VITAMIN B-6 FROM PLANT FOODS CONTAINING PYRIDOXINE GLUCOSIDE

by

Nathan D. Bills

A THESIS

submitted to

Oregon State University

In partial fulfillment of
the requirements for the
degree of
Doctor of Philosophy
Completed June 11, 1990
Commencement 1991

APPROVED:

Professor of Nutrition and Food Management in Charge of Major

Head of Nutrition and Food Management Department

Dean of Graduate School

Date thesis is presented June 11, 1990

ACKNOWLEDGEMENTS

Many people have helped me to achieve this degree. I would like to thank my fellow graduate students in the trenches, Christine Jensen, Jane Moore, Michael Beilstein and many others for their friendship and support. I'd like to also thank Phil Monaco, Karin Hardin, Linda Barstow, and Jim Riddlington for teaching me many "tricks of the trade" in laboratory research and for keeping it all in perspective. The professors in the department of Nutrition and Food Management and elsewhere including Mrs. Peters and Drs. Woodburn, Cerklewski, Gamble, Wander and Miller not only taught me the facts, but also modeled the roles of teacher, administrator and scientist. I'd especially like to thank Dr. James E. Leklem, my major professor, who gave me the opportunity and support to work towards this degree, and who I've come to appreciate is recognized internationally as "Mr. B-6". He has given many hours of his valuable time towards the completion of this thesis. Lastly, I'd like to thank my wife, Linda, and the rest of my family for their support and encouragement during the often times trying process of completing an advanced degree.

CONTRIBUTION OF AUTHOR

Chapter 2 BIOAVAILABILITY TO HUMANS OF VITAMIN B-6 IN 10 PYRIDOXINE GLUCOSIDE CONTAINING PLANT FOODS

Authors: Nathan D. Bills, Lorraine T. Miller & James E. Leklem

Nathan D. Bills assisted in the design of this study and was instrumental in its implementation. He performed most of the sample analyses and was responsible for tabulation, reduction and statistical analysis of the data. He was also primarily responsible for writing the manuscript.

Dr. Lorraine T. Miller provided funding for this research as co-principal investigator, was responsible for its initial design, was consulted often during its implementation and provided laboratory space, reagents and advice during sample and data analysis. She provided valuable editorial advice during the preparation of the manuscript.

Dr. James E. Leklem provided funding for this research as principal investigator, was responsible for its initial design, was primarily responsible for its implementation and provided laboratory space, reagents and advice during sample and data analysis. He provided valuable editorial advice during the preparation of the manuscript.

Chapter 3. A COMPARISON OF THREE IN VITRO METHODS FOR ESTIMATING BIOAVAILABILITY OF VITAMIN B-6 IN FOODS

Authors: Nathan D. Bills & James E. Leklem

Nathan D. Bills was primarily responsible for the design and implementation of this research. He performed the experiment and analyzed the samples. He was also primarily responsible for data tabulation, reduction and statistical analysis and for writing the manuscript.

Dr. James E. Leklem provided funding for this research as principal investigator, assisted in its design, and provided laboratory space, reagents and advice during sample and data analysis. He provided valuable editorial advice during the preparation of the manuscript.

TABLE OF CONTENTS

Chapter 1. Introduction 1

Rationale 1

History of Vitamin B-6 2

Discovery 2

Metabolism 3

Forms 3

Absorption 3

Interconversions 4

Functions 6

Mechanisms 7

Hormone Receptors 8

Transport 9

Storage 9

Excretion and end products 12

Intake Recommendations 19

Recommended Dietary Allowances 19

Status Assessment 22

Clinical 22

Biochemical 23

Dietary Intake 26

Vitamin B-6 Bioavailability 27

Availability of Vitamin B-6 in Foods 27

Absorption of Pure Chemical Forms 29

Plant Sources 30

Dietary Fiber 32
Pyridoxine Glucoside 34
Measurement of Pyridoxine Glucoside 36
Animal Studies of Pyridoxine Glucoside 37
Human Studies of Pyridoxine Glucoside 41
Other Vitamin B-6 Conjugates 44
Other Glucosides 44
B_6X 44
Digestive Enzymes 45
Effects on Requirements and Intake Recommendations 45

Chapter 2. Bioavailability to humans of vitamin B-6 in 10 pyridoxine glucoside containing plant foods. 46

Abstract 47
Introduction 48
Materials and Methods 50
Subjects 50
Experimental Design 50
Basal Diet 52
Procedure 53
Analyses 53
Statistical Analysis 55
Results 56
Baseline 4-pyridoxic acid excretion 57
Response to test doses 57

Response to test foods 58
Discussion 59

Chapter 3. A comparison of three in vitro methods for estimating bioavailability of vitamin B-6 in foods. 73

Abstract 74
Introduction 75
Materials and Methods 77
Food samples 77
Assay procedures 77
Total Vitamin B-6 78
Glycosylated vitamin B-6 78
Glycosylated phosphorylated vitamin B-6 and B_6X 79
Digestive enzyme treatment 79
Controls 80
Food bioavailability 81
4-pyridoxic acid 81
Statistics 82
Results 82
Discussion 86

Chapter 4. Summary and Conclusions 96

Bibliography 99

Appendix 120

A Collection of Urine 132

B Xylose Test 133

C Daily Activity Sheet 134

D. Telephone Interview Form 135

E Interview Form 136

F Informed Consent Form 138

LIST OF FIGURES

Figure

Chapter 2.

2.1 Regression of % bioavailability vs % glycosylated vitamin B-6 content of foods. 72

Chapter 3.

3.1 Comparison of vitamin B-6 values for 10 foods obtained by the methods of Kabir et al. 1983a, and Tadera et al. 1986 94

3.2 Predictive value of various methods for quantifiying bioavailable vitamin B-6 95

LIST OF TABLES

Table

Chapter 2.

2.1 Age, weight and height of the subjects. 66

2.2 % Glycosylated and total vitamin B-6 content and amount fed of the test foods. 67

2.3 Basal diet composition. 68

2.4 Plasma pyridoxal 5'-phosphate concentration over the course of the study. 69

2.5 Urinary 4-pyridoxic acid excretion after the 10-day adaptation, non-test food, non-test dose days. 70

2.6 Mean urinary 4-pyridoxic acid excretion in 9 subjects following pyridoxine or food dose ingestion. 71

Chapter 3.

3.1 Concentration of vitamin B-6 in foods as pyridoxine equivalents after various treatments as measured by *Saccromyces uvarum*. 91

3.2 Amounts and percentages of glycosylated vitamin B-6, glycosylated-phosphorylated, B_6X, and forms not released by digestive enzymes in ten foods. 92

3.3 Mean urinary 4-pyridoxic acid excretion in 9 subjects following pyridoxine or food dose ingestion. 93

LIST OF APPENDIX TABLES

Table

1 Tryptophan metabolites after a 2 gram dose of L-tryptophan. 120

2 Food test dose schedule. 121

3 Test dose schedule. 122

4 Urinary 4-pyridoxic acid excretion during the 10-day adaptation period. 123

5 Daily urinary 4-pyridoxic acid excretion for the 9 subjects on days 11 through 49. 124

6 Vitamin B-6 content of basal diet composites. 125

7 4-pyridoxic acid excretion and increment on pyridoxine dose days for nine subjects. 126

8 4-pyridoxic acid excretion and increment on test food days for nine subjects. 127

9 Clinical blood chemistry of subjects. 128

10 Daily urinary creatinine excretion for 9 subjects. 129

11 Daily weights for the 9 subjects 130

12 Xylose excretion, Percent of 5 g dose. 131

IN VIVO AND IN VITRO DETERMINATION OF THE BIOAVAILABILITY OF VITAMIN B-6 FROM PLANT FOODS CONTAINING PYRIDOXINE GLUCOSIDE

Chapter 1

INTRODUCTION

Rationale The human dietary requirement for any nutrient may be influenced by many factors, including the number and nature of the nutrient's biochemical roles in various tissues and organs, the physiological state, age, gender, overall health, and activity level of an individual, and the intake of other nutrients. In addition, the availability of a nutrient from dietary sources may be influenced by its matrix within a food, processing prior to ingestion, as well as its chemical form.

Bioavailability of a nutrient can be understood as that fraction which is freed by digestion, absorbed, and utilized by the organism. Thus the levels of a nutrient or its metabolites in foodstuffs, body tissues, and excretory products are useful in providing a measure of its bioavailability.

The importance of vitamin B-6 as a nutrient is illustrated by its function in over 100 metabolic reactions (Sauberlich, 1985). Although vitamin B-6 is widely distributed in food, there are significant portions of the population with intakes less than the RDA (FNB, 1989). Vitamin B-6 is one of the nutrients lost in the milling of grains that is not replaced by

fortification. In addition, vitamin B-6 is not fully available in many of its food sources which exacerbates the gap between requirements and intakes. Intelligent recommendations for intake should include estimates of vitamin B-6 bioavailability.

The chemical form of vitamin B-6 (vitamer) found in a food may affect its bioavailability. In vitro prediction of bioavailability from the chemical form of vitamin B-6 found in a food or diet would be a useful tool for setting intake recommendations and for estimating vitamin B-6 nutriture status of individuals or populations.

History of Vitamin B-6

Discovery In the process of trying to establish the identity of "vitamin B_2," a relatively heat stable, water soluble food extract, a nonfluorescent compound was found to be necessary for rat health (Gyorgy, 1971). Rats fed diets supplemented with vitamin B_1 (thiamin) and what was thought to be vitamin B_2 (riboflavin) still needed a yeast extract to prevent a scaly symmetric peripheral dermatitis (rat acrodynia). Within four years the active agent, vitamin B-6 (pyridoxine), was isolated by Lepkovsky at Berkeley, California (Lepkovsky, 1938). At least three other groups, working independently, isolated crystalline pyridoxine at about the same time. Within a year the structure was elucidated, and Gyorgy suggested the name of pyridoxine (Gyorgy & Eckhard, 1939). The understanding that pyridoxine was only one of

several vitamers is credited to Snell (1942). His group also identified pyridoxine and pyridoxal (especially as phosphorylated forms) as the active enzyme cofactors.

Confirmation that vitamin B-6 was essential in human nutrition came from the discovery of pyridoxine responsive seizures in infants receiving heat treated formula (Coursin, 1954). This was also the first indication that the chemical form of vitamin B-6 in foods (in this case altered by heat treatment) was important to its bioavailability (Gregory & Ink, 1985).

Metabolism

Forms Vitamin B-6 consists of three different 2-methyl, 3-hydroxy, 5-hydroxymethyl pyridine derivatives and their 5' phosphate esters. The substituent at the 4- position can be either a methyl alcohol (pyridoxine (PN)), an aldehyde (pyridoxal (PL)) or an amine (pyridoxamine (PM)). The corresponding phosphorylated forms are pyridoxine 5'phosphate (PNP), pyridoxal 5'phosphate (PLP) and pyridoxamine 5'phosphate (PMP). The primary dietary forms are PN and PM (from vegetable sources) and PLP and PMP (from animal sources).

Absorption Absorption of these dietary forms occurs probably by way of a nonsaturable passive process, after intestinal hydrolysis of phosphate esters by alkaline

phosphatase (Middleton, 1978, 1982). After absorption, the vitamin B-6 travels through the portal vein to the liver.

Because highly charged compounds do not readily cross cell membranes, phosphorylated B-6 vitamers are absorbed after hydrolysis by intestinal alkaline phosphatase (Middleton, 1978, 1982). (The relative absorption of the three vitamers will be described in the section on bioavailability). PN, PL and PM are probably absorbed by nonsaturable passive diffusion in the small intestine (Henderson, 1985). While this has been demonstrated for the rat (Buss et al. 1980; Hamm et al. 1979), little information is available for the human. More recent information (Middleton, 1985) suggested that the absorption may be saturable, particularly in the proximal small intestine. This suggests that pharmacological doses of vitamin B-6 may not be absorbed as completely as physiological doses.

Interconversions Although limited interconversion of the three B-6 vitamers occurs in the intestinal cell, the major site of interconversion is in the liver (Henderson, 1984; Lumeng et al. 1985) The enzyme responsible for phosphorylating the vitamers is pyridoxal kinase (ATP: pyridoxal 5'phosphotransferase E.C. 2.7.1.35) which can phosphorylate each of the three nonphosphorylated forms. Dephosphorylation occurs by means of nonspecific alkaline phosphatase. Conversion of PNP and PMP to the primary coenzyme form (PLP) is accomplished by pyridoxine (pyridoxamine) oxidase (E.C. 1.4.3.5) which requires flavin mononucleotide as a cofactor

(Lumeng et al. 1985). .This enzyme is not found in muscle tissue and only in limited amounts in human tissues other than liver or brain. The dead-end metabolite of vitamin B-6 is 4-pyridoxic acid (the 4 carboxylic acid substituted 2-methyl 3-hydroxy-5-hydroxymethyl pyridine derivative), which is formed by the action of aldeyde oxidase and/or NAD-dependent dehydrogenase on PL (Henderson, 1984).

Merrill et al. (1984) measured the activity of hepatic enzymes that interconvert B-6 vitamers in liver biopsies from patients without liver disease. They concluded that pyridoxal kinase (EC 2.7.1.35) is a soluble enzyme (as it is in other mammals and in other human tissues) because it was found in the 105,00 x g supernatant fraction. Pyridoxine (pyridoxamine) 5'-phosphate oxidase (EC 1.4.3.5) and pyridoxal oxidase were also found to be soluble enzymes based on the same criteria. Unlike other mammals (Stanulovic et al. 1976), no NAD-dependent dehydrogenase which oxidizes PL to 4-pyridoxic acid (4-PA) was found in human liver. Although activities measured under optimum in vitro conditions indicate that there is phosphatase activity (an insoluble enzyme as evidenced by its activity exclusively in the 105,000 x g pellet) calculation of the activities under estimated normal in vivo physiological conditions indicate that there was a 10-fold greater activity of the kinase versus the phosphatase. However, since the pyridoxal oxidase activities were higher than kinase activities, protein binding of PLP is necessary to maintain normal levels in tissues. The activities of these

enzymes measured in the five individuals varied from 30% of the means for pyridoxal kinase and pyridoxamine (pyridoxine) 5'-phosphate oxidase to 65-80% of the mean for pyridoxal 5'-phosphate phosphatase and pyridoxal oxidase. Intra-assay variation for replicates was less than 10%. In a subsequent study, Merrill et al. (1986) found that the biopsied liver phosphatase activities in 13 cirrhotic patients was higher (9.55 ± 8.03 versus 3.97 ± 2.36 nmole/min/mg protein (mean ± SD)) than in 11 noncirrhotic controls. Since the pyridoxal kinase and pyridoxine (pyridoxamine) 5'-phosphate oxidase values were similar for patients and controls and since pyridoxal oxidase activities were slightly lower in patients than controls, they concluded that the lower plasma PLP values observed in cirrhotics are due to increased dephosphorylation by hepatic phosphatases.

Functions Vitamin B-6 (as PLP) functions as a coenzyme in over 100 reactions (Sauberlich, 1985). Generally, PLP's role is to function as a Schiff base in transamination and decarboxylation reactions of amino acids which require PLP as a cofactor (Sauberlich, 1968; Martinez-Carrion, 1986). Transamination reactions, where an α-amino group is transferred from one molecule leaving a α-keto analogue to an α-keto group of another molecule to form an α-amino group are essential in the utilization of non-essential amino acids as well as in gluconeogenesis. Decarboxylation reactions involving PLP include the the removal of CO_2 from histidine to

form histamine, a potent vasodilator, steps in the tryptophan pathways for formation of serotonin and niacin, and decarboxylation of dopa (dioxyphenylalanine), a step in the formation of epinephrine and norepinephrine from tyrosine (Dakshinamurti et al. 1985).

In nucleic acid metabolism vitamin B-6 is required for one-carbon transfers. Vitamin B-6 has both a conformational and catalytic function in glycogen phosphorylase, thus playing a role in carbohydrate metabolism (Krebs and Fischer, 1964). Several Japanese researchers have discovered abnormal lipid metabolism in rats deficient in vitamin B-6. These effects include accumulation of lipids in the liver, alterations in cholesterol metabolism, and decreases in lipogenesis (Iwami & Okada, 1982; Suzuki & Okada, 1982; Suzuki et al. 1976; and Okada & Ochi, 1971).

Mechanisms PLP has a highly reactive aldehyde group which binds readily to the ε-lysine amino group in lysine-containing proteins. A major metabolic step where PLP (and PMP) phosphate are involved is in the transfer of α-amino groups from one group to another by a "ping pong" or dual replacement mechanism (Lehninger, 1982). The usual acceptor for the amino group is α-ketoglutarate (yielding glutamate) while the donor can be a number of amino acids (such as alanine, aspartic acid, leucine, or tyrosine). An example of one such reaction is (E = enzyme):

L-alanine + PLP-E ----- pyruvate + PMP-E PMP-E + α-ketoglutarate ---- glutamate + PLP-E

Thus the amino group is transferred from alanine to PLP, yielding PMP and pyruvate, the amino acid group then is transferred to α-ketoglutarate, yielding glutamate and regenerating the PLP.

The reaction between PLP and alanine occurs via the formation of a Schiff base between the incoming amino group and the carbonyl on PLP. This Schiff base undergoes tautomerism to yield an α-keto acid analogue of the amino acid and PMP (Lehninger, 1982).

Hormone Receptors Another function of PLP is in the modulation of the binding of steroid hormones to receptors. The action of a steroid hormone involves binding of hormone to the receptor which is then followed by binding of the steroid receptor complex with DNA and nucleoproteins (Bender et al. 1989). PLP interacts with the lysine moieties in receptors in such a way as to inhibit binding of the hormone to the receptor. This was first demonstrated with the glucocorticoid receptor (Cake et al. 1978) and later extended to other steroid receptors (DiSorbo & Litwack, 1982). Furthermore these interactions appear to occur at physiological concentrations of PLP (Bender et al. 1988). These latter authors demonstrated in vivo that vitamin B-6 deficiency enhanced end organ sensitivity to steroid hormones. Vitamin B-6 deficient female rats were

more sensitive to ethinyl estradiol than vitamin B-6 sufficient rats as evidenced by induction of uterine peroxidase and suppression of leutenizing hormone in ovariectomized rats. Similarly, testosterone given to castrated male vitamin B-6 deficient rats stimulated prostate growth more that it did when given to similarly treated vitamin B-6 sufficient controls. Thus the effective role of steroid hormones may be modulated by deficiency or excess of vitamin B-6.

Transport Vitamin B-6 is found in the blood mainly as PLP and PL, with estimates of the two forms ranging from 52 to 66% for PLP and 21 to 27% for PL (Lumeng et al. 1985; Coburn & Mahuren, 1983). Circulating PLP is mainly bound to albumin. Of current interest is whether it is PLP per se, or PLP hydrolyzed to PL by alkaline phosphatase which is specifically delivered to tissues (Barnard et al. 1987). Another incomplete part of the picture is the extent to which RBCs serve a transport function in plasma. Erythrocytes concentrate vitamin B-6 and hemoglobin has binding sites for both PLP and PL. Because of the tenacity with which hemoglobin binds these vitamers in vitro, the extent to which these two potential sources of vitamin B-6 are available to other tissues in vivo is under investigation (Leklem, 1987).

Storage PLP and PMP are the primary forms of vitamin B-6 in tissues. In rats, the ratio of these forms varies according to tissue, with ratios of PMP/PLP in liver, muscle

and brain being 1, 0.2 and 1.9, respectively (Lyon et al. 1962). A potential storage reservoir for vitamin B-6 is glycogen phosphorylase in muscle. The high ratio of PLP:PMP in muscle can be explained by the fact that glycogen phosphorylase has a high affinity for PLP but not for PMP (Black et al. 1978). Since muscle makes up about 40% of total body mass and 5% of soluble muscle protein is glycogen phosphorylase, muscle contains a large reservoir of PLP (Black et al. 1977). However, its potential as a reservoir during times of reduced vitamin B-6 intake is still being investigated. It is known, that in the rat muscle glycogen phosphorylase does serve as a reservoir during times of caloric deficit, but not a vitamin B-6 deficit per se (Black et al. 1978).

Russell et al. (1985) investigated the effects of feeding 0.45, 2.1, or 83 mg vitamin B-6/day (form not stated) to postpubertal gilts. An additional group was fed 83 mg vitamin B-6/day for 57 days and then switched to 0.45 mg/day for 64 days. They were interested in determining the extent to which muscle PLP content was affected by dietary intake of vitamin B-6 and to what extent PLP could be mobilized from muscle during times of dietary vitamin B-6 deficit. From 60 to 95% of PLP in muscle was bound to glycogen phosphorylase. Swine fed the two lower levels of vitamin B-6 had a loss of whole muscle transaminase activity while total PLP and glycogen phosphorylase content changed little. While swine fed the diet highest in vitamin B-6 had increased levels of PLP and phosphorylase activity, when these animals were switched to

the vitamin B-6 deficient diet no decrease in either muscle glycogen phosphorylase activity or PLP content was observed. Thus, the authors concluded that, as in the rat, muscle PLP is not readily mobilized during times of vitamin B-6 deficiency in growing swine.

Coburn et al. (1988) estimated human vitamin B-6 pools from the assayed vitamer content of muscle biopsies. The total muscle mass was calculated as the mean of a 40% estimated contribution of muscle to total body weight and the muscle mass as calculated from creatinine excretion. Assuming that muscle contains approximately 80% of the total body vitamin B-6 pool, and the measured-calculated values of 917 ± 319 and 850 ± 216 μmoles in the muscles of males and females, respectively, they estimated the total body vitamin B-6 pool to be about 1000 μmoles (169 mg PN equivalents).

While no determinations have been made of the availability of muscle PLP to humans during times of dietary vitamin B-6 deficit, several studies have demonstrated that exercise, a form of acute starvation, causes transient increases in plasma PLP levels. Leklem (1985) summarized the results of five studies on the effects of strenuous exercise on plasma PLP changes. In every case there was a postexercise increase in plasma PLP levels that could not be accounted for by changes in plasma volume alone. Manore & Leklem (1987) and Manore et al. (1988) assessed the effect of carbohydrate and vitamin B-6 on fuel substrates during exercise and vitamin B-6 metabolism in five young trained, five young untrained and five

postmenopausal untrained women. There was no effect of increased carbohydrate, training, or age on vitamin B-6 metabolism. In all groups plasma PLP and total plasma vitamin B-6 increased significantly with exercise and decreased below initial values at 60 min postexercise. Again, the changes in plasma B-6 vitamers could not be explained by changes in plasma volume alone. These data of Leklem (1985), Manore & Leklem (1987) and Manore et al. (1988) suggest that muscle PLP may be mobilized during periods of strenuous exercise. However, further research is needed to furnish direct evidence that the increased postexercise plasma PLP levels are derived from the PLP associated with muscle glycogen phosphorylase.

Excretion and end Products The major form of vitamin B-6 excreted in the urine is 4-PA (Rabinowitz and Snell, 1949) with small amounts of PL, PM and PN (Sauberlich et al. 1972). Small amounts of vitamin B-6 are also found in the feces, either from intestinal gut microflora synthesis or from unabsorbed dietary vitamin B-6.

Perhaps the earliest report on the effects of vitamin B-6 deficiency on excretion of urinary vitamin B-6 metabolites is that of Snyderman et al. (1953) who withdrew vitamin B-6 from the diets of two infants and followed the excretion of urinary vitamin B-6 and 4-PA. The excretion of 4-PA dropped to zero very rapidly while that of total vitamin B-6 fell to near zero but not quite as rapidly.

Baker et al. (1964) found that excretion of urinary vitamin B-6 paralleled dietary intakes of men fed controlled diets. The excretion level decreased to a minimum of 15 µg/24 hr on an intake of 0.06 mg of vitamin B-6/day. Baysal et al. (1966) fed 6 male students a partially purified diet containing 0.16 mg vitamin B-6/day. At the beginning of the study subjects excreted from about 4 to 7 µmoles/24 hours of 4-PA and from about 0.6 to 0.8 µmoles urinary vitamin B-6. After 25 days of depletion subjects had no detectable 4-PA in their urine and from 0.2 to 0.4 µmoles/24 hr of total vitamin B-6. Supplementation with 0.6 or 0.9 mg vitamin B-6/day resulted in a slight increase in urinary 4-PA and total urinary vitamin B-6 excretion that did not reach predepletion levels.

Kelsay et al. (1968) fed young men diets containing either 54 or 150 gm protein/day and 1.66, 0.16, or 0.76 mg vitamin B-6/day, consecutively. The protein content of the diets had no effect on total 24 hr urinary excretion of vitamin B-6. When fed the diets with 1.66 mg (9.82 µmoles) vitamin B-6, the total vitamin B-6 of urine consisted of 60% pyridoxal and 35% pyridoxamine. When the subjects were fed 0.16 mg vitamin B-6/day the total urinary vitamin B-6 decreased rapidly to near zero. At the lower levels of intake the pyridoxal and pyridoxamine concentrations of urine were approximately equal. Four-PA excretion ranged from 0.88 ± 0.11 (SD) mg/24 hr (5.20 ± 0.65 µmole) for the 150 g protein diet to 1.01 ± 0.32 mg/24 hr (5.97 ± 1.89 µmoles) for the 54 g protein diet when subjects were fed 1.66 mg vitamin B-6/day. After 5 days on

the 0.16 mg vitamin B-6/day diet, 4-PA excretion was 20% of its original value and by 25 days it was undetectable.

In another study from the same laboratory, Linkswiler et al. (1967) reported that urinary total vitamin B-6 and 4-PA excretion decreased rapidly when subjects were fed either 55 or 100 g of protein/day. However, it took 25 days for 4-PA excretion to reach approximately 0 in men consuming 100 g of protein/day whereas 40 days were required to reach 0 when subjects were consuming 55 g protein/day. Conversely, total urinary vitamin B-6 values fell more quickly in subjects on the lower protein intake. In every case the 4-PA excretion values were higher for subjects on the lower protein intake while the total urinary vitamin B-6 excretion values were higher in subjects consuming the higher level of dietary protein.

Sauberlich et al. (1972) reviewed the literature on urinary vitamin B-6 excretion and suggested a tentative guide for interpreting urinary levels of vitamin B-6. For adults, levels below 20 μg (118 nmoles)/g creatinine or about 40 μg (237 nmoles)/day were considered unacceptable while levels above this value were deemed acceptable. These authors also reviewed the literature on urinary 4-PA excretion and concluded that 40 to 50% of ingested vitamin B-6 is excreted as 4-PA in the urine.

Brown et al. (1975) fed 15 oral contraceptive (OC) users and 9 control women a diet containing 0.19 mg PN equivalents for 1 month. During the subsequent month OC users were divided into three groups, one group (n=5) received 0.8 mg/day

supplemental PNHCl/day, another group (n=6) received 2.0 mg/day, and the remaining group (n=4) received 20 mg/day. During the 2nd month the controls were divided into 2 groups, one (n=6) receiving 0.8 and the other (n=4) 2.0 mg of supplemental PN.HCl/day. During the depletion period (first month) the percent of vitamin B-6 intake excreted as urinary 4-PA in the control subjects decreased from 263% at time 0,to 96.3% the first week, to 73.1% the second week, to 48.2% the third week, to 37.5% the fourth week. Except for time 0, when it was slightly higher, in every case the OC users' percentage of vitamin B-6 intake excreted as urinary 4-PA was lower than controls. Furthermore, the rate of decrease in percentage was faster, with OC users excreting 45.5% of their intake as 4-PA by the second week. Controls repleted with 0.8 mg supplemental PN.HCl/day gradually increased their percentage of vitamin B-6 intake as urinary 4-PA excretion from 29.5%, to 33.7%, 36.3%, and 39.4% the first, second, third and fourth weeks of repletion, respectively. Similarly, controls receiving 2.0 mg of supplemental PN.HCl/day increased this percentage from 35.6%, to 51.1%, 50.7%, and 55.5% during this same time period. Similar changes were seen in OC users on the same levels of supplementation. OC users receiving 20.0 mg/day supplemental PN·HCl excreted 63.4%, 50.6%, 69.3%, and 80.5% of their vitamin B-6 intake as urinary 4-PA the first, second, third, and fourth weeks, respectively suggesting that subjects supplemented with this level were saturated by the first week.

Donald & Bosse (1979) also compared urinary vitamin B-6 metabolites in OC users and controls fed diets with defined amounts of vitamin B-6. Subjects were depleted of vitamin B-6 for 42 days (controls were fed 0.34 mg and OC users were fed 0.36 mg PN equivalents/day). Urinary total vitamin B-6 excretion of control subjects dropped from 22.9 to 0.4% of intake during this period while OC users dropped from 12.7 to 9.4% of intake. Urinary 4-PA excretion as a percent of total vitamin B-6 intake dropped concomitantly from 79.6 to 34.8% in controls and from 90.6 to 40.0% in OC users during this same period. Percent intake excretion values of vitamin B-6 for controls and OC users fed 0.94 and 0.96 mg PN equivalents/day, respectively, were 3.42 and 4.66%. Analogous values for 4-PA excretion were 22.5% and 28.2% for controls and OC users, respectively. Controls and OC users fed 1.54 and 1.56 mg PN equivalents/day excreted 3.63 and 4.66% of their vitamin B-6 intake as vitamin B-6 and 26.4 and 25.9% as 4-PA, respectively. When OC users were fed 29.9 mg PN equivalents/day they excreted 3.1% and 23.6% of this intake as vitamin B-6 and 4-PA, respectively. The lower percentages of the vitamin B-6 intake excreted as 4-PA in the study of Donald & Bosse are difficult to explain. In contrast to the study of Brown et al. (1975) the urinary 4-PA was only measured following the day of a tryptophan load test in the later study.

Shultz & Leklem (1981) evaluated the urinary excretion of 4-PA and total vitamin B-6 as criteria for assessing vitamin B-6 status and intake in adults. Thirty-five male and 41 female

subjects, either Seventh-day Adventists vegetarians or nonvegetarians, or general population nonvegetarians showed no significant differences between these groups of subjects in urinary 4-PA excretion or total vitamin B-6 excretion. Vitamin B-6 intake and urinary excretion of both 4-PA and vitamin B-6 were higher in males than in females. Dietary vitamin B-6 intake was 2.0 ± 0.8 and 1.6 ± 0.5 mg PN equivalents/day for males and females, respectively. Urinary excretion of 4-PA and total vitamin B-6 was 7.46 ± 4.34 and 0.92 ± 0.49 for men and 5.57 ± 3.09 and 0.76 ± 0.24 for women, respectively. When expressed as a percentage of intake, however, there was no significant difference in excretion of 4-PA by men and women (62 ± 32 and 59 ± 22%, respectively). Total urinary excretion of vitamin B-6 as a percent of intake was also similar in males and females (7.8 and 8.0%, respectively).

Wozenski et al. (1980) studied the metabolism of small doses of vitamin B-6 in 5 apparently healthy men aged 24 to 32 years. Subjects ingested a total of either 6.4, 8.8, 11.2, 16.1, 25.8, or 55 µmoles PN or 25.8 µmoles of either PN, PM, or PL the day following a day where they were fed controlled diet containing 9.46 µmoles vitamin B-6. After an overnight fast, the doses were given and urine was collected in timed intervals from 0-3, 3-8, 8-12, 12-16, and 16-24 hr to determine not only the extent but the rate of excretion of urinary 4-PA and vitamin B-6 as well. The rate of 4-PA and vitamin B-6 excretion was maximal during the first 3 hours. As the dose of

PN was increased from 6.4 to 55 μmoles, the percent of total vitamin B-6 ingested excreted as 4-PA decreased from 63 to 35% while the percent as vitamin B-6 decreased from 9 to 7%. When equimolar doses (25.8 μmoles) of PN, PM, or PL were given 4-PA excretion was 45.9 ± 6.3, 48.6 ± 6.1, and 55.2 ± 20.2% of the dose, respectively, while total urinary vitamin B-6 excretion was 5.7 ± 0.8, 4.7 ± 0.8, and 4.6 ± 0.6%, respectively. PL was more rapidly converted to 4-PA than the other two vitamers, probably because of its availability as a substrate for aldehyde oxidase.

In another dose study performed at the same laboratory, Leklem (1987) gave eight men aged 20-30 years PN,PM, or PL or and equimolar mixture of the three forms after feeding a constant diet containing 9.45 μmoles of vitamin B-6 for two days. Doses were 5.91 or 11.82 μmoles and were fed in addition to a diet containing 8.10 μmoles vitamin B-6. Mean pre-dose day total urinary excretion of 4-PA and vitamin B-6 were 77.19 ± 7.37 and 11.75 ± 0.73% of the 8.10 μmole intake, respectively. There was little vitamer effect on either 4-PA or vitamin B-6 excretion. Mean excretion of 4-PA and vitamin B-6 following the 5.91 μmole doses of all vitamers were 54.2 ± 2.9 and 6.8 ± 0.4% of the total 14.01 μmol intake. Similarly, 46.5 ± 2.0 and 5.3 ± 0.3% of the total 19.92 μmol intake were excreted as 4-PA and vitamin B-6 following the 11.82 μmol doses.

In summary, excretion of total vitamin B-6 (from about 5 to 10%) and 4-PA (from about 40 to 70%) are a fairly constant

percent of dose on an adequate diet. Generally, the percent of intake excreted decreases as the intake increases. When fed a diet inadequate in vitamin B-6, the excretion of these metabolites decrease to nearly undetectable levels.

Intake Recommendations

Recommended Dietary Allowances The National Research Council makes recommendations for dietary intakes of nutrients (FNB, 1980, 1989). For the 1980 recommendations, reviewed clinical measurements which were used to estimate requirements included results from tryptophan load tests, plasma PLP levels, transaminase levels in both serum and red blood cells, and urinary excretion of 4-pyridoxic acid and/or total vitamin B-6 (Sauberlich, 1974). Allowances are made based on the levels of protein intake as this affects requirements (Baker et al. 1964; Linkswiler, 1978). Using a ratio based on the Canadian recommendations (Dietary Standards for Canada, 1975), a level of 0.02 mg of vitamin B-6 per gram protein was deemed sufficient. This ratio resulted in the NRC recommendation of 2.0 mg/day for women and 2.2 mg/day for men based on the assumption (from dietary food consumption surveys) that American women and men ingest an average of 100 gm and 110 gm of protein per day, respectively.

In their most recent recommendations (FNB, 1989), the committee cited evidence (Linkswiler, 1978; Miller & Linkswiler, 1967; Park & Linkswiler, 1970) that 0.010 to 0.015

mg vitamin B-6/gm protein were adequate to prevent or eliminate biochemical indicators of deficiency in men. They cited Shultz & Leklem (1981) who found that urinary excretion of 4-PA and vitamin B-6, and plasma PLP levels were within acceptable ranges in women who were ingesting 1.25 or 1.5 mg of vitamin B-6 (equivalent to 0.0125 and 0.015 mg vitamin B-6/g protein) as evidence that these levels were adequate for women. Accordingly, they recommended that 0.016 mg vitamin B-6 per g protein was an adequate recommendation and based on protein intakes of twice the RDA (126 g/day for men and 100 g/day for women) recommended RDA's of 2.0 and 1.6 mg/day for men and women, respectively. This is a decrease from the 1980 recommendations which were based on 0.020 mg vitamin B-6/g protein rather than 0.016 mg/g. They do state that these lower levels may not be adequate for those on protein intakes at or above the 90th percentile but assume that since vitamin B-6 is associated with protein containing foods, those on higher protein intakes will likely consume adequate vitamin B-6 along with the protein.

According to Bessey et al. (1957), infants consuming 0.3 mg/day of vitamin B-6 are protected against excretion of abnormal levels of tryptophan metabolites following a load test. Proprietary formulas which contain either 0.015 mg/g of protein or 0.04mg/100 kcal provide adequate B-6 for infants (FNB, 1968; AAP, 1976). Thus the NRC recommends levels of 0.3 mg/day for infants under 0.5 years of age and 0.6 mg/day for infants 0.5-1.0 years of age (NRC, 1980).

Limited data were available for the NRC to make recommendations for intake for children and adolescents. Maintaining a level of 0.02 mg/gm of dietary protein results in recommendations of levels of 0.9 mg/day, 1.3 mg/day, and 1.6 mg/day for children aged 1-3, 4-6, and 7-10 years, respectively. Recommendations for males aged 11-14 years are 1.8 mg/day and for males aged 15-18 years are 2.0 mg/day. For females 11-14 years recommendations are 1.8 mg/day and are increased to the adult 2.0 mg day at age 15 years (NRC, 1980).

Several reasons could be cited for increasing vitamin B-6 recommendations during pregnancy. Since, during pregnancy, an additional 30 g of protein/day above the normal 50 g/day are recommended, a concommitant increase in vitamin B-6 intake would be sensible. A second reason is the observation of numerous biochemical indices such as plasma PLP levels, plasma total vitamin B-6 levels and erythrocyte alanine amino transferase levels, that are reduced in pregnancy that are usually indicative of compromised vitamin B-6 status in normal adults (Shane & Contractor, 1975). Third, the increased level of tryptophan oxygenase activity (a vitamin B-6 dependent enzyme) (Rose, 1978), caused by estrogens, is a possible indicator of the need for additional vitamin B-6 during pregnancy. In practice the Food and Nutrition Board elected to increase the allowances only by 0.6 mg/day as dictated by the increased protein recommendation (NRC, 1980).

The additional recommendations for lactation are based on the concentration of vitamin B-6 in human milk. Since

intakes of 2.5-5 mg/day were required to increase vitamin B-6 to protein ratios above 0.02 mg/day in the milk, and this ratio has been deemed appropriate for other age groups, the Council recommends an increase of 0.5 mg/day during lactation (NRC, 1980).

The NRC includes a discussion on the possible benefits of increasing vitamin B-6 recommendations for women taking oral contraceptives but cites Leklem et al. (1975) as indicating there is doubtful clinical significance to the changes in status indicators and thus makes no change in recommendation.

Status Assessment Leklem & Reynolds (1981, 1988) have made recommendations for status assessment of vitamin B-6. They point out that the particular situation being studied influences the assessment methodology used. They also recommend that more than one method of assessment be used. They noted that few clinical studies of therapeutic uses of vitamin B-6 have use multiple indicators of vitamin B-6 status. Leklem and Reynolds (1981) recommend that for definitive determination of vitamin B-6 status plasma PLP, urinary 4-PA and urinary metabolites of tryptophan after a load test be measured.

Clinical Since clinical symptoms of vitamin B-6 deficiency closely match those of other B-vitamin deficiencies, clinical diagnosis of vitamin B-6 deficiency is difficult. Clinical symptoms include weight loss and psychological

symptoms of irritability, depression, apathy and somnolence; external symptoms include oral lesions of cheilosis, glossitis and stomatitis (Sauberlich and Canham, 1980). Other external symptoms are acneform papular rash on the forehead, lesions around the eyes and in nasolabial folds, pellagra-like dermatitis on the thighs, arms and neck. Intertrigo develops under the breasts and in other moist areas. Peripheral neuropathy is sometimes observed, especially as a result of drug interactions (Bhagavan, 1985). Anemia and abnormal electroencephalograms are also observed (Sauberlich, 1981; Ebadi, 1978).

Biochemical Biochemical indices of vitamin B-6 status include both direct measurements of vitamin B-6 compounds in blood or urine or functional tests of metabolic pathways or enzymes which use vitamin B-6 vitamer(s) as cofactors.

The current most accepted measure of vitamin B-6 status is the amount of PLP in plasma. PLP is believed to be the most sensitive and reliable indicator of vitamin B-6 status. In animals plasma PLP levels correlate closely with tissue levels (Lumeng et al. 1978). Plasma PLP is influenced by changes in dietary intake but generally reflects long term status (Brown et al. 1975). Wozenski et al. (1980) found that single oral doses of PN less than 1 mg raised PLP levels transiently. Doses greater than 1 mg resulted in elevation of plasma PLP that were still evident after 24 hr. The plasma levels of PLP, however, remained relatively constant on non-dose days throughout the 7 weeks of this study. After giving a PN dose

of 100 mg, Contractor & Shane, (1968) observed elevations in plasma PLP 96 hours later. PLP is quite reliable as an indicator during fairly constant intakes of vitamin B-6 (Leklem, 1988). Recent work indicates that there may be a shift of vitamin B-6 as PLP in the plasma to PL during normal periods of metabolic adjustment such as pregnancy (Barnard et al. 1987). Leklem & Reynolds (1988) suggested that perhaps total plasma vitamin B-6 aldehyde (whether phosphorylated or not) may be a more specific indicator of vitamin B-6 status, especially in women.

A relatively noninvasive measure of vitamin B-6 status is the excretion of the urinary metabolite, 4-PA. Urinary levels reflect more recent alterations in intake than do changes in plasma PLP levels (Canham et al. 1969) but are generally in close agreement for predicting status (Brown et al. 1975). Often it is not possible to obtain complete 24 hr urine collections. A single plasma sample is far easier to obtain, therefore population studies often use plasma PLP as a vitamin B-6 status assessment criterion.

An indirect functional method of assessing vitamin B-6 status is the measurement of the excretion of tryptophan metabolites before and after an oral dose of L-tryptophan. One key enzyme (kynureninase) in the pathway from tryptophan to niacin requires vitamin B-6 (as PLP) as a cofactor (Leklem, 1971). Although both the enzymes kynurenine aminotransferase and kynureninase require PLP, the former enzyme is less sensitive to deficiency due to its compartmentalization mainly in the mitochondria, and due to its higher affinity for PLP

(Brown, 1981). Thus during vitamin B-6 deficiency, more xanthurenic acid and kynurenic acid are excreted than normal. The tryptophan load test is ususally a sensitive and reliable indicator of the functional adequacy of vitamin B-6 status (Brown, 1981).

However, Hayaishi et al. (1984) reported that indoleamine dioxygenase is capable of producing a precursor of tryptophan metabolites. Brown (1988) reported that this nonhepatic enzyme can be stimulated by several factors, including bacterial or virus infections, bacterial endotoxins or interferons. These recent findings suggest that the tryptophan-load test may have severe limitations as an index of vitamin B-6 deficiency in many clinical conditions.

A test similar to the tryptophan load test, but using a methionine load instead is sometimes used. Cystathione is excreted in increased amounts during vitamin B-6 deficiency. When 6 male subjects were depleted of vitamin B-6 for 3 weeks, pre and post cystathionine urinary excretion after a 3 g loading dose of L-methionine was 1508 and 3719 µmoles/24 hours, respectively (Park & Linkswiler, 1970). Prior to depletion these values were 128 and 163 µmoles, respectively. Supplementation with 2.0 mg PN caused a sharp reduction of these values in 2 days, after 1 week of this supplement values were similar to predepletion levels. However, the test is sensitive to vitamin B-6 status only in cases of severe deficiency (Leklem et al. 1977).

An often used, but because of its variability a probably less sensitive indicator of vitamin B-6 status, is the measurement of erythrocyte aminotransferase activity either as a direct measurement or as percent stimulation of erythrocyte aspartate (or alanine) aminotransferase activity with exogenous PLP (Woodring & Storvick, 1970). These authors reported a wide variation among individuals in erythrocyte glutamic-pyruvic transaminase (EGPT) and concluded that this lessened the value of this vitamin B-6 status indicator. However 50 mg of supplemental PN resulted in higher EGPT indices, even those on adequate vitamin B-6 intakes.

Dietary Intake There has been a relative lack of vitamin B-6 intake data in population studies (Sauberlich, 1981). As food composition tables become more reliable in their estimates of food vitamin B-6 content, and good estimates of food vitamin B-6 bioavailability become available, food intake data, whether obtained by 24 hr recalls, diet histories, food frequency questionnaires, or intake logs, will become a more important means of vitamin B-6 status assessment.

The USDA (1986, 1987) monitors average vitamin B-6 intake in the United States in their Nationwide Food Consumption Survey; Continuing Survey of Food Intakes by Individuals. In 1985 (USDA, 1986) men 19-50 years of age consumed an average of 1.87 mg of vitamin B-6 based on a 1 day survey. Based on a 4 day survey, women (19-50 years of

age) and their children (1-5 years of age), consumed an average of 1.16 and 1.22 mg/day, respectively. This corresponded to a vitamin B-6/protein ratio of 0.019, 0.019, and 0.023 mg/g for men, women, and children, respectively.

The Life Sciences Research Office (LSRO, 1989) prepared a report on nutrition monitoring in the United States. According to this report the per capita intake of vitamin B-6 was about 2.1 mg in 1980, with 41.1% supplied by meats, poultry, and fish, 21.9% supplied by vegetables, 10.7% by dairy products, and 10.6% by fruits. The total per capita intake has not changed appreciably since the early 1900's. The LSRO (1989) concluded "In order to interpret the consequences of these intakes, further study is needed on the content and bioavailability of vitamin B-6 in foods, vitamin B-6 requirements, and biochemical or other techniques for assessing vitamin B-6 nutritional status. Increased monitoring activity may be warranted as progress is made in these areas."

Vitamin B-6 Bioavailability

Availability of Vitamin B-6 in Foods The first indication that food sources of vitamin B-6 were not as bioavailable as crystalline forms was obtained by Samra et al. (1947). Differences were found in the biological effect of vitamin B-6 between microbiological growth response and rat growth bioassays when feeding mixed rations. Results ranged from 5-211% for the rat assay compared to PN. They concluded these

differences were due to differences in activity of pyridoxamine and pyridoxal compared to pyridoxine (PN) in the rat but not in *Saccharomyces uvarum*. However, current research demonstrates that at physiological concentrations all 3 vitamers (PN, PL, and PM) have equal biopotency (based on isolated vascular perfusion in rats) and that the phosphorylated forms are hydrolyzed prior to complete absorption by intestinal phosphatases (Henderson, 1985).

Protein binding has been implicated as an inhibitor of vitamin B-6 bioavailability, mainly through formation of ε-pyridoxyllysine (Gregory and Kirk, 1981). Gregory and Kirk (1978) found that ε-pyridoxyllysine was 60% as bioavailable as PN in rats. In contrast, in vitro systems indicate that this compound is largely converted to PLP and is available (Gregory, 1980). Vitamin B-6 compounds formed either in stored low moisture, protein foods, or protein foods subjected to extreme heat or a combination thereof (probably formed from the reductive binding of PL or PLP to ε-amino groups of lysine) are less bioavailable than nonconjugated PN, PL, PN or their phosphorylated derivatives. Gregory and Ink (1985) concluded that the occurrence of vitamin B-6 responsive seizures in infants receiving commercial unfortified formula (Coursin, 1954), was due to marginal intake plus a slight (15%) reduction in bioavailability from proteinacious products of thermal processing.

Absorption of Pure Chemical Forms Absorption of the various forms of vitamin B-6 (PN, PL, and PM) occurs probably by way of a nonsaturable passive process, after intestinal hydrolysis of phosphate esters by alkaline phosphatase (Middleton, 1978, 1982). After absorption, the vitamin B-6 travels through the portal vein to the liver.

PN, PL and PM are probably absorbed by nonsaturable passive diffusion in the small intestine (Henderson, 1985). While this has been demonstrated for the rat (Buss et al. 1980; Hamm et al. 1979), little information is available for the human. More recent information (Middleton, 1985) suggested that the absorption may be saturable, particularly in the proximal small intestine; this suggests that pharmacological doses of vitamin B-6 may not be absorbed as completely as physiological doses.

Henderson (1985) found there was some interconversion of the vitamers in the intestinal mucosal cell. After a 10 min perfusion of rat intestine, he found that approximately equal amounts (14 to 18% of a 20 nmol dose of ^{3}H- PN, PL, PLP, PM or PMP were) were found in the mucosa. However, with small doses (20 nmol) the substition of the 4-position remained identical to the form given, with large doses (200 nmol) intact PLP and PMP were absorbed when these forms were given. The mucosal cell does appear to metabolize vitamers of B-6, at the low dosage of PN the major form that was found in the perfusate was PLP (32% of the dose), while at the higher doses, much less PLP was found. While this indicates that absorbed

vitamin B-6 can be metabolized in the mucosal cells, the primary site of metabolism is the liver (Merrill et al. 1984).

Plant Sources Nelson et al. (1976) found a significantly higher uptake of synthetic PN in solutions than the vitamin B-6 in orange juice using a triple lumen tube in human subjects. The ratio of PN:PLP:PM were matched to that of orange juice in the synthetic solution. Glucose added to the synthetic solution enhanced this differential uptake. On the basis of equilibrium dialysis, they concluded that there was a vitamin B-6-containing compound in orange juice that had a molecular weight of less than 3000 daltons.

Using chick bioassays, Yen et al. (1976) found the vitamin B-6 in corn and soybeans less available than crystalline vitamin B-6 as measured by growth and serum aminotransferace activity as response indicators. Since they did not quantitate the vitamin B-6 in these foods, their data are difficult to interpret. However, extrapolation from published values of the vitamin B-6 content of these foods indicate that the bioavailability of vitamin B-6 in corn and soybeans was less than that of synthetic vitamin B-6.

Tarr et al. (1981) fed 6 healthy male subjects a semipurified diet containing 1.1 mg/day PN for 35 days. This was followed by a 35 day period where natural food sources ("average American Diet") supplied 2.3 mg PN/day for 21 days. A third period followed in which the subjects were fed a

formula diet providing 2.7 mg PN/day for 21 days. They found that the vitamin B-6 from an average American diet was 61-81% (mean 71%) and 73-92% (mean 79%) less available than pure vitamin B-6 (as PN) when using urinary vitamin B-6 excretion and plasma PLP concentration as response indices, respectively.

In a study designed to assess the efficacy of fortifying wheat flour with vitamin B-6, Leklem et al. (1980) found the vitamin B-6 in whole wheat bread to be 5-10% less available than either the vitamin B-6 in white bread, vitamin B-6 fortified white bread, or PN. Indices that indicated this decrease in bioavailability were a 9.5 μmole/week increase in fecal content of vitamin B-6, a 13% decrease in urinary 4-PA excretion, and a 9.7% decrease in plasma PLP when whole wheat bread was fed. No significant differences were found in either erythrocyte transaminase activity, total excretion of urinary vitamin B-6, or plasma vitamin B-6 levels when the various breads were fed.

Using rat bioassay methods, Gregory (1980) found that the vitamin B-6 from fortified rice breakfast cereal was less available than pure vitamin B-6. In contrast, the vitamin B-6 in nonfat dry milk was found to be 100% available. Growth, feed efficiency and liver PLP concentration all indicated that the vitamin B-6 from fortified rice breakfast cereal had less activity in the rat bioassay than in the microbiological assay. However, no significant differences were observed in erythrocyte aspartate aminotransferase (Asp-AT) activity nor

per cent stimulation by PLP in vitro between rats fed nonfat milk, pure vitamin B-6, or fortified rice breakfast cereal.

Nguyen & Gregory (1983), again using rats, found that the vitamin B-6 in beef was more available than that from either cornmeal, spinach, or potato. This difference was also demonstrated using plasma PLP, but poor correlations with growth and feed efficiency were observed. Possible intestinal synthesis of vitamin B-6 vitamers and subsequent coprophagy may confound rat bioassays.

Thus it appears that vegetable sources of vitamin B-6 are generally less available than either crystalline or animal sources.

Dietary Fiber A possible explanation for the variation in vitamin B-6 bioavailability in plant foods or diets is the type and level of dietary fiber. Nguyen et al. (1981) constructed dose response curves for growth, feed consumption, feed efficiency, liver PLP and Asp-AT in chicks and rats fed diets low in PN and containing 5% of various sources of fiber. Pectin resulted in increased growth and feed efficiency in rats, probably due to microflora synthesis of vitamin B-6 and either intestinal absorption or coprophagy as indicated by higher fecal vitamin B-6 content. In contrast, the chicks showed a decrease in feed efficiency and growth with pectin and exhibited diarrhea. The authors concluded that there were no significant effects of pectin, bran, cellulose or a combination thereof on bioavailability of vitamin B-6.

Using total urinary vitamin B-6 excretion in humans as a measure, Machida and Nagai (1980) administered tablets of PN:HCl compounded with hydroxypropyl cellulose and observed delayed but complete absorption compared to pure PN tablets. In vitro binding experiments with vitamin B-6 vitamers and wheat bran, lignin, cellulose, hydroxypropyl cellulose, carboxymethyl cellulose, methyl cellulose, citrus pectin, xanthan gum, sodium alginate, or gum arabic showed no absorption by the fibers as demonstrated by equilibrium dialysis rates of vitamin B-6 incubated with the various fibers (Nyugen, 1981).

Two human studies investigated the effect on vitamin B-6 bioavailability of pectin or wheat bran. Fifteen grams of pectin fed to 8 men for 18 days had no effect on urinary vitamin B-6, urinary 4-PA excretion, and plasma vitamin B-6 compared to a basal diet containing no pectin. As in the rat, an increase in fecal vitamin B-6 was observed, but this was apparently unavailable for absorption (Miller et al. 1980). In a similar experiment, Lindbeg et al. (1983) fed 10 men, aged 20-35 years, a constant diet with and without 15 grams of cooked wheat bran for successive 18 day periods in a switch-back design. They observed a 17% decrease in bioavailability of vitamin B-6 during the wheat bran periods as evidenced by plasma vitamin B-6 and PLP levels, and urinary vitamin B-6 and 4-PA excretion. Fecal vitamin B-6 excretion was also significantly increased with the bran regimen. However, the basal diet was fortified with the amount of vitamin B-6 in the

wheat bran (0.186 mg) and the observed difference in status indicators could possibly be due to decreased bioavailability of the intrinsic vitamin B-6 in wheat bran.

Using young men as subjects, 4 indices, (urinary 4-PA and total vitamin B-6 excretion, fecal vitamin B-6 excretion, and plasma PLP), Kabir et al. (1983a) demonstrated that the vitamin B-6 in whole wheat bread and peanut butter were an average of 75% and 63%, respectively, as available as the vitamin B-6 in tuna. They used a ten day adjustment period and a 3X3 latin square design. Vitamin B-6 intake was set at 1.6 mg/day and 50% of the vitamin B-6 came from the test food for each of the 14 day periods. Differences in plasma PLP concentrations for the test diets did not agree closely with the differences observed for the other three status indicators. However, plasma PLP concentrations are more indicative of long term changes in vitamin B-6 status than the more immediate responses of the other three indicators (Leklem & Reynolds, 1988).

Pyridoxine Glucoside

Scudi (1942) reported the existence of conjugated forms of vitamin B-6 in rice bran. In 1977 Yasumoto et al. identified a PN-containing compound in rice bran extract as 5'-0-(ß-D-glucopyranosyl) pyridoxine (or pyridoxine glucoside (PNG)) which on hydrolysis gave glucose and PN. PNG may be crucial in explaining the variation in bioavailability of vegetable

sources of vitamin B-6. The compound was extracted from a faintly yellow water extract syrup by repeated ion exchange and paper chromatography. This compound, together with any other compounds which release measurable vitamin B-6 with almond ß-glucosidase treatment, was called glycosylated vitamin B-6 (GB6) by Kabir et al. (1983b) who measured the GB6 content of 30 different foods and found essentially none in animal foods and from 0 to 82% in various plant foods. Percent GB6 of some of these foods are frozen cauliflower (82%), raw filberts (26%), wheat bran (36%), and beef (none detected). Subsequently the GB6 content of many other foods has been measured by workers at Oregon State and has been found to contain a similar range of per cent glycosylation (unpublished observations).

Kabir et al. (1983c) developed regression equations comparing the per cent glycosylation of vitamin B-6 in foods with bioavailability from his tuna, whole wheat and peanut butter study (Kabir et al. 1983a) and two other studies (Tarr et al. 1981; Gonzalez, 1982. With their own data for tuna, whole wheat bread, and peanut butter, they regressed the data from urinary vitamin B-6, urinary 4-PA, plasma PLP and fecal vitamin B-6 against GB6 content and found correlation coefficients of 0.99, 0.94, 0.94, and 0.92, respectively. Using their regression equations and an estimate of percentage of GB6 (10%) in the average American diet fed by Tarr et al. (1981) the bioavailability was estimated to be 82% from urinary vitamin B-6 data and 80% from plasma PLP data. These

are very close to Tarr's bioavailability estimates of 79% and 71%, respectively. Comparing the 4-PA excretion values of Gonzalez (1982) with their regression equation, Kabir et al. (1983c) found the vitamin B-6 in bananas 98% available (with 3% GB6 content), in filberts 96% available (with 4% GB6 content), and in soybeans 41% available (with 57% GB6 content).

These last data suggested that there was a strong inverse correlation between the content of GB6 in a diet or individual food, and the bioavailability of vitamin B-6 in that food.

Measurement of PNG Two published assay procedures (Tadera et al. 1986, Kabir et al. 1983a) for measuring PNG content of samples differ in several respects. Different buffers, temperatures, and times are used for extraction of vitamin B6 and subsequent incubation with ß-glucosidase. In addition, the amounts of "nonconjugated" or "free" forms used in the calculation of PNG content are arrived at by different methods (Tadera et al.1986, Kabir et al. 1983a). The amount of PNG quantitated by these two methods have not been compared in single samples.

Gregory & Ink (1987) developed an HPLC method for the fluorometric measurement of PNG. Samples are extracted using sulfosalicylic acid. The samples are then centrifuged and an aliquot of the aqueous supernatant is removed. The sample is further purified on an anion-exchange column. An aliquot of this mixture is then treated with ß-glucosidase and

deproteinated with trichloroacetic acid. The samples are then assayed fluorometrically (excitation 330 nm and emmission 400 nm) upon elution from an octadecylsilyl column using a gradient of potassium phosphate, octanesulfonic acid, and propanol. Detection was enhanced by postcolumn addition of phosphate-bisulfite reagent.

Animal Studies of PNG Bioavailability Soon after their isolation and characterization of PNG, Tsuji et al. (1977) investigated its functional availability as vitamin B-6 to rats. Using chemically synthesized PNG they investigated the effects of orally administered or intravenously injected PN and PNG on the excretion of xanthurenic acid (XA) in rats fed a vitamin B-6 deficient diet. Forty-five mg of L-tryptophan were given simultaneously with the PN of PNG. Although there was no difference between the orally administered PN and PNG in the normalization of XA excretion, a slight (nonsignificant) difference (25.8 $\pm$ 7.2 versus 17.2 $\pm$ 6.5 mg) was seen in XA excretion when PNG versus PN was administered intravenously, respectively. The activation of glutamate-pyruvate transaminase and cysteine transulfhydrase were restored to the levels of control rats by either PN or PNG. They found ß - glucosidase activity in homogenates of the small intestine and in liver and blood that hydrolyzed PNG to pyridoxine and glucose in vitro. PNG was able to transverse everted sacs of rats jejunum both under anearobic and aerobic conditions. From

these observations the authors concluded that PNG was essentially 100% available to rats.

In contrast to the studies of Tsuji et al. (1977), Gregory and coworkers have performed several studies investigating the bioavailability of PNG and PNG-containing foods to rats and have found that PNG is less available than nonconjugated vitamin B-6. A study was performed by Ink et al. (1986) to determine the bioavailability of PNG to rats. Rats were fed ^{3}H labelled PNG either in purified form or in intrinsically enriched alfalfa sprouts grown in ^{3}H labelled PN. The bioavailability of PNG was compared with the availablity of ^{14}C labelled PN. The vitamin B-6 compounds were administered in a single test meal in calcium alginate gel which the rats had been trained to consume. The rats were killed 24 hours after administration of the test meal. Retention of ^{3}H in ^{3}H-PNG fed rats was 50% or less than controls fed ^{3} labelled PN. No ^{3}H-PNG was found in the livers of the rats. Fully 85% of ^{3}H in urine from rats fed ^{3}H-PNG was found intact as PNG. Approximately half as much PNG as PN was absorbed from the gastrointestinal tract. Using the sum of the ^{3}H labelled 4-PA and its lactone in the urine gave an estimate of 40% for the bioavailability of PNG under these conditions.

In another study, Trumbo et al. (1988) sought to determine the bioavailability of PNG during chronic administration in a depletion-repletion bioassay. Results of this study indicated the PNG was only 10-34% as available as PN based on slope-ratio analysis of growth and plasma PLP of

rats depleted for 14 days on a vitamin B-6 free diet and then repleted with PN or PNG for 17 days. The dose of PNG fed had no effect on its percentage absorption, metabolic utilization or excretion. Seven to nine percent of ingested PNG was excreted intact as PNG in the urine.

Trumbo & Gregory (1988) investigated the utilization of PNG in rats that were either vitamin B-6 deficient or vitamin B-6 adequate. They evaluated the effect of oral versus intaperitoneal injection of PNG on its metabolic fate. Vitamin B-6 deficient and vitamin B-6 sufficient rats were given simultaneous doses of ^{3}H labelled PNG and ^{14}C labelled PN. Results showed that there was little difference in utilization of PNG between vitamin B-6 adequate and deficient rats. In both intraperitoneally injected and orally dosed rats, there was greater ^{14}C than ^{3}H retained in the carcass and livers. This indicated greater metabolic utilization of PN than PNG. Both compounds were well absorbed, with little label (less than 12% or the administered dose) remaining in the intestinal contents and feces following oral doses. However, there was significantly greater retention of both ^{3}H and ^{14}C in orally dosed deficient compared to vitamin B-6 adequate rats. This indicated that vitamin B-6 deficiency increases metabolic utilization of vitamin B-6. ^{3}H-4-PA excreted in the urine was 3.65% versus 0.54% of the dose for oral versus intraperitoneal doses of PNG, indicating that the intestine is probably responsible for the approximately 20% conversion of PNG to PN.

To determine the extent to which PNG could serve as a source of vitamin B-6 to rat pups, and to determine if PNG was excreted intact from mammary glands of rats, lactating rats were administered ^{3}H-PNG and ^{14}C-PN (Trumbo & Gregory, 1989). Analysis of the milk from the dam and the intestinal contents and liver of the pups showed that only 20-25% of the ^{3}H compared to the ^{14}C was secreted into the milk. No PNG was found in the milk, stomach contents, or livers, indicating that PNG was hydrolyzed to PN prior to secretion into milk. PLP was the only metabolite found in the stomach contents, and the relative distribution of ^{3}H and ^{14}C vitamers in the livers of the pups was similar.

Banks & Gregory (1990) fed 150-250 g rats with either 1 mg/kg or 0 mg/kg PN and either 0, 5, 10, or 15 nmol/g PNG to measure the effects of vitamin B-6 level and PNG content of the diet on the utilization of PNG. ß-glucosidase activity for 4-methylumbelliferyl-ß-glucoside or tritiated PNG was measured in intestinal mucosa at 0 and 4 weeks. There was no effect of dietary PN or PNG level on ß-glucosidase activity.

Gilbert & Gregory, (1990) investigated the effects of PNG fed to rats on the metabolism of simultaneously administered ^{14}C labeled PN. Three groups of 6 rats were given an oral dose of either 0, 36 or 72 nmol of PNG given with 240 nmol ^{14}C-PN. The % of the administered ^{14}C found in the urine increased, while the proportion of ^{14}C found in urinary 4-PA decreased with increasing doses of PNG. These results indicate that PNG may decrease the utilization of nonglygosylated vitamin B-6.

Tsuji et al. (1977) observed changes in XA excretion, and normalization of blood transaminases after feeding PNG to deficient rats. These indicators of vitamin B-6 status are less sensitive to changes in status than the indicators (such as tissue distribution and excretion of radioisotopically labelled PNG) obtained in Gregory's laboratory. The latter investigators did find some absorption of PNG and utilization of PNG. However, much of the administered dose was excreted intact as PNG. The finding by Tsuji et al. (1977) that glucosidases are widespread in mammalian systems did not address the question of accessibility of substrate to enzyme due to compartmentalization of enzymes in cells and organelles. The data from Gregory's laboratory indicate that PNG is partially available to the rat as a source of vitamin B-6 activity.

Human Studies of PNG Bioavailability The question of the human bioavailability of PNG still needs to be addressed in more detail. Trumbo et al. (1988; abstract) administered a single oral dose of deuterated PNG. The 4-PA acid excreted after this dose was administered was analyzed by mass spectrometry and determined to be enriched enough with deuterium for the authors to conclude that PNG was better utilized by humans than by rats.

In a subsequent study, Trumbo & Gregory (1989; abstract) found that there was significant ß-glucosidase activity in human small intestinal tissue. The activity in the human small intestine was higher than in the small intestines of rats or

guinea pigs. Enzyme activity was observed in both soluble and particulate cellular fractions, was inhibited by detergents, had a pH optimum of 5.5 - 6.0 and a Km of 2-3 mM.

Gregory et al. (1989; abstract) also gave doses of deuterium labelled PN (d5-PN) and PNG (d2-PNG) to humans. Two trials were performed with fasting male subjects, in the first both compounds were given orally, in the second the d5-PN was given orally while the d2-PNG was given intravenously. The amount of 4-PA excreted as the d2 or d5 forms was used to estimate the availability of vitamin B-6 from these two forms. The authors reported variable but effective utilization from the orally administered d5-PNG while the d2/d5 ratio in the subjects injected with d5-PNG was 5 times lower. The authors concluded that these data indicate that the intestinal microflora or mucosa are responsible for any release of PN from PNG.

Cho et al. (1990, abstract) measured the levels of PNG and other vitamin B-6 vitamers in the breast milk of American and Egyptian women during 6 months of lactation by reverse phase HPLC. The American women were supplemented with either 2.5 or 10 mg of PN-HCl. PNG content of the milk was 11% for Egyptian women, compared to less than 1% for American women. Total vitamin B-6 levels in milk (in Egyptian women) correlated negatively with plant protein intake ($r=-0.49$) and % PNG of the diet ($r=-0.59$). The authors suggest that high levels of plant protein intake are associated with reduced PL and total vitamin B-6 in human milk.

A study of American Seventh-day Adventists observed poorer but not statistically significant vitamin B-6 status among an American population consuming high intakes of plant proteins. While not directly measuring the utilization of PNG, Shultz & Leklem (1987) looked at the vitamin B-6 status of vegetarian women and found no significant differences in plasma PLP, urinary 4-PA and urinary vitamin B-6 between vegetarian subjects who consumed diets high in plant fiber and protein, and controls who had lower (37%) intakes of fiber and higher intakes of animal protein.

The vitamin B-6 status of lactating Nepalese women and their children was evaluated (Reynolds et al. 1988, Reynolds et al. 1986). These vegetarian women consume diets high in PNG. Although the total vitamin B-6 intake of the Nepalese women (1.70 ± 0.65 mg/d) was higher than American women (1.52 ± 0.80 mg/d) (Reynolds et al. 1984), the plasma PLP levels of Nepalese women were lower than those of the American women. Unlike American women (Gregory & Ink, 1987), Nepalese women had substantial amounts of PNG in their breast milk. Furthermore, the frequency distribution of % PNG in the breast milk and the diet composites of these mothers was remarkably similar. The total vitamin B-6 content of the American and Nepalese women's milk was similar. However, the plasma PLP content of the Nepalese children was significantly lower than that of American children. Thus it appears likely that PNG content of the diet can influence vitamin B-6 status of both women and children.

Other Vitamin B-6 Conjugates

Other Glucosides Tadera et al. (1985) found other conjugated PN compounds in the seedlings of *Pisum sativum L*. These compounds were 5'-O-[6-O-(3-hydroxy-3-methyl-4-carboxybutanoyl)-ß-D-glucopyranosyl] pyridoxine and 5'-O-(6-O-malonyl-ß-D-glucopyranosyl) pyridoxine and comprised less than 4% of the pyridoxine-containing compounds. In a subsequent paper the same authors (Tadera et al. 1988) presented evidence for three new pyridoxine-glucosides in rice bran. From 10 kg of rice bran 325 mg of pyridoxine-containing compounds were isolated of which 53 mg was identified as 5'-O-(ß-cellobisyl) pyridoxine, 7.8 mg as 4-'O-(ß-D-glucosyl)-5'-O-(ß-cellobiosyl) pyridoxine, and 5.8 mg as 5'-O-(ß-glucotriosyl) pyridoxine where the cellobiosyl moiety of the first compound was bound to a glucose moiety by a ß-glycosidic linkage. These workers made no speculation as to the mammalian bioavailability of these compounds.

B_6X In 1986 Tadera et al. found evidence for the occurrence of a novel vitamin B-6 conjugate in plant foods. Using chemical and enzymatic analysis they concluded that the compound was probably PNG esterified with an organic acid. The compound was found in peas, soybeans, rice bran, and wheat bran, but not in cauliflower, spinach, pumpkin, or broad beans.

They made no speculation as to this compound's bioavailability as vitamin B-6.

Digestive Enzymes Ekanayake & Nelson (1986) found good agreement between the amounts of vitamin B-6 released by treatment of model processed foods with digestive enzymes, extracted with methanolic HCl and quantitated by HPLC and the amount estimated by rat feed efficiency bioassays. The treatment consisted of slurrying freeze-dried foods in water, acidifying to pH 2.0, digestion with pepsin for 3 hr at 37°C, adjustment to pH 8.0 and digestion with pancreatin for 12 h at 37°C. This mixture was acidified with HCl-methanol and buffered to pH 2.2, centrifuged and analyzed with HPLC using fluorescence detection. Use of digestive enzymes may approximate closely the in vivo availability of vitamin B-6. This represents a new approach to the determination of vitamin B-6 bioavailability.

Effects on Requirements and Intake Recommendations An accurate determination of the bioavailability of vitamin B-6 is necessary to set intake recommendations. Current recommendations are based on experiments using pure vitamers (NRC, 1989). If the bioavailability of vitamin B-6 in foods is significantly different from 100%, concomitant increases in recommendations will need to be made.

Chapter 2

BIOAVAILABILITY TO HUMANS OF VITAMIN B-6 IN 10 PYRIDOXINE GLUCOSIDE CONTAINING PLANT FOODS

Bills, Nathan D., Miller, Lorraine T. & Leklem, James E.

Department of Nutrition and Food Management
Oregon State University
Corvallis, OR 97331

To be submitted to the Journal of Nutrition

Abstract

To test the hypothesis that vitamin B-6 (B6) bioavailability (BA) is inversely correlated with %B6 as pyridoxine glucoside (PNG), the BA of B6 in 10 plant foods was assessed. Nine men aged 20-33 yrs were fed a constant diet containing 3.2 mg of B6 throughout a 49-day study. Incremental urinary 24-hr 4-pyridoxic acid (4-PA) excretion in response to test doses of pyridoxine (PN) (0.2, 0.4, and 0.6 mg) or test foods (0.5 mg B6 equivalents) fed in addition to the diet every third day after a 10-day adaptation period was used to assess differences in B6 BA among foods. Their %BA calculated from a urinary 4-PA excretion response line generated from PN doses was: walnuts 87; bananas 60; tomato juice 43; wheat bran -46; shredded wheat -23; spinach 17; orange juice 19; broccoli 62; cauliflower 56; and carrots -1. Regression analysis showed a strong inverse relationship between %PNG and BA for 6 of the ten foods ($R=-.94$,$p<0.01$). However, wheat bran and shredded wheat had low, negative BA while the two crucifers, broccoli and cauliflower, had high BA despite a high percentage of B6 as PNG. Percentage B6 as PNG predicted some, but not all, of the variation in B6 BA of the foods.

Introduction

In 1977 Yasumoto et al. identified a pyridoxine (PN) containing compound in rice bran. On hydrolysis this compound yielded glucose and PN. The identity proved to be 5'-0-(ß-D-glucopyranosyl) pyridoxine, commonly called pyridoxine ß-glucoside (PNG). This compound may be crucial in explaining the variation in bioavailability of vegetable sources of vitamin B-6. This compound, together with any other compounds which release measurable vitamin B-6 with almond ß-glucosidase treatment, was measured in 30 different foods by Kabir et al. (1983a). They detected no PNG in animal foods and from 0 to 82% in various plant foods.

Several animal studies have been performed to assess the biological availability of PNG. Tsuji et al. (1977) found that PNG was absorbed and biologically available to rats that were made deficient in vitamin B-6. Other rat studies conducted by Gregory and coworkers indicated: 1) only 50% of a single radiolabeled dose of PNG was absorbed in non-deficient rats, 2) the majority of this dose was excreted intact in the urine as PNG (Ink et al. 1986), 3) between 10 and 30% of PNG was biologically available in a depletion/repletion assay as determined by slope-ratio analysis of growth and plasma pyridoxal 5'-phosphate (PLP) values (Trumbo et al. 1988), and 4) PNG was nearly completely absorbed but was poorly metabolized (Trumbo and Gregory, 1988). Trumbo and Gregory

(1989) recently reported that PNG is more readily utilized in vitamin B-6 metabolism by humans than it is by rats.

In human studies, the bioavailability of vitamin B-6 from foods which contain PNG has been assessed. Kabir et al. (1983c) developed regression equations comparing the per cent PNG in foods with their bioavailability using data from a study comparing the bioavailability of tuna, whole wheat and peanut butter (Kabir et al. 1983b). These equations demonstrated an inverse correlation between the bioavailability of vitamin B-6 in these foods and their PNG content when using total urinary vitamin B-6, urinary 4-pyridoxic acid (4-PA), plasma PLP, and fecal vitamin B-6 as bioavailability indices. Reconstructing the percentage of PNG (10%) in the "typical American diet" of Tarr et al. (1981), and using these regression equations, Kabir et al. (1983c) predicted bioavailability values that agreed closely with the results of Tarr et al. (1981). Comparing the urinary 4-PA excretion values of Gonzalez, (1982), with the same regression equations, Kabir et al. (1983c) found the bioavailability of vitamin B-6 in bananas, filberts, and soybeans was predicted by their % PNG content.

The data presented by Kabir et al. (1983c) suggest that there is an inverse correlation between the PNG content of a food and its bioavailability to humans. The research presented here was conducted to further clarify this relationship by assessing the bioavailability of ten plant foods containing a wide range of percent PNG. The specific objective was to

determine if in vitro determination of the %PNG of a food could predict its in vivo availability to humans.

Materials and Methods

Subjects Nine male subjects were recruited by advertisement from the University community. All indices indicated apparent health, and their age, weights and heights are given in Table 1. Approval for the protocol and the informed consent form was obtained from the Oregon State University Human Subjects Committee. Subjects were not engaged in strenuous exercise, took no drugs, and were not vitamin supplement users except subject #8 who joined the study 2 days late because one of the original recruits chose to discontinue participation. Subject #8 stopped vitamin supplement use immediately upon joining the study. All except 2 had normal absorption of a 5 gm dose of xylose (between 24% and 48%) (Sartini, 1961), as measured by the method of Harris, (1969). Subjects 6 and 7 had values slightly above the normal range, absorbing 50.4% and 50.O% of the dose, respectively. A blood chemistry profile performed to further assess their health status indicated that all subjects had normal biochemistry.

Experimental Design The experiment consisted of a 49 day feeding study divided into three periods. A constant basal diet containing 3.29 mg of vitamin B-6/day (19.5 μmoles/day)

was fed for the first 10 day period to adapt subjects to the diet and standardize their vitamin B-6 status. During the second period, lasting 8 days, on the second, fifth and eighth days, doses of 0.2, 0.4, and 0.6 mg pyridoxine were given to each individual in random order as pyridoxine·HCl (1.18, 2.36, 3.55 μmoles). During the final 31 day period, on every third day, test foods were given to each individual in random order such that no two subjects received the same test food on the same day. The quantity of test food given was calculated to contain 0.5 mg of pyridoxine equivalents as determined by measurement with *Saccharomyces uvarum* using pyridoxine as the standard. The foods were chosen to give the widest possible range of glycosylated vitamin B-6 content (see Table 2.) Test doses and foods were divided into two equal doses and given with breakfast and the evening meal. Walnuts, tomato juice, wheat bran and shredded wheat were consumed as purchased. Bananas were pureed prior to the study, aliquots were frozen and consumed after thawing. Frozen spinach, broccoli and cauliflower were microwaved for 2 min prior to consumption. Canned carrots were microwaved for 1 min prior to consumption. All food doses were from the same lot and were mixed and aliquoted prior to the study and kept frozen in individual containers at -20°C.

The increment in urinary 4-pyridoxic acid excretion in response to a test food was the index used to assess differences in the bioavailability of vitamin B-6 from food sources with varying percentages of glycosylated vitamin B-6.

Using data from all subjects, a curve derived from the mean urinary 4-pyridoxic acid excretion response to the 3 test doses was used to estimate the % bioavailability of the test foods.

Basal Diet The basal diet is listed in table 3 and was adequate in all nutrients (Food and Nutrition Board, 1980). The diet was designed to contain 2.2 mg of pyridoxine equivalents (13.0 μmoles) and was determined from 6 individual weekly composites of the vegetable, meat, and dairy components to contain 2.3 ± 0.11 mg (13.6 ± 0.65 μmoles) pyridoxine equivalents by measurement with *S. uvarum* (Haskell & Snell, 1970). The level of PNG in the basal diet was 13.4% (of the 2.30 mg) or 0.307 mg pyridoxine equivalents (1.81 μmoles) per day (Kabir et al.1983a). The diet was supplemented with 1.00 mg of pyridoxine (5.91 μmoles) as pyridoxine·HCl, divided into two equal doses and added to the subject's orange juice in the morning and the milk in the evening. Wozenski et al. (1980) found that dietary intakes of between 3.0 and 4.0 mg. per day pyridoxine equivalents gave measurable urinary 4-PA increments when small doses of supplementary vitamin B-6 were fed. Therefore, the total vitamin B-6 intake was set at approximately 3.2 mg (18.9 μmoles) to approximate a normal dietary intake while providing a measurable increment in 4-PA excretion when additional vitamin B-6 was added to the diet. Each subject's caloric intake was determined using the equation of Cunningham (1982) and individually adjusted by

altering the amounts of soft drinks, margarine, jelly, and mayonnaise to maintain constant weight.

Procedure The subjects maintained their normal activities during the 7 weeks of the study except that all their meals were consumed in the metabolic kitchen at Oregon State University. On a few occasions bag lunches were provided to individual subjects. The subjects' body weights were recorded prior to breakfast each day. Complete 24-hour urine collections were made over toluene for the entire study. Daily urine aliquots were stored frozen at -20°C. Several times during the study, but not on a day that a test dose or food was given, fasting blood samples were drawn from the antecubital vein prior to breakfast. Plasma was isolated by centrifugation and stored frozen at -40°C. On day 4 of the study, 2 gm of L-tryptophan were given with the morning meal to provide an additional measure of vitamin B-6 status.

Analyses Urine was analyzed for creatinine using an automated alkaline picrate assay (Pino et al. 1965) (Technicon Autoanalyzer, Technicon Corp.,. Tarrytown, NY). Urinary xanthurenic and kynurenic acid were measured by an ion-exchange/fluorometric method (Price et al. 1965). Urinary total vitamin B-6 was measured using *S. uvarum* (Miller & Edwards, 1981).

The 4-PA content of the urine was measured by the method of Gregory and Kirk (1979) with several modifications.

The assay employed reverse phase HPLC using a Beckman 114M Solvent Delivery Module, a Model 420 System Controller Programmer, and a Model 210 Sample Injection Valve with a 50 µl injection loop (Beckman Instruments, Inc. Altex Division, San Ramon, California). A Perkin-Elmer LS-3B Fluorescence Spectrophotometer (Perkin-Elmer, Buckinghamshire, England) and a Hewlett Packard Model 3390A Reporting Integrator (Hewlett Packard, Avondale, Pennsylvania) were used for detection and quantification. The mobile phase consisted of 0.034 M potassium phosphate buffer (pH 2.2), 3.5% acetonitrile, and 5% methanol in redistilled water (V:V:V) filtered through a 0.45 micron filter and degassed by vacuum. Samples were chromatographed on an Econosil C18, 10 µ column, with a length of 25 cm and internal diameter of 4.6 mm (Alltech Associates, Inc., Deerfield, Illinois) and water jacketed at 25°C. Four-PA was detected by fluoresence (excitation 320 nm, emission 425 nm) and quantified using the internal standard calibrated peak height mode of the integrator. The standard contained 1µg/ml 4-PA (5.46 µmolar) and 8 µg/ml of pyridoxamine (4.76 µmolar) as an internal standard. (No pyridoxamine peak was detectable under these chromatographic conditions in the urine of one of the subjects who was supplemented after the study for 9 consecutive days, 3 days each with 10 mg (59.1 µmol) of pyridoxine, pyridoxal, or pyridoxamine.) The standard solution was dissolved in 1% urea and 0.45% NaCl. These solutes were used to approximate a normal urine composition and ionic strength. In addition,

including these solutes in standard solutions resulted in a mean recovery of added 4-PA of 98.8% ± 2.3%, n=6, while omitting them resulted in a mean recovery of 116.8% ± 3.6%, n=21. Urine samples were thawed, centrifuged for 10 minutes at 1,800 x g. An aliquot of 0.1% of the total urine volume and 0.4 ml of a 0.1 mg/ml pyridoxamine solution in 2mM HCl were brought to 5 ml with redistilled water. This dilution scheme kept the ionic strength of the samples nearly constant for each subject. The intraassay coefficient of variation (c.v.) for a control urine sample was 8.4% (n=7) while the interassay c.v. was 5.0% (n=6).

Plasma PLP was measured by a modification of the tyrosine decarboxylase method of Chabner and Livingston, (1970). The intraassay c.v. of a control plasma sample was 2.5% (n=10). The interassay c.v. was 3.4% (n=7). Hematocrit and hemoglobin levels did not change significantly during the course of the study.

Statistical analysis Assuming that the 4-PA excretion levels produced by pure pyridoxine represented 100% bioavailability, a dose response curve was constructed from the regression equation of the pyridoxine dose and the mean urinary 4-PA excretion value for that respective dose. The apparent dose was interpolated from the 4-PA excretion response from the test foods. The resulting dose value was divided by 2.96 µmol, (the vitamin B-6 content of the test food dose), and multiplied by 100 to yield % bioavailability. Values more than 1.5 interquartile range from the mean were excluded

from calculations of the standard curve and bioavailability values (Snedecor and Cochran, 1980).

Differences in values were tested for significance using ANOVA. If this proved there were significant differences, further comparisons were made using contrasts of means. A paired t-test was used to compare subjects' weights at the beginning and end of the study with a significance level of $p<0.05$ (Snedecor and Cochran, 1980; Petersen, 1985). All values are as means ± standard deviations unless otherwise indicated.

Results

A paired t-test showed that the subjects maintained their weight while consuming the experimental diet. Mean weight (±SD) on the third day (allowing for initial adaptation to the diet) was 75.9 ± 7.4 kg and was 75.2 ± 7.7 kg at the end of the study. The mean plasma PLP concentration was relatively constant throughout the study, with an average individual c.v. of 6.0% (see Table 4). One individual (subject #8) joined the experiment two days late. His initial plasma PLP value was high (282.2 nmol/L), and he appears to have been taking a vitamin B-6 supplement prior to the start of the study. This subject's plasma PLP concentration remained well above (2 times) the mean of the other eight subjects throughout the 49 day experiment (209% of other subject's means).

The daily coefficient of variation for creatinine excretion ranged between 3.8 and 7.2% (mean 5.3%) indicating good compliance with the urine collection procedure (Table 5). Urinary excretion values for kynurenic and xanthurenic acid in response to the 2 g dose of L-tryptophan given on day 4 indicated normal vitamin B-6 metabolism (68.7 ± 34.3 and 24.7 ± 5.7 µmoles/24hr, respectively).

Baseline 4-pyridoxic acid excretion. After the adaptation period, the mean daily excretion of 4-PA for the 26 nondose, nontest food days was remarkably similar for all the subjects (Table 5). The mean was 11.07 ± 0.43 (SEM) µmol/24hrs (56.9 ± 2.2% of the intake). The majority of the subjects had little change in 4-PA excretion during the 10 day adaptation period. However, the 4-PA excretion of subject #8 was high (17.23 ± 3.42 µmol/24hr) during his first 7 days of participation. Unlike his plasma PLP values, after the adaptation period his 4- PA excretion values dropped to a value comparable to that of the other subjects. His mean 4-PA excretion on nonfood nondose days was 10.46 ± 0.99 µmol/24hr, n=26 days, as compared to the group mean for the remainder of the 8 subjects of 11.14 µmol/24hr.

Response to test doses. The administration of the 0.2 mg (1.18 µmol) pyridoxine dose in addition to the basal diet resulted in a mean 4-PA excretion increment of 0.81 ± 1.21 µmoles (Table 6). The 0.4 and 0.6 mg pyridoxine doses (2.36 and

3.55 μmol) resulted in mean excretion increments with values of 0.91 ± 0.72 and 1.03 ± 0.83, respectively. The wide variation in individual urinary 4-PA response to the doses that was observed is within limits predicted by the coefficient of variation of the urinary collection procedure (5.3%) combined with the variability in the HPLC method for measuring 4-PA (c.v. of 8.4% or 0.93 μmoles, compare with standard deviation of 1.21, 0.72, and 1.03 μmoles for the doses, mean = 0.99 μmoles). Regressing individual incremental 4-pyridoxic acid (μmoles) against dose (μmoles) yielded the equation y = 0.43x - 0.013. Thus, about 43% of the vitamin B-6, given in addition to the basal diet, was excreted as 4-PA.

Response to test foods. Table 6 shows the urinary 4-PA excretion values for the days the test foods were given. An ANOVA of incremental 4-PA excretion indicated highly significant differences between foods ($p<.01$) and nonsignificant differences between subjects.

A plot of least significant differences at a 95% confidence level demonstrated a trend toward lower 4-PA excretion increments with increasing per cent PNG. Significant exceptions were when broccoli and cauliflower were fed, which resulted in high 4-PA excretion increments, and the feeding of wheat bran and shredded wheat, which resulted in negative excretion increments. A contrast between increments in 4-PA excretion resulting when broccoli and cauliflower were fed versus the increments for the rest of the

test foods showed a highly significant difference ($p<.01$). Similarly, the 4-PA excretion values associated with feeding wheat bran and shredded wheat were highly significantly different from the incremental 4-PA excretion values when feeding the other foods ($p<.01$).

The regression equation from the pyridoxine test doses was used to estimate the % bioavailability of the various foods (Table 6). A regression of apparent bioavailability against % glycosylation was than performed after eliminating wheat bran, shredded wheat, broccoli, and cauliflower from the equation (Figure 1.) This gave the equation $y = -1.16x + 75.5$ demonstrating a significant negative correlation between %glycosylation and bioavailability for 6 of the ten foods ($r=-.94$, $p<.01$).

Discussion

Much of the difference in vitamin B-6 bioavailability of the test foods was predicted by the percentage of total vitamin B-6 as PNG in the foods. However, the percentage of vitamin B-6 as PNG was not an adequate predictor of bioavailability in 4 of the ten foods tested. In the only other published study directly evaluating the availability of PNG containing foods to humans, Kabir et al. (1983c) found that the vitamin B-6 in foods containing higher percentages of their vitamin B-6 as PNG was less bioavailable than nonconjugated vitamin B-6. These authors assumed 100% bioavailability for the vitamin

B-6 in tuna, and determined the vitamin B-6 bioavailability of peanut butter and whole wheat bread to be 63 and 75%, respectively. Using the regression equation from the 4-PA data produced in the present study yields predicts bioavailability values of 56% for whole wheat bread and 54% for peanut butter. The lower incremental 4-PA excretion values predicted by data from the present study may be a result of the higher basal dietary level of vitamin B-6 (3.3 mg pyridoxine equivalents per day versus 1.6 mg per day). Wozenski et al. (1980) found that a lower percentage of a 4 mg or a 10 mg dose of PN was excreted as 4-PA as compared to a 1 mg or 2 mg PN dose.

Wheat bran had a low, apparently negative, bioavailability (-45.6%) in the present study. The data of Kies et al. (1984) who used free and total plasma vitamin B-6 as indices, also indicates that the vitamin B-6 from wheat bran is both unavailable and reduces the bioavailability of vitamin B-6 in the rest of a human diet. They found that feeding 20 g/day of wheat bran for 7 days resulted in total urinary excretion of 5.98% of the total vitamin B-6 intake compared with 9.46% during the control periods. Lindberg et al. (1983), using a switchback design, found that the addition of 15 grams of cooked wheat bran to a basal diet resulted in urinary 4-PA excretion that was 87% of the non-wheat bran supplemented periods. In that study PN was added to the basal diet to match the vitamin B-6 content of the bran diet during the non-wheat bran periods. The mean 4-PA excretion value for the wheat

bran dose in the present study (10.5 μmoles) is 85% of the predicted total 4-PA excretion value for a 0.5 mg (2.96 μmol) PN dose added to the basal diet. However, the dose in the present study was 42 gm rather than 15 gm and the bran was ingested raw rather than cooked. Lindberg et al. (1983) also found increased fecal vitamin B-6 when wheat bran was fed. We did not measure this index in the present study, but this observation could explain the negative bioavailability associated with the feeding of wheat bran. An animal study by Hudson et al. 1988 compared the bioavailability of vitamin B-6 in the total diet using rats fed either fiber-free, 20% wheat bran supplemented, or 7% cellulose-supplemented diets. They measured growth, feed intake, urinary excretion of 4-PA and urinary excretion and liver retention of ^{14}C after a dose of labelled PN. In contrast to the human bioavailability studies of Kabir et al. 1983, Lindbergh et al. 1983, and Kies et al. 1984, results from all three indicators were that vitamin B-6 in wheat bran was available and contributed to the vitamin B-6 nutriture of the rat. Hudson et al. 1988 suggest that this species difference may be due to differences in microbial flora in rats and humans.

In vitro studies by Nguyen et al. (1981) showed no binding of vitamin B-6 by wheat bran as demonstrated by equilibrium dialysis. Similarly, Shultz & Slattery (1988) did not observe any in vitro binding of radiolabeled PN to bran. Thus binding of vitamin B-6 in the remainder of the diet by bran does not appear to explain the reduction of total vitamin B-6

bioavailability when wheat bran was fed. However, the effects of the digestive process on the binding of vitamin B-6 by wheat bran may be different.

Leklem et al. (1980) found a significant increase in fecal vitamin B-6 excretion and a significant decrease in 4-PA excretion when whole wheat bread was fed as compared to either white bread or white bread supplemented with vitamin B-6. They estimated that the vitamin B-6 in whole wheat bread was 5 to 10% less available than the vitamin B-6 in white bread or white bread supplemented with vitamin B-6. The similar lack of bioavailability of shredded wheat observed in the present study may be related to the fact that, like whole wheat bread, shredded wheat is a whole wheat product. Since 85% of the vitamin B-6 in whole wheat is in the aleurone and germ fractions (Polansky & Toepfer, (1969), the bran content of the shredded wheat could explain part of its relative unavailability. In another cereal product, Gregory (1980), using rat growth, feed efficiency, liver PLP and erythrocyte aspartate aminotransferase activity as indices, found that the vitamin B-6 bioavailability of a fortified rice-based breakfast cereal was only 18 to 44%.

Another factor that may explain the low bioavailability of the two wheat products is the possible influence of a recently discovered vitamin B-6 conjugate. Tadera et al. (1986) named the vitamin B-6 compound(s) that were measurable by *S. uvarum* only after alkali treatment followed by ß-glucosidase treatment as B_6X. They reported contents of this B_6X

conjugate between 0 and 43% in the 8 foods they measured. We measured the B_6X levels in the ten foods used in this study (unpublished observations). Values ranged from 0 to 12% of the vitamin B-6 content. Our value for wheat bran was 5%, compared to theirs of 19%, and our value for shredded wheat was 12%, while they had no data for this food. If B_6X is biologically unavailable, this may explain part of the reduced bioavailability of the vitamin B-6 in the two wheat products as well as the orange juice, the only other of the ten foods with significant amounts of B_6X (7%).

The two foods, broccoli and cauliflower, had high apparent bioavailability even though they had substantial PNG content. Bioavailability values were 62% and 56% respectivelly. Kabir et al. 1983a, found that these vegetables had little PNG when assayed raw but had levels of 78% for broccoli and 69% for cauliflower when frozen. Furthermore, incubation of frozen cauliflower with a homogenate of raw cauliflower resulted in release of 52% of the conjugate from the frozen cauliflower. As indicated by the differing levels of PNG measured in raw and frozen cauliflower, the blanching before freezing probably inactivates any ß-glucosidase activity present in the raw vegetable. Unless ß-glucosidase activity of the frozen vegetable was somehow restored in the intestinal tract, this does not provide an explanation of the higher apparent bioavailability of the PNG contained in these two vegetables.

Nelson et al. 1976, measured the human intestinal uptake of vitamin B-6 in a 30 cm segment of the jejunum. Using a triple lumen tube, they found uptake of vitamin B-6 from orange juice 46% of that from a synthetic solution. Transfer rate across intestine was only 56% of the synthetic solution. These values are not comparable to the present value of 18.9% orange juice bioavailability because extent of absorption in the whole intestine was not measured in the study of Nelson et al.

Tsuji et al. 1977 found that oral or injected PNG normalized vitamin B-6 status indicators in vitamin B-6 deficient rats. They also found that PNG transversed everted small intestinal sacs. In other rat studies, Gregory and coworkers found half as much of a dose of radiolabelled PNG was absorbed as labelled PN and that most of this dose was excreted in the urine as PNG (Ink et al. 1986). In another study, PNG was found to be only 10 to 30% as bioavailable as PN to rats (Trumbo et al. 1988). Recently, using ^{3}H labelled PNG and ^{14}C labelled PN, given either orally or interperitoneally to both vitamin B-6 and vitamin B-6 deficient rats, they found that vitamin B-6 status had little effect on the utilization of PNG. They suggested that the intestine is the site of limited conversion of PNG to PN in the rat (Trumbo & Gregory, 1988).

Human studies also indicate that PNG is both absorbed intact and excreted intact in the urine (Kabir et al. 1983c). ß-glucosidases exist in various compartments of mammalian cells. However, specificity for, and access of PNG to these enzymes has not been determined. Gregory et al. (1989), using

deuterated and unlabelled PNG and PN, suggest that PNG is more available to humans than it is to rats and that gastrointestinal hydrolysis is required for its utilization.

The extent of the daily intra-individual variation in 4-PA excrection suggests that future tests of food bioavailability in humans should use designs where specific foods are given for several days instead of the single day used in this study. Use of smaller food doses as was done in the present study should be continued as this closely approximates a normal physiological food intake.

The in vitro PNG content successfully predicted the bioavailability of 6 of the 10 foods. Future work should be directed to explain the high bioavailability of the two cruciferous vegetable and the low bioavailibility of the two grain products. Factors in addition to PNG content are necessary to explain variation in plant food vitamin B-6 bioavailability.

Table 2.1 Age, weight and height of the subjects

Subject No.	Age years	Starting[1] body wt kg	Height cm
1	26	71.6	165
2	23	87.5	183
3	28	72.0	183
4	33	84.0	183
5	22	64.8	180
6	26	81.2	183
7	23	83.6	185
8	32	68.9	165
Mean±SD	25.9±4.5	76.6±7.8	178.9±8.0

[1]Weight on the first day of study

Table 2.2 % Glycosylated and total vitamin B-6 content and amount fed of the test foods.

Food	%Glycosylated	mg B-6/100gm	Amount Fed[1] gm	Kcals
Walnuts	1.1	0.673	74	484
Banana	16.4	0.345	45	83
Tomato Juice (canned)	21.4	0.103	485	91
Wheat Bran	26.9	1.182	42	90
Shredded Wheat	30.7	0.244	205	725
Spinach (frozen chopped)	35.4	0.230	215	50
Orange Juice (frozen, reconstituted)	46.8	0.167	299	508
Broccoli (frozen)	53.9	0.124	403	104
Cauliflower (frozen)	63.2	0.151	331	313
Carrots (canned)	75.4	0.060	836	393

Vitamin B-6 analyses were done by microbiological assay using *S. uvarum* (Haskell & Snell, 1970) Glycosylated B-6 measured by method of Kabir et al. (1983).

[1]The amount of each food fed was calculated to contain 0.5 mg pyridoxine equivalents (2.96 µmoles).

Table 2.3. Basal diet composition

FOOD	WEIGHT (gm)
Orange juice, frozen, reconstituted	170
Shredded wheat	50
Milk (2%)	500
Bread, whole wheat	150
Margarine	40
Jelly	30
Tuna, water pack, drained	60
Lettuce, iceberg, raw	60
Pickle, dill	30
Mayonnaise	37
Carrot, raw	70
Pineapple juice, canned	250
Vanilla wafers	16
Pears, canned	160
Turkey breast	125
Red cabbage, raw	15
French dressing	16
Brown rice, before cooking	45
Green beans, canned	165
Ice milk, vanilla	80
Peaches, canned	150
Popcorn, before popping	12

Total Kcal = 2200. Per cent kcal from protein, fat and carbohydrate were 16, 30, & 60 respectively. Diets were adjusted to individual caloric requirments by varying the amounts of additional soft drinks, margarine, jelly, & mayonnaise.

Table 2.4. Plasma pyridoxal 5'-phosphate concentration.

Subject #	Day of Study								
	pre-study	1	5	12	19	32	38	47	Mean[1] ±SD
					nmol/L				
1	74.4	51.7	66.4	71.1	79.8	87.0	77.3	82.3	79.5±5.9
2	58.8	50.9	71.4	73.4	79.4	85.4	81.4	83.5	80.6±4.6
3	53.5	48.4	54.2	56.7	56.9	63.1	59.2	61.2	59.4±2.8
4	-	94.4	87.2	87.4	86.2	90.7	87.6	95.2	89.4±3.6
5	65.7	48.0	74.4	78.6	68.6	61.6	74.6	72.4	71.2±6.5
6	55.8	58.8	57.7	55.4	55.4	60.4	57.7	56.1	57.0±2.1
7	79.2	34.2	41.6	52.2	52.1	55.2	48.7	49.5	51.5±2.6
8	-	288.2	268.0	183.7	149.7	147.6	148.0	143.5	154.5±16.5
9	94.2	69.1	103.1	91.7	98.5	99.6	100.8	97.3	97.6±3.5
									82.3
$\bar{X}$±	68.8	82.6	91.6	83.4	80.7	83.4	81.7	82.3	
SD	14.7	78.9	68.6	40.1	30.2	28.9	29.7	28.4	

[1]Mean ± SD of the 5 observations from day 12 through 47. The 8 samples for each subject were measured in a single assay.

Table 2.5. Urinary 4-pyridoxic acid excretion ater the 10 day adaptation. Non-test food, non-test dose days.

Subject	Average 4-PA[1] (µmoles/24hrs)	% of B6 Intake (%)	Coefficient[2] of Variation for Creatinine
1	10.74±1.13	55.2	5.6
2	11.06±0.92	56.9	5.8
3	11.73±1.16	60.3	4.8
4	11.44±0.54	58.8	3.8
5	11.39±0.86	58.6	4.6
6	10.60±1.00	54.5	7.2
7	11.29±0.61	58.1	4.4
8	10.46±0.99	53.8	6.3
9	10.90±0.58	56.1	5.2
Mean±SEM	11.07±.43	56.9±2.2	5.3±1.0
	C.V.=4.1%[3]	C.V.=4.0%	

[1]Average of 26 days.

[2]Creatinine coefficient of variation (%) over the 49 days of the study.

[3]Coefficient of variation of the nine means.

Table 2.6. Mean urinary 4-pyridoxic acid excretion in 9 subjects following pyridoxine or food dose ingestion

Dose or Food[1]	%Glycosylated[2]	Apparent[3] Bioavailability	4-Pyridoxic Acid[4] (μmoles/24hr)
0 (mg)	- - -	- - -	11.07 ± 0.43
0.2 "	- - -	- - -	11.39 ± 1.21
0.4 "	- - -	- - -	11.98 ± 0.72
0.6 "	- - -	- - -	12.55 ± 0.83
Walnuts	1.1	87.4 (n=8)	12.18 ± 0.66
Bananas	16.4	59.8 (n=7)	11.83 ± 0.56
Tomato juice	21.4	42.5 (n=9)	11.61 ± 0.59
Wheat Bran	26.9	-45.6 (n=8)	10.49 ± 0.76
Shredded Wheat	30.7	-22.8 (n=9)	10.78 ± 0.80
Spinach	35.4	17.3 (n=7)	11.29 ± 0.39
Orange juice	46.8	18.9 (n=7)	11.31 ± 0.81
Broccoli	53.9	62.2 (n=8)	11.86 ± 0.73
Cauliflower	63.2	55.9 (n=9)	11.78 ± 0.70
Carrots	75.4	-1.3 (n=7)	11.02 ± 0.69

[1]Basal Diet (0 mg) contained 3.295 mg (19.47μmol/day) pyridoxine equivalents.
Doses were given as pyridoxine·HCl (0.2 mg = 1.18 μmol, 0.4 mg = 2.36 μmol,
0.6 mg = 3.55 umol pyridoxine). Food doses contained 0.5 mg (2.955 umol) pyridoxine equivalents as measured by *S. uvarum*.
[2]Glycosylated vitamin B-6 % measured by method of Kabir et al. (1983c)
[3]Values for 4PA > 1.5 interquartile range values from the mean not included;
n= number of subjects.
[4]For doses values represent mean ± SD. For foods values represent mean ± SD with n given under apparent bioavailability.

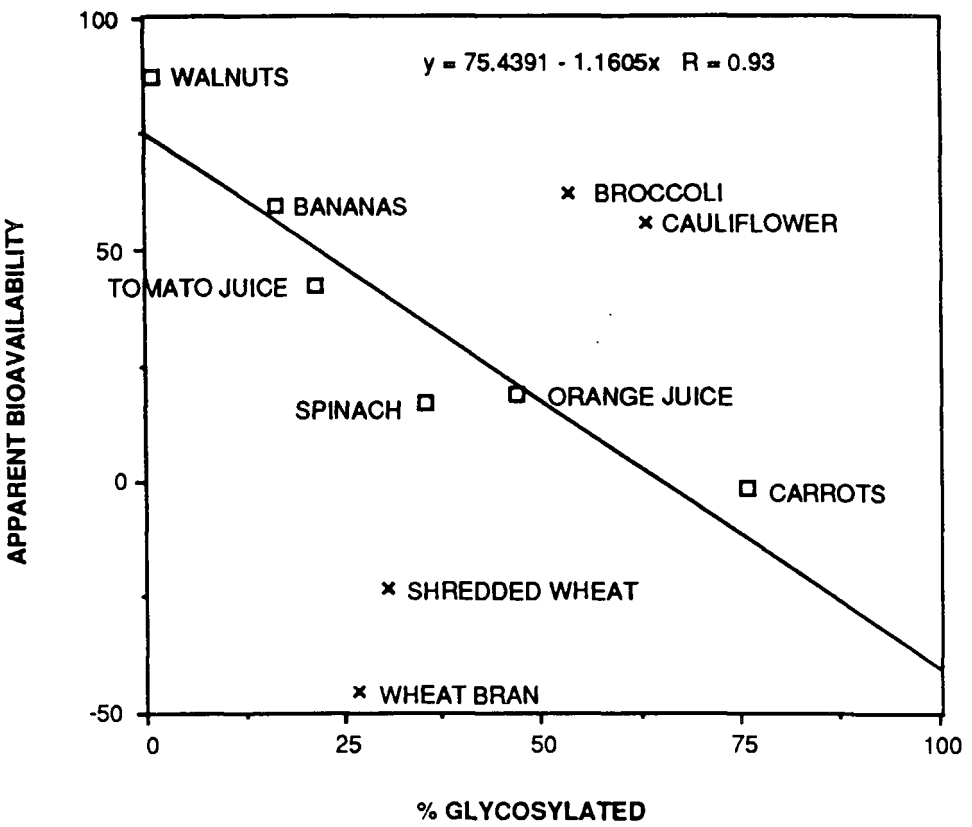

Figure 2.1 Regression of % Bioavailability vs % Glycosylated vitamin B-6

Chapter 3

A COMPARISON OF THREE IN VITRO METHODS FOR ESTIMATING BIOAVAILABILITY OF VITAMIN B-6 IN FOODS

Bills, Nathan D., and Leklem, James E.

Department of Nutrition and Food Management
Oregon State University
Corvallis, OR 97331

To be submitted to the Journal of Food
and Agricultural Chemistry

Abstract

The content of conjugated forms of vitamin B-6 in foods may be important in assessing bioavailable vitamin B-6. To compare methods for measuring available forms of vitamin B-6 in foods and to correlate these values with bioavailability data, ten plant foods were assayed by three different procedures. Pyridoxine glucoside (PNG) was quantified by a differential growth assay using *S. uvarum* after prior incubation with or without ß-glucosidase. PNG content measured by the method of Kabir et al. 1983a was lower (p=.005) than that measured by method of Tadera et al. 1986 which use cold dispersion and hot water extraction of the foods, respectively. Food values for B_6X, an unidentified form of vitamin B-6 from a method of Tadera et al. 1986 were obtained by treating foods with base (0.7 M KOH) prior to glucosidase treatment and measurement with *S. uvarum*. B_6X was detected in wheat products, spinach, orange juice and carrots in small amounts (1-12%). In a third method foods were treated with digestive enzymes (pepsin followed by pancreatin) by the method of Ekanayake and Nelson, 1986, extracted with methanolic HCl and quantified by *S. uvarum*. Of the three procedures, the best correlation with food vitamin B-6 bioavailability obtained in a human feeding study was with vitamin B-6 values obtained by enzymatic treatment of the foods with pepsin followed by pancreatin.

Introduction

Several assay procedures for in vitro assessment of biologically available vitamin B-6 in foods have been developed. In most cases a specific pretreatment, designed to liberate specific bound chemical forms of vitamin B-6, is followed by microbiological assay with *Sacchromyces uvarum* which measures only free (nonconjugated) forms of vitamin B-6 (Polansky, 1985). Emphasis has been placed on the level of 5'-O-(ß-D-glucopyranosyl) pyridoxine, (commonly called pyridoxine glucoside (PNG)), and any other compounds which are released when foods are treated with almond ß-glucosidase. The per cent PNG has been reported to be important in assessing bioavailability of vitamin B-6 containing foods (Kabir et al. 1983b; Ink et al. 1986; Trumbo et al. 1988; Trumbo & Gregory, 1989).

Two published assay procedures (Tadera et al. 1986; Kabir et al. 1983a) for measuring PNG content of foods differ in several respects. Different buffers, temperatures, and times are used for extraction of vitamin B-6 and subsequent incubation with ß-glucosidase. In addition, the amounts of "nonconjugated" or "free" forms used in the calculation of PNG content are arrived at by different methods (Tadera et al.1986, Kabir et al. 1983a).

Another approach that has been used to assess the bioavailability of vitamin B-6 in foods is to measure the amount of vitamin B-6 released after incubating them with digestive enzymes. Ekanayake and Nelson (1986) found close

agreement between the biologically available vitamin B-6 as determined by rat bioassay and the amount of vitamin B-6 released from a food by enzymic incubation with pepsin followed by incubation with pancreatin. This procedure was followed by extraction of vitamin B-6 with acidified methanol and subsequent HPLC quantification. To date, this treatment has only applied to specific casein-based model food systems, and not to simple plant sources of vitamin B-6.

Recently, Tadera et al. (1986), reported evidence for a compound, which they called B_6X, that was released only when a food was treated with alkali (0.7M KOH) prior to incubation with ß-glucosidase. The speculated structure is that of PNG with an unknown constituent esterified to the glucose moiety. They made no speculation as to its bioavailability.

To determine if the results of the quantification procedures for measuring PNG differ substantially when using the procedures of Tadera et al. 1986, versus Kabir et al. 1983a, aliquots of 10 foods were measured by both methods. Simultaneously, quantities of vitamin B-6 liberated by the method of Ekanayake and Nelson (1986) were measured in these same foods. At the same time amounts of B_6X in these foods were quantified. Finally, to see which of these three methods was the best predictor of human vitamin B-6 bioavailability, available vitamin B-6 quantified by each of these methods was compared with human vitamin B-6 bioavailability data recently obtained for these foods. Human bioavailability of vitamin B-6 from these foods was estimated from incremental urinary

4-pyridoxic acid excretion obtained after doses of foods given in addition to a basal diet containing a constant amount of vitamin B-6 were fed.

Materials and Methods

Food Samples Food samples were chosen to contain a wide range of percent PNG values for use as test foods in a human feeding experiment investigating the relationship between % vitamin B-6 as PNG and vitamin B-6 bioavailablity (Kabir et al. 1983). Foods were purchased in one lot, individual packages were opened and contents were mixed. A representative sample was frozen under liquid nitrogen and immediately homogenized with a Waring Blender until a fine powder was formed. The powder was stored frozen at -20°C until assayed. The treatments described below were performed on two separate aliquots of the samples. Results are the means of these two determinations.

Assay Procedures Food samples were subjected to the treatments described below. The individual samples were then adjusted to pH 4.5, filtered through Whatman No. 1 filter paper, diluted to an approximate concentration of 1 to 2 ng/ml pyridoxine equivalents and assayed for vitamin B-6 content by measuring growth of *S. uvarum* (Haskell & Snell, 1970).

Total Vitamin B-6 Total vitamin B-6 content of each sample was determined as follows (Miller and Edwards, 1981). One gram of food was mixed with 100 ml. of 0.44 N HCl was added and autoclaved at 121°C for 2 hr. The sample was then assayed by *S. uvarum* after pH adjustment and appropriate dilution as described above.

Glycosylated Vitamin B-6 The method of Kabir et al. (1983a) was used for the determination of glycosylated vitamin B-6. One gram of food and 100 ml of 0.1 M phosphate buffer (pH 6.8) were combined and stirred for 2 hrs in the dark at room temperature. The pH was then adjusted to 5.0, 60 units of almond ß-D-glucosidase (Sigma Chemicals, St. Louis, Missouri) was added and the sample was incubated for 2 hours in a shaking water bath at 37°C. Ten ml of 1 N HCl was then added and the mixture was steamed for 5 minutes to stop enzyme action. Prior to assay, dilution and pH adjustment were then performed as described.

To measure nonconjugated vitamin B-6, 1 gm of food was subjected to the previous treatment with the exception that no ß-D-glucosidase was added prior to the 2 hr. incubation at 37°C. The per cent glycosylated vitamin B-6 was calculated as the difference between the value for the ß-D-glucosidase treated and nonenzyme treated sample divided by the total hydrolyzed vitamin B-6 value times 100.

Glycosylated/phosphorylated Vitamin B-6 and vitamin B_6X To determine the amount of free vitamin B-6, glycosylated/phosphorylated vitamin B-6 and vitamin B_6X as defined by Tadera et al. (1986), the following treatments were used. For free vitamin B-6, 1 gm of sample suspended in 50 ml of redistilled water was extracted for 15 minutes in a boiling water bath, filtered, and the residue was washed three times with 10 ml of redistilled water. The washings were combined with the filtrate. This solution was adjusted to pH 4.5, diluted appropriately, and assayed for vitamin B-6 by *S. uvarum*. Glycosylated/phosphorylated vitamin B-6 was measured by dispersing a 1 gm sample in 50 ml of 50 mM acetate buffer (pH 5.0) and heating the solution in a boiling water bath for 15 minutes. After cooling, 60 units of ß-D-glucosidase were added, and the mixture was incubated at 37°C for 2 hrs. The sample was then steamed for 5 minutes to stop enzyme action. The pH was adjusted, the mixture was then filtered and diluted appropriately for measurement with *S. uvarum*. Vitamin B_6X was assayed by adding 1 gram of sample to 50 ml 0.7 M KOH and allowing to stand for 3 hrs in the dark. After the pH of the solution was adjusted to 5.0, the sample was incubated for 2 hrs at 37°C with ß-D-glucosidase, then steamed and assayed as was the glycosylated, phophorylated vitamin B-6 sample.

Digestive Enzyme Treatment To determine the vitamin B-6 content of the foods that was released by digestive

anzymes and made available to *S. uvarum* as described by Ekanayake and Nelson (1986), one gram of food was slurried in 20 ml of water, brought to pH 2.0 with 1.5 M HCl and incubated while shaking for 3 hrs at 37°C with 5.0 mg porcine pepsin (Sigma Chemicals, Inc., St. Louis, Missouri). The pH was adjusted to 8.0 with 1 M NaOH and 3.5 mg of pancreatin (Sigma Chemicals, Inc., St. Louis, Missouri) (previously suspended in 2.0 ml of 1.0 M phosphate buffer, pH 8.0) was added and the mixture incubated for 12 hrs at 37°C. The sample was brought to a volume of 100 ml with redistilled water and 50 ml. of 0.12 M HCl in methanol was added. This mixture was filtered and the methanol removed by rotoevaporation at 37°C. The resulting mixture was then autoclaved at 121°C for 30 minutes. The pH of this solution was adjusted to 4.5 and diluted appropriately for assay by *S. uvarum*.

Controls Orange juice concentrate was used as a control for each assay. (Per cent glycosylated vitamin B-6 was 49.9 ± 4.6, and the inter assay coefficient of variation was 9.2%). Turkey breast and canned tuna were also subjected to each of the procedures. No conjugated (PNG or B_6X) forms of vitamin B-6 were found in these animal products. The digestive enzyme treatment of Ekanayake and Nelson (1986), liberated the same amount of vitamin B-6 as standard hydrolysis did for these two foods (Haskell & Snell, 1981).

Food Bioavailability The bioavailability of the ten foods assayed in this study was evaluated in humans in a previous experiment. Nine subjects were fed a basal diet containing a constant amount of vitamin B-6. Complete 24 hour urine collections were obtained throughout the study. After an initial 10 day adaptation period, three levels (0.2, 0.4, and 0.6 mg) of reagent pyridoxine as pyridoxine·HCL were administered in individual random order in addition to the basal diet at three day intervals. Thereafter, test foods were adminstered on every third day. The incremental urinary 4-PA excreted in response to the pyridoxine doses was used to construct a standard curve from which the bioavailability of the foods were determined. The incremental 4-PA excreted after ingestion of 0.5 mg pyridoxine equivalents of each food was used to determine its comparative bioavailability.

4-Pyridoxic Acid 4-PA was analyzed by an HPLC procedure which was a modification of the method of Gregory & Kirk, (1979). Reverse-phase HPLC using a 0.034 M potassium phosphate buffer (pH 2.2) with 3.5% acetonitrile and 5% methanol was employed with fluorescence detection (excitation 320nm, emission 425nm). A solution of 1% urea and 0.45% NaCl was used to dilute standards. This solution approximates the ionic strength of urine and resulted in recovery values for added 4-PA of 100%. Inter- and intra-assay coefficient of variation were 5.0 and 8.4%, respectively.

Statistics Appropriate treatments were compared by means of paired t-tests (Snedechor and Cochran, 1980). Regression analysis (Neter & Wasserman, 1974) was used to compare the amounts of vitamin B-6 released by the various treatments with the bioavailability data obtained previously.

Results

The vitamin B-6 content of the foods as measured by *S. uvarum* after the various treatments is shown in Table 1. These samples were analyzed previously and differences in values were minor after one year in frozen storage (see Table 2.2) The total (acid hydrolyzed) vitamin B-6 content of the 10 foods ranged from 0.086 mg/100 gm for tomato juice to 1.494 mg/100 gm for wheat bran. Mean vitamin B-6 released from these 10 foods by treatment with ß-glucosidase was 0.263 ± 0.247 mg/100 gm when prepared by the procedure of Kabir et al. (1983a) which was lower than 0.278 ± 0.256 mg/100 gm the value for the preparation method of Tadera et al. (1986). A paired t-test showed these values to be significantly different. While the values for orange juice and carrots were only slightly higher for the second method (1.5 and 1.8%, respectively), the values for the rest of the foods were an average of 8.7% higher, ranging from 3.6% for walnuts, to 14.5% for tomato juice. The mean "nonconjugated" (Kabir et al. 1983a) and "free" (Tadera et al. 1986) vitamin B-6 values measured in these 10 foods were 0.168 ± 0.202 (40.1% of hydrolyzed) and 0.151 ± 0.185 (44.7% of

hydrolyzed) mg/100 gm, respectively. Using the paired t-test these values were not significantly different. The mean vitamin B-6 value measured by treatment with pepsin followed by pancreatin (Ekanayake & Nelson, 1986) was 0.174 ± 0.164 mg/100 gm.

In Table 2 are shown the amounts and percentages of glycosylated and glycosylated-phosphorylated vitamin B-6, (calculated as the enzyme treated value minus the "nonconjugated" or "free" values divided by the total (hydrolyzed) vitamin B-6 value), B_6X (calculated as the alkali treated value minus the enzyme treated value divided by the hydrolyzed vitamin B-6 value), and the bound forms not freed for measurement by *S. uvarum* with digestive enzymes and the percent of hydrolyzed vitamin B-6 that was not released by digestive enzyme treatment.

The mean glycosylated vitamin B-6 value as measured by the method of Kabir et al. 1983a was 0.095 ± 0.085 mg/100 gm or 37.8 ± 24.4% of the total. Higher amounts were measured by the method of Tadera et al. (1986), the mean glycosylated-phosphorylated value was 0.128 ± 0.143 mg/100 gm or 46.8 ± 27.3% of the total. These values were significantly different from each other ($p<.001$). The average percentage of vitamin B-6 as PNG was 23.8% higher when measured by the method of Tadera et al. (1986) compared to the method of Kabir et al. (1983). One food (walnuts) had slightly lower %PNG when measured by the former method (2.4% cf 5.9%), two foods (orange juice and carrots) were approximately the same (49.1

and 49.7%, and 78.7 and 76.4%, respectively) while the rest of the foods ranged from 18.2% (for shredded wheat) to 128.8% (for bananas) higher.

The digestive enzyme treatment of Ekanayake & Nelson (pepsin followed by pancreatin) released an average of 0.174 ± 0.164 (46.3 ± 43.6%) mg vitamin B-6/100 gm as measured by *S. uvarum*. The mean bound forms (not released by digestive enzyme treatments) was 42.8 ± 26.5% and is close to that of the percent glycosylated-phosphorylated treatment of Tadera et al. (1986) (46.8%); however, the individual food values were significantly different from each other ($p<0.001$). Of particular interest are the wheat products, wheat bran and shredded wheat, which had around 30% PNG content, but which had bound forms not released by digestive enzymes of 78.4 and 85.4%, respectively. In contrast, broccoli and cauliflower, which had approximately 60% of their vitamin B-6 as PNG, had only 39.0 and 25.5% of their vitamin B-6 unavailable to *S. uvarum* when treated with digestive enzymes, respectively.

B_6X was found in five of the foods (wheat bran, shredded wheat, spinach, orange juice and carrots) with values ranging from 1% in spinach to 11.8% in shredded wheat. In the other 5 foods treatment with 0.7 M KOH prior to incubation with ß-glucosidase resulted in lower vitamin B-6 values than those obtained by enzyme treatment alone.

Figure 2 shows the relationship between the in vitro measures of available vitamin B-6 and the estimated bioavailability of the vitamin B-6 in these foods to humans.

Table 3 shows the urinary 4-pyridoxic acid excretion of 9 human subjects when they were fed either a basal diet, the basal diet plus a dose of pyridoxine, or the basal diet plus an amount of a test food (shown) calculated to contain 0.5 mg (2.96 µmoles) pyridoxine equivalents as measured by *S. uvarum*. The incremental 4-PA acid excreted in response to these test foods was used to calculate the comparative bioavailability of these foods.

The %glycosylated vitamin B-6 measurements obtained by the method of Kabir et al. (1983a) are inversely correlated with the bioavailability data only when the wheat based foods and the cruciferous vegetables are removed from the equation (Figure 2, Top left panel). Similarly the glycosylated-phosphorylated values from the procedure of Tadera et al. (1986) were not well correlated with the bioavailability data (Figure 2, Top right panel). Adding the B_6X content to the glycosylated-phosphorylated content (assuming B_6X is not available to humans) did not improve this relationship significantly (Figure 2, Bottom left panel). Only the quantities of vitamin B-6 released after foods were treated with digestive enzymes gave a significant correlation with the human bioavailability data (R=0.88) (Figure 2, Bottom right panel).

Discussion

Correlations between the specific chemical forms of vitamin B-6 found in foods and the bioavailability of the vitamin B-6 from these same foods would be useful in the assessment of the vitamin B-6 status of an individual or population. If in vitro procedures, designed to liberate vitamin B-6 available for microorganisms, were developed to measure approximately the same amount of vitamin B-6 that is available to humans, the expense of determining the bioavailability of a food or diet would be greatly reduced. Recent studies have indicated (Kabir et al. 1983b; Ink et al. 1986) that the pyridoxine glucoside content of foods may be an important predictor of vitamin B-6 bioavailability. Another approach to assessing bioavailability is to measure the amount of vitamin B-6 released by treatment with digestive enzymes. To date this approach has only been used to estimate the bioavailability of model food systems. In this study we compared the results of two procedures for measuring pyridoxine glucoside content of foods, the amounts of a novel vitamin B-6 conjugate (B_6X) recently found in foods, and the amount of vitamin B-6 available to microorganisms that is released by treatment of foods with digestive enzymes. Finally, correlations between these in vitro values of available and unavailable vitamin B-6 were made with the human bioavailability of the vitamin B-6 from these foods estimated previously.

Significant differences in the pyridoxine glucoside measured in the foods by the two methods were not due to differences in the non-bound forms measured in either cold buffer dispersions (Kabir et al. 1983a) or hot water extracts (Tadera et al. 1986). However, the amount of vitamin B-6 liberated by ß-glucosidase was significantly higher (34.7%) when food samples were extracted in hot water (Tadera et al. 1986) rather than in cold buffer dipersions (Kabir et al. 1983a). The higher values obtained using the former method could be due to more efficient extraction of vitamin B-6 compounds from the food matrix by hot buffer extraction, both by increased solvation and by increased breakdown of the food matrix itself.

Although the amounts of pyridoxine glucoside measured by the method of Tadera et al. 1986 were higher than those of Kabir et al. 1983a, the foods remained in the same order of increasing pyridoxine glucoside content except for spinach and orange juice where the order was reversed. Pyridoxine glucoside, quantified by either method, had little correlation with human bioavailability data (Figure 2, Top panels), except when significant exceptions are made for wheat products and cruciferous vegetables. It is possible that matrix effect, such as nonspecific binding by fibers may contribute to the low bioavailability of vitamin B-6 in the wheat products. The reason for the greater availability of vitamin B-6 in the two crucifers is more difficult to assess. Both foods had high pyridoxine glucoside content, yet for both the vitamin B-6 was

highly available. Both foods were purchased frozen and were therefore blanched, presumably inactivating any ß-glucosidase present that would be capable of deconjugating any pyridoxine glucoside in the rest of the diet. The pyridoxine glucoside content of these foods is low when they are measured fresh (Kabir et al. 1983a); this suggests that there is ß-glucosidase activity in these foods that is inactivated by blanching.

B_6X is a conjugate of vitamin B-6 from which pyridoxine is released only when a food is treated with alkali prior to incubation with ß-glucosidase. A speculative structure of this compound is that of an organic acid esterified to the glucose moiety of pyridoxine glucoside (Tadera et al. 1986) B_6X was found in 5 of the 10 foods (wheat bran, shredded wheat, spinach, orange juice, and carrots). In 4 out of the 5 foods, (walnuts, banana, tomato juice, and cauliflower) that apparently did not contain B_6X, treatment with 0.7 M KOH for 3 hours in the dark resulted in significant destruction of vitamin B-6 such that less was released than by ß-glucosidase treatment alone. Thus, even in those foods where B_6X was found, the content was probably underestimated due to nonspecific base hydrolysis.

The amounts of B_6X we measured in wheat bran and spinach were different than those measured by Tadera et al. (1986). Our sample of wheat bran contained 5% B_6X compared to Tadera's value of 19%, and spinach contained 1% versus none detected. Neither group found B_6X in cauliflower.

Assuming none, or limited bioavailability for B_6X, the B_6X and the glycosylated/phosphorylated values from the method of Tadera et al. 1986 were added. The combination gave no additional predictive value for human vitamin B-6 bioavailability of these foods (Figure 2, Bottom left panel).

The best prediction of vitamin B-6 bioavailability for these foods was the amount released after treatment with digestive enzymes according to the method of Ekanayake et al. (1986) followed by quantification with *S. uvarum*. Although there was a significant positive correlation (R=0.88) with these values, the negative bioavailability (and negative y-intercept of the regression line) of some of the foods is puzzling. Others have found (Kies et al. 1984) that the inclusion of wheat bran in a diet decreases the bioavailability of vitamin B-6 in the rest of the diet. Although present evidence points to its negative effect on the bioavailability of vitamin B-6 in the rest of the diet, it is not clear whether vitamin B-6 would be available from wheat bran when it is the sole source of vitamin B-6 in the diet.

Gregory et al. 1989 have found that PNG is variably but relatively highly available to humans given an oral dose of d2-PNG. Intravenous injection of d2-PNG showed low utilization relative to PN. Thus hydrolysis of PNG by digestive enzymes would account for the extent of its utilization. Other matrix effects could explain the variability in release of bound forms by digestive enzymes and thus the better correlation with

bioavailability found with quantitation with pancreatin and pepsin treatment of foods.

Future research should be directed to explaining the low, apparently negative bioavailability of the vitamin B-6 in wheat products. In addition, the action of digestive enzymes on foods, the vitamin B-6 forms released, and the effect on total dietary vitamin B-6 bioavailability of simultaneous ingestion of vitamin B-6 containing foods of differing bioavailabililty should be investigated.

Table 3.1. Concentration of vitamin B-6 as pyridoxine equivalents after various treatments as measured by *Sacchromyces uvarum*.

mg B-6/100gm food
(pyridoxine equivalents)

TREATMENT[1]

	Method of AOAC, 1980	Method of Kabir et al. 1983a		Method of Tadera et al. 1986			Method of Ekanayake & Nelson,1986
FOOD	Hydrolyzed	Glycosylated	Nonconjugated	Glycosylated	Free	B6X	Digestive Enzymes
Walnuts	.624	.531	.494	.550	.535	.548	.542
Banana	.378	.361	.336	.386	.329	.342	.301
Tomato juice	.086	.076	.059	.087	.059	.081	.061
Wheat bran	1.494	.834	.522	.870	.354	.941	.323
Shredded wheat	.374	.170	.051	.185	.044	.229	.055
Spinach	.198	.165	.076	.188	.064	.190	.146
Orange juice	.263	.203	.072	.206	.077	.224	.094
Broccoli	.143	.115	.034	.125	.023	.125	.087
Cauliflower	.137	.122	.034	.133	.017	.124	.102
Carrots	.066	.054	.004	.055	.003	.057	.028
Mean	.376	.263	.168	.278	.151	.286	.174
±SD	± 428	±.247	± .202	±.256	±.185	±.272	±.164

[1] Hydrolyzed was value measured by method of AOAC, 1980. Glycosylated and nonconjugated were the values measured by method of Kabir et al. (1983) that are used along with the hydrolyzed value to calculate %glycosylated vitamin B-6. Glycosylated, phosphorylated and Free were the values as measured by the method of Tadera et al. (1986) which are used along with hydrolyzed values to calculate %glycosylated, phosphorylated vitamin B-6. B_6X is the value measured by the method of Tadera et al. (1986), to determine the amount of B_6X. Digestive enzymes is the amount of vitamin B-6 measured after a food was treated with digestive enzymes.

Table 3.2. Amounts and percentages of glycosylated vitamin B-6, glycosylated-phosphorylated, B_6X, and forms not released by digestive enzymes in ten foods[1]

	mg/100gm pyridoxine equivalents							
	Kabir et al. 1983		Tadera et al. 1986				Ekanayake & Nelson, 1986	
Food	Glycosylated	(%)	Glycosylated phosphorylated	(%)	B6X	(%)	Digestive Enzymes	(%)
Walnuts	.037	5.9%	.015	2.4%	---[2]	----	.082	13.1%
Bananas	.025	6.6%	.057	15.1%	---	----	.077	20.4%
Tomato juice	.017	20.2%	.028	32.4%	---	----	.025	18.6%
Wheat bran	.312	20.9%	.516	34.5%	.071	4.8%	1.171	78.4%
Shredded wheat	.119	31.8%	.141	37.6%	.044	11.8%	.319	85.4%
Spinach	.089	45.1%	.124	62.5%	.002	1.0%	.052	26.3%
Orange juice	.131	49.7%	.129	49.1%	.018	6.8%	.169	64.2%
Broccoli	.081	56.4%	.102	71.2%	.000	0.0%	.056	39.0%
Cauliflower	.088	64.5%	.116	84.5%	---	----	.035	25.5%
Carrots	.050	76.4%	.052	78.7%	.002	3.0%	.038	57.0%
Mean	.095	37.8%	.128	46.8%	.023	4.6%	.202	56.2%
± SD	±.085	± 24.4	±.143	± 27.3	±.029	± 4.3	±.352	±53.0

[1]Glycosylated vitamin B-6 determined by the method of Kabir et al. (1983), glycosylated-phosphorylated and B_6X determined by the method of Tadera et al. (1986). Digestive enzymes is the amount and percentage of vitamin B-6 not released by treatment with pepsin followed by pancreatin.

[2]Values less than 0.0 are designated with ----.

Table 3.3. Mean urinary 4-pyridoxic acid excretion in 9 subjects following pyridoxine or food dose ingestion

Dose or Food[1]	Amount Fed[2] (gm)	4-Pyridoxic Acid[3] (μmoles/24hr)
0 (mg)	---	11.07 ± 0.43
0.2 "	---	11.39 ± 1.21
0.4 "	---	11.98 ± 0.72
0.6 "	---	12.55 ± 0.83
Walnuts	74	12.18 ± 0.66
Bananas	145	11.83 ± 0.56
Tomato juice	485	11.61 ± 0.59
Wheat Bran	42	10.49 ± 0.76
Shredded Wheat	205	10.78 ± 0.80
Spinach	215	11.29 ± 0.39
Orange juice	299	11.31 ± 0.81
Broccoli	403	11.86 ± 0.73
Cauliflower	331	11.78 ± 0.70
Carrots	836	11.02 ± 0.69

[1]Basal Diet (0 mg) contained 3.295 mg (19.47μmol/day) pyridoxine equivalents. Doses were given as pyridoxine·HCl (0.2 mg = 1.18 μmol, 0.4 mg = 2.36 μmol, 0.6 mg = 3.55 μmol pyridoxine). [2]Food doses contained 0.5 mg (2.955 μmol) pyridoxine equivalents as measured by *S. uvarum*.
[3]Mean ± SD: Values for 4-pyridoxic acid > 1.5 interquartile range values from the mean not included.

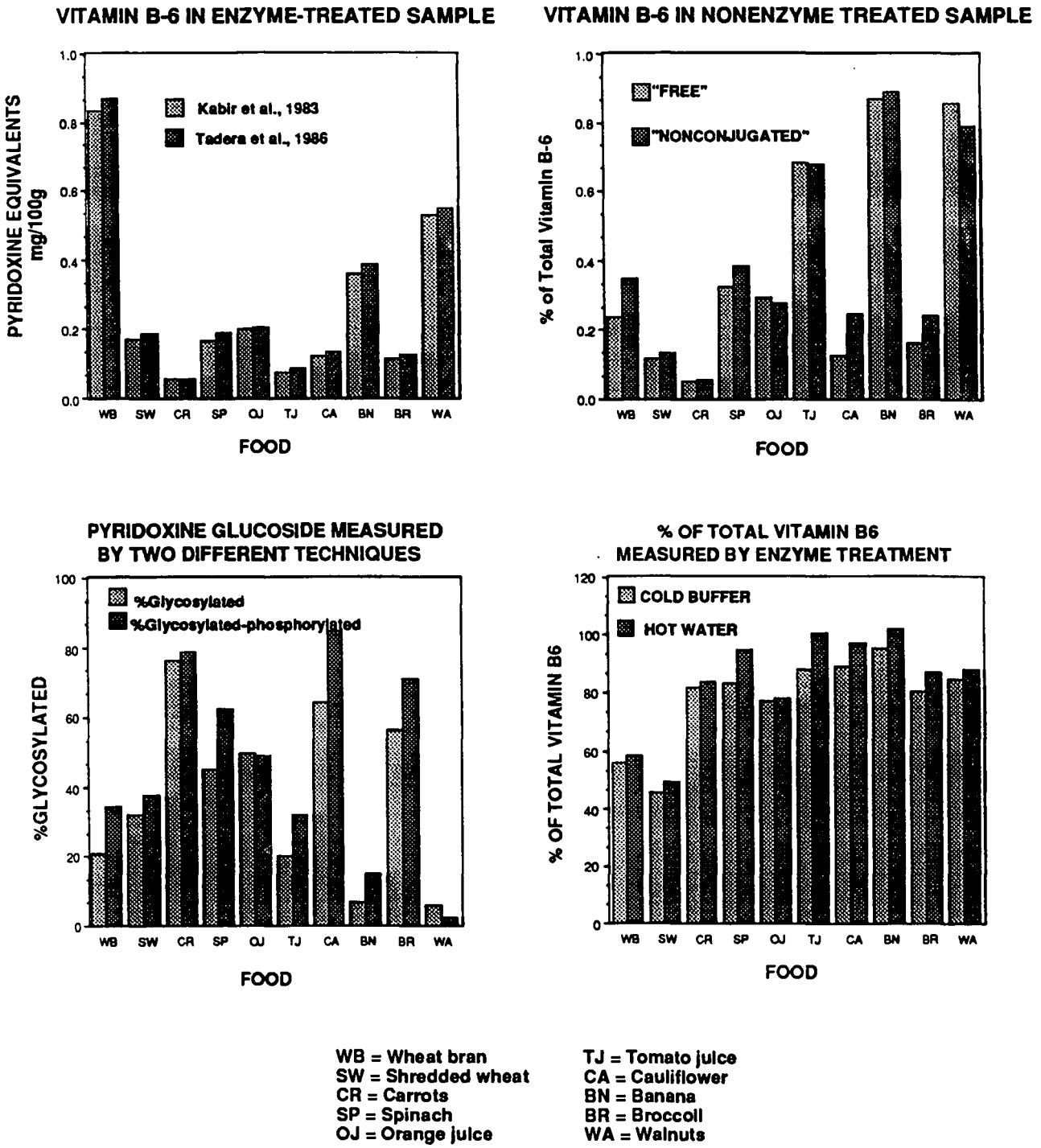

Figure 3.1. Comparison of vitamin B-6 values obtained by methods of Kabir et al. 1983a, and Tadera et al. 1986.

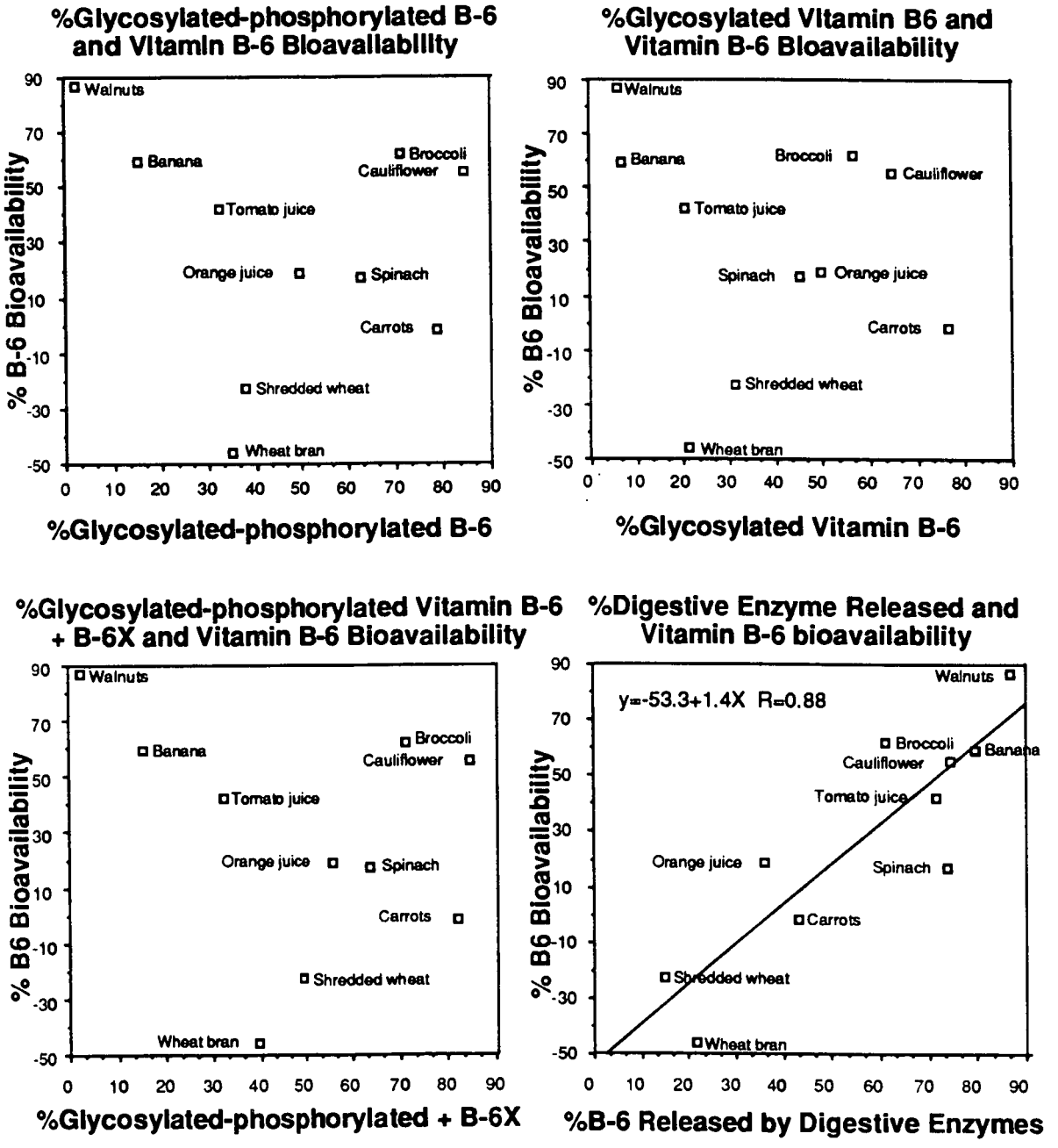

Figure 3.2 Predictive value of various in vitro methods of estimating bioavailable vitamin B-6

Chapter 4

SUMMARY AND CONCLUSIONS

The bioavailability to humans of the vitamin B-6 in foods containing the pyridoxine conjugate, pyridoxine glucoside (5'-0- (ß-D-glucopyranosyl) was investigated. A hypothesis based on earlier research had suggested that vitamin B-6 bioavailability was inversely correlated with % vitamin B-6 as pyridoxine glucoside. To test this hypothesis, the bioavailability of 10 plant foods containing a wide range of vitamin B-6 as pyridoxine glucoside was assessed.

A feeding study utilizing nine men aged 20-33 yrs as subjects was used to test this hypothesis. The subjects were fed a constant basal diet containing 3.2 mg of vitamin B-6 throughout a 49-day study. The first 10 days were used as an adjustment period to normalize the subjects' vitamin B-6 status and to adapt them to the daily procedure. Complete 24 hour urine collections were obtained throughout the study, and the incremental urinary 24-hr 4-pyridoxic acid excretion in response to test doses of pyridoxine (0.2, 0.4, and 0.6 mg) or test foods (0.5 mg pyridoxine equivalents) fed in addition to the basal diet was used to assess differences in vitamin B-6 bioavailability among foods. Immediately after the 10 day adaptation period test doses or foods were given on every third day. The % bioavailability of the foods calculated from a urinary 4-PA excretion response line generated from the

pyridoxine doses were as follows: walnuts 87; bananas 60; tomato juice 43; wheat bran -46; shredded wheat -23; spinach 17; orange juice 19; broccoli 62; cauliflower 56; and carrots -1. Regression analysis showed a strong inverse relationship between %pyridoxine glucoside and bioavailability for 6 of the ten foods ($R=-.94$,$p<0.01$). However, wheat bran and shredded wheat had low, negative bioavailability while the two crucifers, broccoli and cauliflower, had high bioavailability despite a high percentage (62% and 56%, respectively) of vitamin B-6 as pyridoxine glucoside. Thus percentage vitamin B-6 as pyridoxine predicted some, but not all, of the variation in vitamin B-6 bioavailability of the foods.

Since the content of conjugated forms of vitamin B-6 in foods may be important in assessing bioavailable vitamin B-6, methods for measuring available forms of vitamin B-6 in foods were compared. To correlate these values with the bioavailability data obtained in the first study, the same ten plant foods were assayed by three different procedures. The first two methods quantified pyridoxine glucoside by a differential growth assay using *S. uvarum* after prior incubation with or without ß-glucosidase. Pyridoxine glucoside content measured by the method of Kabir et al. (1983a) was lower ($p=.005$) than that measured by the method of Tadera et al. (1986) which used cold dispersion and hot water extraction of the foods, respectively. Food values for B_6X, an unidentified form of vitamin B-6 quantitated by a method of Tadera et al. (1986) were obtained by treating foods

with base (0.7 M KOH) prior to glucosidase treatment and measurement with *S. uvarum*. B_6X was detected in wheat products, spinach, orange juice and carrots in small amounts (1-12%). In a third method, foods were treated with digestive enzymes (pepsin followed by pancreatin) by the method of Ekanayake and Nelson, 1986, extracted with methanolic HCl and quantified by *S. uvarum*. Of the three procedures, the best correlation with food vitamin B-6 bioavailability obtained in the human feeding study was with vitamin B-6 values obtained by enzymatic treatment of the foods.

Further research should be directed towards explaining both the apparently high vitamin B-6 bioavailability of the cruciferous vegetables used in this study, and the apparent negative bioavailability of the vitamin B-6 in the wheat products. Future feeding studies should use longer feeding periods of individual test foods to obtain more accurate estimates of mean incremental 4-pyridoxic acid excretion in response to these foods in spite of the high daily variation in 4-pyridoxic acid excretion.

Prediction of the bioavailability of more foods should be attempted using the extraction method of Ekanayake and Nelson, (1986) to see if this method of available vitamin B-6 estimation is widely useful. The forms of vitamin B-6 found in plant foods, their interaction with digestive processes, and their ultimate disposition also needs further work.

BIBLIOGRAPHY

AAP(American Academy of Pediatrics, Committee on Nutrition). 1976 Commentary on breast-feeding and infant formulas, including proposed standards for formulas. Pediatrics 57:278-285.

Abrahams, H.E. & Robinson, D. 1969 ß-D-glucosidases and related enzymatic activities in pig kidney. Biochem J 111:749-755.

AOAC 1980 Official Methods of Analysis. 13th edition. Washington, DC: Association of Official Analytical Chemists. pp 768-769.

Baker, E.M. Canham, J.E., Nunes, W.T., Sauberlich, H.E. & McDowell, M.E. 1964 Vitamin B-6 requirement for adult men. Am J Clin Nutr 15:59-66.

Banks, M.A. & Gregory III, J.F. 1990 Effects of feeding pyridoxine-5'-ß-D-glucoside on the induction of rat intestinal ß-glucosidase activity. FASEB J 4(3):A366 (Abstract).

Barnard, H.C., DeKock, J.J., Vermaak, J.H. & Potgieter, G.M. 1987 A new perspective in the assessment of vitamin B-6 nutritional status during pregnancy in humans. J Nutr 117:1303-1306.

Barr, W. 1984 Pyridoxine supplements in the premenstrual syndrome. Practitioner 228:425-427.

Bauernfiend, J.C., & Miller, O.N. 1978 Vitamin B-6: Nutritional and pharmaceutical usage, stability, bioavailability, antagonists and safety. In: Human Vitamin B-6 Requirements. National Research Council. National Acad. Sci. Washington, DC pp. 78-110.

Baysal, A., Johnson, B.A., & Linkswiler, H. 1966 Vitamin B-6 depletion in man: Blood vitamin B-6, plasma pyridoxal-phosphate, serum cholesterol, serum transaminases and urinary vitamin B-6 and 4-pyridoxic acid. J Nutr 89:19-23.

Bender, D.A. 1986 Uptake of tryptophan into the brain: dietary influences on serotoninergic function. Bibl Nutr Dieta 38: 82-86.

Bender, D.A., Bowden, J.F, Coulson, W.F, Dewji, M.R., Sutton, J. & Symes E.K. 1988 Vitamin B-6 deficiency enhances end-organ sensitivity to steroid hormones. In: Leklem, J.E., & Reynolds, R.D., eds. Clinical and Physiological Applications of Vitamin B-6. New York; NY: Alan R. Liss, Inc. pp. 45-49.

Bender, D.A., Tagoe, C.E., & Vale, J.A. 1982 Effects of oestrogen administration on vitamin B6 and tryptophan metabolism in the rat. Br J Nutr 47(3): 609-14.

Bender, D.A., & Totoe, L. 1984 High doses of vitamin B-6 in the rat are associated with inhibition of hepatic tryptophan metabolism and increased uptake of tryptophan into the brain. J Neurochem 43(3): 733-736.

Bessey,O.A., Adam, D.J. & Hansen, A.E. 1957 Intake of vitamin B-6 and infantile convulsions: a first approximation of requirements of pyridoxine in infants. Pediatrics 20:33-44.

Bhagavan, H.N. 1985 Interaction between vitamin B-6 and drugs. In: Reynolds, R.D. & Leklem, J.E., eds. Vitamin B-6: Its Role in Health and Disease, New York, NY Alan R. Liss, Inc. 401-415.

Bills, N.D., Leklem, J.E. & Miller, L.T. 1987 Vitamin B-6 bioavailability in plant foods is inversely correlated with % glycosylated vitamin B-6. Fed Proc 46:1487 (abstract).

Black, A.L., Guirard, B.M. & Snell, E.E. 1977 Increased muscle phosphorylase in rats fed high levels of vitamin B-6. J Nutr 107:1962.

Black, A.L., Guirard, B.M. & Snell, E.E. 1978 The behavior of muscle phosphorylase as a reservoir for vitamin B6 in the rat. J Nutr 108:670-677.

Blalock, T.L., Thaxton, J.P., & Garlich, J.D. 1984
Humoral immunity in chicks experiencing marginal vitamin B-6 deficiency. J Nutr 114(2): 312-322.

Brown, R.R. 1988 Possible role of vitamin B-6 in cancer prevention and treatment. In: Leklem, J.E. & Reynolds, R.D., eds. Clinical and Physiological Applications of Vitamin B-6. New York, NY Alan R. Liss, Inc. pp 279-302.

Brown, R.R., Rose, D.P., Leklem, J.E., Linkswiler, H. & Anand, R. 1975 Urinary 4-pyridoxic acid, plasma pyridoxal phosphate, and erythrocyte aminotransferace levels in oral contraceptive users receiving controlled intakes of vitamin B-6. Am J Clin Nutr 28:10-19.

Buss, D.B., Hamm, M.W. & Henderson, L.M. 1979 Transport and metabolism of pyridoxine in the perfused small intestine and the hind limb of the rat. J Nutr 110:1655-1633.

Butler, P. 1985 Turnover of skeletal muscle glycogen phosphorylase studied using the cofactor, PLP as a specific label. Biochim Bioph Acta 847:316-323.

Cake, M.H., DiSorbo, D.M., & Litwack, G. 1978 Effect of pyridoxal phosphate on the DNA-binding site of activated hepatic glucocorticoid receptor. J Biol Chem 253:4886-4891.

Cannell, R.J.P., Kellarn, S.J., Owsiank, A.M., & Walker, J.M. 1987 Microalgae and cyanobacteria as a source of glycosidase inhibitors. J Gen Microbiol 133:1701-1705

Carney, M.W., Ravindran, A., Rinsler, M.G., & Williams, D.G. 1982 Thiamine, riboflavin and pyridoxine deficiency in psychiatric in-patients. Br J Psychiatry 141: 271-272.

Carpenter, K. J., Harris, L.J. & Kodicek, E. 1948 Pyridoxine and riboflavin deficiencies in rats on synthetic diets containing succinyl sulfathiazol. Br J Nutr 2:VII-VIII.

Chabner, B., & Livingston, D. 1970 A simple enzymic assay for pyridoxsal phosphate. Anal Biochem 34:413-423.

Chandra, R.K. 1983 Nutrition and immune responses. Can J Physiol Pharmacol 61(3): 290-294.

Chandra, R.K. & Puri, S. 1985 Vitamin B-6 modulation of immune responses and infections. In: Reynolds, R.D., and Leklem, J.E. eds. Vitamin B-6: Its Role in Health and Disease. New York; NY: Alan R. Liss, Inc. pp. 163-165.

Cho, J.Y., Kirksey, A., Galal, O., Bassily, N. Harrison, G. and Jerome, N. 1990 B-6 vitamers and ß-glucoside conjugate levels in milk of American and Egyptian women during 6 months of lactation. FASEB J 4(3):A668. (Abstract).

Coburn, S.P., Lewis, D.L.N., Fink, W.J., Mahuren, J.D., Schaltenbrand, W.E. & Costill, D.L. 1988 Human vitamin B-6 pools estimated through muscle biopsies. Am J Clin Nutr 48:291-294.

Coburn, S.P. & Mahuren, J.D. 1983 A versatile cation-exchange procedure for measuring the seven major forms of vitamin B-6 in biological samples. Anal Biochem 129:310-317.

Coburn, S.P., Mahuren, J.D. & Guilarte, T.R. 1984 Vitamin B-6 content of plasma of domestic animals determined by HPLC, enzymatic and radiometric microbiological methods. J Nutr 114:2269-2273.

Committee on Enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology. 1974 Recommended methods for the determination of four enzymes in blood. Scand J Clin Lab Invest 33:291.

Contractor, S.F., & Shane, B. 1970 Stability of the tritium label in different positions of the pyridoxol molecule during its metabolism in rats. Biochem Pharmacol 19:1669-1674.

Coursin, D.B. 1954 Convulsive seizure in infants with pyridoxine deficient diet. J Am Med Accoc 154:406-408.

Cunningham, J.J. 1982 An individualization of dietary requirements for energy in adults. J Am Diet Assoc 80:335-338.

Dakshinamurti, K., Paulose, C.S., & Siou, Y.L. 1985 Neurobiology of pyridoxine. In: Reynolds, R.D., and Leklem, J.E. eds. Vitamin

B-6: Its Role in Health and Disease. New York; NY: Alan R. Liss, Inc. pp. 99-121.

Daniels, L.B., Coyle, P.J., Chiao, Y-B. & Glew, R.H. 1981 Purification and characterization of a cytosolic broad specificity B-glucosidase from human liver. J Biol Chem 256(24):13004-13013.

David, S., & Kalyankar, G.D. 1983 Influence of adrenalectomy on vitamin B6 status. Experientia 39(3):329.

Dempsey, W.B., & Christensen, H.N. 1962 The specific binding of pyridoxal 5'-phosphate to bovine plasma albumin. J Biol Chem 237(4):1113-1120.

Dietary Standards for Canada. 1975 Bureau of Nutritional Sciences, Dpartment of National Health and Welfare, Ottawa, Canada.

DiSorbo, D.M., & Litwack, G. 1982 The use of pyridoxal 5-phosphate as a tool in the study of steroid receptors. In: Biochemical Actions of Hormones, Vol 9, Litwack G, ed New York,NY Academic Press, Inc. pp 205-219.

Donald, E.A. & Bosse, T.R. 1979 The vitamin B-6 requirement in oral contraceptive users: II. Assessment by tryptophan metabolites, vitamin B-6, and pyridoxic acid levels in urine. Am J Clin Nutr 32:1024-1032.

Donald, E.A., McBean, L.D., Simpson, M.E., Sun, M.F. & Aly, H.E. 1971 Vitamin B6 requirements of young adult women. Am J Clin Nutr 24:1028-1041.

Doyle, W. Crawford, M.A., Laurance, B.M. & Drury, P. 1982 Dietary survey during pregnancy in a low socio-economic group. Hum Nutr Appl Nutr 36(2): 95-106.

Ebadi, M., Gessert, C.F., & Al-Sayegh, A. 1982 Drug-pyridoxal phosphate interactions. Q Rev Drug Metab Drug Interact 4(4): 289-331.

Edwards, M., Benson, E. & Storvick, C.A. 1963 Collaborative study of vitamin B-6 methodology. J of the AOAC 46(3):396-399.

Ekanayake, A. & Nelson, P. 1986 An in vitro method for estimating biologically available vitamin B-6 in processed foods. Brit J of Nutr 55:235-244.

Evangelopoulos, A.E. 1984 Oestrogen uptake by target tissues and biological actions in vitamin B6 deficiency. In: Evangelopoulos, A.E., (ed) Chemical and Biological Aspects of Vitamin B6 Catalysis, Part A: Metabolism, Structure, and Function of Phosphorylases, Decarboxylases, and other Enzymes., Prog Clin. Biol Res., vol 144A, 1984., pp. 55-62.

FNB (Food and Nutrition Board, National Research Council). 1968 Rcommended dietary allowances, 7th ed. National Academy of Sciences, Washington, D.C.

FNB (Food and Nutrition Board, National Research Council), National Academy of Sciences. 1979 Proposed fortification policy for cereal grain products. Washington, D.C.

FNB (Food and Nutrition Board, National Research Council) 1980 Recommended Dietary Allowances, 9th ed. National Academy of Sciences, Washington, D.C.

FNB (Food and Nutrition Board, National Research Council) 1989 Recommended Dietary Allowances, 10th ed. National Academy of Sciences, Washington, D.C. pp. 142-150.

Fukui, T., Tagaya, M., & Horinishi, N. 1986 Direct phosphate-phosphate interaction between coenzyme and substrate in the catalytic reaction of glycogen phosphorylase. J Protein Chem 5(2): 75-78.

Gilbert, J.A. & Gregory III, J.F. 1990 Effects of pyridoxine-beta-glucoside on the metabolic utilization of pyridoxine in the rat. FASEB J 4(3):A366. (Abstract).

Godfrey, K. 1985 Statistics in practice. Comparing the mean of several groups. New Eng J Med 313:1450-1456.

Gonzalez, P.L. 1982 The bioavailability of vitamin B-6 from selected foods as measured by urinary 4-pyridoxic acid in men saturated with pyridoxine. M.S. Thesis. Oregon State University.

Gregory, J.F. 1980 Effects of ε-pyridoxyllysine bound to dietary protein on the vitamin B6 status of rats. J Nutr 110:995-1005.

Gregory, J.F. 1980 Effects of ε-pyridoxyllysine and related compounds on liver and brain pyridoxal kinase and liver pyridoxamine (pyridoxine) 5'-phosphate oxidase. J Biol Chem 255:2355-2359.

Gregory, J. F. 1980 Bioavailability of vitamin B-6 in nonfat dry milk and a fortified rice breakfast cereal product. J Food Sci 45:84-86.

Gregory, J.F. & Feldstein, D. 1985 Determination of vitamin B-6 in foods and other biological materials by paired-ion high-performance liquid chromatography. J Agric Food Chem 33:359-363.

Gregory, J.F. & Ink, S.L. 1985 The bioavailability of vitamin B-6. In: Reynolds, R.D., and Leklem, J.E. eds. Vitamin B-6: Its Role in Health and Disease. New York; NY: Alan R. Liss, Inc. pp. 3-23.

Gregory, J.F., & Ink, S.L. 1987 Identification and quantification of pyridoxine-ß-glucoside as a major form of vitamin B-6 in plant-derived foods. J Agric Food Chem 35(1):76-82.

Gregory, J.F., & Kirk, J.R. 1978 Vitamin B-6 activity for rats of ε-pyridoxyllysine bound to dietary protein. J Nutr 108:1192-1199.

Gregory III, J.F., & Kirk, J.R. 1979 Determination of urinary 4-pyridoxic acid using high performance liquid chromatography. Am J Clin Nutr 32:879-883.

Gregory, J.F. & Kirk, J.R. 1981 Determination of vitamin B-6 compounds by semiautomated continuous flow and

chromatographic methods. In: Reynolds, R.D., and Leklem, J.E. eds. Vitamin B-6: Its Role in Health and Disease. New York; NY: Alan R. Liss, Inc. pp. 149.

Gregory, J.F. & Litherland, S.A. 1985 Efficacy of the rat bioassay for the determination of biologically available vitamin B-6. J Nutr 116:87-97.

Gregory III, J.F., Trumbo, P.R., Bailey, L.B., Toth, J.P., Cerda, J.J., and Baumgartner, T.G. 1989 Bioavailability of oral and intravenous deuterium-labelled pyridoxine-beta-glucoside in human subjects. FASEB J 3(3): A454. (Abstract).

Guilarte, T.R., Wagner, H.N. Jr., & Frost, J.J. 1987 Effects of perinatal vitamin B6 deficiency on dopaminergic neurochemistry. J Neurochem 48(2): 432-439.

Gyorgy, P. 1971 Developments leading to the metabolic role of vitamin B-6. Am J Clin Nutr 24:1250-1256.

Gyorgy, P., & Eckhardt, R.E. 1939 Vitamin B6 and skin lesions in rats. Nature 133:448-449.

Ha, C., Kewkvliet, N.I., & Miller, L.T. 1984 The effect of vitamin B-6 deficiency on host susceptibility to Moloney sacoma virus-induced tumor growth in mice. J Nutr 114(5): 938-945.

Ha, C., Miller, L.T., & Kerkvliet, N.I. 1984 The effect of vitamin B-6 deficiency on cytotoxic immune responses of T cells, antibodies, and natural killer cells, and phagocytosis by macrophages. Cell Immunol 85(2): 318-329.

Hamm, M.W., Mehansho, H. & Henderson, L.M. 1982 Transport and metabolism of pyridoxamine and pyridoxamine phosphate in the small intestine of the rat. J Nutr 109:1552-1559.

Haskell, B.E. & Snell, E.E. 1970 Microbiological determination of the vitamin B-6 group. Methods Enzymol 18A:512-519.

Harris, A. 1969 Determination of D-xylose in urine for the D-xylose absorption test. Clin Chem 15:65-71.

Hayaishi, O., Yoshida, R., Takikawa, O. & Yasui, H. 1984 Indoleamine-dioxygenase - a possible biological function. In: Schlossberger H.G. Kochen, W. Linzen, B. & Steinhart H. eds. Progress in tryptophan and serotonin research. Berlin, W de Gruyter pp.33-42

Henderson, L.M. 1984 Vitamin B-6. In: Present Knowledge of Nutrition. 5th edition. Washington, DC. The Nutrition Foundation, Inc. pp. 303-317.

Hoes, M.J., Loeffen, T., & Vree, T.B. 1981 Kinetics of L-tryptophan in depressive patients: a possible correlation between the plasma concentrations of L-tryptophan and some psychiatric rating scales. Psychopharmacology 75(4):350-353.

Holley, J., Bender, D.A., Coulson, W.F., & Symes, E.K. 1983 Effects of vitamin B-6 nutritional status on the uptake of 3H-oestradiol into the uterus, liver and hypothalamus of the rat. J Steroid Biochem 18(2):161-165.

Horrobin, D.F. 1983 The role of essential fatty acids and prostaglandins in the premenstrual syndrome. J Reprod Med 28(7):456-456.

Hudson ,C, A, Betschart, A. B., & Oace, S. M. 1988 Bioavailability of vitamin B-6 from rat diets containing wheat bran or cellulose. J Nutr 118:65-71.

Ink, S.L., Gregory III, J.F. & Sartain, D.B. 1986 Determination of vitamin B-6 bioavailability in animal tissues using intrinsic and extrinsic labeling in the rat. J Agr & Food Chem 34:998-1004.

Iwami, K. & Yasumoto, K. 1986 Synthesis of pyridoxine-ß-glucoside by rice bran ß-glucosidase and its *in situ* absorption in rat small intestine. Nutr Res 6:407-414.

Johansson, S., Lindstedt, S. & Register, U. 1966 Metabolism of labeled pyridoxine in the rat. Am J Physiol 210(2):1086-1095.

Johansson, S., Lindstedt, S. & Tiselius, H.G. 1968 Metabolism of $^{3}H_{8}$ pyridoxine in mice. Biochem 7:2327-2332.

Johansson, S. & Tiselius, H.G. 1973 Metabolism of tritium- and 14C-labeled pyridoxine in the rat. Scand J Clin Lab Invest 32:91-14.

Kabir, H., Leklem, J. & Miller, L.T., 1983a Measurement of glycosylated vitamin B-6 in foods. J Food Science 48(5):1422-1425.

Kabir, H., Leklem, J.E., & Miller, L.T. 1983b Relationship of the glycosylated vitamin B-6 content of foods to vitamin B-6 bioavailability in humans. Nutr Rept Int 28:709-716.

Kabir, H., Leklem, J.E. & Miller, L.T. 1983c Comparative vitamin B-6 bioavailability from tuna, whole wheat bread and peanut butter in humans. J Nutr 113:2412-2420.

Kelsay, J., Baysal, A. & Linkswiler, H. 1968 Effect of vitamin B-6 depletion on the pyridoxal, pyridoxame, and pyridoxine content of the blood and urine of men. J Nutr 94:490-494.

Kies, C., Kan, S., & Fox, H.M. 1984 Vitamin B6 availability from wheat, rice and corn bran for humans. Nutr Rep Int 30(2):483-491.

Kubler, W. 1981 Nutritional deficiencies in pregnancy. Bibl Nutr Dieta 30:17-29.

Leklem, J.E. 1971 Quantitative aspects of tryptophan metabolism in humans and other species. Am J Clin Nutr 24:659- 672.

Leklem, J.E. 1985 Physical activity and vitamin B-6 metabolism in men and women: Interrelationship with fuel needs. In: Reynolds, R.D., and Leklem, J.E. eds. Vitamin B-6: Its Role in Health and Disease. New York; NY: Alan R. Liss, Inc. pp. 221-241.

Leklem, J.E., Brown, R.R., Rose, D.P., and Linkswiler, H.M. 1975 Vitamin B-6 requirements of women using oral contraceptives. Am J Clin Nutr 28:535-541.

Leklem, J.E., Miller, L.T., Perera, A.D. & Peffers, D.E. 1980 Bioavailability of vitamin B-6 from wheat bread in humans. J Nutr 110: 1819-1828.

Leklem, J.E. & Reynolds, R.D. 1981 Recommendations for Status Assessment of vitamin B-6. In: Leklem J.E. & Reynolds R.D., eds. Methods in Vitamin B-6 Nutrition. New York, Plenum Press. pp 389-392.

Lehninger, A.L. 1982 Principles of Biochemistry. Worth Publishers, Inc. New York. pp. 258.

Lepkovsky, S. 1938 Crystalline factor 1. Science 87:169.

Lewis, J.S. & Nunn, K.P. 1977 Vitamin B-6 intake and 24-hr 4-pyridoxic acid excretion in children. Am J Clin Nutr 30:2023-2027.

Life Sciences Research Office, Federation of American Societies for Experimental Biology: Nutrition Monitoring in the United States- An Update Report on Nutrition Monitoring. Prepared for the U.S. Department of Agriculture and the U.S. Department of Health and Human Services. DHHS Publication No.(PHS) 89-1255. Public Health Service. Washington. U.S. Government Printing Office. September 1989.

Lindberg, A.S., Leklem, J.E., & Miller, L.T. 1983 The effect of wheat bran on the bioavailability of vitamin B-6 in young men. J Nutr 113:2578-2586.

Linkswiler, H. 1967 Biochemical and physiological changes in vitamin B6 deficiency. Am J Clin Nutr 20:547-555.

Linkswiler, H.M. 1978 Vitamin B-6 requirements of men. In: Human vitamin B-6 requirements. National Academy of Sciences pp. 279-290

Litwack, G., Miller-Diener, A., DiSorbo, D.M., & Schmidt, T.J. 1985 Vitamin B-6 and the glucocorticoid receptor. Reynolds, R.D., and Leklem, J.E. eds. Vitamin B-6: Its Role in Health and Disease. New York; NY: Alan R. Liss, Inc. pp. 177-191.

Loo, G., Goodman, P.J., & Smith, J.T. 1986 Creatinine metabolism in the pyridoxine-deficient rat. J Nutr 116(2):2403-8.

Lui, A., Lumeng, L., & Li, T-K. 1985 The measurement of plasma vitamin B-6 compounds: comparison of a cation-exchange HPLC method with the open-column chrmomatographic method and the L- tyrosine apodecarboxylase assay. Am J Clin Nutr 41:1236-1243.

Lumeng, L., & Li, T-K. 1980 Mammalian vitamin B-6 metabolism: regulatory role of protein-b;inding and the hydrolysis of pyridoxal 5'-phosphate in storage and transport. In: Tryfiates, G.P. ed. Vitamin B-6 Metabolism and Role in Growth. Westport, CT: Food and Nutrition Press.

Lumeng, L., Li, T-K, & Lui, A. 1985 The interorgan transport and metabolism of vitamin B-6. In: Reynolds, R.D., and Leklem, J.E. eds. Vitamin B-6: Its Role in Health and Disease. New York; NY: Alan R. Liss, Inc. pp. 35-54.

Lumeng, L., Lui, A. & Li, T-K. 1980 Plasma content B-6 vitamers and its relationship to hepatic vitamin B-6 metabolism. J Clin Invest 66:688-695.

Lyon, J.B.,Jr. Bain, J.A. & Williams, H.L. 1962 The distribution of vitamin B6 in the tissues of two inbred strains of mice fed complete and vitamin B6-deficient rations. J Biol Chem 237:1989-1991.

Machida, Y. & Nagai, T. 1980 In vitro test and absorption study of a per oral controlled release dosage form containing pyridoxine hydrochloride or sodium riboflavin phosphate with hydroxypropyl cellulose. Chem Pharm Bull 28(4):1082-1089.

Manore, M.M. & Leklem, J.E. 1988 Effect of carbohydrate and vitamin B-6 on fuel substrates during exercise in women. Med Sci Sports Exerc 20(3):233-241.

Manore, M.M., Leklem, J.E. & Walter, M.C. 1987 Vitamin B-6 metabolism as affected by exercise in trained and untrained women fed diets differing in carbohydrate and vitamin B-6 content. Am J Clin Nutr 46:995-1004.

Martinez-Cariion, M. 1986 Pyridoxal phosphate bvinding sites in enzymes. In: Dophin D, Poulson R, Abramovic, O, eds. Vitamin B-6, pyridoxal phosphate. Part B. New York: John Wiley & Sons, 1-22.

Martner-Hewes, P.M., Hunt, I.F., Muarphy, N.J., Swendseld, M.E., & Settlage, R.H. 1986 Vitamin B-6 nutriture and plasma diamine oxidase activity in pregnant Hispanic teenagers. Am J Clin Nutr 44(6): 907-913.

Mattes, J.A., & Martin, D. 1982 Pyridoxine in premenstrual depression. Human Nutr:Applied Nutr 36A:131-133.

McMurray, D. 1984 Cell-mediated immunity in nutritional deficiency. Prog Food Nutr Sci 8(3-4):193-228.

Mehansho, H., Buss, D.P., Hamm, M.W., & Henderson, L.M. 1980 Transport and metabolism of pyridoxine in rat liver. Biochim Biophys Acta 631:112-123.

Mellor, J.D. & Layne, D.S. 1974 Steroid ß-D-glucosidase: Purification of the enzyme from rabbit liver. J Biol Chem 249(2):361-365.

Merrill, A.H. Jr., Henderson, J.M., Wang, E., Codner, M.A., Hollins, B. & Millikan, W.J. 1986 Activities of the hepatic enzymes of vitamin B-6 metabolism for patients with cirrhosis. Am J Clin Nutr 44:461-467.

Merrill, A.H. Jr., Henderson, J.M., Wang, E., McDonald, B.W., & Millikan, W.J. 1984 Metabolism of vitamin B-6 by human liver. J Nutr 114(9):1664-1674.

Middleton, H.M. 1978 Jejunal phosphorylation and dephosphorylation of absorbed pyridoxine.HCl in vitro. Am J Physiol 235:E272-278.

Middleton, H.M. 1982 Characterization of pyridoxal 5'phosphate disappearance from in vivo perfused segments of rat jejunum. J Nutr 12:269-275.

Middleton, H.M. 1985 Uptake of pyridoxine by in vivo perfused segments of rat samll intestine: a possible role for intracellular vitamin metabolism. J Nutr 115:1079-88.

Miller, L.T., & Edwards, M. 1981 Microbiological asay of vitamin B-6 in blood and urine. In: Methods in Vitamin B-6 Nutrition. Leklem, J.E. & Reynolds, R.D., eds. Plenum Press, New York, NY., pp. 45-55.

Miller, L.T., Leklem, J.E., & Shultz, T.D. 1985 The effect of dietary protein on the metabolism of vitamin B-6 in humans. J Nutr 115(12):1663-1672.

Miller, L.T., & Linkswiler, H. 1967 Effect of protein intake on the development of abnormal tryptophan metabolism in males during vitamin B-6 depeletion. J Nutr 93:53-59.

Miller, L.T., Shultz, T.D., & Leklem, J.E. 1980 Influence of citrus pectin on the bioavailability of vitamin B-6 in men. Fed Proc 39:797. (abstract).

Nelson, E.W., Lane, H. & Cerda, J.J. 1976 Comparative human intestinal bioavailability of vitamin B-6 from a synthetic and a natural source. J Nutr 106:1433-1437.

Neter, J., & Wasserman, W. 1974 Applied Linear Statistical Models. Richard D. Irwin, Inc., Homewood, Illinois.

Nguyen, L.B. & Gregory, J.F. 1983 Effects of food composition on the bioavailability of vitamin B-6 in the rat. J Nutr 113:1550-1560.

Nguyen, L.B., Gregory, J.F., Burgin, C.W., & Cerda, J.J. 1981 In vitro biding of vitamin B-6 by selected polysaccharides, lignin, and wheat bran. J Food Sci 46:1860-1862.

Nguyen, L.B., Gregory, J.F. & Damron, B.L. 1981 Effects of selected polysaccarides on the bioavailability of pyridoxine in rats and chicks. J Nutr 111:1403-1440.

Nguyen, L.B., Hiner, M.E., Litherland, S.A., & Gregory, J.F. 1983 Relative biological activity of nonphosphorylated vitaminb B-6 compounds in the rat. J Agric Food Chem 31:1282-1287.

Ottinger, J.M. 1985 Influence of riboflavin doses on the urinary excretion of riboflavin and 4-pyridoxic acid in young men. M.S. Thesis. Oregon State University.

Park, Y.K., & Linkswiler, H. 1970 Effect of vitamin B6 depletion in adult man on the excretion of cystathionine and other methionine metabolites. J Nutr 100:110-116.

Pao, E.M. & Michele, S.J. 1981 Problem nutrients in the United States. Food Tech 35:58-79.

Petersen, R.G. 1985 Design and Analysis of Experiments. Marcel Dekker, Inc. New York, NY.

Pino, S., Bennotte, J. & Gordnja, H. 1965 An automated method for urine creatinine which does not require a dialyzer module. Clin Chem 11:664.

Polansky, M.M., Reynolds, R.D. & J.T. Vanderslice. 1985 Vitamin B-6. In: Methods of Vitamin Assay. 4th ed. John Wiley & Sons. N.Y. ed by J. Augustus, B.P., Klein, D.A., Becker, & P.B. Venigopal. pp 417-443.

Polansky, M.M., & Toepfer, E.W., 1969 Nutrient composition of selected wheats and wheat products. 4. Vitamin B-6 components. Cereal Chem 46:664-674.

Price, J.M., Brown, R.R., & Yess, N. 1965 Testing the functional capacity of the tryptophan-niacin pathway in man by analysis of urinary metabolites. Adv Metab Disorders 2:159.

Reynolds, R.D. & Leklem, J.E. 1981 Recommended methods for vitamin B-6 nutrition. In: Methods in Vitamin B-6 Nutrition. New York, NY Plenum Pub Corp. pp. 383-387.

Reynolds, R.D. 1988 Bioavailability of vitamin B-6 from plant foods. Am J Clin Nutr 48:863-867.

Reynolds, R.D., Acharya, S., Leklem, J.E. & Moser, P.B. 1986 Effects of low maternal dietary intake of calcium, selenium and vitamin B-6 upon breast milk composition in Nepal. In:

Human Lactation 2: Maternal and Environmental Factors. Hamosh, M. & Goldman, A.S., eds. Plenum Press, N.Y., NY.

Reynolds, R.D., Polansky, M. & Moser, P.B. 1984 Analyzed vitamin B-6 intakes of pregnany and postparum lactating and nonlactating women. J Am Diet Assoc 84:1339-44.

Robinson, D. 1956 The fluorometric determination of B-glucosidase: its occurence in the tissues of animals including insects. Biochem J 63:39-44.

Root, E.J., Longenecker, J.B. 1983 Brain cell alterations suggesting premature aging induced by dietary deficiency of vitamin B6 and/or copper. Am J Clin Nutr 37(1): 510-552.

Rose, D.P. 1978 Oral contraceptives and vitamin B6. pp 193-201. In: Human vitamin B-6 requirements. National Academy of Sciences, Washington D.C.

Ruseler van Embden, J.G.H., & van Lieshout, L.M.C. 1987 Increased faecal glycosidases in patients with Crohn's disease. Digestion 37:43-50.

Russell, L.E., Bechtel, P.J. & Easter, R.A. 1985 Effect of deficient and excess dietary vitamin B-6 on amino transaminase and glycogen phosphorylase activity and pyridoxal phosphate content in two muscles from postpubertal gilts. J Nutr 115:1124-1135.

Santini, R., Sheehy, T., and Martinez-de, K. 1961 The xylose tolerance test with a five-gram dose. Gastorenterology 40:772

Sarma, P.S., Snell, E.E., & Elvelyein, C.A. 1947 The bioassay of vitamin B-6 in natural elements. J Nutr 33:121-128.

Sauberlich, H.E. 1968 Vitamin B-6 Group. IX. Biochemical systems and biochemical detection of deficiency. In: The Vitamins. Vol. II. (Sebrell, W.H. & Harris, R.S. eds.) Academic Press. New York, NY pp. 44-80.

Sauberlich, H.E. 1984 Newer laboratory methods for assessing nurtiture of selected B-complex vitamins. Ann Rev Nutr 4:377- 407.

Sauberlich, H.E. 1985 Interaction of vitamin B-6 with other nutrients. In: Reynolds, R.D. & Leklem, J.E. eds. Vitamin B-6: Its Role in Health and Disease. New York, N.Y.: Alan R. Liss, Inc. pp 193-217.

Sauberlich, H.E., Canham, J.E., Baker, E.M., Raica, N. & Herman, Y.F. 1972 Biochemical assessment of the nutritional status of vitamin B-6 in the human. Am J Clin Nutr 25(6):629-642.

Sauberlich, H.E., Shala, J.H., & Dowdy, R.P. 1974 Laboratory tests for the assessment of nurtitional status. pp 37-49. CRC Press, Inc., Cleveland, Ohio.

Scudi, J.V. 1942 Conjugated pyridoxine in rice bran concentrates. J Biol Chem 145:637-9.

Serfontein, W.J., De-Villiers, L.S., Ubbink, J., & Pitout, M.J. 1984 Vitamin B-6 revisited. Evidence of subclinical deficiencies in various segments of the population and possible consequences thereof. S Afr Med J 66(12): 437-441.

Shane, B. 1982 Vitamin B-6 metabolism and turnover in the ethanol-fed rat. J Nutr 112:610-618.

Shane, B., & Contractor, S.F. 1975 Assessment of vitamin B-6 status. Studies on pregnant women and oral contraceptive users. Am J Clin Nutr 28:739.

Shultz, T.D. & Leklem, J.E. 1987 Vitamin B-6 status and bioavailability in vegetarian women. Am J Clin Nutr 46:647-651.

Shultz, T.D. & Leklem, J.E. 1981 Urinary 4-pyridoxic acid, urinary vitamin B6 and plasma pyridoxal phosphate as measures of vitamin B6 status and dietary intake of adults. Pp. 297-320 in J.E. Leklem and R.D. Reynolds, eds. Methods in Vitamin B6 Nutrition: Analysis and Status Assessment. Plenum Press, New York.

Shultz, T.D, & Slattery, C.W. 1988 Binding of pyridoxine in vitro by natural and purified fiber. FASEB J 2(4):848. (Abstract)

Schuster, K., Bailey, L.B. & Mahan, C.S. 1984 Effect of maternal pyridoxine.HCl supplementation on the vitamin B-6 status of mother and infant and on pregnancy outcome. J Nutr 114:977-988.

Siow, Y.L., Dakshinamurti, K. 1985 Effect of pyridoxine deficiency on aromatic L-amino acid decarboxylase in adult rat brain. Exp Brain Res 59(3): 575-581.

Snedechor G.W., & Cochran, W.G. 1980 Statistical Methods. 7th edition. Ames, IA, The Iowa State University Press.

Snell, E.E, Guirard, B.M., & Williams, R.S. 1942 Occurence in natural products of a physiologically active metabolite of pyridoxine. J Biol Chem 143:519-530.

Snell, E.E., & Haskell, B.E. 1971 The metabolism of vitamin B-6. In: Florkin, M., Stotz, E.H., eds. Comprehensive Biochemistry, volume 21. Amsterdam, Elsevier. pp. 47-71.

Snyderman, S.E., Holt, L.E., Carretero, R. & Jacobs, K. 1953 Pyridoxine deficiency in the human infant. Am J Clin Nutr 1:200.

Stanulovic, M. Jeremic, V., Leskovac, V. & Chaykin, S. 1976 New pathway of conversion of pyridoxal to 4-pyridoxic acid. Enzyme 21:357-369.

Stokes, J. & Mendels, J. 1972 Pyridoxine and premenstural tension. Lancet i:1177-1178.

Storvick, C.A., & Peters, J.M. 1964 Methods for the determination of vitamin B-6 in biological materials. Vitamins & Hormones 22:833-854.

Stryer, L. 1981 Biochemistry. San Francisco, Calif. W.H. Freeman & Co. pp. 408-411.

Tadera, K., Arima, M., Yoshino, S., Yagi, F. & Kobayashi, A. 1986 Conversion of pyridoxine into 6-hydropyridoxine by food components, especially ascorbic acid. J Nutr Sci Vitaminol 32:267-277.

Tadera, K., Kaneko, T. & Yagi, F. 1986 Evidence for the occurence and distribution of a new type of viatmin B-6 conjugate in plant foods. Agric Biol Chem 50(11):2933-2934.

Tadera, K. Mori, E., Yagi, F., Kobayashi, A., Imada, K. & Imabeppu, M. 1985 Isolation and structure of a minor metaboliteof pyridoxine in seedlings of *Pisum sativum L*. J Nutr Sci and Vitaminol 31:403-406.

Talbott, M.C., Miller, L.T., and Kerkvliet, N.I. 1987 Pyridoxine supplementation: effect on lymphocyte responses in elderly persons. Am J Clin Nutr 46:659-64.

Tamura, T., & Stokstad, E.L.R. 1973 The availability of food folate in man. Brit J Haemotol pp 513-532.

Tarr, J.B., Tamura, T., & Stokstad, R. 1981 Availability of vitamin B-6 and pantothenate in an average American diet in man. Am J Clin Nutr 34:1328-1337.

Toepfer, E.W., & Polansky, M.M. 1970 Microbiological assay of vitamin B6 and its components. J Assoc Off Anal Chem 53:546- 550.

Toyota, T., Kai, Y., Kakizake, M., Ohtsuka, H., Shibata, Y., & Goto, Y. 1981 The endocrine pancreas in pyridoxine deficient rats. Tohoku J Exp Med 134(3): 331-336.

Trumbo, P.R., Gregory III, J.F. 1988 Metabolic utilization of pyridoxine-beta-glucoside in rats: Influence of vitamin B-6 status and route of administration. J Nutr 118:1336-1342.

Trumbo, P.R. & Gregory III, J.F. 1989 ß-glucosidase activity and the metabolic utilization of dietary pyridoxine-beta-glucoside. FASEB J 3(3):A454 (Abstract).

Trumbo, P.R. & Gregory III, J.F. 1989 The fate of dietary pyridoxine-ß-glucoside in the lactating rat. J Nutr 119:36-39.

Trumbo, P.R., Gregory III, J.F., & Sartain, D.B. 1988 Incomplete utilization of pyridoxine-ß-glucoside as vitamin B-6 in the rat. J Nutr 118:170-175.

Trumbo, P.B., Gregory III, J.F., Sartain, D.B., Toth, J.F., Bailey, L.B. & J.J. Cerda 1988 The bioavailability of pyridoxine-beta-glucoside in the rat and human. FASEB J 2(5):A1086 (Abstract).

Tryfiates, G.P. & Saus, F.L. 1976 Metabolism of pyridoxine in the liver of vitamin B6 deficient rats. Biochim Biophys Acta 451:333-342.

Tsuji, H., Okada, J., Iwami, K., Yasumoto, K. & Mitusda, H. 1977 Availability as vitamin B6 and small intestinal absorption of pyridoxine-beta-D-glucoside in rats. Vitamins (Kyoto) 50:327-333.

Ubbink, J.B., Serfontein, W.J., Becker, P.J. & De Villiers, L. S. 1986 Determination of urinary 4-pyridoxic acid levels as 4-pyridoxic acid lactone, using high-performance liquid chromatography. Am J Clin Nutr 44:698-703.

USDA-Agricultural Research Service, 1975 Nutritive Value of American Foods. Agricultural Handbook #456. Washington, D.C. USDA-ARS.

Vandenberg, H., Bogards, J.J. P., Sinkeldam, E.J. & Schreurs, W.H.P. 1982 Effect of different levels of vitamin B6 in the diet of rats in the content of pyridoxamine-5-phosphate and pyridoxal-5-phosphate in the liver. Int J Vit Nutr Res 52:407-416.

Vandenberg, H., Louwerse, E.S., Bruise, H.W., Thissen, J.T., Schrijver, J. 1986 Vitamin B6 status of women suffering from premenstrual syndrome. Hum Nutr Clin Nutr 40(6): 441-450.

Vanderslice, J.T., Maire, C.E., Doherty, R.F. & Beecher, G.R. 1980 Sulfosalicylic acid as an extraction agent for vitamin B6 in food. J Agric Food Chem 28:1145-1149.

Wasynozuk, A., Kirksay, A., & Morre, D.M. 1983 Effect of maternal vitamin B-6 deficiency on specific regions of developing rat brain: Amino acid metabolism. J Nutr 113(1): 735-745.

Whyte, M.P., Mahuren, J.D., Vrabel, L.A., & Coburn, S.P. 1985 Markedly increased circulating pyridoxal-5'-phosphate levels in hypophosphatasia. Alkaline phosphatase acts in vitamin B6 metabolism. J Clin Invest 76(2): 752-756.

Wozenski, J.R., Leklem, J.E., & Miller, L.T. 1980 The metabolism of small doses of vitamin B-6 in men. J Nutr 110:275-285.

Yasomoto, K., Tsuji, H., Iwami, K., & Mitsuda, H. 1977 Isolation from rice bran of a bound form of vitamin B-6 and its identification as 5'-0-(ß-D-glucosylpyranosyl) pyridoxine. Agric Biol Chem 41(6):1061-1067.

Yen, J.T., Jensen, A.H. & Baker, D.H. 1976 Assessment of the concentration of biologically available vitamin B-6 in corn and soybean meal. J Animal Sci 42(4):866-870.

Young, D.S. 1987 Implementation of SI units for clinical laboratory data. Annals of Internal Med 106:114-129.

APPENDICES

Appendix Table 1. Tryptophan metabolites after 2 gram dose of L-tryptophan

Subject #	Kynurenic Acid (umoles/24hr)	[1]%Conversion	Xanthurenic Acid (umoles/24hr)	[1]%Conversion
1	42.9	0.44	29.2	0.30
2	55.5	0.57	25.7	0.26
3	45.7	0.47	31.7	0.32
4	42.8	0.44	26.9	0.28
5	83.0	0.85	26.4	0.27
6	67.5	0.69	22.3	0.23
7	153.1	1.56	28.8	0.29
8	63.0	0.64	14.2	0.15
9	64.8	0.66	17.4	0.18
Range	42.9-153.1	0.44-1.56	14.2-31.7	0.15-0.32

[1]This value is the molar percentage conversion of tryptophan to this metabolite. Metabolites measured by method of Satok & Price, 1958. Two grams of L-tryptophan were given to subjects who had received 3.2 mg (18.9 μmoles) vitamin B-6 for 3 days.

Appendix Table 2. Food test dose schedule

Subject	Day of Study									
	21	24	27	30	33	36	39	42	45	48
#	food code[1]									
1	1	9	10	5	2	6	7	4	8	3
2	10	8	9	3	1	5	6	2	7	4
3	7	5	6	1	8	2	4	9	3	10
4	2	10	1	6	4	7	8	3	9	5
5	9	7	8	4	10	3	5	1	6	2
6	3	2	4	8	5	9	10	6	1	7
7	8	6	7	2	9	4	3	10	5	1
8	5	4	3	9	6	10	1	7	2	8
9	6	3	5	10	7	1	2	8	4	9

[1] 1=walnuts, 2=banana, 3=tomato juice, 4= wheat bran, 5=shredded wheat, 6=spinach, 7=orange juice, 8=broccoli, 9=cauliflower, 10=carrots

Appendix Table 3. Test dose schedule.

Subject	Day of Study 12	15	18
#	pyridoxine (mg)[1]		
1	0.4	0.6	0.2
2	0.6	0.2	0.4
3	0.2	0.6	0.4
4	0.6	0.4	0.2
5	0.6	0.2	0.4
6	0.6	0.4	0.2
7	0.4	0.2	0.6
8	0.2	0.4	0.6
9	0.4	0.2	0.6

[1]Doses given as pyridoxine·HCl,divided into two equal doses, fed with orange juice at breakfast and with milk at dinner. 0.2 mg = 1.182 µmoles, 0.4 mg = 2.364 µmoles, 0.6 mg = 3.546 µmoles.

Appendix 4. Urinary 4-pyridoxic acid excretion during the 10-day adaptation period.

	Day of Study											
Subject #	1	2	3	4	5	6	7	8	9	10	mean±SD	26-day[1] mean±SD
	umoles/24hr											
1	9.51	12.60	10.26	11.17	11.52	10.49	10.85	12.16	11.86	11.83	11.23±0.95	10.74±1.13
2	9.60	8.31	9.60	9.51	11.92	9.20	9.01	10.86	9.51	9.94	9.75±1.00	11.06±0.92
3	10.03	8.26	9.42	9.81	11.05	9.82	9.49	10.07	11.14	10.78	9.99±0.86	11.73±1.16
4	10.72	11.22	11.19	10.88	10.97	11.77	11.20	11.81	11.67	10.09	11.15±0.53	11.44±0.54
5	8.02	9.13	9.36	9.57	9.44	10.31	9.46	9.49	9.33	11.30	9.54±0.83	11.39±0.86
6	10.02	10.84	9.80	10.44	11.95	10.99	9.04	9.92	9.16	10.28	10.24±0.87	10.60±1.00
7	9.54	10.73	11.23	11.58	10.46	11.72	9.74	9.64	9.69	10.08	10.44±0.84	11.29±0.61
8	-----	-----	23.30	18.55	17.85	16.58	17.50	14.34	12.52	11.77	16.55±3.71	10.46±0.99
9	9.64	0.83	0.60	10.30	10.26	9.98	11.36	11.82	10.52	10.67	10.60±0.64	10.90±0.58
Mean	9.64	10.24	11.64	11.31	11.71	11.21	10.85	11.12	10.60	10.75	11.05	11.07
±SD	0.77	1.52	4.43	2.81	2.44	2.19	2.65	1.58	1.24	0.73	2.13	0.43

[1]Mean 4-pyridoxic acid excretion values for 26 days before and after each test dose or food for each subject.

Appendix Table 5. Daily urinary 4-pyridoxic acid excretion for the 9 subjects on days 11 through 49.

Day of Study[1]	Subject # 1	2	3	4	5	6	7	8	9	Mean ±SD
	µmole 4-pyridoxic acid/day									
11B	12.26	9.27	10.16	10.32	10.08	9.29	10.48	11.08	11.18	10.46±0.95 B
12D	13.46	12.08	12.16	12.78	11.02	11.92	12.02	12.89	11.38	12.19±0.76 D
13A	11.27	10.11	10.52	11.65	10.04	10.20	11.42	12.53	11.18	10.99±0.84 A
14B	11.97	8.39	9.76	11.57	10.25	10.01	11.57	12.20	10.55	10.70±1.24 B
15D	13.20	10.72	11.99	12.08	10.46	11.44	11.91	11.39	11.61	11.64±0.80 D
16A	11.48	10.92	11.38	11.30	10.70	9.81	10.85	9.87	11.20	10.83±0.62 A
17B	11.30	11.03	11.47	11.45	10.59	11.18	10.79	10.90	11.43	11.13±0.32 B
18D	11.74	11.60	11.98	14.00	12.46	6.98	16.38	11.65	11.94	12.08±2.47 D
19A	8.15	12.18	11.95	11.76	12.16	10.00	11.38	11.26	12.32	11.24±1.36 A
20B	13.02	11.55	11.14	11.52	11.83	11.34	13.23	*8.39*	11.33	11.87±0.80 B
21F	12.15	11.38	9.93	12.62	11.09	10.28	12.45	9.09	11.61	1.18±1.20 F
22A	11.77	11.99	11.78	12.28	11.41	12.47	11.42	9.68	10.81	11.51±0.85 A
23B	11.66	11.49	12.07	11.45	11.77	10.69	11.23	11.23	11.26	11.43±0.39 B
24F	11.79	12.54	10.93	16.64	11.41	14.56	10.99	10.58	10.75	12.24±2.06 F
25A	10.11	10.93	11.78	12.01	11.11	10.69	11.36	9.65	10.96	10.96±0.75 A
26B	12.52	12.30	11.47	12.20	11.84	12.22	10.73	9.98	10.52	11.53±0.91 B
27F	10.26	12.94	10.33	12.14	12.42	10.86	10.83	11.35	9.74	11.21±1.09 F
28A	9.58	11.33	12.14	11.50	10.18	9.25	11.31	8.79	10.79	10.54±1.15 A
29B	11.66	11.12	10.40	11.01	13.06	11.01	12.00	12.16	10.58	11.56±0.84 B
30F	11.45	12.37	10.95	11.11	9.95	14.89	12.37	10.28	11.03	11.60±1.45 F
31A	9.66	11.20	14.18	10.83	12.45	10.89	11.47	10.27	11.01	11.33±1.32 A
32B	10.26	11.70	11.62	11.39	12.82	11.10	11.48	9.97	11.01	11.26±0.83 B
33F	12.32	12.01	13.09	10.81	11.43	10.72	12.57	10.87	12.00	11.87±0.98 F
34A	10.67	12.04	14.78	11.40	10.71	9.13	11.45	9.75	11.45	11.26±1.60 A
35B	11.12	11.50	10.84	10.74	11.20	9.79	11.38	9.60	10.30	10.72±0.68 B
36F	11.17	10.78	12.31	11.06	11.47	11.52	11.56	10.75	11.02	11.29±0.49 F
37A	9.88	11.78	12.76	10.96	12.63	9.36	11.40	10.51	10.66	11.10±1.16 A
38B	9.36	11.16	12.34	10.96	11.70	11.58	10.94	10.37	11.48	11.10±0.86 B
39F	8.27	12.61	9.99	12.12	12.23	9.92	11.90	10.91	11.27	11.02±1.41 F
40A	10.96	10.93	12.50	11.38	11.74	10.44	10.29	11.43	10.06	11.08±0.77 A
41B	9.38	11.23	11.92	11.18	12.10	9.40	10.88	10.40	10.93	10.82±0.96 B
42F	9.90	11.68	12.68	11.74	13.33	10.99	16.37	9.81	10.34	11.88±2.07 F
43A	10.29	11.86	12.65	11.25	11.38	12.24	10.92	9.74	10.74	11.23±0.93 A
44B	10.73	9.48	12.75	12.20	11.82	9.57	11.46	8.76	10.14	10.77±1.37 B
45F	10.70	12.43	12.72	11.78	11.59	12.37	11.46	9.49	10.18	11.41±1.09 F
46A	9.98	11.02	10.19	12.22	11.02	11.71	12.30	10.41	10.68	11.06±0.85 A
47B	10.36	10.60	10.90	12.28	10.82	11.36	11.02	10.56	9.61	10.83±0.73 B
48F	11.88	12.70	11.74	10.60	11.29	11.26	12.84	11.67	11.37	11.71±0.71 F
49A	9.99	10.63	11.47	10.55	10.74	11.02	*14.05*	*7.09*	11.69	10.87±0.58 A
Days before Mean±SD	11.20 ±1.14	10.83 ±1.12	11.30 ±0.88	11.41 ±0.58	11.53 ±0.91	10.66 ±0.94	11.29 ±0.74	10.60 ±1.00	10.77 ±0.59	
Days after Mean±SD	10.29 ±0.96	11.30 ±0.63	12.16 ±1.28	11.47 ±0.52	11.25 ±0.82	10.55 ±1.09	11.30 ±0.47	10.32 ±1.00	11.04 ±0.56	
Mean of days before and after	10.74	11.06	11.73	11.44	11.39	10.60	11.29	10.46	10.90	

[1] D=day of pyridoxine dose, F=food dose day, B=day before dose or food, A=day after dose of food, italicized values eliminated from mean calculations. Paired t-test of day before minus day after showed a mean difference of -0.01±0.50 µmol and was not significant at $p<0.01$.

Appendix Table 6. Vitamin B-6 content of basal diet composites.

Day of Study	Vegetable[1]	Dairy	Meat	Total
	mg pyridoxine equivalents			
8	1.178	.369	.736	2.283
16	1.430	.343	.674	2.447
23	1.209	.358	.763	2.330
29	1.294	.359	.723	2.376
37	1.208	.330	.638	2.176
44	1.110	.348	.700	2.158
Mean	1.238	.351	.706	2.295
±SD	±.111	±.013	±.045	±.113

[1]Vegetable composites consisted of all plant foods. Dairy composites consisted ofmilk and ice milk. Meat composites consistedof tuna, mayonnaise, and turkey.

Appendix 7. 4-pyridoxic acid excretion and increment on pyridoxine dose days for nine subjects.

		PYRIDOXINE DOSE µmoles / mg		
Subject #		1.18 0.02	2.36 0.40	3.55 0.66
	Mean 4-PA Excretion[1]	Total µmoles 4-PA Incremental µmoles 4-PA		
1	10.74	11.74 1.00	13.46 2.72	13.20 2.46
2	11.06	10.72 -0.34	11.60 0.54	12.08 1.02
3	11.73	12.16 0.43	11.98 0.25	11.99 0.26
4	11.44	14.00 2.56	12.08 0.64	12.78 1.34
5	11.39	10.46 -0.93	12.46 1.07	11.02 -0.37
6	10.60	6.98 (-3.62)[2]	11.44 0.84	11.92 1.32
7	11.29	11.91 0.62	12.02 0.73	16.38 (5.09)
8	10.46	12.89 2.43	11.39 0.93	11.65 1.19
9	10.90	11.61 0.71	11.38 0.48	11.94 1.04
	Mean ±SD	0.81 ±1.21	0.91 ±0.72	1.03 ±0.83
% of dose		68.5%	38.5%	29.1%

[1]Mean urinary 4-pyridoxic acid excretion of the 26 days before or after each dose or food. [2]Values in parenthesis were eliminated from calculations.

Appendix 8. 4-pyridoxic acid excretion and increment on test food days for nine subjects.

Subject #	mean 4-pyridoxic acid[1]	FOOD walnuts	bananas	tomato juice	wheat bran	shredded wheat	spinach	orange juice	broccoli	cauli-flower	carrots
		umol/24hr[2]									
1	10.74	12.15	12.32	11.88	9.95	11.45	11.17	8.27	10.70	11.79	10.26
		1.41	1.58	1.14	-0.79	0.71	0.43	*-2.47*	-0.04	1.05	-0.48
2	11.06	12.01	11.68	12.37	12.70	10.78	12.61	12.43	12.54	12.94	11.38
		0.95	0.62	1.31	*1.64*	-0.28	*1.55*	1.37	1.48	1.88	0.32
3	11.73	10.95	12.31	12.72	9.99	10.93	10.33	9.93	13.09	12.68	11.74
		-0.78	0.58	0.99	-1.74	-0.80	*-1.40*	*-1.80*	1.36	0.95	0.01
4	11.44	12.14	12.62	11.74	10.81	10.60	11.11	11.06	12.12	11.78	16.64
		0.70	1.18	0.30	-0.63	-0.84	-0.33	-0.38	0.68	0.34	*5.20*
5	11.39	13.33	11.29	11.47	9.95	12.23	11.59	11.41	12.42	11.09	11.43
		1.94	-0.10	0.08	-1.44	0.84	0.20	0.02	1.03	-0.30	0.04
6	10.60	12.37	14.56	10.28	10.86	10.72	10.99	11.26	14.89	11.52	9.92
		1.77	*3.96*	-0.32	0.26	0.12	0.39	0.66	*4.29*	0.92	-0.68
7	11.29	12.84	12.37	11.90	11.56	11.46	10.99	10.83	12.45	12.57	16.37
		1.55	1.08	0.61	0.27	0.17	-0.30	-0.46	1.16	1.28	*5.08*
8	10.46	10.91	9.49	11.35	10.58	9.09	10.87	9.81	11.67	10.28	10.75
		0.45	*-0.97*	0.89	0.12	-1.37	0.41	-0.65	1.21	-0.18	0.29
9	10.90	11.02	11.27	10.75	10.18	9.74	11.61	12.00	10.34	11.37	11.03
		0.12	0.37	-0.15	-0.72	-1.16	0.71	1.10	-0.56	0.47	0.13
Mean of difference 0.05			1.11	0.76	0.54	-0.58	-0.29	0.22	0.24	0.79	0.71
±SD		±0.66	±0.56	±0.59	±0.76	±0.80	±0.39	±0.81	±0.73	±0.70	±0.38

[1]This is the mean daily excretion of 4-pyridoxic acid on the 26 days before and after the pyridoxine and food doses.

[2]The top number is the total 4-pyridoxic acid excretion on the test food day. The bottom number is the difference between the top number and the mean daily excretion of 4-pyridoxic acid for each subject on the 26 days before and after the pyridoxine and food doses for that individual subject.

Italicized values were more than 1.5 interquartile range from the mean and were not used in calculations.

Appendix Table 9. Clinical blood chemistry of subjects

	Subject #								
	1	2	3	4	5	6	7	8	9
Glucose (mg/dL)	86	89	93	88	88	83	95	94	86
Creatinine (mg/dL)	1.0	1.1	1.0	1.2	1.1	1.3	0.9	1.2	1.1
Blood urea nitrogen (mg/dL)	26.3	23.1	23.8	12.9	24.3	22.7	21.0	14.3	24.5
Uric acid (mg/dL)	4.4	4.5	4.5	6.4	4.8	6.2	5.0	7.2	3.7
Total protein (g/dL)	6.7	7.0	7.0	7.6	7.1	6.6	6.7	7.5	6.7
Albumin (g/dL)	4.8	4.4	4.7	4.5	4.8	4.4	4.9	4.4	4.6
Cholesterol (mg/dL)	169	188	172	156	150	156	242	169	134
Triglycerides (mg/dL)	84	73	70	71	94	70	204	103	105
Sodium (Na+) (mEq/L)	138	141	138	140	138	139	137	139	138
Potassium (K+) (mEq/L)	4.1	4.3	4.8	4.4	4.0	4.8	3.9	3.9	4.4
Chloride (Cl-) (mEq/L)	104	100	102	103	102	103	101	99	101
Calcium (Ca++) (mg/dL)	9.0	9.3	9.6	9.1	9.7	9.4	9.2	9.4	9.4
Phosphorus (mg/dL)	3.7	4.0	3.5	3.1	4.0	3.5	4.3	3.6	3.1
Total bilirubin (mg/dL)	0.6	0.6	1.1	0.6	1.1	0.6	1.0	0.4	1.1
Serum glutamic oxaloacetic transaminase (units/ml)	46	29	31	52	22	33	36	29	27
Lactic dehyrdogenase (IU/L)	193	188	159	190	159	267	197	169	240
Serum glutamic pyruvic transaminase (units/ml)	64	21	18	51	18	22	28	47	40
Alkaline phosphatase (IU)	37	34	52	56	47	44	54	46	75
Total iron binding capacity (ug/L)	4983	4984	4985	3141	4989	4987	4988	5869	4991

Appendix Table 10. Daily urinary creatinine excretion for 9 subjects

Day #[1]	Subject # 1	2	3	4	5	6	7	8	9
					mg/day				
1	1991	2844	2083	2037	2387	2542	2842		1784
2	2127	1856	1678	2050	1985	2355	2695		2122
3	1768	2004	1936	2182	1903	2064	2532	1771	1867
4	2062	1973	2080	2146	1826	2610	2605	1884	1921
5	2127	2123	2132	2181	1788	2427	2551	1844	2075
6	2097	1761	1968	2102	1952	2449	2425	1758	1952
7	1913	1794	2006	2232	1803	2387	2484	1918	2045
8	2134	2228	2014	2092	1882	2483	2314	1918	2093
9	2092	1918	1985	2054	1825	2168	2435	1869	2081
10	2079	2286	2067	2095	2077	2438	2530	1879	1866
11B	2108	1883	2067	2146	1879	2088	2450	1924	1768
12D	2322	2323	1902	2192	1843	2402	2580	1906	1767
13A	1964	1836	2276	2170	1939	2424	2439	1964	1831
14B	2066	1758	1766	2270	1917	2316	2523	1864	1802
15D	2024	1935	2165	2167	1782	2510	2556	1975	1876
16A	2053	2117	1856	2256	2037	2113	2451	1869	1816
17B	2008	2188	2038	2103	1888	2490	2547	2017	1923
18D	2460	2043	2043	2233	1915	1656	3199	1851	1758
19A	1762	2143	2188	2130	1887	2530	2550	1907	2062
20B	2579	2142	2049	2064	1914	2196	2978	1383	2007
21F	2065	2134	2108	2162	1838	1997	2526	1632	1995
22A	1961	2100	1904	2157	1706	2489	2598	1812	1976
23B	2203	2077	2115	2085	1872	1789	2480	1972	1955
24F	2182	2157	1725	1975	1917	2691	2462	1806	2018
25A	2086	1930	1987	2111	1856	2258	2396	1712	2008
26B	2340	2025	2208	1958	2028	1450	1454	1890	2010
27F	2218	2155	1878	1985	1848	2637	2960	1930	1722
28A	1937	1824	2003	1794	1962	2187	2581	1444	1888
29B	2312	2000	1987	2065	1923	2496	2654	2427	1917
30F	2152	2077	1971	2168	1422	2947	2792	1876	1989
31A	2146	2106	2103	2184	1872	2182	2557	2010	2050
32B	2104	2226	2054	2336	2015	2269	2700	2121	1882
33F	2363	2027	2412	1825	1902	2468	2551	1985	2096
34A	2162	2110	1946	2024	1757	1901	2532	1890	1992
35B	2336	2083	2033	2012	1906	2205	2594	1872	2112
36F	2232	2232	2088	2115	1978	2557	2519	2198	1668
37A	2192	1994	2147	2080	2166	2290	2430	2117	1796
38B	2299	2010	2016	2237	1901	2493	2592	2056	2077
39F	2033	2218	1904	2035	2096	2110	2526	2119	1870
40A	2374	2062	2363	2030	1917	2534	2470	2184	1915
41B	2001	1980	1839	1938	2034	2429	2123	2078	1996
42F	2171	2002	1752	1923	1725	2314	2839	2037	1933
43A	2094	2139	2188	1894	1883	2498	2587	2105	1782
44B	2195	2070	1938	2044	1892	2152	2544	1901	1878
45F	2156	2085	1875	2080	2064	2279	1787	2025	1804
46A	2207	1823	1906	2043	2012	2151	2516	1972	1854
47B	2259	2116	2038	1912	1917	2275	2455	1937	1970
48F	2040	2062	2360	2027	1887	2558	2599	1951	1847
49A	2170	2046	1885	2090	1892	2329	3120	1810	2009

[1]D=Day of pyridoxine dose, F=food dose day, B=day before dose or food, A=day after dose of food.

Appendix Table 11. Daily weights for the 9 subjects.

Day #	Subject # 1	2	3	4	5	6	7	8	9
					Weight (kg)				
1	71.6	78.5	72.0	84.1	64.8	81.2	83.6		75.7
2	70.3	86.7	71.2	84.0	65.1	81.8	82.3		75.6
3	70.1	86.2	71.9	83.3	64.9	80.5	82.0		75.1
4	69.8	86.6	71.9	83.4	64.8	80.0	82.4	68.9	75.1
5	70.2	86.4	71.4	83.3	64.8	80.2	82.5	68.9	75.0
6	69.5	86.4	71.6	84.3	64.3	80.0	82.3	68.9	74.7
7	69.5	86.7	71.3	83.1	64.3	80.5	81.8	68.9	74.7
8	69.8	86.6	71.7	83.4	64.8	80.9	81.7	68.5	74.9
9	69.3	87.0	71.7	83.2	64.8	80.1	81.6	68.9	74.9
10	69.3	86.7	71.7	83.4	65.1	81.9	81.9	69.2	74.5
11	69.3	86.5	71.6	83.3	65.4	81.9	81.9	69.1	75.2
12	69.8	86.7	70.8	83.3	64.1	80.5	81.4	69.2	74.1
13	69.0	85.7	70.8	83.6	64.7	79.6	81.7	68.7	74.0
14	69.3	86.6	70.4	83.5	64.9	79.1	81.7	69.5	74.1
15	69.1	85.6	71.3	83.2	64.2	79.9	80.8	69.8	74.1
16	68.5	86.5	71.2	83.4	65.5	80.0	81.4	69.2	74.2
17	68.5	86.0	71.1	83.0	64.5	80.7	81.6	69.0	74.0
18	68.4	86.1	71.1	83.0	64.9	81.4	81.4	68.9	73.9
19	68.0	85.6	70.8	82.9	64.9	80.5	81.6	69.3	73.9
20	68.0	85.7	70.8	83.3	65.0	80.0	83.3	69.1	73.6
21	67.8	86.2	70.5	83.6	64.7	80.5	81.7	69.0	75.7
22	68.2	86	70.8	83.1	64.8	80.8	81.7	69.3	75.2
23	68.3	86.6	70.1	83.2	64.3	79.7	81.4	69.2	74.0
24	68.7	86.0	70.5	83.4	65.0	80.2	81.4	68.8	74.0
25	68.3	86.1	71.7	83.4	64.8	80.5	81.7	69.5	73.9
26	68.3	85.7	70.7	83.4	65.0	79.1	82.0	69.3	74.1
27	68.2	85.5	70.9	83.3	64.5	80.1	81.8	68.9	73.6
28	68.5	87.0	70.7	83.3	64.7	80.9	80.9	69.4	73.5
29	68.3	87.0	70.7	83.5	64.1	80.5	81.0	69.4	73.4
30	68.6	86.5	70.4	82.8	65.9	79.5	80.7	69.5	74.2
31	68.5	85.9	70.2	83.3	64.1	80.0	81.2	69.0	73.6
32	68.0	86.0	70.3	83.5	64.8	80.5	81.0	69.4	73.5
33	68.1	85.9	69.9	83.0	64.8	79.5	81.3	69.8	73.4
34	67.8	86.7	70.0	83.1	65.1	80.7	81.4	69.5	73.4
35	68.0	85.4	70.4	83.5	63.9	79.8	80.9	69.5	73.3
36	68.5	86.4	70.1	83.0	64.9	80.0	80.5	69.0	73.2
37	68.3	86.8	70.1	83.3	63.6	79.8	81.1	69.6	73.6
38	68.3	86.3	69.6	82.6	65.2	80.5	80.9	69.3	73.3
39	68.3	85.7	69.5	83.1	65.1	79.8	80.5	69.6	73.4
40	68.6	85.2	69.3	83.0	65.2	80.5	80.9	70.0	73.3
41	69.5	86.3	69.2		65.1	79.5	80.5	69.2	73.3
42	67.7	86.3	69.3	83.2	64.9	79.5	81.0	69.5	73.1
43	68.3	86.8	70.1	83.6	65.8	79.1	80.9	70.1	73.2
44	68.7	86.6	70.2	83.1	67.4	79.8	80.7	70.0	73.0
45	68.2	87.3	69.9	82.8	65.1	79.5	81.3	69.8	73.3
46	68.7	86.7	67.7	82.8	64.7	79.1	81.1	70.0	73.1
47	68.2		70.0	83.2	64.7	79.5	80.8	69.8	
48	69.0		69.3	82.8	64.3	79.3	81.1	69.5	74.5
49	67.5	88.1		84.5	64.2	78.6	81.0	69.8	73.2

Appendix Table 12. Xylose excretion, % of 5 g dose.

Subject #	% of Dose[1] %
1	45.9
2	36.1
3	43.4
4	35.2
5	35.6
6	50.4
7	50.0
8	46.0
9	48.4

[1] Subjects, fasted overnight, voided, consumed 5 g D-xylose, and then collected all urine for the next 5 hr.
% of xylose refers to molar % of 5 g dose excreted in 5 hr.

Appendix A. Collection of Urine

COLLECTION OF URINE

SPRING 1986 DIET STUDY

1. Collect all urine in containers provided (24 hr. urine collection). You will receive clean urine containers each morning.

2. Label all containers carefully and clearly with your initials and date if they are not already labelled. (Be sure and label with the date that the 24 hr. collection started , which is not necessarily today's date).

3. Each day:

Urine collections will be made on a 24-hr. basis and run, for example, from 6:45 AM one day until the same time the next day. Therefore, the collection made on rising in the morning belongs with the urine collected on the previous day and should be dated accordingly . It is important that the collection made on rising is done at the same time each day.

4. Urine will be collected starting with breakfast on the day you start on the diet study. Bring urine samples daily at any time convenient for you to the refrigerator in 5m 10s, Miriam Hall.

5. Store urine in a cool place and protect from light.

6. Please be careful not to spill or lose any urine. If this does happen, however, let us know immediately. The urine collections are a very critical part of this study. If, by chance, you do not have the proper container available try to find another container and save the urine. (It is a good idea to have extra bottle(s) available).

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

Appendix B. Xylose Test

XYLOSE TEST

INSTRUCTIONS FOR SUBJECTS

1) Find bottle with your initials marked "pre". Completely empty your bladder into it.

2) Drink xylose solution and rinse glass with the water supplied. Note time.

3) Collect all urine for the next five hours in the bottle marked "TV = ". (If you need an extra bottle there are some available in Milam room 106). At the end of the five hours completely empty your bladder into this bottle.

4) Return full bottles to the refrigerator in room 106 as soon as possible.

5) IMPORTANT ! NO FOOD OR DRINK ALLOWED FOR THE FIVE HOUR PERIOD ! PLEASE TRY TO DRINK PLENTY OF WATER (A GLASS OR SO EVERY HOUR OR TWO).

Appendix C. Daily Activity Sheet

Dr. Leklem
Diet Study
Food and Nutrition Dept.
Oregon State University

Name _____

Date _____

DAILY ACTIVITY SHEET

1. Record all activity for the previous day and length spent at each.

Activity	Length of Time (fraction of hours)	Time of Day*
Sleep		
Sitting		
Walking		
Physical work		
Other activities		
Other sports or activities (indicate type)		

* M - morning; A - after noon; E - evening; L - late night/early morning

2. Record all "free" foods in exact amounts used. Indicate type also used, decaf, etc.

   Coffee (cups) _____

   Tea (cups) _____

   Diet Pop _____

3. How do you feel today? Excellent _____
   Good _____
   Fair _____
   Poor _____

4. Any medications? (i.e., aspirin, etc.)

5. Other unusual events, exams, injuries, etc.

6. Did you turn your urine bottles in and pick up clean ones? _____

7. Your weight today _____

8. Other comments.

Appendix D. Telephone Interview Form

Vitamin B-6, Spring 1986
Telephone Interview form

Name_____ Telephone #_____

Local Address_____

Date of Birth_____ Height_____ Weight_____

Do you have any physical or metabolic defects?_____ If yes, please describe.

Are you single?_____

Do you have any food allergies?_____ If yes, please describe.

Describe briefly your daily physical activities.

Do you smoke?_____

What is your class and work schedule spring term?

Do you take vitamins?_____ If yes, what kind?_____ How many?_____

Interviewer: Please explain: Compliance to diet, must be at Milam through whole study. 24-hour urine collections. blood draws. Emphasize the study is for researcher to gather scientific information.

Make appointment_____

Appendix E. Interview Form

Dr. Leklem
Diet Study 1986
Food and Nutrition Dept.
Oregon State University

CONFIDENTIAL
Nutrition Project

Name: _____ Date: _____
Last First Middle

Address: Home Phone:

Age: Birth Date: State or Country of Birth:

Predominant State of Residence: City: No. of Years:

Present Employment:

Race (Circle one): a. American Indian e. Chinese
b. Black f. Japenese
c. Caucasian g. Other Oriental (specify) _____
d. Latin American h. Other (specify) _____

Marital Status (Circle one): a. single c. divorced/separated
b. married d. widowed

HEIGHT/WEIGHT: Height (feet and inches) _____ Present Weight _____
Most weighed _____ What Year _____
Length of time you have maintained your current weight _____

MEDICAL HISTORY (Check any conditions for which you have been diagnosed and give the age at diagnosis):

___ a. diabetes
___ b. hypothyroidism
___ c. hyperthyroidism
___ d. goiter
___ e. hypoadrenalism (Addison's disease)
___ f. osteoporosis
___ g. hepatitis
___ h. cirrhosis
___ j. kidney stones
___ k. nephritis
___ l. cystitis
___ m. high blood pressure
___ n. angina
___ o. mental depression requiring medication
___ p. insomnia requiring frequent medication
___ q. ulcers
___ r. pancreatitis
___ s. ulcerative colit
___ t. spastic colon/ diverticulitis
___ u. recurring gastritis
___ v. allergies
___ w. heart problems (specify) _____
___ x. cancer (specify type) _____

Have you ever had a glucose tolerance test? ___ yes ___ no
If yes, please explain the reason and the results:

Do any of your close relatives have diabetes? ___ yes ___ no
If yes, please check who of the relatives listed below had diabetes:
___ a. mother ___ c. sister ___ e. cousin ___ g. uncle ___ i. grandfa
___ b. father ___ d. brother ___ f. aunt ___ h. grandmother

Do you smoke? (Circle one)

Yes No

If yes, circle what you smoke:

Cigarettes Cigars

How many per day_____ Packs per day _____

Do you drink coffee or tea? (Circle one)

Yes No

Do you drink decaffeinated coffee (Circel one)

Yes No

If yes give number of cups per day _____

(Circle one) coffee tea

EXERCISE LEVEL:

Do you have a daily fitness program? _____yes _____no

If yes, describe:

If no, what types of exercise would you get in a typical week:

Appendix F. Informed Consent Form

VITAMIN B-6 BIOAVAILABILITY STUDY
Informed Consent

In this investigation it will be determined whether or not a diet high in glycosyleted vitemin B-6, a form of vitemin B-6 in plent foods, will effect vitemin B-6 stetus in humens. Thera will he 10 mele subjects. This study will he 49 days long, total length depending on time required for suhjects to edept to tha diet. Vitemin B-6 content will he kept conetent et 3.2 mg per dey except for test deys when it will ha 3.7 mg.

Prior to tha study, I will giva 20 ml of blood end a urine collection which will ha usad to determine my health end vitemin statns. I will also teke en orel dose of 5 g of D-xylosa et this time. During the study I will record my dietery inteke end agree to take no vitemin or other nutritional supplement other than tha pyridoxine supplement which I will receive during the study. I egree to consume no elcoholic bevereges during the study. I will inform tha principel invastigetors if I take any prescrihed or unprescrihed drugs.

I will consume only tha foods and bevarages which era provided or ellowed on the constent diet I will racaive daily in the Department of Foods and Nutrition et Oregon Stata University. I understend thet this diet contains 3.2 mg vitemin B-6, is adeqnate in ell other nutrients, end meets my minimum requiremant for energy so thet I will neither gein nor lose weight.

I will collect 24-honr nrina specimens deily throughout thie investigation. My nnderstanding of consecutive 24-hour nrine collectione is es follows: After rising in tha morning of tha first day of this investigetion, I will completely empty my bleddar. I will discard this nrina. After this, I will collect ell of my nrina in the conteiners provided. Each morning I will completely empty my bladder et tha same time es the dey hefore and lebel, this nrine es belonging to thet collected on the preceding dey (24-honr period). I will continne collecting my nrina in this mennor aach day throughont this study. I will hring my nrine to the leboretory aach morning. I will report eny eccidental loss of nrine to ona of the investigators.

I egree to heva 20 ml of blood drewn from my foreerm hy e registered medical technologist on deys 1, 5, 12, 20, 26, 32, 38, and 47 of the study.

I understend thet there ara no risks involved in this experiment except those essociated with hlood withdrawal. I understend thet thera are no risks associated with the ingestion of 5 g of D-xylose or 2 g of L-tryptophan. I understend thet ona of the principal invastigators will he available to me as the investigetion proceeds.

I heva hed this experiment expleined to my satisfection end ell of my questions heva heen enswered. I recognize thet, other than the frea food and finenciel renumeretion of $150.00, I will receive no direct medical henefit by perticipeting in this project. I undarstend thet my records will he kept confidentiel.

I egrea to perticipate in this reseerch with the understending thet I em frea to withdrew from this project et any time. I understend that if, basad on the resnlts of the urinery enalysas, my urina collections era either incomplete or indicete thet I am not edharing to tha diet, the invastigetors heve the right to remove me from this study or to reduca the remuneretion I receive for participating in this investigation. I will receive remunerstion only after setisfactory completion of the study.

_____
Subject

_____
Deta

_____
Witness