AN ABSTRACT OF THE THESIS OF

Christine M. Hansen for the degree of Doctor of Philosophy presented on September 21, 1995. Title: Effect of Vitamin B-6 Intake, Protein Intake and Bioavailability on Vitamin B-6 Status of Women.

Abstract approved: ______________________________

James E. Leklem

Four studies were conducted to evaluate the effect of varying levels of vitamin B-6 (B6), protein and pyridoxine glucoside (PNG) on B6 status and requirements of women. In the first two studies, women were fed a constant protein diet and vitamin B-6 intakes of 0.84 to 2.39 mg/d during 10- to 15-day experimental periods. Significant differences among intake levels were found in urinary 4-pyridoxic acid (4PA) and total vitamin B-6 (UB6), plasma pyridoxal 5′-phosphate (PLP) and total vitamin B-6 (TB6), and urinary xanthurenic acid (XA) following a tryptophan load. Significant correlations were found between B6 intake and 4PA, UB6, plasma PLP, TB6, erythrocyte alanine aminotransferase (EALT) percent stimulation, and postload urinary XA and volatile amines (VA, kynurenine plus acetylkynurenine). More than 1.33 mg B6/d (> 0.016 mg B6/g dietary protein) was required for adequate B6 status. In a third study, nine women were fed diets providing 1.25 mg B6/d and three levels of protein (0.5, 1.0 and 2.0 g/kg body weight), for 14 days each. Significant differences in urinary 4PA, plasma PLP, and postload urinary VA were found among

protein levels. Nitrogen intake was significantly negatively correlated with urinary 4PA and plasma PLP, and positively correlated with EALT percent stimulation and postload urinary kynurenic acid (KA), XA and VA. Compared to men in a previous study, women excreted a greater percentage of B6 intake as 4PA, had lower plasma PLP and greater amounts of postload urinary tryptophan metabolites. At least 0.020 mg B6/g protein was required for adequate status. In a fourth study, nine women were fed diets with a high (27%) or low (9%) percentage of the B6 intake as pyridoxine glucoside, a form known to have reduced bioavailability, for 18 days each. Urinary 4PA and UB6, plasma TB6 and red blood cell PLP were significantly lower, and fecal B6 was significantly higher during the high PNG diet. The decrease in B6 status indicators on the high PNG diet suggested a loss of 15 to 18% of the total B6 intake. Taking into account bioavailability and gender differences in the effect of dietary protein, and including a safety margin, the RDA for B6 for women should be at least 0.020 mg/g dietary protein.

Effect of Vitamin B-6 Intake, Protein Intake and Bioavailability on Vitamin B-6 Status of Women

by

Christine M. Hansen

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Doctor of Philosophy

Completed September 21, 1995
Commencement June 1996

Doctor of Philosophy thesis of Christine M. Hansen presented on September 21, 1995.

APPROVED:

____

Major Professor, representing Nutrition and Food Management

____

Head of Department of Nutrition and Food Management

____

Dean of Graduate School

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

____

Christine M. Hansen, Author

ACKNOWLEDGEMENTS

I would like to thank the following people:

Dr. James Leklem, my Major Professor, for the innumerable things he did to support me in completing this thesis and throughout my graduate studies.

The members of my Doctoral Committee, for their time, energy and careful consideration of my work.

Jim Ridlington and Karin Hardin, for their help in the lab and their companionship.

My fellow graduate students, especially Daisy Wan, Julie Gordon, Dan Smith, Kerry Martinson, Christine Jensen, Nancy Dunton, Ricky Virk, Jenny Young, Xu Wang, Wen-Shan Chou Chen, Wei Ding, Terri Hoos and all the others whose friendship and encouragement made graduate study more enjoyable.

My immediate and extended family, especially my husband Brian and my children, Matthew, Emily, Jacob, Nathaniel and Sarah, for their support, patience and understanding.

My church family at Calvary Baptist Church in Newport, especially Harry and Wilma Earles and Jackie Stam, for their prayers, support and encouragement.

The studies described in Chapters 3, 4 and 5 were funded by USDA grant # 88-37200-3520.

DEDICATION

This thesis is dedicated to the memory of my brother

James L. Suydam

(October 4, 1948 - October 9, 1969)

and to the glory of my Lord and Savior, Jesus Christ.

"I can do all things through Christ which strengthens me."

Phillipians 4:13

CONTRIBUTION OF AUTHORS

Dr. James Leklem and Dr. Lorraine Miller were the co-principal investigators for the grant that funded these studies. They were involved in the design, data collection and analysis for the studies and assisted in the writing of the manuscripts.

TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

TABLE OF CONTENTS (continued)

LIST OF FIGURES

LIST OF TABLES

LIST OF TABLES (Continued)

LIST OF APPENDIX FIGURES

LIST OF APPENDIX TABLES

LIST OF APPENDIX TABLES (Continued)

LIST OF APPENDIX TABLES (Continued)

LIST OF ABBREVIATIONS

4PA	4-pyridoxic acid
B6	Vitamin B-6
EALT	Erythrocyte alanine aminotransferase activity
EAST	Erythrocyte aspartate aminotransferase activity
FB6	Fecal total vitamin B-6
FNB	Food and Nutrition Board
KA	Kynurenic acid
NRC	National Research Council
PL	Pyridoxal
PLP	Pyridoxal 5′-phosphate
PM	Pyridoxamine
PN	Pyridoxine
PNG	Pyridoxine glucoside
RDA	Recommended Dietary Allowance
TB6	Total plasma vitamin B-6
UB6	Urinary total vitamin B-6
VA	Volatile amines (kynurenine plus acetylkynurenine)
XA	Xanthurenic acid

EFFECT OF VITAMIN B-6 INTAKE, PROTEIN INTAKE AND BIOAVAILABILITY ON VITAMIN B-6 STATUS OF WOMEN

CHAPTER 1

INTRODUCTION

Rationale

Since its discovery and the identification of its forms more than half a century ago, vitamin B-6 has been the subject of hundreds of scientific studies. Much has been learned about the metabolism and function of vitamin B-6, and the importance of vitamin B-6 in human nutrition is well established. The first Recommended Dietary Allowance (RDA) for vitamin B-6 was set in 1968 (NRC 1968), and in each subsequent publication of the RDAs, the Food and Nutrition Board (FNB) re-examined the existing research literature to evaluate its recommendations.

In 1989, the RDA for vitamin B-6 was reduced from 2.2 to 2.0 mg/d for men, and from 2.0 to 1.6 mg/d for women (NRC 1989). The recommendation was based on a dietary vitamin B-6 to protein ratio of 0.016 mg/g and was calculated assuming a protein intake of 100 g/d for women. The same ratio was used in setting the RDA for both men and women, although the studies that established the link between dietary protein and vitamin B-6 requirement were done in men (Baker et al. 1964, Miller and Linkswiler 1967, Miller et al. 1985). There is a need for more studies in women because some gender-related differences in vitamin B-6 metabolism have been reported (Leklem et al. 1975, Ribaya-Mercado et al. 1991, Shin and Linkswiler 1974, Shultz and Leklem 1981).

Some of the issues considered by the FNB in the establishment of an RDA for vitamin B-6 were published in a statement in the Journal of Nutrition in 1986, and included the following questions: What are the most reliable biochemical and physiological indicators of vitamin B-6 nutriture? Should the RDA for vitamin B-6 be linked to protein *intake* or *requirement*? Is pyridoxine requirement linearly related to protein intake? The Board also suggested types of data that should be considered when establishing RDAs, including: (1) data from experiments on people in controlled experimental environments and (2) information on bioavailability (FNB 1986). The controlled metabolic studies described here were designed to answer the some of the questions posed by the FNB and provide the types of data mentioned above, to be used in evaluating the RDAs for vitamin B-6 for women.

The hypotheses tested by the studies were: (1) the dietary ratio of vitamin B-6 to protein influences vitamin B-6 status indicators in women, (2) the effect of protein intake on B6 status in women is different than the effect in men, and (3) the consumption of foods high in pyridoxine glucoside reduces vitamin B-6 status. Our objectives were: (1) to determine the effect of varying vitamin B-6 to protein ratios on several indices of vitamin B-6 status in women under controlled metabolic conditions, (2) to compare the results in women to a similar study done in men, (3) to determine the effect of bioavailability of a diet high in pyridoxine glucoside on vitamin B-6 status compared to a diet low in pyridoxine glucoside, and (4) to provide a recommendation for a vitamin B-6 allowance in women based on studies of dietary B6 to protein ratio and bioavailability.

Because there are many excellent general reviews of vitamin B-6 available (Bender 1992, Leklem 1991, Dakshinamurti 1990), this chapter will provide only a brief discussion of vitamin B-6 chemistry, metabolism and function, food sources of vitamin B-6, its absorption and bioavailability, and status assessment and requirements. Chapter 2 is a review of the literature of studies in women that have evaluated vitamin B-6 status and/or requirements. Two studies that examined the effect of varying levels of vitamin B-6 intake and constant protein intake on the vitamin B-6 status of women are presented in Chapter 3. A third study in which a constant vitamin B-6 intake and three levels of protein were fed is described in Chapter 4. The results of a study in which women were fed diets that were high and low in a less bioavailable form of vitamin B-6, pyridoxine glucoside, are presented in Chapter 5. The final chapter is a summary of the research described in Chapters 3 through 6 and conclusions based on their results are presented.

Vitamin B-6 chemistry, metabolism and function

Vitamin B-6 is the commonly used term for all 3-hydroxy-2-methylpyridine derivatives. The three primary forms of vitamin B-6 are pyridoxine (PN), pyridoxal (PL) and pyridoxamine (PM), and each of these may also be phosphorylated at the 5′ position. The liver is primarily responsible for the metabolism of vitamin B-6 (Merrill and Henderson 1990). Pyridoxine kinase converts the nonphosphorylated forms to their phosphorylated forms in an ATP- and zinc-requiring reaction. Pyridoxamine 5′-phosphate (PMP) and pyridoxine 5′-phosphate are converted by a flavin mononucleotide (FNM)-dependent oxidase reaction to pyridoxal 5′-phosphate (PLP), the metabolically active form. The phosphorylated forms are dephosphorylated by alkaline phosphatase, and pyridoxal can be irreversibly converted to 4-pyridoxic acid (4PA), the major metabolic end-product, by a FAD-requiring aldehyde oxidase or a FAD-dependent dehydrogenase (Leklem 1991).

Pyridoxal phosphate reacts with ϵ-amino groups of lysine residues in proteins, forming a Schiff's base, and functions as a coenzyme for aminotransferases, decarboxylases, racemases and enzymes involved in side-chain elimination and replacement reactions (Bender 1992). The cellular processes in which PLP plays a role include amino acid metabolism, immune function, gluconeogenesis, niacin formation, red cell metabolism and function, lipid metabolism, neurotransmitter synthesis and hormone modulation (Bender 1992, Leklem 1991). Most of the body's pool of vitamin B-6 is found in muscle, with the majority of it bound to glycogen phosphorylase (Coburn et al. 1988).

Food sources, absorption and bioavailability

Vitamin B-6 is found in a variety of fruits, vegetables, legumes, nuts, meat and dairy products. The major form in animal products is pyridoxal phosphate, while pyridoxine and pyridoxamine and their phosphorylated forms are the major ones in plant foods (Leklem 1991). In the intestinal mucosa, phosphorylated forms are dephosphorylated by alkaline phosphatase, and PN, PL and PM are rapidly absorbed by passive diffusion. The predominate forms found in blood are PLP and PL, which circulate bound to albumin in plasma and hemoglobin in erythrocytes (Bender 1992).

An additional form of vitamin B-6 found in plants is pyridoxine glucoside (PNG). From 5 to 80% of the total vitamin B-6 content of various plant foods may be in the form of pyridoxine glucoside (Gregory and Ink 1987, Kabir et al. 1983b). This form has been found to have a reduced bioavailability, estimated at 58% of the bioavailability of PN (Gregory et al. 1991). In human bioassays, there was an inverse relationship between vitamin B-6 bioavailability and the pyridoxine glucoside content of food (Bills 1991, Kabir et al. 1983c).

Status assessment and requirements

Vitamin B-6 status can be evaluated by a variety of methods. Direct methods include measurement of PLP, PL or total vitamin B-6 concentrations in plasma or erythrocytes and urinary 4PA excretion. Indirect methods include measurement of erythrocyte alanine and aspartate aminotransferase (EALT, EAST) activities with and without added PLP, and excretion of tryptophan or methionine metabolites after an oral load. Values indicating adequate vitamin B-6 status have been suggested for some of the most common indicators used (Leklem 1990, Shultz and Leklem 1981).

Requirements for vitamin B-6 have been evaluated primarily through metabolic studies in which subjects are depleted of vitamin B-6 and then repleted with increasing intakes of the vitamin. The criterion most commonly used to judge requirement is the vitamin B-6 intake necessary to restore status indicator values to predepletion levels. The discussion of status and requirements continues in Chapter 2 with a review of the literature of research studies done in women.

CHAPTER 2

VITAMIN B-6 STATUS AND REQUIREMENTS OF WOMEN OF CHILD-BEARING AGE

Christine M. Hansen and James E. Leklem

In: *Vitamin B-6 Metabolism in Pregnancy, Lactation and Infancy* (1995) Raiten, D.J., ed., pp.141-158. Used by permission of CRC Press, Boca Raton, FL.

Introduction

Many studies assessing vitamin B-6 status or requirements have been done in men but fewer studies have been done in women. In the latter case, the majority of the studies focused on women using oral contraceptives, pregnant women, or women with premenstrual syndrome. Several studies have suggested that women have lower values for some vitamin B-6 status indicators than men with the same vitamin B-6 intake or dietary vitamin B-6 to protein ratio.[1-3]

Data from the second National Health and Nutrition Examination Survey (NHANES II) indicate that women have a lower average daily intake of vitamin B-6 than men (1.14 $\pm$ 0.01 compared to 1.85 $\pm$ 0.02 mg/d) and that 90% of females consume less than the 1980 Recommended Dietary Allowance (RDA) for vitamin B-6.[4] Forty-eight percent of white females and 58% of black females had intakes less than 50% of the RDA. Although the RDA for vitamin B-6 was lowered in 1989 from 2.2 to 2.0 mg/d for men and from 2.0 to 1.6 mg/d for women,[5] the mean intake for women found in NHANES II was still only 71% of the new RDA.

This review will discuss the results of studies that have investigated the vitamin B-6 status or requirements of healthy, normal adult women and the implications for the establishment of recommended dietary intake.

Status assessment

Studies that assess the vitamin B-6 status of women may be divided into those in which subjects consumed self-selected diets and those in which subjects were fed controlled diets. The focus of many of these studies was the potential relationship between vitamin B-6 and the impact of oral contraceptives, pregnancy or premenstrual syndrome. Most of these reports have included normal subjects as controls. Some of the controlled diet studies have been depletion/repletion studies which use restoration of status indicator values to predepletion levels to evaluate requirements.

A variety of status indicators have been used, both direct and indirect.[6] Direct indicators include measurements of the B-6 vitamers or the metabolite 4-pyridoxic acid (4PA) in various biological fluids and erythrocytes. The status indicators used most frequently are plasma pyridoxal 5′-phosphate (PLP) and urinary 4PA. Indirect, or functional, status measures include the activity, and stimulation by the coenzyme PLP, of erythrocyte alanine (EALT) or aspartate aminotransferase (EAST). Urinary excretion of tryptophan metabolites after an oral tryptophan load has also been used to evaluate status. The methionine load test is a less commonly employed indicator of PLP function. The relative strengths and weaknesses of the various measures of vitamin B-6 status have been discussed by Reynolds in a previous chapter of this book.

Using only dietary intake of vitamin B-6 (or the B-6 to protein ratio of the diet) to evaluate status is not advisable because of the difficulties in obtaining accurate reports, and because most nutrient data bases used in evaluating dietary intakes lack complete data for vitamin B-6 content of foods (see Trumbo in this text for a more complete discussion of this topic). Studies in which biochemical status indicators were measured in subjects consuming controlled diets provide a standard by which to evaluate the accuracy of estimating vitamin B-6 intake from the food records of individuals consuming self-selected diets.

To properly assess vitamin B-6 status, several status indicators should be used including both direct and indirect measures in addition to an evaluation of both vitamin B-6 and protein intake,[6] the latter because increased dietary protein has been observed to reduce plasma PLP concentration and urinary 4PA excretion.[3,7-9]

Studies utilizing direct status indices

Pyridoxal 5′-phosphate

The most commonly used direct measure of vitamin B-6 status is plasma PLP. In rats, plasma PLP concentration has been found to be significantly correlated with tissue concentrations of PLP,[10] and studies in humans show that plasma PLP is significantly correlated with vitamin B-6 intake.[2] **Table 2.1**[2,3,11-34] is a compilation of PLP values reported for healthy adult women including both those on self-selected diets and those fed controlled intakes of vitamin B-6. Data for subjects who were reported to be taking a vitamin supplement were not included. The range of mean PLP values found in these studies is from 22.9 to 92.8 nmol/L, but the number of studies in which intake was controlled is small.

In the studies in which intake was controlled (range of 0.8 to 2.4 mg B-6/d) there was a significant correlation between vitamin B-6 and plasma PLP concentration ($r=0.881$, $p<0.001$). This suggests that plasma PLP is a valid index of vitamin B-6 nutriture in healthy women over the range of normal vitamin B-6 intakes. Leklem has

Table 2.1. Mean plasma PLP concentrations in normal adult women.[a]

Reference	Subjects (n)	Age (yrs)	PLP (nmol/L)	Method
Self-selected diets				
Wachstein, 1960[11]	20	--	34.0±10.1	TDC[b]
Chabner and Livingston, 1970[12]	12	20-34	67.9±14.6	TDC
	7	35-49	46.1±13.8	TDC
Reinken and Gant, 1974[13]	29	--	36.8±8.9	TDC
Lumeng et al., 1974[10]	77	29±8	38.0±17.0	TDC
Shane and Contractor, 1975[14]	12	18-38	38.9±6.9	TDC
Miller et al., 1975[15]	11	20-29	25.9±15.4	TDC
Brophy and Sliter, 1975[16]	4	20-34	68.4	TDC
Cleary et al., 1975[17]	58	20-34	43.4	TDC
Shultz and Leklem, 1981[2]	41	25-79	37.7±14.7	TDC
Shultz and Leklem, 1982[18]	4	24-32	38.4±15.8	TDC
Guilland et al., 1984[19]	23	27±2	92.8±7.3	LC/FL[c]
Schuster et al., 1984[20]	12	18-33	32.5±8.4	TDC
Lee and Leklem, 1985[21]	5	24±3	35.5±14.8	TDC
	5	55±4	31.3±13.3	TDC
van den Berg et al., 1986[22]	19	35±7	38-44	TDC
Ritchie and Singkameni, 1986[23]	85	35±7	40.5±23.3	AAT[d]
Ubbink et al., 1987[24]	9	--	31.7±19.4	HPLC[e]

Continued

Table 2.1. (continued)

Shultz and Leklem, 1987[25]	20	50-83	30.8±11.2 (V)[f]	TDC
			39.3±16.3 (NV)[g]	TDC
Driskell et al., 1989[26]	15	21-46	63.1±22.3	TDC
Driskell et al., 1991[27]	21	21-27	78.4±18.8	HPLC
Kang-Yoon et al., 1992[28]	10	20-30	45±2	TDC
Controlled diets				
Brown et al., 1975[29]				
(0.83, 78 g/d)[h]				
(1.83, 78 g/d)	6	22±2	22.9±13.9	TDC
	3	22±2	60.7±20.2	TDC
Lee and Leklem, 1985[21]				
(2.3, 72 g/d)	5	24±3	61.7±25.6	TDC
(2.3, 72 g/d)	8	55±4	40.5±12.2	TDC
Kretsch et al., 1991[30]				
(1.8, 1.6 g/kg)	8	21-34	25.4±10.6	TDC
Hansen et al., 1991[31]				
(1.0, 90 g/d)	10	21-39	27.9±11.4	TDC
(1.3, 90 g/d)	10	21-39	32.4±11.5	TDC
(1.7, 90 g/d)	10	21-39	40.7±14.8	TDC
(2.4, 90 g/d)	10	21-39	58.4±25.4	TDC
Leklem et al., 1991[3]				
(1.3, 0.5 g/kg)	9	19-36	42±12	TDC
(1.3, 1.0 g/kg)	9	19-36	33±8	TDC
(1.3, 2.0 g/kg)	9	19-36	28±9	TDC

Continued

Table 2.1. (continued)

Hansen et al., 1992[32]				
(1.5 HG, 90 g/d)	9	21-39	29.3±9.8	TDC
(1.4 LG, 84 g/d)	9	21-39	33.1±13.3	TDC
Hansen et al., 1993[33]				
(0.8, 90 g/d)	6	25-33	26.5±12.4	TDC
(1.1, 90 g/d)	6	25-33	29.4±12.5	TDC
(2.3, 90 g/d)	6	25-33	58.9±22.3	TDC
Trumbo and Wang, 1993[34]				
(2.5, 107 g/d)	8	28±5	56.4±34.5	HPLC

[a] Mean±SD
[b] TDC=Tyrosine decarboxylase
[c] LC/FL=Liquid chromatography/fluorometric detection
[d] AAT=Alanine aminotransferase
[e] HPLC=High performance liquid chromatography
[f] V=Vegetarian
[g] NV=Nonvegetarian
[h] Vitamin B-6 intake (mg/day), protein intake (g/day or g/kg body weight)

proposed that a plasma PLP concentration greater than 30 nmol/L is indicative of adequate vitamin B-6 status in adults.[6] Using this value, the limited data in Table 2.1 suggest that an intake greater than 1.6 mg of vitamin B-6 is needed before an adequate plasma PLP concentration is achieved.

As discussed by Reynolds in a previous chapter and noted by Whyte et al., alkaline phosphatase activity can influence the plasma PLP concentration.[35] The extent to which this influences PLP levels in a healthy population is not fully understood. Males have higher alkaline phosphatase activity than females,[36] but this difference appears to be only in the 20-29 year age range.[37]

Other direct status indicators in blood include plasma pyridoxal (PL), plasma total vitamin B-6 and erythrocyte PLP. There are only a few studies in which these indices have been measured in adults, or specifically in women. Therefore, it is not possible to arrive at standards for women based on these measures.

The data for plasma PLP concentrations in adult women is somewhat limited, especially under conditions of controlled vitamin B-6 and protein intake. Further investigations in which intake of other nutrients involved in vitamin B-6 metabolism (i.e., riboflavin and magnesium) are considered are needed to help clarify vitamin B-6 metabolism and the importance of plasma PLP as an appropriate index of vitamin B-6 status.

4-Pyridoxic acid

The excretion of 4-pyridoxic acid and total vitamin B-6 (UB6) in urine have been used as status indicators. Urinary 4PA is the major metabolic excretion product of vitamin B-6 and is considered to be a good short-term indicator of vitamin B-6 status. Urinary 4PA has been shown to respond rapidly to a change in vitamin B-6 intake and the fluctuations in urinary 4PA levels parallel those seen in plasma PLP in response to changes in B-6 intake.[29]

Table 2.2[2,3,13,20,21,24,25,29-34,38-46] lists reported values for urinary 4-PA and total vitamin B-6 excretion in adult women. For women consuming self-selected diets, 4-PA excretion ranged from 3.9 to 8.3 μmol/day. In a study of vegetarians and non-vegetarians, 4-PA excretion was slightly lower in vegetarians. This lower excretion may be related to a higher intake of the glucoside form of pyridoxine in vegetarians and a resultant lower bioavailability.[47,48]

The 4-PA excretion associated with defined intakes of vitamin B-6 are also given in Table 2.2. The intakes of vitamin B-6 in these metabolic studies ranged from 0.34 to 2.50 mg/d (2.0 to 14.8 μmol/d) and were associated with a urinary 4-PA excretion of 0.63 to 12.5 μmol/d. There was a highly significant correlation between vitamin B-6 intake and urinary 4-PA excretion for the 21 levels of intake ($r=.859$, $p<0.001$). For these levels of intake, the percentage of the ingested vitamin B-6 excreted as 4-PA ranged from 24 to 85%, with a majority of the values clustering around 50-55%. A daily intake greater than 1.0 mg is needed to achieve the level of 4-PA excretion indicative of adequate status (>3.0 μmol/d).[6] This interpretation is tempered by the observation that one must consider the level of protein consumed when evaluating urinary 4-PA excretion. As an example, a vitamin B-6 intake of 1.3 mg/d and a 1.0 g/kg protein resulted in a mean 4-PA excretion of 3.38 μmol/d, while

Table 2.2. Mean urinary 4-pyridoxic acid and vitamin B-6 excretion in normal adult women.[a]

Reference	Subjects (n)	Age (yrs)	4PA (μmol/day)	UB6 (μmol/day)
Self-selected diets				
Contractor and Shane, 1970[38]	26	--	6.62±4.6	
Mikai-Devic and Tomanic, 1972[39]	15	18-47	4.5±0.9	
Adams and Rose, 1973[40]	28	--	3.9±0.7	
Reinken and Gant, 1974[13]	29	25	8.32±1.30	
Shultz nd Leklem, 1981[2]	41	50±14	5.57±3.09	0.76±0.24
Wirth and Lohman, 1982[41]	10	18-33	3.82±1.63	
Schuster et al., 1984[20]	12	13-33	8.56±4.3	
Ubbink et al., 1987[24]	9	--	5.48±2.93	
Shultz and Leklem, 1987[25]	20	50-83	5.20±2.55 (V)[b] 5.81±2.52 (NV)[c]	
Coburn et al., 1988[42]	5	19-58	8.2±2.0	
Controlled diets				
Donald et al., 1971[43]				
(.34, 57 g/d)[d]	8	21-30	0.63±0.09	0.09±0.02
(.9, 57 g/d)	8	21-30	1.17±0.09	0.20±0.01
(1.5, 57 g/d)	8	21-30	2.16±0.09	0.33±0.03
Aly et al., 1971[44]				
(1.3, 81 g/d)	5	21-31		0.24±0.03

Continued

Table 2.2 (continued)

Miller et al., 1974[45]				
(1.9, 127 g/d)	2	22,31	5.80±1.38	0.96±0.16
Brown et al., 1975[29]				
(.8, 78 g/d)	6	2±2	1.98±0.81	
(1.8, 78 g/d)	3	22±2	6.03±2.04	
Donald et al., 1978[46]				
(1.5, 65 g/d)	8	18-23	2.4±0.1	
(2.1, 65 g/d)	8	18-23	3.78±0.41	
Lee and Leklem, 1985[21]				
(2.3, 72 g/d)	5	24±3	6.89±0.55	1.12±0.29
(2.3, 72 g/d)	5	55±4	7.35±0.80	0.89±0.19
Kretsch et al., 1991[30]				
(1.8, 1.6 g/kg)	8	21-30	5.48±3.37	
Hansen et al., 1991[31]				
(1.0, 90 g/d)	10	21-39	3.22±0.66	
(1.3, 90 g/d)	10	21-39	4.12±0.86	
(1.7, 90 g/d)	10	21-39	5.87±0.77	
(2.4, 90 g/d)	10	21-39	9.55±0.86	
Leklem et al., 1991[3]				
(1.3, .5 g/kg)	9	19-36	3.95±0.72	
(1.3, 1.0 g/kg)	9	19-36	3.38±0.53	
(1.3, 2.0 g/kg)	9	19-36	2.71±0.51	
Hansen et al., 1992[32]				
(1.5 HG[e], 90 g/d)	9	21-39	3.64±0.8	0.59±0.08
(1.4 LG[f], 84 g/d)	9	21-39	4.05±0.58	0.67±0.12

Continued

Table 2.2 (continued)

Hansen et al., 1993[33]			
(0.8, 90 g/d)	6	25-33	2.90±0.43
(1.1, 90 g/d)	6	25-33	3.44±0.49
(2.3, 90 g/d)	6	25-33	7.91±0.43
Trumbo et al., 1993[34]			
(2.5, 107 g/d)	8	20±2	12.5±2.3

[a] Mean±SD
[b] V=Vegetarian
[c] NV=Nonvegetarian
[d] Vitamin B-6 intake (mg/d), protein intake (g/d or g/kg body weight)
[e] HG=High in pyridoxine glucoside
[f] LG=Low in pyridoxine glucoside

at the same vitamin B-6 intake and a 2.0 g/kg protein intake the 4-PA excretion fell below 3.0 μmol/d to a mean of 2.71 μmol/d.[3]

Under appropriate conditions and with knowledge of both vitamin B-6 and protein intake, 4-pyridoxic acid excretion in women is useful in assessing short term vitamin B-6 status. Urinary vitamin B-6 measurement can complement 4PA excretion and aid in evaluating vitamin B-6 intake data in women. As was pointed out for plasma PLP, additional nutrient interrelationship studies in which 4-PA excretion is measured would add to our overall understanding of vitamin B-6 nutrition. Such data will also help in better setting a vitamin B-6 requirement for adult women.

Studies utilizing indirect status indices

Aminotransferases

The activity and associated stimulation tests of the erythrocyte aminotransferases, EALT and EAST, are among the most frequently used indirect indices for vitamin B-6 status.[6] However, the different ways in which aminotransferase activity is expressed (per ml blood, per mg hemoglobin, per ml packed red cells, or per number of erythrocytes) make it difficult to compare one study with another. Woodring and Storvick suggested that the use of activity coefficient is a more useful comparative approach.[49] To properly assess status with aminotransferase activity both basal activity and stimulation with excess PLP in the assay should be determined. Also, it should not be assumed that both aminotransferases give the same information about status. The genetic polymorphism of EALT,[50] but not EAST, complicates the interpretation of the former.

Table 2.3[14,20,22,29,31,43,44,51-63] lists values for aminotransferase activity coefficient and percent stimulation reported for women consuming self-selected diets and those whose vitamin B-6 intake was controlled. On self-selected diets EAST activity coefficients ranged from 1.2 to 2.0 and percent stimulation from almost 6% to 78%, and EALT from 4% to 22%. In general, the eleven studies in which EAST was measured had mean values which were under the value of 1.8 (80%) suggested as the upper limit of normal.[6] For the eight studies in which EALT activity was determined, all means were below the 1.25 value suggested as a cut off.[6] As illustrated in Table 3, a relatively small number of studies can be found in which both EAST and EALT activities were determined. An additional observation relative to the limited number of studies is that there are insufficient data to define the relationship between B-6/protein ratios and aminotransferase activity.

The erythrocyte aminotransferases are considered long term indicators of status, based in part on the 120 day lifespan of the erythrocyte. The depletion/repletion study of Donald, et al.[43] illustrates the long-term nature of the aspartate aminotransferase as a status indicator: EAST activity dropped during the 43-day depletion phase but had recovered to only 65% of baseline following 11 days of repletion.

Several recent studies have also raised questions about the validity of both aminotransferases as status assessment tools. Kang-Yoon and Kirksey reported that in metabolic studies neither EAST nor EALT stimulation were reliable indices of status in women.[28] This was particularly true for EAST. Cinnamon and Beaton reported

Table 2.3. Aminotransferase activity coefficient (α) or percent stimulation (%) in normal adult women.[a]

Reference	Subjects (n)	EAST	EALT
Self-selected diets			
Doberenz et al., 1971[51]	11		22.4±3.6 (%)[b]
Rose et al., 1973[52]	50	78±15 (%)	17±14 (%)
Salkeld et al., 1973[53]	76	1.5±0.2 (α)[c]	
Shane and Contractor, 1975[14]	12	1.69±0.16 (α)	
Driskell et.al., 1976[54]	73		7.6±5.2 (%)
Salih et al., 1976[55]	30	63±55 (%)	
Miller et al., 1978[56]	4	52±20 (%)	10±3 (%)
Wien, 1978[57]	94		1.1±0.1 (α)
Chrisley and Driskell, 1979[58]	83		6±3 (%)
Vir and Love, 1979[59]	20		12.0±12.8 (%)
Schuster et al., 1984[20]	12	77±67 (%)	
Ramaswamy and Natarajan, 1984[60]	20	5.8±2.1 (%)	4.5±3.1 (%)
Tovar et al., 1985[61]	45(U)[d] 45(R)[e]	1.2±0.1 (α) 1.8±0.3 (α)	
van den Berg et al., 1986[22]	19	2.00-2.07 (α)	
Mira et al., 1988[62]	23	61±34 (%) (F)[f] 52±27 (%) (L)[g]	

Continued

Table 2.3. (continued)

Millet et al., 1989[63]	26	2.03±0.20 (α) (V)[h]	
	36	2.23±0.27 (α) (NV)[i]	
Controlled diets			
Aly et al., 1971[44]			
(1.3, 81 g)[j]	5	32.6 (%)	
Donald et al., 1971[43]			
(.34, 57 g)	8	184±11 (%)	127±9 (%)
(.9, 57 g)	8	100±11 (%)	95±26 (%)
(1.5, 57 g)	8	95±8 (%)	174±27 (%)
Brown et al., 1975[29]			
(.83, 78 g)	6	65 (%)	5 (%)
(1.83, 78 g)	3	55 (%)	0 (%)
Hansen et al., 1991[31]			
(1.03, 90 g)	10	43±6 (%)	27±2 (%)
(1.33, 90 g)	10	40±3 (%)	24±6 (%)
(1.73, 90 g)	10	35±5 (%)	21±4 (%)
(2.39, 90 g)	10	37±3 (%)	17±3 (%)

[a] Mean±SD
[b] % = [(stimulated activity - unstimulated activity)/unstimulated activity] x 100%
[c] α = stimulated activity/unstimulated activity
[d] U=Urban
[e] R=Rural
[f] F=Follicular phase
[g] L=Luteal phase
[h] V=Vegetarian
[i] NV=Non-vegetarian
[j] Vitamin B-6 intake (mg/d), protein intake (g/d)

similar findings in men.[64] However, Donald and coworkers[43] found that EAST, but not EALT was responsive to depletion of vitamin B-6. In a study by Jacobs et al, basal and stimulated activity of EALT, but not EAST, was found to correlate with age in women (slight decrease with age).[65]

Aminotransferase activity (and stimulation) data for both EAST and EALT should continue to be measured in adult women, so a better understanding of the relationship between these indices and others can be realized.

Tryptophan metabolism

Historically, tryptophan metabolism has been considered one of the best indicators of vitamin B-6 status.[6,66] In fact, one can argue that the excretion of tryptophan metabolites was once considered the "gold standard" for vitamin B-6 status. The determination of xanthurenic acid excretion in urine following a tryptophan load has been used to assess vitamin B-6 status in numerous populations.[6,66,67]

Tryptophan metabolism in healthy women has received significant attention. This has occurred largely in the context of the investigation of tryptophan metabolism by oral contraceptive users. Interest in the relationship of the tryptophan metabolite, serotonin, to neurologic conditions[68] has also resulted in a number of studies which yielded information about tryptophan metabolism by healthy control women.

Listed in **Table 2.4**[1,21,22,43-45,69-72] are representative studies in women where tryptophan loads have been given and urinary metabolites measured. Not listed are excretion values of tryptophan metabolites when dietary vitamin B-6 was dramatically

Table 2.4. Excretion of xanthurenic acid and other tryptophan metabolites in women following an L-tryptophan load.[a]

Reference	No. of Subj. (n)	Age (yr)	Tryp Load (g)	Vitamin B-6 Intake (mg/d)	Xanthurenic Acid (μmol/24hr)	Kynurenic Acid (μmol/24hr)	Kynurenine (μmol/24hr)
Cheslock and McCully, 1960[69]	7	college	5	SS[b]	38-206	--	--
Aly et al., 1971[44]	5	21-31	2	1.3	83±4	56±8	25±7
Donald et al., 1971[43]	8	college	2	2.06	32±4	55±8	16±3
Luhby et al., 1971[70]	10	21-42	2	NR[c] (SS)	9-18	--	--
Miller et al., 1974[45]	2	22,31	2	1.9	30	56	37
Shin and Linkswiler, 1974[1]	5	19-23	2	2.13	5±1	41±5	49±12
Leklem et al., 1975[71]	10 (6)	22	2	0.83	24±4	51±10	34±26
	(4)			1.83	19±5	49±11	18±9
Lee and Leklem, 1985[71]	5	24±3	2	2.3	32±3	78±10	--
van den Berg et al., 1986[22]	19	35±7	2	1.2±0.03	20	--	--
Hrboticky et al., 1989[72]	8	24±2	3	NR (SS)	--	--	64±13(F)[d] 82±14(L)[e]

[a] Mean±SD
[b] SS=Self-selected diet
[c] NR=Not reported
[d] F=Follicular phase
[e] L=Luteal phase

reduced. Reduction in vitamin B-6 intake to 0.16 to 0.40 mg/d has been associated with 40- to 100-fold increase in urinary levels of xanthurenic acid and a 5- to 20-fold increase in kynurenic acid and kynurenine.[1,69] While xanthurenic acid excretion responds most dramatically to a low level of vitamin B-6 intake, kynurenine excretion appears to be more responsive to the normal range of vitamin B-6 intakes.[3,71] Because of various factors which can influence tryptophan metabolism, and in particular the initial step (tryptophan pyrrolase and indole oxygenase) of tryptophan degradation, there is a question of the utility of the tryptophan load test as a status indicator[67] unless one is assured the population studied is in good health.

Tryptophan metabolism via the kynurenine pathway has been found to be affected by the phase of menstrual cycle, with a 28% greater excretion of kynurenine in the luteal phase as compared to the follicular phase.[72] This suggests that use of the tryptophan load in assessing vitamin B-6 status should take into account the phase of the menstrual cycle. While there may be concerns about the safety of tryptophan loads, past experience of this author[71] and others[66] demonstrate that the use of a 2-gram L-tryptophan load administered with a meal is safe and without significant complications.

Methionine metabolism

Another indirect test which has been utilized to assess vitamin B-6 status in women is the measurement of methionine metabolites following a methionine load (usually 3 g).[1,73] Two metabolic studies utilizing a vitamin B-6 depletion and repletion protocol have been conducted in women age 19 to 31. In both studies tryptophan loads were also used to assess status. The study by Shin and Linkswiler[1] had a 14-day deficiency period (0.16 mg B-6/d) and a 14 day repletion (2.16 mg B-6/d). The study by Leklem, et al.[73] utilized a longer deficiency period (28 days; 0.019 mg B-6/d) and repletion period (28 days; 0.83 and 1.83 mg B-6/d). In the latter study, cystathionine was the only methionine metabolite in the urine which increased significantly consequent to the vitamin B-6 deficiency. The base excretion increased three-fold while the post load increase was eight-fold. The 1.83 mg vitamin B-6 repletion levels, but not the 0.83 mg vitamin B-6 level, restored cystathionine excretion to normal levels. Thus, this level of repletion restored both tryptophan and methionine metabolism to normal. The methionine load test provides an alternative to the tryptophan load test and may not suffer the drawbacks associated with use and interpretation of the tryptophan load test.[67]

Requirement

The establishment of a vitamin B-6 requirement for women of child-bearing age is critical not only for the health of this group, but also for the evaluation of national surveys of nutrient status and intake of healthy women, and as a foundation for studies of vitamin B-6 needs relative to various disease states. The prior discussion of research on the indices of vitamin B-6 status for women provides a basis for an evaluation of the research that has been used to make recommendations of vitamin B-6 needs for adult women. While several publications have focused on vitamin B-6 requirements of humans,[74-77] few have focused specifically on women.[46]

Whether the focus for vitamin B-6 need is on women or men, an appreciation of the various factors which may effect vitamin B-6 requirement is important. **Table 2.5** categorizes several factors (grouped into four general categories: dietary; delivery to tissues; increased loss or metabolism; apoenzyme defects) which may influence the need for vitamin B-6. The research base for some of these factors is more complete than for others. Those factors which are most likely to affect requirement in adult women are dietary and tissue delivery.

Table 2.5. Factors affecting vitamin B-6 requirements (some factors are speculative and have not necessarily been shown to significantly affect requirement).

1. Dietary
   a. Forms of vitamin B-6 in the diet: pyridoxine, pyridoxal, pyridoxamine, glucoside
   b. Inactivation prior to consumption: cooking, reaction with other nutrients or food constituents
2. Delivery to tissues
   a. Impairment of intestinal absorption
   b. Impairment of transport into cells
   c. Impaired conversion of the forms of vitamin B-6 to pyridoxal 5'-phosphate
3. Increased loss and metabolic turnover
   a. Increased renal clearance - diuretics, renal damage
   b. Increased oxidation of pyridoxal to 4-pyridoxic acid
   c. Protein intake increased; elevated cellular levels of amino acid metabolizing enzymes (i.e., aminotransferases)
   d. Increased alkaline phosphatase activity
   e. Loss via sweat or other secretions
   f. Physical activity - increased 4PA excretion
4. Apoenzyme defects that alter binding of pyridoxal 5'-phosphate (genetic disorders)

The dietary factors affecting requirement include the relative proportion of each of the three major forms of vitamin B-6. Studies have shown that ingestion of pyridoxal as compared to pyridoxine or pyridoxamine results in a greater conversion to 4-pyridoxic acid.[78,79] As a result, a diet which has a higher proportion of pyridoxal (animal products vs. plant products) would result in a greater proportion of the total vitamin B-6 being converted to the dead-end product 4-PA. Balancing this possibility is the presence of pyridoxine-ß-glucoside (PNG) in plant foods as compared to animal foods.[80] As reviewed by Trumbo in an earlier chapter, PNG has a low bioavailability[47] and thus a high proportion of plant foods containing PNG in the diet would in effect increase the requirement for vitamin B-6. The impact of a lower vitamin B-6 bioavailability on requirement is more significant when one considers (as discussed in more detail below) that most studies of vitamin B-6 requirements were conducted with diets in which the vitamin B-6 was present in forms that were of high bioavailability.

A further consideration related to diet but more directly to metabolism and cellular transport is the level of protein in the diet. As protein intake increases, the circulating concentrations of PLP and total vitamin B-6 decrease and urinary 4-PA excretion decreases.[3,9] In addition, tryptophan metabolism at a given level of vitamin B-6 intake becomes more abnormal as protein is increased.[8,9] These protein-B6 interrelationships have been demonstrated in both men[9] and women.[3] Other nutrients which may affect vitamin B-6 metabolism include zinc (via alkaline phosphatase) and riboflavin (as a cofactor for pyridoxamine phosphate oxidase).[68] While some of these factors have been considered in establishing an RDA for vitamin B-6, historically, few of these have received serious or extensive study, especially in women.

When the RDA for vitamin B-6 was first established (1968),[81] the adult RDA was based primarily on deficiency-repletion studies (see **Table 2.6**[1,2,40,43,69,82]) and the level of protein in the diet. The RDA of 2.0 mg/d was applied to adults and did not distinguish between women and men. Similarly, the next RDA (1974)[83] kept the recommended amount at 2.0 mg/d, did not present a specific amount for women per

Table 2.6. Studies which have been utilized in setting vitamin B-6 requirements in women[a].

Reference	No. of Subj. (n)	Age (yr)	B6[b] (mg)	Diet Pro. (g)	Food Type	Adj. Period (days)	Defic. Period (days)	Repletion (B6,mg)	Status Tests	Suggest. Require.
Cheslock, 1960[69]	7	18-20	0.41	26	N[c]	None	52	None	5g L-Try; XA;Blood B6	>0.5 mg/d
Aly, 1971[44]	5	21-31	1.3	81	L/S	6	--	--	2g L-Try; XA, Urinary B6; Amino Acids	None suggested
Donald, 1971[43]	8	21-31	0.34	57	N	None	44	7(0.94) 3(1.54)	Urinary B6, 4PA; Erythrocyte B6, EGOT; Amino Acids	1.5 mg/d
Shin, 1974[1]	5	23	0.16	109	SS	7 (2.16 mg B6)	14	14(2.16)	2g L-Try, TM; Urinary B6; 3g L-Methionine	None suggested
Leklem, 1975,1975[71,82]	10	22±2	0.19	78	SS	4	28	28(0.83) 28(1.83)	2g L-Try, TM; Urinary 4PA; Plasma PLP, EGOT, EGPT; 3g L-methionine	>0.83 mg/d
Shultz, 1981[2]	41	49±14	1.6 ±0.5	62±12	SlfS	None	None	None	Urinary 4PA & B6; plasma PLP	None suggested

[a] Mean±SD

[b] Daily intake from diet

[c] Abbreviations: L/S, liquid/solid; N, natural; NR, not reported; SlfS, self-selected; SS, semi-synthetic; TM, tryptophan metabolites; XA, xanthurenic acid.

se, and as with the previous RDA, linked the recommended B6 intake with protein intake.

With the publication of the 1980 RDA[84] came the first discussion of a specific recommendation for women. For women, the RDA for vitamin B-6, 2.0 mg/d, was based upon results from deficiency-repletion studies[1,43,82] and depended on protein intake. A protein intake level of 100 g/d was chosen and was based on an average intake, as determined by food consumption surveys (no explanation was given for the establishment of 100 g/d protein intake as the reference value).

The committee which established the 1989 RDA relied extensively on studies utilizing intakes of 0.0125 to 0.016 mg of vitamin B-6 per gram of protein, interpreting the observed plasma PLP concentrations and urinary 4PA levels to be indicative of adequate status. One of the studies cited in the 1989 RDA was a controlled metabolic study in which women were fed defined diets containing 0.19 mg of vitamin B-6 and 78 g of high quality protein, relatively refined forms of carbohydrate and 85 g of fat.[29,71] The vitamin B-6 used during repletion was as pyridoxine hydrochloride (note, the actual level of the two intakes of PN were 0.83 and 1.83 mg/d). Thus, the vitamin B-6 was in a form that was of higher bioavailability than in diets commonly consumed by women. Therefore the data from this study would underestimate the requirement for vitamin B-6.

The other study referred to by the RDA committee was one in which the intake of both vitamin B-6 and protein were determined from 3-day diet records.[2] Because of the inherent errors in such diet intake data and the incomplete data base for food values for vitamin B-6, there exists the possibility that vitamin B-6 intake was underestimated, in which case the associations between the estimated levels of vitamin B-6 intake and the observed biochemical indices indicative of adequate status would be misleading. This study by Shultz and Leklem[2] utilized a range of vitamin B-6 to protein ratios (0.0125-0.015) which represent a lower range of intakes associated with normalizing tryptophan metabolism in women fed controlled intakes of vitamin B-6. It should be emphasized that these intakes in turn reflected amounts associated with lower limits of adequate vitamin B-6 status. Therefore, the amounts of vitamin B-6 needed to provide a safety margin and cover 95% of the population is probably greater than the ratio of 0.0125-0.015 mg B-6 per gram protein and thus greater than 1.6 mg of vitamin B-6 (assuming an upper level of protein intake of 100 g/d).

The metabolic studies which have been utilized in the evaluation of vitamin B-6 requirements for women are summarized in Table 2.6. A common characteristic of these studies is the use of diets (foods) which are not totally representative of those commonly consumed. In some of the studies semi-synthetic and/or liquid diets were used. Also, as mentioned above, the form of vitamin B-6 used during the repletion phase of these studies was PN, a form of vitamin B-6 considered highly available. An additional consideration is the variable amount of protein fed to the women. A range of 26 to 100 g of protein was fed with a resultant B6 to protein ratio during the repletion phase of 0.016 to 0.027. Thus, this range does not include values below 0.016 (the presently accepted ratio used in setting the current RDA), values which provide insight into the extent to which status indices are affected by low B-6/protein ratios.

The limited range of B6 to protein ratios, the use of semi-synthetic foods and liquid based diets and diets in which a majority of the vitamin B-6 is highly available limits the application of these studies in setting vitamin B-6 requirements for adult women. Given these limitations, the existing RDA may be too low. Support for this conclusion comes from the higher requirement suggested for older women[85] as based on normalization of plasma PLP concentration and xanthurenic acid excretion. An additional factor in adult women which may have implications for requirement is physical activity. An exercise study in women suggests that there was an increased loss of vitamin B-6 (4%) associated with 20 minutes of cycling at 80% VO_2 max.[86] However, studies in trained and sedentary men suggested retention of vitamin B-6 in trained vs. sedentary men.[87]

The studies utilized by the 1989 RDA committee for setting vitamin B-6 requirements in adults were in our opinion, inappropriately interpreted. As discussed above, more consideration should be given to the effect of bioavailability, the overall diet composition, nutrient interaction, and physical activity.

The 1989 RDA states, "The RDA for vitamin B-6 in this edition is somewhat lower than in the ninth edition, being based on a figure of 0.016 mg/g protein rather than 0.020 mg/g". The subcommittee based this figure (0.016 mg/g) on studies "cited above". It is our opinion that the two studies cited[2,29] do not, by themselves, provide compelling evidence for reduction of the RDA for vitamin B-6 for women.
No sound explanation or data for the 0.004 mg/g reduction was provided in the 1989 edition of the RDA. Simply stating that "the dietary vitamin B-6 ratio appears to ensure acceptable values for most indices in adults of both sexes", does not, in itself,

make this figure definitive. As illustrated in the present review, there are limited data that can be utilized in establishing a range of values for selected vitamin B-6 status indices for adult women, but there are limitations in these studies (as discussed above) which must be considered.

Conclusion

The quantitative value for the vitamin B-6 requirement of adult women of child-bearing age remains an open question. Based on both past and recent human metabolic studies an intake of at least 2.0 mg of vitamin B-6 per day more realistically reflects an amount associated with normal vitamin B-6 indices in 95% of healthy adult women. In particular, little justification may be found to support the recent lowering of the RDA for vitamin B-6 to 1.6 mg/d for women.

The assessment of vitamin B-6 status of women in a free-living setting still presents a challenge. Proper evaluation of dietary intake of both vitamin B-6 and protein is necessary. Attention to medication use (eg. steroid hormones, cortisone, isoniazid) and exercise habits are also important. Use of at least two indices of status (preferably a direct measure and an indirect measure reflective of function) is also recommended. The incorporation of additional criteria for status assessment, especially one or more functional tests, and utilizing stable isotopes and modeling experiments in women[88] (see chapter by Coburn) may help in the establishment of a more metabolically based requirement.

References

1. Shin, H.K., Linkswiler, H., Tryptophan and methionine metabolism of adult females as affected by vitamin B-6 deficiency, *J. Nutr.*, 104, 1348, 1974.

2. Shultz, T.D., Leklem, J.E., Urinary 4-pyridoxic acid, urinary vitamin B-6 and plasma pyridoxal phosphate as measures of vitamin B-6 status and dietary intake in adults, in *Methods in Vitamin B-6 Nutrition*, Leklem, J.E., Reynolds, R.D., Eds., Plenum Press, New York, 1981, 297.

3. Leklem, J., Hansen, C., Miller, L., Effect of three levels of protein on vitamin B-6 status of adult women, *FASEB J.*, 5, A557 (abst.), 1991.

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

5. National Research Council, Vitamin B-6, in *Recommended Dietary Allowances, 10th Ed.*, National Academy of Sciences, Washington, D.C., 1989, 142.

6. Leklem, J.E., Vitamin B-6: a status report, *J. Nutr.*, 120, 1503, 1990.

7. Miller, L.T., Linkswiler, H., Effect of protein on the development of abnormal tryptophan metabolism by men during vitamin B-6 depletion, *J. Nutr.*, 93, 53, 1967.

8. Canham, J.E., Baker, E.M., Harding, R.S., Sauberlich, H.E., Plough, I.C., Dietary protein - its relationship to vitamin B6 requirements and function, *Ann. N.Y. Acad. Sci.*, 166, 16, 1969.

9. Miller, L.T., Leklem, J.E., Shultz, T.D., The effect of dietary protein on the metabolism of vitamin B-6 in humans, *J. Nutr.*, 115, 1663, 1985.

10. Lumeng, L., Cleary, R.E., Li, T.-K., Effect of oral contraceptives on the plasma concentration of pyridoxal phosphate, *Am. J. Clin. Nutr.*, 27, 326, 1974.

11. Wachstein, M., Kellner, J.D., Ortiz, J.M., Pyridoxal phosphate in plasma and leukocytes of normal and pregnant subjects following B-6 load tests, *Proc. Soc. Exp. Biol. Med.*, 103, 350, 1960.

12. Chabner, B., Livingston, D., A simple enzymic assay for pyridoxal phosphate, *Anal. Biochem.*, 34, 413, 1970.

13. Reinken, L., Gant, H., Vitamin B-6 nutrition in women with hyperemesis gravidarum during the first trimester of pregnancy, *Clin. Chim. Acta*, 55, 101, 1974.

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

15. Miller, L.T., Johnson, A., Benson, E.M., Woodring, M.J., Effect of oral contraceptives and pyridoxine on the metabolism of vitamin B-6 and on plasma tryptophan and α-amino nitrogen, *Am. J. Clin. Nutr.*, 28, 846, 1975.

16. Brophy, M.H., Siiteri, P.K., Pyridoxal phosphate and hypertensive disorders of pregnancy, *Am. J. Obstet. Gynecol.*, 121, 1075, 1975.

17. Cleary, R.E., Lumeng, L., Li, T-K., Maternal and fetal plasma levels of pyridoxal phosphate at term: Adequacy of vitamin B-6 supplementation during pregnancy, *Am. J. Obstet. Gynecol.*, 121, 25,

18. Shultz, T.D., Leklem, J.E., Effect of high dose ascorbic acid on vitamin B-6 metabolism, *Am. J. Clin. Nutr.*, 35, 1400, 1982.

19. Guilland, J.C., Bereksi-Reguig, B., Lequeu, B., Evaluation of pyridoxine intake and pyridoxine status among aged institutionalised people, *Internat. J. Vit. Nutr.*, 54, 185, 1984.

20. Schuster, K., Bailey, L.B., Cerda, J.J., Gregory, J.F., Urinary 4-pyridoxic acid excretion in 24-hour versus random urine samples as a measurement of vitamin B-6 status in humans, *Am. J. Clin. Nutr.*, 39, 466, 1984.

21. Lee, C.M., Leklem, J.E., Differences in vitamin B-6 status indicator responses between young and middle-aged women fed constant diets with two levels of vitamin B-6, *Am. J. Clin. Nutr.*, 42, 226, 1985.

22. van den Berg, H., Louwerse, E.S., Bruinse, H.W., Thissen, J.T.N.M., Schrijver, J. Vitamin B-6 status of women suffering from the premenstrual syndrome, *Hum. Nutr. Clin. Nutr.*, 40C, 441-50, 1986.

23. Ritchie, C.D., Singkamani, R., Plasma pyridoxal 5'-phosphate in women with the premenstrual syndrome, *Hum. Nutr. Clin. Nutr.*, 40C, 75, 1986.

24. Ubbink, J.B., Serfontein, W.J., Becker, P.J., de Villiers, L.S., Effect of different levels of oral pyridoxine supplementation on plasma pyridoxal 5'-phosphate and pyridoxal levels and urinary vitamin B-6 excretion, *Am. J. Clin. Nutr.*, 46, 78, 1987.

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

26. Driskell, J.A., Chrisley, B.M., Reynolds L.K., Moak, S.W., Plasma pyridoxal 5'-phosphate concentrations in obese and nonobese black women residing near Petersburg, VA, *Am. J. Clin. Nutr.*, 50, 37, 1989.

27. Driskell, J.A., Chrisley, B. Mc., Plasma B-6 vitamer and plasma and urinary 4-pyridoxic acid concentrations in young women as determined using high performance liquid chromatography, *Biomed. Chromatog.*, 5, 198, 1991.

28. Kang-Yoon, S.A., Kirksey, A., Relation of short-term pyridoxine supplementation to plasma vitamin B-6 vitamers and amino acid concentrations in young women, *Am. J. Clin. Nutr.*, 55, 865, 1992.

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

30. Kretsch, M.J., Sauberlich, H.E., Newbrun, E., Electroencephalographic changes and periodontal status during short-term vitamin B-6 depletion of young nonpregnant women, *Am. J. Clin. Nutr.*, 53, 1266, 1991.

31. Hansen, C., Leklem, J., Hardin, K., Miller L., Vitamin B-6 status of women with constant protein intake and four levels of vitamin B-6, *FASEB J.*, 5, A556 (abstr.), 1991.

32. Hansen, C., Leklem, J., Hardin, K., Miller, L., Chen, W., Effect of feeding diets of high and low pyridoxine glucoside content on vitamin B-6 status of women, *FASEB J.*, 6, A1374 (abstr.), 1992.

33. Hansen, C., Leklem, J., Miller, L., Hardin, K., Ridlington, J., Response of red blood cell pyridoxal 5'-phosphate to short term changes in vitamin B-6 intake, *FASEB J.*, 7, A728 (abstr.), 1993.

34. Trumbo, P.R., Wang, J.W., Vitamin B-6 status indices are lower in pregnant than in nonpregnant women but urinary excretion of 4-pyridoxic acid does not differ, *J. Nutr.*, 123, 2137, 1993.

35. Whyte, M.P., Mahuren, J.D., Vrabel, L.A., Coburn, S.P., Markedly increased circulating pyridoxal-5'-phosphate levels in hypophosphatasia, *J. Clin. Invest.*, 76, 752, 1985.

36. Eastman, J.R., Bixler, D., Serum alkaline phosphatase: normal values by sex and age, *Clin. Chem.*, 23, 1769, 1977.

37. Reed A.H., Cannon, D.C., Winkelman, J.W., Bhasin, Y.P., Henry, R.J., Pileggi, V.J., Estimation of normal ranges from a controlled sample survey. I. sex-and-age related influence on the SMA 12/60 screening groups of tests, *Clin. Chem.*, 18, 57, 1972.

38. Contractor, S.F., Shane, B., Blood and urine levels of vitamin B-6 in the mother and fetus before and after loading of the mother with vitamin B-6, *Am. J. Obstet. Gynecol.*, 107, 635, 1970.

39. Mikai-Devic, D., Tomanic, C., Determination of 4-pyridoxic acid in urine by a fluorimetric method, *Clin. Chim. Acta*, 38, 235, 1972.

40. Adams, P.W., Rose, D.P., Effects of pyridoxine hydrochloride (vitamin B-6) upon depression associated with oral contraception, *Lancet*, i, 897, 1973.

41. Wirth, J.C., Lohman, T.G., The relationship of static muscle function to use of oral contraceptives, *Med. Sci. Sports Exer.*, 14, 16, 1982.

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

43. Donald, A.E., McBean, L.D., Simpson, M.H.W., Sun, M.F., Aly, H.E., Vitamin B-6 requirement of young adult women, *Am. J. Clin. Nutr.*, 24, 1028, 1971.

44. Aly, H.E., Donald, E.A., Simpson M.H.W., Oral contraceptives and vitamin B-6 metabolism, *Am. J. Clin. Nutr.*, 24, 297, 1971.

45. Miller, L.T., Benson, E.M., Edwards, M.A., Young, J. Vitamin B-6 metabolism in women using oral contraceptives, *Am. J. Clin. Nutr.*, 27, 797, 1974.

46. Donald, E.A., Vitamin B-6 requirement of young women, in *Human Vitamin B-6 Requirements*, National Academy of Sciences, Washington, D.C., 1978, 226.

47. Leklem, J.E., Vitamin B-6 bioavailability and its application to human nutrition, *Food Technol.*, 42, 194, 1988.

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

49. Woodring, M.J., Storvick, C.A., Effect of pyridoxine supplementation on glutamic-pyruvic transaminase and in vitro stimulation in erythrocytes of normal women, *Am. J. Clin. Nutr.*, 23, 1385, 1970.

50. Ubbink, J.B., Bissbort, S., van den Berg, I., de Villiers, L.S., Becker, P.J., Genetic polymorphism of glutamate-pyruvate transaminase (alanine aminotransferase): influence on erythrocyte activity as a marker of vitamin B-6 nutritional status, *Am. J. Clin. Nutr.*, 50, 1420, 1989.

51. Doberenz, A.R., Miller J.P.V., Green, J.R., Beaton, J.R., Vitamin B-6 depletion in women using oral contraceptives as determined by erythrocyte glutamic-pyruvic transaminase activities, *Proc. Soc. Exp. Biol. Med.*, 137, 1100, 1971.

52. Rose, D.P., Strong, R., Folkard, J., Adams, P.W., Erythrocyte aminotransferase activities in women using oral contraceptives and the effect of vitamin B6 supplementation, *Am. J. Clin. Nutr.*, 26, 48, 1973.

53. Salkeld, R.M., Knörr, K., Körner, W.F., The effect of oral contraceptives on vitamin B_6 status, *Clin. Chim. Acta*, 49, 195, 1973.

54. Driskell, J.A., Geders, J.M., Urban, M.C., Vitamin B-6 status of young men, women, and women using oral contraceptives, *J. Lab. Clin. Med.*, 87, 813, 1976.

55. Salih, E.Y., Zein, A.A., Bayoumi, R.A., The effect of oral contraceptives on the apparent vitamin B_6 status in some Sudanese women, *Br. J. Nutr.*, 56, 363, 1986.

56. Miller, L.T., Dow, M.J., Kokkeler, S.C., Methionine metabolism and vitamin B-6 status in women using oral contraceptives, *Am. J. Clin. Nutr.*, 31, 619, 1978.

57. Wien, E.M., Vitamin B_6 status of Nigerian women using various methods of contraception, *Am. J. Clin. Nutr.*, 31, 1392, 1978.

58. Chrisley, B.M., Driskell, J.A., Vitamin B-6 status of adults in Virginia, *Nutr. Rept. Int.*, 19, 553, 1979.

59. Vir, S.C., Love A.H.G, Effect of oral contraceptives on vitamin B-6 nutriture of young women, *Internat. J. Vit. Nutr. Res.*, 50, 29, 1980.

60. Ramaswamy, P.G., Natarajan, R., Vitamin B-6 status in patients with cancer of the uterine cervix, *Nutr. Cancer.*, 6, 176, 1984.

61. Tovar, A., Bourges, H., Canto, T., Torres, N., López-Castro, B.R., Effect of oral contraceptive use on the erythrocytic glutathione reductase and aspartate aminotransferase activities in women without clinical signs of vitamin deficiency, *Nutr. Rept. Int.*, 32, 199, 1985.

62. Mira, M., Stewart, P.M., Abraham, S.F., Vitamin and trace element status in premenstrual syndrome, *Am. J. Clin. Nutr.*, 47, 636, 1988.

63. Millet, P., Guilland, J.C., Fuchs, F., Klepping, J., Nutrient intake and vitamin status of healthy French vegetarians and nonvegetarians, *Am. J. Clin. Nutr.*, 50, 718, 1989.

64. Cinnamon, A.D., Beaton, J.R., Biochemical assessment of vitamin B6 status in man, *Am. J. Clin. Nutr.*, 23, 696, 1970.

65. Jacobs, A., Cavill, I.A., Hughes, J.N.P., Erythrocyte transaminase activity. effect of age, sex, and vitamin B-6 supplementation, *Am. J. Clin. Nutr.*, 21, 502, 1968.

66. Brown, R.R., The tryptophan load test as an index of vitamin B-6 nutrition, in *Methods in Vitamin B-6 Nutrition*, Leklem, J.E., Reynolds, R.D., Eds., Plenum Press, New York, 1981, 321.

67. Brown, R.R., Possible role for vitamin B-6 in cancer prevention and treatment, in *Clinical and Physiological Applications of Vitamin B-6*, Leklem, J.E., Reynolds, R.D., Eds., AR Liss, New York, 1988, 279.

68. Leklem, J.E., Vitamin B-6, in *Handbook of Vitamins, 2nd ed.*, Machlin, L., Ed., Marcel Dekker, New York, 1991, 341.

69. Cheslock, K.E., McCully, M.T., Response of human beings to a low-vitamin B-6 diet, *J. Nutr.*, 70, 507, 1960.

70. Luhby, A.L., Brin, M., Gordon, M., Davis, P., Murphy, M., Spiegel, H., Vitamin B6 metabolism in users of oral contraceptive agents. I. Abnormal urinary xanthurenic acid excretion and its correction by pyridoxine, *Am. J. Clin. Nutr.*, 24, 684, 1971.

71. Leklem, J.E., Brown, R.R., Rose, D.P., Linkswiler, H., Arend, R.A., Metabolism of tryptophan and niacin in oral contraceptive users receiving controlled intakes of vitamin B-6, *Am. J. Clin. Nutr.*, 28, 146, 1975.

72. Hrboticky, N., Leiter, L.A., Anderson, G.H., Menstrual cycle affects on the metabolism of tryptophan loads, *Am. J. Clin. Nutr.*, 50, 46, 1989.

73. Leklem, J.E., Linkswiler, H.M., Brown, R.R., Rose, D.P., Anand, C.R., Metabolism of methionine in oral contraceptive users and control women receiving controlled intakes of vitamin B-6, *Am. J. Clin. Nutr.*, 30, 1122, 1977.

74. Leitch, I., Hepburn, A., Pyridoxine: metabolism and requirement, *Nutr. Abst. Rev.*, 31, 389, 1961.

75. Committee on Dietary Allowances, Food and Nutrition Board, National Research Council, Ed. *Human Vitamin B-6 Requirements*, National Academy of Sciences, Washington, D.C., 1978.

76. Leklem, J.E., Reynolds, R.D., *Methods in Vitamin B-6 Nutrition*, Plenum Press, New York, 1981.

77. Reynolds, R.D., Leklem, J.E., *Vitamin B-6: Its Role in Health and Disease*, AR Liss, New York, 1985.

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

79. Leklem, J.E., Vitamin B-6 metabolism and function in humans, in *Clinical and Physiological Applications of Vitamin B-6*, Leklem, J.E., Reynolds, R.D., Eds., AR Liss, New York, 1988, 3.

80. Kabir, H., Leklem, J.E., Miller, L.T., Measurement of glycosylated vitamin B-6 in foods, *J. Food Sci.*, 48, 1422, 1983.

81. National Research Council, Vitamin B-6, in *Recommended Dietary Allowances, 7th Ed.*, National Academy of Sciences, Washington, D.C., 1968, 43.

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

83. National Research Council, Vitamin B-6, in *Recommended Dietary Allowances, 8th Ed.*, National Academy of Sciences, Washington, D.C., 1974, 74.

84. National Research Council, Vitamin B-6, in *Recommended Dietary Allowances, 9th Ed.*, National Academy of Sciences, Washington, D.C., 1980, 96.

85. Ribaya-Mercado J.D., Russell R.M., Sahyoun N., Morrow, F.D., Gershoff, S.N., Vitamin B-6 requirements of elderly men and women, *J. Nutr.*, 121, 1062, 1991.

86. Manore, M.M., Leklem, J.E., Walter, M.C., 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, 1987.

87. Dreon, D.M., Butterfield, G.E., Vitamin B_6 utilization in active and inactive young men, *Am. J. Clin. Nutr.*, 43, 816, 1986.

88. Coburn, S.P., Location and turnover of vitamin B_6 pools and vitamin B_6 requirements of humans, *Ann. N.Y. Acad. Sci.*, 585, 76, 1990.

CHAPTER 3

CHANGES IN VITAMIN B-6 STATUS INDICATORS OF WOMEN FED A CONSTANT PROTEIN DIET WITH VARYING LEVELS OF VITAMIN B-6

Christine M. Hansen, James E. Leklem and Lorraine T. Miller

To be submitted to the *American Journal of Clinical Nutrition*,
American Society for Clinical Nutrition,
Bethesda, MD

Abstract

To evaluate changes in vitamin B-6 (B6) status indicators in subjects not depleted of B6, ten young women consumed diets providing 85 g protein/d and 1.03, 1.33. 1.73 and 2.39 mg B6/d for 12 or 15 d; in a second study, six women were fed diets providing 85 g protein/d and 0.84, 1.14 and 2.34 mg B6/d for 10 or 12 d. Vitamin B-6 status indicators showing significant differences among intake levels included urinary excretion of 4-pyridoxic acid (U4PA) and total B6 (UB6), plasma pyridoxal 5′-phosphate (PLP) and total B6 (TB6), and urinary xanthurenic acid (XA) excretion following a 2 g L-tryptophan load. Significant correlations were found between B6 intake and U4PA, UB6, plasma PLP, TB6, erythrocyte alanine aminotransferase (EALT) percent stimulation and post-load excretion of XA and volatile amines (kynurenine plus acetylkynurenine). Depending on the indicator, between 20 and 70% of the subjects had inadequate values for U4PA, UB6, plasma PLP and EALT percent stimulation at a B6 intake of 1.33 mg/d (0.016 mg B6/g protein). A dietary B6 to protein ratio > 0.016 mg/g is required for adequate B6 status in women.

Introduction

The present Recommended Daily Allowance (RDA) of vitamin B-6 for women, 1.6 mg/day, is based on a dietary ratio of 0.016 mg vitamin B-6 (B6) per gram of protein (1). The research upon which this recommendation is based (1,2) was done primarily in men (3-5) or in women who were taking oral contraceptives (6-9), studies which also included normal control women. Other than these types of investigations, the few studies that have been done in healthy women, not pregnant or using oral contraceptives, were depletion/repletion studies which used a return to predepletion values to determine when B6 status had normalized (10-12). Also, the dietary B6 to protein ratios used in these studies, varied over a wide range.

According to data from the Second National Health and Nutrition Examination Survey (NHANES II), 40% of black females and 33% of white females consumed less than 0.015 mg vitamin B-6 per g protein, suggesting women may be at risk for inadequate vitamin B-6 status (13). Thus, there is a need to investigate the vitamin B-6 status of women who have not been depleted of B6 and are consuming a ratio of B6 to protein less than that used to set the RDA for vitamin B-6.

To assess accurately the requirement for vitamin B-6 in normal adult women, the values of several vitamin B-6 status indicators at varying B6 intake levels and B6 to protein ratios should be determined (14). The objective of the two studies described here was to evaluate the extent of changes in vitamin B-6 status indicators in women fed a controlled diet of constant protein and varied levels of vitamin B-6, over a normal range of intakes, using several direct and indirect measures of vitamin B-6 status. An additional objective was to examine the interrelationships among the indices of vitamin B-6 status under controlled metabolic conditions.

Subjects and methods

Subjects

The subjects, ten in Study 1 and six in Study 2, were healthy women, between the ages of 21 and 39 years, who were nonsmokers and not using oral contraceptives. They were recruited from the local community. Subjects were screened for history of intestinal, renal or metabolic disorders that could affect absorption, metabolism or excretion of vitamin B-6 (15) and for previous vitamin supplementation. None of the subjects was taking vitamin B-6 supplements on a regular basis for three months prior to their participation in the studies. Three subjects who reported occasional multivitamin use had not taken any supplements for at least three weeks prior to the beginning of the studies. Normal blood chemistry, including hematocrit, hemoglobin, tests of liver function, glucose, protein and lipids, and a normal xylose absorption test (16) were required for selection. Subjects were instructed to maintain their usual activity level throughout the study. The study protocol was approved by Oregon State University's Committee for the Protection of Human Subjects, and informed consent was obtained from each subject.

Experimental design

In Study 1, the first experimental period was 15 days long to allow for adaptation to the diet and was followed by three 12-day experimental periods. Because some of the blood samples from this study were lost due to a freezer failure before the plasma total B6 and red blood cell pyridoxal 5′-phosphate analyses were completed, a second study was done. In Study 2, there were three experimental periods, 12, 10 and 10 days long, respectively. We used periods of this length because during previous research in our laboratory, several indices of B6 status had plateaued by 7-8 days at B6 intakes of 1.6 to 2.3 mg/d (17-20).

The basal diet used in both studies (**Table 3.1**) was prepared and served in our metabolic kitchen and provided approximately 2000 kcal. Additional energy was supplied by hard candy, soda pop, sugar, margarine and salad dressing to maintain the subjects' body weight. In Study 1, the basal diet fed throughout the four experimental periods provided 1.03 mg (6.09 μmoles) vitamin B-6 and 85 g of crude protein (g N x 6.25) per day. Vitamin B-6 intake was increased in Periods 2, 3 and 4 by oral administration of an equally divided dose of pyridoxine hydrochloride solution at breakfast and dinner to provide a total of 1.33, 1.73 and 2.39 mg (7.87, 10.24, and 14.14 μmol) vitamin B-6 per day, respectively. Vitamin B-6 to protein ratios for the four successive periods were 0.012, 0.016, 0.020 and 0.028 mg/g.

The basal diet in Study 2 was the same as in Study 1, except that Life cereal (Quaker Oats, Chicago, IL) was substituted for shredded wheat at breakfast. Based on microbiological analysis in our laboratory, 30 g of shredded wheat provides 0.07 mg and Life cereal 0.08 mg of vitamin B-6. The basal diet in Study 2 provided 85 g crude protein and 0.84 mg (4.96 μmol) vitamin B-6 per day. The difference in vitamin B-6 content of the diet between Study 1 and Study 2 was due to a difference in the analyzed B6 content of the animal products (determined after the feeding part of each study). In the second and third periods an oral pyridoxine hydrochloride solution increased the intake to 1.14 and 2.34 mg (6.74 and 13.83 μmol) vitamin B-6

Table 3.1 Composition of basal diet for both studies[1]

Food item	Amount
Breakfast	
Orange juice, frozen, reconstituted	160 g
Shredded wheat cereal	30 g
Blueberries, frozen	40 g
Milk, 2% milkfat	180 g
Biscuit	1/3 recipe[2]
Lunch	
Gelatin drink	1 cup[3]
Cheddar cheese	45 g
Lettuce, iceberg	50 g
Carrot, raw	20 g
Red cabbage, raw	10 g
Celery, raw	30 g
French dressing	20 g
Pears, canned, light syrup	100 g
Apple, raw	70 g
Biscuit	1/3 recipe[2]
Dinner	
Turkey, precooked frozen	50 g
Rice, white (uncooked wt.)	45 g[4]
Green beans, canned	100 g
Cottage cheese	40 g
Peaches, canned, light syrup	100 g
Milk, 2% milkfat	180 g
Biscuit	1/3 recipe[2]
Snack	
Graham crackers	40 g

[1] The diet for Study 2 substituted Life cereal (Quaker Oats, Chicago, IL) for shredded wheat.

[2] Biscuit recipe: 75 g flour, 20 g corn oil, 10 g sugar, 15 g vitamin-free casein, 5 g baking powder, 2 g salt, 80 mL water; divided into three portions and baked at 350° F for 10 min.

[3] 14 g gelatin plus 240 mL prepared drink mix.

[4] Rice was combined with 120 mL water and baked in a covered glass casserole at 350° F for 25 min.

per day, respectively. This resulted in vitamin B-6 to protein ratios of 0.010, 0.013, and 0.028 for the three experimental periods.

In Study 1, an oral 2 g L-tryptophan load (four tablets of 500 mg each) was administered with breakfast on Day 15 of Period 1, Day 12 of Periods 2 and 3, and Day 11 of the final period. We determined that the tablets contained an average of 500 ± 10 mg of L-tryptophan. Two tablets from each of four different bottles of Bi-Mart brand (Eugene, OR) L-tryptophan tablets were dissolved in 0.1 N HCl and the UV spectra compared with one obtained from pure L-tryptophan (Sigma Chemical, St. Louis, MO). Because of the reported cases of eosinophilia in people taking tryptophan (21) and the subsequent recall of tryptophan from retail stores, the tryptophan load test was not done in the second study.

Sample collection and analyses

Composites of the daily diet were made weekly during both studies and analyzed for vitamin B-6 by the AOAC procedure (22), omitting the chromatographic step, using *Saccharomyces uvarum* and for total nitrogen by a boric acid modification of the Kjeldahl method (23). The diet composite in Study 2 was also analyzed for pyridoxine glucoside (PNG) content by the method of Kabir et al (24). Pyridoxine glucoside was measured to provide data that can be used to compare PNG effect on status data obtained in prior and future studies.

For both studies, twenty-four hour urine collections from each day were assessed for completeness and evidence of adherence to the diet by measurement of creatinine (25) and urea nitrogen excretion (26) by automated procedures (Technicon

Autoanalyzer, Technicon Corp., Tarrytown, NY). Urinary 4-pyridoxic acid (4PA) excretion was determined by an HPLC method (27); recovery of added crystalline 4PA averaged 91±6%. Total urinary B6 (UB6) was assayed by a microbiological procedure (28) using *Saccharomyces uvarum*. If the 24 h collections were judged complete based on creatinine excretion, urinary 4PA and UB6 excretion was averaged over the last three days of each period before doing the statistical analyses. Urines from the day before and the day of the tryptophan load were analyzed for xanthurenic acid (XA) and kynurenic acid (KA) by the method of Price et al (29), and for volatile amines (VA, kynurenine plus acetylkynurenine) (30). The volatile amine assay was performed by Raymond R. Brown, Ph.D., University of Wisconsin, Madison, WI. Measurement of KA and VA provide a more complete picture of tryptophan metabolism than measurement of XA alone.

In Study 1, fasting blood was drawn on Days 1, 4 and 8 of each period, on Day 12 of Period 1 and on the morning after Day 12 of Period 4. In Study 2, fasting blood was drawn on Days 1 and 5 of each period, Day 9 of the first period and on the morning following the final day of the third period. After whole blood was removed for determination of hematocrit and hemoglobin concentration, samples were centrifuged at 4°. Plasma and red blood cells were frozen at -30° until analysis.

Plasma and red blood cell pyridoxal 5′-phosphate (PLP) concentrations were determined by a tyrosine decarboxylase apoenzyme/isotopic procedure (31). Samples were assayed in duplicate and any duplicates that varied more than 5% from their mean were repeated. Interassay coefficients of variation were 5.7% and 2.3% for the plasma PLP assays in Study 1 and 2, respectively, and 8.0% for the RBC PLP assay in Study 2. Recovery of PLP added to plasma ranged from 82-109%, and that added to red blood cells was 54-74%. Values were not corrected for recovery.

Plasma alkaline phosphatase activity was determined by a colorimetric procedure (32). Erythrocyte aspartate and alanine aminotransferase (EAST and EALT) activity with and without PLP added in vitro were determined by the method of Woodring and Storvick (33), using a 0.033 M Tris buffer (pH 7.4) instead of 0.1 M potassium phosphate. Percent stimulation was calculated [% stimulation = (activity w/added PLP - activity w/o added PLP) ÷ activity w/o added PLP]. Plasma total B6 (TB6) was determined by a microbiological procedure (28) using *Saccharomyces uvarum*. For all blood B6 status indicators, the value for the final sample in each period was used in the statistical analysis.

Near the end of each experimental period in Study 1, subjects were given a fecal marker (50 mg FD&C Blue #1 mixed with 200 mg methylcellulose in a gelatin capsule) with breakfast on the first and last day of a 5-day period. Complete 5-day fecal collections were analyzed for total vitamin B-6 (FB6) by a microbiological procedure (28).

Means and individual values for several of the status indicators measured were compared to values suggested for adequate B6 status (14,34). Statistical analyses were done with the STATGRAPHICS computer program (Statistical Graphics Corporation, Rockville, MD). Analysis of variance (ANOVA) was used to compare the means of status indicators at the end of the experimental periods, and Newman-Keuls multiple range analysis was used to determine significant differences. Differences were considered statistically significant if $P < 0.05$. Simple regression analysis of vitamin

B-6 intake and the various status indicators using linear, multiplicative, exponential and reciprocal models was performed, and Pearson correlation coefficients were determined.

Results

Characteristics of the subjects, including weight at the beginning and end of each study, are given in **Table 3.2**. Mean urinary excretion of creatinine and urea nitrogen, hematocrit and hemoglobin concentration did not differ among experimental periods in either study and are not reported here.

The pyridoxine glucoside content of the diet in Study 2 was 0.145 mg or about 6-17% of the total B6 intake. The pyridoxine glucoside content of the diet in Study 1 was not measured, but the only difference from the diet in Study 2 was the cereal eaten at breakfast (Table 3.1). Based on the pyridoxine glucoside content of shredded wheat (35) and assuming negligible pyridoxine glucoside in Life cereal (Quaker Oats, Chicago, IL), the estimated pyridoxine glucoside content of the diet in Study 1 was 0.140 mg or 6-14% of the total B6 intake.

Direct measures of vitamin B-6 status

The mean urinary 4PA excretion over the last three days of each period in Study 1 and Study 2 is shown in **Table 3.3**. In both studies there were significant increases ($P<0.05$) in the means at the end of each successive experimental period. The suggested value for urinary 4PA excretion indicating adequate status is

Table 3.2 Characteristics of subjects

Subject	Age (y)	Height (cm)	Weight at beginning (kg)	Weight at end of study (kg)
Study 1				
1	22	155	45.5	45.9
2	36	162	61.4	61.4
3	28	169	83.6	81.4
4	23	164	54.8	54.8
5	27	145	69.5	68.6
6	35	162	58.2	59.1
7	21	180	78.2	77.3
8	39	151	93.6	91.8
9	23	167	65.2	63.9
10	21	175	125.5	123.6
$\bar{x}\pm$SD	27.5±6.8	163.3±10.5	73.6±23.2	72.8±22.4
Study 2				
1	28	162	68.2	67.7
2	28	185	64.5	63.2
3	27	162	94.1	95.0
4	33	183	68.6	68.4
5	28	170	62.7	61.8
6	25	173	57.7	57.7
$\bar{x}\pm$SD	28.2±2.6	172.5±9.9	69.3±12.8	69.0±13.4

>3.0 μmol/day (14,34) and the means exceeded this value at the end of all four periods of Study 1 and the final two periods of Study 2. However, in Study 1 three of the ten subjects had values less than 3.0 μmol/day with a B6 intake of 1.03 mg/d, and one subject was still excreting less than adequate amounts with an intake of 1.33 mg B6/d (**Table 3.4**). In Study 2, one subject had a 4PA excretion of less than 3.0

Table 3.3 Mean values of vitamin B-6 status indicators at the end of each experimental period of Study 1 and Study 2[1]

	Study 1 Period 1	**(*n*=10)** Period 2	Period 3	Period 4	**Study 2** Period 1	**(*n*=6)** Period 2	Period 3
B6 intake, mg/d (μmol/d)	1.03 (6.09)	1.33 (7.87)	1.73 (10.24)	2.39 (14.14)	0.84 (4.96)	1.14 (6.74)	2.34 (13.83)
B6:protein, mg/g	0.012	0.016	0.020	0.028	0.010	0.013	0.028
Urine 4PA[2], μmol/d	3.23±0.73[a]	4.00±0.91[b]	5.89±0.77[c]	9.51±1.08[d]	2.87±0.47[a]	3.35±0.65[b]	7.91±0.81[c]
Urine B6, μmol/d	0.54±0.12[a]	0.61±0.10[b]	0.75±0.09[c]	0.95±0.14[d]	0.48±0.08[a]	0.53±0.09[a]	0.85±0.11[b]
Plasma PLP, nmol/L	27.9±11.4[a]	32.4±11.6[a]	41.0±14.8[a]	58.9±25.3[b]	26.5±12.4[a]	29.4±12.5[a]	58.9±22.3[b]
Plasma TB6, nmol/L	---	---	---	---	39.9±13.4[a]	43.9±10.2[a]	72.4±17.7[b]
RBC PLP, nmol/L	---	---	---	---	40.2±9.8	45.5±10.1	47.2±14.0
EALT, % stim	26±8[ab]	34±13[b]	17±4[a]	17±9[a]	13±8	---	15±3
EAST, % stim	37±11	39±11	41±15	39±7	52±6	---	53±7
Fecal B6, μmol/5 d	10.2±2.9	11.4±2.8	11.4±3.0	13.0±3.5	---	---	---
KA, μmol/d	102±36.1	86.8±32.3	80.1±26.6	72.9±36.3	---	---	---
XA, μmol/d	55.4±25.7[a]	35.0±13.2[b]	29.1±9.5[b]	25.0±19.8[b]	---	---	---
VA, μmol/d	89.4±93.4	46.1±42.7	35.6±30.1	28.4±19.9	---	---	---

[1] $\bar{x}$ ± SD. Means of indicators within a study not sharing a common superscript letter are significantly different, $P<0.05$ (ANOVA, Newman-Keuls multiple range analysis).

[2] Abbreviations: 4PA, 4-pyridoxic acid; EALT, erythrocyte alanine aminotransferase; EAST, erythrocyte aspartate aminotransferase; KA, kynurenic acid; PLP, pyridoxal 5'-phosphate; TB6, total B6; VA, volatile amines (kynurenine plus acetylkynurenine); XA, xanthurenic acid.

Table 3.4 Number of subjects not reaching indicator values suggested by Leklem (13) for adequate status at each intake level

		Study 1	**(n=10)**		**Study 2**	**(n=6)**	
B6 Intake,	Period 1	Period 2	Period 3	Period 4	Period 1	Period 2	Period 3
mg/d	1.03	1.33	1.73	2.39	0.84	1.14	2.34
(μmol/d)	(6.09)	(7.87)	(10.24)	(14.14)	(4.96)	(6.74)	(13.83)
B6:protein, mg/g	0.012	0.016	0.020	0.028	0.010	0.013	0.028
Urine 4PA[1]	3	1	0	0	3	1	0
Urine B6	2	2	0	0	4	2	0
Plasma PLP	7	4	3	0	4	4	0
Plasma TB6	-	-	-	-	4	2	0
EALT	5	7	0	1	0	-	0
EAST	0	0	0	0	0	-	0
XA	3	0	0	0	-	-	-

[1] Abbreviations: 4PA, 4-pyridoxic acid; PLP, pyridoxal 5′-phosphate; TB6, total vitamin B-6; EALT, erythrocyte alanine aminotransferase % stimulation; EAST, erythrocyte aspartate aminotransferase % stimulation; XA, xanthurenic acid excretion after an oral 2 g L-tryptophan load.

μmol/d with a B6 intake of 1.14 mg/d (Table 3.4). Mean urinary 4PA excretion represented 60, 52, 54 and 69% of total B6 intake in the four respective periods of Study 1 and 58, 50 and 57% of intake, respectively, in the three periods of Study 2.

Mean total urinary B6 (UB6) over the last three days of each period was significantly higher ($P<0.05$) in each successive period in Study 1. In Study 2, the mean at the end of the third period was significantly higher ($P<0.05$) than those at the end of the first two periods (Table 3.3). The suggested value for adequate status is >0.5 μmol/day (14,34), and the means exceeded this value at the end of all four periods of Study 1 and at the end of the final two periods in Study 2. In both Study 1 and Study 2, however, two subjects still had values lower than 0.5 μmol/d with intakes of 1.33 and 1.14 mg B6/d, respectively (Table 3.4). Total urinary B6 excretion was 8.9, 7.8, 7.3, and 6.7% of total B6 intake, respectively, in the four periods of Study 1 and 9.7, 7.9 and 6.1% in the three respective periods of Study 2.

Although the mean plasma PLP concentration increased with each successive period, the differences were not statistically significant until the end of the final period in both studies (Table 3.3). A value greater than 30 nmol/L is considered indicative of adequate status (14,34). The mean exceeded this value with a B6 intake of 1.33 mg/d in Study 1, although three of the ten subjects still had concentrations less than 30 nmol/L with an intake of 1.73 mg B6/d (Table 3.4). In Study 2, the mean exceeded 30 nmol/L when the intake was 2.34 mg/d; four of six subjects still had less than adequate plasma PLP concentrations when the B6 intake was 1.14 mg/d (Table 3.4).

In Study 2, the mean plasma total B6 concentration (TB6) at the end of Period 3 was significantly different from the means at the end of Periods 1 and 2 (Table 3.3). The means exceeded the suggested value for adequate status of >40 nmol/L (14) with B6 intakes of 1.14 and 2.34 mg/d, although two subjects still had concentrations less than this with an intake of 1.14 mg/d (Table 3.4). Plasma PLP accounted for 66, 67 and 81% of TB6 in the three respective periods.

While there was a trend for RBC PLP concentration to increase as B6 intake increased, no significant differences were found in mean RBC PLP concentrations among the three periods of Study 2 (Table 3.3). Suggested values for adequate status have not been established for RBC PLP. Fecal vitamin B-6 excretion was not significantly different among the four experimental periods in Study 1 (Table 3.3) and was not measured in Study 2.

Indirect measures of vitamin B-6 status

There were no significant differences in mean percent stimulation of EAST activity at the end of the four periods of Study 1, but the mean percent stimulation in EALT activity was significantly lower at the end of Period 3 and 4 than at the end of Period 2 (Table 3.3). In Study 2, mean EAST and EALT percent stimulation did not differ significantly between the beginning of the first period and the end of the final period (Table 3.3). The suggested value for EALT percent stimulation indicating adequate B6 status is <25% (14), which was achieved by the end of Period 3 (1.73 mg B6/d) in Study 1. For EAST, the suggested value is <80% (14) and all subjects in all periods of Study 1 had percent stimulation values less than 80%. In Study 2, all subjects had acceptable values for EAST and EALT percent stimulation at the beginning of Period 1 and at the end of the study.

There were no significant differences in KA, XA or VA excretion the day before the tryptophan load among all four periods in Study 1. The day before the tryptophan load, urinary KA excretion averaged 13.0 ± 1.9 μmol/d, XA excretion averaged 12.3 ± 3.8 μmol/d and VA excretion averaged 4.4 ± 1.2 μmol/d. Table 3.3 shows the mean urinary excretion of tryptophan metabolites after an oral 2 g L-tryptophan load in the four experimental periods of Study 1. Xanthurenic acid excretion was significantly higher after the tryptophan load in Period 1 than in the subsequent three periods. Excretion of KA and VA showed a downward trend as B6 intake increased, but the variation among subjects was so high that the differences between periods were not statistically significant. For adequate status, the suggested value for XA excretion after a 2 g tryptophan load is <65 μmol/d (14), and three subjects had post-tryptophan excretions higher than this when consuming a B6 intake of 1.03 mg/d (Table 3.4), although the means in all four periods were less than the suggested value.

One subject's tryptophan metabolites data were excluded from the statistical analysis because after the experimental periods ended, she was found to have an infection. Her post-load tryptophan metabolite excretion was several-fold higher than the means of the other subjects and the infection may have contributed to this increased excretion (36).

Although not a true indirect measure of vitamin B-6 status, plasma alkaline phosphatase activity has been found to have an effect on plasma PLP concentrations

(37). Mean plasma alkaline phosphatase activity in our subjects was 25.5 $\pm$ 4.9 $\mu mol \cdot min^{-1} \cdot L^{-1}$ (range: 15.4-35.7 $\mu mol \cdot min^{-1} \cdot L^{-1}$) in Study 1 and 24.2 $\pm$ 6.1 $\mu mol \cdot min^{-1} \cdot L^{-1}$ (range: 18.2-37.7 $\mu mol \cdot min^{-1} \cdot L^{-1}$) in Study 2 and did not differ significantly among the four periods of Study 1 or the three periods of Study 2. Although no significant correlation was found between alkaline phosphatase activity and plasma PLP concentrations at any level of B6 intake, a significant positive correlation was found between plasma alkaline phosphatase activity and RBC PLP concentrations in Periods 2 and 3 of Study 2 (r=0.938, 0.817, respectively; P<0.05).

Correlations among vitamin B-6 status indicators

Table 3.5 lists the correlation coefficients (r) among the vitamin B-6 status indicators calculated using the combined data from both studies. All of the direct indicators showed a significant positive correlation with vitamin B-6 intake and with one another except RBC PLP concentration, which was not significantly correlated with intake or any of the other B6 status indicators. Erythrocyte alanine aminotransferase percent stimulation was inversely correlated with B6 intake, U4PA, UB6, plasma PLP and EAST percent stimulation. Among the urinary tryptophan metabolites excreted after a tryptophan load, VA excretion was inversely correlated with B6 intake, 4PA and UB6 excretion and plasma PLP concentration. Excretion of XA was inversely correlated with B6 intake, 4PA and UB6 excretion. Post-load KA

Table 3.5 Correlation coefficients (r) among vitamin B-6 status indicators for the two studies combined[1]

(n)	U4PA (16)	UB6 (16)	TB6 (6)	Plasma PLP (16)	RBC PLP (6)	VA (9)	XA (9)	KA (9)	EAST (10)	EALT (10)
B6 Intake	**0.946**[2,3]	**0.858**[2,3]	**0.747**[3,4]	**0.643**[2,5]	0.224[3]	**-0.351**[3,6]	**-0.583**[4,7]	0.326[8]	0.069[3]	**-0.304**[3,6]
U4PA		**0.875**[2,7]	**0.730**[3,4]	**0.540**[2,3]	-0.338[8]	**0.526**[4,8]	**-0.453**[5,9]	-0.205[7]	0.015[5]	**-0.349**[3,6]
UB6			**0.676**[3,9]	**0.587**[2,7]	-0.168[8]	**0.634**[2,8]	**-0.431**[5,9]	0.318[8]	-0.094[8]	**0.336**[6,8]
TB6				**0.951**[2,3]	0.400[7]	---	---	---	---	---
Plasma PLP					0.271[7]	**0.562**[4,8]	-0.172[7]	**-0.702**[2,5]	**-0.304**[6,8]	**-0.278**[5,6]
VA							0.289[3]	**0.768**[2,5]	-0.223[5]	0.308[5]
XA								0.302[7]	-0.091[3]	0.107[7]
KA									-0.327[5]	0.234[7]
EAST										**-0.314**[5,6]

[1] Excluding one subject's VA, KA and XA values (See text); r values in **bold** are statistically significant. Abbreviations: EAST, erythrocyte aspartate aminotransferase % stimulation; EALT, erythrocyte alanine aminotransferase % stimulation; KA, kynurenic acid; PLP, pyridoxal phosphate; TB6, plasma total B6; U4PA, 4-pyridoxic acid; UB6, urinary total B6; VA, volatile amines; XA, xanthurenic acid.

[2] $P<0.0001$

[3] Linear model: $Y=a+bX$

[4] $P<0.001$

[5] Exponential model: $\ln Y=a+bX$

[6] $P<0.05$

[7] Multiplicative model: $Y=aX^b$

[8] Reciprocal model: $1/Y=a+bX$

[9] $P<0.01$

excretion was inversely correlated with plasma PLP concentration and positively correlated with VA excretion.

Discussion

Our purpose was to evaluate the changes in several direct and indirect vitamin B-6 status indicators in women with a constant protein intake and B6 intakes varying over the normal range found in this population. Data from the Nationwide Food Consumption Survey conducted in 1985 show that average vitamin B-6 intakes for the majority of women ages 19-50, excluding those below the 10th percentile and those above the 90th percentile of intake, range from 0.62 to 1.81 mg/d (38). There have been few studies done in which more than one or two B6 status indicators were measured in healthy women who were not B6-depleted, using a range of B6 intakes and known protein intake. The studies reported here provide extensive data on vitamin B-6 status indicator values in women fed controlled diets at several levels within the normal range of vitamin B-6 intakes and dietary B6:protein ratios. The data also show the strong interrelationships between the status indicators under controlled metabolic conditions.

The PNG content of the diet was within the range of intakes reported in other studies (39,40) and is reported here for completeness and comparison with prior and future studies. The level of PNG in the diet can affect excretion of 4PA and total B6 (41). We feel it is important to determine dietary PNG in studies such as this so that potential effects on B6 status indicators can be considered in comparisons with other studies.

Direct measures of vitamin B-6 status

Because 4-pyridoxic acid is the major end-product of vitamin B-6 metabolism, urinary 4PA excretion has often been used as a direct indicator of vitamin B-6 status and is considered to be indicative of recent B6 intake (14). In three previous studies in B6-depleted young women (7,10,12), it took a B6 intake of 1.5 to 1.8 mg/d (0.015 to 0.026 mg B6/g protein) to restore 4PA excretion to predepletion levels. In a study of elderly men and women (42), it took 1.90 to 1.95 mg B6/d to bring 4PA excretion back to predepletion levels in women consuming 78 or 54 g protein/d (0.024-0.036 mg B6/g protein).

In the studies reported here, intakes of 1.33 mg B6/d in Study 1 and 1.14 mg B6/d in Study 2 were sufficient to raise urinary 4PA excretion above adequate levels in all but one subject in each study. Urinary 4PA excretion reached values indicative of adequate status in all subjects at a B6 intake level of 1.73 mg/d.

Like urinary 4PA excretion, urinary total vitamin B-6 is considered a short term indicator of vitamin B-6 status. In a depletion/repletion study by Donald et al (10), urinary total B6 excretion nearly reached predepletion levels after three days of an intake of 1.54 mg (0.026 mg B6/g protein), but in a recent study by Kretsch et al (12), even 2.0 mg B6/d (0.020 mg B6/g protein) was not sufficient to restore baseline urinary total vitamin B-6 excretion in the B6-depleted subjects. In our studies, a B6 intake of 1.03 mg/d was sufficient to raise the mean UB6 excretion above 0.50 μmol/d, but even at an intake of 1.33 mg/d, 2 of the 10 subjects still were excreting less than adequate amounts. At an intake of 1.73 mg/d, all the subjects excreted UB6 above the value indicative of adequate status. The difference between our results and those of Kretsch et al (12) may be because their subjects were depleted of vitamin B-6, or because their baseline value was unusually high (0.92 μmol/d).

Although the use of plasma PLP concentration as a status indicator has been questioned (43), plasma PLP is probably the most frequently used direct indicator of vitamin B-6 status (14). In two previous studies (7,12), plasma PLP concentrations were restored to their predepletion levels with B6 intakes of 1.0 to 1.84 mg/d (0.010 to 0.023 mg B6/g protein). In elderly women (42), an intake of 1.90 mg B6/d (0.024 mg B6/g protein) did not quite restore their plasma PLP concentrations to predepletion levels, but in a second group consuming less protein 1.33 mg B6/d (0.025 mg B6/g protein) normalized their plasma PLP values .

At daily B6 intakes of 0.84, 1.03 and 1.14 mg (0.10, 0.012 and 0.013 mg B6/g protein) the mean plasma PLP concentrations of the subjects in the present studies were less than the value suggested for adequate status. While the mean plasma PLP concentrations at these intakes may not be statistically less than the recommended cut-off of 30 nmol/L, a majority of the subjects were below 30 nmol/L. Use of a cut-off value such as this is based on experimental data (34), but it is important to recognize that cut-off values are best used for individual data and are less useful for comparison of mean values. When the B6 intake was 1.33 mg/d, the mean plasma PLP concentration was greater than 30 nmol/L, but 4 of the 10 subjects still had concentrations less than this level. Three subjects still were below 30 nmol/L with a B6 intake of 1.73 mg/d, but all had plasma PLP values indicative of adequate status at 2.34 or 2.39 mg B6/d.

Shultz and Leklem (34) used 3-day dietary intake records to estimate B6 intake in 41 females, 25-79 years old, and developed equations relating fasting plasma PLP concentrations and urinary 4PA and total B6 excretion to vitamin B-6 intake and dietary B6 to protein ratio. When using vitamin B-6 intakes from these studies, the equations closely predicted the respective values for plasma PLP concentrations and urinary 4PA and total B6 excretion. This suggests that these indices can be used in a free-living population to estimate B6 intake when it falls within the normal range of intakes.

Red blood cell PLP concentration has been suggested as a useful indicator of vitamin B-6 status at marginal and adequate intakes (44), but no significant correlation between RBC PLP concentration and B6 intake or any of the other B6 status indicators was found in Study 2. Limited data are available on RBC PLP concentrations, but in men consuming a daily B6 intake of 2.1 $\pm$ 0.1 mg/d RBC PLP concentrations averaged 85 $\pm$ 6 nmol/L (45) which is higher than the 47.2 $\pm$ 14.0 nmol/L we measured in women with an intake of 2.34 mg B6/d in Study 2. The difference may be due, in part, to the different methods used to measure RBC PLP. We measured it in red blood cells, while in the study in men (45) PLP concentrations were determined in whole blood and in plasma, and the RBC PLP concentration calculated from them using hematocrit. We had poor recovery of added PLP in the RBC PLP assay, suggesting incomplete extraction. Our results suggest RBC PLP concentration is not useful as a status indicator in healthy subjects consuming intakes within the normal range, at least over short time periods as in Study 2.

Whyte et al (37) and Kant et al (45) found a significant inverse correlation between serum alkaline phosphatase activity and plasma PLP concentration. We found no significant correlation between plasma PLP concentration and alkaline phosphatase activity in these studies, but a significant direct correlation was found between RBC PLP concentration and plasma alkaline phosphatase activity. Since pyridoxal (PL) is the form of vitamin B-6 that most readily crosses membranes, it may be that a higher activity of plasma alkaline phosphatase is reflective of cellular membrane alkaline phosphatase activity, which converts PLP to PL, allowing more PL to enter the red blood cell where it may then be converted to PLP (43).

Mean fecal B6 excretion did not vary among the periods of Study 1 and was similar to values found by Kabir et al (17) in men consuming a diet providing 1.6 mg B6/d. The amount excreted represents 18-33% of the total B6 intake. From this data we can not distinguish between the amount not absorbed from the diet and the amount produced by synthesis by intestinal microflora.

Indirect measures of vitamin B-6 status

Erythrocyte aminotransferase activity (EAST and EALT) percent stimulation is considered a long term measure of B6 status because of the length of the lifespan of erythrocytes (14). In two previous depletion/repletion studies (7,12), intakes of 1.5 to 1.84 mg B6/d (0.015 to 0.023 mg B6/g protein) restored EALT percent stimulation or activity coefficient values to their predepletion levels, and the EAST activity coefficient was restored at 2.0 mg 6/d (0.020 mg B6/g protein). Ribaya-Mercado et al (42) found that the EAST percent simulation in their B6 depleted elderly subjects displayed a lag time in response to an increase in vitamin B-6 intake. For their women subjects, 1.90 to 1.95 mg B6/d were required to restore this indicator to baseline levels.

The subjects in the studies reported here were consuming each level of B6 intake for 10-15 days and only the EALT percent stimulation showed significant changes. Mean values above the <25% suggested for adequate status at B6 intakes of 1.03 and 1.33 mg/d and achieved a better than adequate value at a B6 intake of 1.73 mg/d. The length of the diet periods in relation to the lifespan of red blood cells may have affected the response of the EALT to a change in intake. This was especially evident at the 1.33 mg/d level. No significant changes were seen in EAST among the four experimental periods of Study 1. These results suggest that EALT responds more readily to changes in B6 intake than EAST. Of the two measures, EALT is considered to be the more sensitive to changes in B6 intake (14).

The primary urinary tryptophan metabolite which has been used to assess B6 status is the urinary excretion of xanthurenic acid following a 2 g L-tryptophan load (14). Leklem et al (47) reported that a B6 intake of 0.83 mg/d for four weeks by B6 depleted subjects was sufficient to return mean xanthurenic acid excretion after a 2 g L-tryptophan load to its predepletion value, which was better than the <65 μmol/d suggested for adequate status. Kretsch et al (12) found that normalization of postload XA excretion occurred at 1.5 mg B6/d (0.015 mg B6/g protein). In the study by Ribaya-Mercado et al (42), elderly women consuming 78 g protein required 1.90 mg B6/d to restore predepletion levels of XA excretion after a 5 g L-tryptophan load.

In Study 1, mean xanthurenic acid excretion following a 2 g L-tryptophan load was below the suggested value for adequate status (<65 μmol/d) at all four levels of B6 intake (Table 3.4), but with an intake of 1.03 mg/d 30% of the subjects (3 of 10) had post-load excretions of XA greater than 65 μmol/d, indicating inadequate status. Although mean post-load urinary excretion of KA and VA was not significantly different at any of the four levels of intake in Study 1, both measures showed a progressively downward trend as intake increased (Table 3.3). Both XA and VA excretion were inversely correlated with B6 intake and urinary 4PA and UB6 excretion (Table 3.5), suggesting that in healthy women either measure is a good non-invasive functional measure of B6 status. The one subject whose post-load excretion of KA and VA was elevated, possibly due to infection, had normal values for XA excretion. This suggests post-load XA excretion may be the preferred measure when infection may be present, as long as the infection is not serious enough to increase stress hormones.

Correlations between vitamin B-6 status indicators

Comparing the mean values of status indicators in the two studies (Table 3.3), similar values were measured with similar intakes and B6 to protein ratios. This shows the results were reproducible since the studies were conducted more than two years apart with two different groups of subjects.

Significant correlations between B6 intake and urinary 4PA, UB6 and plasma PLP concentration have been reported in free-living subjects whose intake was estimated from dietary records (34). As shown in Table 3.6, the status indicators showing the strongest correlation with B6 intake were urinary 4PA excretion, UB6 excretion, plasma TB6 concentration, plasma PLP concentration and XA excretion after an oral 2 g L-tryptophan load. Urinary 4PA excretion was strongly correlated with UB6 excretion, and plasma TB6 and PLP concentrations. Excretion of urinary 4PA and UB6 change rapidly with changes in intake and so may be considered short-term indicators of B6 status.

The strong correlation between plasma TB6 and PLP concentrations was expected, since 66-81% of the plasma TB6 was as PLP. There was also a strong inverse correlation between plasma PLP concentration and post-tryptophan load urinary KA excretion. The aminotransferase which converts kynurenine to KA and requires PLP as a coenzyme is a cytosolic enzyme which is more responsive to changes in PLP concentration than the mitochondrial aminotransferase which converts 3-hydroxykynurenine to xanthurenic acid (48). These data indicate that urinary 4PA and UB6 excretion, plasma TB6 and PLP concentrations and XA excretion after a tryptophan load are all reflective of vitamin B-6 intake and can be used as reliable indicators of B6 status in healthy women. Based on the correlation data, it appears that one measure is sufficient to assess B6 status. However, because of the factors that can influence B6 status indicators, many of which were controlled or constant in this study, as well as differences in analysis methods, we believe at least three measures should be used, as previously recommended (49).

Vitamin B-6 requirements

The vitamin B-6 requirement suggested by the studies of Donald et al (10) and Brown et al (7) may underestimate the B6 intake necessary to achieve adequate B6 status considering most of the B6 intake was as crystalline pyridoxine-HCl, a form considered to be highly bioavailable, and does not reflect the bioavailability of vitamin B6 in a typical diet composed of natural foods (17,24,35,39,50).

The diets in the studies reported here were composed mostly of natural foods with 60-100% of the total B6 intake derived from the diet at the five lowest levels of intake (43 and 36% at the two highest levels). The percent of the total B6 intake as pyridoxine glucoside (PNG), a form found to have reduced bioavailability compared to pyridoxine (41), was 6 to 17% in Study 2, which may be comparable to intakes in a typical diet (39,40). PNG is not present in animal products, but constitutes 5-80% of the total vitamin B-6 in various fruits and vegetables (24,40). Therefore, individuals who consume few or no animal products may have a higher percentage of the less bioavailable PNG in their diets and may need a higher intake of B6 to meet their requirement. Although Shultz and Leklem (51) found no significant differences in plasma PLP, urinary 4PA or UB6 between vegetarian (n=7) and nonvegetarian women (n=13) with similar B6 intakes, means of all the indicators were lower in the vegetarians. It is possible that with a larger sample size the differences could become significant.

Although some status indicators reached values indicative of adequate status at lower intakes, it took a B6 intake greater than 1.33 mg/d (>0.016 mg B6/g protein) for all status indicators measured to achieve the values suggested for adequate status.

This suggests that the minimal B6 requirement for young adult women is in the range of 1.33 to 1.73 mg/d with a protein intake of 85 g/d (0.016-0.020 mg B6/g protein). Kretsch et al (12) concluded that the requirement for vitamin B-6 is between 0.015 and 0.020 mg/g protein, and suggested that adding a margin of safety to account for bioavailability differences and variance in the requirement of individuals would raise the vitamin B-6 RDA above the currently recommended amount of 0.016 mg/g protein. Our results confirm and reinforce their conclusions.

Acknowledgements

The authors thank Karin Hardin and Jim Ridlington for their technical assistance.

References

1. National Research Council. Recommended dietary allowances. 10th ed. Washington, DC: National Academy Press, 1989:142-50.

2. National Research Council. Recommended dietary allowances. 9th ed. Washington, DC: National Academy Press, 1980:96-106.

3. Linkswiler HM. Vitamin B_6 requirements of men. In: National Academy of Sciences. Human vitamin B_6 requirements. Washington, DC: National Academy Press, 1978:279-90.

4. Miller, LT, Linkswiler, HM. Effect of protein intake on the development of abnormal tryptophan metabolism by men during vitamin B_6 depletion. J Nutr 1967; 93:53-9.

5. Park YK and Linkswiler H. Effect of vitamin B_6 depletion in adult man on the excretion of cystathionine and other methionine metabolites. J Nutr 1970;100:110-6.

6. Aly HE, Donald EA, Simpson MHW. Oral contraceptives and vitamin B_6 metabolism. Am J Clin Nutr 1971;24:297-303.

7. Brown RR, Rose DP, Leklem JE, Linkswiler H, Arand R. Urinary 4-pyridoxic acid, plasma pyridoxal phosphate, and erythrocyte aminotransferase levels in oral contraceptive users receiving controlled intakes of vitamin B_6. Am J Clin Nutr 1975; 28:10-19.

8. Leklem JE, Brown RR, Rose DP, Linkswiler HM. Vitamin B_6 requirements of women using oral contraceptives. Am J Clin Nutr 1975;28:535-41.

9. Bosse TR, Donald EA. The vitamin B_6 requirement in oral contraceptive users. I. Assessment by pyridoxal level and aminotransferase activities in erythrocytes. Am J Clin Nutr 1979;32:1015-23.

10. Donald EA, McBean LD, Simpson MHW, Sun MF, Aly HE. Vitamin B_6 requirement of young adult women. Am J Clin Nutr 1971;24:1028-41.

11. Shin HK, Linkswiler H. Tryptophan and methionine metabolism of adult females as affected by vitamin B_6 deficiency. J Nutr 1974;104:1348-55.

12. Kretsch MJ, Sauberlich HE, Skala JH, Johnson HL. Vitamin B-6 requirement and assessment: young women fed a depletion diet followed by plant- or animal-protein diet with graded amounts of vitamin B-6. Am J Clin Nutr 1995;61:1091-101.

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

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

15. Merrill AH, Henderson JM. Diseases associated with defects in vitamin B-6 metabolism or utilization. Ann Rev Nutr 1987;7:137-56.

16. Harris AL. Determination of D-xylose in urine for the D-xylose absorption test. Clin Chem 1969;15:65.

17. Kabir H, Leklem JE, Miller LT. Comparative vitamin B-6 bioavailability from tuna, whole wheat bread and peanut butter in humans. J Nutr 1983;113:2412-20.

18. Lindberg AS, Leklem JE, Miller LT. The effect of wheat bran on the bioavailability of vitamin B-6 in young men. J Nutr 1983;2578-86.

19. Leklem JE, Shultz TD, Miller LT. Comparative bioavailability of vitamin B-6 from soybeans and beef. Fed Proc 1980;38:452 (abstr).

20. Lee CM, Leklem JE. Differences in vitamin B-6 status indicator responses between young and middle-aged women fed constant diets with two levels of vitamin B-6. Amer J Clin Nutr 1985;42:226-34.

21. Belongia EA, Hedberg CW, Gleich GJ, White KE, Mayeno AN, Loegering DA, Dunnette SL, Pirie PL, MacDonald KL, Osterholm MT. An investigation of the cause of the eosinophilia-myalgia syndrome associated with tryptophan use. N Eng J Med 1990;323:357-65.

22. Horwitz GW, ed. Official methods of analysis of the AOAC. 13th ed. Washington DC, 1980:768.

23. Scales FM, Harrison AP. Boric acid modification of the Kjeldahl method for crop and soil analysis. Ind Eng Chem 1929;12:350.

24. Kabir H, Leklem JE, Miller LT. Measurement of glycosylated vitamin B-6 in foods. J Food Sci 1983;48:1422-1425.

25. Pino S, Bennotte J, Gordnya H. An automated method for urine creatinine which does not require a dialyzer module. Clin Chem 1965;11:664-66.

26. Georgia JD. Nonprotein nitrogenous constituents. In: Henry RJ, Cannon DC, Winkleman JW. Clinical chemistry: principles and technics. 2nd edition. New York: Harper and Row, 1974:504-63.

27. Gregory JF, Kirk JR. Determination of urinary 4-pyridoxic acid using high performance liquid chromatography. Am J Clin Nutr 1979;32:879-93.

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

29. Price JM, Brown RR, Yess N. Testing the functional capacity of the tryptophan-niacin pathway in man by analysis of urinary metabolites. Adv Metab Disorders 1965;2:159-225.

30. Arend RA, Leklem JE, Brown RR. Direct and steam distillation autoanalyzer methods for assay of diazotizable aromatic amine metabolites of tryptophan in urine and in serum. Biochem Med 1970;4:457-68.

31. Chabner BA, Livingston DM. A simple enzymatic assay for pyridoxal phosphate. Anal Biochem 1970;34:413-423.

32. Roy AV. Rapid method for determining alkaline phosphatase activity in serum with thymolphthalein monophosphate. Clin Chem 1970;16:431-6.

33. Woodring MJ, Storvick, CA. Effect of pyridoxine supplementation on glutamic-pyruvic transaminase and in vitro stimulation in erythrocytes of normal women. Am J Clin Nutr 1970;23:1385-95.

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

35. Bills ND. In vivo and in vitro determination of the bioavailability of vitamin B-6 from plant foods containing pyridoxine glucoside. Doctoral thesis 1991; Oregon State University.

36. Brown RR. Possible role for vitamin B-6 in cancer prevention and treatment. In: Leklem JE, Reynolds RD, eds. Clinical and physiological applications of vitamin B-6. New York:AR Liss, 1988:279-301.

37. Whyte MP, Mahuren JD, Vrabel LA, Coburn SP. Markedly increased circulating pyridoxal-5'-phosphate levels in hypophosphatasia: alkaline phosphatase acts in vitamin B_6 metabolism. J Clin Invest 1985;76:752-6.

38. Human Nutrition Information Service, US Department of Agriculture. Nationwide food consumption survey, continuing survey of food intakes by individuals, 1985. Hyattsville MD: US Department of Agriculture, 1987 (Report 85-4).

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

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

41. Gregory JF, Trumbo PR, Bailey LB, Toth JP, Baumgartner TG, Cerda JJ. Bioavailability of pyridoxine-5'-ß-D-glucoside determined in humans by stable-isotopic methods. J Nutr 1991;121:177-86.

42. Ribaya-Mercado JD, Russell RM, Sahyoun N, Morrow FD, Gershoff SN. Vitamin B-6 requirements of elderly men and women. J Nutr 1991;121:1062-74.

43. Leklem JE, Reynolds RD. Challenges and directions in the search for clinical applications of vitamin B-6. In: Leklem JE, Reynolds RD, eds. Clinical and physiological applications of vitamin B-6. New York: AR Liss, 1988:437-54.

44. Sampson DA, Schaeffer MC, Skala JH, O'Connor DK. B-6 vitamers in erythrocytes and muscle of rats fed graded levels of vitamin B-6. FASEB J 1989; 3:A454(abstr).

45. Kant AK, Moser-Veillon PB, Reynolds RD. Effect of age on changes in plasma, erythrocyte, and urinary B-6 vitamers after an oral vitamin B-6 load. Am J Clin Nutr 1988;48:1284-90.

46. Anderson BB, Fulford-Jones CE, Child JA, Beard MEJ, Bateman CJT. Conversion of vitamin B_6 compounds to active forms in the red blood cell. J Clin Invest 1971; 50:1901-9.

47. Leklem JE, Brown RR, Rose DP, Linkswiler H, Arend RA. Metabolism of tryptophan and niacin in oral contraceptive users receiving controlled intakes of vitamin B_6. Am J Clin Nutr 1975;28:146-56.

48. Henderson LM, Hulse JD. Vitamin B-6 relationships in tryptophan metabolism. In: National Academy of Sciences. Human vitamin B_6 requirements. Washington, DC: National Academy Press, 1978:21-36.

49. Leklem JL, Reynolds RD. Recommendations for status assessment of vitamin B-6. In: Leklem JE, Reynolds RD, eds. Methods in vitamin B-6 nutrition. New York: Plenum Press, 1981:389-392.

50. Tarr JB, Tamura T, Stokstad ELR. Availability of vitamin B-6 and pantothenate in an average diet in man. Am J Clin Nutr 1981;34:1328-37.

51. Shultz TD, Leklem JE. Vitamin B-6 status and bioavailability in vegetarian women. Am J Clin Nutr 1987:46:647-51.

CHAPTER 4

VITAMIN B-6 STATUS OF WOMEN FED A CONSTANT INTAKE OF VITAMIN B-6 AND THREE LEVELS OF PROTEIN

Christine M. Hansen, James E. Leklem and Lorraine T. Miller

To be submitted to the *Journal of Nutrition,*
American Institute of Nutrition,
Bethesda, MD

Abstract

To determine the effect of varying levels of dietary protein with a constant intake of vitamin B-6 (B6) on B6 status, nine women were fed diets providing daily intakes of 1.25 mg B6 and 0.5, 1.0 and 2.0 g protein/kg body weight. After an 8-day adjustment period, the women consumed each level of dietary protein for 14 d in a Latin square design. Several direct and indirect B6 status indicators were measured in blood and urine. Significant differences among protein levels were found for urinary 4-pyridoxic acid (4PA) excretion ($P < 0.01$), plasma pyridoxal 5′-phosphate (PLP) concentration ($P < 0.05$), and urinary excretion of volatile amines (VA, kynurenine plus acetylkynurenine) after a 2 g L-tryptophan load ($P < 0.05$). Nitrogen intake was significantly negatively correlated with urinary 4PA excretion ($P < 0.001$) and plasma PLP concentration ($P < 0.01$), and positively correlated with erythrocyte alanine aminotransferase percent stimulation ($P < 0.05$) and urinary post-tryptophan load excretion of xanthurenic acid ($P < 0.05$), kynurenic acid ($P < 0.05$) and VA ($P < 0.01$). Compared to men consuming diets with a similar B6 to protein ratio in a previous study, the women excreted a greater percentage of the B6 intake as 4PA, had lower plasma PLP concentrations and excreted greater amounts of postload urinary tryptophan metabolites at all three protein levels. If the Recommended Daily Allowance (RDA) of vitamin B-6 is to be based on the dietary B6 to protein ratio, gender differences in response to varying protein intakes should be taken into consideration. For the levels of protein intake used in this study and a B6 intake of 1.25 mg/d, a B6 to protein ratio of at least 0.020 mg/g is required for adequate vitamin B-6 status in women.

Introduction

The relationship between dietary protein and the requirement for vitamin B-6 has been well established in young men (Baker et al. 1964, Miller and Linkswiler 1967, Miller et al. 1985). When fed a vitamin B-6 deficient diet, men with a high protein intake (100 g/d) exhibited abnormal tryptophan metabolism sooner than men with a low protein intake (30 g/d). They also required a higher intake of vitamin B-6 than the low protein group, i.e. 1.75-2.0 mg/d (10.36-11.83 μmol/d) vs. 1.25-1.5 mg/d (7.40-8.88 μmol/d), to normalize urinary excretion of tryptophan metabolites (Baker et al. 1964). The concentration of vitamin B-6 compounds in blood and their excretion in urine were inversely related to protein intake in men fed three levels of protein with a constant vitamin B-6 intake (Miller et al. 1985). The relationship between vitamin B-6 requirement and protein intake is thought to be due to the increased activities of pyridoxal 5′-phosphate (PLP)-dependent enzymes which catabolize excess amino acids when dietary protein intake is high. In animals, the activities of hepatic PLP-dependent enzymes involved in protein catabolism increase as dietary protein increases (Harper 1968, Waldorf et al. 1963).

In elderly subjects, however, the association between protein intake and vitamin B-6 requirement is less clear. When vitamin B-6 depleted elderly subjects were repleted, there was no difference in the daily intake of vitamin B-6 required to normalize xanthurenic acid (XA) excretion after a 5 g L-tryptophan load between groups with a high (120 g/d for men; 78 g/d for women) or low (69 g/d for men; 54 g/d for women) protein intake (Ribaya-Mercado et al. 1991). However, elderly women fed the lower protein diet required 1.33 mg vitamin B-6/d to attain predepletion plasma PLP concentrations, while the women given the higher protein diet did not return to predepletion PLP concentrations even with 1.90 mg vitamin B-6/d. In another recent study, no effect of protein intake on indices of vitamin B-6 status [urinary 4-pyridoxic acid (4PA) excretion as a percentage of intake, plasma pyridoxal and total vitamin B6 concentrations and erythrocyte aspartate aminotransferase (EAST) activity coefficient] was found in elderly subjects fed two levels of dietary protein. In young adults, however, urinary excretion of 4PA as a percentage of vitamin B-6 intake decreased with increased protein intake (Pannemanns et al. 1994).

There have been few studies of the effect of varying levels of dietary protein with a constant vitamin B-6 intake on indices of vitamin B-6 status in young women. Some gender differences in vitamin B-6 metabolism have been reported. Women depleted of vitamin B-6 exhibited abnormal tryptophan metabolism sooner than men (Miller and Linkswiler 1967, Shin and Linkswiler 1974). Women had lower plasma PLP concentrations than men with similar dietary vitamin B-6 to protein ratios (Shultz and Leklem 1981). During vitamin B-6 repletion, a higher dietary vitamin B-6 to protein ratio was required to normalize post-tryptophan load xanthurenic acid excretion in elderly women than in elderly men (0.0244 vs. 0.0163 mg/g) (Ribaya-Mercado et al. 1991).

The present Recommended Dietary Allowance (RDA) for vitamin B-6 is based on a dietary vitamin B-6 to protein ratio of 0.016 mg/g (NRC 1989). Because the same dietary ratio of vitamin B-6 to protein was used in calculating the RDA for vitamin B-6 for both men and women, and the studies on which the ratio is based were done in men, data are needed on the response of women to varying dietary vitamin B-6 to protein ratios using typical diets with vitamin B-6 intakes in the normal range. The objective of the research reported here was to determine the effect of diets low, medium and high in protein content (0.5, 1.0 and 2.0 g protein/kg body weight) on several direct and indirect vitamin B-6 status indicators in young women with a constant intake of vitamin B-6. The results can be used to evaluate the dietary vitamin B-6 to protein ratio necessary to meet the vitamin B-6 requirements of women. An additional objective was to compare these results with those from a similar study conducted in men (Miller et al. 1985) to determine possible gender differences in vitamin B-6 metabolism.

Materials and methods

Subjects

Nine healthy young women, described in **Table 4.1**, were recruited from the local community. They were nonsmokers who were not using oral contraceptives or any medications known to affect vitamin B-6 metabolism (Bhagavan 1985). Subjects were screened for diseases that could affect absorption, metabolism or excretion of vitamin B-6 (Merrill and Henderson 1987), and a normal blood chemistry test was required for selection. Subjects were instructed to maintain their usual activity level throughout the study. The study protocol and informed consent form, which was signed by each subject prior to participation in the study, were approved by Oregon State University's Committee for the Protection of Human Subjects.

Table 4.1 Characteristics of subjects and order of protein feeding

Subject	Age (y)	Height (cm)	Weight[1] (kg)	Protein intake[2] for Periods[3] 1 2 3
1	38	150	56.4	H M L
2	26	165	62.7	M L H
3	36	163	62.8	L H M
4	22	173	64.4	H L M
5	30	173	55.5	M H L
6	19	173	63.4	L M H
7	25	157	53.0	H M L
8	23	163	53.0	M L H
9	22	170	57.5	L H M
$\bar{x}$ ± SD	26.8 ± 6.6	165 ± 8.0	58.7 ± 4.6	

[1] Weight at beginning of study.

[2] L, M and H refer to diets calculated to contain, respectively, 0.5, 1 and 2 g protein/kg body weight.

[3] Experimental periods were 14 days long and followed an initial adjustment period of 8 d during which protein intake was 72 g/d and vitamin B-6 intake was 1.32 mg/d. A diet providing negligible protein intake was fed on the first day of a period during which a lower level of protein was fed than during the preceding one.

Experimental design

All meals were prepared and served in the metabolic kitchen in the Department of Nutrition and Food Management at Oregon State University. There was an 8-d adjustment period during which subjects were fed a diet providing 0.95 mg vitamin B-6 and 72 g protein per day. They also received an oral pyridoxine hydrochloride supplement to increase the daily vitamin B-6 intake to 1.32 mg (7.81 μmoles) during the adjustment period. The adjustment period was followed by three experimental periods of 14 d each, during which subjects received a diet calculated to contain 0.5, 1.0 or 2.0 g protein per kg body weight (low, medium and high protein, respectively). Vitamin B-6 intake was held constant at 1.25 mg/d (7.32 μmol/d) during all three experimental periods. The order in which the subjects received these diets is listed in Table 4.1.

Table 4.2 lists the types and amounts of food in the experimental diet. Different protein levels were achieved by varying the amounts of vitamin-free casein, soy protein concentrate, gelatin and cheddar cheese fed to the subjects. A variable amount of pyridoxine hydrochloride was baked into the biscuits to keep the vitamin B-6 intake constant. Based on laboratory analysis of the biscuits, there was no measurable loss of vitamin B-6 by baking. The subjects consumed varying amounts of margarine, salad dressing, jellies, sugar, soda pop and hard candy (foods which contain less than 0.001 mg vitamin B-6/100 g) to meet their energy needs and maintain their body weight.

To shorten the time of adjustment to the reduced nitrogen intake when changing from a higher to a lower protein intake, subjects were fed a diet of negligible protein content on the first day of an experimental period during which their protein intake was lower than the previous period. This negligible protein diet consisted of fruits, vegetables and low protein bread (Unimix™, Kingsmill Foods, Scarborough, Ontario, Canada) and pasta (Aglutella®, Gentili, Pisa, Italy) and provided 0.92 mg/d of vitamin B-6 (calculated by computer analysis using Food Processor II, ESHA Research, Salem, OR). An oral pyridoxine HCl supplement was

Table 4.2 Composition of diets

Food	Amount (g)	N (g)	Vitamin B-6 (mg)
A. Nonvariable[1]			
Orange juice, frozen, reconstituted	200		
Shredded wheat cereal	30		
Milk, 2% milkfat	100		
Grapefruit sections, canned	115		
Dill pickles	35		
Lettuce	35		
Carrot, raw, grated	10		
Red cabbage, raw	10		
Applesauce, canned	120		
Peach halves, canned	120		
Rice casserole[2]			
Brown rice, uncooked wt.	40		
Carrot, raw	25		
Celery, raw	25		
Onion, dried	5		
Tomato juice, canned	100		
Pear halves, canned	120		
Popcorn, air-popped	20		
Raisins	70		
Subtotal		2.73	0.849
B. Variable[3]			
Gelatin[4,5]	0-40.2	0-6.49	0-0.003
Cheese, cheddar	0-29	0-0.99	0-0.016
Salad dressing	Variable	0	0
Sugar, carbonated beverages, candies, jellies	Variable	0	0
Margarine	Variable	0	0

Continued

Table 4.2, continued

Biscuits[6]			
Flour, enriched	75	1.24	0.156
Sugar	15	0	0
Salt	2	0	0
Baking powder	5	0	0
Corn oil	20	0	0
Wheat bran	10	0.25	0.130
Casein, vitamin-free[5]	0-46.1	0-6.68	0-0.22
Soy protein concentrate[5,7]	0-26.1	0-3.59	0-0.015
Pyridoxine[8]	Variable	0	0.241-0.280
Vitamin-mineral mix[9]	Variable	0	0
Subtotal		1.73-18.91	0.383-0.415
Total		4.46-21.64	1.23-1.26

[1] Each subject received these foods and amounts daily. Subtotals of N and vitamin B-6 content are from laboratory analysis of food composites.

[2] Ingredients for the rice casserole were mixed, 120 mL water was added and the covered casseroles were baked at 375° F for 60 min.

[3] Each subject received variable amounts of the items indicated to achieve appropriate nitrogen, vitamin B-6 and energy intake. Values for individual food items are from computer analysis (Food Processor II) or provided by product manufacturer. Subtotals and total N and vitamin B-6 content are from laboratory analysis of food composites.

[4] Gelatin was dissolved in water and flavored with a powdered drink mix concentrate.

[5] Amounts used for each subject were varied proportionately for medium and high levels of nitrogen so that 46% of the additional N came from casein, 38% from gelatin and 16% from soy protein concentrate.

[6] Water was added to daily batches for each subject, divided into three biscuits (one for each meal), baked and stored frozen.

[7] Procon Plus, Central Soya Company Inc, Fort Wayne, IN.

[8] Pyridoxine HCl was mixed with sugar and variable amounts baked into the biscuits to keep the diets equal in vitamin B-6 content.

[9] Vitamin and mineral mix supplied 600 mg Ca, 6 mg Zn, 150 μg KI, 0.5 mg riboflavin and 1.4 μg vitamin B-12 per day.

given with the negligible protein diet to increase the daily vitamin B-6 intake to 1.29 mg (7.63 μmoles).

Based on analyses of weekly food composites and taking into account the variable food amounts that adjusted nitrogen intake according to body weight, the low, medium and high protein diets provided daily mean intakes ($\pm$ SD) of 4.94 $\pm$

0.34, 9.71 ± 0.58 and 20.2 ± 1.30 g of nitrogen, respectively. Using food composition tables, the diet was planned to provide 1.4 mg vitamin B-6 daily throughout the adjustment and three experimental periods, but microbiological analysis of the diet measured about 0.15 mg less vitamin B-6 in the diet than that planned. The analyzed vitamin B-6 intake for the three experimental periods was 1.26 ± 0.01, 1.24 ± 0.02 and 1.25 ± 0.01 mg (7.44 ± 0.03, 7.32 ± 0.09, 7.42 ± 0.03 μmoles) of vitamin B-6 daily, respectively. Approximately 20% of the vitamin B-6 intake was pyridoxine glucoside (PNG), based on data for foods analyzed in our laboratory (Leklem 1990 and unpublished). The mean dietary vitamin B-6 to protein ratios for the low, medium and high protein diets were 0.041 ± 0.003, 0.020 ± 0.001 and 0.010 ± 0.001 mg/g, respectively. The vitamin B-6 to protein ratios of the low, medium and high protein diets were, however, similar to those provided by the diet in the previous study done in men (i.e., 0.048 ± 0.005, 0.024 ± 0.002, and 0.011 ± 0.001 mg/g, respectively) (Miller et al. 1985).

An oral 2 g L-tryptophan load was administered with breakfast on the final day of Periods 2 and 3. There was no tryptophan load test in experimental Period 1 because we had a limited amount of pure L-tryptophan (provided by Annie P. Prince, Ph.D., Child Development and Rehabilitation Center Metabolic Clinic Program, Oregon Health Sciences University, Portland, OR). Each subject, therefore, received L-tryptophan loads, given in gelatin capsules, at two of the three levels of protein intake, and thus there was an *n* of 6 for each of the urinary tryptophan metabolites measured. Potential menstrual cycle effects on tryptophan metabolism were not taken into account (Hrboticky et al. 1989); all subjects began the study on the same date.

Sample collection and analyses

Composites of the experimental diet were made weekly and analyzed for vitamin B-6 content using *Saccharomyces uvarum* (Horwitz 1980) and for nitrogen by a boric acid modification of the Kjeldahl method (Scales and Harrison 1929). Variable foods and the biscuits were analyzed separately.

Twenty four hour urine collections from each day of the study were assayed for creatinine (Pino et al. 1965) and urea nitrogen (Georgia 1974) by an automated process (Technicon Autoanalyzer, Technicon Corp., Tarrytown, NY). Urinary nitrogen was analyzed by a boric acid modification of the Kjeldahl method (Scales and Harrison 1929). Urinary 4-pyridoxic acid (4PA) was determined by an HPLC procedure (Gregory and Kirk 1979). The interassay coefficient of variation for urinary 4PA was less than 5% and the recovery of added 4PA averaged 91 $\pm$ 8%. Urinary total B6 (UB6) was measured by a microbiological procedure using *Saccharomyces uvarum* (Miller and Edwards 1981). Urinary 4PA was averaged over the last three days and UB6 excretion over the last two days of each period for each subject. These values are reported here and were used for statistical analyses. Urine collections from the day before and the day of the L-tryptophan load were analyzed for xanthurenic acid (XA) and kynurenic acid (KA) (Price et al. 1965) and for volatile amines (VA, kynurenine plus acetylkynurenine) (Arend et al. 1970). The volatile amine assay was performed by Raymond Brown, Ph.D., University of Wisconsin, Madison, WI.

Fasting blood was drawn on Days 1 and 6 of the adjustment period and on Days 1, 7 and 12 of the three experimental periods, as well as on the morning after the final day of experimental Period 3. Whole blood was removed for determination of hematocrit and hemoglobin concentration and then samples were centrifuged at 4° . Plasma was frozen at -30° until analysis. Because of a freezer failure some blood samples were lost before all the analyses were completed. As a result, analysis for plasma total vitamin B-6 and red blood cell pyridoxal 5'-phosphate (PLP) could not be done.

Plasma PLP concentrations were determined by a tyrosine decarboxylase apoenzyme/isotopic procedure (Chabner and Livingston 1970). Samples were assayed in duplicate and any duplicates with a coefficient of variation of more than 5% were assayed again. Interassay coefficient of variation was 8% and recovery of PLP added to samples averaged 87 $\pm$ 14%. Plasma alkaline phosphatase activity was determined by a colorimetric procedure (Roy 1970). Erythrocyte aspartate and alanine aminotransferase (EAST and EALT) activity, with and without PLP added in vitro, were determined by the method of Woodring and Storvick (1970), using a 0.033 M Tris buffer (pH 7.4) instead of 0.1 M potassium phosphate. Percent stimulation was calculated by the equation: % stimulation = (activity w/added PLP - activity w/o added PLP) $\div$ activity w/o added PLP. The aminotransferase assays were performed on fresh samples within 24 hours of sampling. For all blood vitamin B-6 status indicators, the value for the final sample in each period was used in the statistical analyses.

After 7 to 9 days at each level of dietary protein, subjects were given a fecal marker (50 mg FD&C Brilliant Blue No. 1 mixed with 200 mg methylcellulose in a gelatin capsule) with breakfast at the beginning and end of a 5-day period. Complete

5-day fecal collections were analyzed for total vitamin B-6 (FB6) by a microbiological procedure using *Saccharomyces uvarum* (Miller and Edwards 1981).

Statistical analyses were done with STATGRAPHICS (Statistical Graphics Corp., Rockville, MD). Means for each status indicator at the three levels of dietary protein were compared using ANOVA. There was no significant effect of order of protein feeding. When ANOVA indicated significant *F*-values ($P < 0.05$), significant differences between means were determined using Newman-Keuls multiple range analysis. Simple regression analysis of each status indicator vs. nitrogen intake was performed and Pearson correlation coefficients were calculated.

Results

The means for hematocrit, hemoglobin concentration, urinary creatinine excretion and subjects' body weight did not differ significantly among the three levels of dietary protein and are not reported here. Urinary urea nitrogen excretion increased significantly ($P < 0.001$) as dietary protein increased, with means of 3.38 ± 0.79, 6.47 ± 0.85 and 14.52 ± 1.98 g/d for the low, medium and high protein diets, respectively.

As the level of dietary protein increased, the urinary excretion of 4PA (**Figure 4.1**) decreased significantly. The mean urinary excretion of 4PA at the highest level of dietary protein was significantly lower ($P < 0.01$) than the mean excretion during either the low or medium protein periods. Eight of the nine subjects (all except subject #3) excreted the lowest amount of 4PA in response to the high protein diet, and 7 of 9 (all except #1 and #8) excreted the highest amount of 4PA in response to

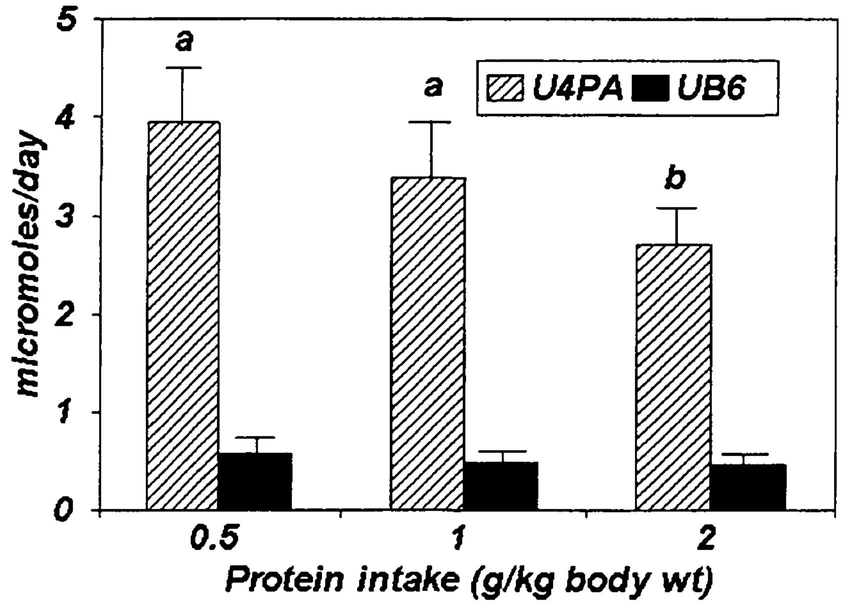

Figure 4.1 Urinary 4-PA and UB6 excretion (means ± SD). Different letters denote means that are significantly different among protein levels ($P < 0.01$).

the low protein diet. The mean percentage of vitamin B-6 intake excreted as 4PA on the low, medium and high protein diets was 53 ± 10, 46 ± 7 and 36 ± 7%, respectively.

The mean urinary total B6 excretion decreased as dietary protein increased (Fig. 4.1), but the differences were not statistically significant. The mean percentage of vitamin B-6 intake excreted as UB6 on the low, medium and high protein diets was 7.6 ± 2.0, 6.6 ± 1.2 and 6.2 ± 0.8%, respectively.

The mean concentration of plasma PLP also decreased as dietary protein increased (**Fig. 4.2**), but only the difference in mean concentration of plasma PLP between the high and low protein diets was statistically significant ($P < .04$). Plasma PLP concentrations in all subjects except #9 were lowest in response to the high

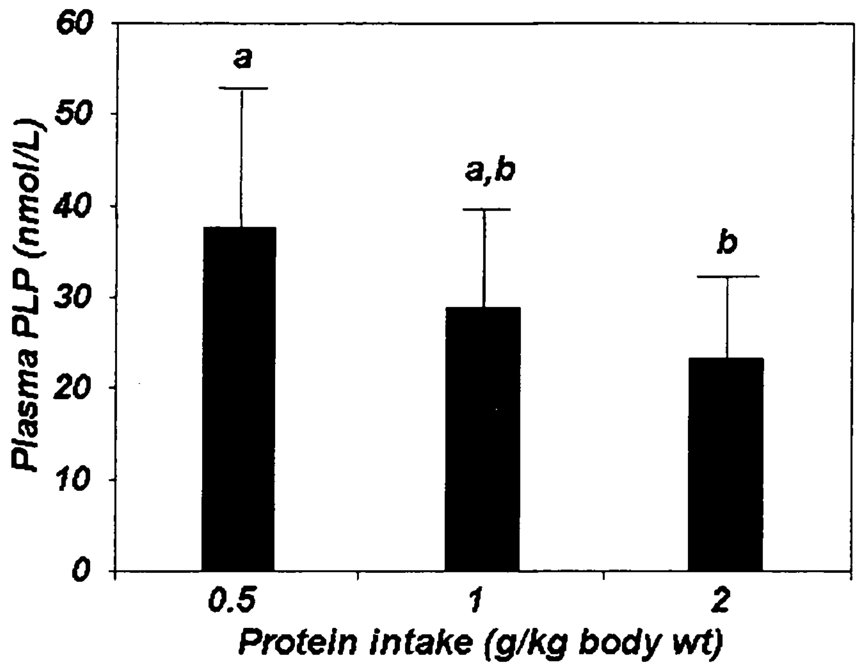

Figure 4.2 Plasma PLP concentrations (means $\pm$ SD). Different letters denote means that are significantly different among protein levels ($P < 0.05$).

protein diet, and in all subjects except #1 they were highest in response to the low protein diet.

Plasma alkaline phosphatase activity has been reported to affect plasma PLP concentrations (Whyte et al. 1985). Plasma alkaline phosphatase activity was measured at the beginning and end of the study and the means were not significantly different. There was no significant correlation between plasma alkaline phosphatase activity and plasma PLP concentration at either time point..

Because the tryptophan load test was done only in the last two periods, at each level of protein intake only 6 of the 9 subjects received a tryptophan load. Although the means of all the urinary tryptophan metabolites excreted after a 2 g L-tryptophan load increased as dietary protein increased (**Fig. 4.3**), only the volatile amine (VA)

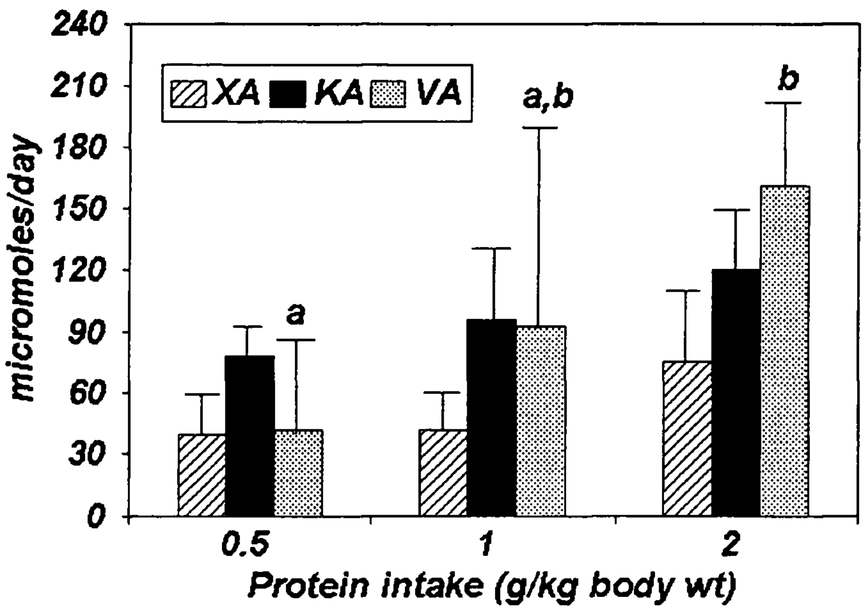

Figure 4.3 Postload urinary excretion of XA, KA and VA (means ± SD). Different letters denote means that are significantly different among protein levels ($P < 0.05$).

excretion was significantly different between the high and low protein diets ($P < 0.05$). There was no effect of protein intake on the basal excretion of tryptophan metabolites (Fig. 4.3).

The mean percent stimulation of EALT and EAST increased as protein level increased (**Fig. 4.4**), but there were no significant differences among the three experimental periods. Basal aminotransferase activities also were unaffected by protein intake. Fecal vitamin B-6 excretion was not affected by protein intake: the means (± SD) on the low, medium and high protein diets were 3.97 ± 0.81, 4.37 ± 0.76 and 4.32 ± 0.55 μmol/d, respectively.

Table 4.3 shows the number of subjects with status indicator values indicating less than adequate vitamin B-6 status, using values suggested by Leklem (1990), at

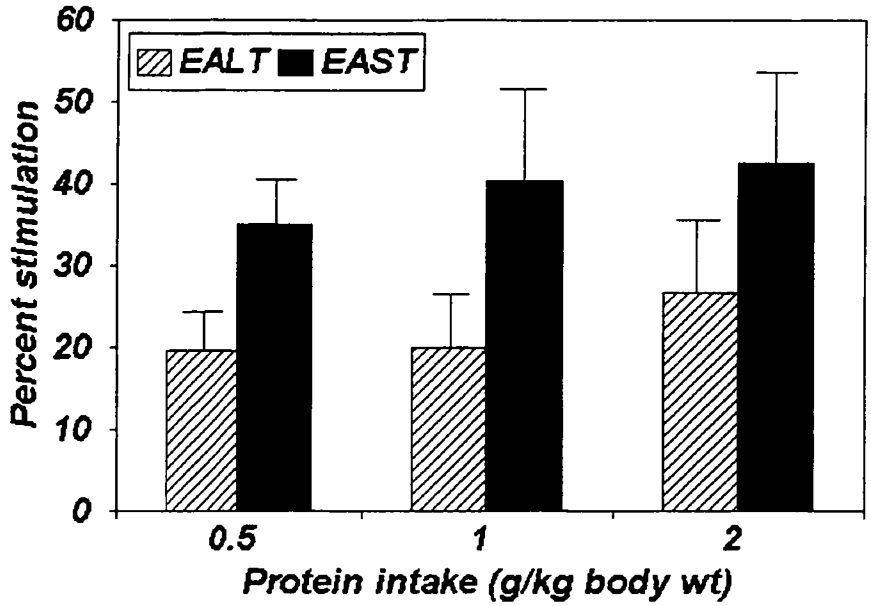

Figure 4.4 Percent stimulation of EALT and EAST (means ± SD).

Table 4.3 Percentage of subjects with vitamin B-6 status indicator values indicating less than adequate status at each level of dietary protein[1,2]

Protein intake, g/kg body wt. B6[3]:protein, mg/g	0.5 0.041	1.0 0.020	2.0 0.010
Urinary 4PA	11	22	78
Urinary B6	56	56	67
Plasma PLP	33	67	78
EALT	0	33	44
EAST	0	0	0
XA	11	11	44

[1] Using values suggested by Leklem (1990).
[2] Total $n=9$ for all indicators except $n=6$ for XA.
[3] Abbreviations: 4PA, 4-pyridoxic acid; B6, total vitamin B-6; EALT, erythrocyte alanine aminotransferase % stimulation; EAST, erythrocyte aspartate aminotransferase % stimulation; PLP, pyridoxal 5′-phosphate; XA, urinary xanthurenic acid excretion after an oral 2 g L-tryptophan load.

each level of dietary protein. At the lowest protein intake, the majority of subjects had adequate vitamin B-6 status indicator values, except for urinary total B6. At the highest level of protein intake, the majority of subjects had inadequate vitamin B-6 status indicator values.

The correlations between dietary nitrogen and the vitamin B-6 status indicators measured in this study are shown in **Table 4.4**. All of the status indicators measured, except UB6 and EAST percent stimulation, were significantly correlated with nitrogen intake. The strongest negative correlation ($P < 0.001$) was between dietary nitrogen and urinary 4PA excretion. Plasma PLP concentrations were also significantly negatively correlated ($P < 0.01$) with nitrogen intake. Urinary excretion of VA after a tryptophan load showed a strong positive correlation ($P < 0.01$) with nitrogen intake. Postload urinary excretion of XA and KA and EALT percent stimulation were also positively correlated ($P < 0.05$) with nitrogen intake.

Table 4.4 Correlation coefficients (r) between vitamin B-6 status indicators and dietary nitrogen[1]

	U4PA[2]	UB6	PLP	XA	KA	VA	EALT	EAST
r	-0.619[3,4]	-0.371[4]	-0.549[4,5]	0.535[6,7]	0.563[4,6]	0.626[5,7]	0.418[6,8]	0.344[8]

[1] n = 27 (9 subjects x 3 intake levels)

[2] Abbreviations: U4PA, urinary 4-pyridoxic acid; UB6, urinary total vitamin B-6; PLP, plasma pyridoxal 5'-phosphate; XA, urinary xanthurenic acid excretion following a 2 g L-tryptophan load; KA, post-load urinary excretion of kynurenic acid; VA, post-load urinary excretion of volatile amines; EALT, erythrocyte alanine aminotransferase % stimulation; EAST, erythrocyte aspartate aminotransferase % stimulation.

[3] $P \leq 0.001$

[4] Multiplicative model: $Y=aX^b$

[5] $P \leq 0.01$

[6] $P \leq 0.05$

[7] Exponential model: $\ln Y=aX+b$

[8] Linear model: $Y=aX+b$

The mean nitrogen balance (nitrogen intake minus urinary and fecal nitrogen excretion) of the subjects was -0.64 $\pm$ 0.41 g/d on the low protein diet, 0.51 $\pm$ 0.63 g/d on the medium protein diet and 2.65 $\pm$ 1.72 g/d on the high protein diet. Nitrogen losses other than urinary and fecal were not taken into account. All subjects were in negative nitrogen balance on the low protein diet. All but one subject were in nitrogen equilibrium or positive balance during the period of medium protein intake, and all were in positive nitrogen balance on the high protein diet.

Discussion

The effect of dietary protein on the vitamin B-6 indicators measured in this study in young women is qualitatively similar to the effect seen in the previous study in men (Miller et al. 1985). As in the study in men, urinary excretion of 4PA was inversely correlated with protein intake (Table 4.4). The percentage of dietary vitamin B-6 excreted as urinary 4PA and vitamin B-6 decreased as protein intake increased, just as it did in men (Miller et al. 1985). This suggests that when the subjects were fed higher protein diets, more vitamin B-6 was being retained in the tissues to supply PLP for the PLP-dependent enzymes that catabolize excess dietary amino acids. This is in agreement with the results of the study by Pannemanns et al. (1994), who also found a lower urinary 4PA excretion in young adults fed a higher protein diet. However, in the older subjects in their study urinary excretion of 4PA was significantly higher when fed a higher level of protein.

Compared to men, the women subjects excreted a greater percentage of the vitamin B-6 intake as 4PA (53, 46 and 36% vs. 45, 38 and 30% for the low, medium and high protein diets, respectively). At a vitamin B-6 intake of 1.25 mg/d (7.42 μmol/d), a dietary vitamin B-6 to protein ratio of 0.010 mg/g was not sufficient to produce levels of excretion indicative of adequate status (Leklem 1990). In elderly women subjects (Ribaya-Mercado et al. 1991), a dietary vitamin B-6 to protein ratio of 0.017 mg/g was not sufficient to return urinary 4PA excretion to predepletion levels, but 0.024 mg/g did normalize urinary 4PA excretion. In vitamin B-6 depleted young women (Kretsch et al. 1995), a B6 to protein ratio of 0.015 mg/g was required to restore urinary 4PA excretion to predepletion levels. At the level of vitamin B-6 intake used in this study, a dietary vitamin B-6 to protein ratio of 0.020 mg/g was sufficient to achieve urinary 4PA excretion that indicates adequate vitamin B-6 status. Urinary total vitamin B-6 excretion was not responsive to changes in protein intake in this study nor in the study in men (Miller et al. 1985), which may limit its usefulness as an indicator of vitamin B-6 status.

The plasma concentration of PLP was negatively correlated with protein intake in women, as was seen previously in men. The mean plasma PLP concentrations at the three levels of dietary protein were, however, lower than plasma PLP concentrations in men with similar dietary vitamin B-6 to protein ratios (37.6, 28.8 and 23.4 vs. 44, 33 and 28 nmol/L on the low, medium and high protein diets, respectively), but the vitamin B-6 intake of the men was 28% higher [1.6 mg (9.47 μmol)/d]. Elderly women have also exhibited lower PLP concentrations than elderly men at similar dietary vitamin B-6 to protein ratios (Ribaya-Mercado et al. 1991). Shultz and Leklem (1981) found that plasma PLP concentrations in women with self-selected diets were lower than those of men with similar dietary vitamin B-6 to protein ratios. Women and men respond similarly to changes in protein intake, but plasma PLP concentrations of women are lower than those of men with similar dietary vitamin B-6 to protein ratios.

Although the use of plasma PLP concentration as a vitamin B-6 status indicator has been questioned (Leklem and Reynolds 1988), the significant correlation with dietary vitamin B-6 to protein ratio found in this investigation supports its use. The mean plasma PLP concentrations on the medium and high protein diet were lower than the value considered indicative of adequate vitamin B-6 status (Leklem 1990). In addition, a dietary vitamin B-6 to protein ratio of 0.020 mg/g was insufficient to achieve plasma PLP concentrations of greater than 30 nmol/L in 6 of the 9 subjects.

In elderly women (Ribaya-Mercado et al. 1991), a dietary vitamin B-6 to protein ratio of 0.017 mg/g was not enough to restore PLP concentrations to predepletion levels, but 0.024 mg/g was sufficient. A dietary B6 to protein ratio of 0.010 to 0.015 mg/g was required to restore baseline plasma PLP concentrations (25.4 $\pm$ 10.6 nmol/L) in B6-depleted young women (Kretsch et al. 1995). In the present study, based on plasma PLP concentrations, a dietary vitamin B-6 to protein ratio of greater than 0.020 mg/g was necessary to achieve adequate vitamin B-6 status with a vitamin B-6 intake of 1.25 mg/day (7.42 μmol/d). However, in a previous study in women (Hansen et al. 1991), with an intake of 1.7 mg vitamin B-6, a dietary vitamin B-6 to protein ratio of 0.020 mg/g was sufficient to produce mean plasma PLP concentrations indicative of adequate status. This suggests that with vitamin B-6 intakes at or above the RDA, a dietary vitamin B-6 to protein ratio of 0.020 mg/g is sufficient to produce plasma PLP values indicative of adequate vitamin B-6 status, but with lower vitamin B-6 intakes, a higher dietary vitamin B-6 to protein ratio may be required.

While plasma PLP is considered a direct measure of vitamin B-6 status, tryptophan metabolites have been used frequently as a functional and indirect indicator of vitamin B-6 status (Baker et al. 1964, Leklem et al. 1975, Miller & Linkswiler 1967, Shin & Linkswiler 1974, Yess et al. 1964). Although no effect of protein on excretion of tryptophan metabolites following a 2 g L-tryptophan load was found in men (Miller et al. 1985), in women postload excretion of XA, KA and VA were all were positively correlated with protein intake (Table 4.4). The women also excreted significantly greater amounts of VA after a 2 g L-tryptophan load on the high protein diet than on the low protein diet.

In men, only 1 of 8 subjects exhibited abnormal tryptophan metabolism in response to a 2 g L-tryptophan load on the high protein diet (Miller et al. 1985), but 4 of 6 women subjects exhibited abnormal tryptophan metabolism (excretion of > 65 μmol XA/d) at a similar dietary vitamin B-6 to protein ratio. Other investigators have reported that women excreted larger amounts of XA than men and developed abnormalities in tryptophan metabolism faster than men when fed a vitamin B-6 deficient diet (Leklem et al. 1975, Miller and Linkswiler 1967, Ribaya-Mercado et al. 1991, Shin and Linkswiler 1974, Yess et al. 1964). Compared to men (Miller et al. 1985), the women subjects excreted larger amounts of tryptophan metabolites after a 2 g L-tryptophan load than men, at all three levels of dietary protein. It is possible this may be a consequence of body weight differences between men and women (the tryptophan load was not adjusted for body weight) or menstrual cycle effects, which were not taken into account. Of the tryptophan metabolites measured, postload urinary excretion of VA was the most responsive to changes in protein intake.

With respect to requirement, post-tryptophan load excretion of XA in young women depleted of vitamin B-6 (Kretsch et al. 1995) was restored to predepletion levels with repletion of 0.015 mg B6/g protein. At the vitamin B-6 intake used in this study, a dietary vitamin B-6 to protein ratio of 0.020 mg/g was required to produce a mean postload excretion of XA indicating adequate vitamin B-6 status.

Another indirect measure that has been used to evaluate requirements and status is erythrocyte aminotransferase activity coefficient or percent stimulation (Aly et al. 1971, Donald et al. 1971, Brown et al. 1975). Erythrocyte aminotransferase activities were not measured in the study in men. In this study, EALT percent stimulation was positively correlated with protein intake (Table 4.4), even with relatively short experimental periods.

In B6-depleted young women (Kretsch et al. 1995), EALT activity coefficient was restored to predepletion levels when a dietary B6 to protein ratio of 0.015 mg/g as fed for 21 days, but the EAST activity coefficient was not normalized until 0.020 mg B6/g protein was fed. In the present study, the mean EALT percent stimulation indicated less than adequate status at a vitamin B-6 to protein ratio of 0.010 mg/g, but indicated adequate status at a dietary vitamin B-6 to protein ratio of 0.020 mg/g. In contrast, the EAST percent stimulation was not affected by protein intake. The EALT percent stimulation is considered to be more sensitive than EAST percent stimulation as an indicator of vitamin B-6 status (Leklem 1990), which is supported by these results. Because of the length of the lifespan of a red blood cell, there may have been further change seen in the aminotransferase indicators if the experimental periods had been longer. Basal activities did not differ with changes in protein intake, and so do not account for the observed differences in percent stimulation.

The women subjects' mean fecal excretion of vitamin B-6 was about 20% greater than the men's (Miller et al. 1985). This may be accounted for, in part, by the inclusion of shredded wheat in the women's diet, which has been reported to reduce the bioavailability of vitamin B-6 (Bills 1991). The effect of this potential reduction in bioavailability, however, would have been the same across all three levels of protein intake. Reduced urinary 4PA excretion would be an expected effect of reduced bioavailability, which makes the greater 4PA excretion of the women compared to men even more noteworthy.

In summary, compared to men, the women subjects excreted a greater percentage of vitamin B-6 intake as 4PA, had lower plasma PLP concentrations and excreted greater amounts of postload urinary tryptophan metabolites at all three levels of protein intake. In addition, women exhibited abnormal tryptophan metabolism on the high protein diet and the men did not. These results suggest that increased protein intake has a greater effect on the vitamin B-6 status of women than it does on the status of men. This calls into question using the same dietary vitamin B-6 to protein ratio for men and women when setting the RDA for vitamin B-6.

The status indicators most responsive to changes in protein intake were urinary excretion of 4PA, plasma PLP concentration and urinary excretion of VA following a 2 g L-tryptophan load. At a dietary vitamin B-6 to protein ratio of 0.010 mg/g, plasma PLP concentrations and urinary 4PA acid excretion indicated less than adequate status in a majority of the subjects. When the dietary vitamin B-6 to protein ratio was 0.020 mg/g, 6 of the 9 subjects still had inadequate PLP concentrations. If plasma PLP alone was used as an indicator of vitamin B-6 status, this would suggest that even 0.020 mg vitamin B-6/g dietary protein did not meet the subjects' vitamin B-6 requirements.

Application of the results presented here to the vitamin B-6 requirements of women must take into account average vitamin B-6 intakes and the bioavailability of vitamin B-6 in typical diets. A recent survey (Kant and Block 1990) reported the average vitamin B-6 intake of women was 1.14 mg/d (71% of the RDA), and that more than one-third of women had dietary vitamin B-6 to protein ratios of less than 0.015 mg/g. The vitamin B-6 intake in this study was about 78% of the RDA for women and thus is similar to the average intake reported by Kant and Block (1990). About 20% of the vitamin B-6 intake during the experimental diet periods was pyridoxine glucoside, a form of vitamin B-6 with a reported 58% bioavailability in humans (Gregory et al. 1991). The estimated percentage of PNG in a normal mixed diet is 10-15% (Andon et al. 1989, Gregory et al. 1991). The higher percentage of the vitamin B-6 as PNG in the diet may account, in part, for the low urinary total vitamin B-6 excretion reported here. The results of this study suggest that with a vitamin B-6 intake of 1.25 mg/d, a dietary vitamin B-6 to protein ratio of at least 0.020 mg/g is required to produce levels of vitamin B-6 status indicators indicating adequate vitamin B-6 status in young women.

Further research evaluating the vitamin B-6 status of women with vitamin B-6 intakes at or above the RDA and varying dietary vitamin B-6 to protein ratios is needed to determine the relative quantitative importance of the effect of vitamin B-6 intake vs. the effect of dietary vitamin B-6 to protein ratio on vitamin B-6 status in women.

Acknowledgements

We thank Jim Ridlington and Karin Hardin for their technical assistance.

Literature cited

Aly, H.E., Donald, E.A. & Simpson, M.H.W. (1971) Oral contraceptives and vitamin B-6 metabolism. Am. J. Clin. Nutr. 24: 297-303.

Andon, M.B., Reynolds, R.D., Moser-Veillon, P.B. & Howard, M.P. (1989) Dietary intake of total and glycosylated vitamin B-6 and vitamin B-6 nutritional status of unsupplemented lactating women and their infants. Am. J. Clin. Nutr. 50: 1050-1058.

Arend, R.A., Leklem, J.E. & Brown, R.R. (1970) Direct and steam distillation autoanalyzer methods for assay of diazotizable aromatic amine metabolites of tryptophan in urine and in serum. Biochem. Med. 4: 457-468.

Baker, E.M., Canham, J.E., Nunes, W.T., Sauberlich, H.E. & McDowell, M.E. (1964) Vitamin B_6 requirements for adult men. Am. J. Clin. Nutr. 15: 59-66.

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

Bills, N.D. (1991) In Vivo and In Vitro Determination of the Bioavailability of Vitamin B-6 from Plant Foods Containing Pyridoxine Glucoside. Doctoral thesis, Oregon State University.

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

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

Donald, E.A., McBean, L.D., Simpson, M.H.W., Sun, M.F. & Aly, H.E. (1971) Vitamin B-6 requirement of young adult women. Am. J. Clin. Nutr. 24: 1028-1041.

Georgia, J.D. (1974) Nonprotein nitrogenous constituents. In: Clinical Chemistry: Principles and Technics, 2nd ed. (Henry, R.J., Cannon, D.C. & Winkleman, J.W., eds.), pp. 504-563. Harper and Row, New York, N.Y.

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. Agri. Food Chem. 35: 76-82.

Gregory, J.F. & Kirk, J.R. (1979) Determination of urinary 4-pyridoxic acid using high performance liquid chromatography. Am. J. Clin. Nutr. 32: 879-893.

Gregory, J.F., Trumbo, P.R., Bailey, L.B., Toth, J.P., Baumgartner, T.B. & Cerda, J.J. (1991) Bioavailability of pyridoxine-5-ß-D-glucoside determined in humans by stable isotopic methods. J. Nutr. 121: 177-186.

Hansen, C., Leklem, J., Hardin, K. & Miller, L. (1991) Vitamin B-6 status of women with constant protein intake and four levels of vitamin B-6. FASEB J 6:A1374 (abstr).

Harper, A.E. (1968) Diet and plasma amino acids. Am. J. Clin. Nutr. 21: 358-366.

Horwitz, G.W., ed. (1980) Official methods of analysis of the AOAC, 13th ed., pp.768. Washington, D.C.

Hrboticky, N., Leiter, L.A. & Anderson, G.H. (1989) Menstrual cycle effects on the metabolism of tryptophan loads. Am. J. Clin. Nutr. 50: 46-52.

Kretsch, M.J., Sauberlich, H.E., Skala, J.H. & Johnson, H.L. (1995) Vitamin B-6 requirement and status assessment: young women fed a depletion diet followed by a plant- or animal-protein diet with graded levels of vitamin B-6. Am. J. Clin. Nutr. 61: 1091-1101.

Leklem, J.E. (1990) Vitamin B-6: a status report. J. Nutr. 120: 1503-1507.

Leklem, J.E. (1991) Vitamin B-6. In: Handbook of Vitamins, 2nd edition (Machlin, L., ed.), pp. 341-392. Marcel Dekker, New York, N.Y.

Leklem, J.E., Brown, R.R., Rose, D.P., Linkswiler, H. & Arend, R.A. (1975) Metabolism of tryptophan and niacin in oral contraceptive users receiving controlled intakes of vitamin B_6. Am. J. Clin. Nutr. 28: 146-156.

Leklem, J.E. & Reynolds, R.D. (1988) Challenges and directions in the search for clinical applications of vitamin B-6. In: Clinical and Physiological Applications of Vitamin B-6 (Leklem, J.E. & Reynolds, R.D., eds.), pp. 437-454. A.R. Liss, New York, N.Y.

Merrill, A.H. & Henderson, J.M. (1987) Diseases associated with defects in vitamin B-6 metabolism or utilization. Ann. Rev. Nutr. 7: 137-156.

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

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: 1663-72.

Miller, L.T. & Linkswiler, H. (1967) Effect of protein intake on the development of abnormal tryptophan metabolism by men during vitamin B_6 depletion. J. Nutr. 93: 53-59.

National Research Council (1980) Recommended Dietary Allowances, 9th edition, pp. 96-106. National Academy Press, Washington, D.C.

National Research Council (1989) Recommended Dietary Allowances, 10th edition, pp.142-50. National Academy Press, Washington, D.C.

Pannemanns, D.L.E., van den Berg, H. & Westerterp, K.R. (1994) The influence of protein intake in vitamin B-6 metabolism differs in young and elderly humans. J. Nutr. 124: 1207-1214.

Pino, S., Bennotte, J. & Gordnya, H. (1965) An automated method for urine creatinine which does not require a dialyzer module. Clin. Chem. 11: 664-666.

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: 159-225.

Ribaya-Mercado, J.D., Russell, R.M., Sahyoun, N., Morrow, F.D. & Gershoff, S.N. (1991) Vitamin B-6 requirements of elderly men and women. J. Nutr. 121: 1062-1074.

Roy, A.V. (1970) Rapid method for determining alkaline phosphatase activity in serum with thymolpthalein monophosphate. Clin. Chem. 16: 431-436.

Scales, F.M. & Harrison, A.P. (1929) Boric acid modification of the Kjeldahl method for crop and soil analysis. Ind. Eng. Chem. 12: 350.

Shin, H.K. & Linkswiler H.M. (1974) Tryptophan and methionine metabolism of adult females as affected by vitamin B-6 deficiency. J. Nutr. 104: 1348-1355.

Shultz, T.D. & Leklem, J.E. (1981) Urinary 4-pyridoxic acid, urinary vitamin B-6 and plasma pyridoxal phosphate as measures of vitamin B-6 status and dietary intake of adults. In: Methods in Vitamin B-6 Nutrition (Leklem, J.E. & Reynolds, R.D., eds.), pp.297-320. Plenum Press, New York, N.Y.

Waldorf, M.A., Kirk, M.C., Linkswiler, H. & Harper, A.E. (1963) Metabolic adaptations in higher animals. VII. Responses of glutamate-oxaloacetate and glutamate-pyruvate transaminases to diet. Proc. Soc. Exp. Biol. Med. 112: 764-768.

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 B_6 metabolism. J. Clin. Invest. 76: 752-756.

Woodring, M.J. & Storvick, C.A. (1970) Effect of pyridoxine supplementation on glutamic-pyruvic transaminase and in vitro stimulation in erythrocytes of normal women. Am. J. Clin. Nutr. 23: 1385-1395.

Yess, N., Price, J.M., Brown, R.R., Swan, P.B. & Linkswiler, H. (1964) Vitamin B_6 depletion in man: urinary excretion of tryptophan metabolites. J. Nutr. 84: 229-236.

CHAPTER 5

VITAMIN B-6 STATUS OF WOMEN FED DIETS OF HIGH AND LOW PYRIDOXINE GLUCOSIDE CONTENT

Christine M. Hansen, James E. Leklem and Lorraine T. Miller

To be submitted to the *Journal of Nutrition,*
American Institute of Nutrition,
Bethesda, MD

Abstract

Previous research has shown that the pyridoxine glucoside (PNG) form of vitamin B-6 has a reduced bioavailability compared to pyridoxine, but its effect on vitamin B-6 status has not been assessed. Following a 8-day adjustment period, 9 women were fed diets containing a high or low amount of PNG for 18 days each, in a crossover design. The high and low PNG diets provided 1.52 mg/d (8.98 μmol/d) and 1.44 mg/d (8.57 μmol/d) vitamin B-6, of which 27% and 9% was PNG, respectively. The dietary vitamin B-6 to protein ratio of both diets was 0.017 mg/g. Urinary excretion of 4-pyridoxic acid and total vitamin B-6 were significantly lower ($P < 0.05$) during the high PNG diet period than when the low PNG diet was fed. Urinary PNG excretion was equal to about 9% of the total PNG intake during both periods. Plasma total vitamin B-6 ($P < 0.01$) and red blood cell pyridoxal 5′-phosphate (PLP) ($P < 0.05$) were significantly lower when the high PNG diet was fed than during the low PNG diet period. Fecal total vitamin B-6 excretion was significantly higher ($P < 0.001$) when the high PNG diet was fed. Women fed a diet containing a higher percentage of the total vitamin B-6 intake as PNG exhibited a decrease in vitamin B-6 status indicators, consistent with the reduced bioavailability of PNG demonstrated in other studies, equal to a loss of 15-18% of the total vitamin B-6 intake. When setting Recommended Dietary Allowances, the reduced bioavailability of PNG and its presence in higher amounts in some diets should be taken into consideration.

Introduction

Pyridoxine 5′-ß-D-glucoside (PNG) has been identified as a form of vitamin B-6 comprising 5-80% of the total vitamin B-6 content of various fruits and vegetables (Gregory and Ink 1987, Kabir et al. 1983a). In non-vegetarian mixed diets, an estimated 10-15% of the total vitamin B-6 content may be PNG (Andon et al. 1989, Gregory et al. 1991).

In rats, this form of vitamin B-6 has a reported bioavailability of about 20-30% (Ink et al. 1986, Trumbo and Gregory 1988 and 1989, Trumbo et al. 1988). Furthermore, in a recent report of a study in rats, Nakano and Gregory (1995) concluded that PNG alters the metabolism of coingested pyridoxine, possibly through competition at the site of transport into tissues. Using stable isotope methods to determine the bioavailability of PNG in humans, Gregory et al. (1991) concluded that, although the bioavailability of PNG in humans is greater than in rats, it may be as low as 58% of the bioavailability of free pyridoxine. Previous human bioassays in our laboratory indicated that the bioavailability of vitamin B-6 in food is inversely related to its pyridoxine glucoside content (Bills 1991, Kabir et al. 1983b).

Reduced bioavailability of vitamin B-6 may be a concern for women in particular, because the average vitamin B-6 intake of women is reported to be about 71% of the RDA (Kant and Block 1990). The effect of increased pyridoxine glucoside content and, consequently, reduced bioavailability on indices of vitamin B-6 status in women has not been determined. The objective of this study was to determine the effect of diets of low and high pyridoxine glucoside content, composed of commonly consumed foods, on several direct and indirect vitamin B-6 status indicators in women.

Materials and methods

Ten healthy women subjects were recruited from the local community. Subjects were all nonsmokers who were not taking oral contraceptives or any medications known to have an effect on vitamin B-6 metabolism (Bhagavan 1985). Their mean age ($\pm$ SD) was 29 $\pm$ 6 years, mean height was 160 $\pm$ 8 cm and mean weight was 55.4 $\pm$ 10.1 kg. They were instructed to maintain their usual activity level and kept records of daily activity throughout the study. The study protocol was approved by Oregon State University's Committee for the Protection of Human Subjects and each subject gave informed consent before participating in the study. One subject withdrew from the study after 9 days because of illness.

All meals were prepared and served in our metabolic kitchen. After an 8-day adjustment period, subjects were divided into two groups (one group of 4 and one group of 5 subjects) and fed a diet that was either low or high in pyridoxine glucoside for 18 d each, in a crossover design. The length of the periods was chosen to allow time for vitamin B-6 indicators to plateau, which has taken 7 or 8 days in previous studies with similar vitamin B-6 intakes (Kabir et al. 1983c, Lee and Leklem 1985, Leklem et al. 1980b, Lindberg et al. 1983).

The foods comprising the low and high pyridoxine glucoside diets are given in **Table 5.1**. In addition to the foods listed, subjects consumed varying amounts of

Table 5.1 Experimental diets

Food	Amount
HIGH PYRIDOXINE GLUCOSIDE DIET[1]	g
Breakfast	
Orange juice, prepared from frozen	200
Oat bran cereal, uncooked weight	30
Whole wheat bread	14
Milk, 2% milkfat	200
Gelatin[2]	12.5
Lunch	
Whole wheat bread	75
Peanut butter	50
Salad	
Lettuce, iceberg	20
Red cabbage, raw	10
Celery, raw	40
Carrot, raw	20
Peaches, canned, light syrup	100
Carrot, raw	30
Tomato juice, canned	100
Dinner	
Potato, dehydrated flakes	25
Turkey, breast meat	40
Apricots, dried	30
Red kidney beans, canned	80
Gelatin[2]	12.5
Milk, 2% milkfat	220
Vanilla wafers	12

Continued

Table 5.1, continued

LOW PYRIDOXINE GLUCOSIDE DIET[3]

Breakfast	
Orange juice, prepared from frozen	200
Life cereal[4]	30
Milk, 2% milkfat	200
White bread	37
Pineapple, canned, light syrup	100
Lunch	
Sandwich	
White bread	75
Tuna, water pack, drained	50
Mayonnaise	20
Dill pickle, chopped	10
Egg white, cooked	30
Salad	
Lettuce, iceberg	20
Celery	40
Red cabbage	10
Carrot	10
Apple juice, prepared from frozen	180
Dinner	
White rice, uncooked weight	40
Turkey, breast meat	100
Peas, frozen	80
Pears, canned, light syrup	100
Milk, 2% milkfat	250
Gelatin[2]	12
Vanilla wafers	12
Popcorn	15

[1] High pyridoxine glucoside diet provided 90 g protein, 249 g carbohydrate, 45 g fat, 1.52 mg vitamin B-6 (27% as pyridoxine glucoside).

[2] Gelatin was dissolved in water and flavored with a powdered drink mix concentrate.

[3] Low pyridoxine glucoside diet provided 84 g protein, 260 g carbohydrate, 42 g fat, 1.44 mg vitamin B-6 (9% as pyridoxine glucoside).

[4] Quaker Oats, Chicago, IL.

margarine, salad dressing, sugar and hard candy to meet their energy needs and maintain a constant body weight. The adjustment diet provided 72 g protein and 0.958 mg vitamin B-6. An oral pyridoxine hydrochloride solution given in equally divided doses at breakfast and dinner brought the total vitamin intake to 1.33 mg/d (7.86 μmoles/d), 13% of which was pyridoxine glucoside (0.99 μmoles). The experimental diets were designed to provide 90 g protein and 1.6 mg (9.46 μmoles) vitamin B-6 using food composition tables (Food Processor II, ESHA research, Salem, OR). By laboratory analysis, the high pyridoxine glucoside diet contained 90 g protein (g N X 6.25) and 1.52 mg (8.98 μmoles) vitamin B-6 [27% pyridoxine glucoside (2.42 μmoles)], and the low pyridoxine glucoside diet provided 84 g protein and 1.44 mg (8.57 μmoles) vitamin B-6 [9% pyridoxine glucoside (0.76 μmoles)]. The vitamin B-6 to protein ratio for both experimental diets was 0.017 mg/g. Based on values calculated using Food Processor II (ESHA Research, Salem, OR), the dietary fiber content of the adjustment and low PNG diets was 18 g, while the high PNG diet provided 34 g dietary fiber. The adjustment, high PNG and low PNG diets provided 1.64, 1.59 and 2.32 mg riboflavin, respectively.

Composites of the diets were made weekly and analyzed for vitamin B-6 (Horowitz 1980) and pyridoxine glucoside (Kabir et al. 1983a) content using *Saccharomyces uvarum*. Twenty four hour urine collections from each day of the study were assessed for completeness and evidence of adherence to the diet by measurement of creatinine (Pino et al. 1965) and urea nitrogen (Georgia 1974) by automated procedures. Urinary 4-pyridoxic acid (4PA) was determined by a modification of an HPLC procedure (Gregory and Kirk 1979). Interassay coefficient of variation was 9% and recovery of added 4PA averaged 94.5 ± 5.0%. Each subject's samples were run in one assay or two consecutive assays to minimize the effect of interassay variation. Urinary total vitamin B-6 (UB6) (Miller and Edwards 1981) and pyridoxine glucoside (PNG) (Kabir et al. 1983a) were measured by microbiological procedures using *Saccharomyces uvarum*. Urinary 4PA excretion was averaged over the last three days and UB6 excretion over the last two days of each period to compensate for any day-to-day variation.

Fasting blood was drawn every 3 to 6 days, for a total of two times during the adjustment period and four times during each of the experimental periods. Whole blood was removed immediately for determination of hematocrit and hemoglobin concentration. Samples were then centrifuged at 4°, and the plasma and red blood cells separated. Red blood cells were washed three times with ice-cold saline, samples were removed for the aminotransferase activity assay, and the plasma and remaining red blood cells were frozen separately at -30° until analysis.

Plasma and red blood cell pyridoxal 5′-phosphate (PLP) concentration was determined by a tyrosine decarboxylase apoenzyme-isotopic procedure (Chabner & Livingston 1970). Samples were assayed in duplicate and if values for the same sample differed by more than 5%, analysis was repeated. Interassay coefficient of variation was 7% for plasma and 8.6% for red blood cells. Recovery of added PLP averaged 91.3 ± 7.0% for plasma and 66.5 ± 8.2% for red blood cells. Plasma total vitamin B-6 was assayed by a microbiological procedure using *Saccharomyces uvarum* (Miller and Edwards 1981).

Erythrocyte aspartate and alanine aminotransferase (EAST, EALT) activity with and without PLP stimulation was measured on fresh erythrocytes by the method of Woodring and Storvick (1970), using a 0.033 M Tris buffer (pH 7.4) instead of 0.1 M potassium phosphate. Percent stimulation was calculated by the equation: % stimulation = (activity w/added PLP - activity w/o added PLP) ÷ activity w/o added PLP. For all blood vitamin B-6 status indicators, the last measurement in each period was used in the statistical analyses.

Twice during each of the two experimental periods, subjects were given a fecal marker (50 g FD&C Brilliant Blue No. 1 mixed with 200 mg methylcellulose in a gelatin capsule) with breakfast at the beginning and end of a 3- or 4-day period. Complete 3- or 4-day fecal collections were analyzed for total vitamin B-6 (Miller and Edwards 1981) using *Saccharomyces uvarum* and the average daily fecal total vitamin B-6 excretion was calculated for each subject.

Statistical analyses were done using STATGRAPHICS (Statistical Graphics Corp., Rockville MD). Analysis of variance showed no significant effect of order of diets on any of the status indicators measured. Means of status indicators at the end of the periods of high and low PNG intake were compared using paired t tests and were considered significantly different at $P \leq 0.05$. Regression analysis was performed and Pearson correlation coefficients between vitamin B-6 status indicators were calculated.

Results

The means for hematocrit and hemoglobin concentration, and subjects' body weight were not significantly different between the two experimental diet periods (data not reported here). The mean urinary creatinine excretion was 1.10 $\pm$ 0.17 g/d on the high PNG diet and 1.21 $\pm$ 0.13 g/d on the low PNG diet (averaged over the entire diet period), reflecting the higher meat intake on the low PNG diet. The mean urea nitrogen excretion was 9.80 $\pm$ 1.25 g/d on the high PNG diet and 8.87 $\pm$ 0.83 g/d on the low PNG diet (averaged over the entire diet period), reflecting the slightly higher protein intake on the high PNG diet.

The mean urinary 4PA excretion was 10% lower (0.42 μmoles less) when the high PNG diet was fed than when the low PNG diet was fed (**Table 5.2**), and this difference was statistically significant ($P < 0.05$). Eight of the 9 subjects were excreting less urinary 4PA at the end of the high PNG diet period than at the end of the low PNG diet period. The mean percentage of vitamin B-6 intake excreted as urinary 4PA and as urinary total B6, and the percentage of PNG intake excreted as urinary PNG in each of the experimental periods is listed in Table 5.2.

The mean urinary total vitamin B-6 excretion was 12% lower (0.07 μmoles less) with the high PNG diet than with the low PNG diet (Table 5.2), and this difference was statistically significant ($P < 0.04$). Seven of the 9 subjects excreted less urinary total vitamin B-6 at the end of the high PNG diet period than at the end of the low PNG diet period.

Table 5.2 Urinary vitamin B-6 status indicators

Indicator		Low PNG[1,2]	High PNG[2]	Expected High PNG[3]	Difference from expected[4]	% of intake loss[5]
U4PA						
	μmol/d	4.02 ± 0.58	3.60 ± 0.84	4.26	0.62	
	% B6 intake	47.5 ± 6.8	40.1 ± 9.3			15
UB6						
	μmol/d	0.660 ± 0.116	0.587 ± 0.082	0.692	0.105	
	% B6 intake	7.7 ± 1.3	6.5 ± 0.9			15
UPNG						
	μmol/d	0.075 ± 0.024	0.216 ± 0.046	0.239		
	% PNG intake	9.9 ± 3.2	8.8 ± 2.0			
Non-PNG UB6[6]						
	μmol/d	0.586 ± 0.103	0.371 ± 0.085	0.492	0.121	
	% non-PNG B6 intake	7.5 ± 1.3	5.7 ± 1.3			18

[1] Abbreviations: B6, vitamin B-6; PNG, pyridoxine glucoside; U4PA, urinary 4-pyridoxic acid excretion; UB6, urinary total vitamin B-6 excretion; UPNG, urinary PNG excretion.

[2] Means ± SD.

[3] % of B6 intake excreted on Low PNG diet X B6 intake on High PNG diet.

[4] Expected excretion minus actual excretion during High PNG diet.

[5] Difference from expected ÷ % B6 intake excreted on Low PNG diet.

[6] UB6 minus UPNG.

The mean urinary excretion of PNG on the high PNG diet was nearly three times the excretion on the low PNG diet (Table 5.2), reflecting the three-fold difference in PNG intake, and the difference was statistically significant ($P < 0.001$). The mean percentage of PNG intake excreted as urinary PNG was similar for the two diets (Table 5.2).

The mean plasma total vitamin B-6 concentration was 18% lower at the end of the high PNG diet period than at the end of the low PNG diet period (**Figure 5.1**), and the difference was statistically significant ($P < 0.01$). Eight of the 9 subjects had plasma total vitamin B-6 concentrations that were lower on the high PNG diet than on the low PNG diet.

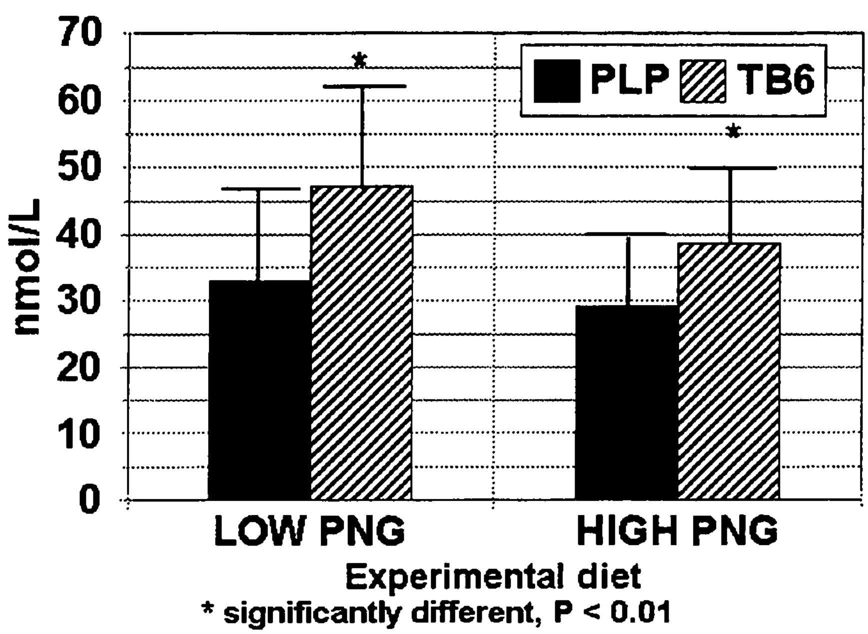

Figure 5.1 Plasma PLP and TB6 concentrations (means ± SD).

The mean plasma PLP concentration at the end of the high PNG diet period was 12% lower than the concentration at the end of the low PNG diet period (Figure 5.1), but the difference was not statistically significant. Seven of the 9 subjects had a lower plasma PLP concentration on the high PNG diet than on the low PNG diet. Plasma PLP was 74.5 ± 10.8% of the plasma total vitamin B-6 concentration on the high PNG diet and 69.3 ± 8.4% on the low PNG diet.

The mean red blood cell PLP concentration was 17% lower at the end of the high PNG diet period than at the end of the low PNG diet period (**Figure 5.2**), and the difference was statistically significant ($P < 0.05$). Seven of the 9 subjects had a lower red blood cell PLP concentration on the high PNG diet than on the low PNG

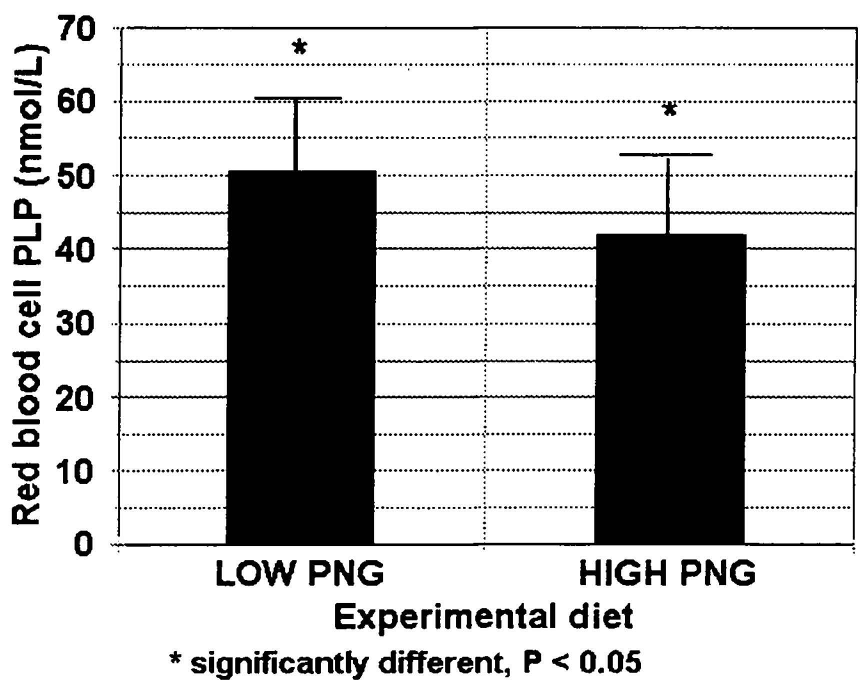

Figure 5.2 Red blood cell PLP concentrations (means ± SD).

diet. The means of EALT and EAST percent stimulation were not different between the two experimental diets (**Figure 5.3**).

The mean daily fecal total vitamin B-6 excretion on the high PNG diet was about 50% greater than the excretion on the low PNG diet (**Figure 5.4**). This difference was statistically significant ($P < 0.001$). All nine subjects had a greater fecal excretion of total vitamin B-6 when fed the high PNG diet compared to fecal excretion during the low PNG diet period. There was no statistically significant difference between the samples collected near the midpoint and the samples collected at the end of either the high or low PNG diet period.

Discussion

The amount of pyridoxine glucoside present in typical mixed diets has been estimated at 10-15% of the total vitamin B-6 content (Andon et al. 1989, Gregory et al. 1991). The effect of the reduced bioavailability of pyridoxine glucoside on vitamin B-6 status is important to consider in determining requirements for vitamin B-6. The bioavailability of pyridoxine glucoside may be nutritionally important in individuals with marginal vitamin B-6 intakes and in vegetarians and others whose vitamin B-6 intake is primarily from plant foods. A recent survey estimated that there are 12 million vegetarians in the United States, an eightfold increase from 1979 to 1992 (Johnston 1994). In a study comparing the vitamin B-6 status of vegetarians and non-vegetarians with similar vitamin B-6 intakes (Shultz and Leklem 1987), mean urinary 4PA and total vitamin B-6 excretion and plasma PLP were lower in the vegetarians (although these differences were not statistically significant). The two experimental

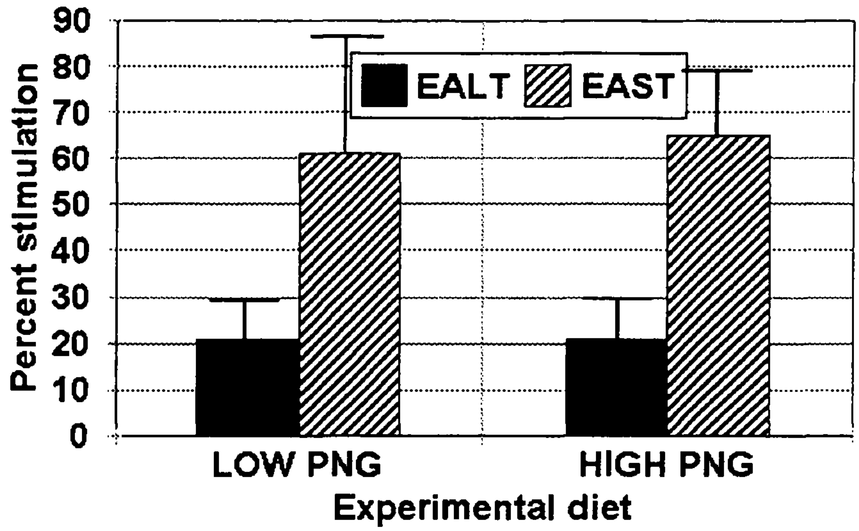

Figure 5.3 Percent stimulation of EALT and EAST (means ± SD).

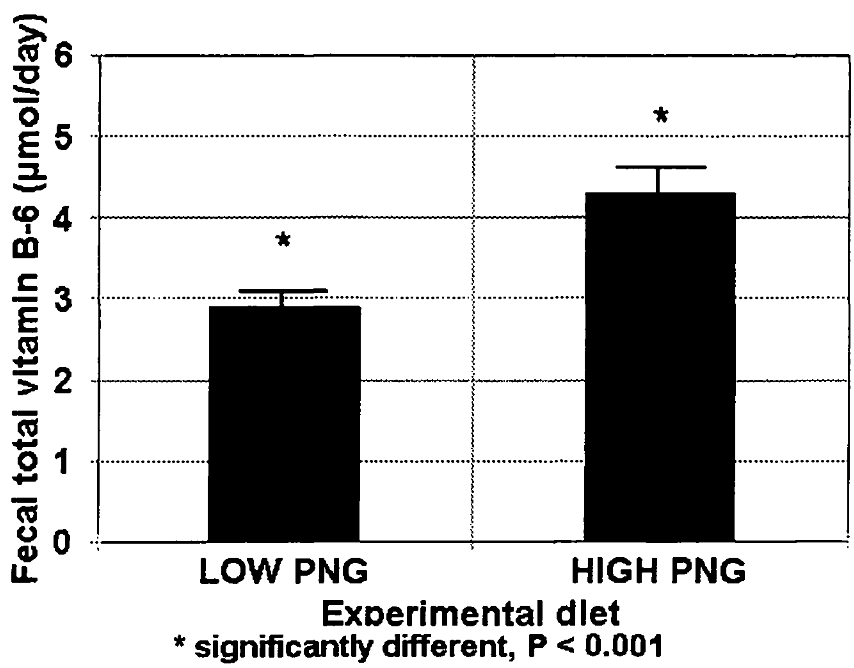

Figure 5.4 Daily fecal total vitamin B-6 (means ± SD).

diets used in this study represent pyridoxine glucoside intakes that should encompass the range that may be found in normal diets, including vegetarian or near-vegetarian diets.

Urinary 4PA is often used as a short term indicator of vitamin B-6 status. As the end-product of vitamin B-6 metabolism, urinary 4PA excretion reflects the major portion of intake that has been absorbed and metabolized. Urinary 4PA excretion was reduced by about 0.42 μmoles/d on the high PNG diet compared to the low PNG diet, even though the vitamin B-6 intake was 0.21 μmoles/d higher on the high PNG diet. Urinary 4-pyridoxic acid excretion on the low PNG diet was 47.5% of the total vitamin B-6 intake, which is within the range that we have seen in previous studies (Hansen et al. 1991 and 1993, Lee and Leklem 1985, Leklem et al. 1991). If a similar amount had been excreted on the high PNG diet, we would have expected an average urinary 4PA excretion of about 4.26 μmoles/d. Thus, urinary 4PA excretion was 0.62 μmoles/d lower than we would have expected, representing a loss of 1.31 μmoles vitamin B-6 intake/d, or 15% of the total vitamin B-6 intake on the high PNG diet (Table 5.2).

Urinary total vitamin B-6 excretion also reflects vitamin B-6 that was absorbed and metabolized (Leklem et. al 1980a). Total urinary B6 excretion was reduced by about 0.073 μmoles/d, even though urinary PNG excretion increased 0.141 μmoles/d. Considering that urinary total vitamin B-6 excretion accounted for about 7.7% of the total vitamin B-6 intake on the low PNG diet, this reduction represents a loss of 1.36 μmoles/d or 15% of the total vitamin B-6 intake (Table 5.2).

About 9-10% of the pyridoxine glucoside intake on both the high and low PNG diets was excreted unchanged in urine. This represented 2.4% of the total vitamin B-6 intake on the high PNG diet and 0.9% of the total intake on the low PNG diet. About 7.5% of the non-PNG vitamin B-6 intake was excreted as non-PNG urinary vitamin B-6 when the low PNG diet was fed and 5.7% during the high PNG diet period. This represents a reduction in non-PNG urinary total vitamin B-6 of 0.119 μmoles/d when the high PNG diet was fed, and suggests that less of the non-PNG portion of the vitamin B-6 intake was being absorbed and/or metabolized. This reduction in non-PNG urinary vitamin B-6 excretion represents a loss of 18% of the total vitamin B-6 intake. If we consider 58% of the PNG to be bioavailable, as Gregory et al (1991) estimated, the increase in PNG intake during the high PNG diet would represent a loss of bioavailability of 0.57 μmoles vitamin B-6 (42% of the 1.36 μmol/d increase). The losses estimated in this study using urinary 4-pyridoxic acid excretion and urinary total vitamin B-6 excretion exceed the loss estimated using a figure of 58% bioavailability and may reflect the competitive effect of PNG on PN utilization reported by Nakano and Gregory (1995).

Another reflection of reduced bioavailability on the PNG diet was the 18% reduction in total plasma vitamin B-6 and the 17% reduction in RBC PLP concentration on the high PNG diet, both of which were statistically significant. The degree of change in RBC PLP was not expected based on other work we have done in which RBC PLP did not change significantly when women were fed 0.8 to 2.4 mg of vitamin B-6 (Hansen et. al 1993). The reduction in red blood cell PLP could be explained, in part, by the reported competition for uptake into tissues between co-ingested PN and PNG found in rats (Nakano and Gregory 1995). The 12% reduction in plasma PLP concentration, though not statistically significant, provides further evidence of the effect of reduced bioavailability on vitamin B-6 status.

When fed the high PNG diet, the subjects' fecal excretion of total vitamin B-6 was 1.41 μmoles more than the fecal excretion on the low PNG diet. The difference in fecal vitamin B-6 excretion between the two experimental diets represents 16% of the total vitamin B-6 intake on the high PNG diet. The source of this difference could be reduced absorption of vitamin B-6 and/or increased synthesis by intestinal microflora. If the difference was due to a change in intestinal microflora, the change would be expected to be gradual over time. However, we found that there was no difference in total fecal vitamin B-6 content between samples taken near the midpoint of the experimental period and those taken near the end, which is evidence against a change in intestinal microflora.

A confounding factor is the difference in dietary fiber between the two experimental diets. The high PNG diet had about twice the dietary fiber of the low PNG diet, a consequence of the increased whole grains, legumes, fruit and vegetables. Previous work in our laboratory suggested the bioavailability of vitamin B-6 could be decreased by up to 17% with the addition of 15 g of wheat bran to the diet (Lindberg et al. 1983) and that the bioavailability of vitamin B-6 from whole wheat bread is 5 to 10% lower than that in white bread with added crystalline pyridoxine (Leklem et al. 1980a). In another study, pectin appeared to stimulate the synthesis of vitamin B-6 by intestinal microorganisms (Miller et al. 1980). This synthesized vitamin B-6 is probably not available to the subjects because their status indices (plasma PLP and total vitamin B-6, urinary 4PA and total vitamin B-6) did not increase while fecal excretion increased.

Other studies in our laboratory have shown that very little of the fecal excretion of vitamin B-6 is as PNG, indicating hydrolysis by intestinal microflora (Kabir et al. 1983b). Evidence of ß-glucosidase activity in human gut microflora has been reported (Mallett et al. 1989). For this reason, we can not determine from our results how much of the PNG intake was not absorbed and was excreted as fecal vitamin B-6.

Only one of the status indicators measured, erythrocyte aminotransferase percent stimulation, failed to respond to an increase in PNG intake. It is possible that a response would have been seen with a longer term change in diet, and this is a question that needs further investigation. Whether a similar change in vitamin B-6 status indicators would be seen in men also needs to be determined. Because we only tested two levels of intake, it cannot be determined from these results whether the changes in status indicators are linear over a range of PNG intake levels.

In summary, a diet composed of natural foods with a high pyridoxine glucoside content reduced urinary total vitamin B-6 and 4PA excretion, plasma total vitamin B-6 and red blood cell PLP, and increased fecal total vitamin B-6 excretion in women, compared to a diet low in pyridoxine glucoside. The decrease in urinary 4PA and urinary total vitamin B-6 excretion on the high PNG diet represent a loss of bioavailability of about 15% of the total vitamin B-6 intake. The reduction in non-PNG urinary total B6 excretion indicates a loss of about 18% of the total vitamin B-6 intake during the high PNG diet period. Thus, there may be a loss of between 15 and

18% of the total vitamin B-6 intake when a high PNG diet is fed. Because diets that are high in pyridoxine glucoside are also high in dietary fiber, which may also reduce the bioavailability of vitamin B-6, the effect on vitamin B-6 status may be important for people with marginal intakes or those consuming diets in which most of the vitamin B-6 intake is from plant foods, especially those foods which are high in PNG content. The long term effect of consuming diets high in pyridoxine glucoside on vitamin B-6 status needs to be investigated. Our results suggest that the RDA for vitamin B-6 needs to take into account the PNG content of the diet.

Acknowledgements

We gratefully acknowledge the technical assistance of Karin Hardin and Jim Ridlington.

Literature cited

Andon, M.B., Reynolds, R.D., Moser-Veillon, P.B. & Howard, M.P. (1989) Dietary intake of total and glycosylated vitamin B-6 and the vitamin B-6 nutritional status of unsupplemented lactating women and their infants. Am. J. Clin. Nutr. 50: 1050-1058.

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

Bills, N.D. (1991) In Vivo and In Vitro Determination of the Bioavailability of Vitamin B-6 from Plant Foods Containing Pyridoxine Glucoside. Doctoral thesis, Oregon State University.

Georgia, J.D. (1974) Nonprotein nitrogenous constituents. In: Clinical Chemistry: Principles and Technics (Henry, R.J., Cannon, D.C. & Winkleman, J.W., eds.), 2nd ed., pp 504-63. Harper and Row, New York, NY.

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: 76-82.

Gregory, J.F. & Kirk, J.R. (1979) Determination of urinary 4-pyridoxic acid using high performance liquid chromatography. Am. J. Clin. Nutr. 32: 879-893.

Gregory, J.F., Trumbo, P.R., Bailey, L.B., Toth, J.P., Baumgartner, T.G. & Cerda, J.J. (1991) Bioavailability of pyridoxine-5′-ß-D-glucoside determined in humans by stable-isotopic methods. J. Nutr. 121: 177-186.

Hansen, C., Leklem, J., Hardin, K. and Miller, L. (1991) Vitamin B-6 status of women with constant protein intake and four levels of vitamin B-6. FASEB J 5: A556(abstr 1080).

Hansen, C., Leklem, J., Miller, L., Hardin, K. and Ridlington, J. (1993) Response of red blood cell pyridoxal 5′-phosphate to short term changes in vitamin B-6 intake. FASEB J 7(4):A728(abstr 4204).

Horwitz, G.W., ed. (1980) Official Methods of Analysis of the AOAC, 13th ed., p. 768. Washington, D.C.

Ink, S.L., Gregory, J.F. & Sartain, D.B. (1986) Determination of pyridoxine ß-glucoside bioavailability using intrinsic and extrinsic labeling in the rat. J Agric. Food Chem. 34: 857-862.

Johnston, P.K. (1994) Second International Congress on Vegetarian Nutrition. Am. J. Clin. Nutr. 59(suppl.): vii.

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

Kabir, H., Leklem J.E. & Miller, L.T. (1983b) Relationship of glycosylated vitamin B-6 content of foods to vitamin B-6 bioavailability in humans. Nutr. Reports Intl. 28: 709-715.

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.

Lee, C.M. & Leklem, J.E. (1985) Differences in vitamin B-6 status indicator responses between young and middle-aged women fed constant diets with two levels of vitamin B-6. Am. J. Clin. Nutr. 42: 226-234.

Leklem, J., Hansen, C. and Miller, L. (1991) Effect of three levels of protein on vitamin B-6 status of adult women. FASEB J 5: A557(abstr 1081).

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., Shultz, T.D. & Miller, L.T. (1980) Comparative bioavailability of vitamin B-6 from soybeans and beef. Fed. Proc. 38: 452(abstr).

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.

Mallett, A.K., Bearne, C.A. & Rowland, I.R. (1989) The influence of incubation pH on the activity of rat and human gut flora enzymes. J. Appl. Bacteriol. 66: 433-437.

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

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

Nakano, H & Gregory, J.F. (1995) Pyridoxine-5′-ß-D-glucoside influences the short-term metabolic utilization of pyridoxine in rats. J. Nutr. 125: 926-932.

Pino, S., Bennotte, J. & Gordnya, H. (1965) An automated method for urine creatinine which does not require a dialyzer module. Clin. Chem. 11: 664-66.

Reddy. S.K., Reynolds, M.S. & Price, J.M. (1985) The determination of 4-pyridoxic acid in human urine. J. Biol. Chem. 233: 691-696.

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

Trumbo, P.R. & Gregory, J.F. (1988) Metabolic utilization of pyridoxine-ß-glucoside in rats: influence of vitamin B6 status and route of administration. J. Nutr. 118: 1336-1342.

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

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

Woodring, M.J. & Storvick, C.A. (1970) Effect of pyridoxine supplementation on glutamic-pyruvic transaminase and in vitro stimulation in erythrocytes of normal women. Am. J. Clin. Nutr. 23: 1385-1395.

CHAPTER 6

SUMMARY AND CONCLUSIONS

To determine the effect of vitamin B-6 intake, protein intake and pyridoxine glucoside intake on the vitamin B-6 status of women, four studies were conducted. Our hypotheses were that the dietary ratio of B6 to protein would influence the vitamin B-6 status of women, and that the effect would be different than the effect in men. We also hypothesized that a diet high in pyridoxine glucoside would result in reduced B6 status compared to a diet low in PNG.

In the first two studies, protein intake was held constant and the vitamin B-6 intake was ranged from 0.84 to 2.39 mg/d. Ten women were fed diets providing 85 g protein and 1.03, 1.33, 1.73 and 2.39 mg vitamin B-6/d for 12- to 15-d periods. Six women were fed diets providing 85 g protein and 0.84, 1.14 and 2.34 mg B6 for 10- to 12-d periods. There were significant differences among B6 intake levels for means of urinary excretion of 4PA and UB6, plasma PLP and TB6, and urinary XA excretion following an oral 2 g L-tryptophan load. Vitamin B-6 status indicators showing a significant linear relationship with vitamin B-6 intake included urinary 4PA and UB6 excretion, plasma PLP and TB6, EAST percent stimulation and post-tryptophan load excretion of XA and VA. A vitamin B-6 intake greater than 1.33 mg/d (> 0.016 mg B6/g dietary protein) was required to produce status indicator values indicative of adequate vitamin B-6 status. If the minimum requirement for adequate status is more than 0.016 mg B6/g dietary protein, the RDA for women should be based on a higher ratio in order to include a safety margin.

In a third study, vitamin B-6 intake was held constant at 1.25 mg/d and three levels of protein were fed. Nine women consumed diets providing 0.5, 1.0 and 2.0 g protein/kg body weight for 14 d each in a Latin square design. There were significant differences among protein levels for means of urinary 4PA excretion, plasma PLP concentration and post-tryptophan load excretion of VA. There were significant linear relationships between nitrogen intake and urinary 4PA excretion, plasma PLP concentration, EALT percent stimulation, and post-tryptophan load excretion of XA, KA and VA. Compared to men consuming diets with similar B6 to protein ratios in a previous study, the women excreted a greater percentage of the B6 intake as urinary 4PA, had lower plasma PLP concentrations and excreted greater amounts of post-load tryptophan metabolites at all three protein levels. With a B6 intake of 1.25 mg/d, a B6 to protein ratio of at least 0.020 mg/g was required to produce vitamin B6 status indicator values indicative of adequate status. Women need a higher dietary ratio of vitamin B-6 to protein than men to achieve similar levels of B6 status indicators, and this should be considered when setting RDAs for women.

The effect of pyridoxine glucoside, a form of B6 which is less bioavailable than other commonly consumed forms of B6, on vitamin B-6 status was examined in a fourth study. Nine women were fed diets containing a high (27% of the total B6 intake) or low (9% of the total B6 intake) amount of PNG for 18 d each, in a crossover design. The dietary vitamin B-6 to protein ratio of both experimental diets was 0.017 mg/g. Vitamin B-6 status indicators showing significant differences between the high and low PNG diets included urinary 4PA and UB6, plasma TB6, red blood cell PLP and fecal B6. The decrease in vitamin B-6 status indicators when the high PNG diet was fed suggested a loss of 15-18% of the total B6 intake. The RDA for vitamin B-6 should take into account the reduced vitamin B-6 bioavailability of a diet high in pyridoxine glucoside.

In 1989, the RDA for vitamin B-6 for women was lowered from 2.0 to 1.6 mg/d, based on a dietary vitamin B-6 to protein ratio of 0.016 mg/g. The same B6 to protein ratio was used to calculate the RDA for both men and women, although the studies that linked vitamin B-6 requirement to protein intake were conducted in men. In the studies discussed above, a dietary vitamin B-6 to protein ratio of 0.016 to 0.020 mg/g was required to produce vitamin B-6 status indicator values above the level indicating adequate status. In addition, consuming a high pyridoxine glucoside diet may result in a loss of bioavailability of 15-18% of the total vitamin B-6 intake. These results suggest that if gender differences in the effect of dietary protein on B6 status are considered, bioavailability is taken into account, and a safety margin is included, the current RDA for women is set too low. We recommend an RDA for women based on a dietary vitamin B-6 to protein ratio of at least 0.020 mg/g.

More research is needed to clarify the quantitative effect of vitamin B-6 intake vs. protein intake on vitamin B-6 status. The study in which the protein intake was varied had only one level of vitamin B-6 intake which was relatively low (1.25 mg/d). A study in which protein is varied and several levels of vitamin B-6 within the normal range of intakes are fed would provide more data to evaluate the relationship between protein intake and vitamin B-6 intake and their relative effects on status. The long-term effect on vitamin B-6 status of eating a diet high in pyridoxine glucoside also needs to be investigated. Another limitation of this research is that only women between 19 and 40 years old were studied, and so the results may not apply to children, adolescents, or older adults. Our subjects were also all nonsmokers and refrained from strenuous exercise. Women who are athletes or exercise regularly, and smokers may have different requirements for vitamin B-6.

BIBLIOGRAPHY

Adams, P.W. & Rose, D.P. (1973) Effects of pyridoxine hydrochloride (vitamin B-6) upon depression associated with oral contraception. *Lancet* i: 897-904.

Aly, H.E., Donald, E.A. & Simpson, M.H.W. (1971) Oral contraceptives and vitamin B_6 metabolism. *Am. J. Clin. Nutr.* 24: 297-303.

Anderson, B.B., Fulford-Jones, C.E., Child, J.A., Beard, M.E.J. & Bateman, C.J.T. (1971) Conversion of vitamin B_6 compounds to active forms in the red blood cell. *J. Clin. Invest.* 50: 1901-1909.

Andon, M.B., Reynolds, R.D., Moser-Veillon, P.B. & Howard, M.P. (1989) Dietary intake of total and glycosylated vitamin B-6 and vitamin B-6 nutritional status of unsupplemented lactating women and their infants. *Am. J. Clin. Nutr.* 50: 1050-1058.

Arend, R.A., Leklem, J.E. & Brown, R.R. (1970) Direct and steam distillation autoanalyzer methods for assay of diazotizable aromatic amine metabolites of tryptophan in urine and in serum. *Biochem. Med.* 4: 457-468.

Baker, E.M., Canham, J.E., Nunes, W.T., Sauberlich, H.E. & McDowell, M.E. (1964) Vitamin B_6 requirements for adult men. *Am. J. Clin. Nutr.* 15: 59-66.

Belongia, E.A., Hedberg, C.W., Gleich, G.J., White, K.E., Mayeno, A.N., Loegering, D.A., Dunnette, S.L., Pirie, P.L., MacDonald, K.L. & Osterholm M.T. (1990) An investigation of the cause of the eosinophilia-myalgia syndrome associated with tryptophan use. *N. Eng. J. Med.* 323: 357-65.

Bender, D.A. (1992) Vitamin B-6. In: *Nutritional Biochemistry of the Vitamins*, pp. 223-268. Cambridge University Press, Cambridge, G.B.

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

Bills, N.D. (1991) *In Vivo and In Vitro Determination of the Bioavailability of Vitamin B-6 from Plant Foods Containing Pyridoxine Glucoside.* Doctoral thesis, Oregon State University.

Bosse, T.R. & Donald, E.A. (1979) The vitamin B_6 requirement in oral contraceptive users. I. Assessment by pyridoxal level and transferase activities in erythrocytes. *Am. J. Clin. Nutr.* 32: 1015-1023.

Brophy, M.H. & Siiteri, P.K. (1975) Pyridoxal phosphate and hypertensive disorders of pregnancy. *Am. J. Obstet. Gynecol.* 121: 1075-1029.

Brown, R.R. (1981) The tryptophan load test as an index of vitamin B-6 nutrition. In: *Methods in Vitamin B-6 Nutrition* (Leklem, J.E.& Reynolds, R.D., eds.), pp. 321-340. Plenum Press, New York, N.Y.

Brown, R.R. (1988) Possible role for vitamin B-6 in cancer prevention and treatment. In: *Clinical and Physiological Applications of Vitamin B-6* (Leklem, J.E. & Reynolds, R.D., eds.), pp. 279-301. Alan R. Liss, New York, N.Y.

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

Canham, J.E., Baker, E.M., Harding, R.S., Sauberlich, H.E. & Plough, I.C. (1969) Dietary protein - its relationship to vitamin B6 requirements and function. *Ann. N.Y. Acad. Sci.* 166: 16-29.

Chabner, B.A. & Livingston, D.M. (1970) A simple enzymic assay for pyridoxal phosphate. *Anal. Biochem.* 34: 413-423.

Cheslock, K.E.& McCully, M.T. (1960) Response of human beings to a low-vitamin B-6 diet. *J. Nutr.* 70: 507-512 .

Chrisley, B.Mc. & Driskell, J.A. (1979) Vitamin B-6 status of adults in Virginia. *Nutr. Rept. Int.* 19: 553-559.

Cinnamon, A.D. & Beaton, J.R. (1970) Biochemical assessment of vitamin B6 status in man. *Am. J. Clin. Nutr.* 23: 696-702.

Cleary, R.E., Lumeng, L. & Li, T-K. (1975) Maternal and fetal plasma levels of pyridoxal phosphate at term: adequacy of vitamin B-6 supplementation during pregnancy. *Am. J. Obstet. Gynecol.* 121: 25-28.

Coburn, S.P. (1990) Location and turnover of vitamin B_6 pools and vitamin B_6 requirements of humans. *Ann. N.Y. Acad. Sci.* 585: 76-85.

Coburn, S.P., Lewis, D.L.N., Fink, W.J., Mauhren, 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.

Committee on Dietary Allowances, Food and Nutrition Board, National Research Council, ed. (1978) *Human Vitamin B-6 Requirements*. National Academy of Sciences, Washington, D.C.

Contractor, S.F. & Shane, B. (1970) Blood and urine levels of vitamin B-6 in the mother and fetus before and after loading of the mother with vitamin B-6. *Am. J. Obstet. Gynecol.* 107: 635-640.

Dakshinamurti, K., ed. Vitamin B_6. *Ann. N.Y. Acad. Sci.* 585.

Doberenz, A.R., Miller, J.P.V., Green, J.R. & Beaton, J.R. (1971) Vitamin B-6 depletion in women using oral contraceptives as determined by erythrocyte glutamic-pyruvic transaminase activities. *Proc. Soc. Exp. Biol. Med.* 137: 1100-1103.

Donald, E.A. (1978) Vitamin B-6 requirement of young women. In: *Human Vitamin B-6 Requirements* (National Academy of Sciences), pp. 226-237. National Academy Press, Washington, D.C.

Donald, E.A., McBean, L.D., Simpson, M.H.W., Sun, M.F. & Aly, H.E. (1971) Vitamin B-6 requirement of young adult women. *Am. J. Clin. Nutr.* 24: 1028-1041.

Dreon, D.M. & Butterfield, G.E. (1986) Vitamin B_6 utilization in active and inactive young men. *Am. J. Clin. Nutr.* 43: 816-824.

Driskell, J.A. & Chrisley, B.Mc. (1991) Plasma B-6 vitamer and plasma and urinary 4-pyridoxic acid concentrations in young women as determined using high performance liquid chromatography, *Biomed. Chromatog.* 5: 198-201.

Driskell, J.A., Chrisley, B.Mc., Reynolds, L.K. & Moak, S.W. (1989) Plasma pyridoxal 5′-phosphate concentrations in obese and nonobese black women residing near Petersburg, VA. *Am. J. Clin. Nutr.* 50: 37-40.

Driskell, J.A., Geders, J.M. & Urban, M.C. (1976) Vitamin B-6 status of young men, women, and women using oral contraceptives. *J. Lab. Clin. Med.* 87: 813-821.

Eastman, J.R. & Bixler, D. (1977) Serum alkaline phosphatase: normal values by sex and age. *Clin. Chem.* 23: 1769-1770.

Food and Nutrition Board (1986) Recommended dietary allowances: scientific issues and process for the future. *J. Nutr.* 116: 482-488.

Georgia, J.D. (1974) Nonprotein nitrogenous constituents. In: *Clinical Chemistry: Principles and Technics*, 2nd ed. (Henry, R.J., Cannon, D.C. & Winkleman, J.W., eds.), pp. 504-563. Harper and Row, New York, N.Y.

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. Agri. Food Chem.* 35: 76-82.

Gregory, J.F. & Kirk, J.R. (1979) Determination of urinary 4-pyridoxic acid using high performance liquid chromatography. *Am. J. Clin. Nutr.* 32: 879-893.

Gregory, J.F., Trumbo, P.R., Bailey, L.B., Toth, J.P., Baumgartner, T.B. & Cerda, J.J. (1991) Bioavailability of pyridoxine-5-ß-D-glucoside determined in humans by stable isotopic methods. *J. Nutr.* 121: 177-186.

Guilland, J.C., Bereksi-Reguig, B. & Lequeu, B. (1984) Evaluation of pyridoxine intake and pyridoxine status among aged institutionalised people. *Internat. J. Vit. Nutr.* 54: 185-193.

Hansen, C., Leklem, J., Hardin, K. & Miller, L. (1991) Vitamin B-6 status of women with constant protein intake and four levels of vitamin B-6. *FASEB J.* 5:A556 (abstr).

Hansen, C., Leklem, J., Hardin, K., Miller, L., Chen, W. (1992) Effect of feeding diets of high and low pyridoxine glucoside content on vitamin B-6 status of women. *FASEB J.* 6: A1374 (abstr).

Hansen, C., Leklem, J., Miller, L., Hardin, K. & Ridlington, J. (1993) Response of red blood cell pyridoxal 5'-phosphate to short term changes in vitamin B-6 intake. *FASEB J.* 7:A728 (abstr).

Harper, A.E. (1968) Diet and plasma amino acids. *Am. J. Clin. Nutr.* 21: 358-366.

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

Henderson, L.M. & Hulse, J.D. (1978) Vitamin B-6 relationships in tryptophan metabolism. In: *Human Vitamin B_6 Requirements* (National Academy of Sciences), pp. 21-36. National Academy Press, Washington, D.C.

Horwitz, G.W., ed. (1980) *Official Methods of Analysis of the AOAC*, 13th ed., pp.768. Washington, D.C.

Hrboticky, N., Leiter, L.A. & Anderson, G.H. (1989) Menstrual cycle effects on the metabolism of tryptophan loads. *Am. J. Clin. Nutr.* 50: 46-52.

Human Nutrition Information Service, U.S. Department of Agriculture (1987) *Nationwide food consumption survey, continuing survey of food intakes by individuals, 1985*. Hyattsville, MD: U.S. Department of Agriculture (Report 85-4).

Ink, S.L., Gregory, J.F. & Sartain, D.B. (1986) Determination of pyridoxine ß-glucoside bioavailability using intrinsic and extrinsic labeling in the rat. *J. Agric. Food Chem.* 34: 857-862.

Jacobs, A., Cavill, I.A. & Hughes, J.N.P. (1968) Erythrocyte transaminase activity: effect of age, sex, and vitamin B-6 supplementation. *Am. J. Clin. Nutr.* 21: 502-507.

Johnston, P.K. (1994) Second International Congress on Vegetarian Nutrition. *Am. J. Clin. Nutr.* 59 (suppl.): vii.

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

Kabir, H., Leklem, J.E. & Miller, L.T. (1983b) Measurement of glycosylated vitamin B-6 in foods. *J. Food Sci.* 48: 1422-1425.

Kabir, H., Leklem J.E. & Miller, L.T. (1983c) Relationship of glycosylated vitamin B-6 content of foods to vitamin B-6 bioavailability in humans. *Nutr. Reports Intl.* 28: 709-715.

Kang-Yoon, S.A. & Kirksey, A. (1992) Relation of short-term pyridoxine supplementation to plasma vitamin B-6 vitamers and amino acid concentrations in young women. *Am. J. Clin. Nutr.* 55: 865-872.

Kant, A.K, Moser-Veillon, P.B. & Reynolds, R.D. (1988) Effect of age on changes in plasma, erythrocyte, and urinary B-6 vitamers after an oral vitamin B-6 load. *Am. J. Clin. Nutr.* 48: 1284-1290.

Kant, A.K. & Block, G. (1990) Dietary vitamin B-6 intake and food sources in the US population: NHANES II, 1976-1980. *Am. J. Clin. Nutr.* 52: 707-16.

Kretsch, M.J., Sauberlich, H.E. & Newbrun, E. (1991) Electroencephalographic changes and periodontal status during short-term vitamin B-6 depletion of young nonpregnant women. *Am. J. Clin. Nutr.* 53: 1266-1274.

Kretsch, M.J., Sauberlich, H.E., Skala, J.H. & Johnson, H.L. (1995) Vitamin B-6 requirement and status assessment: young women fed a depletion diet followed by a plant- or animal-protein diet with graded amounts of vitamin B-6. *Am. J. Clin. Nutr.* 61: 1091-1101.

Lee, C.M. & Leklem, J.E. (1985) Differences in vitamin B-6 status indicator responses between young and middle-aged women fed constant diets with two levels of vitamin B-6. *Amer. J. Clin. Nutr.* 42: 226-234.

Leitch, I. & Hepburn, A. (1961) Pyridoxine: metabolism and requirement. *Nutr. Abst. Rev.* 31: 389-401.

Leklem, J.E. (1988a) Vitamin B-6 bioavailability and its application to human nutrition. *Food Technol.* 42: 194-196.

Leklem, J.E. (1988b) Vitamin B-6 metabolism and function in humans. In: *Clinical and physiological applications of vitamin B-6* (Leklem, J.E. & Reynolds, R.D., eds.), pp. 3-26. Alan R. Liss, New York, N.Y.

Leklem, J.E. (1990) Vitamin B-6: a status report. *J. Nutr.* 120: 1503-1507.

Leklem, J.E. (1991) Vitamin B-6. In: *Handbook of vitamins*, 2nd ed. (Machlin, L., ed.), pp. 341-392. Marcel Dekker, New York, N.Y.

Leklem, J.E., Brown, R.R., Rose, D.P. & Linkswiler, H.M. (1975) Vitamin B_6 requirements of women using oral contraceptives. *Am. J. Clin. Nutr.* 28: 535-541.

Leklem, J.E., Brown, R.R., Rose, D.P., Linkswiler, H. & Arend, R.A. (1975) Metabolism of tryptophan and niacin in oral contraceptive users receiving controlled intakes of vitamin B_6. *Am. J. Clin. Nutr.* 28: 146-156.

Leklem, J., Hansen, C. & Miller, L. (1991) Effect of three levels of protein on vitamin B-6 status of adult women. *FASEB J.* 5: A557 (abst).

Leklem, J.E., Linkswiler, H.M., Brown, R.R., Rose, D.P. & Anand, C.R. (1977) Metabolism of methionine in oral contraceptive users and control women receiving controlled intakes of vitamin B-6. *Am. J. Clin. Nutr.* 30: 1122-1128.

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., eds. (1981) *Methods in Vitamin B-6 Nutrition.* Plenum Press, New York, N.Y.

Leklem, J.E. & Reynolds, R.D. (1988) Challenges and directions in the search for clinical applications of vitamin B-6. In: *Clinical and Physiological Applications of Vitamin B-6* (Leklem, J.E. & Reynolds, R.D., eds.), pp. 437-54. Alan R. Liss, New York, N.Y.

Leklem, J.E., Shultz, T.D. & Miller, L.T. (1980) Comparative bioavailability of vitamin B-6 from soybeans and beef. *Fed. Proc.* 38: 452 (abstr).

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.M. (1978) Vitamin B_6 requirements of men. In: *Human vitamin B_6 requirements* (National Academy of Sciences), pp.279-290. National Academy Press, Washington, DC.

Luhby, A.L., Brin, M., Gordon, M., Davis, P., Murphy, M. & Spiegel, H. (1971) Vitamin B6 metabolism in users of oral contraceptive agents. I. Abnormal urinary xanthurenic acid excretion and its correction by pyridoxine. *Am. J. Clin. Nutr.* 24: 684-693.

Lumeng, L., Cleary, R.E. & Li, T.-K. (1974) Effect of oral contraceptives on the plasma concentration of pyridoxal phosphate. *Am. J. Clin. Nutr.* 27: 326-333.

Mallett, A.K., Bearne, C.A. & Rowland, I.R. (1989) The influence of incubation pH on the activity of rat and human gut flora enzymes. *J. Appl. Bacteriol.* 66: 433-437.

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.

Merrill, A.H. & Henderson, J.M. (1990) Vitamin B-6 metabolism by human liver. *Ann. N.Y. Acad. Sci.* 585: 110-117.

Merrill, A.H. & Henderson, J.M. (1987) Diseases associated with defects in vitamin B-6 metabolism or utilization. *Ann. Rev. Nutr.* 7: 137-156.

Mikai-Devic, D. & Tomanic, C. (1972) Determination of 4-pyridoxic acid in urine by a fluorimetric method. *Clin. Chim. Acta* 38: 235-238.

Miller, L.T., Benson, E.M., Edwards, M.A. & Young, J. (1974) Vitamin B-6 metabolism in women using oral contraceptives. *Am. J. Clin. Nutr.* 27: 797-805.

Miller, L.T., Dow, M.J & Kokkeler, S.C. (1978) Methionine metabolism and vitamin B-6 status in women using oral contraceptives. *Am. J. Clin. Nutr.* 31: 619-625.

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

Miller, L.T., Johnson, A., Benson, E.M. & Woodring, M.J. (1975) Effect of oral contraceptives and pyridoxine on the metabolism of vitamin B-6 and on plasma tryptophan and α-amino nitrogen. *Am. J. Clin. Nutr.* 28: 846-853.

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: 1663-1672.

Miller, L.T. & Linkswiler, H.M. (1967) Effect of protein intake on the development of abnormal tryptophan metabolism by men during vitamin B_6 depletion. *J. Nutr.* 93: 53-59.

Miller, L.T., Shultz, T.D. & Leklem, J.E. (1980) The influence of citrus pectin on the bioavailability of vitamin B-6 to men. *Fed. Proc.* 39: 797 (abstr).

Millet, P., Guilland, J.C., Fuchs, F. & Klepping, J. (1989) Nutrient intake and vitamin status of healthy French vegetarians and nonvegetarians. *Am. J. Clin. Nutr.* 50: 718-727.

Mira, M., Stewart, P.M. & Abraham, S.F. (1988) Vitamin and trace element status in premenstrual syndrome. *Am. J. Clin. Nutr.* 47: 636-641.

Nakano, H. & Gregory, J.F. (1995) Pyridoxine-5′-ß-D-glucoside influences the short-term metabolic utilization of pyridoxine in rats. *J. Nutr.* 125: 926-932.

National Research Council (1968) Vitamin B-6. In: *Recommended Dietary Allowances*, 7th ed. pp. 43-47. National Academy Press, Washington, D.C.

National Research Council (1974) Vitamin B-6. In: *Recommended Dietary Allowances*, 8th ed., pp. 74-77. National Academy Press, Washington, D.C.

National Research Council (1980) Vitamin B-6. In: *Recommended Dietary Allowances*, 9th ed., pp. 96-106. National Academy Press, Washington, D.C.

National Research Council (1989) Vitamin B-6. In: *Recommended Dietary Allowances*, 10th ed., pp.142-50. National Academy Press, Washington, D.C.

Pannemanns, D.L.E., van den Berg, H. & Westerterp, K.R. (1994) The influence of protein intake in vitamin B-6 metabolism differs in young and elderly humans. *J. Nutr.* 124: 1207-1214.

Park, Y.K. & Linkswiler, H. (1970) Effect of vitamin B_6 depletion in adult man on the excretion of cystathionine and other methionine metabolites. *J. Nutr.* 100: 110-106.

Pino, S., Bennotte, J. & Gordnya, H. (1965) An automated method for urine creatinine which does not require a dialyzer module. *Clin. Chem.* 11: 664-666.

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: 159-225.

Ramaswamy, P.G. & Natarajan, R. (1984) Vitamin B-6 status in patients with cancer of the uterine cervix. *Nutr. Cancer* 6: 176-180.

Reddy, S.K., Reynolds, M.S. & Price, J.M. (1985) The determination of 4-pyridoxic acid in human urine. *J. Biol. Chem.* 233: 691-696.

Reed, A.H., Cannon, D.C., Winkelman, J.W., Bhasin, Y.P., Henry, R.J. & Pileggi, V.J. (1972) Estimation of normal ranges from a controlled sample survey. I. Sex- and age-related influence on the SMA 12/60 screening groups of tests. *Clin. Chem.* 18: 57-66.

Reinken, L. & Gant, H. (1974) Vitamin B-6 nutrition in women with hyperemesis gravidarum during the first trimester of pregnancy. *Clin. Chim. Acta.* 55: 101-102.

Reynolds, R.D. & Leklem, J.E. (1985) *Vitamin B-6: its role in health and disease.* Alan R. Liss, New York, N.Y.

Ribaya-Mercado, J.D., Russell, R.M., Sahyoun, N., Morrow, F.D. & Gershoff, S.N. (1991) Vitamin B-6 requirements of elderly men and women. *J. Nutr.* 121: 1062-1074.

Ritchie, C.D. & Singkamani, R. (1986) Plasma pyridoxal 5'-phosphate in women with the premenstrual syndrome. *Hum. Nutr. Clin. Nutr.*40C: 75-80.

Rose, D.P., Strong, R., Folkard, J. & Adams, P.W. (1973) Erythrocyte aminotransferase activities in women using oral contraceptives and the effect of vitamin B6 supplementation, *Am. J. Clin. Nutr.* 26: 48-52.

+Roy, A.V. (1970) Rapid method for determining alkaline phosphatase activity in serum with thymolpthalein monophosphate. *Clin. Chem.* 16: 431-436.

Salih, E.Y., Zein, A.A & Bayoumi, R.A. (1986) The effect of oral contraceptives on the apparent vitamin B_6 status in some Sudanese women. *Br. J. Nutr.* 56: 363-367.

Salkeld, R.M., Knörr, K. & Körner, W.F. (1973) The effect of oral contraceptives on vitamin B_6 status. *Clin. Chim. Acta* 49: 195-199.

Sampson, D.A., Schaeffer, M.C., Skala, J.H. & O'Connor, D.K. (1989) B-6 vitamers in erythrocytes and muscle of rats fed graded levels of vitamin B-6. *FASEB J.* 3: A454(abstr).

Scales, F.M. & Harrison, A.P. (1929) Boric acid modification of the Kjeldahl method for crop and soil analysis. *Ind. Eng. Chem.* 12: 350.

Schuster, K., Bailey, L.B., Cerda, J.J. & Gregory, J.F. (1984) Urinary 4-pyridoxic acid excretion in 24-hour versus random urine samples as a measurement of vitamin B-6 status in humans. *Am. J. Clin. Nutr.* 39: 466-470.

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: 739-742.

Shin, H.K. & Linkswiler H.M. (1974) Tryptophan and methionine metabolism of adult females as affected by vitamin B-6 deficiency. *J. Nutr.* 104: 1348-1355.

Shultz, T.D. & Leklem, J.E. (1981) Urinary 4-pyridoxic acid, urinary vitamin B-6 and plasma pyridoxal phosphate as measures of vitamin B-6 status and dietary intake of adults. In: *Methods in Vitamin B-6 Nutrition* (Leklem, J.E. & Reynolds, R.D., eds.), pp.297-320. Plenum Press, New York, N.Y.

Shultz, T.D. & Leklem, J.E. (1982) Effect of high dose ascorbic acid on vitamin B-6 metabolism. *Am. J. Clin. Nutr.* 35: 1400-1407.

Shultz, T.D. & Leklem, J.E. (1987) Vitamin B-6 status and bioavailability in vegetarian women. *Am. J. Clin. Nutr.* 46: 647-51.

Tarr, J.B., Tamura, T. & Stokstad, E.L.R. (1981) Availability of vitamin B-6 and pantothenate in an average diet in man. *Am. J. Clin. Nutr.* 34: 1328-37.

Tovar, A., Bourges, H., Canto, T., Torres, N. & López-Castro, B.R. (1985) Effect of oral contraceptive use on the erythrocytic glutathione reductase and aspartate aminotransferase activities in women without clinical signs of vitamin deficiency. *Nutr. Rept. Int.* 32: 199-209.

Trumbo, P.R. & Gregory, J.F. (1988) Metabolic utilization of pyridoxine-ß-glucoside in rats: influence of vitamin B6 status and route of administration. *J. Nutr.* 118: 1336-1342.

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

Trumbo, P.R., Gregory, 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.R. & Wang, J.W. (1993) Vitamin B-6 status indices are lower in pregnant than in nonpregnant women but urinary excretion of 4-pyridoxic acid does not differ. *J. Nutr.* 123: 2137-2141.

Ubbink, J.B., Serfontein, W.J., Becker, P.J. & de Villiers, L.S. (1987) Effect of different levels of oral pyridoxine supplementation on plasma pyridoxal 5'-phosphate and pyridoxal levels and urinary vitamin B-6 excretion. *Am. J. Clin. Nutr.* 46: 78-85.

Ubbink, J.B., Bissbort, S., van den Berg, I., de Villiers, L.S. & Becker, P.J. (1989) Genetic polymorphism of glutamate-pyruvate transaminase (alanine aminotransferase): influence on erythrocyte activity as a marker of vitamin B-6 nutritional status. *Am. J. Clin. Nutr.* 50: 1420-1428.

van den Berg, H., Louwerse, E.S., Bruinse, H.W., Thissen, J.T.N.M. & Schrijver, J. (1986) Vitamin B-6 status of women suffering from the premenstrual syndrome. *Hum. Nutr. Clin. Nutr.* 40C: 441-50.

Vir, S.C. & Love, A.H.G. (1980) Effect of oral contraceptives on vitamin B-6 nutriture of young women. *Internat. J. Vit. Nutr. Res.* 50: 29-34.

Wachstein, M., Kellner, J.D. & Ortiz, J.M. (1960) Pyridoxal phosphate in plasma and leukocytes of normal and pregnant subjects following B-6 load tests. *Proc. Soc. Exp. Biol. Med.* 103: 350-352.

Waldorf, M.A., Kirk, M.C., Linkswiler, H. & Harper, A.E. (1963) Metabolic adaptations in higher animals. VII. Responses of glutamate-oxaloacetate and glutamate-pyruvate transaminases to diet. *Proc. Soc. Exp. Biol. Med.* 112: 764-768.

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 B_6 metabolism. *J. Clin. Invest.* 76: 752-756.

Wien, E.M. (1978) Vitamin B_6 status of Nigerian women using various methods of contraception. *Am. J. Clin. Nutr.* 31: 1392-1396.

Wirth, J.C. & Lohman, T.G. (1982) The relationship of static muscle function to use of oral contraceptives. *Med. Sci. Sports Exer.* 14: 16-20.

Woodring, M.J. & Storvick, C.A. (1970) Effect of pyridoxine supplementation on glutamic-pyruvic transaminase and in vitro stimulation in erythrocytes of normal women. *Am. J. Clin. Nutr.* 23: 1385-1395.

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.

Yess, N., Price, J.M., Brown, R.R., Swan, P.B. & Linkswiler, H. (1964) Vitamin B_6 depletion in man: urinary excretion of tryptophan metabolites. *J. Nutr.* 84: 229-236.

APPENDICES

APPENDIX A

DATA FROM THE STUDIES DESCRIBED IN CHAPTER 3 (FALL 1989 AND SPRING 1992)

Table A.1 Urinary creatinine excretion (g/day) Fall 1989 (Study 1)

	Subjects									
Date	1	2	3	4	5	6	7	8	9	10
10/2	0.943	1.325	1.659	1.097	0.931	1.026	1.630	1.385	1.072	1.674
10/3	0.960	1.232	1.322	1.019	0.981	1.067	1.608	1.320	1.100	1.748
10/4	0.978	1.232	1.518	1.095	1.110	1.070	1.657	1.447	1.112	1.779
10/5	0.939	1.231	1.663	1.076	0.997	1.096	1.654	1.416	1.172	1.635
10/6	0.978	1.325	1.709	1.081	1.229	1.023	1.362	1.557	1.247	1.874
10/7	0.994	1.160	1.568	1.096	1.194	1.143	1.704	1.359	1.137	1.759
10/8	1.010	1.165	1.609	1.049	1.070	0.908	1.633	1.395	1.171	1.422
10/9	0.964	1.568	1.608	1.156	1.310	1.368	1.472	1.358	1.184	0.730
10/10	0.954	1.060	1.600	1.092	1.188	1.116	1.794	1.470	1.199	1.747
10/11	0.954	1.062	1.648	1.044	1.223	0.988	1.689	1.262	1.273	1.833
10/12	1.004	1.234	1.641	1.093	0.995	1.126	1.690	1.363	1.123	1.776
10/13	1.028	1.234	1.607	1.110	1.240	1.120	1.680	1.437	1.237	1.794
10/14	1.023	1.088	1.649	1.066	1.274	1.105	1.736	1.319	1.188	1.747
10/15	1.056	1.193	1.262	1.116	0.807	1.117	1.736	1.409	1.176	1.675
10/16	1.011	1.208	1.621	1.109	1.211	1.055	1.711	1.373	1.211	1.809

Cont.

Table A.1, cont.

10/17	1.083	1.174	1.497	1.147	1.145	1.032	1.840	1.394	1.274	1.775
10/18	1.025	1.191	1.381	1.153	1.153	1.166	1.532	1.577	1.245	1.796
10/19	1.061	1.238	1.683	1.147	1.128	0.958	1.673	1.316	1.200	1.755
10/20	1.082	1.226	1.539	1.149	1.173	1.136	1.688	1.512	1.257	1.796
10/21	1.092	1.220	1.736	1.190	0.926	1.106	1.906	1.506	1.252	1.699
10/22	1.104	1.236	1.588	1.152	1.038	1.058	1.656	1.394	1.271	1.733
10/23	1.028	1.287	1.577	1.197	1.065	1.138	1.635	1.397	1.252	1.644
10/24	0.947	1.292	1.350	1.026	0.966	1.053	1.737	1.356	1.109	1.698
10/25	0.994	1.212	1.590	1.117	0.952	1.018	1.580	1.351	1.310	1.703
10/26	0.994	1.298	1.521	1.070	1.216	1.077	1.651	1.431	1.004	1.808
10/27	0.984	1.268	1.309	1.014	1.337	1.003	1.538	1.380	1.276	1.232
10/28	1.028	1.351	1.613	1.010	1.264	1.095	1.600	1.322	1.154	1.757
10/29	0.988	1.170	1.654	1.084	1.209	1.012	1.405	1.360	1.210	1.586
10/30	0.994	1.172	1.492	1.074	0.906	0.986	1.550	1.431	1.223	1.784
10/31	0.976	1.309	1.513	1.083	0.704	1.008	1.662	1.197	1.185	1.762
11/1	1.012	1.206	1.563	1.140	1.101	1.097	1.650	1.375	1.183	1.851

Cont.

Table A.1, cont.

11/2	0.997	1.217	1.580	1.124	1.145	1.145	1.558	1.344	1.243	1.749
11/3	0.998	1.231	1.509	1.098	1.288	1.104	1.088	1.287	1.193	2.024
11/4	1.032	1.139	1.712	1.112	1.117	1.090	1.312	1.417	1.185	1.654
11/5	0.955	1.062	1.527	1.097	1.254	0.990	0.960	1.828	1.197	1.774
11/6	1.015	1.228	1.406	1.111	1.205	1.034	1.622	1.422	1.226	1.807
11/7	1.001	1.106	1.494	1.085	1.210	1.178	1.699	1.226	1.212	1.615
11/8	0.989	1.264	1.564	1.116	1.150	1.115	1.584	1.365	1.232	1.845
11/9	1.030	1.201	1.556	1.105	1.204	0.987	1.623	1.229	1.153	1.642
11/10	1.011	1.248	1.571	1.175	1.211	1.137	1.399	1.266	1.238	1.672
11/11	0.963	1.300	1.688	1.138	1.222	1.138	1.526	1.302	1.193	1.717
11/12	1.013	1.082	1.538	1.083	1.040	0.846	1.034	1.310	1.236	1.614
11/13	0.972	1.253	1.578	1.076	1.012	1.216	1.685	1.416	1.231	1.739
11/14	0.956	1.272	1.536	1.014	1.217	1.013	1.204	1.276	1.200	1.785
11/15	0.976	1.142	1.676	1.109	1.260	1.081	1.400	1.344	1.138	1.869
11/16	0.970	1.187	1.584	1.127	1.365	1.067	1.242	1.425	1.134	1.653
11/17	0.989	1.078	1.562	1.123	1.320	1.138	1.460	1.391	1.189	1.830

Cont.

Table A.1, cont.

11/18	1.011	1.154	1.608	1.142	1.285	1.065	1.562	1.583	1.220	1.758
11/19	0.972	1.134	1.469	1.167	1.204	1.030	1.624	1.410	1.318	1.416
11/20	1.031	1.171	1.562	1.201	1.171	1.160	1.146	1.450	1.247	1.599
11/21	1.012	1.240	1.543	1.122	1.071	1.051	1.623	1.332	1.129	1.536

Table A.2 Urinary creatinine excretion (g/day) Spring 1992 (Study 2)

	Subjects					
Date	1	2	3	4	5	6
4/20	1.21	1.15	----	1.18	1.17	1.06
4/21	1.17	1.25	----	1.28	1.12	1.09
4/22	1.31	1.10	----	1.26	0.97	1.09
4/23	1.32	1.24	----	1.36	1.19	1.19
4/24	1.41	1.38	----	1.29	1.24	1.27
4/25	1.51	1.14	1.73	1.26	1.17	1.13
4/26	1.24	1.13	1.23	1.32	1.11	1.23
4/27	1.38	1.24	1.18	1.19	1.08	1.15
4/28	1.36	1.28	0.96	1.30	1.17	1.18
4/29	1.38	1.35	1.28	1.27	1.17	1.02
4/30	1.32	1.34	1.06	1.23	1.11	1.13
5/1	1.33	1.25	1.16	1.18	1.07	1.19
5/2	1.29	1.02	1.08	1.31	1.19	1.33
5/3	1.30	0.57	1.08	1.35	1.23	1.00
5/4	1.36	1.46	1.18	1.19	1.23	1.27
5/5	1.16	1.36	1.13	1.34	1.17	1.25
5/6	1.34	1.28	1.07	1.26	1.19	1.22
5/7	1.18	1.26	1.29	1.31	1.19	1.13
5/8	1.31	0.90	1.12	1.21	1.19	1.26
5/9	1.20	0.96	1.30	1.32	1.15	1.36
5/10	1.16	1.21	1.18	1.31	1.14	1.13
5/11	1.29	1.19	1.04	1.31	1.15	0.94
5/12	1.28	1.24	1.19	1.22	1.19	0.88
5/13	1.37	1.38	1.22	1.33	1.17	1.14

Cont.

Table A.2, cont.

5/14	1.31	1.27	1.19	1.45	1.18	1.11
5/15	1.32	1.18	1.25	1.33	1.16	1.26
5/16	1.22	1.29	1.27	1.35	1.19	1.07
5/17	1.34	1.19	1.25	1.22	1.12	1.08
5/18	1.31	1.23	1.15	1.38	1.14	1.15
5/19	1.29	1.24	1.21	1.29	1.14	1.13
5/21	1.22	1.29	1.02	1.40	1.11	1.06

Table A.3 Urinary urea nitrogen (g/day) Fall 1989 (Study 1)

	Subjects									
Date	1	2	3	4	5	6	7	8	9	10
10/2	10.56	9.35	10.39	8.65	9.64	9.17	8.61	10.01	8.16	10.64
10/3	9.44	9.95	9.09	9.76	8.95	9.70	9.55	10.19	9.32	11.24
10/4	10.06	9.22	9.68	9.00	9.61	10.40	10.31	11.34	9.55	9.93
10/5	9.67	8.93	10.02	9.25	8.79	9.94	9.50	10.97	9.44	9.57
10/6	10.23	9.02	9.95	9.24	9.41	10.03	7.40	11.56	10.10	11.09
10/7	9.43	8.10	9.44	9.24	9.73	10.59	9.96	10.70	7.91	9.51
10/8	9.77	7.66	9.59	8.79	8.35	8.13	9.27	10.56	9.77	7.00
10/9	9.03	10.71	10.15	9.90	10.23	12.81	8.19	11.02	9.59	4.04
10/10	9.88	8.51	10.32	10.61	9.34	10.14	9.51	11.52	8.20	10.64
10/11	9.60	8.22	10.37	9.41	9.20	10.00	8.70	9.95	9.62	10.66
10/12	9.71	10.51	9.93	10.23	8.33	11.55	8.95	10.23	8.12	9.58
10/13	10.50	8.69	10.70	9.72	10.82	11.07	10.13	10.91	12.19	9.62
10/14	10.11	7.80	9.29	9.75	10.35	10.74	12.64	10.48	9.31	9.93
10/15	9.87	9.30	7.26	9.81	-----	10.56	10.52	10.22	9.56	8.62
10/16	10.59	9.82	9.74	9.92	10.43	10.59	10.87	9.37	10.04	8.98

Cont.

Table A.3, cont.

10/17	10.36	8.61	9.71	10.08	9.48	10.15	11.76	10.09	10.20	8.81
10/18	9.27	9.25	7.39	10.29	8.44	9.60	10.12	10.87	8.89	9.27
10/19	9.88	9.14	9.59	9.82	10.08	9.28	9.76	9.14	9.94	9.57
10/20	9.34	8.32	8.77	9.95	10.18	10.25	9.16	10.74	9.14	9.84
10/21	9.88	8.73	11.05	9.92	7.95	11.14	10.65	11.29	8.59	8.78
10/22	8.66	8.78	9.32	9.69	8.52	10.28	9.11	9.86	9.38	8.87
10/23	8.69	9.89	9.34	10.16	9.36	10.60	8.32	9.94	8.80	9.29
10/24	8.69	11.24	8.51	10.27	8.43	9.71	9.42	10.43	7.87	10.00
10/25	9.43	10.24	9.25	10.18	9.53	10.16	8.87	10.48	9.72	7.86
10/26	9.50	11.08	8.85	10.37	9.82	10.17	9.69	11.02	7.17	9.79
10/27	9.48	9.80	7.98	10.55	10.68	10.51	8.51	10.38	8.72	6.66
10/28	10.86	10.88	9.74	10.31	11.96	11.37	10.37	9.28	9.93	8.26
10/29	9.51	8.82	9.49	9.88	12.78	9.98	9.06	9.28	9.03	7.71
10/30	10.42	9.48	8.32	10.31	10.22	8.81	9.27	10.23	8.93	9.80
10/31	10.14	10.03	9.33	9.41	7.85	9.63	7.11	8.76	9.21	8.99
11/1	7.23	10.17	9.85	9.90	10.39	11.18	10.62	10.09	8.10	8.71

Cont.

Table A.3, cont

11/2	10.04	9.69	9.65	9.74	10.46	11.78	9.98	9.69	8.46	9.10
11/3	9.15	9.59	8.56	10.45	11.77	11.19	6.34	8.83	8.52	10.56
11/4	10.73	8.48	10.14	9.92	9.29	11.33	8.78	9.52	8.33	8.05
11/5	9.51	8.55	8.94	10.01	10.28	9.89	6.51	11.78	9.95	9.92
11/6	9.79	9.47	7.99	10.26	9.74	10.30	10.90	11.28	9.82	10.29
11/7	9.84	9.58	7.97	10.18	10.08	11.85	10.73	9.71	10.88	9.31
11/8	10.09	10.51	9.33	10.20	9.33	10.39	10.26	11.32	9.40	8.78
11/9	9.73	9.94	10.46	11.01	9.51	10.11	10.31	9.67	9.32	9.75
11/10	9.92	9.61	10.36	9.62	10.38	10.41	10.04	10.58	8.57	10.32
11/11	10.42	10.15	9.74	10.47	9.43	9.57	10.56	9.20	9.17	9.14
11/12	9.77	8.97	8.86	9.92	9.49	6.28	6.20	8.96	7.62	7.68
11/13	9.45	10.18	8.75	10.12	9.31	11.10	10.51	8.90	8.92	11.04
11/14	9.94	9.97	9.16	9.99	10.70	9.28	7.15	8.90	9.21	10.05
11/15	10.17	8.75	9.74	10.37	11.14	9.71	8.02	8.94	7.70	9.70
11/16	9.68	9.66	9.66	11.49	10.38	9.24	5.99	8.46	9.55	9.61
11/17	10.04	11.93	9.58	11.17	10.78	10.35	9.05	8.88	10.91	11.80

Cont.

Table A.3, cont.

11/18	10.44	9.31	9.49	10.15	10.39	11.44	9.89	9.87	8.79	10.00
11/19	9.23	9.54	8.59	11.73	10.31	9.69	12.63	9.37	9.59	9.62
11/20	9.91	10.71	10.14	10.61	10.37	10.51	6.66	8.74	8.93	10.85
11/21	9.39	10.62	9.59	10.32	9.94	9.65	10.82	8.32	9.88	8.79

Table A.4 Urinary urea nitrogen (g/day) Spring 1992 (Study 2)

	Subjects					
Date	1	2	3	4	5	6
4/20	8.52	7.98	----	3.14	11.71	8.65
4/21	8.93	8.97	----	8.24	11.93	8.58
4/22	11.00	8.65	----	7.57	12.00	9.34
4/23	11.87	8.67	----	10.15	10.06	9.81
4/24	10.52	8.02	----	9.54	11.90	9.42
4/25	10.52	7.70	7.83	9.47	11.06	8.69
4/26	10.20	6.85	7.76	8.50	11.46	8.79
4/27	10.97	6.71	6.42	8.10	10.43	8.15
4/28	10.07	8.48	9.20	9.28	10.54	8.18
4/29	10.95	7.73	9.33	9.54	10.68	11.05
4/30	11.21	11.14	10.22	8.95	9.96	9.06
5/1	9.93	10.79	7.74	7.91	9.43	9.67
5/2	10.32	8.96	8.41	9.35	10.54	11.42
5/3	10.54	5.64	7.24	7.77	8.67	7.97
5/4	1.36	1.46	1.18	1.19	1.23	1.27
5/5	1.16	1.36	1.13	1.34	1.17	1.25
5/6	9.98	9.52	9.04	6.91	10.10	8.85
5/7	9.97	9.11	8.23	8.63	11.18	8.36
5/8	9.45	5.80	8.59	8.17	11.37	10.72
5/9	8.00	7.04	8.09	9.75	9.49	11.67
5/10	8.00	8.28	8.09	8.61	9.75	8.93
5/11	7.88	8.02	7.27	8.75	9.34	7.93
5/12	9.21	---	7.54	9.21	10.41	6.93
5/13	9.79	8.96	8.75	10.35	9.86	8.76

Cont.

Table A.4, cont.

5/14	9.18	9.66	8.07	10.50	10.44	9.45
5/15	10.49	8.63	9.02	9.19	9.38	9.90
5/16	9.32	10.17	9.08	9.67	9.29	9.35
5/17	10.24	9.96	9.46	8.43	10.10	9.88
5/18	9.45	8.11	9.56	9.74	9.53	9.38
5/19	10.36	---	10.07	9.08	9.67	9.34
5/20	9.67	7.29	10.09	8.43	9.34	9.34
5/21	10.49	9.13	7.64	10.07	8.75	8.29

Table A.5 Urinary 4-pyridoxic acid excretion (μmoles/day) Fall 1989 (Study 1)

	Subjects									
Date	1	2	3	4	5	6	7	8	9	10
10/2	2.24	4.99	4.91	10.64	3.29	6.17	6.52	6.72	6.78	5.21
10/3	2.46	3.39	7.31	8.17	3.46	6.09	6.59	5.80	6.41	5.08
10/4	2.13	3.04	4.83	6.47	3.38	5.17	5.93	5.97	6.43	4.31
10/5	2.13	3.38	4.15	4.95	3.09	4.65	6.05	5.25	5.69	3.98
10/6	2.29	2.72	3.97	4.81	3.49	4.11	5.11	5.67	5.03	4.09
10/7	2.07	2.25	3.29	4.33	3.43	4.42	4.69	4.66	4.67	3.49
10/8	2.24	2.50	3.28	4.59	2.96	3.28	4.82	4.94	5.22	2.88
10/9	2.24	4.02	2.99	4.35	3.27	4.38	4.00	4.64	4.63	2.45
10/10	1.31	4.18	3.25	4.06	3.08	3.38	4.14	4.76	4.76	3.18
10/11	1.97	2.24	3.21	3.66	3.18	3.54	3.51	4.15	4.33	3.16
10/12	1.47	2.73	3.10	4.86	2.71	4.02	3.34	4.68	4.39	3.14
10/13	2.79	2.78	3.07	3.88	3.09	3.95	3.23	4.96	4.27	3.01
10/14	1.84	2.28	3.16	3.80	3.25	3.29	3.31	4.66	3.74	3.25
10/15	2.29	2.40	2.45	3.65	2.17	3.74	2.59	4.53	3.49	2.64
10/16	2.02	3.24	3.26	3.50	2.82	3.58	3.07	4.38	3.76	2.49

Cont.

Table A.5, cont.

10/17	2.18	2.80	2.95	3.86	2.63	3.85	3.10	5.49	4.03	2.77
10/18	2.51	3.21	3.33	4.10	3.60	4.09	4.22	6.64	4.16	3.35
10/19	2.29	3.28	2.88	4.20	3.04	4.20	3.60	4.99	3.21	2.94
10/20	2.56	2.95	3.22	3.97	3.18	3.82	3.32	5.88	3.99	3.01
10/21	2.67	3.29	4.07	4.31	2.78	4.24	4.04	5.47	3.62	3.06
10/22	2.95	3.21	3.74	4.01	3.17	4.10	4.21	5.03	3.51	3.22
10/23	2.80	3.29	3.82	4.34	3.10	4.76	2.17	5.31	3.72	3.08
10/24	3.48	3.35	3.53	4.46	3.12	4.38	3.69	5.40	4.14	3.42
10/25	3.08	3.51	3.84	4.43	3.21	4.61	3.60	5.25	3.95	3.39
10/26	3.00	3.79	3.98	4.18	4.01	4.83	4.18	5.15	3.18	3.45
10/27	2.62	3.76	3.39	4.29	4.48	4.55	4.14	4.14	3.38	2.63
10/28	2.22	3.71	4.19	4.63	3.80	5.39	4.39	5.55	3.66	3.04
10/29	2.96	3.82	4.33	4.61	4.78	4.96	5.09	5.83	4.29	3.12
10/30	3.62	4.37	4.43	4.86	3.05	7.95	5.19	4.93	4.42	4.30
10/31	4.05	5.32	5.91	5.18	3.65	5.34	5.92	5.87	4.67	4.38

Cont.

Table
A.5,
cont.

11/1	4.03	4.98	5.29	6.63	4.67	6.36	6.58	6.81	5.54	4.59
11/2	4.31	5.45	5.16	5.63	5.17	6.33	6.49	6.63	5.13	4.16
11/3	4.15	5.53	6.24	5.23	5.47	5.80	5.36	6.43	5.43	5.14
11/4	4.47	5.79	5.25	5.57	5.40	6.19	5.51	6.59	5.70	4.79
11/5	4.38	5.47	6.08	5.75	5.89	5.80	4.58	7.26	5.34	5.07
11/6	4.45	5.98	6.38	5.71	5.63	5.85	6.10	8.41	5.10	5.35
11/7	4.60	6.81	6.97	5.98	5.98	6.77	6.60	7.10	5.40	4.80
11/8	4.64	6.98	6.38	5.80	5.93	6.31	6.53	8.34	5.38	5.29
11/9	5.02	5.90	6.95	5.91	5.62	6.32	6.36	6.64	6.90	4.92
11/10	6.44	8.37	7.79	7.03	7.99	7.68	6.95	8.10	6.57	6.45
11/11	7.01	8.90	8.71	8.06	8.23	9.31	8.46	9.35	7.07	7.26
11/12	7.37	9.67	8.25	7.62	6.86	7.20	6.46	8.99	6.86	7.39
11/13	7.11	9.49	8.70	8.85	7.17	10.43	9.71	11.38	7.91	7.71
11/14	8.23	11.30	9.14	8.90	8.66	8.97	7.60	10.63	8.00	8.45
11/15	7.92	11.63	9.87	8.60	10.09	8.93	8.77	10.80	7.90	8.59
11/16	7.94	10.00	9.53	8.36	9.21	9.61	7.79	11.48	10.51	7.92

Cont.

Table A.5, cont.

11/17	8.57	11.03	9.44	9.42	9.88	10.52	10.08	11.29	11.23	9.19
11/18	8.70	9.04	9.40	9.25	9.55	10.29	9.02	12.66	11.83	9.14
11/19	8.40	8.95	9.08	9.57	9.05	9.78	9.55	11.55	11.17	8.20
11/20	8.54	9.49	9.03	9.85	8.43	10.14	7.72	11.08	10.69	8.69
11/21	8.47	11.12	10.42	9.26	10.66	10.29	9.10	10.87	8.40	7.81

Table A.6 Urinary 4-pyridoxic acid excretion (μmoles/day) Spring 1992 (Study 2)

	Subjects					
Date	1	2	3	4	5	6
4/20	3.73	4.74	----	3.50	4.78	2.93
4/21	3.17	4.25	----	3.14	4.19	2.71
4/22	3.02	3.28	----	3.08	3.77	3.71
4/23	2.83	3.34	----	3.23	3.38	3.79
4/24	2.77	3.61	----	3.14	3.06	3.90
4/25	2.93	3.08	3.71	3.04	3.24	2.75
4/26	3.06	2.91	3.44	3.32	3.29	2.87
4/27	3.08	2.98	2.94	3.30	2.86	2.39
4/28	2.96	3.05	3.82	3.27	2.55	2.34
4/29	2.89	3.26	3.49	2.88	2.76	2.33
4/30	3.52	3.03	3.32	2.28	2.91	2.31
5/1	3.89	2.77	2.84	2.28	3.29	2.20
5/2	3.90	2.17	2.89	2.93	2.70	2.90
5/3	4.06	1.30	3.27	2.48	2.87	2.01
5/4	4.65	3.14	3.11	2.45	3.01	2.65
5/5	2.97	3.63	3.38	2.54	3.17	2.24
5/6	4.56	3.69	3.43	2.65	2.96	2.84
5/7	4.59	3.62	3.32	2.72	2.82	2.89
5/8	4.67	2.46	4.00	2.78	3.33	3.05
5/9	4.61	2.66	4.44	3.12	3.00	3.80
5/10	3.72	2.83	3.29	3.15	2.85	3.04
5/11	4.43	3.49	3.64	3.09	2.82	2.25
5/12	5.53	5.09	5.11	4.45	4.75	3.12
5/13	6.24	7.12	6.11	5.88	5.96	5.66

Cont.

Table A.6, cont.

5/14	6.05	7.62	6.92	6.99	6.05	6.14
5/15	7.47	6.06	7.47	7.05	6.21	6.84
5/16	7.58	8.06	8.23	7.25	7.12	7.20
5/17	7.99	7.67	8.15	6.78	9.12	6.88
5/18	8.21	8.34	8.77	6.26	7.10	8.71
5/19	7.35	8.53	7.92	6.88	6.64	8.65
5/20	9.87	8.76	8.64	6.99	7.96	8.23
5/21	7.94	7.61	7.16	7.89	8.08	7.23

Table A.7 Urinary total vitamin B-6 (μmoles/day) Fall 1989 (Study 1)

	Subjects									
Date	1	2	3	4	5	6	7	8	9	10
10/2	0.406	0.533	0.769	0.955	0.528	0.915	0.681	0.924	0.939	0.878
10/3	0.388	0.537	0.602	0.878	0.551	0.850	0.618	0.815	0.887	0.882
10/4	0.299	0.472	0.648	0.778	0.560	0.795	0.621	0.790	0.855	0.882
10/8	0.350	0.372	0.749	0.617	0.475	0.513	0.548	0.604	0.663	0.535
10/9	0.266	0.594	0.660	0.648	0.578	0.794	0.541	0.586	0.659	0.273
10/14	0.294	0.302	0.455	0.577	0.520	0.625	0.650	0.623	0.556	0.702
10/15	0.318	0.420	0.417	0.632	0.335	0.604	0.483	0.649	0.526	0.597
10/16	0.442	0.471	0.677	0.617	0.555	0.639	0.544	0.622	0.556	0.649
10/17	0.467	0.421	0.640	0.685	0.481	0.695	0.567	0.643	0.561	0.676
10/19	0.462	0.462	0.725	0.726	0.492	0.680	0.495	0.666	0.662	0.667
10/23	0.448	0.467	0.669	0.773	0.426	0.790	0.521	0.691	0.646	0.672
10/26	0.523	0.444	0.692	0.693	0.499	0.739	0.586	0.612	0.528	0.713
10/27	0.476	0.433	0.513	0.682	0.648	0.654	0.531	0.661	0.629	0.487
10/28	0.489	-----	0.664	0.792	0.635	0.756	0.550	0.634	0.613	0.736

Cont.

Table A.7, cont.

10/29	0.576	0.426	0.671	0.761	0.584	0.742	0.590	0.716	0.631	0.655
10/31	0.660	0.517	0.665	0.829	0.397	0.735	0.590	0.558	0.624	0.838
11/5	0.648	0.444	0.780	0.896	0.684	0.755	0.394	1.203	0.846	-----
11/7	0.686	0.579	0.754	0.880	0.647	0.892	0.659	0.775	0.811	-----
11/8	0.711	0.673	0.735	0.883	0.645	0.813	0.642	0.935	0.813	-----
11/9	0.670	0.776	0.779	0.895	0.655	0.732	0.703	0.756	0.765	-----
11/10	0.879	0.708	0.829	0.967	0.702	0.987	0.572	0.895	0.900	-----
11/12	0.953	0.668	0.865	1.011	0.657	0.787	0.544	0.868	0.905	-----
11/16	0.944	0.805	1.007	1.173	0.704	1.022	0.624	1.029	0.945	-----
11/19	1.028	0.850	0.910	1.189	0.879	1.066	0.826	0.937	1.016	0.864
11/20	0.976	0.908	0.974	1.258	0.743	1.077	0.549	0.916	1.050	1.049
11/21	0.959	0.893	0.889	1.234	0.798	1.069	0.820	0.952	1.008	0.878

Table A.8 Urinary total vitamin B-6 (μmoles/day) Spring 1992 (Study 2)

	Subjects					
Date	1	2	3	4	5	6
4/20	0.527	0.702	----	0.696	0.754	0.502
4/22	0.523	0.644	----	0.521	0.695	0.518
4/26	0.430	0.468	0.537	0.500	0.562	0.387
4/29	0.509	0.486	0.525	0.473	0.592	0.382
4/30	0.458	0.487	0.448	0.599	0.620	0.362
5/1	0.481	0.365	0.422	0.525	0.532	0.331
5/2	0.437	-----	0.429	0.534	0.685	0.392
5/3	0.401	0.196	0.415	0.576	0.624	0.360
5/6	0.440	0.469	0.471	0.587	0.600	0.393
5/9	0.486	0.443	0.503	0.642	0.691	0.464
5/10	0.415	0.463	0.585	0.586	0.710	0.466
5/11	0.627	0.437	0.521	0.609	0.632	0.383
5/12	0.633	0.606	0.711	0.671	0.841	0.411
5/13	0.726	0.812	0.784	0.892	0.825	0.572
5/16	0.728	0.885	0.890	0.918	0.929	0.652
5/19	0.855	0.940	0.773	0.901	1.02	0.663
5/20	0.807	0.832	0.839	0.842	1.03	0.774
5/21	0.747	0.911	0.800	0.825	1.09	0.719

Table A.9 Urinary xanthurenic acid excretion (μmoles/day) the day before and the day of a 2 g L-tryptophan load Fall 1989 (Study 1)

	Subjects									
Date	1	2	3	4	5	6	7	8	9	10
10/15	5.12	8.11	12.99	14.60	8.37	12.70	9.92	11.81	18.25	13.19
10/16	76.27	20.87	78.24	42.80	58.70	40.22	49.26	58.22	101.48	31.16
10/27	6.38	8.30	12.73	12.87	11.26	11.57	11.61	12.73	19.46	9.23
10/28	17.46	33.75	63.30	30.80	38.01	29.13	29.61	37.43	46.66	26.74
11/8	5.94	7.49	17.86	14.79	12.77	15.55	11.56	13.83	17.83	16.16
11/9	25.92	12.22	42.40	35.95	44.64	29.79	26.56	24.06	41.21	23.64
11/19	4.58	10.59	14.08	12.22	11.87	10.38	14.71	14.06	17.31	11.71
11/20	19.20	12.80	42.77	23.35	28.60	23.35	13.40	26.13	36.48	27.30

Table A.10 Urinary kynurenic acid excretion (μmoles/day) the day before and the day of a 2 g L-tryptophan load Fall 1989 (Study 1)

	Subjects									
Date	1	2	3	4	5	6	7	8	9	10
10/15	12.35	13.88	12.84	10.61	20.10	15.82	14.73	11.58	11.63	12.00
10/16	128.71	109.39	122.81	61.99	503.51	145.38	143.69	83.48	75.16	47.96
10/27	11.96	15.80	12.74	11.16	25.65	13.07	15.01	12.28	10.25	9.96
10/28	78.58	109.33	112.04	69.97	536.45	140.56	102.90	78.58	40.55	48.26
11/8	10.30	15.44	14.36	11.97	27.17	12.91	16.82	12.38	10.48	14.80
11/9	78.36	96.81	97.12	78.73	406.74	132.94	77.48	59.48	45.09	54.78
11/19	11.28	14.52	14.36	11.97	27.17	12.91	16.82	12.38	10.48	14.80
11/20	71.79	94.01	137.26	74.85	416.12	107.84	19.16	65.37	44.21	42.06

Table A.11 Urinary volatile amines (kynurenine plus acetylkynurenine, μmoles/day) the day before and the day of a 2 g L-tryptophan load Fall 1989 (Study 1)

	Subjects									
Date	1	2	3	4	5	6	7	8	9	10
10/15	3.7	5.1	5.8	4.3	3.5	4.3	4.7	2.7	3.2	4.4
10/16	290.1	116.9	96.7	34.3	995.8	175.4	38.5	12.3	31.3	9.3
10/27	4.1	6.3	3.4	4.7	3.8	5.3	3.0	3.3	4.2	2.6
10/28	67.4	129.4	30.5	39.5	1007.6	96.1	13.1	13.9	17.5	7.8
11/8	3.9	5.4	5.7	4.4	4.2	7.0	4.2	3.5	4.2	5.1
11/9	34.1	97.9	41.5	43.1	660.6	62.2	11.2	9.9	11.6	8.9
11/19	2.7	2.0	6.8	5.4	5.1	4.4	6.1	3.3	4.7	4.7
11/20	23.9	61.4	39.5	38.7	634.1	49.9	4.7	11.0	16.1	10.4

Table A.12 Hematocrit (%) Fall 1989 (Study 1)

	Subjects									
Date	1	2	3	4	5	6	7	8	9	10
10/2	41.0	41.0	43.5	42.8	39.5	42.5	41.7	42.3	42.5	41.3
10/5	38.8	39.5	40.0	42.2	40.5	42.5	42.2	39.8	41.2	40.7
10/9	40.5	37.5	39.8	43.3	39.7	41.5	39.5	39.3	39.7	40.7
10/13	38.3	36.7	41.0	43.8	40.3	41.0	41.2	41.0	42.2	39.8
10/17	38.7	37.5	40.5	42.7	41.5	43.0	41.8	39.3	43.5	41.5
10/20	37.8	39.3	42.5	41.1	41.3	43.2	41.5	44.0	41.8	40.0
10/24	39.2	37.7	41.1	41.8	39.2	41.4	42.9	40.3	41.6	40.8
10/29	39.6	37.5	41.7	41.6	41.5	42.7	41.2	40.6	41.0	40.7
11/1	39.5	37.4	41.2	43.2	40.9	40.5	41.6	37.6	40.6	39.9
11/5	38.6	38.6	40.8	40.9	40.9	43.0	41.2	38.8	40.8	37.9
11/10	38.4	37.7	40.0	41.7	40.3	40.8	41.8	39.6	38.6	38.9
11/13	40.7	39.6	40.7	40.9	40.4	38.7	43.6	39.6	39.6	39.5
11/17	39.5	38.4	40.6	40.7	39.7	40.3	40.9	39.7	42.6	38.8
11/22	39.3	40.7	40.1	40.8	40.1	41.2	41.6	39.7	38.1	38.5

Table A.13 Hemoglobin concentration (g/100mL) Fall 1989 (Study 1)

	Subjects									
Date	1	2	3	4	5	6	7	8	9	10
10/2	13.5	13.1	14.4	14.0	12.8	14.3	13.6	14.2	14.4	13.4
10/5	13.2	12.8	13.8	14.0	12.9	14.5	14.6	13.5	14.3	13.5
10/9	13.1	12.3	13.5	14.6	12.9	14.0	13.2	13.4	13.6	13.5
10/13	12.3	11.6	13.8	14.5	13.2	14.0	13.5	13.7	14.3	13.9
10/17	12.7	12.0	13.3	13.8	13.4	14.2	13.6	13.2	14.6	13.6
10/20	11.9	12.3	13.8	13.1	12.9	14.2	13.3	12.7	14.3	13.2
10/24	12.5	11.8	13.7	13.4	12.6	13.8	13.8	13.2	13.9	13.2
10/29	13.0	12.1	14.0	13.6	13.1	14.3	13.6	13.5	14.3	13.5
11/1	12.4	11.7	13.8	13.9	12.8	13.6	13.7	12.7	13.4	13.0
11/5	13.0	12.5	13.4	14.0	13.8	14.7	14.5	13.1	14.6	13.0
11/10	12.8	12.3	13.3	13.8	12.8	13.0	14.1	13.8	13.4	12.8
11/13	13.5	12.9	13.5	13.4	13.0	13.3	14.6	13.2	13.5	12.8
11/17	13.1	12.5	13.6	13.4	13.0	13.9	14.1	13.4	14.6	12.9
11/22	12.9	12.9	13.5	13.2	12.7	14.1	13.5	13.3	13.3	12.7

Table A.14 Hematocrit (%) Spring 1992 (Study 2)

	Subjects					
Date	1	2	3	4	5	6
4/20	39.8	45.3	---	44.0	39.5	43.8
4/24	37.5	43.5	40.0	40.8	41.8	41.5
4/28	37.5	42.5	38.5	40.5	40.8	41.0
5/2	38.5	44.5	37.5	38.0	40.5	44.0
5/6	38.5	42.5	39.0	38.5	40.0	41.0
5/12	37.0	43.5	38.0	39.5	39.5	42.0
5/16	38.5	43.5	36.5	37.5	38.3	41.5
5/22	38.8	43.0	36.5	40.5	39.5	42.0

Table A.15 Hemoglobin concentration (g/100mL) Spring 1992 (Study 2)

	Subjects					
Date	1	2	3	4	5	6
4/20	12.7	14.2	13.7	13.1	12.6	14.1
4/24	12.3	13.2	13.4	12.7	13.0	13.4
4/28	12.5	13.3	12.9	12.5	12.7	13.6
5/2	12.4	13.8	12.4	11.7	12.3	13.9
5/6	12.6	13.8	13.0	11.9	12.8	13.3
5/12	12.0	13.5	12.7	12.1	12.5	13.9
5/16	14.7	14.2	13.3	13.2	14.0	13.6
5/22	12.5	13.2	12.5	12.5	12.5	13.7

Table A.16 Plasma pyridoxal 5′-phosphate (nmoles/L) Fall 1989 (Study 1)

	Subjects									
Date	1	2	3	4	5	6	7	8	9	10
10/2	45.1	20.3	25.1	109	48.7	38.4	30.3	45.1	105	40.1
10/5	43.5	19.1	22.8	55.8	37.5	23.4	32.4	46.5	97.7	39.9
10/9	31.0	16.5	20.5	45.8	29.1	20.3	26.4	30.5	63.4	35.3
10/13	26.6	13.0	20.2	39.6	27.1	15.9	38.9	31.8	51.3	30.0
10/17	28.1	13.7	18.9	37.9	27.2	16.5	24.3	27.4	53.0	31.9
10/20	30.5	16.5	21.8	37.6	33.0	18.0	26.0	27.5	52.3	41.2
10/24	31.9	18.0	20.0	37.6	35.1	18.3	29.1	30.5	50.1	46.2
10/29	32.9	17.4	21.0	37.4	47.3	19.3	30.1	28.0	51.2	39.5
11/1	34.8	20.1	25.4	41.2	57.4	22.5	30.4	34.1	61.9	33.7
11/5	48.0	24.5	26.0	45.9	56.1	24.0	34.1	41.1	58.5	34.1
11/10	61.0	24.6	29.4	45.8	51.1	22.7	38.6	36.6	66.2	34.3
11/13	69.6	32.6	33.9	59.3	63.7	28.2	50.0	44.9	85.0	48.3
11/17	79.9	25.0	38.3	59.3	93.3	36.8	53.4	37.9	85.3	52.8
11/22	86.5	35.5	38.6	68.4	105	30.4	56.2	38.5	81.0	49.1

Table A.17 Plasma pyridoxal 5′-phosphate (nmoles/L) Spring 1992 (Study 2)

	Subjects					
Date	1	2	3	4	5	6
4/20	37.6	39.7	---	28.0	71.6	60.0
4/24	22.2	21.7	23.1	23.3	58.9	47.0
4/28	21.5	21.7	17.8	19.1	49.5	38.4
5/2	21.1	19.5	17.3	19.2	49.2	32.8
5/6	23.0	21.4	19.8	20.3	52.3	40.1
5/12	25.4	22.8	18.1	20.3	48.2	41.8
5/16	36.2	36.2	34.7	37.7	68.9	79.6
5/22	45.2	39.8	44.4	49.4	84.0	90.6

Table A.18 Red blood cell pyridoxal 5′-phosphate (nmoles/L) Spring 1992 (Study 2)

	Subjects					
Date	1	2	3	4	5	6
4/20	51.1	25.7	---	28.3	49.2	38.7
4/24	51.8	29.0	59.7	31.6	50.6	44.2
4/28	52.4	34.1	55.3	32.5	48.7	46.4
5/2	50.1	27.8	48.5	28.5	43.5	42.4
5/6	48.5	30.3	54.1	30.1	43.6	45.0
5/12	52.4	33.4	60.1	35.6	47.7	43.9
5/16	56.5	31.0	69.6	35.6	51.2	48.5
5/22	52.5	27.0	67.0	36.1	48.2	52.6

Table A.19 Plasma total vitamin B-6 (nmoles/L) Spring 1992 (Study 2)

	Subjects					
Date	1	2	3	4	5	6
4/2	67.6	50.0	---	37.2	138	72.5
4/20	66.1	47.9	---	32.0	88.4	71.9
4/24	51.8	41.8	38.8	33.8	64.4	54.1
4/28	41.8	29.6	31.9	34.6	57.8	45.6
5/2	37.2	35.1	32.6	23.6	60.7	50.4
5/6	57.6	31.8	40.4	30.4	55.9	52.2
5/12	54.2	32.3	33.2	40.1	48.7	55.1
5/16	58.2	50.4	51.2	43.5	73.9	102
5/22	67.3	54.5	61.8	61.5	93.0	96.0

Table A.20 Plasma alkaline phosphatase activity (μmoles/min/L) Fall 1989 (Study 1)

	Subjects									
Date	1	2	3	4	5	6	7	8	9	10
10/2	27.9	25.9	28.4	15.4	24.2	19.2	27.9	27.0	42.5	34.8
10/9	25.7	24.0	26.1	15.6	27.9	20.6	26.4	27.9	19.2	32.9
10/17	24.7	23.8	24.6	16.2	28.9	22.2	26.7	27.2	19.7	34.6
10/24	24.3	22.6	26.9	15.4	28.4	22.1	27.1	27.2	21.1	33.5
11/1	24.8	22.9	28.0	16.5	28.7	21.4	30.0	27.7	22.4	34.5
11/10	26.1	22.9	26.1	17.7	28.7	21.9	31.6	29.1	20.5	34.8
11/17	26.1	23.7	25.7	20.5	27.3	22.9	29.4	30.7	24.0	35.7

Table A.21 Plasma alkaline phosphatase activity (μmoles/min/L) Spring 1992 (Study 2)

	Subjects					
Date	1	2	3	4	5	6
Pre	28.6	18.1	---	23.4	21.5	17.9
4/20	28.1	18.2	---	23.4	21.5	17.9
4/24	29.0	16.2	34.2	18.2	23.0	18.0
4/28	28.9	16.5	31.7	18.9	22.2	17.8
5/2	28.3	18.2	32.3	18.0	22.6	19.0
5/6	29.5	18.1	34.9	18.2	24.1	19.4
5/12	28.1	19.3	32.6	19.4	25.6	19.9
5/16	31.9	20.0	35.3	18.7	26.1	20.7
5/22	30.0	19.0	37.7	19.2	25.2	20.5

Table A.22 Erythrocyte aspartate aminotransferase activity (μg pyruvate/mg Hgb/hr) with (stim) and without (unstim) added pyridoxal phosphate, and percent stimulation (%) Fall 1989 (Study 1)

		Subjects									
Date		1	2	3	4	5	6	7	8	9	10
	unstim	23.15	23.74	24.74	9.97	31.43	25.42	22.60	34.42	27.63	9.76
10/2	stim	35.09	37.05	35.62	35.05	40.81	32.10	34.25	42.12	38.32	18.01
	%	52	56	44	252	30	26	52	22	39	85
	unstim	22.67	25.40	22.12	21.77	33.16	26.40	20.87	36.31	28.34	29.91
10/5	stim	33.33	34.08	34.27	35.48	43.10	35.87	30.74	46.50	43.00	44.24
	%	47	34	55	63	30	36	47	28	52	48
	unstim	20.78	27.74	29.29	28.27	31.93	29.29	21.96	29.37	29.55	33.33
10/9	stim	34.63	38.01	38.72	39.93	47.19	43.77	39.19	43.89	39.61	46.88
	%	67	37	32	41	48	49	78	49	34	41
	unstim	20.38	20.00	26.60	12.07	---	29.24	28.04	38.75	30.43	30.88
10/13	stim	34.39	32.90	39.06	40.52	---	44.35	36.99	51.21	42.47	47.71
	%	69	65	47	236	---	52	32	32	40	55
	unstim	---	30.22	33.10	32.97	40.99	34.28	25.80	34.59	32.76	32.37
10/17	stim	---	36.87	41.64	46.34	51.06	48.76	38.87	45.72	49.15	46.40
	%	---	22	26	41	25	42	51	32	50	43
	unstim	24.48	27.82	24.92	24.31	35.38	19.38	19.58	27.57	25.68	29.02
10/20	stim	31.29	32.04	33.00	33.33	40.77	31.83	37.41	41.96	40.41	43.71
	%	28	15	32	37	15	64	91	52	57	51

Continued

Table A.22, continued

	unstim	25.93	30.51	22.11	25.72	28.57	30.03	27.30	29.10	30.82	37.40
10/24	stim	36.11	34.74	37.41	34.06	41.81	36.52	35.49	30.00	45.21	53.70
	%	39	14	69	32	46	22	30	34	47	44
	unstim	24.83	24.64	25.85	25.17	33.33	27.31	27.68	31.58	26.59	30.00
10/29	stim	34.90	34.64	30.95	36.01	44.09	29.42	34.42	43.86	41.81	43.16
	%	41	41	20	43	32	44	24	39	57	44
	unstim	21.05	20.07	21.43	19.50	31.52	29.41	24.32	21.92	28.67	30.80
11/1	stim	27.63	28.87	30.61	25.18	42.03	35.64	31.51	33.22	36.52	35.64
	%	31	44	43	29	33	21	30	52	27	16
	unstim	20.45	20.14	27.00	23.61	30.52	27.27	21.55	26.99	29.97	53.39
11/5	stim	32.95	30.20	29.33	29.59	34.39	35.02	31.65	41.18	34.36	40.29
	%	61	50	9	25	13	28	47	53	15	21
	unstim	18.33	22.89	21.88	20.49	31.10	36.88	31.56	35.08	38.69	41.53
11/10	stim	27.33	32.04	21.88	29.51	44.17	54.49	45.51	54.42	57.70	58.79
	%	49	40	0	44	42	48	44	55	49	42
	unstim	29.33	26.25	26.09	22.24	36.18	31.06	24.17	32.32	26.99	30.80
11/13	stim	38.67	29.10	35.79	32.11	51.45	39.53	34.44	45.79	35.76	39.13
	%	32	11	37	44	42	27	42	42	32	27

Continued

Table A.22, continued

	unstim	28.36	28.09	25.57	24.66	35.86	31.00	24.50	29.04	32.58	34.14
11/17	stim	40.66	38.46	38.03	31.76	44.48	43.00	34.23	45.05	45.16	46.21
	%	43	37	49	29	24	39	40	55	39	35
	unstim	27.61	32.99	30.87	31.96	44.40	37.41	21.92	33.45	28.28	37.50
11/22	stim	40.91	45.14	40.94	44.33	56.68	48.62	29.79	47.93	41.08	55.71
	%	48	37	33	39	28	30	36	43	45	49

Table A.23 Erythrocyte alanine aminotransferase activity (μg pyruvate/mg Hgb/hr) with (stim) and without (unstim) added pyridoxal phosphate, and percent stimulation (%) Fall 1989 (Study 1)

		Subjects									
Date		1	2	3	4	5	6	7	8	9	10
	unstim	1.25	1.86	2.66	1.92	2.12	4.24	2.46	3.05	4.69	4.04
10/2	stim	1.47	2.43	3.10	2.01	2.31	4.51	2.96	3.39	4.80	4.36
	%	18	31	17	5	9	6	20	11	2	8
	unstim	0.93	1.04	2.05	1.47	1.24	2.39	1.28	1.80	2.92	1.81
10/5	stim	1.29	1.20	2.32	1.53	1.46	2.78	1.62	1.84	2.27	2.16
	%	38	15	13	4	18	16	27	2	0	19
	unstim	1.11	1.04	1.96	1.14	1.82	2.78	1.50	1.85	2.80	1.54
10/9	stim	1.43	1.45	2.49	1.39	2.20	3.37	1.79	2.19	3.23	2.71
	%	29	39	27	22	21	21	19	18	15	76
	unstim	1.36	1.17	2.28	1.26	1.96	3.25	2.02	2.81	3.25	2.30
10/13	stim	1.81	1.83	2.82	1.52	2.41	3.86	2.41	3.35	3.81	2.73
	%	33	56	24	21	23	19	19	19	17	19
	unstim	1.23	1.07	1.89	1.02	1.72	2.73	1.68	1.92	---	2.46
10/17	stim	1.59	1.47	2.42	1.38	2.34	3.42	2.10	2.19	---	2.88
	%	29	37	28	35	36	25	25	14	---	17
	unstim	1.33	1.41	2.31	1.15	1.66	2.91	2.00	2.34	3.65	3.16
10/20	stim	1.71	1.74	2.87	1.45	2.02	3.69	2.42	2.56	3.85	3.33
	%	29	23	24	26	22	27	21	9	5	5

Continued

Table A.23,
continued

	unstim	1.29	1.59	1.76	1.19	1.68	2.55	1.58	1.81	3.44	2.93
10/24	stim	1.68	1.85	2.28	1.40	2.19	3.06	2.03	2.20	3.94	3.26
	%	30	16	30	18	30	20	28	22	15	11
	unstim	0.92	1.44	1.63	0.75	1.88	2.50	1.57	1.78	3.16	2.70
10/29	stim	1.42	1.99	2.14	1.19	2.44	3.12	2.08	2.33	3.78	3.28
	%	54	38	31	58	30	25	32	31	20	21
	unstim	1.09	1.22	1.71	0.97	1.66	2.16	1.56	1.23	3.15	2.35
11/1	stim	1.42	1.50	2.22	1.18	1.83	2.69	1.88	1.61	3.48	2.61
	%	30	23	30	22	10	25	21	31	10	11
	unstim	1.09	1.02	2.21	0.89	1.68	1.94	1.35	1.67	2.79	2.51
11/5	stim	1.41	1.50	2.91	1.03	2.00	2.46	1.66	2.06	3.33	2.90
	%	29	47	32	15	19	27	23	23	19	16
	unstim	1.38	1.48	1.85	0.98	2.00	2.28	1.46	1.68	3.41	1.85
11/10	stim	1.64	1.68	2.19	1.12	2.37	2.68	1.72	2.08	3.66	2.19
	%	19	14	18	14	19	18	18	24	7	18
	unstim	1.26	1.05	1.82	0.83	2.09	2.34	1.45	1.82	2.88	2.17
11/13	stim	1.56	1.23	2.26	1.11	2.31	2.84	1.75	2.20	3.21	2.58
	%	24	17	24	34	11	21	21	21	11	19
	unstim	1.44	1.53	1.77	0.71	1.79	2.31	1.47	1.50	3.19	2.05
11/17	stim	1.60	1.93	2.12	0.96	1.89	2.60	1.62	1.64	3.27	2.32
	%	11	26	20	34	6	13	10	9	3	13
	unstim	1.70	1.81	1.88	0.70	1.98	2.52	1.72	1.65	3.33	2.54
11/22	stim	1.81	2.01	2.18	0.96	2.33	2.81	2.06	2.00	3.60	3.02
	%	6	11	16	38	18	12	20	21	8	19

Table A.24 Erythrocyte aspartate aminotransferase activity (μg pyruvate/mg Hgb/hr) with (stim) and without (unstim) added pyridoxal phosphate, and percent stimulation (%) Spring 1992 (Study 2)

		Subjects					
Date		1	2	3	4	5	6
	unstim	35.06	29.93	---	28.77	27.08	28.57
4/20	stim	53.25	43.20	---	43.84	43.40	42.86
	%	52	44	---	52	60	50
	unstim	31.85	27.67	39.17	26.80	34.51	28.87
5/22	stim	51.59	43.00	58.75	40.89	48.59	45.70
	%	62	55	50	53	41	58

Table A.25 Erythrocyte alanine aminotransferase activity (μg pyruvate/g Hgb/hr) with (stim) and without (unstim) added pyridoxal phosphate, and percent stimulation (%) Spring 1992 (Study 2)

		Subjects					
Date		1	2	3	4	5	6
	unstim	4.03	1.17	---	2.86	2.63	2.77
4/20	stim	4.43	1.35	---	2.88	3.03	3.37
	%	10	15	---	0.6	15	22
	unstim	2.81	0.89	1.60	1.78	1.90	2.65
5/22	stim	3.32	1.03	1.88	1.97	2.22	2.97
	%	18	16	18	10	17	12

Table A.26 Fecal total vitamin B-6 (μmoles/5 days) Fall 1989 (Study 1)

	Subjects									
Period	1	2	3	4	5	6	7	8	9	10
I	9.07	14.72	7.26	13.72	8.38	12.97	11.59	5.73	9.77	10.07
II	9.03	14.97	9.38	13.16	6.28	11.03	9.69	15.26	12.42	12.49
III	10.03	14.55	10.97	11.98	11.84	10.45	6.54	15.93	14.34	7.83
IV	12.09	11.24	14.55	17.77	9.89	13.85	---	18.06	11.96	7.60

Table A.27 Subjects' weight (lbs) Fall 1989 (Study 1)

Date	Subjects 1	2	3	4	5	6	7	8	9	10
10/2	100	135	184	120.5	153	128	172	206	143.5	281
10/3	100	133.75	185	119.5	153	128	173	206	141.5	276
10/4	101	134.5	185	120	154.5	128.5	172	205	140	276
10/5	101.25	134.5	184	120.5	153.5	128.5	173	204	139	276
10/6	101	134.5	184	120.5	153.5	128.5	173	204	139.5	275
10/7	101	134	185	120.5	153.5	129	172	204	139	274
10/8	100	133.5	185	120.5	154	127.75	173	204	139	275
10/9	100.5	134.75	185	120.75	154	127.5	173	204	139.5	275
10/10	100.25	134	184	119.75	154	127.75	174	204.25	139.5	278
10/11	101.75	134.25	185	120.0	154.5	127.75	174	204	139	274
10/12	101.25	134	185	120.5	154.5	129	173	204	139	274
10/13	101.75	133.5	183	120.5	154	128.25	172	204	139	274
10/14	101.5	133.75	184	121.5	154	128.75	171	203.5	139.5	274
10/15	101	133.75	182	120.0	154	127.5	173	203.5	139.5	274
10/16	101.25	136	182	121.5	152.5	128.25	172	203	139	274

Cont.

Table A.27, cont.

10/17	101.75	133.5	182	121.25	152.5	128	171.5	204	139	273
10/18	101	133.25	182	119.75	152.25	131	172	204	139	273
10/19	101.75	133.25	183	120	152.25	128.75	171	205	139	273
10/20	102	134.5	181	120.5	152.25	128.25	171	205	139	271.5
10/21	101.75	134.25	180	121.5	152.5	129.25	172	204.25	138.5	271.5
10/22	101.25	133.75	180	120.5	152.25	128.5	171	204.25	139	272
10/23	1101.25	134.25	180	120.25	152	130	172	205	139	271.5
10/24	102.5	134.5	1811	121	152	129	172	205	139	271
10/25	101.75	133.25	180	121.5	152	128.25	173	205	139	271.5
10/26	101.75	133	181	121	152	128	173	206	140	271.5
10/27	102	133.75	182	121	152	131	172	206	140	272
10/28	101.75	133.5	180	121	152	129.25	172	206.5	140	271.5
10/29	101.5	132	179	120.5	152	129	171	207	140	271.5
10/30	100.75	132.5	181	120.5	151	130	172	206.5	139.5	271
10/31	100.75	135	181	119	151	129.25	172.5	206.5	139.5	270.5
11/1	101.5	132.75	180	119.5	151	130	172	207	139.5	271

Cont.

Table
A.27,
cont.

11/2	101.25	133.75	180	121	151	129.5	171	206.5	140	271
11/3	100.75	134.5	181	121	152.5	129.75	172	206	140	273.5
11/4	101	135	181	120.5	152	128.75	173	206	140.25	272
11/5	100.5	134	180	120	152	129	173	206	140	271.5
11/6	101.25	134.25	180	120.5	151	130.25	172	206	139.5	273.5
11/7	101.75	134	182	120.25	152	130	172	206	140	272.5
11/8	102.25	136	182	120	152	130.25	172	206	140	273
11/9	101.25	133.75	181	119.75	152	129.5	171	206	140	273
11/10	102	133.5	179	119	151	130	171	206	139.5	273
11/11	101.25	133.75	180	120	151	131	171	206.5	139	272.5
11/12	101.5	132.5	180	120	152	129	172	206	140	272.5
11/13	101.25	132.75	181	120	152	129.5	172	206.5	140	273
11/14	101.5	132.5	182	121	151	130	173	204	140	273
11/15	101.75	132.5	181	121	151	131	172	204.5	140.5	273
11/16	101.75	133	182	120.5	151	129.5	172	203	140.25	272.5
11/17	101.75	133.5	181	120.5	151	128.25	171	203	140	269.5

Cont.

Table A.27, cont.

11/18	101.25	135	179	120.5	151	130	172	202	140	271
11/19	101.25	133.75	178	120	151	128.75	172	202.5	140.5	270.5
11/20	101.5	134	179	121.5	151	130	171	203	140.5	270.5
11/21	101	135	179	120.5	151	130	170	---	140.5	272

Table A.28 Subjects' weights (lbs) Spring 1992 (Study 2)

	Subjects					
Date	1	2	3	4	5	6
4/20	150	142	---	151	142	127
4/21	150	138	---	151.5	138	126
4/22	150	139	---	152	137	127
4/23	150	139.5	209	153	137	127
4/24	150	140	210	152	137	127
4/25	150	140	210	151.5	136	126
4/26	150	141	210.5	152.5	136	126
4/27	150	138	210.5	151.5	136	128
4/28	149.5	140	210.5	152	137	127
4/29	149.5	140.75	211	151	135.5	126
4/30	149.25	140.25	211	152	135.5	126.5
5/1	148.5	139	211	153.25	134.5	126
5/2	149.5	139.75	211	151	136	126
5/3	149.75	138	210	152	134.5	126
5/4	150	138	210	152.25	136.5	126.5
5/5	150	137.5	209	152.5	136	126
5/6	149.75	138.25	209.5	150.5	136.5	126
5/7	148.5	137	209.25	151.5	136.5	126.5
5/8	148.75	139	209.75	152	136.5	127.5
5/9	149.5	140.25	210.75	152.5	136.5	128
5/10	149.75	138	211.25	153	136	126.5
5/11	148.5	138	211.75	151.75	136.5	128
5/12	149.5	138.25	211.75	152.5	137	128
5/13	149.5	138	210.75	152.75	137.75	128
5/14	149	138	211.25	153	136.25	127

Cont.

Table A.28, cont.

5/15	149	138.25	211.5	151.5	135	128
5/16	147.75	139	211.5	150.5	136.75	127
5/17	148.5	136	210	152	136	127.5
5/18	148	137.75	209.75	151.5	136.25	127.5
5/19	148	139	208	151.75	136.5	126
5/20	148.25	139	209.5	152	137	127
5/21	148.5	140.5	210.25	152.5	137	126
5/22	149	139	209.5	150.5	136	127

APPENDIX B

DATA FROM THE STUDY DESCRIBED IN CHAPTER 4 (SPRING 1990)

Table B.1 Urinary creatinine excretion (g/day) Spring 1990

	Subjects								
Date or day of diet	1	2	3	4	5	6	7	8	9
4/4	0.90	0.93	1.05	1.28	1.14	1.14	1.00	1.51	0.94
4/5	0.88	1.02	1.08	1.42	1.15	1.15	1.03	1.16	1.05
4/6	0.92	1.08	1.10	1.34	1.13	1.13	0.99	1.18	0.87
4/7	0.92	1.06	1.12	1.46	1.15	1.19	0.84	1.18	1.09
4/8	0.86	0.91	1.11	1.22	1.15	1.11	1.16	1.18	0.94
4/9	0.93	1.11	1.11	1.14	1.16	1.13	0.99	1.23	0.90
4/10	0.90	1.08	1.07	1.32	1.18	1.15	1.03	1.21	0.99
4/11	0.92	1.07	1.13	1.19	1.16	1.15	0.95	1.15	0.93
LOW 1	0.86	1.07	0.80	1.33	1.16	1.05	1.00	1.11	0.75
LOW 2	0.92	0.99	1.05	1.35	1.14	1.16	1.06	0.59	0.67
LOW 3	0.87	1.02	0.94	0.78	1.12	1.17	1.04	1.06	1.10
LOW 4	0.93	0.95	1.07	1.36	1.13	1.28	0.99	1.04	0.62
LOW 5	0.88	1.06	0.99	1.29	1.07	1.11	1.00	1.06	1.27
LOW 6	0.82	1.00	0.96	1.34	1.08	1.13	1.00	1.00	0.87
LOW 7	0.89	1.02	1.03	1.34	1.10	1.13	0.96	1.05	0.74
LOW 8	0.75	1.05	0.97	1.28	1.00	0.98	0.96	1.05	0.94
LOW 9	1.03	1.05	0.85	1.38	1.04	1.04	0.98	1.09	1.03
LOW 10	0.88	1.01	1.02	1.38	1.08	1.08	0.95	1.05	0.85
LOW 11	0.85	1.00	0.98	1.30	1.02	1.08	0.94	0.97	1.05
LOW 12	0.86	0.99	1.00	1.33	0.99	0.98	0.95	1.01	0.84
LOW 13	0.88	0.94	0.93	1.35	0.98	1.04	0.75	1.04	1.04
LOW 14	0.77	1.03	0.97	1.31	0.98	1.06	0.95	0.95	1.07
MED 1	0.90	1.04	1.10	1.29	1.07	1.09	0.97	1.22	1.18
MED 2	0.88	1.04	1.08	1.38	1.16	1.03	0.96	0.85	1.21

Continued

Table B.1, continued

MED 3	0.90	1.06	0.90	1.34	1.03	0.98	1.53	1.21	0.93
MED 4	0.88	1.02	1.06	1.26	0.78	1.09	0.97	1.17	1.14
MED 5	0.84	1.06	1.20	1.23	1.10	1.08	0.98	1.10	0.90
MED 6	0.88	1.00	0.89	1.02	1.12	1.07	1.03	1.13	0.65
MED 7	0.87	1.08	0.97	1.00	1.08	1.06	1.04	1.16	0.75
MED 8	0.88	1.02	0.95	1.27	1.12	1.09	1.03	1.10	0.58
MED 9	0.91	1.13	0.98	1.36	1.19	1.03	1.02	1.11	1.05
MED 10	0.82	1.06	1.05	1.17	0.98	1.15	1.02	1.09	0.96
MED 11	0.98	1.04	0.94	1.32	1.08	1.03	1.02	1.07	1.03
MED 12	0.89	1.12	0.99	1.12	1.10	1.02	1.02	1.14	1.07
MED 13	0.84	1.11	1.03	1.22	1.11	1.10	1.02	1.09	1.03
MED 14	0.86	1.06	0.96	1.14	1.03	1.08	1.02	1.08	0.80
HIGH 1	0.87	0.92	1.01	1.17	1.04	1.09	0.97	1.03	0.99
HIGH 2	0.80	1.00	0.96	1.42	1.10	1.08	0.87	1.08	0.96
HIGH 3	0.76	0.92	0.95	0.86	0.98	1.09	0.87	1.00	0.57
HIGH 4	0.88	0.94	0.94	1.37	1.14	1.07	1.09	0.97	0.77
HIGH 5	0.92	0.96	1.13	1.40	1.07	1.13	0.99	1.00	0.99
HIGH 6	0.91	0.97	1.02	1.29	1.16	1.02	1.01	0.97	1.01
HIGH 7	0.94	1.08	1.10	1.39	1.15	1.05	0.98	1.06	1.06
HIGH 8	0.92	0.96	1.01	1.17	1.14	1.05	1.00	0.48	0.70
HIGH 9	0.90	0.94	1.02	1.37	1.17	1.08	0.97	1.59	0.88
HIGH 10	0.96	1.02	0.91	1.40	1.13	1.08	0.97	1.04	0.91
HIGH 11	0.90	1.12	0.89	1.19	1.14	1.13	1.01	1.05	0.89
HIGH 12	0.95	1.00	1.03	1.32	1.12	1.09	0.99	1.04	1.22
HIGH 13	0.95	1.11	0.97	1.25	1.15	1.11	0.98	1.03	0.97
HIGH 14	0.94	1.00	1.01	1.34	1.20	1.14	0.97	0.97	0.69

Table B.2 Urinary urea nitrogen (g/d) Spring 1990

Date or day of diet	Subjects 1	2	3	4	5	6	7	8	9
4/4	7.13	8.75	10.98	9.78	12.17	9.31	7.41	8.97	6.26
4/5	8.69	9.92	9.93	12.35	111.60	9.44	7.77	8.23	8.64
4/6	8.55	10.07	9.57	8.87	11.33	9.67	7.19	8.99	8.25
4/7	8.98	9.37	8.77	10.41	9.87	9.88	7.38	8.06	7.53
4/8	7.46	8.71	8.05	9.29	10.57	10.09	8.73	8.28	7.77
4/9	8.41	8.75	8.22	7.72	9.83	8.90	8.80	9.73	5.98
4/10	8.38	8.17	7.45	7.84	8.00	7.90	7.45	8.46	7.87
4/11	7.82	8.04	7.85	9.00	8.23	8.38	7.96	8.14	7.28
LOW 1	4.05	5.62	3.76	9.37	7.06	7.02	3.92	4.45	3.58
LOW 2	2.64	2.73	2.39	5.23	3.88	3.37	2.59	1.84	2.21
LOW 3	2.20	2.51	2.75	2.29	3.41	4.17	2.81	3.38	3.46
LOW 4	2.62	2.49	3.55	4.65	4.22	4.53	2.68	3.35	1.94
LOW 6	3.19	3.33	3.16	3.72	3.85	3.76	2.35	2.66	2.80
LOW 7	3.38	4.14	3.35	4.56	3.75	4.31	2.74	3.27	1.62

Cont.

Table
B.2. cont.

LOW 8	3.37	3.15	3.25	3.96	4.24	3.32	3.03	3.21	2.04
LOW 9	3.83	2.78	3.22	3.46	3.83	3.67	2.79	3.62	2.53
LOW 10	3.36	3.91	3.20	4.35	3.57	3.64	2.98	2.93	2.26
LOW 11	2.95	3.51	3.24	4.39	3.56	3.42	2.95	2.61	2.44
LOW 12	2.99	3.38	3.84	4.60	3.36	3.59	3.59	2.54	1.97
LOW 13	3.29	3.41	4.28	4.79	3.47	3.43	2.27	2.77	2.36
LOW 14	2.80	3.96	3.91	4.58	3.22	4.37	3.93	2.66	2.04
MED 1	6.84	5.68	6.76	6.54	4.93	5.41	6.28	5.05	7.30
MED 2	5.55	9.84	6.15	6.83	4.95	6.17	3.98	4.38	5.16
MED 3	6.49	3.08	6.39	7.17	5.34	5.57	7.11	5.15	4.75
MED 4	6.37	5.76	6.82	7.62	4.49	5.93	5.09	5.49	5.32
MED 5	6.16	6.87	6.19	6.50	5.60	6.90	5.52	5.11	3.38
MED 6	6.99	7.80	6.26	6.13	7.21	6.86	5.92	4.59	4.21
MED 7	6.45	10.10	6.33	6.57	6.43	7.16	5.88	5.61	5.36
MED 8	5.64	9.65	5.98	6.99	6.24	6.77	5.49	5.90	4.87
MED 9	5.87	7.82	5.94	8.64	7.11	6.73	5.97	6.32	5.10
MED 10	5.91	7.21	6.79	7.43	6.14	6.79	5.68	7.44	4.61

Cont.

Table
B.2, cont.

MED 11	5.51	6.58	6.09	6.36	7.12	7.26	5.85	5.95	5.53
MED 12	5.80	7.23	6.29	6.45	7.24	7.51	5.85	6.27	5.87
MED 13	5.58	6.98	7.16	7.74	6.47	7.29	6.31	6.60	4.33
MED 14	5.52	6.94	7.09	7.96	6.73	6.68	5.77	6.25	4.96
HIGH 1	11.12	9.43	10.52	10.63	11.39	11.83	10.76	7.24	6.13
HIGH 2	12.05	12.41	14.33	16.69	14.47	14.25	10.37	12.02	9.91
HIGH 3	12.48	13.02	14.79	10.24	15.19	13.87	10.78	10.49	5.34
HIGH 4	11.89	12.49	14.06	15.98	18.59	15.40	12.31	12.31	8.48
HIGH 5	12.78	14.82	15.71	16.62	16.84	15.66	12.19	12.00	9.80
HIGH 6	12.93	18.14	14.28	16.34	14.98	14.11	12.12	11.14	10.59
HIGH 7	13.67	18.67	14.39	16.73	15.67	15.25	12.28	12.75	13.04
HIGH 8	13.82	14.73	13.11	15.47	15.85	14.82	12.25	6.28	9.91
HIGH 9	13.30	14.34	13.15	17.85	17.38	16.33	11.88	19.05	9.78
HIGH 10	14.49	14.93	13.14	16.57	13.59	17.37	12.79	12.39	8.06
HIGH 11	14.67	16.43	11.91	15.07	15.10	16.88	12.32	13.14	8.67
HIGH 12	14.62	15.34	13.64	15.93	15.11	17.44	12.02	13.37	14.58
HIGH 13	14.40	17.73	13.72	16.34	14.44	17.34	12.63	13.55	11.96
HIGH 14	15.50	16.13	14.07	16.56	13.76	16.75	13.20	13.21	8.91

Table B.3 Urinary and fecal total nitrogen (g/5 days) Spring 1990

Diet		Subjects 1	2	3	4	5	6	7	8	9
LOW	urine	16.50	18.17	18.47	22.71	18.56	18.45	15.34	22.10	11.07
	feces	9.43	7.65	7.08	6.60	6.14	6.60	7.27	8.16	9.98
MED	urine	29.67	40.29	31.09	35.87	33.70	35.53	29.94	32.51	25.78
	feces	10.23	10.02	8.96	9.08	8.95	7.93	8.40	9.91	9.43
HIGH	urine	73.68	90.11	66.48	84.54	72.00	82.84	62.96	64.22	52.18
	feces	13.38	17.96	9.17	10.10	8.94	11.65	9.96	10.11	10.78

Table B.4 Urinary 4-pyridoxic acid excretion (μmoles/day) Spring 1990

	Subjects								
Date or day of diet	1	2	3	4	5	6	7	8	9
4/4	3.41	6.81	4.23	5.64	6.01	7.00	4.20	3.51	3.80
4/5	3.99	5.73	4.56	7.02	5.67	6.75	3.80	3.47	4.60
4/6	3.79	5.75	4.31	7.63	5.25	6.61	3.59	4.22	4.56
4/7	4.39	4.68	4.32	6.71	5.63	6.79	3.45	2.87	4.38
4/8	3.87	4.79	4.28	7.06	4.23	6.38	4.89	3.39	4.29
4/9	4.27	4.65	4.15	4.69	4.70	6.01	3.70	3.70	3.94
4/10	3.89	4.75	4.23	7.25	4.49	6.38	3.67	3.62	4.05
4/11	3.78	4.46	4.17	5.82	4.84	6.16	4.20	3.35	4.14
LOW 1	4.63	5.18	5.21	7.39	5.14	6.17	4.60	4.07	4.57
LOW 2	4.82	3.73	5.07	6.26	4.68	6.70	4.39	1.83	3.26
LOW 3	3.68	3.87	4.05	3.36	4.29	6.78	4.32	3.01	4.73
LOW 4	3.23	3.61	4.30	4.24	4.37	5.12	4.73	2.68	2.57
LOW 5	3.24	4.12	4.31	3.84	4.13	5.26	3.45	3.42	5.23
LOW 6	3.35	4.67	4.70	4.14	4.11	5.51	3.51	2.91	3.46
LOW 7	3.36	5.20	4.68	4.25	4.58	5.59	3.72	2.84	3.29
LOW 8	3.40	4.55	4.79	3.85	4.43	4.44	3.46	3.14	3.27
LOW 9	3.71	6.41	4.18	4.01	4.23	4.44	3.67	3.13	4.49
LOW 10	2.78	6.42	3.85	4.64	4.55	5.05	3.57	2.61	4.27
LOW 11	3.49	6.39	3.83	4.12	4.37	4.99	4.55	2.47	4.34
LOW 12	3.71	5.28	3.66	4.51	4.44	4.69	3.99	2.54	3.17
LOW 13	3.38	4.34	3.50	4.74	3.97	5.19	3.38	2.86	3.64
LOW 14	3.12	4.81	3.47	4.22	3.62	4.64	4.29	2.56	3.66
MED 1	4.10	5.51	4.57	4.23	5.90	4.39	4.60	4.50	5.27
MED 2	3.38	4.06	3.64	4.32	4.63	4.24	3.30	3.38	2.94

Cont.

Table B.4, cont.

MED 3	3.24	4.68	3.47	4.62	3.69	3.97	4.31	3.03	2.47
MED 4	3.39	4.16	3.29	3.84	2.42	4.00	2.74	3.15	2.96
MED 5	3.08	4.05	3.62	3.89	3.59	4.07	2.83	3.35	2.51
MED 6	3.26	5.00	3.44	3.32	3.34	3.80	3.05	3.82	1.69
MED 7	3.22	4.67	3.81	3.85	3.61	3.91	3.21	4.23	2.64
MED 8	2.89	4.29	4.02	4.35	3.81	3.74	3.13	4.07	2.35
MED 9	3.01	4.12	4.01	4.52	3.78	3.75	2.90	3.85	2.84
MED 10	3.38	3.70	3.85	4.03	2.96	3.83	3.14	4.25	2.69
MED 11	2.29	3.40	3.44	3.79	3.58	3.84	3.28	2.92	2.67
MED 12	3.43	3.57	2.93	3.53	3.79	3.79	3.02	3.32	2.63
MED 13	3.17	3.57	3.09	3.43	3.88	4.74	2.99	2.81	2.45
MED 14	4.76	3.75	3.24	3.67	3.95	3.63	3.29	2.85	2.06
HIGH 1	2.94	3.06	2.92	4.71	3.41	2.98	3.40	1.89	2.87
HIGH 2	2.71	2.75	2.40	4.86	2.83	3.46	3.20	1.94	2.41
HIGH 3	3.31	2.79	2.50	2.90	2.27	2.24	3.28	2.04	1.81
HIGH 4	2.34	2.69	2.36	4.08	2.67	2.38	2.93	2.01	1.60
HIGH 5	2.39	2.59	2.53	3.60	2.78	2.53	2.31	1.92	1.98
HIGH 6	2.44	2.35	2.41	2.73	2.50	2.25	2.68	2.03	2.14
HIGH 7	2.20	2.80	2.68	3.61	2.68	2.57	2.21	1.99	2.59
HIGH 8	2.59	2.49	2.65	3.81	2.64	2.48	2.70	0.83	1.84
HIGH 9	2.61	2.43	2.90	3.78	3.13	2.74	2.68	2.69	2.05
HIGH 10	2.39	2.85	2.79	2.96	2.96	2.84	2.38	1.98	1.69
HIGH 11	2.31	2.53	2.66	3.58	2.95	2.65	2.21	2.02	1.87
HIGH 12	2.37	2.57	2.92	3.79	3.37	2.49	1.83	2.01	2.09
HIGH 13	2.27	2.90	2.93	3.88	2.91	2.32	2.91	1.96	2.55
HIGH 14	2.58	2.71	2.84	3.95	3.17	2.24	2.60	2.02	1.89

Table B.5 Urinary total vitamin B-6 excretion (μmoles/day) Spring 1990

	Subjects								
Date or day of diet	1	2	3	4	5	6	7	8	9
4/4	0.560	0.595	0.594	0.802	0.563	0.767	0.709	1.080	1.114
4/5	0.567	0.634	0.608	0.789	0.480	0.815	0.707	0.942	0.547
4/10	0.536	0.609	0.546	0.582	0.402	0.736	0.685	0.877	0.497
4/11	0.567	0.567	0.600	0.539	0.454	0.691	0.618	0.854	0.500
LOW 1	0.457	0.636	0.591	0.440	0.260	0.556	0.543	0.641	0.374
LOW 2	0.451	0.674	0.504	0.422	0.388	0.517	0.506	0.239	0.299
LOW 3	0.420	0.426	0.410	0.209	0.432	0.529	0.469	0.675	0.652
LOW 4	0.418	0.446	0.502	0.675	0.469	0.538	0.497	0.597	0.482
LOW 5	0.427	0.478	0.428	0.496	0.481	0.581	0.535	0.678	0.388
LOW 6	0.346	0.505	0.516	0.429	0.361	0.468	0.468	0.736	0.456
LOW 12	-----	0.512	1.171	0.496	-----	0.424	-----	0.641	0.403
LOW 13	0.472	0.462	0.913	0.443	0.481	0.471	0.710	0.692	0.455
LOW 14	0.409	0.586	0.833	0.433	0.495	0.472	0.737	0.624	0.486
MED 1	0.490	0.624	0.525	0.436	0.431	0.540	0.705	0.911	0.578

Continued

Table
B.5, cont.

MED 2	0.451	0.656	0.503	0.314	0.422	0.468	0.553	0.782	0.602
MED 3	0.467	0.612	0.432	0.400	0.324	0.532	0.951	0.762	0.426
MED 4	0.441	0.601	0.470	0.324	0.340	0.652	0.553	0.750	0.368
MED 5	0.433	0.619	0.503	0.376	0.355	0.572	0.515	0.677	0.409
MED 6	0.450	0.744	0.367	0.166	0.377	0.721	0.567	0.640	0.281
MED 12	0.496	0.670	-----	0.396	0.386	0.736	0.556	0.620	0.395
MED 13	0.456	0.537	0.462	0.396	0.389	0.679	0.519	0.581	0.395
MED 14	0.460	0.500	0.413	0.401	0.382	0.620	0.486	0.561	0.408
HIGH 1	0.616	0.546	0.801	0.549	0.431	0.692	0.683	0.632	0.529
HIGH 2	0.590	0.545	0.724	0.617	0.422	0.683	0.587	0.614	0.537
HIGH 3	0.469	0.476	0.802	0.299	0.324	0.545	0.521	0.564	0.328
HIGH 4	0.504	0.450	0.428	0.387	0.340	0.451	0.652	0.565	0.518
HIGH 5	0.491	0.509	0.504	0.449	0.355	0.463	0.664	0.663	0.560
HIGH 6	0.486	0.318	0.491	0.321	0.377	0.455	0.564	0.199	0.549
HIGH 12	0.438	-----	0.442	0.381	0.386	-----	0.438	0.526	0.549
HIGH 13	0.428	0.446	0.406	0.376	0.389	0.538	0.546	0.526	0.549
HIGH 14	0.441	0.407	0.444	0.488	0.383	-----	0.580	0.519	0.385

Table B.6 Urinary xanthurenic acid excretion (μmoles/day) the day before (basal) and the day of (load) a 2 g L-tryptophan load Spring 1990

		Subjects								
Diet		1	2	3	4	5	6	7	8	9
LOW	basal	13.9	12.1	----	11.3	8.7	----	10.2	12.0	----
	load	82.9	30.4	----	28.6	29.5	----	38.3	30.2	----
MED	basal	13.1	----	17.1	10.6	----	12.2	8.8	----	13.2
	load	70.4	----	42.8	34.1	----	51.6	17.5	----	34.7
HIGH	basal	----	16.1	15.6	----	10.7	11.7	----	12.7	13.6
	load	----	78.8	48.2	----	81.3	76.9	----	134	33.0

Table B.7 Urinary kynurenic acid excretion (μmoles/day) the day before (basal) and the day of (load) a 2 g L-tryptophan load Spring 1990

		Subjects								
Diet		1	2	3	4	5	6	7	8	9
LOW	basal	6.0	9.3	----	9.3	6.0	----	6.7	5.4	----
	load	95.2	95.2	----	80.0	63.8	----	85.1	47.4	----
MED	basal	6.4	----	12.0	9.3	----	8.6	9.6	----	7.2
	load	82.8	----	111	82.3	----	63.7	161	----	74.8
HIGH	basal	----	21.3	15.9	----	11.6	14.4	----	9.3	16.1
	load	----	151	105	----	111	112	----	81.0	161

Table B.8 Urinary excretion of volatile amines (kynurenine plus acetylkynurenine, μmoles/day) the day before (basal) and the day of (load) a 2 g L-tryptophan load Spring 1990

		Subjects								
Diet		1	2	3	4	5	6	7	8	9
LOW	basal	3.4	2.3	----	2.4	1.6	----	1.0	1.5	----
	load	13.3	15.9	----	8.9	24.9	----	53.5	12.8	----
MED	basal	3.7	----	4.8	2.1	----	4.6	3.5	----	2.1
	load	82.6	----	119	9.6	----	24.2	276	----	42.6
HIGH	basal	----	6.2	6.4	----	5.0	3.5	----	2.5	2.3
	load	----	120	145	----	202	223	----	116	160

Table B.9 Hematocrit (%) Spring 1990

	Subjects								
Date	1	2	3	4	5	6	7	8	9
Pre	44.7	39.5	42.5	41.5	37.2	40.5	42.5	45.2	39.5
4/4	41.8	39.5	43.2	43.8	37.8	40.0	42.5	45.2	38.8
4/9	44.0	42.3	39.0	43.5	36.7	40.5	41.3	45.7	38.5
4/12	44.5	40.2	37.7	39.8	35.7	38.8	40.5	44.3	36.5
4/18	45.0	40.8	39.5	40.2	35.8	39.5	38.8	44.2	39.2
4/23	44.5	40.6	39.5	39.8	37.5	39.0	41.0	44.5	38.0
4/26	44.0	40.8	38.5	40.2	38.0	39.5	40.5	44.8	38.5
5/2	43.0	41.5	37.0	41.0	38.0	39.0	40.0	44.3	38.0
5/7	44.0	40.0	35.2	41.5	39.2	39.2	40.5	42.5	37.8
5/10	42.6	40.5	35.5	41.0	38.3	38.5	39.8	43.0	38.5
5/21	42.5	40.8	39.0	40.0	41.8	39.0	40.5	42.5	38.0
5/24	40.0	39.0	39.0	39.0	40.5	39.0	40.8	43.0	37.8

Table B.10 Hemoglobin concentration (g/100mL) Spring 1990

	Subjects								
Date	1	2	3	4	5	6	7	8	9
Pre	15.4	13.4	13.5	13.7	12.0	13.2	13.8	14.6	14.0
4/4	14.3	14.0	14.0	14.6	11.8	13.2	13.9	15.3	13.9
4/9	15.4	14.6	12.8	14.5	14.6	13.2	14.9	15.7	13.7
4/12	18.7	14.1	12.6	13.5	11.5	13.1	14.0	14.7	13.2
4/18	15.8	14.0	13.3	13.5	11.6	13.2	13.9	15.2	14.0
4/23	15.5	14.1	12.7	14.1	11.7	12.5	13.8	14.8	13.4
4/26	15.0	14.0	12.5	13.2	12.2	12.8	14.1	15.3	13.3
5/2	14.8	14.1	12.0	13.6	11.9	14.5	13.5	14.4	13.2
5/7	15.3	13.7	11.7	14.0	12.8	12.8	13.8	14.4	13.8
5/10	14.9	14.0	11.8	13.7	12.4	12.4	13.2	14.8	13.4
5/16	14.6	13.9	12.2	13.1	12.5	12.7	13.4	14.0	13.3
5/21	14.8	13.9	12.5	13.7	13.3	12.4	13.7	14.4	13.1
5/24	14.2	13.5	12.9	12.8	12.8	12.8	14.0	13.7	13.1

Table B.11 Plasma pyridoxal 5′-phosphate (nmoles/L) Spring 1990

	Subjects								
Day of diet	1	2	3	4	5	6	7	8	9
ADJ 1	26.1	37.8	31.2	34.6	46.0	63.3	40.7	70.5	33.6
ADJ 6	35.0	40.3	21.7	40.0	29.5	45.4	40.3	77.4	38.3
ADJ POST	40.4	39.9	22.0	36.0	28.1	45.3	41.3	66.9	32.4
LOW 7	31.4	26.4	23.3	31.8	23.9	48.0	38.5	69.4	46.2
LOW 12	29.1	29.0	16.9	32.0	23.2	38.1	40.0	61.4	40.6
LOW POST	27.1	38.8	18.8	32.6	27.6	44.8	51.5	62.9	34.1
MED 7	30.4	33.2	16.5	28.9	26.6	40.7	31.5	41.9	22.3
MED 12	31.4	28.4	15.8	37.6	26.4	33.5	23.1	49.1	20.9
MED POST	27.1	26.5	16.9	27.5	23.9	32.2	34.7	51.3	19.1
HIGH 7	32.2	26.0	13.5	23.9	12.1	27.4	30.1	43.4	28.6
HIGH 12	28.9	16.8	13.0	28.6	14.4	20.8	32.8	34.2	29.0
HIGH POST	21.9	21.8	14.2	23.0	14.1	20.5	30.4	42.9	21.9

Table B.12 Plasma alkaline phosphatase activity (μmoles/min/L) Spring 1990

	Subjects								
Date	1	2	3	4	5	6	7	8	9
4/4	17.9	15.9	27.0	28.7	23.1	35.3	17.5	19.8	20.8
5/24	23.3	16.0	25.1	31.3	22.3	34.5	19.0	20.7	19.1

Table B.13 Erythrocyte aspartate aminotransferase activity (μg pyruvate/mg Hgb/hr) with (stim) and without (unstim) added pyridoxal phosphate, and percent stimulation (%) Spring 1990

		Subjects								
Day of diet		1	2	3	4	5	6	7	8	9
	unstim	20.19	11.30	17.42	23.00	22.78	22.38	18.00	27.97	29.26
ADJ 1	stim	49.36	25.68	23.34	35.00	28.47	30.07	29.67	36.01	40.19
	%	144	127	34	52	16	34	65	29	37
	unstim	28.17	19.79	29.33	44.02	38.93	25.81	23.16	31.10	29.41
ADJ 6	stim	42.96	37.10	38.52	59.46	53.44	38.71	40.00	41.70	46.73
	%	53	88	31	35	37	50	73	34	59
	unstim	25.81	26.28	27.48	30.04	29.54	21.69	26.01	29.84	35.28
ADJ POST	stim	35.80	38.57	41.22	43.22	38.79	32.93	42.23	48.69	48.16
	%	39	47	50	44	31	52	62	63	37
	unstim	42.26	27.99	24.67	44.44	47.72	32.29	42.86	43.68	22.40
LOW 7	stim	59.35	39.93	29.33	63.80	60.70	46.53	56.48	63.90	34.70
	%	40	43	19	44	27	44	32	46	55
	unstim	39.12	25.16	30.69	38.72	44.83	31.31	37.70	45.24	35.49
LOW 12	stim	56.78	39.49	43.23	54.55	60.54	42.35	51.48	56.80	44.44
	%	45	57	44	41	35	35	37	26	25

Continued

Table B.13,
continued

	unstim	37.84	27.78	31.40	43.24	45.55	31.99	45.78	28.30	36.21
LOW POST	stim	51.35	40.52	40.96	54.73	59.43	49.63	61.36	39.85	51.92
	%	36	39	30	27	30	55	34	41	43
	unstim	34.93	24.41	35.43	40.73	26.15	31.99	42.20	29.68	43.73
MED 7	stim	50.00	34.24	48.68	58.28	42.31	49.63	57.09	37.46	57.49
	%	43	40	37	43	62	55	35	26	31
	unstim	30.03	24.77	33.79	40.61	35.38	30.34	34.56	35.28	38.11
MED 12	stim	46.01	36.25	45.52	52.90	47.65	45.17	52.68	47.95	54.72
	%	53	46	35	30	35	49	52	36	44
	unstim	33.43	25.23	34.52	39.46	37.50	29.49	30.22	35.23	48.75
MED POST	stim	49.86	36.62	50.49	47.83	48.93	46.44	37.45	49.11	65.63
	%	49	45	46	21	30	57	24	39	35
	unstim	28.52	29.30	34.63	28.62	45.59	35.59	24.37	39.74	44.88
HIGH 7	stim	41.28	46.82	42.40	37.93	59.00	48.47	40.50]	54.30	59.74
	%	45	60	22	33	29	36	66	37	33
	unstim	33.65	25.41	28.62	31.70	43.37	30.86	31.65	45.02	38.91
HIGH 12	stim	44.03	40.39	43.10	46.41	59.86	48.33	45.57	60.14	53.70
	%	31	59	51	46	38	57	44	34	38
	unstim	29.69	26.11	33.11	31.66	40.99	31.85	31.94	46.05	26.09
HIGH POST	stim	43.13	40.76	47.30	46.71	49.12	50.00	43.87	61.51	39.72
	%	45	56	43	48	20	57	37	34	52

Table B.14 Erythrocyte alanine aminotransferase activity (μg pyruvate/mg Hgb/hr) with (stim) and without (unstim) added pyridoxal phosphate, and percent stimulation (%) Spring 1990

		Subjects								
Day of diet		1	2	3	4	5	6	7	8	9
	unstim	1.25	0.972	1.99	2.96	3.41	4.61	3.14	3.23	1.79
ADJ 1	stim	2.92	1.18	2.60	3.75	3.70	5.10	3.30	3.58	2.18
	%	134	21	31	27	9	11	5	11	22
	unstim	1.91	0.588	1.81	2.30	3.02	3.38	2.20	2.54	1.16
ADJ 6	stim	2.29	0.773	2.11	3.06	3.65	4.01	2.55	2.74	1.52
	%	20	31	16	33	21	19	16	8	32
	unstim	2.24	0.783	2.17	2.19	2.84	3.94	2.44	2.76	----
ADJ POST	stim	2.71	1.09	2.39	2.87	3.70	4.81	2.93	3.06	----
	%	21	39	10	31	30	22	20	11	----
	unstim	2.75	0.638	2.00	2.13	3.36	3.67	2.76	2.51	1.29
LOW 7	stim	3.31	0.925	2.12	2.61	4.04	3.26	3.36	2.80	2.06
	%	20	45	21	23	20	13	22	11	60
	unstim	2.52	0.762	2.12	2.10	2.71	3.43	2.31	2.37	1.64
LOW 12	stim	3.06	1.05	2.55	2.74	3.32	3.76	2.80	3.42	1.90
	%	22	38	20	30	23	9	21	45	16

Continued

Table B.14,
continued

	unstim	2.27	0.909	2.55	2.32	3.00	3.65	2.53	1.79	2.01
LOW POST	stim	2.78	1.12	3.15	2.78	3.68	4.35	3.01	2.14	2.17
	%	23	24	24	20	23	19	19	20	8
	unstim	1.96	0.915	2.30	2.09	3.02	2.97	2.41	3.09	1.81
MED 7	stim	2.49	1.19	2.85	2.70	3.78	3.78	2.76	3.52	2.17
	%	27	30	24	30	25	27	15	14	20
	unstim	2.19	1.09	2.21	1.73	3.22	3.37	2.94	2.48	1.56
MED 12	stim	2.66	1.25	2.82	2.34	3.58	3.63	3.51	2.82	1.85
	%	22	15	28	35	11	8	19	14	18
	unstim	2.41	1.06	2.15	1.74	4.45	3.41	2.59	2.82	1.63
MED POST	stim	3.05	1.18	2.72	2.27	4.93	4.00	3.02	3.48	2.08
	%	27	12	26	31	12	17	17	23	28
	unstim	2.64	0.870	2.12	1.95	4.04	3.56	2.40	3.22	1.90
HIGH 7	stim	3.08	1.19	2.73	2.44	4.38	4.42	2.91	4.02	2.24
	%	17	36	29	25	8	24	21	25	18
	unstim	2.45	0.752	1.85	2.06	3.33	2.89	2.34	2.85	1.84
HIGH 12	stim	2.65	0.944	2.41	2.60	3.55	3.56	2.68	3.25	2.22
	%	8	26	30	26	6	23	15	14	21
	unstim	2.58	0.714	2.09	2.38	2.90	2.91	2.65	2.90	3.25
HIGH POST	stim	3.13	1.06	2.62	2.92	3.78	3.52	3.27	3.49	3.60
	%	21	49	25	22	31	21	23	20	11

Table B.15 Fecal total vitamin B-6 (μmoles/d) Spring 1990

	Subjects								
Diet	1	2	3	4	5	6	7	8	9
LOW	5.23	3.01	4.39	3.55	3.29	3.48	3.84	3.72	5.26
MED	5.20	3.76	4.11	5.13	3.87	3.22	4.45	5.49	4.06
HIGH	5.15	3.62	4.47	4.74	3.37	4.57	4.49	4.32	4.16

Table B.16 Subjects' weight (lbs) Spring 1990

	Subjects								
Date or day of diet	1	2	3	4	5	6	7	8	9
4/4	124	139	138.5	144	122	140.5	117	118	127
4/5	124	138	138.75	141.75	122	139.5	116.5	116.5	126.5
4/6	123.75	138	138	141.75	122	138.5	116	116.25	124
4/7	124	137	138	142	121.5	137.5	116.75	116	125.5
4/8	122.5	136.5	137.75	141.75	120.5	137	116.5	116	125.5
4/9	122.5	137.75	138.5	141.75	119.5	138.5	116.5	116	126
4/10	123.25	139	138.75	141.75	121.5	137.25	116.5	115.75	126
4/11	123.25	138.75	138.5	143	121	137	116	115.75	125
LOW 1	125.5	138	138.75	143.5	121	139	118	114.5	126
LOW 2	126	139	140.5	143	122	137.75	119	114.5	125
LOW 3	125.5	139	139.5	145	122	137.5	118.5	114.75	126.5
LOW 4	125	137	139.5	144	121	137	118.5	115	123.5
LOW 5	124.75	139	139.5	144	120.5	136.5	118.25	114.5	126
LOW 6	124.75	138	139	145	120	137	118.5	114	124.5

Continued

Table
B.16,
cont.

LOW 7	124.5	136.25	137.75	143.75	118.5	136	119	114	125
LOW 8	124	135.75	137.25	142.5	119.5	135.25	117.25	114.25	125.5
LOW 9	123.25	136.5	137.25	142.75	118.5	135.5	116.25	114	125.25
LOW 10	123.75	135.75	137	141.25	117.5	136	116	113	125.5
LOW 11	123.75	137	137	143	117	136	115.5	114	127.5
LOW 12	123.25	138.5	137.5	142.25	118	135.75	116.5	114	123.5
LOW 13	123.25	136.5	137	142.25	118.5	134.75	115.25	113.5	124.5
LOW 14	123	137	136.75	143.75	117	134.5	115	114	124.5
MED 1	125	138	138.5	140.75	122	134	118.25	116.5	128.5
MED 2	125	138	138.5	140.5	121	134	117.75	116.5	127.5
MED 3	125.25	138	138.5	140.5	120.5	134	117	116	129
MED 4	124.5	137.75	138.5	144.75	119.5	133.75	117	116	128
MED 5	124.25	138	139.5	142.25	119.5	134.25	118.75	116.25	128
MED 6	124.25	137.75	138	142.25	119	133.25	118.75	116.5	130
MED 7	125	137	137.75	142.25	119.5	133.75	118.5	116	127.5
MED 8	125.25	138	138.5	143.25	119.5	133	118.25	115.75	128

Continued

Table B.16, cont.

MED 9	124.75	138	139	142.25	119	134.25	117.5	115.5	127
MED 10	124.25	136.25	137.25	142.25	119.5	134.75	118.25	115	127.5
MED 11	124	138.75	137.75	142.5	119.5	135	117.75	114	127.5
MED 12	124	138.25	139	141	119	134.25	118.5	114	128
MED 13	123.75	138	138.75	144.25	119.5	134.25	117.5	114	127.5
MED 14	125.25	138	138.25	142.25	120	134.5	118	114.5	127.5
HIGH 1	123.5	136	136.25	142	119.5	134.75	116	113	125.5
HIGH 2	124.25	136	136	141.75	120	134.5	116	113.5	125
HIGH 3	124.25	136.25	136.5	143	121	135.5	116	114	127
HIGH 4	124.75	139.75	136.25	141.75	121	135.5	116.75	114.5	125
HIGH 5	125.5	139	136.75	143.75	120	136.5	117.5	115	128
HIGH 6	125	139	137.75	143.75	120	136	117.5	115	127
HIGH 7	125	138.5	138.5	145.75	120.5	136.25	117	115.25	125.5
HIGH 8	125.75	137	139.5	143	121	136.5	116.75	115.5	126
HIGH 9	124.75	136.75	139	143.75	121	136.75	117.5	115	127
HIGH 10	124.25	137.75	139	145	119	137	117.75	115	126

Continued

Table B.16, cont.

HIGH 11	124.75	138	141	144.75	122	136.5	118.5	115.5	128
HIGH 12	124.5	139	140.75	144.5	121.5	135.5	118	115.5	128.5
HIGH 13	124.5	139.25	140.75	144	122	136.75	117.25	115.5	130
HIGH 14	125	139	139.5	143.75	121	135	118.5	115.5	127

APPENDIX C

DATA FROM THE STUDY DESCRIBED IN CHAPTER 5 (SPRING 1991)

Table C.1 Urinary creatinine excretion (g/day) Spring 1991

	Subjects								
Date or day of diet	1	2	3	4	6	7	8	9	10
4/8	1.08	0.76	1.01	1.24	1.12	---	1.03	1.24	1.30
4/9	1.08	1.08	1.06	1.26	1.13	1.15	1.12	1.23	1.33
4/10	1.00	1.08	0.80	1.07	1.01	1.35	1.06	1.30	1.35
4/11	1.11	0.98	1.07	1.21	0.59	1.34	1.49	1.26	1.23
4/12	1.22	0.96	1.10	1.18	1.02	1.35	1.01	1.32	1.46
4/13	1.10	0.98	1.07	1.26	1.02	1.22	1.22	1.24	1.24
4/14	1.08	0.88	0.99	1.21	0.85	1.51	1.09	1.29	1.12
4/15	0.99	0.77	1.08	1.23	0.95	1.33	1.06	1.29	1.23
LOW 1	1.07	1.03	1.19	1.27	1.09	1.18	1.17	1.33	1.33
LOW 2	1.11	1.02	1.20	1.19	1.08	1.32	1.14	1.31	1.36
LOW 3	1.15	1.02	1.15	1.39	1.09	1.20	1.18	1.32	1.33
LOW 4	1.18	1.01	1.21	1.22	1.09	1.29	1.18	1.29	1.53
LOW 5	1.23	1.01	1.19	1.46	1.10	1.43	1.09	1.48	1.09
LOW 6	1.19	1.00	1.24	1.38	1.08	1.27	1.14	1.26	1.49
LOW 7	1.23	0.99	1.22	1.37	1.10	1.17	1.16	1.34	1.34
LOW 8	1.22	0.98	1.14	1.28	1.08	1.04	1.15	1.34	1.22
LOW 9	1.16	1.01	0.83	1.34	1.07	1.37	1.11	1.14	0.97
LOW 10	1.22	1.01	1.11	1.40	0.99	1.43	1.15	1.38	1.37
LOW 11	1.20	1.08	1.18	1.47	1.06	1.35	1.16	1.25	1.34
LOW 12	1.23	1.02	1.07	1.38	0.98	1.43	1.14	1.31	1.31
LOW 13	1.10	1.06	1.02	1.20	1.12	1.63	0.98	1.83	1.33
LOW 14	1.22	1.05	1.16	1.41	1.06	1.51	1.15	1.37	1.38
LOW 15	1.25	1.04	1.18	1.34	1.11	1.43	1.15	1.37	1.41
LOW 16	1.23	1.04	1.11	1.36	1.04	1.26	1.22	1.22	1.16

Continued

Table C.1,
continued

LOW 17	1.25	1.06	1.09	1.38	1.13	1.41	1.11	1.39	1.36
LOW 18	1.15	1.01	1.11	1.30	1.10	1.53	1.20	1.22	1.34
HIGH 1	0.97	1.00	0.99	1.35	0.92	1.36	1.20	1.32	1.04
HIGH 2	1.21	0.89	1.03	1.22	0.95	1.30	1.11	1.31	1.19
HIGH 3	1.08	0.90	1.00	1.21	0.86	1.38	0.99	1.29	1.04
HIGH 4	1.13	0.94	1.00	1.22	0.95	1.31	1.04	1.25	1.24
HIGH 5	1.08	0.94	1.05	1.26	0.99	1.39	1.20	1.24	1.21
HIGH 6	1.08	0.91	1.03	1.25	0.95	1.36	0.96	1.12	1.15
HIGH 7	1.07	1.00	1.02	1.23	0.98	1.36	1.14	1.23	1.16
HIGH 8	1.11	0.95	1.15	1.13	0.92	1.07	1.15	1.11	1.08
HIGH 9	1.09	0.88	0.93	1.30	0.98	1.34	1.09	1.08	1.07
HIGH 10	1.06	0.96	1.06	1.24	0.93	1.38	1.17	1.31	1.09
HIGH 11	1.08	0.95	1.11	1.25	0.97	1.30	1.14	1.30	1.34
HIGH 12	1.10	0.91	1.03	1.21	0.95	1.30	1.11	1.23	1.31
HIGH 13	0.77	0.93	1.04	1.05	0.97	1.21	1.05	1.13	1.21
HIGH 14	0.94	0.99	1.10	1.60	0.98	1.45	1.03	1.16	1.27
HIGH 15	0.74	0.97	1.06	1.22	0.92	1.34	1.13	1.21	0.76
HIGH 16	0.88	0.93	1.04	1.22	1.00	1.40	1.08	1.26	1.26
HIGH 17	1.01	0.92	1.03	1.31	0.92	1.34	1.05	1.17	1.25
HIGH 18	1.01	0.80	1.01	1.24	0.90	1.34	1.10	1.32	1.04

Table C.2 Urinary urea nitrogen excretion (g/day) Spring 1991

	Subjects								
Date or day of diet	1	2	3	4	6	7	8	9	10
4/8	12.83	6.98	5.86	7.69	9.89	---	10.38	7.48	10.53
4/9	8.96	8.15	7.65	6.83	9.25	8.77	10.08	7.51	10.56
4/10	7.86	8.15	6.50	7.34	9.09	9.65	9.82	7.95	9.65
4/11	8.03	7.12	9.10	7.28	4.85	9.13	11.62	7.20	9.36
4/12	8.37	9.30	8.24	8.07	8.35	9.65	8.00	8.91	9.33
4/13	8.03	7.86	8.17	7.27	7.42	8.42	10.08	8.58	8.61
4/14	7.70	7.67	7.62	7.28	6.90	8.82	8.49	7.41	6.97
4/15	7.73	6.95	8.51	6.05	6.98	8.47	8.75	7.75	7.38
LO 1	7.52	6.13	7.69	7.60	7.71	7.26	9.86	10.60	9.00
LO 2	8.34	9.01	10.56	7.92	9.57	7.51	10.30	9.05	8.97
LO 3	8.22	8.42	10.80	8.39	8.06	8.22	9.11	9.68	8.69
LO 4	9.18	7.73	9.30	8.49	8.20	8.11	9.59	7.60	10.60
LO 5	8.91	9.29	9.32	7.18	8.88	9.52	10.29	9.46	6.80
LO 6	9.26	9.15	8.04	8.76	8.60	8.77	8.76	9.08	9.44
LO 7	8.72	8.95	9.31	8.37	7.90	8.80	9.32	9.00	8.75
LO 8	9.79	8.26	8.69	8.38	8.31	3.72	9.74	8.58	7.23
LO 9	9.57	8.03	7.14	8.53	7.70	10.16	8.75	7.97	6.53
LO 10	8.86	9.29	8.85	8.77	6.25	9.09	8.40	7.64	8.55
LO 11	9.84	9.58	8.25	9.38	7.04	9.21	9.13	6.88	8.14
LO 12	8.71	9.38	7.97	8.39	8.67	9.40	9.64	7.74	7.80
LO 13	9.32	9.88	8.33	8.85	8.83	10.19	9.23	8.82	9.14
LO 14	9.07	9.24	8.82	8.15	8.46	9.34	9.33	6.94	8.57
LO 15	8.95	9.02	10.06	9.31	8.27	9.07	8.88	6.94	9.29

Cont.

Table C.2, cont.

LO 16	8.62	9.31	10.34	8.70	8.31	7.89	9.60	9.60	9.75
LO 17	8.84	8.90	10.00	8.80	8.03	8.37	8.46	8.02	7.90
LO 18	6.13	8.98	9.51	8.92	7.55	9.52	9.74	7.21	9.74
HI 1	9.15	10.81	10.17	10.90	7.12	11.06	11.66	10.19	8.61
HI 2	10.06	8.95	10.00	9.17	11.08	9.74	11.58	10.08	10.81
HI 3	8.69	9.91	9.68	9.53	10.80	10.78	11.10	9.88	10.07
HI 4	10.52	10.34	10.84	9.60	11.37	10.63	12.19	10.22	11.69
HI 5	10.32	10.06	9.52	9.34	10.27	10.72	10.85	10.08	10.93
HI 6	10.32	10.34	10.34	10.20	10.72	9.45	10.91	10.10	10.79
HI 7	10.02	9.37	9.75	10.76	10.22	9.22	10.68	9.56	10.85
HI 8	11.08	10.57	10.90	9.63	10.37	8.66	9.77	10.63	9.83
HI 9	11.58	9.18	12.27	12.54	9.90	10.12	11.87	8.40	9.00
HI 10	10.63	11.02	11.07	11.21	10.97	10.59	10.27	10.71	10.00
HI 11	8.16	12.05	11.80	10.57	10.85	9.84	10.35	9.99	10.79
HI 12	10.41	10.13	9.99	10.76	9.89	9.84	9.45	9.27	9.81
HI 13	9.98	10.57	10.48	9.54	9.89	10.36	9.82	8.08	8.69
HI 14	10.52	10.67	10.97	10.71	9.95	9.41	10.66	7.48	9.20
HI 15	10.69	9.22	10.67	10.87	9.63	9.58	10.77	9.15	5.62
HI 16	9.97	9.88	9.13	9.93	9.10	9.86	9.89	8.79	7.75
HI 17	8.85	10.37	10.32	11.24	9.37	11.64	10.65	9.16	10.01
HI 18	8.00	8.62	10.19	11.40	9.60	11.83	10.27	7.91	10.18

Table C.3 Urinary 4-pyridoxic acid excretion (μmoles/day) Spring 1991

	Subjects								
Date or day of diet	1	2	3	4	6	7	8	9	10
4/8	5.46	2.63	2.42	5.93	5.21	---	5.58	4.65	4.77
4/9	5.00	3.13	3.01	4.79	4.49	3.09	5.47	5.13	4.88
4/10	4.34	4.23	2.78	4.32	4.05	3.42	5.81	4.85	4.90
4/11	4.45	3.32	3.90	4.39	2.20	3.57	5.88	4.87	4.29
4/12	4.75	3.16	4.10	4.90	4.03	3.37	5.88	4.88	4.73
4/13	5.10	3.32	3.87	4.81	4.46	3.09	5.73	4.94	4.85
4/14	4.29	2.55	3.71	5.05	3.94	3.44	5.17	4.46	3.70
4/15	4.35	2.41	3.71	4.65	3.72	3.48	4.98	4.35	4.34
LOW 1	3.97	3.43	4.14	5.06	3.14	3.11	4.48	5.37	2.65
LOW 2	4.08	3.39	4.46	3.82	3.19	3.72	4.30	5.11	2.91
LOW 3	4.18	2.46	4.74	4.67	3.46	3.28	4.20	5.10	3.24
LOW 4	4.14	3.55	4.81	5.34	3.43	3.64	4.49	5.01	4.20
LOW 5	4.86	3.33	4.51	4.78	3.71	4.02	5.11	4.88	3.40
LOW 6	4.48	3.46	4.05	4.32	3.55	3.39	4.91	4.89	4.33
LOW 7	4.32	3.86	4.59	4.28	3.50	3.47	4.91	5.83	3.15
LOW 8	4.36	3.59	4.60	3.94	3.76	2.84	4.62	5.63	3.80
LOW 9	3.83	3.60	5.86	4.11	3.70	3.38	4.61	4.67	3.12
LOW 10	4.41	3.68	3.87	4.65	3.22	3.73	4.62	5.63	3.98
LOW 11	3.91	2.72	4.17	4.82	3.58	3.87	5.08	5.13	3.81
LOW 12	4.05	3.46	4.13	4.78	3.19	3.92	5.29	4.90	3.60
LOW 13	3.94	3.60	3.68	4.37	3.31	3.82	4.86	4.75	3.89
LOW 14	4.71	3.29	4.24	4.23	2.90	4.01	4.89	4.24	3.90
LOW 15	4.63	3.06	4.78	4.27	2.94	3.97	4.49	4.24	4.19
LOW 16	4.26	3.19	4.56	4.44	3.24	3.10	4.62	5.22	3.54

Continued

Table C.3,
continued

LOW 17	3.98	3.02	3.57	4.49	3.65	3.53	4.91	4.77	3.84
LOW 18	3.97	3.08	3.97	4.46	3.18	3.96	4.74	4.31	4.37
HIGH 1	3.45	3.25	3.57	4.74	3.30	3.05	4.93	4.64	3.29
HIGH 2	4.40	2.97	4.67	4.36	3.30	3.39	4.51	4.84	3.61
HIGH 3	3.51	2.70	3.71	4.39	3.33	3.40	4.38	4.87	3.61
HIGH 4	3.70	2.78	3.33	4.33	3.03	3.32	4.42	4.89	4.06
HIGH 5	3.22	2.91	3.71	4.44	3.24	3.68	4.66	4.95	4.48
HIGH 6	3.22	2.77	3.33	4.13	3.34	3.59	4.38	4.89	4.26
HIGH 7	3.38	3.06	3.13	4.26	3.27	3.20	4.51	5.19	4.46
HIGH 8	3.35	3.48	2.88	3.91	3.59	2.62	4.70	5.40	3.87
HIGH 9	3.38	2.67	2.32	3.93	3.41	2.98	4.85	5.00	3.64
HIGH 10	3.47	2.78	2.82	4.31	3.13	3.32	4.63	5.87	3.52
HIGH 11	3.34	2.85	3.01	4.25	2.74	2.99	3.94	5.21	3.88
HIGH 12	3.32	2.78	2.73	4.67	3.14	2.99	4.25	4.81	3.37
HIGH 13	3.32	2.84	3.03	4.37	3.02	2.83	4.23	4.73	3.43
HIGH 14	3.46	2.96	3.38	4.97	3.01	3.11	4.04	5.11	3.56
HIGH 15	3.32	2.69	2.93	4.76	3.30	3.28	4.07	5.19	2.21
HIGH 16	3.12	2.79	2.67	4.00	3.15	3.41	4.08	5.25	3.36
HIGH 17	3.36	3.03	3.00	4.44	3.00	3.63	4.30	4.71	3.13
HIGH 18	3.35	2.55	2.85	4.63	3.18	3.96	4.74	4.31	4.37

Table C.4 Urinary total vitamin B-6 (μmoles/day) Spring 1991

Date or day of diet	Subjects 1	2	3	4	6	7	8	9	10
4/14	0.621	0.692	0.564	0.689	0.534	----	0.612	0.657	0.789
4/15	0.568	0.541	0.604	0.632	0.585	0.768	0.619	0.573	0.690
LO 17	0.676	0.785	0.775	0.663	0.510	0.806	0.505	0.707	0.619
LO 18	0.637	0.750	0.715	0.668	0.462	0.853	0.481	0.527	0.736
HI 17	0.626	0.543	0.619	0.513	0.500	0.730	0.519	0.683	0.604
HI 18	0.620	0.557	0.579	0.501	0.496	0.773	0.498	0.613	0.594

Table C.5 Urinary pyridoxine glucoside (μmoles/day) Spring 1991

Date or day of diet	Subjects 1	2	3	4	6	7	8	9	10
4/14	0.130	0.093	0.151	0.078	0.060	0.116	0.043	0.041	0.131
4/15	0.023	0.097	0.092	0.082	0.066	0.097	0.080	0.065	0.070
LO 17	0.069	0.068	0.039	0.070	0.096	0.061	----	0.060	0.047
LO 18	0.053	0.127	0.076	0.109	0.072	0.166	0.038	0.081	0.111
HI 17	0.241	0.276	0.300	0.199	0.189	0.221	0.184	0.161	----
HI 18	0.204	0.233	0.292	0.191	0.228	0.260	0.121	0.142	0.223

Table C.6 Hematocrit (%) Spring 1991

	Subjects								
Date or day of diet	1	2	3	4	6	7	8	9	10
4/8	39.0	43.3	44.0	42.5	39.5	41.5	39.5	40.5	45.8
4/16	38.8	42.5	43.5	43.0	38.0	42.0	40.0	38.3	39.5
LOW 7	40.0	44.5	44.5	42.5	39.8	41.5	43.3	38.5	41.8
LOW 11	39.0	44.5	45.0	45.5	38.8	41.5	42.0	38.0	41.5
LOW 15	39.5	44.5	42.5	45.5	37.5	39.5	43.0	38.8	38.5
POST	39.5	45.0	43.5	44.0	38.5	41.0	41.5	39.0	40.0
HIGH 7	40.0	44.5	43.5	45.0	39.0	42.5	41.5	40.0	41.0
HIGH 11	38.5	43.5	45.0	43.5	39.8	42.0	40.5	35.3	41.3
HIGH 15	40.0	41.5	43.8	44.5	37.5	42.3	42.5	38.5	40.0
POST	39.5	44.0	44.0	45.5	43.0	41.0	41.3	36.5	41.5

Table C.7 Hemoglobin concentration (g/100 mL) Spring 1991

	Subjects								
Date or day of diet	1	2	3	4	6	7	8	9	10
4/8	---	13.8	---	13.8	12.8	13.7	12.4	12.4	14.7
4/16	13.5	14.2	15.1	14.1	12.7	14.3	12.8	12.1	14.0
LOW 7	14.7	16.1	14.8	13.7	13.0	13.4	13.7	12.2	13.9
LOW 11	12.8	14.1	14.8	14.0	12.3	13.2	12.9	11.8	13.7
LOW 15	13.1	14.1	14.0	14.0	11.9	16.5	13.5	12.2	13.2
POST	13.1	14.5	14.7	13.8	12.0	13.3	12.9	12.4	13.8
HIGH 7	13.5	14.6	14.7	14.3	13.0	13.8	13.1	12.2	13.9
HIGH 11	12.9	14.0	16.0	13.6	12.3	13.4	12.5	10.7	13.4
HIGH 15	13.6	15.4	15.1	14.2	11.8	13.4	13.0	12.9	14.3
POST	13.3	14.7	15.1	14.6	11.7	13.2	12.9	11.2	13.4

Table C.8 Plasma pyridoxal 5′-phosphate (nmoles/L) Spring 1991

	Subjects								
Date or day of diet	1	2	3	4	6	7	8	9	10
pre	54.3	45.5	26.9	33.3	34.0	---	16.1	16.9	---
4/8	63.4	64.2	18.7	39.0	34.5	30.5	20.9	25.3	70.0
4/16	47.3	69.9	36.6	44.6	28.9	44.7	22.7	26.0	47.1
LOW 7	38.9	66.8	28.4	27.8	26.6	36.1	19.7	28.6	34.6
LOW 11	39.6	66.1	27.9	29.1	30.1	35.5	15.0	26.1	34.4
LOW 15	38.0	65.6	32.0	26.1	27.4	37.9	16.8	25.3	31.9
POST	30.8	65.4	32.2	26.3	29.4	37.1	17.3	27.1	32.2
HIGH 7	30.3	49.7	24.6	27.8	29.8	35.8	20.9	24.3	37.1
HIGH 11	33.5	49.0	25.2	20.4	30.8	28.2	18.3	19.6	37.7
HIGH 15	31.8	48.8	25.8	25.2	24.0	31.0	18.3	24.9	29.8
POST	26.2	50.4	25.8	22.0	23.1	34.4	20.6	23.0	37.9

Table C.9 Plasma total vitamin B-6 (nmoles/L) Spring 1991

	Subjects								
Date or day of diet	1	2	3	4	6	7	8	9	10
pre	74.5	65.7	46.7	50.6	55.0	---	26.2	27.4	---
4/8	77.7	67.3	33.1	52.4	55.7	51.5	30.5	30.3	79.3
4/16	59.8	79.7	42.8	53.2	47.7	62.0	31.3	30.5	58.1
LOW 7	53.2	78.7	41.0	39.5	44.6	55.2	32.5	35.0	44.3
LOW 11	50.4	73.1	37.7	40.9	41.8	56.6	25.7	28.1	43.3
LOW 15	50.7	74.9	41.6	38.7	42.7	58.4	25.6	34.5	46.2
POST	56.7	78.7	42.8	39.2	45.1	56.1	27.2	36.1	43.7
HIGH 7	72.2	67.6	34.8	36.1	48.0	54.3	28.5	28.9	46.2
HIGH 11	75.5	60.5	35.9	29.4	48.6	48.4	23.8	22.1	47.0
HIGH 15	52.8	62.2	34.0	32.3	42.7	58.4	25.6	34.5	46.2
POST	43.2	56.4	34.9	28.6	37.7	46.6	30.8	27.8	42.6

Table C.10 Red blood cell pyridoxal 5′-phosphate (nmoles/L) Spring 1991

Date or day of diet	Subjects 1	2	3	4	6	7	8	9	10
4/8	35.8	45.2	40.1	47.2	37.7	60.3	55.0	45.4	54.4
4/16	34.1	45.6	45.4	48.0	47.6	47.9	56.3	56.5	47.1
LOW 7	41.0	48.6	45.3	41.8	45.0	56.8	49.9	53.7	39.2
LOW 11	39.1	49.5	45.3	36.6	44.8	50.5	44.3	50.8	40.5
LOW 15	37.8	45.9	51.8	38.3	47.9	61.4	51.4	50.4	46.9
POST	42.5	46.4	59.7	38.1	50.1	71.9	55.5	48.4	43.0
HIGH 7	43.9	49.8	48.5	40.6	43.3	55.7	51.8	51.6	37.0
HIGH 11	37.4	49.7	55.7	37.2	46.5	59.4	44.4	45.8	36.8
HIGH 15	37.5	48.1	51.3	40.7	37.5	53.4	39.3	52.2	39.4
POST	36.9	47.8	51.0	34.6	38.4	50.2	34.4	56.8	27.2

Table C.11 Plasma alkaline phosphatase activity (μmoles/min/L) Spring 1991

Date or day of diet	Subjects 1	2	3	4	6	7	8	9	10
4/8	17.1	20.4	20.1	17.3	24.4	29.9	22.6	23.0	29.1
5/14	16.3	19.6	18.5	20.7	25.5	23.9	26.3	21.4	28.3

Table C.12 Erythrocyte aspartate aminotransferase activity (μg pyruvate/mg Hgb/hr) with (stim) and without (unstim) added pyridoxal phosphate, and percent stimulation (%) Spring 1991

		Subjects								
Date or diet period		1	2	3	4	6	7	8	9	10
	unstim	42.07	32.32	35.74	35.17	37.15	---	29.90	35.96	34.83
4/8	stim	67.16	55.22	54.10	51.38	50.87	---	47.51	56.35	52.07
	%	60	71	51	46	37	---	59	57	50
	unstim	14.19	29.11	30.85	29.19	31.75	30.96	31.71	25.84	24.01
4/16	stim	43.23	44.80	44.41	34.56	37.50	48.04	46.34	33.33	37.28
	%	205	54	44	18	18	55	46	30	55
	unstim	31.43	37.42	28.43	23.75	25.94	18.05	28.21	22.14	16.33
LOW	stim	64.44	46.69	44.06	36.36	37.88	29.60	42.86	41.79	28.00
	%	105	25	55	53	61	64	52	89	71
	unstim	19.71	21.10	20.71	16.10	22.63	24.93	22.49	23.17	25.57
HIGH	stim	32.57	36.13	33.14	30.97	36.50	39.94	39.21	35.98	43.28
	%	65	71	60	92	46	60	74	55	69

Table C.13 Erythrocyte alanine aminotransferase activity (μg pyruvate/mg Hgb/hr) with (stim) and without (unstim) added pyridoxal phosphate, and percent stimulation (%) Spring 1991

Date or diet period		Subjects 1	2	3	4	6	7	8	9	10
4/8	unstim	1.17	1.84	1.78	1.68	1.52	---	3.42	1.65	1.65
	stim	1.39	2.08	2.20	1.94	1.94	---	3.72	2.17	1.87
	%	18	13	24	16	28	---	9	31	13
4/16	unstim	1.17	2.31	1.95	1.31	0.97	1.58	3.45	1.76	1.01
	stim	1.46	2.50	2.12	1.53	1.47	2.02	3.83	2.16	1.40
	%	25	8	9	17	52	28	11	23	39
LOW	unstim	1.18	2.19	1.87	1.18	1.12	1.48	2.57	1.43	1.23
	stim	1.47	2.41	2.18	1.52	1.28	1.92	2.87	1.78	1.42
	%	24	10	17	28	14	29	12	25	26
HIGH	unstim	1.10	2.02	1.58	0.87	1.11	1.85	2.50	1.85	1.08
	stim	1.39	2.36	1.92	1.17	1.48	2.04	2.80	2.22	1.36
	%	26	17	21	35	33	10	12	20	15

Table C.14 Fecal total vitamin B-6 (μmoles/day) Spring 1991

	Subjects								
Diet period	1	2	3	4	6	7	8	9	10
LOW 1	2.69	2.77	3.37	3.09	0.82	1.89	2.92	4.07	2.64
LOW 2	3.43	2.65	3.77	2.43	4.49	3.71	2.65	3.10	1.60
HIGH 1	4.51	4.58	5.06	4.17	4.72	3.67	4.07	6.91	3.88
HIGH 2	3.52	5.18	5.79	3.57	2.42	4.85	3.93	3.03	3.52

Table C.15 Subjects' weight (lbs) Spring 1991

	Subjects								
Date	1	2	3	4	6	7	8	9	10
4/8	108	96.5	120.75	121.5	105	---	120.5	134.5	131
4/9	104	96	120.25	121.5	105	172	119	135	131
4/10	104	95.25	120.75	121.75	105	170.75	119	135	130.5
4/11	103.5	96.25	119.75	122	105	169.25	120	133.5	131
4/12	101.5	96	119.75	121	104	168.75	119.25	135	131.5
4/13	101	95	119.75	121.5	105	169	120.5	134.5	130.5
4/14	101	95	120.25	121.5	105	168.25	120	134.5	128
4/15	101.5	94.5	120.25	122.5	104.5	168.5	119	134.5	130
4/16	101	95	119	121.5	105	168.25	118.25	133.75	131
4/17	101.25	95	120	122.75	105	168.25	118	134.25	130.5
4/18	100.75	95.5	119	123.5	105	168.25	118	134.5	129
4/19	101	96	119.25	122.5	104.75	168.25	118	134.5	130
4/20	100.75	96	119.25	122.5	105	168.25	117.5	133.5	130
4/21	102	96	118.25	123	105	168.5	116.25	134	129.5
4/22	101.25	96.25	119	123.5	105.5	169	116.25	135	129
4/23	102.25	96.25	119.5	122	105	169.5	117.5	134.5	129
4/24	101.25	96	119	121.5	104.75	169.25	118	136.25	128.5
4/25	101	95.75	119	121	105	169	117.25	135.25	130
4/26	101.25	95.5	119	121	104.75	168	117	136	130
4/27	100.5	96	120.75	120.75	104	169.5	118	135	129
4/28	100.5	96.5	120.25	121	104	168.25	119.5	134.75	128.5
4/29	101.25	96.25	119.25	122.5	105	168.25	117.25	135.25	131
4/30	100.5	97	119.25	121	104	167.75	117	134.75	130
5/1	100.5	96	118.75	---	104	167	117	135.5	131

Cont.

Table C.15, cont.

5/2	101	96	117.75	123	104	166.75	117.75	134.5	130
5/3	102	95.75	117.5	121.5	104	166.5	117	133.75	129
5/4	102	96	116.25	122.75	104	167.5	117.75	133.75	129
5/5	102	95.75	117	122.5	104	166.75	116.25	133.75	127.5
5/6	101.5	95.75	117.75	122.5	104.5	167	117.5	133.5	128
5/7	102	96	118	122.75	104.75	167.25	118	135	127
5/8	101.5	96.25	118.75	124	103.75	168	118.75	135	127.5
5/9	101	96.25	118	122	104.5	167.75	117.25	132.5	128
5/10	101.5	96.5	118.5	122.5	104	168.25	117.5	133	128
5/11	102	95.5	118.25	122.5	104	167.25	117.5	134	128
5/12	101.5	95.5	118.25	123	103.75	167.5	117.75	132.5	128
5/13	102	95.25	117.75	122.25	104.55	167.5	118	134.5	129
5/14	101.5	95.25	117	122.75	104.5	166.5	119.5	134.5	128
5/15	101.5	95	117	122.5	104	167.25	120	133	128
5/16	100.25	95	118	121.75	104	167.25	119.75	133	128
5/17	101.5	96	118.5	120.25	103.5	166.5	119.5	133.5	128
5/18	101	96	119	121.5	104	167	119	---	128
5/19	101.5	95.5	117.75	123	104	167	118.5	---	128
5/20	101.5	95.5	117.75	124.25	104	167.75	120	134.5	127
5/21	101.75	96.5	117.5	123.75	103	167.75	119.5	133	128
5/22	102	96	117.5	121.75	102	166.75	119	132	127

APPENDIX D

FORMS FROM THE STUDIES DESCRIBED IN CHAPTERS 3, 4 AND 5

Department of Nutrition and Food Management
Oregon State University

Informed Consent

I am interested in being considered as a possible participant in a human metabolic study on the interrelationship between dietary protein and vitamin B-6. To determine my eligibility for participating in this investigation, I understand that I will be asked to do the following:

1. Answer a questionnaire.

2. Come to 105 MLM on _________ between 7 and 8 am without having eaten or drunk anything except water since 7 pm the night before. This includes vitamins or any other nutritional supplements. A registered medical technologist will draw 20 mL of blood from a vein in my forearm. Following blood drawing, I will empty my bladder and consume a 5-g dose of D-xylose. During the subsequent 5-hour period I will collect all of my urine and not eat or drink anything except water.

I also understand:

1. That I will receive no direct benefits except information on my vitamin B-6 status, carbohydrate (xylose) absorption and results of the chemistry screen (glucose, electrolytes, enzymes, etc.).

2. That I may receive a slight bruise at the site of blood drawing. A sterile, disposable needle will be used to draw blood into evacuated tubes.

3. Any information obtained from me will be confidential. The only persons who have access to information obtained from me are the two principal investigators. All information will be filed in one of the investigator's office.

4. That I am not committed to participate in any metabolic study even if I meet all of the criteria.

5. Persons at risk for hepatitis B or HTLV III (commonly called AIDS) should not participate in this preliminary investigation (screening) since the donation of both and blood and urine are required to determine my eligibility.

All of my questions have been answered to my satisfaction. Should I have any further questions I can contact either Dr. Jim Leklem or Dr. Lorraine Miller between 8 and 5 weekdays at 737-3561.

Name____

Date____

Principal Investigator____________________________________

Figure D.1 Informed consent form for initial subject screening

Department of Nutrition and Food Management
Oregon State University

Informed Consent

The purpose of this investigation is to examine the effect of varying dietary vitamin B-6 to protein ratios on vitamin B-6 status in women. In this study vitamin B-6 intake will be varied while dietary protein intake is kept constant.

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

1. I will not have taken any vitamin supplement for at least three weeks before the beginning of this project.

2. I am not to take any vitamin or any other nutritional supplement except the vitamin B-6 (pyridoxine) that I will receive during the last three periods of this investigation.

3. This is a 51-day study, ending the morning after day 51.

4. I will consume only those foods and beverages that are served to me or permitted.

5. During this study I will not engage in any strenuous physical activity such as running more than one mile per day or bicycling more than 6 miles per day. I will maintain my normal daily physical activity throughout this study.

6. I will keep a record of my daily activities which I will turn in each day.

7. I will collect complete 24-hour urine specimens in the containers that are provided for me. If I accidentally lose some urine I will immediately report this to a member of the research staff.

8. During 5 consecutive days of each of the 4 experimental periods, I will collect all of my feces in the containers provided for me. If I accidentally lose a specimen I will immediately report this to a member of the research staff. I will receive a fecal marker (FDC Blue No. 1) at the beginning and end of these 5 days of each experimental period.

9. At regular intervals, a total of 14 times, a registered medical technologist will draw 20 mL of blood (equivalent to less than two tablespoons) from my forearm. On the days that blood is drawn, between 7 and 8 a.m., I am not to eat or drink anything except water since 7 p.m. the night before. I understand that this procedure may cause a slight bruise.

Figure D.2 Informed consent form for Fall 1989 study

10. A 2-g test dose of L-tryptophan will be administered to me with breakfast at the end of each period, a total of 4 times. L-Tryptophan is an amino acid which occurs naturally in protein-containing foods.

11. All information obtained from me will be confidential. My data will be identified by a code number. The only persons who will have access to my data are the two principal investigators, the medical technologist and the graduate students who are assisting in this research.

12. My participation in this research is voluntary and I can withdraw at any time without loss of benefits.

13. At the end of this investigation I will receive in a lump sum $3.92 for each day I participated. (For successfully completing this study, the total amount is $200.)

14. The principal investigators reserve the right to remove me from this project and/or reduce my monetary reward if I do not adhere to the requirements of this research. Examples include not showing up for meals, not adhering strictly to the diet, failure to report losses of urine or feces to a member of the research staff. Urine specimens will be assayed daily to determine completeness of collection and adherence to the diet.

15. I will incur no medical risks from participating in this research. I will receive some benefits: a nutritionally balanced diet furnished free of charge for 51 days; $3.92 for each day I participate; and results of my vitamin B-6 status and other laboratory analyses will be available to me if I request.

16. Persons who are at increased risk for hepatitis B or HTLV III (commonly called AIDS) should not participate in this investigation since they will be required to give their blood and excreta.

17. This study will be conducted from Oct. 2, 1989 to Nov. 22, 1989.

All of my questions have been answered to my satisfaction. If I have any questions I will call either Dr. Lorraine T. Miller or Dr. James E. Leklem at 737-3561.

Signed______________________________________Date______________

Present address_____________________________Phone no.________

Principal investigator______________________________________

Figure D.2, continued

Department of Nutrition and Food Management
Oregon State University

Informed Consent

The purpose of this investigation is to examine the effect of varying intakes of vitamin B-6 to protein ratios on vitamin B-6 status in women. In this study vitamin B-6 intake will be varied while protein intake is kept constant.

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

1. I will not have taken any vitamin or any other nutritional supplement for at least four weeks before the beginning of this project.

2. I am not to take any vitamin or any other nutritional supplement except the vitamin B-6 (pyridoxine) that I will receive during this investigation.

3. If I take any medication I will report this to one of the co-principal investigators.

4. This is a 32-day study, ending the morning after day 32.

5. I will consume only those foods and beverages that are served to me or permitted.

6. During this study I will not engage in any strenuous physical activity such as running more than one mile per day or bicycling more than 6 miles per day. I will maintain my normal daily physical activity throughout this study.

7. I will keep a record of my daily activities, including exercise and sleep, which I will turn in each day.

8. Daily throughout this investigation I will collect complete 24-hour urine specimens in the containers that are provided for me. If I accidentally lose some urine I will immediately report this to a member of the research staff.

9. At regular intervals, a total of 8 times, during this 32-day stidy a registered medical technologist will draw 30 mL of blood (equivalent to two tablespoons) from my forearm. On the days that blood is drawn, between 7 and 8 a.m., I am not to eat or drink anything except water since 7 p.m. the night before. I understand that this procedure may cause a slight bruise.

10. All information obtained from me will be confidential. My data will be identified by a code number. The only persons who will have access to my data are the two principal investigators, the medical technologist and the

Figure D.3 Informed consent form for Spring 1992 study

graduate students who are assisting in this research.

11. My participation in this research is voluntary and I can withdraw at any time without loss of benefits.

12. At the end of this investigation I will receive in a lump sum $150 or $4.65 for each day I participated.

13. It is important to note that strict daily adherence to the requirements of this project is necessary to my participation in this study. Failure to do so may result in the discontinuance of my participation as a subject. Examples include not showing up for meals, not adhering strictly to the diet, failure to report loss of urine to a member of the research staff. Urine specimens will be assayed daily to determine completeness of collection and adherence to the diet.

14. I will incur no medical or health risks from participating in this research. I will receive some benefits: a nutritionally balanced diet furnished free of charge for 32 days; $4.68 for each day I participate; and results of my vitamin B-6 status and other laboratory analyses will be available to me if I request.

15. Persons who are at increased risk for hepatitis B or HIV III (commonly called AIDS) should not donate blood or any other body fluids and therefore should not paraticipate in this investigation. Persons at increased risk include men who have had sexual contact with another man since 1977, persons who have used intravenous drugs. and persons who have had sexual contact with either a member of one of these groups, a person who has AIDS, or a person who is HIV infected.

16. This study will be conducted from April 20, 1992 to May 22, 1992.

17. I understand the University does not provide a research subject with compensation or medical treatment in the event the subject is injured as a result of participation in this research project.

All of my questions have been answered to my satisfaction. If I have any questions I will call either Dr. Lorraine T. Miller or Dr. James E. Leklem at 737-3561.

Signed______________________________Date__________

Present address________________________Phone no._______

Co-principal investigator______________________________

Figure D.3, continued

Department of Nutrition and Food Management
Oregon State University

Informed Consent

The purpose of this investigation is to examine the effect of varying dietary vitamin B-6 to protein ratios on vitamin B-6 status in women. In this study dietary vitamin B-6 will be kept constant and protein will be varied.

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

1. I will not have taken any vitamin or any other nutritional supplement for at least three weeks before the beginning of this project.

2. This is a 50-day study. The study ends the morning after the last day of the study. To shorten the adjustment from a higher to a lower protein intake, the period of lower protein intake will be preceded by a diet containing a negligible amount of protein for one day.

3. I will consume only those foods and beverages that are served to me or permitted.

4. I understand that to vary protein without changing the level of vitamin B-6 in the diet, some purified proteins such as vitamin-free casein, gelatin and isolated soy protein will be included in the experimental diet. I have been given the opportunity to taste the foods prepared with these purified proteins.

5. During this study I will not engage in any strenuous physical activity such as running more than one mile per day or bicycling more than 6 miles per day.

6. I will keep a record of my daily activities which I will turn in each day.

7. For each of the 50 days, I will collect complete 24-hour urine specimens in the containers that are provided for me. If I accidentally lose some urine I will immediately report this to a member of the research staff.

8. During the last 5 days of each of the three experimental periods, I will collect all of my feces in the containers provided for me. If I accidentally lose a specimen I will immediately report this to a member of the research staff. I will be administered a fecal marker (FDC Blue No. 1) at regular intervals during the last 5 days of each experimental period.

9. At regular intervals, a total of 12 times, during this

Figure D.4 Informed consent form for Spring 1990 study

50-day study, a registered medical technologist will draw 20 mL (equivalent to less than two tablespoons) of blood from my forearm. I understand that this procedure may cause a slight bruise. On the days that blood is drawn, between 7 and 8 a.m., I am not to eat or drink anything except water since 7 p.m. the night before.

10. A 2-g test dose of L-tryptophan will be administered to me with breakfast at the end of the adjustment period and each experimental period, a total of 4 times. L-tryptophan is an amino acid which occurs naturally in protein-containing foods.

11. All information obtained from me will be confidential. My data will be identified by a code number. The only persons who will have access to my data are the two principal investigators, the medical technologist and the graduate students who are assisting in this research.

12. My participation in this research is voluntary and I can withdraw at any time without loss of benefits.

13. At the end of this investigation I will receive in a lump sum $6.00 for each day I participated.

14. The principal investigators reserve the right to remove me from this project if I do not adhere to the requirements of this research. Examples include not showing up for meals, not adhering strictly to the diet, failure to report losses of urine or feces to a member of the research staff. Urine specimens will be assayed daily to determine completeness of collection and adherence to the diet.

15. I will incur no medical risks from participating in this research. I will receive some benefits: a nutritionally balanced diet furnished free of charge for 50 days; $6.00 for each day I participated; and results of my vitamin B-6 status and other laboratory analyses will be available to me if I request.

16. Persons who are at increased risk for hepatitis B or HTLV III (commonly called AIDS) should not participate in this investigation since they will be required to give their blood and excreta,

17. This study will be conducted from April 4 to May 24.

All of my questions have been answered to my satisfaction. If I have any questions I will call either Dr. Lorraine T. Miller or Dr. James E. Leklem at 737-3561.

Signed________________________________Date____________________

Present address____________________________Telephone___________

Principal investigator______________________________________

Figure D.4, continued

Department of Nutrition and Food Management

Oregon State University

Informed Consent

The purpose of this investigation is to compare the bioavailability of vitamin B-6 from a diet high in glycosylated vitamin B-6 to a diet low in glycosylated vitamin B-6 in women. Glycosylated vitamin B-6 is a form of vitamin B-6 in plants.

I have received a thorough explanation of this study. I understand the following:

1. I will not have taken any vitamin or any other nutritional supplement for at least three weeks before the beginning of this project.

2. This is a 44-day study. The study ends the morning after the last day of the study.

3. I will consume only those foods and beverages that are served to me or permitted.

4. During this study I will not engage in any strenuous physical activity such as running more than one mile per day or bicycling more than 6 miles per day.

5. I will keep a record of my daily activities which I will turn in each day.

6. I will collect complete 24-hour urine specimens in the containers that are provided for me. If I accidentally lose some urine I will immediately report this to a member of the research staff.

7. During the last 8 days of each of the two experimental periods, I will collect all of my feces in the containers provided for me. If I accidentally lose a specimen I will immediately report this to a member of the research staff. I will be administered a fecal marker (FDC Blue No. 1) at regular intervals during the last 8 days of each experimental period.

8. At regular intervals, a total of 9 times, a registered medical technologist will draw 20 mL (equivalent to less than two tablespoons) of blood from my forearm. On the last day of this investigation the medical techologist will withdraw an additional 10 mL for a chem screen which will be done at Good Samaritan Hospital. On the days that blood is drawn, between 7 and 8 a.m., I am not to eat or drink anything except water since 7 p.m. the night before. I understand that this procedure may cause a slight bruise.

Figure D.5 Informed consent form for Spring 1991 study

9. All information obtained from me will be confidential. My data will be identified by a code number. The only persons who will have access to may data are the two principal investigators, the medical technologist and the graduate students who are assisting in this research.

10. My participation in this research is voluntary and I can withdraw at any time without loss of benefits.

11. At the end of this investigation I will receive in a lump sum $4.50 for each day that I participated.

12. The co-principal investigators reserve the right to remove me from this project if I do not adhere to the requirements of this research. Examples include not showing up for meals, not adhering strictly to the diet, failure to report losses of urine or feces to a member of the research staff. Urine specimens will be assayed daily to determine completeness of collection and adherence to the diet.

13. I will incur no medical risks from participating in this research. I will receive some benefits: a nutritially balanced diet furnished free of charge for 44 days; $4.50 for each day I participated; and results of my vitamin B-6 status and other laboratory analyses will be available to me if I request.

14. Persons who are at increased risk for hepatitis B or HTLV III (commonly called AIDS) should not participate in this investigation since they will be requested to give their blood and excreta.

15. This study will be conducted from April 8 to May 22 (morning).

All of my questions have been answered to my satisfaction. If I have any questions I will call either Dr. Lorraine T. Miller at 737-0970 or Dr. James E. Leklem at 737-0969.

Name____

Present address__________________________________

Date____

Co-Principal Investigator____________________________

Figure D.5, continued

Code number__________________ Date ________

Age______________ Height______________ Weight________________

Marital status Single______________ Married_______________

Are you under the care of a physician? Yes ____ No____

If yes, for what reason(s)

____

____

____

Do you have or have you had any kidney, intestinal or metabolic condition (e.g., diabetes, nephrosis)? Yes ____ No ____

If yes, please describe

____

____

Are you taking any drugs? Yes ____ No ____

If yes, please list below and indicate amount(s) and length of time taken. List both prescribed and over the counter (such as aspirin)

____

____

____

Are you presently taking vitamins or any other nutritional supplement(s)?

Yes ____ No ____

If yes, indicate what brand you are taking, the amounts per day and how regularly. When was the last time you had taken them?

Figure D.6 Health questionnaire

____

____

____

____

Have you taken vitamins in the past three months but have stopped recently? Yes ____ No ____

If yes, please list when you stopped, what brand you were using and the amounts taken daily.

____

____

____

____

Do drink alcoholic beverages? Yes ____ No ____

If yes, indicate the kind(s) and the amounts you consume in one week.

____

____

____

____

Do you smoke? Yes____ No ____

If yes, indicate what you smoke and how much in one week.

____

____

Have you stopped smoking in the past 6 months?

Yes ____ No ____

Do you have asthma? Yes ____ No ____

If yes, list medications and amounts you have been taking for this condition

____

Figure D.6, continued

____

____

Have you taken oral contraceptives in the past six months?

Yes ____ No ____

If yes, are you currently taking them?

Yes ____ No ____

Do you have any food allergies or other allergies?

Yes ____ No ____

If yes, please describe

____

____

____

Describe your food dislikes, if any.

____

____

Do you exercise regularly? Yes ____ No ____

If yes, describe your daily program, including the intensity of your exercise and length of time you exercise.

____

____

____

____

Do you have regular menstrual cycles? Yes ____ No ____

If yes, how many days are they usually apart? ________

Figure D.6, continued

Spring '91

General Instructions for [redacted] Diet Study.

COLLECTION OF URINE:

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.

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. Return urine samples daily at any time convenient for you to Rm 106, Mlm Hall.

5. Store urine in a cool place and protected 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.

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

OTHER

1. Eat all food given to you each day. Let us know if you are receiving too much or too little food and we will adjust your diet accordingly.

2. Record all activities every day. A journal will be provided for you at breakfast to fill in for the previous day's activities (# hrs. slept, working, exercising, etc.).

3. No alcoholic beverages, including beer and wine, are to be consumed during the study.

4. No vitamin or mineral supplements are to be consumed during the study.

5. No smoking or use of nicotine during the study.

Figure D.7 General instructions for subjects

Fecal Collections

Due to the variability in the time taken for feces to move through the intestinal tract, fecal collections are different from urine collections. You will receive a fecal marker this morning with breakfast and another one with breakfast 5 days later. Fecal markers are often food colors which are safe and not absorbed from the gastrointestinal tract. The fecal marker that you will receive is FDC Blue #1, or Brilliant Blue. This is a food color, and is the dye that gives Creme de Menthe liquor its bright green color. Let this be a warning so that you are not alarmed when about 12 or even up to 48-72 hours later you will see a bright, grass-green stool. This is marks the breakfast you had eaten when you had taken the first fecal marker by mouth.

Instructions for collecting feces

Beginning this morning, after you have received the first fecal marker, collect all of your stools in the containers that we provide for you until the second marker appears. The stool showing the second marker is to collected also. Remember, collect all of your feces. Complete fecal collections are just as important to this study as complete urine collections.

Use the plastic containers that are provided for you. These will be available in Room 106, the same room where you find your urine bottles.

Start collecting all of your stools immediately after you have taken the first fecal dye marker. Use a new container for each collection.

Using a wax or water-proof marker, indicate on the <u>cover</u> and <u>side</u> of each container: (These markers are available in Room 106.)

Your initials and number

<u>Date</u> and <u>time</u> of collection, including a.m. or p.m.

As soon as possible after each collection, place your stool(s) in the refrigerator in Room 104 (next to Room 106).

Continue to collect all of your stools until the second fecal dye marker appears. Collect this marked stool.

Remember, collect all stools. Complete stool collections are as important to this investigation as are complete urine collections. Do not take a laxative.

If you have any questions, please ask Dr. Miller, Dr. Leklem or Christine. We appreciate your splendid cooperation.

Figure D.8 Fecal collection instructions

Diet Study [redacted] 1990-SP
Dept. of Nutrition and Food Management
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/Regular Pop ____________ Candy / Sugar ____________

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. Did you weigh yourself today? ________ Your weight today. ________

8. Other comments.

Figure D.9 Subjects' daily activity form

Figure D.10 Menstrual chart

Fall '89 Women's Diet Study

Subject ______________________________

No. __________

Menstrual Chart

Yr	Month	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	No. of days from start of period to beginning of next
'89	March [redacted].																																
	April [redacted].																																
	May [redacted]																																
	[redacted].																																

Type of flow: $\boxed{X}$ normal

$\boxed{O}$ exceptionally light

exceptionally heavy