AN ABSTRACT OF THE THESIS OF

Ying-Hui Huang for the degree of Master of Science in Nutrition and Food Management presented on January 11, 2000. Title: The Effect of Two Levels of Glucose Ingestion on Plasma Pyridoxal 5′-Phosphate Concentration.

Abstract approved by: _____

James E. Leklem

This study was designed to evaluate the effect of glucose on plasma pyridoxal 5′-phosphate (PLP) concentration. The objective was to determine whether there was a negative relationship between glucose ingestion and plasma PLP concentration and to evaluate the possible mechanism of decreased PLP after acute glucose ingestion.

Seven healthy subjects (three males and four females) completed the oral glucose tolerance test (OGTT) on three separate occasions over a period of three weeks. Each week, subjects ingested the assigned solutions (a water solution with artificial sweetener equivalent to 25g glucose, a 25g glucose or a 75g glucose load) in a randomized order. Plasma PLP, pyridoxal (PL), 4-pyridoxic acid (4-PA), pyridoxine (PN), glucose, insulin, alkaline phosphatase (AP) activity and red blood cell PLP concentrations were measured at 0 (fasting) (T0), 1 (T1), 2 (T2) and 3 (T3) hours.

The mean vitamin B-6 intake based on two 3-day dietary records was 1.57 ± 0.34 mg/day. All subjects had normal glucose tolerance. There were gender differences among the three solutions. Both the water solution and the 75g glucose load showed a

significant decrease in the mean plasma PLP concentration was observed at T3 for males and at T2 for females ($p<0.05$). An overall mean decrease of 20% (9nmol/L) and 15% (7 nmol/L) was observed for males and females, respectively, after the 75g glucose load. The 25g glucose load resulted in a lower decrease in the mean plasma PLP concentration at each time point compared with the 75g glucose load, but no significant difference was found in the level of decrease between the two glucose loads.

Both genders had a non-significant increase in the mean plasma PL and PN concentrations for the three solutions. Mean plasma 4-PA concentration was decreased at T1 with the three solutions. There was no significant change in the plasma AP activity at any time points after the three solutions. In addition, no significant increase in mean red blood cell PLP concentration was observed at all time points after the three solutions. This study found a negative relationship between glucose ingestion and plasma PLP concentration. However, it did not provide clear evidence for the hypothesized mechanism of the decreased plasma PLP concentration after acute glucose load. Further studies are required to determine the mechanism by which glucose decreases plasma PLP concentration.

The Effect of Two Levels of Glucose Ingestion on Plasma Pyridoxal 5′-Phosphate Concentration

by

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APPROVED:

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

Ying-Hui Huang, Author

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LIST OF ABBREVIATIONS

4-PA	4-pyridoxic acid
AP	Alkaline phosphatase
EALT	Erythrocyte alanine aminotransferase
EAST	Erythrocyte aspartic aminotransferase
HPLC	High performance liquid chromatography
PL	Pyridoxal
PLP	Pyridoxal 5′-phosphate
PM	Pyridoxamine
PMP	Pyridoxamine 5′-phosphate
PN	Pyridoxine
PNP	Pyridoxine 5′-phosphate
PNG	Pyridoxamine glucoside
RBC	Red Blood Cell
RDA	Recommended Dietary Allowance
TDC	Tyrosine apodecarboxylase

DEDICATION

This thesis is dedicated to my parents who have put

all the effort to bring their children the best

THE EFFECT OF TWO LEVELS OF GLUCOSE INGESTION ON PLASMA PYRIDOXAL 5′-PHOSPHATE CONCENTRATION

INTRODUCTION

Typically, people with diabetes have a high plasma glucose concentration (>10 mmol/ L) either chronically or during intermittent periods (Davis, 1976). Several studies have shown that people with diabetes have depressed plasma pyridoxal 5′-phosphate (PLP) and pyridoxal (PL) concentrations (Davis et al, 1976; Hollenbeck et al, 1983; Leklem et al, 1985; Hollenbeck and Leklem, 1985). Another way of looking at this is that under an adequate intake of vitamin B-6, a chronic period of high plasma glucose concentration could decrease plasma PLP concentration. This could lead to a potential adverse effect of high carbohydrate diet on vitamin B-6 status.

PLP, the active form of vitamin B-6, participates in more than 100 enzymatic reactions. Two of the important processes that PLP involves in are gluconeogenesis and glycogenolysis. In the process of gluconeogenesis, alanine aminotransferase, a PLP dependent enzyme, converts alanine to pyruvate in both liver and muscle (Sauberlich, 1968). Pyruvate is then converted to glucose for energy. In the process of glycogenolysis, glycogen phosphorylase, another PLP dependent enzyme, converts glycogen to glucose-1-phosphate in liver and muscle (Krebs and Fischer, 1964), which is also further converted to glucose for energy.

Studies have found an inverse relationship between glucose ingestion and plasma PLP concentration (Leklem and Hollenbeck, 1990; Hofmann et al, 1991). In a study by Leklem and Hollenbeck (1990), nine healthy subjects were given an oral glucose load

(1g/kg body wt.). Plasma PLP and total vitamin B-6 concentrations were measured at 0, 0.5, 1, 2, 3, 4, 5 hours after the glucose load. They found a continuous drop in plasma PLP concentration through five hours in five subjects and through three or four hours in the other four subjects. A significant mean decrease of 17.6% plasma PLP was seen at two hours. The decrease in plasma PLP concentration was greatest through three hours and slowed down after this time.

One of the possible mechanisms leading to decreased plasma PLP concentration after glucose ingestion is the uptake of PL (PLP converted to PL) by specific tissues (Leklem, 1990). Acute glucose ingestion increases plasma glucose concentration, which leads to an increase in plasma insulin level (Castro et al, 1970; Forster et al, 1972; Bratusch et al, 1980). Insulin elevates alkaline phosphatase (AP) activity (Romero et al, 1988; Ognibene et al, 1997), which could in turn increase conversion of PLP to PL (Coburn and Whyte, 1988). Since phosphorylated compounds do not cross cell membranes, hydrolysis of PLP to PL by AP would be consistent with the uptake of PL into cells. Several studies have demonstrated a rapid uptake of PL and PN by the red blood cell (RBC) (Anderson, 1980; Mehansho and Henderson, 1980; Fonda and Harker, 1982; Ink et al, 1982; Ink and Henderson, 1984; Anderson et al, 1989). In addition, two in vitro studies (Yamada et al, 1968; Ink et al, 1982). found an increased uptake of PL by the RBC after 60 minutes of glucose incubation. Because glucose in the RBC provides ATP, which is necessary for the enzyme pyridoxal kinase to convert PL to PLP, an increase in glucose may enhance the conversion of PL to PLP in the RBC.

In summary, the purpose of this study was to evaluate the effect of glucose on plasma PLP concentration. If the change of plasma level of PLP is dependent upon

glucose level, an increase in glucose intake, due to a high intake of refined carbohydrate diets, will decrease vitamin B-6 status. Thus, people typically consuming high simple carbohydrate diets would require a higher intake of vitamin B-6.

Hypotheses

1. Acute glucose ingestion causes a significant decrease in plasma PLP concentration.
2. Compared to low glucose ingestion (25g), higher glucose ingestion (75g) results in a lower plasma PLP concentration.
3. An increase in plasma insulin concentration elevates alkaline phosphatase activity, which increases the conversion of PLP to PL.
4. Acute glucose ingestion increases PLP concentration in red blood cells.

Objectives

1. To independently confirm that there is a negative relationship between glucose ingestion and plasma PLP concentration.
2. To determine whether there is a negative relationship between plasma AP activity and plasma PLP concentration.
3. To determine whether there is an increase in red blood cell PLP concentration after an acute glucose load.

4. To evaluate the possible mechanism of decreased PLP after acute glucose ingestion.

LITERATURE REVIEW

Vitamin B-6

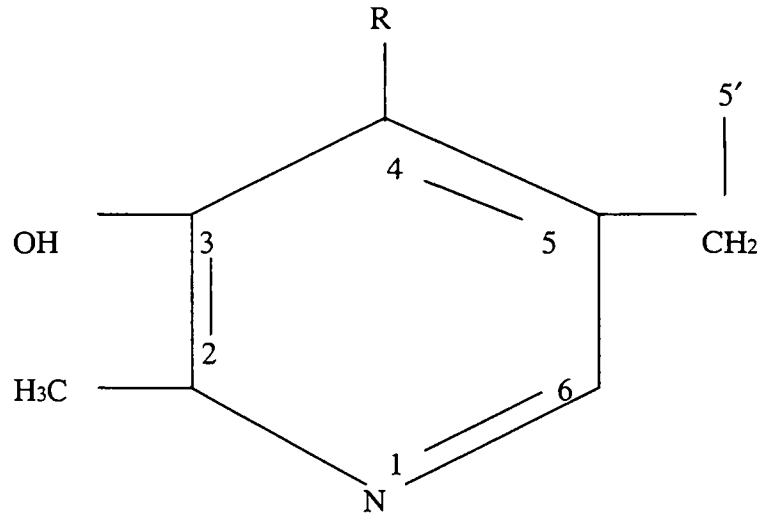
History

Vitamin B-6 was first discovered and named by Paul Gyorgy in 1934. Four years later, Gyorgy (1938) and Lepkovsky (1938) and three other research groups (Kuhn and Wendt, 1938; Keresztesy and Stevens, 1938; Ichiba and Michi, 1938) isolated pure crystalline vitamin B-6. In 1939, Harris and Folkers (1939) and Kuhn et al (1939) identified the chemical structure of vitamin B-6 as 3-hydroxy-4,5-dihydroxymethyl-2-methylpyridine and determined vitamin B-6 to be a pyridine derivative. Gyorgy was the first person to introduce the term pyridoxine (1939). Later, in 1942, Snell and his co-workers, Guirard and Williams, discovered other forms of pyridoxine, including pyridoxamine and pyridoxal, and also developed techniques for measuring pyridoxine through the use of microorganisms (Snell et al, 1942).

Structure and Chemistry

Vitamin B-6 is the generic name for all 3-hydroxy-2-methylpyridine derivatives (IUPAC-IUB, 1973). Figure 1 shows the structure of the different forms of vitamin B6. The structures for the three main forms of vitamin B-6 differ at the 4 position. The hydroxymethyl in this position forms pyridoxine (PN), the aldehyde forms pyridoxal (PL), and the methylamine forms pyridoxamine (PM). In addition, all three

Figure 1: Structure of the forms of vitamin B-6



3-hydroxy-2-methylpyridine derivatives

R	**5′ = -OH**	**5′ = -$OPO_3^=$**
-CH_2OH	Pyridoxine (PN)	Pyridoxine 5′-phosphate (PNP)
-CHO	Pyridoxal (PL)	Pyridoxal 5′-phosphate (PLP)
-CH_2NH_2	Pyridoxamine (PM)	Pyridoxamine 5′-phosphate(PMP)

Adapted from Leklem, 1996

phosphorylated forms exist as pyridoxine 5′-phosphate (PNP), pyridoxal 5′-phosphate (PLP), and pyridoxamine 5′-phosphate (PMP). 4-pyridoxic acid (4-PA) is the irreversible metabolic end product of vitamin B-6, and occurs mainly as a lactone. It has a –COOH group at the 4 position.

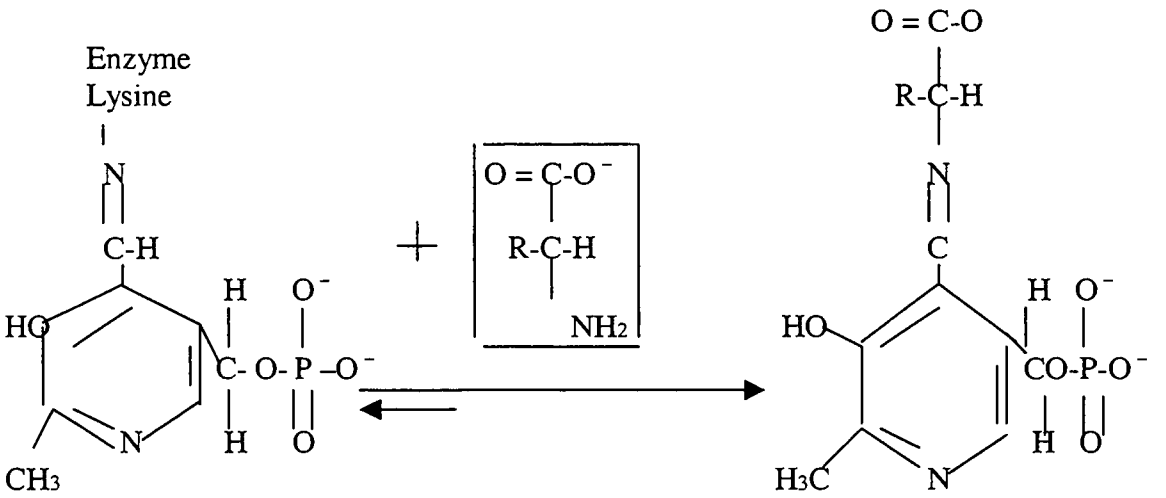
Although there are different degrees of degradation of B-6 vitamers, generally, the three free forms of vitamin B-6 are labile. They are light sensitive in solution (Ang, 1979; Schaltenbrand et al, 1987), and the sensitivity is dependent upon the pH value of the solution. PN, PL, and PM are relatively heat-stable in acid solution and heat-labile in alkaline solution (Leklem, 1991).

Both PLP and PMP are the active coenzyme forms of vitamin B-6, with PLP being the main coenzyme form. PLP is covalently bound to enzymes via a *Schiff* base with an ε-amino group of lysine (reviewed in Leklem, 1991). The *Schiff* base formation between PLP and an amino acid is shown in Figure 2. In enzymatic reactions, one way of forming a *Schiff* base with PLP and an amino acid for the given enzyme is through a transamination reaction. Over 100 enzymatic reactions require PLP with transaminase-type reactions accounting for nearly half of this total (Sauberlich, 1985). PLP catalyzes three types of enzyme reactions which are categorized according to reactions occurring at different positions on the α-, β-, or γ- carbon (Leklem, 1991).

Absorption, Metabolism and Transport

Vitamin B-6 is one of the unique water-soluble vitamins. It exists in foods as the three main forms, pyridoxine (PN), pyridoxal (PL) and pyridoxamine (PM) (Leklem,

Figure 2. *Schiff* base formation between pyridoxal 5′-phosphate and an amino acid



PLP Bound to an enzyme as a *Schiff* base

Schiff base with an amino acid

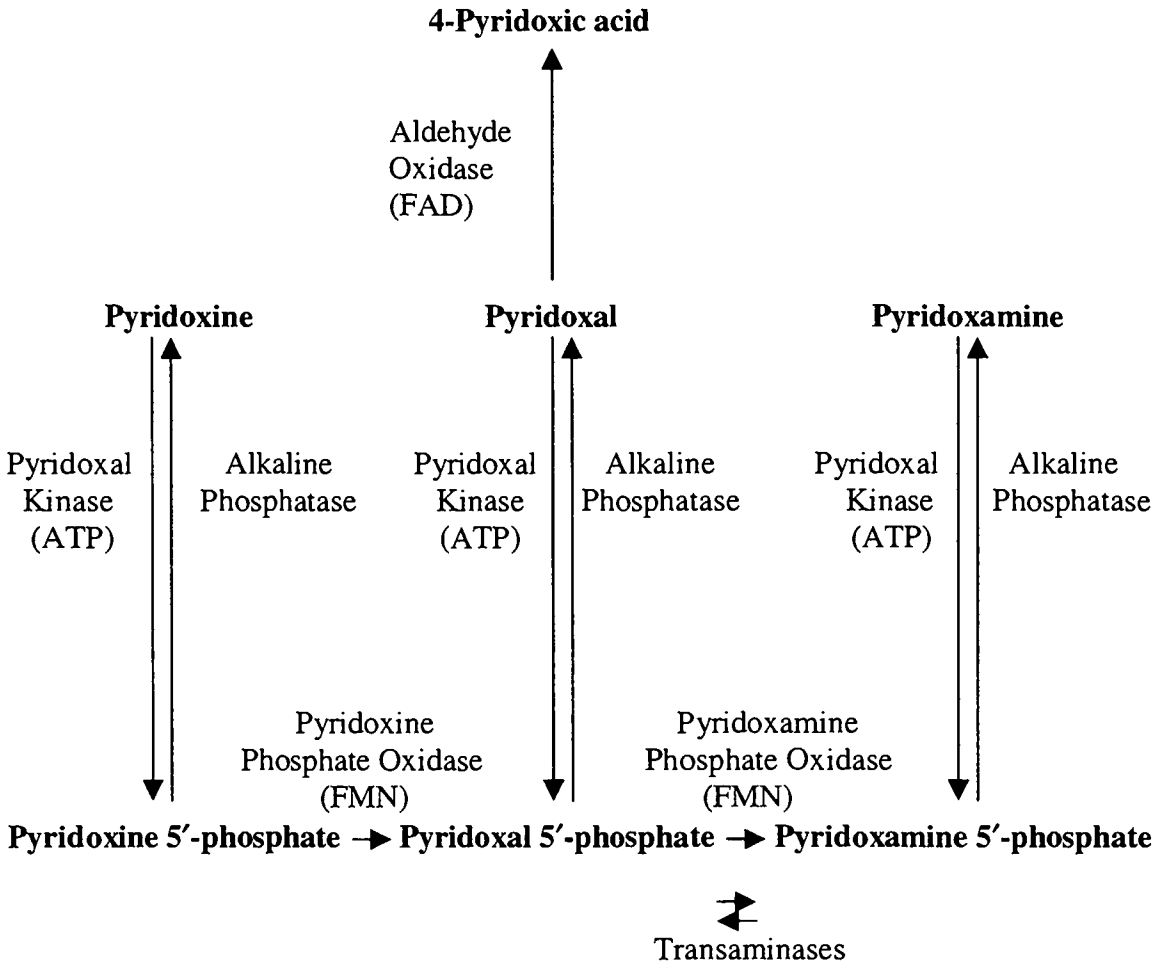
Adapted from Leklem, 1991

1991). The intestinal absorption of various B-6 vitamers has been examined mainly in animals. In rats, the three forms of vitamin B-6 are absorbed mainly in the jejunum by an unsaturable, passive process (Henderson, 1985). Absorption of the phosphorylated forms of vitamin B-6 can also occur via hydrolysis mainly by alkaline phosphatase in the gastrointestinal tract (Middleton, 1982; Hamm et al, 1979). Once the B-6 vitamins are absorbed into intestinal cells, they can be converted to phosphorylated forms and retained in the cell. However, nonphosphorylated B-6 vitamers, mainly PL, are the primary forms that leave the intestinal cells by facilitated diffusion and are transported to the liver (Hamm et al, 1979; Mehansho et al, 1979).

The primary metabolism of vitamin B-6 occurs in the liver (Merrill and Henderson, 1990). Figure 3 shows the metabolic interconversions of the B-6 vitamers. Once the free forms of vitamin B-6 are in the liver, they can be converted to phosphorylated form by pyridoxal kinase with the presence of zinc and adenosine triphosphate (ATP) (Merrill et al, 1984). Both PNP and PMP can be further converted to the active form, PLP, by pyridoxine phosphate oxidase, an enzyme requiring flavin mononucleotide (FMN) (Wada and Snell, 1961). PLP can also be hydrolyzed back to PL by alkaline phosphatase (AP), an enzyme that is consider to be an ectoenzyme (i.e. on outer cell membrane) (Coburn and Whyte, 1988). Excess vitamin B-6 in the form of PL is converted by an irreversible reaction to 4-pyridoxic acid (4-PA), by either an NAD-dependent dehydrogenase or a FAD-dependent oxidase and excreted in the urine (Merrill et al, 1984).

PLP is the most abundant form of vitamin B-6 in our body. In plasma, PLP accounts for 60-70 % of the total vitamin B-6, and PL is the second most abundant form

Figure 3. Metabolic interconversion of B-6 vitamers



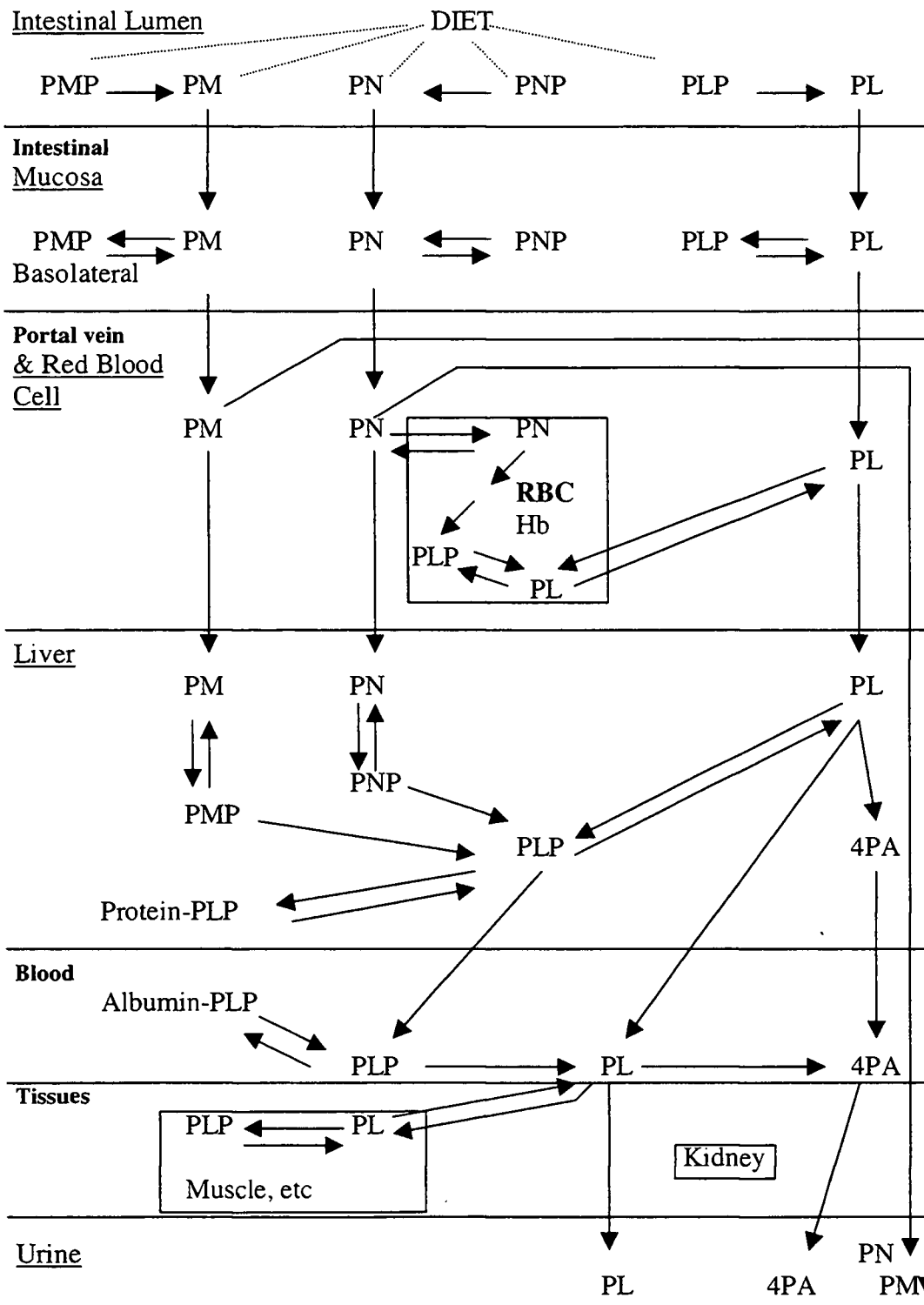
Adapted from Leklem, 1996

(Merrill and Henderson, 1990). PLP in plasma is bound to albumin. The binding of PLP to albumin is tighter than that of PL. In addition to their presence in plasma, PLP and PL are also found in the RBC. Both PLP and PL bind to hemoglobin. In this case, PL binds more tightly to hemoglobin than to albumin. As a result, the concentration of PL in RBC is 4 to 5 times higher than in plasma (Ink and Henderson, 1984). Since phosphorylated compounds do not cross cell membranes, hydrolysis of PLP to PL by a hydrolase enzyme , such as AP, is necessary for the uptake of PL into cells. After PL is taken up by the target cells, such as muscle and RBC, it can be converted back to PLP. Overall, PLP and PL in plasma (and probably PL in RBC) are the major forms of vitamin B-6 that are available to tissues (Leklem, 1988). An overview of the uptake, transport, metabolism and excretion of vitamin B-6 in different organs is summarized in Figure 4.

Alkaline Phosphatase

Alkaline phosphatase (AP) plays an important role in the regulation of blood and tissue PLP levels. Among the phosphatase enzymes, AP is the enzyme that hydrolyzes plasma PLP to PL. It is found mainly in liver, bone and kidney. Many studies have found an inverse relationship between AP activity and plasma PLP concentration. In a rat study, Wan et al (1993) found that AP activity was influenced by low zinc status condition. Because AP is a zinc metalloenzyme, zinc deficiency decreased AP activity, which was correlated with an increase in plasma PLP concentration. Some studies also found that patients with liver diseases had higher AP activity and lower plasma PLP level (Anderson et al, 1980, 1989; Merrill et al, 1986). In addition, plasma PLP is elevated in

Figure 4. Overiew of uptake, transport, metabolism and excretion of vitamin B-6



Adapted from Leklem, 1996

patients with hypophosphatasia (Iqbal et al, 1998). Furthermore, Nielsen et al (1988) found that AP activity in patients taking synthetic glucocorticoid (40mg/day) was 6 % lower compared to that of the controls. Plasma PLP concentration was not measured in these patients. Golik et al (1991) also observed an increased AP activity in about 50 % of the obese male subjects and 20 % of the obese female subjects. Moreover, elevated AP activity has been observed in about 25 % of persons with diabetes (Goldberg et al, 1977).

Body Pools/Stores

Coburn et al (1988) estimated vitamin B-6 pools in humans by obtaining approximately 50 mg of muscle tissue from 7 men and 5 women by needle biopsy of the gastrocnemius muscle and measuring the amount of B-6 vitamers. Creatinine excretion was also measured to estimate total muscle mass. A total of 800-900 μmol of vitamin B-6 was found in the muscle. Based on their data, Coburn et al (1988) estimated that there were approximately 1000 μmol of vitamin B-6 in the body, indicating that muscle is the main reservoir for vitamin B-6 in the body. The turnover time of various vitamin B-6 pools differs depending on the metabolic state and nutritional well-being of the organism (Leklem, 1991). The estimate of the PLP turnover in the plasma is related to a two-compartment model: one that has a rapid turnover of about 12 hours and one that has a slower turnover of 25-37 days (Shane, 1978). A later study by Coburn (1990) suggested that there may be multiple pools of vitamin B-6 in the body.

Functions

Vitamin B-6 participates in more than 100 enzymatic reactions. These enzymatic reactions make vitamin B-6 important in several cellular processes, including immune system function, gluconeogenesis, glycogenolysis, erythrocyte function, niacin formation, nervous system function, lipid metabolism, and hormone modulation (Leklem, 1991). The various systems and cellular processes in which PLP functions are shown in Table 1. The various functions of PLP are shown in Figure 5.

Immune system: PLP serves as a coenzyme for serine transhydroxymethylase, which is one of the main enzymes participating in 1-carbon metabolism in nucleic acid synthesis (Schirch and Jenkins, 1964). A deficiency of vitamin B-6 can cause abnormal 1-carbon metabolism, which in turn affects DNA and RNA synthesis. Decreased DNA and RNA synthesis can then decrease cell multiplication and antibody production, and therefore impair immune function (Axelrod and Trakatelles, 1964; Chandra and Puri,1985). In animal studies, lymphocyte production (van den Berg et al, 1988) and antibody response to antigens (Chandra and Puri, 1985) were strongly affected by vitamin B-6 deficiency. Further animal studies also showed an effect of vitamin B-6 on cell mediated immunity (Cheslock and McCully, 1960). Talbott et al (1987) gave 11 elderly females 50 mg pyridoxine/day for two months to evaluate its effect on the immune system. By looking at lymphocyte response, they found an improvement in the subjects' immune system after the vitamin B-6 supplement was given.

Similar results were found in a study of elderly female subjects by Meydani et al (1991). Furthermore, a study of vitamin B-6 status and immune function found a significant correlation between EAST activity and immunity in patients with

Table 1. The various systems and cellular processes in which pyridoxal 5′-phosphate functions

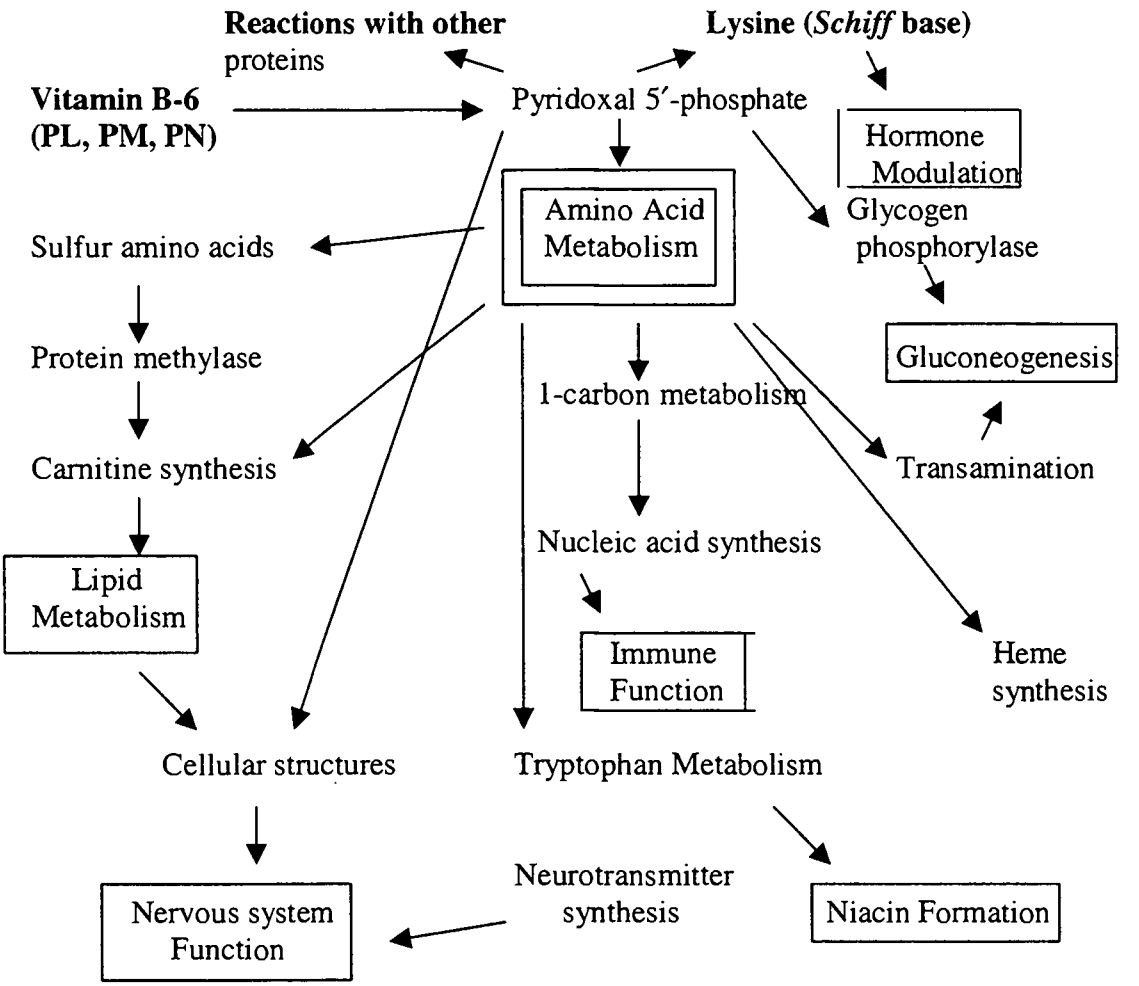
Cellular process or enzyme system	System/ Function
1-Carbon metabolism, steroid modulation	Immune
Transaminase, glycogen phosphorylase	Gluconeogenesis
Tryptophan metabolism	Niacin formation
Heme synthesis, O_2 affinity, transaminases	Red cell metabolism
Lipid and neurotransmitter synthesis	Nervous system
Binding of PLP to lysine of steroid receptor	Steroid (hormone) function

Adapted from Leklem, 1991

asymptomatic human immunodeficiency virus type 1 (HIV-1) infection (Baum et al, 1991). Moreover, Salhany and Schopfer (1993) have found that PLP binds to a protein called soluble CD4 protein, which is a HIV-1 receptor, indicating PLP may possibly be an effective anti-viral agent.

Gluconeogenesis and Glycogenolysis: Two of the important processes that PLP is involved as a coenzyme are glycogenolysis and gluconeogenesis. Both of the processes help in maintaining adequate glucose levels in the body. When the blood glucose level drops, there is an increase in gluconeogenesis. Alanine transaminase converts alanine to pyruvate in both liver and muscle (Sauberlich, 1968). Pyruvate can then be converted to glucose for energy. Meanwhile, glycogenolysis also occurs. In the liver and muscle, in response to an acute need of glucose, glycogen is converted to glucose-1-phosphate (G-1-P) by the PLP-dependent enzyme, glycogen phosphorylase (Krebs and Fischer, 1964). G-1-P in liver is then further converted to glucose for energy. In a study by Angel

Figure 5. Function of pyridoxal 5′-phosphate



Adapted from Leklem, 1993

(1980), male rats were given either a vitamin B-6 deficient or a pair-fed control diet for 8 weeks, and a significant decrease in liver alanine and aspartate transaminase activities was observed in the vitamin B6 deficient rats. Other enzyme activities, such as glutamate dehydrogenase, pyruvate carboxylase, phosphoenolpyruvate carboxykinase and pyruvate kinase, were not influenced by feeding the vitamin B-6 deficient diet, showing the importance of PLP in alanine and aspartate transaminase activities (Angel, 1980).

The major B-6 vitamer in muscle is PLP, which is bound to glycogen phosphorylase (Krebs and Fischer, 1964). Studies by Black et al (1977) found an increase of glycogen phosphorylase in the muscle (muscle phosphorylase) in the rats given 70 mg vitamin B-6/ kg of purified diet. However, in a subsequent study, Black et al (1978) found that the amount of muscle phosphorylase was not affected by B-6 deficiency. Instead, under conditions of partial and total starvation, there was a rapid and significant decrease in muscle phosphorylase in rats, suggesting that the reservoir of PLP in muscle is used only when there is a need for glucose (Black et al, 1978). In addition, Leklem and Shultz (1983; 1987) found an increase in plasma PLP and vitamin B-6 in men and women after strenuous exercise, an acute form of caloric deficit, suggesting that during caloric deficit, the reservoir of PLP in muscle is released from glycogen phosphorylase into the circulation.

Erythrocyte Function: Vitamin B-6 plays an important role in erythropoiesis. In the erythrocytes, PLP acts as a coenzyme for transaminases. Both PLP and PL bind to hemoglobin (Mehansho and Henderson, 1980; Ink et al, 1982). PL binds to the α-chain of hemoglobin which enhances O_2 binding affinity (Benesch et al, 1977), and PLP binds to β-chain of hemoglobin which decreases O_2 binding affinity (Maeda et al, 1976). The

difference in O_2 binding affinity of PLP and PL may be involved in diseases, such as sickle-cell anemia (Reynolds and Natta, 1985).

δ-aminolevulinic acid is an initial precursor in heme synthesis (Bottomley, 1983). The enzyme δ-aminolevulinic acid synthetase, which triggers the condensation between glycine and succinyl-CoA to form δ-aminolevulinic acid, requires PLP as a cofactor (Kikuchi, 1958). As a result, a serious deficiency in vitamin B-6 could theoretically cause hypochromic and microcytic anemia, although the occurance is rare. In human studies, some researchers have reported a response of pyridoxine therapy in patients with sideroblastic anemia (a shortage of δ-aminolevulinic acid synthetase) and other anemias (Harris et al, 1956; Horrigan and Harris, 1968), but others have reported no response to pyridoxine therapy in sideroblastic anemia (Pasanen et al, 1982).

Niacin Formation: One of the important roles PLP plays is in the conversion of tryptophan to niacin (Brown, 1985). In the tryptophan-niacin pathway, PLP is required in at least four enzymatic reactions. One of these four reactions is the conversion of 3-hydroxykynurenine to 3-hydroxyanthranilic acid by the enzyme kynureninase. A deficiency in vitamin B-6 can decrease the activity of kynureninase, which then impairs the conversion of 3-hydroxykynurenine to 3-hydroxyanthranilic acid and results in accumulation of tryptophan metabolites (Leklem, 1991).

A study by Leklem et al (1975) looked at the effect of vitamin B-6 deficiency on the conversion of tryptophan to niacin. They gave female subjects a low vitamin B-6 diet for 4 weeks and followed by a 2 g L-tryptophan load. Total urinary excretion of two major metabolites of niacin, N'-methylnicotinamide and N'-methyl-2-pyridone-5-carboxamide, were compared between subjects fed a normal diet and subjects fed a low

vitamin B-6 diet. Subjects fed a low vitamin B-6 diet had a total urinary excretion of these two metabolites less than subjects with a normal diet. This indicates a moderate effect of vitamin B-6 deficiency on the conversion of tryptophan to niacin.

Nervous System: PLP participates in the synthesis of some neurotransmitters, such as serotonin, taurine, dopamine, norepinephrine, histamine and γ-aminobutyric acid (Dakshinamurti, 1982). For example, in the formation of serotonin, the enzyme 5-hydroxytryptophan decarboxylase requires PLP as a coenzyme to convert 5-hydroxytryptophan to 5-hydroxytryptamine (Dakshinamurti, 1982). Two human infant studies (Coursin, 1954; Maloney and Parmalee, 1954) and two animal studies (Dakshinamurti, 1982; Alton-Mackey and Walker, 1973) reported neurological abnormalities in the subjects deficient in vitamin B-6. These studies support the role of vitamin B-6 in nervous system. In a study by Coursin (1954) infants were inadvertently fed with a formula low in vitamin B-6 and abnormal electroencephalogram (EEG) tracings and convulsions were observed. However, after treatment with 100mg of pyridoxine, both abnormal EEG patterns and convulsions disappeared. This phenomenon was also found in adults with a vitamin B-6 deficiency. Canham et al (1969) reported abnormal EEGs in adults fed a low vitamin B-6 (0.06 mg/d) and a high protein (100 g/d) diet. However, Grabow and Linkswiler (1969) found no abnormality in EEGs in male subjects fed a diet with 0.16 mg/d vitamin B-6 and 150 g/d protein for a shorter period of time (21 days). Coursin (1954; 1969) reported that a deficiency in vitamin B-6 and the abnormal symptoms seemed to be related with the amount of protein in the diet. From these two studies, it appears that the length of vitamin B-6 deficiency is another factor determining whether abnormal EEGs are seen in humans (Leklem, 1991).

Another important aspect of vitamin B-6 and the nervous system is its involvement in brain development. Several studies have reported a relationship between different levels of vitamin B-6 intake and the development of the brain. Aycock et al (1976) studied the effect of feeding different vitamin B-6 levels during the critical period of brain development in rats. They found lower brain weights of progeny and a drop in glutamic acid decarboxylase and alanine transaminase activity in rats without dietary vitamin B-6. Another rat study by Kurtz et al (1972) reported a drop of 30-50 % in cerebral sphingolipid levels of progeny fed a vitamin B-6 deficient diet. Other studies also reported an effect of vitamin B-6 deficiency on the development of cerebellum (Morre et al, 1978a) and Purkinje cells (Chang et al, 1981), fatty acid levels in the cerebellum and cerebrum in the myelination process (Morre et al, 1978b), extrapyramidal motor function (Wasynczuk et al, 1983a), and on changes in fatty acid (Thomas and Kirksey, 1976) and amino acid levels in specific regions of the brain (Kurtz et al, 1972; Wasynczuk et al, 1983b).

Lipid metabolism: The role of vitamin B-6 in lipid metabolism is still unclear (Mueller, 1964). Some earlier animal studies have found a relationship between vitamin B-6 deficiency and lipid metabolism. In one study, rats with a vitamin B-6 deficiency had lower body fat (McHenry and Gauvin, 1938), and another study found vitamin B-6 deficient rats had a significant decrease in liver lipid levels compared to those of pair-fed rats (Audet and Lupien, 1974). The effect of vitamin B-6 on plasma cholesterol in animal studies is not consistent. An increase (Sabo et al, 1971), no change (Desikachar and MeHenry, 1954; Angel, 1975), or a decrease (Angel and Song, 1973) in plasma

cholesterol level have been observed. These variable results may possibly due to different feeding conditions (Okada and Iwami, 1977).

A human study by Mueller and Iacono (1963) found a slight difference in fatty acid levels in both plasma and red blood cells. Studies done in rats have found a decreased conversion of linoleic acid to arachidonic acid (Witten and Holman, 1952; Delrome and Lupien, 1976; Cunnane et al, 1984) and decreased phospholipid methylation in microsomes (Loo and Smith, 1986). The results indicate that vitamin B-6 deficiency may have an inhibitory effect on arachidonic and linolenic acid metabolism and methylation metabolism.

An important role of vitamin B-6 related to lipid metabolism is its involvement in carnitine synthesis (Cunnane et al, 1985; Cho and Leklem, 1990). Carnitine is synthesized from methionine and lysine and is required in the process of β-oxidation of fatty acid. A study by Cho and Leklem (1990) found rats with a vitamin B-6 deficiency had lower carnitine values in plasma, liver, skeletal, muscle, heart, and urine. In addition, after repletion with vitamin B-6, the carnitine levels returned back to normal compared to those values in vitamin B-6 adequate rats. Further studies are needed to solidify the relationship between vitamin B-6 and lipid metabolism.

Hormone modulation: PLP may play a role in steroid modulation (Cidlowski and Thanassi, 1981; Litwack et al, 1985; Bender 1987; Allgood and Cidlowski, 1992). PLP forms a *Schiff* base with a lysine residue on the steroid receptor. Some studies have found reversible reactions between PLP and receptors for estrogen (Muldoon and Cidlowski, 1980), androgen (Hiipakka and Liao, 1980), progesterone (Nishigori et al, 1978), and glucocorticoids (Disorbo et al, 1980). Other studies have demonstrated that

PLP binds to steroid hormone receptor sites, inhibiting the binding of the hormone receptor with DNA. The results indicate that PLP may slow down or stop the action of steroid hormone (Cidlowski and Thanassi, 1981; Litwack et al, 1985; Bender, 1987).

Studies in female rats have found that moderate vitamin B-6 deficient rats given ^{3}H-estradiol shows a greater accumulation of the steroid in the uterine tissues, suggesting a higher end-organ sensitivity to estrogen in vitamin B-6 deficiency (Holly et al, 1983). The evidence for a vitamin B-6 steroid interaction is further supported by studies of Symes et al (1984) and Bunce and Vessal (1987). A subsequent in vitro study has found that a vitamin B-6 deficiency leads to higher hormone induced gene expression, and after repletion with vitamin B-6, the hormone induced gene expression decreased (Allgood and Cidlowski, 1992).

Status Assessment

There are various methods for assessing vitamin B-6 status. These methods can be categorized into direct, indirect, and diet intake methods. Methods for evaluating vitamin B-6 status and suggested values for adequate status in adults are shown in Table 2 (Leklem, 1990). Direct methods of vitamin B-6 status measure three main indices, PLP, PL and total vitamin B-6, or the metabolite, 4-PA, from the blood and urine. Indirect methods of vitamin B-6 status measure the PLP dependent enzyme activity or the concentration of compounds whose metabolism is PLP dependent, in both blood and urine. Diet intake methods of vitamin B-6 status estimate vitamin B-6 intakes, the vitamin B6:protein ratio and the intake of pyridoxine-β-glucoside. From these different

Table 2. Methods for evaluating vitamin B-6 status and suggested values for adequate status in adults

Methods	Suggested values for adequate status
Direct	
Blood	
Plasma PLP	> 30 nmol/L
Plasma PL	NV
Plasma total B-6	> 40 nmol/L
RBC PLP	NV
Urine	
4-PA	> 3.0 μmol/day
Total B-6	> 0.5 μmol/day
Indirect	
Blood	
EGOT (transaminase)	< 1.25, activity coefficient
EAST (transaminase)	< 1.80, activity coefficient
Urine	
2 gram tryptophan load	< 65 μmol/day
3 gram methionine load	< 350 μmol/day
Oxalate excretion	NV
Diet intake	
Vitamin B-6, weekly average	> 1.2-1.5 mg/day
Vitamin B-6:protein ratio	> 0.020
Other	
EEG pattern	NV

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

methods of assessment, it is recommended that at least 3 different measures should be used for vitamin B-6 status assessment (Leklem, 1990).

Plasma PLP, plasma total vitamin B-6 and urinary 4-PA are the three primary direct measures used in the research area of vitamin B-6. Erythrocyte PLP is sometimes used as an additional indicator to provide a more clear picture of vitamin B-6 status. The most popular measure that researchers use today is the measurement of plasma PLP (Leklem, 1990), which was confirmed as a valid status indicator by Lumeng et al (1978). This study has shown that plasma PLP reflected dietary vitamin B-6 intake and vitamin B-6 concentration in liver, brain and muscle.

Protein intake may affect vitamin B-6 status indices. Shultz and Leklem (1981) conducted a human study and found that plasma PLP was altered by both protein intake and vitamin B-6 intake. Miller et al (1985) also found an inverse relationship between plasma PLP, total vitamin B-6, and protein intake in males fed protein ranging from 0.5–2.0 g/kg/day. Furthermore, a recent study by Hansen et al (1996a) fed women three levels of dietary protein (0.5, 1.0 and 2.0 g/kg/body weight) and a constant level of vitamin B-6. They found a negative relationship between plasma PLP values and protein intakes.

Dietary vitamin B-6 intake may also affect vitamin B-6 status indices. A study by Lee and Leklem (1985) found that young female subjects receiving a vitamin B-6 dose of 2.3 or 10.3 mg/day had mean plasma PLP concentrations of 62.4 ± 20.2 and 210 ± 52 nmol/L, respectively. A separate study found plasma PLP concentration was altered by dietary vitamin B-6 intake until reaching a new stable level in 3-5 weeks (Brown et al, 1975). Other factors that may affect plasma PLP concentration include age (Rose et al,

1976; Lee and Leklem, 1985; Hamfelt and Soderhjelm, 1988), exercise (Leklem, 1985), and pregnancy (Lumeng, 1974).

Since plasma PLP accounts for approximately 50–75 % of the total plasma vitamin B-6 (Lumeng and Li, 1980), fasting plasma PLP values are considered a valid status indicator. However, studies have shown that plasma PLP is affected by alkaline phosphatase (AP) activity (Anderson et al, 1980; Merrill et al, 1986; Anderson et al, 1989). As stated earlier, AP activity is influenced by many factors. Therefore, for an accurate measurement of plasma PLP, the factors affecting AP activity would need to be taken into consideration.

The recommended value of plasma PLP for adequate vitamin B-6 status is >30 nmol/L. Mean plasma PLP values for males range from 27-75 nmol/L and for females range from 26-93 nmol/L (Leklem, 1990). Because PLP and PL account for approximately 90 % of total vitamin B-6 in plasma, measurement of plasma total vitamin B-6 and PLP concentration can provide a better estimate of plasma PL (Leklem, 1990), which accounts for 8-30 % of total vitamin B-6 concentration in plasma (Coburn and Mahuren, 1983; Lumeng et al, 1985; Hollins and Hendersons, 1986). Plasma total vitamin B-6 is affected by factors that change PLP values. Therefore, plasma total vitamin B-6 should not be the only vitamin B-6 status indicator. The recommended value of plasma total vitamin B-6 for adequate vitamin B-6 status is >40 nmol/L (Leklem, 1990).

Excretion of urinary 4-PA, a major metabolite of vitamin B-6, is usually used for a short-term vitamin B-6 status indicator because it is rapidly altered by the change of vitamin B-6 intake (Brown et al, 1975; Leklem, 1990). Urinary 4-PA accounts for 40-60

% of the vitamin B-6 intake. The recommended value of urinary 4-PA for adequate vitamin B-6 status is >3.0 μmol/day (Leklem, 1990). Urinary total vitamin B-6 can be considered a good indicator only when there is a low vitamin B-6 intake (Kelsey et al, 1968). The recommended value of urinary total vitamin B-6 for adequate vitamin B-6 status is >0.5 μmol/day (Leklem, 1990). Both male (Kelsay et al, 1968) and female (Brown et al, 1975) studies have shown a parallel relationship between plasma PLP concentration and urinary 4-PA excretion. A study by Miller et al (1985) found that a higher protein intake or lower vitamin B-6 intake led to lower urinary 4-PA excretion. This finding is further supported by Hansen et al (1996a) who looked at the effect of 3 levels of protein intakes on urinary 4-PA excretion in women.

Indirect methods of vitamin B-6 status include erythrocyte alanine aminotransferase (EALT), erythrocyte aspartic aminotransferase (EAST), a 2 g tryptophan load test and a 3 g methionine load test (Leklem, 1990). Of these measures, erythrocyte transferase activity is considered by some to be the most useful indirect measure of vitamin B-6 status (Rose et al, 1976; Guilland et al, 1984; Driskell and Moak, 1986). It is considered a long-term vitamin B-6 status indicator. The recommended values of EALT and EAST activity for adequate vitamin B-6 status are <1.25 and <1.80, respectively (percent stimulation) (Leklem, 1990). The activity is measured with and without excess PLP. Of these two enzyme activities, EALT is considered a better vitamin B-6 status indicator compared to EAST. Several studies found that vitamin B-6 deficiency caused a decrease in EALT activity and an increase in vitro EALT stimulation (Baysal et al, 1966; Sauberlich, 1972; Brown et al, 1975; Solomon and Hillman, 1979). However, some studies found this measure was not as accurate as other measures

(Kirksey and Keaton, 1978; Shultz and Leklem, 1981). The reason for this difference is probably because of the life span of the erythrocyte and the strong binding of PLP to hemoglobin, which lead to a variation and a lack of a significant correlation between plasma PLP and transferase activity coefficient.

Measurement of urinary xanthurenic acid (XA) excretion after a 2 g tryptophan load was a popular measure of vitamin B-6 status (Leklem, 1971; Brown, 1985). With low vitamin B-6 status, excess tryptophan results in higher XA excretion. Studies have found that using the tryptophan load test in people who have specific disease states or who take steroid hormones interferes with the tryptophan metabolism and changes the relationship with vitamin B-6 metabolism (Bender, 1987). The average excretion of XA from a 2 g tryptophan load is 30-40 μmol/day, and the recommended value of XA for adequate vitamin B-6 status is <65 μmol/day (Leklem, 1990). Other indirect measures, such as methionine load test and oxalate excretion, are less popular for vitamin B-6 status assessment (Linkswiler, 1981).

Diet intake of vitamin B-6 by itself is not a good vitamin B-6 status indicator because of the inaccuracy of the dietary intake values. Protein intake also needs to be measured. In addition, a long period of dietary records is necessary for estimating vitamin B-6 status. Furthermore, the nutrient data bases for vitamin B-6 for food are not always complete. As a result, the measurement of diet intake alone is not sufficient as an indicator of vitamin B-6 status (Leklem, 1990).

Food Sources and Bioavailability

There are six different forms of vitamin B-6 in foods, including pyridoxal (PL), pyridoxine (PN), pyridoxamine (PM), and their phosphorylated forms. The predominant forms of vitamin B-6 in plant foods are PN and PM (and their phosphorylated forms) and in animal foods is PL (and its phosphorylated form). In 1943, Atkin et al developed a microbiological method for measuring the vitamin B-6 content of foods. This method has continued to be used as the main method in determining vitamin B-6 content and has provided most of the data available on the vitamin B-6 content of foods. Table 3 lists the vitamin B-6 content of selected foods and percentages of the three forms.

The major dietary sources of vitamin B-6 from a 1980 survey reported by Sauberlich (1981) are meats 40.0 %, vegetables 22.2 %, dairy items 11.6 %, cereals 10.2 %, fruits 8.2 %, legumes 5.4 % and eggs 2.1 %. The second National Health and Nutrition Examination Survey (NHANES II, 1976-1990) found that meats 28.0%, vegetable 14.5 %, breads and cereals 13.2 %, dairy 8.0 %, fruits 7.0 %, alcohol 6.0 %, nuts and legumes 3.8 %, and eggs 2.0 % were sources of vitamin B-6. In addition to the phosphorylated forms of vitamin B-6 in foods, a conjugated form, pyridoxine β-glucoside, has also been discovered in some plant foods. The β-glycosylated form of PN has been identified in rice bran (Yasumoto et al, 1977) and in certain other foods (Kabir et al, 1983b). This β-glycosylated form of PN (PNG) is found only in plant foods. It is absorbed (Kabir et al, 1983a) but not fully utilized (Trumbo and Gregory, 1988; Gregory et al, 1991) in the body. In isolated rat liver cells, the uptake of PNG is approximately 20 % of the uptake of PN, and the cellular conversion of PNG to PLP is only 0.2 % of the cellular conversion of PN to PLP (Zhang et al, 1993).

Table 3. Vitamin B-6 content of selected foods and percentages of the three forms

Food	Vitamin B-6 (mg/100gm)	PN (%)	PL (%)	PM (%)
Vegetables				
Beans lima, frozen	0.150	45	30	25
Cabbage, raw	0.160	61	31	8
Carrots, raw	0.150	75	19	6
Peas, green, raw	0.160	47	47	6
Potatoes, raw	0.250	68	18	14
Tomatoes, raw	0.100	38	29	33
Spinach, raw	0.280	36	49	15
Broccoli, raw	0.195	29	65	6
Cauliflower, raw	0.210	16	79	5
Corn, sweet	0.161	6	68	26
Fruits				
Apples, red delicious	0.030	61	31	8
Apricots, raw	0.070	58	20	22
Apricots, dried	0.169	81	11	8
Avocados, raw	0.420	56	29	15
Bananas, raw	0.510	61	10	29
Oranges, raw	0.060	59	26	15
Peaches, canned	0.019	61	30	9
Raisins, seedless	0.240	83	11	6
Grapefruit, raw	0.034	-	-	-
Legumes				
Beans, white, raw	0.560	62	20	18
Beans, lima, canned	0.090	75	15	10
Lentils	0.600	69	13	18
Peanut butter	0.330	74	9	17
Peas, green, raw	0.160	69	17	14
Soybeans, dry, raw	0.810	44	44	12
Nuts				
Almonds, without skin, shelled	0.100	52	28	20
Pecans	0.183	71	12	17
Filberts	0.545	29	68	3
Walnuts	0.730	31	65	4

Table 3. Continued

Food	Vitamin B-6 (mg/100gm)	PN (%)	PL (%)	PM (%)
Cereals/Grains				
Barley, pearled	0.224	53	42	6
Rice, brown	0.550	78	12	10
Rice, white, regular	0.170	64	19	17
Rye flour, light	0.090	64	22	14
Wheat, cereal, flakes	0.292	79	11	10
Wheat flour, whole	0.340	71	16	13
Wheat flour, all purpose, white	0.060	55	24	21
Oatmeal, dry	0.140	12	49	39
Cornmeal, white and yellow	0.250	11	51	38
Bread, white	0.040	-	-	-
Bread, whole wheat	0.180	-	-	-
Meat/Poultry/Fish				
Beef, raw	0.330	16	53	31
Chicken breast	0.683	7	74	19
Pork, ham, canned	0.320	8	8	84
Flounder fillet	0.170	7	71	22
Salmon, canned	0.300	2	9	89
Sardine, Pacific canned, oil	0.280	13	58	29
Tuna, canned	0.425	19	69	12
Halibut	0.430	-	-	-
Milk/Eggs/Cheese				
Milk, cow, homog.	0.040	3	76	21
Milk, human	0.010	0	50	50
Cheddar	0.080	4	8	88
Egg, whole	0.110	0	85	15

All values taken from Orr, 1969

It is clear that bioavailability of a nutrient is the amount absorbed and utilized by cells compared to the total amount present. A study of vegetarian females by Shultz and Leklem (1987) suggested that the bioavailability of PNG was lower compared to the primary forms of vitamin B-6. A later study found that PNG was only 58 % as available as that of PN (Gregory, 1991). Another study by Bills et al (1987) found an inverse relationship between urinary 4-pyridoxic acid excretion and PNG content in the foods. Moreover, Gilbert and Gregory (1992) found that PNG not only reduced the bioavailability of vitamin B-6 but also impaired the utilization of non-glycosylated forms of vitamin B-6. Furthermore, Hansen et al (1996b) found that feeding a high pyridoxine glucoside diet (2.42μmol/day) compared to a low pyridoxine glucoside diet (0.76μmol/day) resulted in higher excretion of the unmetabolized glucoside in the urine and lower vitamin B-6 status in the subjects.

Leklem et al (1980a) found that vitamin B-6 was 6-7 % more available from beef than from soybeans. Kabir et al (1983b) also found a higher bioavailability of vitamin B-6 from tuna (considered to be 100 %) than that in whole wheat bread (75 %) and peanut butter (65 %). Both studies indicated that vitamin B-6 in animal foods had a higher bioavailability than the vitamin B-6 in plant foods. Leklem et al (1980b) also looked at the bioavailability of vitamin B-6 in different kinds of breads in nine male subjects. They found higher urinary 4-pyridoxic acid excretion and lower fecal vitamin B-6 excretion in men fed whole wheat bread, compared to the other kinds of bread. This study showed that whole wheat bread had a lower vitamin B-6 bioavailability compared to white bread or crystalline vitamin B-6 added to white bread. Several studies also found lower urinary

4-pyridoxic acid excretion in diets with different types of bran, compared to diets without bran added (Lindberg et al, 1983; Kies et al, 1984).

The vitamin B-6 content of food may also be affected by food processing and storage (Woodring and Storvick, 1960; Gregory and Kirk, 1978), which can result in the formation of new compounds in the food. These studies on different kinds of foods have reported a loss of 10-50 % of vitamin B-6 after food processing and storage. Studies by Gregory and Kirk (1977; 1978) tested vitamin B-6 bioavailability of foods exposed to thermal processing and storage in low-moisture condition. They found a decreased binding of PL and PLP to the ε-amino groups of protein or peptide lysyl residues. In addition, the ε-pyridoxallysine bound to protein showed lower vitamin B-6 activity and resistent to hydrolysis. Other studies on different types of food processing on vitamin B-6 content of milk and milk products resulted in a loss of vitamin B-6 of 0-70 % (Woodring and Storvick, 1960). A HPLC method for determining different forms of vitamin B-6 in milk by Vanderslice et al (1984) provides additional information on the effects of food processing on the vitamin B-6 content of milk and milk products.

In addition to food processing and storage, heating of foods also causes a loss of vitamin B-6. Gregory and Kirk (1981) found a loss of 50-70 % vitamin B-6 after toasting bread. Heating results in a formation of a bond between PL and amino acid lysine, which can not be hydrolyzed by digestive enzymes, and therefore reduces its bioavailability. Bernhart et al (1960) found that PL was converted to PM after heat sterilization of commercial milk, and that vitamin B-6 content was decreased after storage of heat sterilized milk because of the formation of bis-4-pyridoxal-disulfide. Furthermore,

Tadera et al (1986) found that food high in vitamin C resulted in the conversion of PL to 6-hydroxypyridoxine, which further decreased the bioavailability.

The bioavailability of vitamin B-6 may also be affected by other factors, such as the amount of fiber, which causes incomplete digestion and decreased absorption of vitamin B-6 (Leklem, 1991). Considering all the possible factors that affect vitamin B-6 bioavailability into account, one can estimate the bioavailability of foods that make up average American diet to be about 71-79 % (Tarr et al, 1981).

Deficiency

Since vitamin B-6 is widely distributed in foods, a deficiency in the B-complex vitamins is more common in the population than a deficiency of only vitamin B-6 (Leklem, 1991). Vitamin B-6 deficiency can be assessed through clinical signs of deficiency and some functional/biochemical tests. Primarily clinical signs of deficiency in infants are abnormal eletroencephalogram pattern and convulsions. In adults, the clinical signs of deficiency include stomatitis, cheilosis, glossitis, irritability, and depression and confusion (Leklem, 1988). Indices of early vitamin B-6 deficiency through direct and indirect measures of vitamin B-6 status include low plasma PLP and total vitamin B-6 concentration, low urinary 4PA and total vitamin B-6 excretion, abnormal tryptophan and methionine metabolism, decreased EALT and EAST activity, increased urinary oxalate excretion, and microcytic hypochromic anemia (Leklem, 1988).

The clinical signs of vitamin B-6 deficiency in infants were first found by Coursin (1954) in infants who were fed pyridoxine-deficient diet due to an inappropriate food

processing of formula. Another study by Mueller and Vilter (1950) found adults given desoxypyridoxine also developed a deficiency of vitamin B-6 because desoxypyridoxine, an analog of PN, antagonizes PN metabolism. A recent study found young women fed vitamin B-6 deficient diet had negative calcium and magnesium balances. Vitamin B-6 deficiency may alter calcium and magnesium metabolism (Turnlund et al, 1992). Miller et al (1992) also looked at the relationship between vitamin B-6 and fasting plasma homocysteine concentration. They found only 1 in 11 elderly subjects had increased fasting plasma homocysteine level, indicating vitamin B-6 deficiency does not directly affect fasting plasma homocysteine level.

Toxicity

The toxicity of vitamin B-6 occurs when people take a large amount of vitamin B-6 dose for a long period of time. Pyridoxine hydrochloride, a common form of vitamin B-6 found in nutritional supplements, has been commonly used as a preventive or therapeutic agent for various diseases, such as Down's syndrome, autism, hyperoxaluria, gestational diabetes, premenstrual syndrome, carpal tunnel syndrome, depression and diabetic neuropathy (Leklem, 1991). Some studies have found that a chronic high dose of PN is related to neurotoxicity (Schaumberg et al, 1983) and photosensitivity (Leklem, 1991). This effect on the nervous system is further supported by Parry and Bredesen (1985) and Dalton (1987). These studies found that a daily dose of >500 mg of PN could cause vitamin B-6 toxicity. Cohen and Bendich (1986) reported that doses of 2-250 mg/day were safe for most people to use for extended periods of time.

Recommended Dietary Allowances

Many factors can influence vitamin B-6 requirements (see Table 4). Of these factors, only a few have been tested and discussed in detail, such as protein intake (Miller and Linkswiler, 1967; Canham et al, 1969; Miller et al, 1985). A study by Miller et al (1985) found an inverse relationship between protein intake and the levels of plasma PLP concentration and urinary 4PA excretion. As protein intake increases, a higher transaminase level is needed to metabolize the excess amino acids. Therefore, more PLP is required. Examples of physiologic conditions requiring higher vitamin B-6 intakes are pregnancy and lactation. Vitamin B-6 requirements related to protein intakes for infants and children have been conducted in only a few studies (Snyderman et al, 1953; Bessey et al, 1957; McCoy 1978), while vitamin B-6 requirement for adults has been well studied by many researchers. The RDA for vitamin B-6 takes into account some factors (listed in Table 4), and it covers 97.5 % of the population. The current RDA for both males and females is 1.3 mg/day (National Research Council, 1999).

Bioavailability of vitamin B-6 is another factor affecting vitamin B-6 requirements. Other factors, such as gender (Leklem, 1991), age (Rose et al, 1976; Lee and Leklem, 1985) and exercise (Leklem and Shultz, 1983), have been conducted in only a few studies. A summary of different studies on the gender differences shown that females had lower plasma PLP and total vitamin B-6 concentration, lower urinary 4-PA and total vitamin B-6 excretion, compared to males when both genders were fed the same diet (Leklem, 1991). One study of age differences found that middle-aged women had significantly lower plasma PLP, plasma total vitamin B-6, urinary total vitamin B-6, and higher urinary 4-PA excretion than young women when given the same diet, indicating a

negative relationship between age and vitamin B-6 status (Lee and Leklem, 1985). This evidence for the negative relationship between age and vitamin B-6 status is further supported by Ribaya-Mercado et al (1991), suggesting a need for a higher vitamin B-6 intake for the elderly.

The tryptophan load test, urinary 4-PA excretion, transaminase activity and plasma PLP concentration are the main tests used in most of the metabolic studies. However, studies on different factors that affect requirements have used different lengths of time, amounts of vitamin B-6, and types of food. As a result, it is not easy to set specific vitamin B-6 requirements. Therefore, more studies with well-controlled and consistent experimental designs would be necessary for a better evaluation and recommendation of vitamin B-6 requirements (Leklem, 1991).

Carbohydrate Metabolism

Relationship Between Glucose Ingestion and Plasma Insulin Response

Insulin plays an indispensable role in carbohydrate metabolism. It acts as a hypoglycemic hormone in blood glucose regulation. Insulin is secreted from the β-cells of pancreas via the β-cell glucoreceptor (Hedeskov, 1980). Under normal conditions, increasing glucose elevates plasma glucose level. When the blood glucose level increases, it stimulates release of insulin. The release of insulin lowers the blood glucose level by increasing receptors that transfer glucose into the cells. Once glucose enters

Table 4. Factors affecting an individual's vitamin B-6 requirement

1. Dietary

a. Physical structure of a food
b. Forms of vitamin B-6; those due to processing
c. Binding of forms of vitamin B-6

2. Defect in delivery to tissues

a. Impaired g.i. absorption
b. Impaired transport-albumin, synthesis, and binding, phosphatase activity

3. Physiological/Biochemical

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

4. Genetic

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

5. Disease prevention/treatment

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

Adapted from Leklem, 1993

cells, insulin enhances the oxidation of glucose for energy and the conversion of glucose to glycogen for storage, while inhibiting other processes that increase blood glucose levels, such as gluconeogenesis and glycogenolysis. The insulin level then returns back to the baseline level.

Many factors can cause a difference in insulin sensitivity, secretion and production. Studies have found that both obesity (Rodin et al, 1985; Peiris et al, 1986; Craig et al, 1987; Bhatherna et al, 1987) and age (Craig et al, 1987; Meneilly et al, 1989) cause lower insulin sensitivity, while exercise (Wright et al, 1983; Craig et al, 1987; Mikines et al, 1989) causes higher insulin sensitivity. A study by Hale et al (1985) looked at the gender differences in blood glucose and insulin levels. They found that female subjects required more insulin secretion to have the same level of decrease in blood glucose, compared to male subjects. Some animal and human studies have found that release of insulin is enhanced by sight, smell, and/or taste (Sjostrom et al, 1980; Simon et al, 1986; Bruce et al, 1987). Other studies have found a variation of changes in blood glucose levels in females during the different phases of the menstrual cycle, with a highest blood glucose level at Day 13-18 (Jarrett and Graver, 1968; MacDonald and Crossley, 1970). Oral contraceptives were also found to increase blood glucose and insulin levels in females (Behall et al, 1980).

A standardized oral glucose tolerance test (OGTT) has long been used in testing glucose and insulin response. Castro et al (1970) found that 75 g of glucose is the best dose for an OGTT. In the Castro et al (1970) study, they found no significant difference in plasma glucose and insulin levels for males and females after 75 g and 100 g glucose ingestions. In contrast, giving a 50g glucose load resulted in a significant decrease in

plasma glucose and insulin responses, compared to that when the 100 g glucose load was administered. Another study by Torsdottir and Anderson (1989) found that subjects consuming water with their meals had significantly higher plasma glucose and insulin levels due to faster gastric emptying with water, compared to those taking meals without consuming water. The types of sugar used in testing glucose and insulin levels also affect the results (Bohannon et al, 1980).

In order to eliminate factors that can influence the results of OGTT, such as the different levels of glucose load and the volumn of water ingested, the standardized OGTT is set at 75 g of glucose in a total 300 ml volume of water (National Diabetes Data Group, 1979). Other factors that need to be taken into consideration include time of the test (Carroll and Nestel, 1973), rate of the glucose ingestion (Heine et al, 1983), amount of carbohydrate in prior diet (Reiser et al, 1979; Fleming and Shaheen, 1988; Wursch et al, 1988), smoking (Wingerd and Duffy, 1977), and factors mentioned earlier: age, gender, body weight, exercise, sight, smell and/or taste of food, menstrual cycle and oral contraceptives.

Several studies have found a positive relationship between glucose ingestion and the plasma insulin level after a glucose load (Castro et al, 1970; Forster et al, 1972; Bratusch et al, 1980). In a study by Castro et al (1970), 12 healthy, nonobese adult subjects were given oral glucose loads of 50 g, 75 g, 100 g and 1.75 g/kg body weight and changes in plasma glucose and insulin levels were measured. They found that the mean peak increases above fasting levels for plasma glucose and insulin were not significantly different in 4 different glucose loads, and the peaks occurred either within 30 minutes or between 30 to 60 minutes in the 3-hour study. The 50g glucose load

resulted in the smallest plasma glucose and insulin responses, compared to that for the 100 g and 1.75 g/kg glucose loads, but there was a significant difference of plasma glucose and insulin responses (defined as the area under the curve) between the 50 g glucose load and the 100 g and 1.75 g/kg glucose loads. Insulin response increased approximately 35 % when the dose increased from 50 g to 75 g glucose and approximately 27 % for the 75 g to 100 g glucose increment. Increasing the glucose load to 120 g did not further increase insulin response, indicating that maximal insulin response was achieved with the 100 g glucose load. In addition, both the 50 g and 75 g glucose loads resulted in a return of plasma insulin to baseline levels at the end of 3-hour study, but the insulin levels with the other two glucose loads still exceeded fasting levels after three hours. This study also showed a wide range of plasma glucose and insulin levels at every time point and different glucose loads among healthy subjects. This was possibly due to the biological variation among individuals. The study further demonstrated that increasing glucose loads resulted in a more prolonged insulin stimulus over time rather than acute release of a greater amount of insulin at one time.

A study by Bratusch et al (1980) looked at insulin response after different glucose loads (Table 5). Sixteen healthy males ingested different levels of glucose solutions, 12.5, 25, 50, 75, and 100 g glucose, after a 12- to 14-hour overnight fast. Catheters were inserted into a peripheral vein and a right-sided hepatic vein under fluoroscopic control. The insulin concentration in hepatic venous plasma was measured at 15, 30, 45, 60, 90, and 120 minutes. They found that the maximal plasma insulin level occurred at different times depending on the amount of glucose: 15 min (12.5 g and 25 g), 45 min (50 g), 60 min (75 g), and 75 min (100 g). For the groups ingesting 12.5 g and 25 g of glucose, the

insulin level rose moderately. However, for the groups ingesting 50, 75, and 100 g glucose, insulin level increased sixfold to ninefold, indicating that 50-100 g glucose loads enhanced insulin secretion to its maximal amount.

Relationship Between Plasma Insulin Concentration and Alkaline Phosphatase Activity

There are only two studies that have looked at the relation between plasma insulin concentration and alkaline phosphatase (AP) activity (Romero et al, 1988; Ognibene et al, 1997). A study by Romero et al (1988) examined the relation of plasma insulin concentration to changes in AP activity. In this vitro study, they treated BC_3H1 myocytes with insulin and found that insulin stimulated the release of AP from BC_3H1 myocytes into the extracellular medium and rapidly increased AP activity, suggesting that insulin stimulates the release of glycophospholipid-anchored proteins from cell membranes. Another study by Ognibene et al (1997) looked at the relation between intestinal alkaline phosphatase (IAP) activity and insulin secretion in obese people. Serum AP contains a small portion of IAP. Elevated IAP is usually found in people with chronic renal failure (55 %), liver cirrhosis (46 %) and diabetes (54 %) (Ognibene et al, 1997). A study by Goldberg et al (1977) also found an elevated AP activity in 25 % of diabetic people. In the Ognibene et al (1997) study, 76 healthy obese subjects were given a standard OGTT (75 g glucose). Serum insulin levels were higher in the IAP-positive groups (serum contained IAP activity) than in the IAP-negative groups (serum contained no IAP activity). In addition, there was a positive relationship between the serum containing IAP variant activity (IAP+/variant) and insulin secretion during the OGTT. Further studies

Table 5. Effect of glucose ingestion (12.5, 25, 50, 75, and 100 g) on hepatic venous insulin concentration (Bratusch et al, 1980)

		Time (min)						
	N	Basal	15	30	45	60	90	120
Glucose dose		Insulin (μU/ml)						
12.5g	3	11±2	29±8	20±3	16±2	15±4	14±4	21±1
25g	3	14±1	38±12	35±3	32±7	22±8	31±22	17±7
50g	3	11±1	54±17	69±14	70±19	32±7	38±3	38±15
75g	1	19	90	90	112	117	86	65
100g	5	15±2	77±21	91±27	100±28	102±28	135±30	82±22

are needed to understand the relation between plasma insulin concentration and AP activity.

Relationship Between Glucose Ingestion and Plasma B-6 Vitamer Concentration

Previous studies have found an inverse relationship between glucose ingestion and plasma PLP concentration (Leklem and Hollenbeck, 1990; Hofmann et al, 1991). Studies conducted by Leklem and Hollenbeck (1990) looked at the relationship between glucose and plasma PLP concentration in nine subjects (five males and four females). All subjects were in good health, were nonsmokers and did not take vitamin B-6 supplements or any drugs which would affect the results. After a 12-h overnight fast, subjects were given an oral glucose load (1 g/kg body wt.). Plasma PLP and total vitamin B-6 concentrations were measured at 0, 0.5, 1, 2, 3, 4, 5 hours after administering the glucose. There was a continuous drop in plasma PLP concentration through 5 hours in

five subjects and 3-4 hours in the other four subjects. A total of a 18-21 % decrease in plasma PLP concentration from fasting level was seen at 5 hours. A significant mean decrease of 17.6 % plasma PLP was seen at 2 hours. The decrease in plasma PLP concentration was greatest through 3 hours and slowed down after this time. Decreased PLP was paralleled by a decrease in plasma total vitamin B-6, suggesting that a change in total vitamin B-6 was mainly due to the change in plasma PLP. In an exercise study by Hofmann et al (1991), subjects were divided into exercise and non-exercise groups. The non-exercise group was then divided into two groups. One group ingested 200ml glucose polymer (GP) containing 46 g GP and 25 g fructose, while the other group ingested only water. Both groups remained standing throughout the experiment. Plasma PLP concentration decreased 13% over the 3 hour period after ingestion of glucose polymer in the non-exercise group. Plasma PL concentration also tended to decrease in the glucose polymer group. However, this change in plasma PL concentration was small and not significant. In a study by Parker et al (1979), dogs were divided into two groups. One group was administered 15g glucose/ kg body weight, and the other group was administered nothing. Two hours later, 1 mg PL/ kg or 2.5 mg PLP/kg body weight was administered intravenously to both groups. They found a small but statistically significant increase ($p<0.01$) in plasma PLP concentration at 2 hour (32.19 ± 3.14 ng/ml) after glucose ingestion and before the iv administration of PL or PLP, compared to 0 time baseline value (28.28 ± 3.24 ng/ml). In the group administered nothing, plasma PLP concentration was 14.39 ± 2.13 ng/ml at time 0 and 16.96 ± 2.58 ng/ml two hours later ($p>0.05$). The reason for the increase in plasma PLP concentration in both groups was unknown. There was no significant difference in net plasma PLP accumulation in both

groups after the iv administration of 1 mg PL/ kg body weight. However, there was a significant decrease in the half-life of plasma PLP in the glucose group compared with the control group after the iv administration of 2.5 mg PLP/ kg body weight. The reason is probably because of the increase in volume of distribution (V_d) in the control group. Since the experimental design in this study was different from that of the previous glucose-plasma PLP studies, it is difficult to compare the data with the previous studies.

Based on these few studies, plasma PLP concentration appears to be decreased under the condition of an acute increase in carbohydrate intake. Several studies have found that people with diabetes tend to have lower vitamin B-6 status compared to non-diabetic people (Davis et al, 1976; Hollenbeck et al, 1983; Leklem et al, 1985; Hollenbeck et al, 1985). Davis et al (1976) conducted a study of a large sample of people without and with diabetes (n=518) on the differences of plasma PLP and folate concentration. Dietary vitamin B-6 intake was not reported in this study, but serum folate concentration was measured and reported adequate for all except 20 subjects. The result showed that people with diabetes had lower mean plasma PLP values (males: 32.3 nmol/L; females: 26.6 nmol/L), compared to the mean plasma PLP values in the normal controls (males: 50.1 nmol/L; females: 42.4 nmol/L), indicating that diabetic people had lower plasma PLP values than the people without diabetes. This study is further supported by Leklem et al (1985). A total of 85 subjects participated in this study. Forty-two subjects were non-vitamin users, aged 70 ± 6 years. By looking at only the non-vitamin users, mean plasma PLP values were lower in those with non-insulin dependent diabetes mellitus (NIDDM) (25 ± 14 nmol/L) compared to the controls (33 ± 20 nmol/L).

Another study by Hollenbeck et al (1983) randomly assigned six women with insulin dependent diabetes mellitus (IDDM) aged 26 $\pm$ 4 years to begin either a control diet (45 % carbohydrate, 40 % fat) for four weeks or a high carbohydrate, low fat experimental diet (65 % carbohydrate, 20 % fat) for six weeks. Subjects completed the two dietary periods in a cross-over experimental design. All nutrients, including vitamin B-6 (2.0 mg/day) in both control and experimental diets, met the 1980 recommended dietary allowance (RDA). Mean plasma PLP values during the control diet and the experimental diet were 30 $\pm$ 15 nmol/L and 30 $\pm$ 17 nmol/L, respectively. The normal laboratory value of plasma PLP is >30 nmol/L (Leklem, 1990). Four of six subjects had plasma PLP concentrations lower than the normal plasma PLP value, indicating a lower vitamin B-6 status in diabetic people under the condition of adequate vitamin B-6 intake. From these studies (Hollenbeck et al, 1983; Davis et al, 1976; Leklem et al, 1985), plasma PLP concentration in diabetic people were found to be either lower than the normal value or the control values. A study by Hollenbeck and Leklem (1985) looked at the changes of plasma PLP concentration during an OGTT in people with NIDDM and normal subjects. There were ten subjects in each group. A significant decrease in plasma PLP was found in both groups during the OGTT, with a greater decrease in controls than in subjects with NIDDM. In addition, they found that subjects with the higher fasting plasma PLP values had the greatest decrease in plasma PLP during the OGTT. Furthermore, the subjects with NIDDM had the lower fasting plasma PLP values than the controls. Typically, there is a high concentration of blood glucose (>10mmol/ L) level in diabetic people either chronically or during transmittent periods (Davis, 1976), and people with diabetes have depressed plasma PLP and PL concentrations. Based on the

above mentioned studies, with an adequate intake of vitamin B-6 and a long period of high plasma glucose concentration, a decrease in plasma PLP concentration is observed.

Effect of Glucose Ingestion on Transport of Pyridoxal and Pyridoxine

Ingestion of high carbohydrate causes a decrease in plasma PLP concentration. Possible places plasma PLP could go are the liver, muscle, red blood cells (RBC), or other tissues. Liver is unlikely to be the place where PLP goes, since PLP is not needed there (Leklem, 1985). In the previous glucose-plasma PLP studies, Leklem (1990) addressed two possible mechanisms of decreased plasma PLP concentration after glucose ingestion. One possibility is that liver reduces PLP synthesis or inhibits PLP release to the circulation. Another possibility, which has more support, is the uptake of PL (PLP converted to PL) by specific tissues. Several studies have demonstrated a rapid uptake of PL and PN by RBC (Anderson, 1980; Mehansho and Henderson, 1980; Fonda and Harker, 1982; Ink et al, 1982; Ink and Henderson, 1984; Anderson et al, 1989). In addition, human RBC have all the enzymes required for interconversions of different forms of vitamin B-6 (Anderson et al, 1971; Lumeng and Li, 1974) and glucose that provides ATP, which is necessary in the pyridoxal kinase reaction (the enzyme that converts PL to PLP). Therefore, an increase in glucose may enhance the conversion of PL to PLP in RBC. PLP is then trapped in the cells. An in vitro study by Yamada et al (1968) investigated the effect of glucose on the transport of vitamin B-6 into both RBC and ascites sarcoma cells. They found a twofold increment of total vitamin B-6 accumulation in ascites sarcoma cells after 60 minutes of glucose (concentration

unknown) incubation. Of the free forms of vitamin B-6, PL was rapidly transported, and the amount transported was considerably higher compared to that for PN and PM. Much smaller amounts of the phosphorylated forms of vitamin B-6 were transported into RBC, since the phosphorylated compounds do not cross the cell membranes. In addition, they found that once PL and PN were in the cells, they were rapidly converted to the phosphorylated forms. In another in vitro study, Ink et al (1982) looked at the effect of two levels of glucose concentration (5 mmol/L, 10 mmol/L) on the uptake of PL by human RBC in the incubation medium. They found a slightly increased uptake of PL by RBC during 60 minutes of 10 mmol/L (180 mg/100ml) glucose incubation, compared to the 5 mmol/L (90 mg/100ml) glucose concentration and the control. They suggested that increased uptake of PL by RBC may be due to the intracellular binding of PL to hemoglobin.

MATERIALS AND METHODS

Subject Selection

The study was approved by the Oregon State University Committee for the Protection of Human Subjects (see Appendix B). The sample size was determined by using a power calculation (Hall, 1983) based on changes in plasma PLP seen in a previous study (Leklem and Hollenbeck, 1990). Advertisement listing basic selection criteria was displayed on bulletin boards on campus and through e-mail. To eliminate individuals who may have glucose intolerance, pre-selection criteria for both males and females was set (a) age of 20 to 40 years; (b) body weight within $\pm$ 20 % of ideal body weight; (c) non-smoker; (d) moderate or light physical activity; (e) self-report of not taking drugs, vitamin supplements or oral contraceptives at least six weeks prior to the experiment; (f) in good health. Potential subjects who met pre-selection criteria were confirmed through an informal telephone interview.

A total of eight healthy subjects (four of each gender) were recruited. Four subjects were Asian, and four were Caucasians. A formal interview was set up. Subjects were asked to complete a health history questionnaire (see Appendix B). Upon acceptance into the study, an informed consent was obtained from each subject (see Appendix B). Body weight and height were measured at each meeting. Each subject's health condition was assessed based on the health history questionnaire and other criteria. All subjects were acceptable for the study.

Evaluation of Sweetness of the Control Solution

One month prior to the study, six volunteers in the department of Nutrition and Food Management participated in the evaluation of a control solution (water with artificial sweetener equivalent to 25 g glucose). Four different kinds of solutions (water, water with artificial sweetener (1 packet of artificial sweetener = 2 teaspoons of sugar = 10 g glucose) (17 mg/ml), 25 g glucose (170 mg/ml) and 75 g glucose (500 mg/ml)) and an evaluation form were prepared for each participant. Water with artificial sweetener was made by measuring the amount of artificial sweetener required for an equivalent of 25 g glucose. A small amount of concentrate lemon juice (~1.0 ml) was added for flavoring. Five milliliters of each solution was transferred to small cups and randomly placed on each participant's tray. Participants were then asked to taste the solutions and evaluate the degree of sweetness by circling a number from 0 to 10 on a scale (non sweet to extremely sweet). A cup of tap water was also provided for each participant to rinse the mouth before tasting the next solution. Using the data obtained, it was concluded that the amount of artificial sweetener used in the water solution had the sweetness closest to the 25g glucose. Therefore, this amount of sweetener was used for the control solution.

Sampling

Each subject had 7 ml of blood drawn to measure fasting plasma glucose and PLP as screening criteria two weeks prior to study. Fasting plasma glucose and PLP levels for each subject were within normal ranges (Young, 1987; Leklem, 1990, respectively) for all subjects, except that two subjects (#6 and #8) had fasting plasma PLP concentration

slightly below the normal ranges. A 3-day dietary record (see Appendix B) was completed at least ten days prior to the study by each subject to evaluate nutrient intake, especially vitamin B-6. A form for recording the 3-day dietary record and a detailed instruction for recording food were provided for each subject. Food models were also shown to help subjects better estimate portion sizes of food before starting their dietary record. In addition, subjects were encouraged to bring either food labels or recipes for better nutrient intake assessment. During the study, participants were asked to maintain their usual diets. They were also asked to complete one more 3-day dietary record the week before trial one, two or three. Furthermore, at least three days prior to each test, a diet of more than 200 g carbohydrate/day was required to ensure proper utilization of glucose load. Each subject was also asked to maintain his or her body weight and usual level of physical activity during the study.

Subjects were tested on three separate occasions over a period of 3 weeks. At one of the three occasions, subjects ingested the assigned solutions (300 ml water with artificial sweetner (equivalent to 25 g glucose based on taste), 25g glucose in 300 ml water, or 75 g glucose in 300 ml water) in a randomized order. An intake of no more than 250 ml water two hours prior to experiment was permitted, and no exercise was allowed 24 hours prior to experiment. All subjects fasted overnight (10-12 hours). A blood sample was collected at 8:00am in the morning. Then, each subject was given the assigned solution on that particular day. After subjects ingested the solution, the cup was rinsed with an additional 150 ml of tap water to make a total volume of 300 ml. Fourteen milliliters of blood was drawn from the subject's forearm at time 0, 1, 2, and 3 hours. All

subjects remained seated during each test. Body weight and height were measured at the beginning of each test. Meals were provided at the end of the experiment.

Preparation of Glucose Solution

The 25 g glucose solution (25 g/150 ml) was prepared by dissolving 200 g glucose in 1200 ml water, and 75 g glucose solution (75 g/150 ml) was prepared by dissolving 600 g glucose in 1200 ml water. Water was first warmed up to 30-35 °C, and the appropriate amount of glucose was then slowly added until it dissolved. The solution was taken up to 1200 ml and then transferred to several glass jars, labeled and frozen for later use. The night before the experiment, the frozen solution was taken out and thawed at room temperature. The following morning, 150 ml portions of assigned solutions for eight subjects were measured, and 5 ml of concentrate lemon juice was added to each for flavoring. The process for water solution was the same, except it was made on the day of the experiment.

Methods

All blood samples were collected in heparinized tubes. Whole blood was used for hemoglobin and hematocrit analysis. The remaining blood was placed on ice for no more than 20 minutes until centrifuged for 15 minutes at 2100x g at 4 °C. Plasma was separated and stored in freezer vials at –40 °C. The remaining red blood cells were washed three times with 5 ml of normal saline (0.9 %) and stored at –80 °C until analysis. All samples were analyzed in duplicate.

Plasma pyridoxal phosphate (PLP), pyridoxal (PL), pyridoxine (PN) and 4-pyridoxic acid (4-PA) were measured by HPLC based on a modified method of Sharma and Dakshinamurti (1992). The equipment for HPLC consisted of a Shimadzu SCL-10H controller, two LC-10AD pumps, a 250 µl injection loop, a Rainin #86200E3 C18 3U 4.6x100 ion-pair analytical column, a RF-10A spectrofluorometric detector, and a CR501 recorder/integrator. Reagents used for the first and second mobile phase were 0.033 M phosphate with 8mM octane sulfonic acid, pH=2.3 (Solvent A) and 0.033 M phosphate with 18 % (v/v) isopropanol, pH=2.3 (Solvent B), respectively. The pump rate was 1.0 ml/min. The reagent for the post column was 1.0 g/L sodium bisulfite in 1M KH_2PO_4, pH=7.5. The pump rate for the post column was 0.1 ml/min. The spectrofluorometric detector was set at an excitation wavelength of 330 nm and an emission wavelength of 400 nm. All samples were prepared under yellow light to minimize photodegradation of vitamin B-6. Plasma sample (0.5 ml) was mixed with 0.3 ml nanopure water, 0.1 ml 100 % trichloroacetic acid (TCA) and 0.1 ml 1 mM deoxypyridoxine (DPN). The solution was mixed and was then centrifuged by using a micro centrifuge to precipitate protein. The supernatant (protein-free solution) was filtered with a 4 mm nylon syringe filter (0.2 µ). A standard solution (0.05 µM) of PLP, 4-PA, PL and PN and a control plasma sample were analyzed in each daily assay. The mean plasma PLP concentration and the interassay coefficient of variation (CV) for the control sample were 93.0 ± 7.6 nmol/L and 8.8 %, respectively (n=14).

Red blood cell PLP was measured by a tyrosine apodecarboxylase method (Chabner and Livingston, 1970). The method is based on the conversion of 1-^{14}C-L-tyrosine to tyramine and $^{14}CO_2$ by tyrosine decarboxylase, a PLP dependent enzyme.

The red blood cell (RBC) sample was thawed and kept in a 0 °C ice water bath. Two milliliter of water and 0.25 ml of RBC sample were briefly mixed together. Then, 0.5ml 75 % TCA was added to deproteinize the red cell solution, and the tubes were set in the dark at room temperature for one hour. TCA was later removed by extracting the red cell solution with 8ml water-saturated ethyl ether three times. A control sample was analyzed in every assay. The mean red blood cell PLP concentration and the interassay coefficient of variation (CV) for the control sample were 121 ± 7.9 nmol/L and 6.5 %, respectively (n=12). The mean recovery of red blood cell PLP for the control sample was 69.9 % ± 14.37 %.

Plasma glucose was determined by a glucose oxidase method (Trinder, 1969). The enzyme glucose oxidase converts glucose to gluconic acid and hydrogen peroxide (H_2O_2). H_2O_2 is then reacted with 4-aminoantipyrine and phenol in the presence of peroxidase and produces a red dye and H_2O. The intensity of the red dye is measured photometrically at 550 nm. All samples were analyzed on the Alpkem Autoanalyzer II System. The mean plasma glucose concentration and the intraassay CV for the control sample were 4.49 ± 0.21 mmol/L and 4.7 %, respectively (n=8).

Plasma insulin was determined by an insulin-antibody immunoassay method (Hales and Randle, 1963). A double antibody radioimmunoassay kit (Coat-A-Count; Diagnostic Products Corporation) was used to measure plasma insulin concentration. The amount of bound ^{125}I-labeled insulin was then measured in a Packard 5230 Auto-Gamma Scintillation Spectrometer. The mean plasma insulin concentration and the intraassay CV for the control sample were 26.25 ± 0.35 μU/ml and 1.3 %, respectively (n=2).

Plasma alkaline phosphatase (AP) activity was determined by a colormetric method (Roy, 1970). The principle of the method is based on the reaction of plasma AP with thymolpthaline monophosphate. A control plasma was run each time at the end of each trial. The mean plasma AP activity and the intraassay CV for the control sample were 15.5 ± 1.2 U/L and 7.9 %, respectively (n=9).

Whole blood was used to analyze hemoglobin (Hgb) and hematocrit (Hct). Hemoglobin was determined by cyanomethemoglobin method, and hematocrit was determined by microhematocrit method. All samples were analyzed in duplicate.

Statistical Analysis

All data were analyzed by using the JMP Start Statistics (Sall and Lehman, 1996) program. A paired t-test (two tailed) was used to determine if there was a difference at two time points (time 1, 2, and 3 from time 0) for each dependent variable (plasma glucose, insulin, AP, PLP, PL, 4PA, PN, and red blood cell PLP). Analysis of variance (ANOVA) was used to determine if differences occurred over time within and among gender and /or solution for each variable. The comparisons included gender, solution, gender x solution, time, gender x time, solution x time, gender x solution x time. Simple linear regression was used to calculate correlation coefficient between variables. Means and standard deviations (mean $\pm$ SD) were calculated from the individual data for each variable. Differences were considered to be statistically significant at the $P<0.05$ level.

RESULTS

Characteristics of Subjects

The characteristics of the subjects are given in Table 6. The body weight for each subject was consistent during the 3-week study. Hemoglobin and hematocrit values were within the normal ranges (Table 7). There were no significant differences in the mean fasting plasma glucose and PLP concentrations between males and females prior to and during the three test periods (Table 8). The mean fasting plasma glucose concentration prior to the study was significantly lower than that in the first ($P<0.04$) and third ($P<0.02$) experimental periods. The mean fasting plasma PLP concentration in the second experimental period (33.2 ± 12.3 nmol/L) was significantly lower than that in the third period (44.8 ± 22.4 nmol/L) ($P<0.05$). All subjects had normal fasting plasma glucose and PLP values prior to and during the three test periods, except that one male subject #8 (prior: 23.52 nmol/L; water solution: 27.10 nmol/L) and one female subject #6 (prior: 20.16 nmol/L; 25g glucose: 22.50 nmol/L) had fasting plasma PLP values less than 30 nmol/L.

Summaries of individual's dietary records of protein, vitamin B-6, and vitamin B-6: protein ratio prior to the study and during the three week experimental periods are shown in Table 9. There were no significant differences in the mean intakes of calories, carbohydrate, protein and vitamin B-6 between the two dietary records for both males and females. The mean protein (M 84 g; F 64 g)and vitamin B-6 (M 1.69 mg; F 1.49 mg) intakes met current recommended dietary allowances (RDAs) (National Research Council, 1999). However, there was no significant correlation between mean vitamin B-

Table 6. Subject characteristics

Subject	Gender	Age (yr)	Weight (kg)	Height (cm)
1	F	28	58	167
2	M	28	70	179
4	F	28	59	173
5	F	34	46	158
6	F	37	52	170
7	M	28	75	182
8	M	28	79	183
X± SD	M	28±0.0	74.7±4.5	181±2
X± SD	F	31.8±4.5	53.8 ±6.0	167±6

Table 7. Mean fasting hemoglobin and hematocrit prior to the study and on the three experimental days

	Prior	Trial 1	Trial 2	Trial 3
Hemoglobin (g/L)				
M	158±11	163±5	ND	168±17
F	150±10	145±2	ND	137±2
Hematocrit (%)				
M	44.3±5.4	47.5±1.5	ND	47.6±3.8
F	45.4±1.3	41.5±0.6	ND	39.4±0.4

X± SD
ND: not determined
Prior: 10 days before the first experiment
Trial 1: day 1 of experiment
Trial 2: day 8 of experiment
Trial 3: day 15 of experiment

Table 8. Mean fasting plasma glucose and PLP concentrations prior to the study and on the three experimental days

	Prior	Trial 1	Trial 2	Trial 3
Plasma glucose (mmol/L)	4.4 ± 0.3	5.0 ± 0.6[a]	4.6 ± 0.3	4.8 ± 0.3[a]
Plasma PLP (nmol/L)	43.5 ± 18.9	37.1 ± 18.3	33.2 ± 12.3	44.8 ± 22.4[b]

X ± SD
Prior: 10 days before the first experiment
Trial 1: day 1 of experiment
Trial 2: day 8 of experiment
Trial 3: day 15 of experiment
a: significantly different from Prior, $p<0.05$
b: significantly different from Trial 2, $p<0.05$

6 intake and mean fasting plasma PLP value, or vitamin B-6: protein ratio and mean fasting plasma PLP value prior to and during the study for all subjects.

Plasma Glucose

The mean plasma glucose concentrations at one hour (T1), two hours (T2), and three hours (T3) from time zero (T0) after water (with artificial sweetener equivalent to 25 g glucose), 25 g glucose and 75 g glucose ingestion are given in Table 10. There were overall significant gender ($P<0.03$) and time ($P<0.0003$) effects and time by solution ($P<0.0004$) interactions. A significant decrease in the mean plasma glucose concentration was observed at T2 and T3 from T0 (-1.0 ± 0.2 mmol/L; -0.6 ± 0.2 mmol/L, respectively) with 25 g glucose load for males (Table 11), and at T2 from T0 (-

Table 9. Individual dietary intake of protein, vitamin B-6, and the vitamin B-6:protein ratio prior to and during the study

Subject	Gender	Protein (g) Prior	During	Vit B-6 (mg) Prior	During	B-6:Pro (mg/g) Prior	During
1	F	103.2	80.2	1.60	1.98	0.016	0.025
2	M	78.4	60.7	2.23	1.35	0.028	0.022
4	F	76.0	59.6	1.18	1.11	0.016	0.019
5	F	67.5	49.3	1.35	2.46	0.020	0.050
6	F	35.0	42.4	1.01	1.25	0.029	0.029
7	M	99.4	81.9	1.66	1.97	0.017	0.024
8	M	89.0	93.3	1.42	1.49	0.016	0.016
X ± SD	M	88.9± 10.5	78.6± 16.5	1.77± 0.42	1.60± 0.33	0.020± 0.007	0.021± 0.004
X ± SD	F	70.4± 28.1	57.9± 16.5	1.29± 0.25	1.70± 0.63	0.020± 0.006	0.031± 0.013
X ± SD	ALL	78.4± 23.0	66.8± 18.7	1.49± 0.40	1.66± 0.49	0.020± 0.006	0.026± 0.011

Prior: 10 days before the first experiment
During: during the three-week experimental periods (day1 to day15)

Table 10. Mean plasma glucose concentrations after water, 25g and 75g glucose ingestion

	N	T0	T1	T2	T3
Glucose (mmol/L)					
H2O					
Males	3	5.0 ± 0.1	4.6 ± 0.2	4.8 ± 0.2	4.9 ± 0.2
Females	4	4.6 ± 0.3	4.5 ± 0.4	4.6 ± 0.3	4.7 ± 0.3
25g glucose					
Males	3	5.3 ± 0.6	5.9 ± 2.2	4.3 ± 0.4*	4.7 ± 0.4*
Females	4	4.7 ± 0.5	4.9 ± 1.5	4.1 ± 0.3*	4.5 ± 0.3
75g glucose					
Males	3	4.7 ± 0.4	6.0 ± 0.8	5.1 ± 1.3	3.3 ± 1.3
Females	4	4.6 ± 0.3	5.5 ± 0.4*	4.3 ± 0.6	3.8 ± 1.2

X ± SD
T0: time zero hour; T1: time one hour; T2: time two hour; T3: time three hour
*Significantly different from the zero-time value, $p<0.05$ (paired t-test)

Table 11. Change in mean plasma glucose concentrations from fasting values after water, 25g and 75g glucose ingestion

	N	T1-T0	T2-T0	T3-T0
Glucose (mmol/L)				
H2O				
Males	3	-0.4 ± 0.2	-0.2 ± 0.2	-0.1 ± 0.3
Females	4	-0.1 ± 0.1	-0.0 ± 0.2	0.1 ± 0.2
25g glucose				
Males	3	0.6 ± 1.8	-1.0 ± 0.2	-0.6 ± 0.2
Females	4	0.2 ± 1.1	-0.7 ± 0.4	-0.2 ± 0.5
75g glucose				
Males	3	1.3 ± 0.8	0.4 ± 0.9	-1.4 ± 1.0
Females	4	0.9 ± 0.5	-0.2 ± 0.5	-0.8 ± 1.3

X ± SD
The change represents the difference from the zero-time (fasting) value

0.7 ± 0.4 mmol/L) for females (P<0.05). With the 75 g glucose load, the mean plasma glucose concentration significantly increased from T0 to T1 (0.9 ± 0.5 mmol/L) for females only. An overall mean increase at T1 for the 25 g and 75 g glucose loads was 11 % and 27 % for the males and 3 % and 19 % for the females, respectively. Of the three solutions, the 75 g glucose load resulted in the greatest magnitude of change over time in the mean plasma glucose concentration. The water solution had the lowest change at all time points. Four out of seven subjects showed stable plasma glucose concentration over time for the water solution. Mean pre-dose fasting plasma glucose values were similar for all three trials for both male and female subjects.

Both genders showed a trend for an increase at T1, a decrease below the baseline values at T2 and then an increase at T3 in the mean plasma glucose concentration for the 25 g glucose load, while the 75 g glucose load showed a further decrease at T3. Compared to changes seen with the 25 g glucose load, the changes in the mean plasma glucose concentration at T1, T2 and T3 from T0 in the 75 g glucose load were higher, but this difference was not statistically significant.

Plasma Insulin

Table 12 gives the mean plasma insulin concentrations observed for the three solutions. There were overall significant gender (P<0.001), solution (P<0.0001) and time (P<0.0001) effects and time by solution (P<0.0001) interaction for plasma insulin values between the 25 g and 75 g glucose loads. The data for the water solution were excluded in the above analysis because plasma insulin concentration at T1 and T2 with the water

Table 12. Mean plasma insulin concentrations after water, 25g and 75g glucose ingestion

	N	T0	T1	T2	T3
Insulin (μU/ml)					
H2O					
Males	3	10.2 ± 1.6[a]	ND	ND	8.6 ± 0.7[a]
Females	4	7.9 ± 2.6	ND	ND	5.4 ± 1.5*
25g glucose					
Males	3	9.8 ± 1.3	31.3 ± 5.5*[a]	9.4 ± 2.3	8.7 ± 1.1*[a]
Females	4	6.5 ± 1.2	19.4 ± 10.8	4.8 ± 0.6	4.9 ± 0.8
75g glucose					
Males	3	8.6 ± 1.0	46.3 ± 7.2*	53.3 ± 15.1*	15.8 ± 12.2
Females	4	6.9 ± 2.7	41.8 ± 6.7*	26.6 ± 8.0*	13.0 ± 10.4

X ± SD
T0: time zero hour; T1: time one hour; T2: time two hour; T3: time three hour
ND: not determined
*Significantly different from the zero-time value, $p<0.05$ (paired t-test)
a: male value significantly higher than female value, $p<0.05$

solution was not measured. Compared to values for females, males had significantly higher mean plasma insulin concentration with three solutions ($P<0.05$). An increase in mean plasma insulin concentration at T1 was observed with both glucose loads for both males and females. Both genders given the 25 g glucose load showed an increase in the mean plasma insulin concentration at T1, a decrease at T2, and no further change at T3. The mean plasma insulin concentration for males with the 75 g glucose load increased at T1, continued to increase at T2 and then decreased at T3, while females had an increase at T1, a decrease at T2 and a further decrease at T3. With the water solution, a significant decrease was observed at T3 from T0 for females ($P<0.05$) (Table 13), but not for males. With the 25 g glucose load, males had a significant increase in plasma insulin concentration at T1 from T0 and a significant decrease at T3 from T0. The 75 g glucose

Table 13. Change in mean plasma insulin concentrations from fasting values after water, 25g and 75g glucose ingestion

	N	T1-T0	T2-T0	T3-T0
Insulin (μU/ml)				
H2O				
Males	3	ND	ND	-1.6 ± 1.1
Females	4	ND	ND	-2.5 ± 1.5
25g glucose				
Males	3	21.6 ± 6.7	-0.4 ± 1.8	-1.1 ± 0.3
Females	4	12.9 ± 10.4	-1.7 ± 1.5	-1.6 ± 1.4
75g glucose				
Males	3	37.7 ± 7.6	44.7 ± 14.2	7.2 ± 11.8
Females	4	34.9 ± 7.6	19.7 ± 7.9	6.1 ± 10.8

X ± SD
ND: not determined
The change represents the difference from the zero-time (fasting) value

load for both males and females resulted in a mean plasma insulin concentration that was significantly increased from T0 to T1 and significantly decreased from T0 to T2. Of the three solutions, the 75 g glucose load for both genders had the greatest magnitude of changes over time in the mean plasma insulin concentration. The mean insulin peak for both genders with the 25 g glucose load and for females given the 75 g glucose load occurred at T1, while for males given the 75 g glucose load, the insulin mean peak occurred at T2. An overall mean increase of 21.6 ± 6.7 μU/ml and 44.7 ± 14.2 μU/ml were observed with the 25 g and 75 g glucose loads, respectively, for males, and 12.9 ± 10.4 μU/ml and 34.9 ± 7.6 μU/ml, respectively, for females. The changes in the mean plasma insulin concentration from T0 to T2 with the 75 g glucose load were

significantly different from those with the 25 g glucose load for both males and females (M $P<0.05$; F $P<0.02$).

Plasma Alkaline Phosphatase Activity

The mean plasma alkaline phosphatase (AP) activity values at all time points are shown in Table 14. Males had a significantly higher mean plasma AP activity at all time points for the three solutions compared to those of females ($P<0.0001$). No significant differences in the mean plasma AP activity were observed at T1, T2, and T3 from T0 for the three solutions for both males and females. All subjects given the three solutions displayed consistent plasma AP activity at all time points.

Plasma Pyridoxal 5′-Phosphate

Table 15 lists the mean plasma pyridoxal 5′-phosphate (PLP) concentrations at all time points after the water solution, and the 25 g and 75 g glucose loads. There were overall significant gender, solution and time effects and solution by gender interaction for plasma PLP values ($P<0.0001$). Mean fasting plasma PLP values were not significantly different between males and females for the three solutions. Males had significantly higher plasma PLP concentrations over time with the water solution and 25 g glucose load than those of females ($P<0.0001$). The mean fasting plasma PLP values for both genders with the 25 g glucose load were coincidentally lower (M 43.7 nmol/L; F 36.1 nmol/L) than the values with the water solution (M 53.1 nmol/L; F 37.0 nmol/L) and 75g

Table 14. Mean plasma alkaline phosphatase (AP) activity after water, 25g and 75g glucose ingestion

	N	T0	T1	T2	T3
AP (U/L)					
H2O					
Males	3	25.9 ± 10.3	25.5 ± 9.5	25.6 ± 10.6	25.6 ± 10.4
Females	4	18.2 ± 3.7	17.9 ± 3.6	18.1 ± 3.4	18.6 ± 3.1
25g glucose					
Males	3	25.9 ± 11.3	25.4 ± 9.7	27.7 ± 12.6	26.5 ± 11.0
Females	4	18.5 ± 2.8	18.1 ± 3.0	18.2 ± 3.1	18.1 ± 2.8
75g glucose					
Males	3	25.7 ± 9.0	25.6 ± 9.3	25.5 ± 9.2	25.7 ± 9.3
Females	4	19.9 ± 6.0	19.0 ± 5.3	19.3 ± 5.4	19.3 ± 4.2

X ± SD
T0: time zero hour; T1: time one hour; T2: time two hour; T3: time three hour

Table 15. Mean plasma pyridoxal 5′-phosphate (PLP) concentration after water, 25g and 75g glucose ingestion

	N	T0	T1	T2	T3
HPLC					
PLP (nmol/L)					
H2O					
Males	3	53.1 ± 29.3	48.4 ± 28.0	45.4 ± 24.4	43.0 ± 26.0*
Females	4	37.0 ± 6.0	33.1 ± 8.2	31.2 ± 7.2*	33.2 ± 2.8
25g glucose					
Males	3	43.7 ± 20.0	37.2 ± 16.2	34.3 ± 14.7	37.9 ± 19.1*
Females	4	36.1 ± 11.3	32.9 ± 10.9	32.3 ± 11.1	30.0 ± 10.3*
75g glucose					
Males	3	48.6 ± 23.2	43.1 ± 23.2	37.9 ± 18.1	39.2 ± 21.8*
Females	4	47.7 ± 13.0	40.9 ± 13.9	37.8 ± 13.6*	40.3 ± 10.7

X ± SD
T0: time zero hour; T1: time one hour; T2: time two hour; T3: time three hour
*Significantly different from the zero-time value, $p<0.05$ (paired t-test)

glucose load (M 48.6 nmol/L; F 47.7 nmol/L). An overall decrease in the mean plasma PLP concentration was observed with all three solutions for both males and females. A significant decrease in the mean plasma PLP concentration was observed at T3 from T0 for males and at T2 from T0 for females for both the water solution and the 75 g glucose load ($P<0.05$). With the 25 g glucose load, both males and females had a significant decrease in the mean plasma PLP concentration at T3 from T0 ($P<0.05$). With the three solutions, both genders showed a drop in the mean plasma PLP concentration at T1, a continuous decline at T2, and then an increase at T3. The males given the water solution had a further drop at T3. Of the three solutions, the 75 g glucose load for both genders resulted in the greatest decrease over time in the mean plasma PLP concentration. Subjects with the highest fasting plasma PLP values tended to have more of a decline in plasma PLP concentration than subjects with lower fasting plasma PLP values. However, no significant correlation was found between the fasting plasma PLP values and the changes in plasma PLP values (T3-T0) for all three solutions.

The range of decrease over time in the mean plasma PLP concentration for males with the water solution, 25 g and 75 g glucose loads were from -4.8 to -10.1 nmol/L, -6.5 to -9.4 nmol/L, and –5.0 to -10.7 nmol/L, respectively, and for females were from -3.9 to -5.8 nmol/L, -3.2 to -6.1 nmol/L, and -6.9 to -9.9 nmol/L, respectively (Table 16). These changes represented an overall mean decrease of 19 %, 13 %, and 20 % , respectively, in the mean plasma PLP concentration at T3 from T0 for males. The respective changes for females were 10 %, 17 %, and 15 % . Due to the small sample size and large standard deviation, the subjects did not show a significant difference in decreased levels between the different solutions as expected. In terms of the responses to the three solutions,

Table 16. Change in mean plasma pyridoxal 5′-phosphate (PLP) concentrations from fasting values after water, 25g and 75g glucose ingestion

	N	T1-T0	T2-T0	T3-T0
HPLC				
PLP (nmol/L)				
H2O				
Males	3	-4.8 ± 3.0	-7.7 ± 5.0	-10.1 ± 3.3
Females	4	-3.9 ± 4.0	-5.8 ± 3.6	-3.8 ± 5.9
25g glucose				
Males	3	-6.5 ± 4.1	-9.4 ± 5.6	-5.8 ± 1.0
Females	4	-3.2 ± 3.3	-3.8 ± 3.0	-6.1 ± 1.6
75g glucose				
Males	3	-5.0 ± 4.4	-10.7 ± 5.1	-9.4 ± 1.5
Females	4	-6.9 ± 4.7	-9.9 ± 3.3	-7.4 ± 5.3

X ± SD
The change represents the difference from the zero-time (fasting) values.

female subjects generally showed a larger but non-significant change in mean plasma PLP concentration over time than that for the males. For the female subjects, the 25 g glucose load showed smaller changes at each time point than the changes with the 75 g glucose load, but the differences were not significant. Males given the 25 g glucose load also showed a smaller but not significant changes in plasma PLP concentration over time compared to those with the 75 g glucose load, except that at T3 from T0, males given the 75 g glucose load showed significantly greater changes than when the 25 g glucose load was given ($P<0.04$).

Plasma Pyridoxal

Table 17 shows the mean plasma pyridoxal (PL) concentrations at all time points. There were overall significant solution by gender ($P<0.01$) interaction for plasma PL values. There were also significant gender differences with the 75 g glucose load ($P<0.03$). Mean fasting plasma PL values were similar for both male and female subjects with the three solutions. A significant increase in the mean plasma PL concentration was observed from T0 (10.2 ± 3.1 nmol/L) to T1 (15.1 ± 5.7 nmol/L) for the water solution, and a significant decrease from T0 (11.2 ± 2.4 nmol/L) to T3 (7.8 ± 2.7 nmol/L) with the 75 g glucose load for males ($P<0.05$). In contrast to plasma PLP, there was an increase in plasma PL at T1 for both males and females given the water solution and 75 g glucose load. However, due to the large standard deviation, the change at T1 for both genders with the 75 g glucose load was not significant. Mean plasma PL concentration with the 25 g glucose load was relatively constant over time. With the water solution, there was a significant increase at T1 ($P<0.05$), a decrease at T2, and an increase at T3 for all subjects (Table 18). The same trend was observed for the 75 g glucose load, except that there was a further drop at T3 for males ($P<0.05$) and for females. When looking at each individual value, all subjects displayed an increase in the plasma PL concentration at either T1 or T2 after the 75 g glucose load. Five of the seven subjects had a range of increase of 20-57 % (2 nmol/L to 14 nmol/L) in the plasma PL concentration after the 75 g glucose load. One male had a maximal increase of only 5 % (1 nmol/L), while one female had a maximal increase of 190 % (9 nmol/L). Table 18 shows the changes in the mean plasma PL concentration from fasting values after water, 25 g and 75 g glucose ingestions.

Table 17. Mean plasma pyridoxal (PL) concentrations after water, 25g and 75g glucose ingestion

	N	T0	T1	T2	T3
HPLC PL (nmol/L)					
H2O	7	10.2 ± 3.1	15.1 ± 5.7*	10.1 ± 3.4	12.5 ± 2.0
25g glucose	7	11.5 ± 3.8	10.1 ± 3.2	9.1 ± 4.1	10.6 ± 2.6
75g glucose					
Males	3	11.2 ± 2.4	12.8 ± 7.6	10.4 ± 4.2	7.8 ± 2.7*
Females	4	13.1 ± 8.5	18.7 ± 14.1	15.1 ± 2.3	12.7 ± 4.9

X ± SD
T0: time zero hour; T1: time one hour; T2: time two hour; T3: time three hour
*Significantly different from the zero-time value, $p<0.05$ (paired t-test)

Table 18. Change in mean plasma pyridoxal (PL) concentrations from fasting values after water, 25g and 75g glucose ingestion

	N	T1-T0	T2-T0	T3-T0
HPLC PL (nmol/L)				
H2O	7	4.9 ± 4.2	-0.1 ± 3.5	2.3 ± 3.5
25g glucose	7	-0.6 ± 2.3	-1.8 ± 6.4	0.5 ± 2.5
75g glucose				
Males	3	1.6 ± 5.1	-0.8 ± 3.0	-3.5 ± 0.7
Females	4	5.6 ± 5.8	2.0 ± 6.5	-0.4 ± 5.5

X ± SD
The change represents the difference from the zero-time (fasting) value

Plasma 4-Pyridoxic Acid

Table 19 lists the mean plasma 4-pyridoxic acid (4-PA) concentrations. There were overall significant gender, solution and time effects and solution by gender Plasma 4-Pyridoxic Acid interaction for plasma 4-PA values ($P<0.0001$). As was seen for PLP, males had slightly higher plasma 4-PA values at all time points among the three solutions than those of females. However, the differences were not significant. In addition, mean fasting plasma 4-PA values for males given the water solution were coincidentally higher than the fasting values for the 25 g and 75 g glucose loads. A drop in the mean plasma 4-PA concentration at T1 was observed for all three solutions for both males and females. A significant decrease in the mean plasma 4-PA concentration was observed from T0 (13.1 ± 3.2 nmol/L) to T1 (11.5 ± 3.6 nmol/L) and T3 (10.0 ± 2.5 nmol/L) for females given the water solution, and from T0 (13.8 ± 3.4 nmol/L) to T3 (12.7 ± 3.2 nmol/L) for males given the 75 g glucose load ($P<0.05$). When looking at each individual value, the maximal decrease for the 75 g glucose load ranged from 7 to 35 % (1 to 5 nmol/L) mainly at T2 or T3 for all subjects. Table 20 shows the changes in the mean plasma 4-PA concentrations from fasting values after water, 25 g and 75 g glucose ingestion.

Plasma Pyridoxine

Table 21 shows the mean plasma pyridoxine (PN) concentrations. There were overall significant solution ($P<0.05$) effects for plasma PN values. There were also gender differences for the 75 g glucose load ($P<0.04$). Mean fasting plasma PN values

Table 19. Mean plasma 4-pyridoxic acid (4-PA) concentrations after water, 25g and 75g glucose ingestion

	N	T0	T1	T2	T3
HPLC					
4-PA (nmol/L)					
H2O					
Males	3	28.3 ± 15.9	23.2 ± 10.5	24.4 ± 9.8	23.7 ± 11.9
Females	4	13.1 ± 3.2	11.5 ± 3.6*	10.3 ± 2.1	10.0 ± 2.5*
25g glucose					
Males	3	20.6 ± 10.1	15.6 ± 6.2	18.2 ± 4.9	19.3 ± 6.7
Females	4	13.9 ± 2.3	10.3 ± 2.1	11.5 ± 1.6	11.3 ± 2.3
75g glucose					
Males	3	20.0 ± 4.8	18.2 ± 3.7[a]	19.5 ± 6.5	16.8 ± 2.4[a]
Females	4	13.8 ± 3.4	12.0 ± 3.2	12.2 ± 4.2	12.7 ± 3.2*

X ± SD
T0: time zero hour; T1: time one hour; T2: time two hour; T3: time three hour
*Significantly different from the zero-time value, $p<0.05$ (paired t-test)
a: male value significantly higher than female value

were not significantly different for both males and females for the three solutions. No significant differences in the mean plasma PN concentration over time were observed for the three solutions. Four out of seven subjects had an increased in the plasma PN concentration that ranged from 16 % to 69 % (2 nmol/L to 6 nmol/L) at different time points for the 75 g glucose load.

Red Blood Cell Pyridoxal 5′-Phosphate

Table 22 gives the mean red blood cell pyridoxal 5′-phosphate (PLP) concentrations at all time points. There were overall significant gender ($P<0.0001$) and

Table 20. Change in mean plasma 4-pyridoxic acid (4-PA) concentrations from fasting values after water, 25g and 75g glucose ingestion

	N	T1-T0	T2-T0	T3-T0
HPLC				
4-PA (nmol/L)				
H2O				
Males	3	-5.2 ± 5.4	-3.9 ± 6.2	-4.6 ± 4.2
Females	4	-1.6 ± 0.6	-2.9 ± 2.0	-3.1 ± 0.8
25g glucose				
Males	3	-5.0 ± 4.4	-2.4 ± 5.4	-1.3 ± 3.4
Females	4	-3.6 ± 3.1	-2.3 ± 3.2	-2.6 ± 4.2
75g glucose				
Males	3	-1.7 ± 1.4	-0.5 ± 2.3	-3.1 ± 2.4
Females	4	-1.9 ± 2.0	-1.7 ± 1.9	-1.1 ± 0.5

X ± SD
The change represents the difference from the zero-time (fasting) value

Table 21. Mean plasma pyridoxine (PN) concentrations after water, 25g and 75g glucose ingestion

	N	T0	T1	T2	T3
HPLC					
PN (nmol/L)					
H2O	7	10.0 ± 2.3	9.0 ± 1.5	9.2 ± 3.0	10.4 ± 3.1
25g glucose	7	7.5 ± 1.7	10.5 ± 4.2	10.3 ± 3.7	9.0 ± 1.7
75g glucose					
Males	3	10.8 ± 3.7	9.0 ± 2.8	8.9 ± 4.0	10.3 ± 1.6
Females	4	13.3 ± 3.8	13.4 ± 3.8	11.4 ± 3.2	10.2 ± 3.0

X ± SD
T0: time zero hour; T1: time one hour; T2: time two hour; T3: time three hour

Table 22. Mean red blood cell PLP concentrations after water, 25g and 75g glucose ingestion

	N	T0	T1	T2	T3
TDC					
RBC PLP (nmol/L)					
H2O					
Males	3	42.3 ± 13.1	40.8 ± 9.7	39.6 ± 8.7	39.5 ± 9.2
Females	4	44.2 ± 15.5	44.8 ± 12.0	43.4 ± 11.7	46.1 ± 12.1
25g glucose					
Males	3	39.5 ± 8.8	40.8 ± 8.4	40.8 ± 10.9	39.5 ± 6.6
Females	4	43.4 ± 9.8	45.5 ± 8.4	48.6 ± 8.2*	48.8 ± 7.1
75g glucose					
Males	3	44.2 ± 14.6	41.3 ± 8.6	43.0 ± 9.2	47.4 ± 10.7
Females	4	47.2 ± 13.2	46.2 ± 12.3	53.8 ± 11.4	51.6 ± 11.1

X ± SD
T0: time zero hour; T1: time one hour; T2: time two hour; T3: time three hour
*Significantly different from the zero-time value, $p<0.05$ (paired t-test)

solution ($P<0.0002$) effects for red blood cell PLP values. In contrast to plasma PLP concentration, females had significantly higher red blood cell PLP values over time for the three solutions than those of males ($P<0.0001$). Mean fasting red blood cell PLP values were not significantly different for both genders for the three solutions. A significant change in the mean red blood cell PLP concentration over time was observed for the 25 g glucose load for females ($P<0.05$). There was a significant increase from T0 (43.4 ± 9.8 nmol/L) to T2 (48.6 ± 8.2 nmol/L) with the 25 g glucose load for females ($P<0.05$). There was a tendency of an overall non-significant increase with the 75 g glucose load for both males and females. The percent increase in the red blood cell PLP ranged from 6 to 46% (2nmol/L to 14 nmol/L) at either T2 or T3 after the 75 g glucose load.

Correlation Between Variables

There was a negative correlation between plasma glucose and PLP at T1, T2 and T3 with the 25 g glucose load for both genders, and at T1 and T3 with the 75 g glucose load for males. However, a significant correlation was observed only at T2 for females given the 25 g glucose load ($r=-0.9792$, $P<0.05$).

A positive, but non-significant, correlation was observed between plasma insulin and AP activity at all time points for females with the three solutions, except that there was a negative correlation between plasma insulin and AP activity at T1 with the 75 g glucose load. For males, a positive correlation was found only at T1 ($r=0.9979$, $P<0.05$) with the 25 g glucose load, and at T2 and T3 with the 75 g glucose load.

There was a consistent negative, but non-significant, correlation between plasma PLP and AP activity at all time points with the three solutions for both genders. A positive, but non-significant, correlation was observed between plasma PL and AP activity for only females at T1, T2 and T3 with the water solution, and at T3 with the 25 g glucose load. There was also a positive, but non-significant, correlation between plasma PLP and plasma PL at all time points with the three solutions for both genders, except for females at T1 and T3 with the 25 g glucose load. There was a significant positive correlation between plasma PLP and PL at T2 for males given the 75 g glucose load ($r=0.9972$, $P<0.05$). A consistent positive correlation was also observed between plasma PLP and red blood cell PLP at all time points for both genders with the three solutions. However, the only significant correlation between plasma PLP and red blood cell PLP concentration was found at T1 for males given the 75 g glucose load ($r=0.9998$,

P<0.02). A summary of changes in mean plasma PLP, 4PA, PL, glucose, insulin concentrations and plasma AP activity is shown in Table 23.

Table 23. A summary of changes in mean plasma PLP, 4PA, PL, glucose, insulin concentrations and plasma AP activity from fasting values after the water, 25g and 75g glucose ingestion

	PLP			4-PA			PL			
	T1	T2	T3	T1	T2	T3		T1	T2	T3
Water										
M	↓	↓	↓*	↓	↓	↓	ALL	↑*	NC	↑
F	↓	↓*	↓	↓*	↓	↓*				
25g Glucose										
M	↓	↓	↓*	↓	↓	↓	ALL	↓	↓	↑
F	↓	↓	↓*	↓	↓	↓				
75g Glucose										
M	↓	↓	↓*	↓	NC	↓		↑	↓	↓*
F	↓	↓*	↓	↓	↓	↓*		↑	↑	NC

	GLUCOSE			INSULIN			AP		
	T1	T2	T3	T1	T2	T3	T1	T2	T3
Water									
M	↓	NC	NC	ND	ND	↓	NC	NC	NC
F	NC	NC	NC	ND	ND	↓*	NC	NC	NC
25g Glucose									
M	↑	↓*	↓*	↑*	NC	↓*	NC	↑	NC
F	NC	↓*	NC	↑	↓	↓	NC	NC	NC
75g Glucose									
M	↑	↑	↓	↑*	↑*	↑	NC	NC	NC
F	↑*	↓	↓	↑*	↑*	↑	NC	NC	NC

T0: time zero hour (fasting); T1: time one hour; T2: time two hour; T3: time three hour
↑ : ≥5% mean increase from the fasting value; ↓ : ≥5% mean decrease from the fasting value; NC: no change; ND: not determined
* : Significantly different from the zero time value, $p<0.05$ (paired t-test)

DISCUSSION

The current study was similar to the previous glucose load study (Leklem and Hollenbeck, 1990), except that this experiment was designed to measure the effect of two levels of glucose (25 g and 75 g). The hypothesis was that acute glucose ingestion would cause an increase in plasma glucose and insulin, and the resulting elevated plasma insulin level would stimulate plasma AP activity. This in turn would increase the conversion of plasma PLP to PL. Then, plasma PL would be taken up by the red blood cell. PL could then be converted back to PLP in the red blood cell. If the mechanism held true, a decrease in plasma PLP concentration, an increase in plasma alkaline phosphatase activity and an increase in red blood cell PLP concentration should be observed.

Subject Characteristics

Several studies have observed that obesity (Rodin et al, 1985; Peiris et al, 1986; Craig et al, 1987; Bhatherna et al, 1987) causes lower insulin sensitivity, which leads to glucose intolerance. In the current study, obesity was not a factor based on the body mass index (BMI). Five of the seven subjects were within their normal range of BMI, and the other two subjects were slightly below their normal range of BMI. Hemoglobin and hematocrit were measured to assess possible changes in plasma PLP concentration during the experimental period. A change in hemoglobin and hematocrit values could affect the plasma volume, which would alter plasma PLP concentration (Leklem and Hollenbeck, 1990). During the experimental period, no significant changes in

hemoglobin and hematocrit were observed. The dietary intakes based on two 3-day dietary records were adequate. Mean vitamin B-6 intake was 1.57 ± 0.34 mg/day. Five subjects met the recommended daily intakes (DRI: 1.3 mg, 1999), and two subjects had vitamin B-6 intake slightly below the DRI. A study by Smith (1991) and Hansen et al (1996a) reported a relationship between the vitamin B-6:protein ratio and the fasting plasma PLP value in healthy subjects. In the current study, no correlation was found between mean vitamin B-6 intake and mean fasting plasma PLP value or between mean vitamin B-6:protein ratio and mean fasting plasma PLP value. Because the subjects' dietary intake was based on two 3-day dietary records and was from their own estimate of what they ate, the estimated vitamin B-6 and protein intakes would not be as accurate as the measured plasma PLP concentration. In addition, subjects were asked to complete the second dietary record during the three-week experimental period. As a result, subjects reported their dietary intakes in different test periods. All of these factors could weaken the significant relationship that has been seen between the vitamin B-6:protein ratio and the fasting plasma PLP concentration (Shultz and Leklem, 1981; Miller et al, 1985; Hansen et al, 1996a).

All subjects displayed normal oral glucose tolerance (Young, 1987). As observed in previous studies (Castro et al, 1970; Forster et al, 1972; Bratusch et al, 1980; Leklem and Hollenbeck, 1990), the current study also showed an increase in the plasma glucose and insulin concentrations after the 25 g and 75 g glucose loads. However, compared to the Leklem and Hollenbeck (1990) and Bratusch et al (1980) studies, the current study displayed a smaller increase in the mean plasma glucose concentration. Previous studies (Peiris et al, 1986; Craig et al, 1987) demonstrated a negative relationship between body

weight and insulin sensitivity. In the present study, since two subjects were underweight and since some of the subjects were close to underweight, these subjects might be very glucose tolerant during the OGTT test, and thus might have contribute to a smaller increase in the mean plasma glucose concentration. The maximal increase in plasma glucose concentration may have occurred at 30 minutes, and plasma glucose concentration was not measured at 30 minutes. Compared with the 75 g glucose load, the 25 g glucose load showed a smaller increase in the mean plasma glucose and insulin concentrations at T1 as expected.

Plasma Pyridoxal 5'-Phosphate During the OGTT

In the previous studies by Leklem and Hollenbeck (1990) and Hofmann et al (1991), an inverse relationship between glucose ingestion and plasma PLP concentration was found. The current study showed a significant decrease in the mean plasma PLP concentration at only one time point for males (T3) and females (T2) after the 75 g glucose load. Overall mean decreases of 20 % (9 nmol/L) and 15 % (7 nmol/L) were observed at T3 for males and females, respectively, after the 75 g glucose load. The result of the percent change was similar to the previous Leklem and Hollenbeck (1990) study, in which they found an overall decrease of 18 % to 21 % at five hours. In the current study, five out of the seven subjects had the greatest decrease of the plasma PLP concentration at T2, while the Leklem and Hollenbeck (1990) study showed the greatest decrease through three hours. It was observed that the higher fasting plasma PLP values were associated with a greater change in the plasma PLP concentration after the glucose

load. Subjects with lower fasting plasma PLP values had less of an absolute decrease in plasma PLP concentration. A possible reason for this may be related to the various pools of PLP in the body (Coburn, 1990). In the plasma, one pool has a rapid turnover rate of about 12 hours and one has a slower turnover rate of 25 to 35 days (Shane, 1978; Leklem, 1990). The acute glucose load may have caused a depletion of plasma PLP in the pool with a rapid turnover rate and caused only a minor effect in the pool with a slower turnover rate. No significant correlation was found between the fasting plasma PLP value and changes in the plasma PLP concentration in the current study.

In addition to the 75 g glucose load, a water solution and a 25 g glucose load were also administered. Compared to results from the Leklem and Hollenbeck (1990) study, when water was given to subjects in the current study there was an unexpectedly larger and significant decrease in the mean plasma PLP concentration at T3 for males and at T2 for females. The difference between the previous and the current studies was that the current study utilized a water solution with artificial sweetener equivalent to 25 g glucose. Previous studies demonstrated a positive effect of a combination of sight, smell and taste on insulin secretion (Simon et al, 1986; Bruce et al, 1987). However, Bruce et al (1987) did not find an increase in plasma insulin concentration after sipping the sweetened water solution (38 mg aspartame in 15 ml water) for five minutes. The difference between the Bruce et al (1987) study and the current study was that the subjects in the current study ingested a larger amount of sweetened water solution (90 mg Saccharin in 150 ml water) in a shorter period of time (one to two minutes). This taste of the water solution with sweetener may have enhanced insulin production and sensitivity, which in turn may have resulted in a decrease in plasma PLP concentration. Mean

plasma glucose concentration was not significantly decreased for both genders with the water solution. Because we did not measure the plasma insulin concentration at T1 and T2 for the water solution, the mechanism for the decreased plasma PLP concentration with the water solution is unknown. Although the decrease in plasma PLP concentration with the water solution was different in these two studies, they both showed a continuous decrease over time. Compared with the 75 g glucose load, the mean plasma PLP concentration with the 25 g glucose load had a lower decrease at each time point as hypothesized. However, the level of decrease between the two glucose loads was not significantly different. There were gender differences in the plasma PLP concentration for the water solution, 25 g and 75 g glucose loads as expected based on the Leklem and Hollenbeck (1990) study, but the level of change at each time point for the three solutions was not significantly different between males and females.

Plasma Pyridoxal, 4-Pyridoxic Acid, and Pyridoxine During the OGTT

In addition to plasma PLP concentration, plasma PL, 4-PA and PN concentrations at each time point for the three solutions were also determined in this study. Hofmann et al (1991) found a non-significant decrease in plasma PL concentration after the glucose polymer ingestion. In the current study, with the 75 g glucose load, both genders showed an increase in the mean plasma PL concentration at T1 and a return back to baseline value by T3, while subjects with the 25 g glucose load, the mean plasma PL concentration did not change over time. When the water solution was given, mean plasma PL concentration was significantly increased at T1. However, the change in the

mean plasma PL concentration was not significant at T1 for the 75 g glucose load, possibly because of the small sample size and large standard deviation. The significant decrease in mean plasma PLP concentration after the water solution and the 75 g glucose load showed a similar increase in plasma PL concentration. This suggests that decreased plasma PLP concentration during the OGTT was correlated with the increase in plasma PL concentration. However, compared to the change in plasma PLP concentration, plasma PL showed a smaller change over time for the three solutions. This may be because plasma PL was rapidly taken up by the red blood cells, and therefore one would not be as likely to see as large a change in plasma PL concentration.

PLP accounts for 60-70 % of the total vitamin B-6 in plasma (Merrill and Henderson, 1990). Leklem and Hollenbeck (1990) stated that plasma PLP appeared to be the major vitamin B-6 that had the greatest change during the OGTT. In the current study, compared with the other free forms of vitamin B-6 (PL, 4-PA, and PN), plasma PLP did show the largest molar changes over time after the glucose load. There was a significant positive correlation between the change in plasma PL and PLP from T0 to T3 for females with the water solution ($r=0.9751$, $P<0.03$). This further supports the PLP-PL relation during the OGTT.

Mean plasma 4-PA concentration was decreased at T1 with the three solutions. A significant decrease was observed for females with the water solution and the 75 g glucose load. The percent decrease in the plasma 4-PA concentration was similar for the water solution, the 25 g glucose and the 75 g glucose loads. Interestingly, both mean plasma PLP and mean plasma 4-PA concentrations were higher for males than for females with the three solutions. However, in contrast to plasma PLP, mean plasma PL

concentration was higher for females than for males with the 75 g glucose load. But when comparing the seven subjects given the 75 g glucose load, one female subject (#5) had higher fasting plasma PL concentration (24.58 nmol/L) and larger plasma PL concentraion over time, which contributed to the higher mean plasma PL concentration observed for females (n=4). If data for this female subject (#5) is excluded, there was no gender differences in mean plasma PL concentration over time. No consistent correlation was found between plasma 4-PA and PLP concentration. Decreased plasma PLP concentration did not contribute to increased plasma 4-PA concentration during the OGTT. This further supports the probability that some plasma PL was taken up by the red blood cell, and thus less PL would be converted to 4-PA in the liver.

There was no significant difference in the mean plasma PN concentration at all time points for the three solutions. Compared to the maximal molar increase of plasma PL, plasma PN showed less of an increase over time after the three solutions. Although both plasma PL and PN concentrations were increased at certain times after the glucose load, none of these increases were significant. As explained earlier, it is possible that plasma PL and PN were rapidly taken up by the red blood cell, and therefore, a significant change in plasma PL and PN concentrations was not observed.

Plasma Alkaline Phosphatase Activity During OGTT

Alkaline phosphatase (AP) is considered an ectoenzyme (i.e. on outer membrane) and hydrolyzes plasma PLP to PL (Coburn and Whyte, 1988). Several studies have demonstrated a negative relation between plasma AP activity and PLP concentration

(Wan et al, 1993; Okada et al, 1997; Iqbal et al, 1998). In the Okada et al (1997) study, there was a significant inverse correlation between AP activity and plasma PLP/PL ratio in rats with or without injection of intraperitoneal streptozotocin (80 mg/kg stz). Patients with liver disease also have a higher AP activity and lower plasma PLP concentration (Anderson et al, 1980; Merrill et al, 1986, Anderson et al, 1989). Moreover, elevated AP activity was observed in about 25 % persons with diabetes (Goldberg et al, 1977; Stephan et al, 1980; Maxwell et al, 1986), but the plasma PLP concentration was not measured in these diabetic people. Other studies (Leklem et al, 1985; Rogers and Mohan, 1994) have shown decreased plasma PLP concentration in persons with diabetes.

The hypothesis was that elevated AP activity would increase the conversion of PLP to PL, which would lead to decreased plasma PLP and increased plasma PL concentrations. However, the current study did not find a significant change in the plasma AP activity at any time points after the 75 g glucose load, nor did the water solution and the 25 g glucose load showed any significant changes in the plasma AP activity. All subjects had normal plasma AP activity during the OGTT (Roy, 1970). The result was similar to that of the Hofmann et al (1991) study, in which they found that serum AP activity was within the normal range at one, two and three hours after the water and the glucose polymer (200 ml containing 46 g GP and 25 g fructose) ingestions. One possible reason that plasma AP activity was not increased during the OGTT may be because the increased plasma insulin concentration elevated AP activity mainly in other tissues, rather than the red blood cell. The change in plasma AP activity had a non-significant positive correlation with the change in plasma PLP concentration from T0 to T1, T2, and T3 for both genders with the 75 g glucose load. The positive correlation

between the changes in plasma AP activity and PLP concentration was stronger and more consistent than the correlation between the changes in plasma AP activity and PL concentration. A significant positive correlation between a change in mean plasma AP activity and a change in plasma PL concentration (2.15 ± 2.99 nmol/L) was observed from T0 to T1 only for females with the 25 g glucose load ($r=0.9835$, $P<0.02$). The change in mean plasma PL concentration from T0 to T1 for both males (1.57 ± 5.14 nmol/L)and females (-1.02 ± 5.34 nmol/L) with the 75 g glucose load probably did not show a significant correlation with the change in mean plasma AP activity because of the large standard deviations observed in the change of plasma PL concentration. Since there was a drop in plasma PLP concentration at T1 with the three solutions, it is more likely that a correlation between the change in plasma PL concentration and the change in plasma AP activity would be seen at T1.

Previous studies (Romero et al, 1988; Ognibene et al, 1997) have examined the relationship between plasma insulin and AP activity. In an in vitro study, Romero et al (1988) found that an increase in plasma insulin levels stimulated the release of glycophospholipid-anchored proteins, such as AP, from cell membranes. Another study (Ognibene et al, 1997) also found a positive relationship between serum containing intestinal alkaline phosphatase (IAP) activity and insulin secretion during the OGTT for healthy obese subjects. In the current study, a positive, but non-significant, correlation was also observed between plasma insulin and AP activity for females given the three solutions. A significant positive correlation was seen only at T1 for males with the 25 g glucose load ($r=0.9979$, $P<0.05$). In addition, like plasma PLP, both mean plasma insulin concentration and AP activity were significantly higher for males than for females with

the three solutions. Overall, the results did not demonstrate a strong relationship between plasma insulin and AP activity during the OGTT.

Red Blood Cell Pyridoxal 5'-Phosphate During the OGTT

Previous studies have observed a rapid uptake of PL and PN by RBC (Anderson, 1980; Mehansho and Henderson, 1980; Fonda and Harker, 1982; Ink et al, 1982; Ink and Henderson, 1984; Anderson et al, 1989). Several studies found red blood cells to contain the required enzymes for conversions of different forms of vitamin B-6 (Anderson et al, 1971; Lumeng and Li, 1974). An in vitro study by Yamada et al (1968) found a twofold increment of total vitamin B-6 accumulation in ascites sarcoma cells after 60 minutes of incubation in a solution containing glucose (concentration unknown). In addition, they observed a higher amount of PL transported into the RBC, compared with PN and PM. In another in vitro study, Ink et al (1982) also found a slightly higher uptake of PL by RBC during 60 minutes of incubation in a 10 mmol/L glucose solution, compared to the 5 mmol/L (90 mg/100 ml) glucose concentration and the control.

The hypothesis for the decreased plasma PLP concentration after the glucose load was that plasma PLP would be converted to plasma PL and PN by AP. The increased plasma PL and PN would then be taken up by the red blood cell. Once PL and PN were in the red blood cell, they could then be rapidly converted to the phosphorylated forms. This would eventually increase the red blood cell PLP concentration. A previous study by Martinson (thesis, 1994) found no changes in the red blood cell PLP concentration after a 75 g glucose load. In the current study, acute glucose ingestion also did not

increase the mean red blood cell PLP concentration significantly. However, compared to the percent mean increase (M 2 %; F 0 %) in the red blood cell PLP concentration of the previous Martinson study (1994), the current study had a mean increase of 8 % (2 nmol/L) and 14 % (5 nmol/L) for males and females, respectively, after the 75 g glucose load. The maximal mean increase was observed at T3 for males and at T2 for females. In contrast to mean plasma PLP, insulin, and AP activity, mean red blood cell PLP concentration was significantly higher over time for females than for males with the three solutions. The reason for the difference is unknown. An increase in the red blood cell PLP concentration with the water, 25 g glucose and 75 g glucose loads ranged from 15 % to 53 % (n=2), 8 % to 26 % (n=6) and 6 % to 46 % (n=7), respectively. The tyrosine enzymatic method (Chabner and Livingston, 1970) used in the current study for measuring red blood cell PLP may also have caused a problem because of the variable recoveries observed. Compared with the tyrosine method for plasma PLP, the method for the red blood cell PLP had much lower recoveries. This may have been because it was difficult to release the PLP from the deproteinized hemoglobin complex (Benesch et al, 1972; Fonda and Harker, 1982). The mean recovery of the red blood cell PLP for the control sample in the current study was 69.9 % ± 14.4 %. The red blood cell PLP values were not corrected for percent recoveries.

For the 75 g glucose load, each individual did show an increase in red blood cell PLP concentration in response to the decreased plasma PLP concentration as expected, while five out of the seven subjects with the water solution did not show this relation. A similar relation between plasma PLP and red blood cell PLP for the 75 g glucose load was also observed with the 25 g glucose load. The mechanism for the relation between

plasma PLP and red blood cell PLP observed with the water solution is unknown. One possibility for the different trends observed in the red blood cell PLP concentration for the water solution and the 75 g glucose load may be because of the supply of ATP in the red blood cell. In this study, the glucose load may have provided a source of energy in the red blood cell (Brewer, 1974), which may have increased the conversion of PL to PLP, while for the water solution, there was only a limited amount of ATP available for this conversion. As a result, an increase in red blood cell PLP concentration would be more likely after the glucose load.

In conclusion, this study suggests that acute glucose ingestion resulted in an increase in plasma glucose and insulin concentration and a decrease in plasma PLP concentration. Plasma AP activity was not significantly changed during the OGTT. However, plasma PL and PN did increase to a limited extent, although the changes were not statistically significant. Because of the small sample size, the red blood cell PLP concentration probably did not show a significant increase during the OGTT.

For further studies, a larger sample size would be helpful to demonstrate if there is a significant relationship among plasma PLP, PL and red blood cell PLP concentration and to evaluate the hypothesized mechanism of decreased plasma PLP after acute glucose load. The current study did not provide solid evidence for the hypothesized mechanism perhaps in part because of the small sample size and large standard deviation for some of variables measured. Several important data, such as plasma PL, PN and red blood cell PLP concentrations showed a trend as hypothesized. More frequent measurements at 15, 30 and 45 minutes, may help in identifying the peak in plasma glucose and insulin after the glucose load. In addition, a longer period of time might be helpful to see the changes

in the red blood cell PLP concentration, since the current study showed the largest increase in the red blood cell PLP concentration at T3 with the 75 g glucose load. Furthermore, a more precise method for measuring the red blood cell PLP concentration also may help to determine if there is a stronger relationship with plasma PLP concentration. Moreover, a significant decrease in plasma PLP concentration was found after the water solution with artificial sweetner equivalent to 25 g glucose. Based on these results, it might be better to use a water solution without the sweetner as a control. It is hoped that this study shows a better picture of the possible mechanism of decreased plasma PLP concentration, and that further study can solidify the mechanism and eventually contribute the finding to people with higher carbohydrate intake and to people with diabetes.

SUMMARY AND CONCLUSION

Summary

This study was designed to measure the effect of two levels of glucose ingestion on plasma pyridoxal 5′-phosphate concentration Three males and four females participated in this study. Each subject was tested on three separate occasions over a period of three weeks. Subjects ingested the assigned solutions (water with artificial sweetner equivalent to 25 g glucose, 25 g glucose or 75 g glucose) in a randomized order. Plasma PLP, PL, 4-PA, PN, glucose, insulin, AP activity and red blood cell PLP were measured at time zero (T0), time one (T1), time two (T2), and time three (T3) hour. Hemoglobin and hematocrit were also measured at T0 in the first and last week of the study. In addition, each subject was asked to complete one 3-day dietary record before and during the study to assess vitamin B-6 and protein intake.

No correlation was found between mean vitamin B-6 intake and mean fasting plasma PLP value or between mean vitamin B-6:protein ratio and mean fasting plasma PLP value. There was an increase in the mean plasma glucose and insulin concentrations after the glucose loads. Mean plasma glucose and insulin concentrations for the water solution were constant at all time points. The 25 g glucose load showed a smaller increase in plasma glucose and insulin concentrations compared to the changes with 75 g glucose load. As expected, based on the previous published research, the higher glucose load (75 g) resulted in higher plasma glucose and insulin concentrations.

A significant decrease in the mean plasma PLP concentration was observed for males and females after the 75 g glucose load as hypothesized. Subjects with higher

fasting plasma PLP value had a greater change in the plasma PLP concentration after the glucose load, but no significant correlation was found for this relation. An unexpected larger and significant decrease in the mean plasma PLP concentration was observed for the water solution with artificial sweetner equivalent to 25 g glucose. As hypothesized, the 25 g glucose load showed a lower decrease in the mean plasma PLP concentration at each time point compared with the 75 g glucose load. No significant difference was found in the level of decrease between the two glucose loads. There were gender differences in the plasma PLP concentration for the three solutions as expected, but the level of change at each time point was not significantly different between males and females. Overall, the 75 g glucose load resulted in the greatest change in the mean plasma PLP concentration.

Unlike what was hypothesized, there was no significant change in the plasma AP activity at all time points after the three solutions. In addition, there was a positive correlation between plasma insulin and AP activity after the glucose loads, although the elevated plasma insulin concentration did not show a significant increase in the plasma AP activity as hypothesized.

There was a non-significant increase in the mean plasma PL and PN concentrations for all subjects with the 25 g and 75 g glucose loads. A positive, but non-significant, correlation between the change in plasma PL and the change in plasma PLP concentration was observed at all time points for females with the three solutions. In general, plasma 4-PA concentration was not significantly changed for the three solutions. Acute glucose ingestion also did not increase the red blood cell PLP concentration

significantly. In addition, there was a positive, but non-significant, relation between plasma PLP and red blood cell PLP in all subjects with the 25 g and 75 g glucose loads.

Conclusion

Acute glucose ingestion caused a significant decrease in plasma PLP concentration in healthy people. Compared to the 25 g glucose load, the 75 g glucose load led to a lower mean plasma PLP concentration. Plasma PLP concentrations were different between genders during the OGTT. In addition, the change in the plasma PLP concentration was different at different time points for the glucose load. Although elevated plasma insulin did not increase AP activity as hypothesized, there was a positive, but non-significant, correlation between plasma insulin concentration and plasma AP activity. There was a non-significant increase in plasma PL and PN concentrations during the OGTT. Red blood cell PLP concentration was also increased after the 75 g glucose load, but due to the small sample size and large standard deviation, the increase was not significant. The results show that acute glucose ingestion caused an increase in plasma glucose and insulin concentrations, a decrease in plasma PLP concentration, and a small increase in plasma PL, PN and red blood cell PLP concentrations.

REFERENCES

Allgood VE, and Cidlowski JA. 1992. Vitamin B-6 modulates transcriptional activation by multiple members of the steroid hormone receptor superfamily. J Biol Chem 267: 3819-3824

Alton-Mackey MG, and Walker BL. 1973. Graded levels of pyridoxine in the rat during gestation and the physical and neuromotor development of offspring. Am J Clin Nutr 26: 420-428

Anderson BB. 1980. Red-cell metabolism of vitamin B-6. In: Tryfiates GP, ed. Vitamin B-6 metabolism and role in growth. Food and Nutrition Press, Westport, CN. Page, 53-83

Anderson BB, Fulford-Jones CE, Child JA, Beard MEJ, and Bateman CJT. 1971. Conversion of vitamin B-6 compounds to active forms in the red blood cell. J Clin Invest 50: 1901-1909

Anderson BB, O'Brien H, Griffin GE, and Mollin DL. 1980. Hydrolysis of pyridoxal 5'-phosphate in plasma in conditions with raised alkaline phosphatase. Gut 21: 192-194

Anderson BB, Perry GM, Clements JE, and Greany MF. 1989. Rapid uptake and clearance of pyridoxine by red blood cells in vivo. Am J Clin Nutr 50: 1059-1063

Ang CYW. 1979. Stability of three forms of vitamin B-6 to laboratory light conditions. J Assoc Off Anal Chem 62: 1170-1173

Angel JF. 1975. Lipogenesis by hepatic and adipose tissues from meal-fed pyridoxine-deprived rats. Nutr Rept Int 11: 369-378

Angel JF. 1980. Gluconeogenesis in meal-fed, vitamin B-6 deficient rats. J Nutr 110: 262-269

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

Atkin L, Schultz AS, Williams WL, and Frey CN. 1943. Yeast microbiological methods for determination of vitamins-pyridoxine. Ind and Eng Chem Anal Ed 15: 141-144

Audet A, and Lupien PJ. 1974. Triglyceride metabolism in pyridoxine-deficient rats. J Nutr 104: 91-100

Axelrod AE, and Trakatelles AC. 1964. Relationship of pyridoxine to immunological phenomen. Vitamin Horm 22: 591-607

Aycock JE, and Kirksey A. 1976. Influence of different levels of dietary pyridoxine on certain parameters of developing and mature brains in rats. J Nutr 106: 680-688

Baum MK, Mantero-Atienza E, Shor-Posner G, et al. 1991. Association of vitamin B-6 status with parameters of immune function in early HIV-1 infection. J Acquired Imm Def Syndromes 4: 1122-1132

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

Behall KM, Moser PB, Kelsay JL, and Prather ES. 1980. The effect of kind of carbohydrate in the diet and use of oral contraceptives on metabolism of young women. III. serum glucose, insulin, and glucagon. Am J Clin Nutr 33: 1041-1048

Bender DA. 1987. Oestrogens and vitamin B-6 actions and interactions. Wld Rev Nutr Diet 51: 140-188

Benesch R, Benesch RE, Edalji R, and Suzuki T. 1977. 5'-Deoxypyridoxal as a potential anti-sickling agent. Proc Natl Acad Sci. USA 74: 1721-1723

Bernhart FW, D'Amato E, and Tomarelli RM. 1960. The vitamin B-6 activity of heat-sterilized milk. Arch Biochem Biophys 88: 267-269

Bessey OA, Adam DJD, and Hansen AE. 1957. Intake of vitamin B-6 and infantile convulsions: a first approximation of requirements of pyridoxine in infants. Pediatr 20:33-44

Bhathena SJ, Aparicio P, Revett K, Voyles N, and Recant L. 1987. Effect of dietary carbohydrates on glucagon and insulin receptors in genetically obese female Zucker rats. J Nutr 117: 1291-1297

Bills ND, Leklem JE, and Miller LT. 1987. Vitamin B-6 bioavailability in plant foods is inversely correlated with % glycosylated vitamin B-6. Fed Proc 46: 1487 (abstract)

Black AL, Guirard BM, and Snell EE. 1977. Increased muscle phosphorylase in rats fed high levels of vitamin B-6. J Nutr 107: 1962-1968

Black AL, Guirard BM, and Snell EE. 1978. The behavior of muscle phosphorylase as a reservoir for vitamin B-6 in the rat. J Nutr 108: 670-677

Bohannon NV, Karam JH, and Forsham PH. 1980. Endocrine responses to sugar ingestion in man. J Am Diet Assoc 76: 555-560

Bottomley SS. 1983. Iron and Vitamin B-6 metabolism in the sideroblastic anemias. In: Lindenbaum, ed. Nutrition in Hematology, Churchill Livingston, New York. Page 203-223

Bratusch PR, Waldhausel W, Gasic S, Korn A, and Nowotny P. 1980. Oral glucose tolerance test: effect of different glucose loads on splanchinc carbohydrate and substrate metabolism in healthy men. Metabolism 29: 289-295

Brewer GJ. 1974. The pathways and enzymes of the red cell. In: Surgenor DM, 2nd ed. The Red Blood Cell. New York, Academic Press, page 391-405.

Brown RR. 1985. The tryptophan load test as an index of vitamin B-6 nutrition. In: Leklem JE and Reynolds RD, eds. Methods in Vitamin B-6 Nutrition, Plenum Press, New York. Page 321-340.

Brown RR, Rose DP, Leklem JE, Linkswiler H, and 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

Bruce DG, Storlien LH, Furler SM, and Chisholm DJ. 1987. Cephalic phase metabolic responses in normal weight adults. Metabolism 36: 721-725

Bunce GE, and Vessal M. 1987. Effect of zinc and/or pyridoxine deficiency upon oestrogen retention and oestrogen receptor distribution in the rat uterus. J Steroid Biochem 26: 303-308

Canham JE, Baker EM, Harding RS, Sauberlich HE, and Plough IC. 1969. Dietary protein-its relationship to vitamin B-6 requirements and function. Ann NY Acad Sci 166: 16-29

Carroll KF, and Nestel PJ. 1973. Diurnal variation in glucose tolerance and in insulin secretion in man. Diabetes 22: 333-348

Castro A, Scott JP, Grettie DP, Macfarlane D, and Bailey RE. 1970. Plasma insulin and glucose responses of healthy subjects to varying glucose loads during three-hour oral glucose tolerance tests. Diabetes 19: 842-851

Chabner B, and Livingston D. 1970. A simple enzymatic assay for pyridoxal phosphate. Anal Biochem 34: 412-423

Chandra RK, and Puri S. 1985. Vitamin B-6 modulation of immune responses and infection. In: Reynolds RD and Leklem JE, eds. Vitamin B-6: Its Role in Human and Disease. A. R. Liss, New York. Page 147-155

Chang SJ, Kirksey A, and Morre DM. 1981. Effects of vitamin B-6 deficiency on morphological changes in dentritic trees of purkinje cells in developing cerebelum of rats. J Nutr 111: 848-857

Cheslock K, and McCully MT. 1960. Response of human beings to a low-vitamin B-6 diet. J Nutr 70: 507-513

Cho Y, and Leklem JE. 1990. In vivo evidence for a vitamin B-6 requirement in carnitine synthesis. J Nutr 120: 258-265

Cidlowski JA, and Thanassi JW. 1981. Pyridoxal phosphate: a possible cofactor in steroid hormone action. J Steroid Biochem 15: 11-16

Coburn SP. 1990. Location and turnover of vitamin B-6 pools and vitamin B-6 requirements of humans. Ann NY Acad Sci 585: 76-85

Coburn SP, Lewis DL, and Fink WJ. et al. 1988. Human vitamin B-6 pools estimated through muscle biopsies. Am J Clin Nutr 48: 291-294

Coburn SP, and Mahuren JD. 1983. A versatile cation-exchange procedure for measuring the seven major forms of vitamin B-6 in biological samples. Anal Biochem 129: 310-317

Coburn SP, and Whyte M.P. 1988. Role of phosphatases in the regulation of vitamin B-6 metabolism in hypophosphatasia and other disorders. In: Leklem JE and Reynolds RD, eds. Clinical and Physiological Applications of vitamin B6. A. R. Liss, New York: page 65-93

Cohen M, and Bendich A. 1986. Safety of pyridoxine – a review of human and animal studies. Toxicol Letters 34: 129-139

Coursin DB. 1954. Convulsive seizures in infants with pyridoxine-deficient diet. J Am Med Assoc 154: 406-408

Coursin DB. 1969. Vitamin B-6 and brain function in animals and man. Ann NY Acad Sci 166: 7-15

Craig BW, Garthwaite SM, and Holloszy JO. 1987. Adipocyte insulin resistance: effects of aging, obesity, exercise, and food restriction. J Appl Physiol 62: 95-100

Cunnane SC, Manku MS, and Horrobin DF. 1984. Accumulation of linoleic and α-linolenic acids in tissue lipids of pyridoxine-deficient rats. J Nutr 114: 1754-1761

Cunnane SC, Manku MS, and Horrobin DF. 1985. Effect of vitamin B-6 deficiency on essential fatty acid metabolism. In: Reynolds RD and Leklem JE, eds. Vitamin B-6: Its Role in Health and Disease. A. R. Liss, New York, page 447-451

Dakshinamurti K. 1982. Neurobiology of pyridoxine. In: Draper HH, ed. Advances in Nutritional Research. Vol. 4, Plenum Press, New York. Page 143-179

Dalton K, and Dalton MJT. 1987. Characteristics of pyridoxine overdose neuropathy syndrome. Acta Neurol Scand 76: 81-91

Davis RE, Calder JS, and Curnow DH. 1976. Serum pyridoxal and folate concentrations in diabetics. Pathology 8: 151-156

Delrome CB, and Lupien PJ. 1976. The effect of vitamin B-6 deficiency on the fatty acid composition of the major phospholipids in the rats. J Nutr 106: 169-180

Desikachar HSR, and McHenry EW. 1954. Some effects of vitamin B-6 deficiency on fat metabolism in the rat. Biochem J 56: 544-547

Disorbo DM, Phelps DS, Ohl VS, and Litwack G. 1980. Pyridoxine deficiency influences the behavior of the glucocorticord receptor complex. J Biol Chem 255: 3866-3870

Driskell JA, and Moak SW. 1986. Plasma pyridoxal phosphate concentrations and coenzyme stimulation of erythrocyte alanine aminotransferase activities of white and black adolescent girls. Am J Clin Nutr 43: 599-603

Fleming SE, and Shaheen SM. 1988. Repeated consumption of high-fiber breakfasts: effects on postprandial glucose and insulin responses after breakfast and lunch. Am J Clin Nutr 47: 859-967

Fonda ML, and Harker CW. 1982. Metabolism of pyridoxine and protein binding of the metabolites in human erythrocytes. Am J Clin Nutr 35: 1391-1399

Forster H, Haslbeck M, and Mehnert H. 1972. Metabolic studies following the oral ingestion of different doses of glucose. Diabetes 21: 1102-1108

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

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

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

Grabow JD, and Linkswiler H. 1969. Electroencephalographic and nerve-conduction studies in experimental vitamin B-6 deficiency in adults. Am J Clin Nutr 22: 1429-1434

Gregory JF. 1988. Methods for determination of vitamin B-6 in foods and other biological materials: a critical review. J Food Composit Anal 1: 105-123

Gregory JF, and Kirk JR. 1977. Interaction of pyridoxal and pyridoxal phosphate with peptides in a model food system during thermal processing. J Food Sci 42: 1554-1561

Gregory JF, and Kirk JR. 1978. Assessment of roasting effects on vitamin B-6 stability and bioavailability in dehydrated food systems. J Food Sci 43: 1585-1589

Gregory JF and Kirk JR. 1981. The bioavailability of vitamin B-6 in foods. Nutr Rev 39:1

Gregory JF III, Trumbo P, Baily LB, Toth JP, Baumgartner TG, and Corda JJ. 1991. Bioavailability of pyridoxine 5'-β-D glucoside determined in humans by stable-isotopic methods. J Nutr 121: 177-186

Guilland JC, Berekski-Regung B, Lequeu B, Moreau D, and Klepping J. 1984. Evaluation of pyridoxine intake and pyridoxine status among aged institutionalized people. Internat J Vit Nutr Res 54: 185-193

Gyorgy P. 1934. Vitamin B-2 and the pellagra-like dermatitis of rats. Nature 133: 448-449

Gyorgy P. 1938. Crystalline vitamin B-6. J Am Chem Soc 60: 983-984

Gyorgy P, and Eckhardt RE. 1939. Vitamin B-6 and skin lesions in rats. Nature 144: 512

Hale PJ, Wright JV, and Nattrass M. 1985. Differences in insulin sensitivity between normal men and women. Metabolism 34: 1133-1138

Hales CN, and Randle PJ. 1963. Immunoassay of insulin with insulin-antibody precipitate. Biochem J 88: 137-146

Hall CJ. 1983. A method for the rapid assessment of sample size in dietary studies. Am J Clin Nutr 37: 473-477

Hamfelt A, and Soderhjelm L. 1988. Vitamin B-6 and aging. In: Leklem JE and Reynold RD, eds., Clinical and Physiological Applications of Vitamin B-6, A. R. Liss, New York, page 95-107

Hamm MW, Mehansho H, and Henderson, LM. 1979. Transport and metabolism of pyridoxal and pyridoxal phosphate in the small intestine of the rat. J Nutr 109: 1552-1559

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

Hansen CM, Leklem JE, and Miller LT. 1996b. Vitamin B-6 status indicators decrease in women consuming a diet high in pyridoxine glucose. J Nutr 126: 2512-2518

Harris JW, Wittington RM, Weisman R, Jr., and Horrigan DL. 1956. Pyridoxine responsive anemia in the human adult. Proc Soc Exp Biol Med 91: 427-432

Harris SA, and Folkers K. 1939. Synthesis of vitamin B-6. J Am Chem Soc 61: 1245-1247

Hedeskov CJ. 1980. Mechanism of glucose-induced insulin secretion. Physiol Rev 60: 442-509

Heine RJ, Hanning I, Morgan L, and Alberti KGMM. 1983. The oral glucose tolerance test (OGTT): effect of rate of ingestion of carbohydrate and different carbohydrate preparations. Diabetes Care 6: 441-445

Henderson LM. 1985. Intestinal absorption of B-6 vitamers. In: Reynold RD and Leklem JE, eds. Vitamin B-6 Its Role in Health and Disease, A. R. Liss, New York, page 22-33

Hiipakka RA, and Liao S. 1980. Effect of pyridoxal phosphate on the androgen receptor from rat prostate: inhibition of receptor aggregation and receptor binding to nuclei and to DNA-cellulose. J Steroid Biochem 13: 841-846

Hofmann A, Reynold RD, Smoak BL, and Villanueva VG, et al. 1991. Plasma pyridoxal and pyridoxal 5'-phosphate concentration on response to ingestion of water or glucose polymer during a 2-h run. Am J Clin Nutr 53: 84-89

Hollenbeck CB, Donner CC, and Leklem JE. 1985. Evidence that the acute fall in plasma pyridoxal-5'-PO4 in response to an oral glucose challenge is associated with insulin stimulated glucose utilization. Clin Res 33: 61A

Hollenbeck CB, Leklem JE, Riddle MC, and Connor WE. 1983. The composition and nutritional adequacy of subject-selected high carbohydrate, low fat diets in insulin-dependent diabetes mellitus. Am J Clin Nutr 38: 41-51

Holley J, Bender, DA, Coulson WF, and Symes EK. 1983. Effects of vitamin B-6 nutritional status on the uptake of (3H) oestradiol into the uterus, liver and hypothalamus of the rat. J steroid Biochem 18: 161-165

Hollins B, and Herderson JM. 1986. Analysis of B-6 vitamers in plasma by reversed-phase column lipid chromatography. J Chromatogr 380: 67-75

Horrigan DL, and Harris JW. 1968. Pyridoxine responsive anemia in man. Vitam Horm 26: 549-568

Ichiba A, and Michi K. 1938. Isolation of vitamin B-6. Soc Papers Inst Phys Chem Res (Tokyo) 34: 623-626

Ink SL, and Henderson LM. 1984. Effect of binding to hemoglobin and albumin on pyridoxal transport and metabolism. J Biol Chem 259: 5833-5837

Ink SL, Mehansho H, and Henderson LM. 1982. The binding of pyridoxal to hemoglobin. J Biol Chem 257: 4753-4757

Iqbal SJ, BrainA, Reynold TM, and Penny M, et al. 1998. Relationship between serum alkaline phosphatase and pyridoxal-5'-phosphate levels in hypophosphatasia. Clin Sci 94: 203-206

IUPAC-IUB Commission on Biochemical Nomenclature. 1973. Nomenclature for vitamin B-6 and related compounds. Eur J Biochem 40: 325-327

Jarrett RJ, and Graver HJ. 1968. Changes in oral glucose tolerance during the menstrual cycle. Br Med J 2: 528-529

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

Kabir H, Leklem JE, and Miller LT. 1983b. Comparative vitamin B-6 bioavailability from tuna, whole wheat bread and peanut butter in humans. J Nutr 113: 2412-2420

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

Keresztesy JC, and Stevens JR. 1938. Vitamin B-6. Proc Soc Exp Biol Med 38: 64-65

Kies C, Kan S, and Fox HM. 1984. Vitamin B-6 availability from wheat, rice, corn brans for humans. Nutr Repts Int 30: 483-491

Kikuchi G, KumarA, and Talmage P. 1958. The enzymatic synthesis of δ-aminolevulinic acid. J Biol Chem 233: 1214-1219

Kirksey A, Keaton K, Abernathy RP, and Greger JL. 1978. Vitamin B-6 nutritional status of a group of female adolescents. Am J Clin Nutr 31: 946-954

Krebs EG, and Fischer EH. 1964. Phosphorylase and related enzymes of glycogen metabolism. In: Harris RS, Wool IG, and Lovaine JA, eds. Vitamins and Hormones. Academic press, New York: 22: 399-410

Kuhn R, and Wendt G. 1938. Uber das antidermatitische Vitamin der Hefe. Ber Deut Chem Ges 71B: 780-782

Kuhn R, Westphal K, Wendt G, and Westphal O. 1939. Synthesis of adermin. Naturwissenschaften 27: 469-470

Kurtz DJ, Levy H, and Kanfer JN. 1972. Cerebral lipids and amino acids in the vitamin B-6 deficient suckling rat. J Nutr 102: 291-298

Lee CM, and Leklem JE. 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 JE. 1971. Quantitative aspects of tryptophan metabolism in humans and other species: a review. Am J Clin Nutr 24: 659-671

Leklem JE. 1985. Physical activity and vitamin B-6 metabolism in men and women: interrelationship with fuel needs. In: Reynold RD and Leklem JE, eds. Vitamin B6: Its Role in Health and Disease. New York: AR Liss, page 221-241

Leklem JE. 1988. Vitamin B-6 metabolism and function in humans. In: Reynold RD and Leklem JE, eds. Clinical and Physiological Applications of Vitamin B-6. New York: Alan R. Liss, page 3-28

Leklem JE. 1990. Vitamin B-6: a status report. J Nutr 120:1503-1507

Leklem JE. 1991. Vitamin B-6. In: Machlin LJ, eds. Handbook of Vitamins. 2nd Ed. New York: Marcel Dekker, page 341-392

Leklem JE. 1996. Vitamin B-6. In: Ziegler EE and Filer LJ, eds. Present Knowledge in Nutrition. Washington D.C., ILSI Press, page 174-183.

Leklem JE, Brown RR, Rose DP, Linkswiler H, and Arend RA. 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 JE, and Hollenbeck CB. 1990. Acute ingestion of glucose decreases plasma pyridoxal 5'-phosphate and total vitamin B6 concentration. Am J Clin Nutr 51: 832-836

Leklem JE, Kingsley L, Pratt C, and Wilson W. 1985. Low plasma pyridoxal 5'-phosphate in persons with non-insulin dependent diabetes. Fed Proc 44: 775

Leklem JE, Miller LT, Perera AD, and Peffers DE. 1980b. Bioavailability of vitamin B-6 from wheat bread in humans. J Nutr. 110: 1819-1828

Leklem JE, and Shultz TD. 1983. Increased plasma pyridoxal 5'-phosphate and vitamin B-6 in male adolescents after a 4500-meter run. Am J Clin Nutr 38: 541-548

Leklem JE, Shultz TD, and Miller LT. 1980a. Comparative bioavailability of vitamin B-6 from soybeans and beef. Fed Proc 39: 558 (abstract)

Lepkovsky S. 1938. Crystalline factor I. Science 87: 169-170

Lindberg AS, Leklem JE, and Miller LT. 1983. The effect of wheat bran on the broavailability of vitamin B-6 in young men. J Nutr 113: 2578-2586

Linkswiler HM. 1981. Methionine metabolite excretion as affected by a vitamin B-6 deficiency. In: Leklem JE and Reynold RD, eds., Methods in Vitamin B-6 Nutrition, Plenum Press, New York, page 373-381

Litwack G, Miller-Diener A, DiSorbo DM, and Schmidt TJ. 1985. Vitamin B-6 and the glucocorticord receptor. In: Reynold RD and Leklem JE, eds., Vitamin B-6: Its Role in Health and Disease. A. R. Liss, New York, page 177-191

Loo G, and Smith JT. 1986. Effect of pyridoxine deficiency on phospholipid methylation in rat liver microsomes. Lipids 21: 409-412

Lumeng L, Cleary RE, and Li T-K. 1974. Effect of oral contraceptives on the plasma concentration of pyridoxal phosphate. Am J Clin Nutr 27: 326-333

Lumeng L, and Li T-K. 1974. Vitamin B-6 metabolism in chronic alcohol abuse. Pyridoxal phosphate levels in plasma and the effects of acetaldehyde on pyridoxal phosphate synthesis and degradation in human erythrocytes. J Clin Invest 53: 693-704

Lumeng L, and Li T-K. 1980. Mammalian vitamin B-6 metabolism: regulatory role of protein-binding and the hydrolysis of pyridoxal 5'-phosphate in storage and transport. In: Tryfiates GP, ed., Vitamin B-6 Metabolism and Role in Growth, Food and Nutrition Press, Wesport, CT, page 27-51

Lumeng L, Li T-K, and Lui A. 1985. The interorgan transport and metabolism of vitamin B-6. In: Reynolds RD and Leklem JE, eds., Vitamin B-6: Its Role in Health and Disease A. R. Liss, New York, page 35-54

Lumeng L, Ryan MP, and Li T-K. 1978. Validation of the diagnostic value of plasma pyridoxal 5'-phosphate measurements in vitamin B-6 nutrition of the rat. J Nutr 108: 545-552

MacDonald I, and Crossley JN. 1970. Glucose tolerance during the menstrual cycle. Diabetes 19: 450-452

Maeda N, Takahashi K, Aono K, and Shiga T. 1976. Effect of pyridoxal 5'-phosphate on the oxygen affinity of human erythrocytes. Br J Haematol 34: 501-509

Maloney CJ, and Parmalee AH. 1954. Convulsions in young infants as a result of pyridoxine deficiency. J Am Med Assoc 154: 405-406

Manore M, Leklem JE, and Walter MC. 1987. Vitamin B-6 metabolism as affected by exercise in trained and untrained women fed diets differing in carbohydrate and vitamin B6 content. Am J Clin Nutr 46:995-1004

Martinson KE. 1994. Changes in plasma pyridoxal 5'-phosphate and red blood cell pyridoxal 5'-phosphate concentration during an oral glucose tolerance test in persons with diebetes mellitus. (MS thesis)

Maxwell DB, Fisher EA, Ross-Clunic HA, and Estep HL. 1989. Serum alkaline phosphatase in diabetes mellitus. J Am Coll Nutr 5: 55-59

McCoy EE. 1978. Vitamin B-6 requirements for infants and children. Human vitamin B-6 requirements. Natl Res Council, WA, D. C., page 257-271

McHenry EW, and Gauvin G. 1938. The B vitamins and fat metabolism, I. Effects of thiamine, riboflavin and rice polish concentrate upon body fat. J Biol Chem 125: 653-660

Mehansho H, Hamm MW, and Henderson LM. 1979. Transport and metabolism of pyridoxamine and pyridoxamine phosphate in the small intestine of the rat. J Nutr 109: 1542-1551

Mehansho H, and Henderson LM. 1980. Transport and accumulation of pyridoxine and pyridoxal by erythrocytes. J Biol Chem 255: 11901-11907

Meneilly GS, Elahi D, Minaker KL, Sclater AL, and Rowe JW. 1989. Impairment of noninsulin-mediated glucose disposal in the elderly. J Clin Endocrinol Metab 68: 566-571

Merrill A, and Henderson JM. 1990. Vitamin B-6 metabolism by human liver. Ann NY Acad Sci 585: 110-117

Merrill AH, Henderson JM, and Wang E. et al. 1984. Metabolism of vitamin B-6 by human liver. J Nutr 114: 1664-1674

Merrill AH, Henderson JM, Wang E, Codner MA, Hollins B, and Millikan WJ. 1986. Activities of the hepatic enzymes of vitamin B-6 metabolism for patients with cirrhosis. Am J Clin Nutr 44: 461-467

Meydani SW, Ribaya-Mercado JD, Russell RM, Sahyoun N, Morrow PD, Gershoff SN. 1991. Vitamin B-6 deficiency impairs interleukin-2 production and lymphocyte proliferation in elderly adults. Am J Clin Nutr 53: 1275-1280

Middleton HM. 1982. Characterization of pyridoxal 5'-phosphate disappearance from in vivo perfused segments of rat jejunum. J Nutr 112: 269-275

Mikines KJ, Dela F, Tronier B, and Galbo H. 1989. Effect of 7 days of bed rest on dose-response relation between plasma glucose and insulin secretion. Am J Physiol 257: E43-E48

Miller JW, Ribaya-Mercado JD, Russel RM, Shepard DC, et al. 1992. Effect of vitamin B-6 deficiency on fasting plasma homocysteine concentrations. Am J Clin Nutr 55:1154-1160

Miller LT, Leklem, JE, and Shultz TD. 1985. The effect of dietary protein on the metabolism of vitamin B-6 in humans. J Nutr 115: 1663-1672

Miller LT, and Linkswiler H. 1967. Effect of protein intake on the development of abnormal tryptophan metabolism by men during vitamin B-6 depletion. J Nutr 93: 53-67

Morre DM, Kirksey A, and Das GD. 1978a. Effects of vitamin B-6 deficiency on the developing central nervous system of the rat. Gross measurements and cytoarchitectural alterations. J Nutr 108: 1250-1259

Morre DM, Kirksey A, and Das GD. 1978b. Effects of vitamin B-6 on the developing central nervous system of the rat. Myelination. J Nutr 108: 1260-1265

Mueller JF. 1964. Vitamin B-6 in fat metabolism. Vit Horm 22: 787-796

Mueller JF, and Iacono JM. 1963. Effect of desoxypyridoxine-induced vitamin B-6 deficiency on polyunsaturated fatty acid metabolism in human beings. Am J Clin Nutr 12: 358-367

Mueller JF, and Vilter RW. 1950. Pyridoxine deficiency in human beings induced with desoxypyridoxine. J Clin Invest 29: 193-201

Muldoon TG, and Cidlowski JA. 1980. Specific modification of rat uterine estrogen receptor by pyridoxal 5'-phosphate. J Biol Chem 255: 3100-3107

National Diabetes Data Group. 1979. Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. Diabetes 28: 1039-1057

National Research Council. 1999. Recommended Dietary Allowances 9th ed. WA, D.C. National Academy Press, page 155-160

NHANES II. National Health and Nutrition Examination Survey. 1976-1990.

Nielsen HK, Thomsen K, Eriksen EF, Charles P, Storm T, and Mosekilde L. 1988. The effects of high-does glucocorticoid administration on serum bone gamma carboxyglutamic acid-containing protein, serum alkaline phosphatase and vitamin D metabolites in normal subjects. Bone and Mineral 4: 105-113

Nishigori H, Moudgil VK, and Taft D. 1978. Inactivation of avian progesterone receptor binding to ATP-sepharose by pyridoxal 5'-phosphate. Biochem Biophys Res Comm 80: 112-118

Ognibene A, Pala L, Messeri G, Rotella CM, and Berti P. 1997. Relations between intestinal alkaline phosphatase activity and insulin secretion in obese patients. Clin Chem 43: 1672-1673

Okada M, and Iwami T. 1977. Effect of pyridoxine deficiency on cholesterogenesis in rats fed different levels of protein. J Nutr Sci Vitaminol 23: 505-512

Okada M, Miyamoto E, Nishida T, Tomida T, and Shibuya M. 1997. Effect of vitamin B-6 nutrition and diabetes on vitamin B-6 metabolism. J Clin Biochem 8: 44-48

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

Parker TH, Marshall JP, and Roberts RK, et al. 1979. Effect of acute alcohol ingestion on plasma pyridoxal 5'-phosphate. Am J Clin Nutr 32: 1246-1252

Parry GJ, and Bredesen DE. 1985. Sensory neuropathy with low-dose pyridoxine. Neurol 35: 1466-1468

Pasanen AVO, Salmi M, Tenhunen R, and Vuopio P. 1982. Haem synthesis during pyridoxine therapy in two families with different types of hereditary sideroblastic anemia. Ann Clin Res 14: 61-65

Peiris AN, Mueller RA, Smith GA, Struve MF, and Kissebah AH. 1986. Splanchnic insulin metabolism in obesity. J Clin Invest 78: 1648-1657

Reiser S, Handler HB, Gardner LB, Hallfrisch JG, Michaelis OE, and Prather ES. 1979. Isocaloric exchange of dietary starch and sucrose in humans: effect of fasting blood insulin, glucose, and glucagon and on insulin and glucose response to a sucrose load. Am J Clin Nutr 32: 2206-2216

Reynolds RD, and Natta CL. 1985. Vitamin B-6 and sickle cell anemia. In: Reynolds RD and Leklem JE, eds. Vitamin B-6: Its Role and in Health and Disease. A. R. Liss, New York, page 301-306

Ribaya-Mercado JD, Russell RM, Sahyoun N, and et al. 1991. Vitamin B-6 requirements for elderly men and women. J Nutr 121: 1062-1074

Rodin J, Wack J, Ferrannini E, and DeFronzo RA. 1985. Effect of insulin and glucose on feeding behavior. Metabolism 34: 826-831

Rogers KS, and Mohan C. 1994. Vitamin B-6 metabolism and diabetes. Biochemical Medicine and Metabolic Biology 52: 10-17

Romero G, Luttrell L, Rogol A, and Zeller K, et al. 1988. Phosphatidylinositol-glycan anchors of membrane proteins: potential precursors of insulin mediators. Science 240: 509-511

Rose CS, Gyorgy P, Butler M, Andres R, Norris, AH, Shock NW, Tobin J, Brin M, and Spiegel H. 1976. Age differences in vitamin B-6 status of 617 men. Am J Clin Nutr 29: 847-853

Roy AV. 1970. Rapid method for determining alkaline phosphatase activity in serum with thymolpthalien monophosphate. Clin Chem 16: 431-436

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

Salhany JM, Schopfer LM. 1993. Pyridoxal 5'-phosphate binds specifically to soluble CD4 protein, the HIV-1 receptor. J Biol Chem 268: 7643-7645

Sall J, and Lehman A. 1996. JMP Start Statistics: A guide to statistics and data analysis using JMP and JMP in software.

Sauberlich HE. 1968. Section IX. Biochemical systems and biochemical detection of deficiency. In: Sebrell WH and Harris RS, eds. The vitamins: Chemistry, Physiology, Pathology, Assay. 2nd ed. Academic Press, New York: 2: 44-80

Sauberlich HE. 1981. Vitamin B-6 status assessment: past and present. In: Leklem JE and Reynolds RD, eds., Methods in Vitamin B-6 Nutrition. Plenum Press, New York., page 203-239

Sauberlich HE. 1985. Interaction of vitamin B-6 with other nutrients. In: Reynolds RD and Leklem JE, eds. Vitamin B-6: Its Role in Health and Disease. A. R. Liss, New York, page 193-217

Sauberlich HE, Canham JE, Baker EM, Raica N, and Herman YF. 1972. Biochemical assessment of the nutritional status of vitamin B-6 in the human. Am J Clin Nutr 25: 629-642

Schaltenbrand WE, Kennedy MS, and Coburn SP. 1987. Low-ultraviolet "white" fluorescent lamps fail to protect pyridoxal phosphate from photolysis. Clin Chem 33: 631

Schaumburg H, Kaplan J, Windebank A, Vick N, Rasmus S, Pleasure D, and Brown MJ. 1983. Sensory neuropathy from pyridoxine abuse: a new megavitamin syndrome. New Engl J Med 309: 445-448

Schirch L, and Jenkins WT. 1964. Serine transhydroxymethylase. J Biol Chem 239: 3797-3800

Shane B. 1978. Vitamin B6 and blood. In: Human Vitamin B-6 Requirements. Washington, D.C., National Academy press, page 111-128

Sharma SK ,and Dakshinamurti K. 1992. Determination of vitamin B-6 vitamers and pyridoxic acid in biological samples. J Chromatography 578: 45-51

Shultz TD, and Leklem JE. 1981. Supplementation and vitamin B-6 metabolism. In: Reynold RD and Leklem JE, eds., Vitamin B-6: Its Role in Health and Disease, A. R. Liss, New York, page 295-305

Shultz TD, and Leklem JE. 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: Leklem JE and Reynolds RD, eds., Methods in vitamin B-6 Nutrition, Plenum, New York, page 297-320

Shultz TD and Leklem JE. 1987. Vitamin B-6 status and bioavailability in vegetarian women. Am J Clin Nutr 46: 647-651

Simon C, Schlienger JL, Sapin R, and Imler M. 1986. Cephalic phase insulin secretion in relation to food presentation in normal and overweight subjects. Physiol Behav 36: 465-469

Sjostrom L, Garellick G, Krotkiewski M, and Luycks A. 1980. Peripheral insulin in response to the sight and smell of food. Metabolism 29: 901-909

Smith DE. 1991. Vitamin B-6 status of persons with diabetes mellitus. (MS thesis)

Snell EE. 1981. Vitamin B-6 analysis: some historical aspects. In: Leklem JE and Reynolds RD, eds. Methods in Vitamin B-6 Nutrition. Plenum, New York. page 1-19

Snell EE, Guirard BM, and Williams RJ. 1942. Occurrence in natural products of a physiologically active metabolite of pyridoxine. J Biol Chem 143: 519-530

Snyderman SE, Holt EH Jr, Carreters R, and Jacobs KG. 1953. Pyridoxine deficiency in the human infant. Am J Clin Nutr 1:200-207

Solomon LR, and Hillman RS. 1979. Regulation of vitamin B-6 metabolism in human red cells. Am J Clin Nutr 32: 1824-1831

Stephan J, Havranek T, Formankova J, Shrha J, Shrha F, and Pacovsky. 1980. Bone isoenzyme of serum alkaline phosphatase in diabetes mellitus. Clin Chim Acta 105: 75-81

Symes EK, Bender DA, Bowden JF, and Coulson WF. 1984. Increased target tissue uptake of, the sensitivity to, testosterone in the vitamin B-6 deficient rat. J Steriod Biochem 20: 1089-1093

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

Talbott MC, Miller LT, and Kerkviet NI. 1987. Pyridoxine supplementation: effect on lymphocyte responses in elderly persons. Am J Clin Nutr 46: 659-664

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

Thomas MR, and Kirksey A. 1976. A postnatal patterns of fatty acids in brain of progeny for vitamin B-6 deficient rats before and after pyridoxine supplementation. J Nutr 106: 1415-1420

Torsdottir I, and Anderson H. 1989. Effect on the postprandial glycaemic level of the addition of water to a meal ingested by healthy subjects and type 2 (non-insulin-dependant) diabetic patients. Diabetologia 32: 231-235

Trinder PJ. 1969. Determination of blood glucose using an oxidase-peroxidase system with a non-carcinogenic chromogen. J Clin Pathol 22: 158-161

Trumbo PR and Gregory JF III. 1988. Incomplete utilization of pyridoxine β-glucoside as vitamin B-6 in the rat. J Nutr 118: 170-175

Turnlund JR, Botschart AA, Liebman M, Kretsch MJ, and Sauberlich HE. 1992. Vitamin B-6 depletion followed by repletion with animal- or plant-source diets and calcium and magnesium metabolism in young women. Am J Clin Nutr 56:905-910

van den Berg H, Mulder J, Spanhaak S, van Dokkum W, and Ockhuizen T. 1988. The influence of marginal vitamin B-6 status on immunological indices. In: Leklem JE and Reynolds RD, eds. Clinical and Physiological Applications of Vitamin B-6. A. R. Liss, New York. Page 147-155

Vanderslice JT, Brownlee SR, and Cortissoy ME. 1984. Liquid chromatographic determination of vitamin B-6 in foods. J Assoc Off Anal Chem 67: 999-1007

Wada H, and Snell EE. 1961. The enzymatic oxidation of pyridoxine and pyridoxamine phosphates. J Biol Chem 236: 2089-2095

Wan DYY, Cerklewski FL, and Leklem JL. 1993. Increased plasma pyridoxal 5'-phosphate when alkaline phosphatase activity is reduced in moderately zinc-deficient rats. Biol Trace Elem Res 39: 203-210

Wasynczuk A, Kirksey A, and Morre DM. 1983a. Effects of vitamin B-6 deficiency on specific regions of developing rat brain: the extrapyramidal motor system. J Nutr 113: 746-754

Wasynczuk A, Kirksey A, and Morre DM. 1983b. Effect of maternal vitamin B-6 deficiency on specific regions of developing rat brain: amino acid metabolism. J Nutr 113: 735-745

Wingerd J, and Duffy T. 1977. Oral contraceptive use and other factors in the standard glucose tolerance test. Diabetes 26: 1024-1033

Witten PW, and Holman RT. 1952. Polyethenoid fatty acid metabolism, VI. Effect of pyridoxine on essential fatty acid conversions. Arc Biochem Biophys 41: 266-273

Woodring MJ, and Storvick CA. 1960. Vitamin B-6 in milk: review of literature. J Assoc Off Agric Chem 43: 63-80

Wright DW, Hansen RI, Mondon CE, and Reaven GM. 1983. Sucrose-induced insulin resistance in the rat: modulation by exercise and diet. Am J Clin Nutr 38: 879-883

Wursch P, Acheson K, Koellreutter B, and Jequier E. 1988. Metabolic effects of instant bean and potato over 6 hours. Am J Clin Nutr 48: 1418-1423

Yamada K, Okuyama S, and Kuzuya H, et al. 1968. Pathophysiological significance of vitamin B6 and its clinical application. In: Yamada K, Katunuma N, and Wada H, eds. Symposium on pyridoxal enzymes. Tokyo: Maruzen Co. page, 197-207

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

Young, DS. 1987. Implementation of SI units for clinical labortary data. Annal Int Med 106: 114-129

Zhang Z, Gregory JF III, and McCormick DB. 1993. Pyridoxine-5-β-glucoside competitively inhibits uptake of vitamin B-6 into isolated rat liver cells. J Nutr 123: 85-89

APPENDICES

Appendix A: Tables

Table 1. Summary of two 3-day 24-hour dietary records for subject #1

YKK-1 (F)

	Calories (Kcal)	Protein (g)	CHO (g)	B1 (mg)	B2 (mg)	B3 (mg)	B6 (mg)	Zn (mg)
Day 1 (1^{st})	2267.78	124.22	221.83	2.48	3.77	34.79	1.57	11.72
(2^{nd})	2201.16	73.10	307.62	1.92	1.59	32.62	1.82	6.00
Day 2	1795.45	68.56	310.36	2.08	2.08	26.19	1.52	10.13
	2587.03	81.36	435.88	2.66	1.73	25.47	2.09	12.07
Day 3	2211.93	116.89	190.60	2.52	2.37	19.74	1.70	11.61
	1643.00	86.11	208.23	1.55	3.14	23.53	2.03	10.37
Ave.	2091.72	103.22	241.26	2.36	2.74	26.91	1.60	11.16
	2143.73	80.19	317.24	2.04	2.15	27.21	1.98	9.48

(1^{st}): before the experiment; (2^{nd}): during the experiment

Table 2. Summary of two 3-day 24-hour dietary records for subject #2

JT-2 (M)

	Calories (Kcal)	Protein (g)	CHO (g)	B1 (mg)	B2 (mg)	B3 (mg)	B6 (mg)	Zn (mg)
Day 1 (1^{st})	904.97	28.05	109.33	1.14	5.18	14.64	0.65	3.81
(2^{nd})	2169.44	48.11	278.92	1.05	0.73	17.53	1.27	4.72
Day 2	3772.73	84.86	578.24	4.41	2.13	39.98	3.55	15.38
	2451.68	75.07	350.01	2.65	1.04	33.45	1.53	7.94
Day 3	3884.60	122.22	455.91	4.04	1.40	45.39	2.49	14.07
	1608.90	58.85	297.81	2.05	0.62	28.87	1.24	6.26
Ave.	2854.10	78.38	381.16	3.20	2.91	33.34	2.23	11.09
	2076.67	60.68	308.91	1.91	0.80	26.62	1.35	6.31

(1^{st}): before the experiment; (2^{nd}): during the experiment

Table 3. Summary of two 3-day 24-hour dietary records for subject #4

DM-4 (F)

	Calories (Kcal)	Protein (g)	CHO (g)	B1 (mg)	B2 (mg)	B3 (mg)	B6 (mg)	Zn (mg)
Day 1 (1^{st})	2861.75	89.52	418.42	1.89	2.86	20.52	1.25	8.86
(2^{nd})	2226.49	77.23	240.37	1.53	1.92	13.77	1.23	7.63
Day 2	1583	59	124.00	1.11	1.38	12.19	1.15	9.12
	1887.07	53.15	174.90	0.98	1.34	9.57	1.08	5.06
Day 3	1831.55	78.50	171.30	1.21	1.52	20.88	1.17	12.59
	1252.56	48.48	190.13	1.55	1.99	6.21	1.03	7.30
Ave.	2092	76	240.00	1.30	1.87	16.91	1.18	10.2
	1788.71	59.62	201.80	1.35	1.75	9.85	1.11	6.67

(1^{st}): before the experiment; (2^{nd}): during the experiment

Table 4. Summary of two 3-day 24-hour dietary records for subject #5

SI-5 (F)

	Calories (Kcal)	Protein (g)	CHO (g)	B1 (mg)	B2 (mg)	B3 (mg)	B6 (mg)	Zn (mg)
Day 1 (1^{st})	2171.19	76.88	355.15	1.93	1.97	28.71	2.17	11.48
(2^{nd})	1602.35	45.14	230.85	2.34	3.60	25.58	2.19	15.37
Day 2	2019.06	74.66	250.73	1.54	1.86	22.22	1.15	11.55
	1796.37	52.17	321.09	2.55	2.24	29.69	2.06	16.07
Day 3	1135.15	50.86	188.09	2.12	2.48	21.77	0.74	6.74
	1376.36	50.15	224.66	2.50	3.50	28.99	3.14	21.36
Ave.	1775.13	67.46	264.66	1.86	2.10	24.23	1.35	9.92
	1591.69	49.33	258.87	2.46	3.12	28.09	2.46	17.60

(1^{st}): before the experiment; (2^{nd}): during the experiment

Table 5. Summary of two 3-day 24-hour dietary records for subject #6

FM-6 (F)

	Calories (Kcal)	Protein (g)	CHO (g)	B1 (mg)	B2 (mg)	B3 (mg)	B6 (mg)	Zn (mg)
Day 1 (1^{st})	852	22	129.2	0.90	0.61	8.98	0.70	2.52
(2^{nd})	1633.51	58.24	295.94	1.09	1.40	12.83	1.83	8.24
Day 2	1362	46.57	198.00	1.55	1.18	14.66	1.30	4.32
	551.47	19.78	124.64	1.23	0.83	9.43	0.80	3.49
Day 3	1747	37.8	217.00	1.47	1.80	13.61	1.01	5.40
	1490.04	49.17	260.21	1.32	4.95	9.75	1.12	8.02
Ave.	1320.33	35	181.33	1.31	1.20	12.42	1.01	4.08
	1225.01	42.40	226.93	1.21	2.39	10.67	1.25	6.58

(1^{st}): before the experiment; (2^{nd}): during the experiment

Table 6. Summary of two 3-day 24-hour dietary records for subject #7

JJ-7 (M)

	Calories (Kcal)	Protein (g)	CHO (g)	B1 (mg)	B2 (mg)	B3 (mg)	B6 (mg)	Zn (mg)
Day 1 (1^{st})	3068.71	124.15	291.63	2.92	7.59	34.63	2.24	17.35
(2^{nd})	3764.01	62.43	297.94	1.83	2.48	17.37	1.66	8.30
Day 2	2234.94	100.85	257.07	2.21	6.69	27.00	1.86	14.56
	2513.86	99.12	304.16	2.09	3.26	24.76	2.98	12.48
Day 3	1635.14	73.18	183.42	1.99	4.51	14.66	0.88	7.53
	1509.50	84.12	141.87	1.21	1.81	22.74	1.28	6.57
Ave.	2312.93	99.39	244.04	2.38	6.27	25.43	1.66	13.15
	2595.79	81.89	247.99	1.71	2.52	21.62	1.97	9.12

(1^{st}): before the experiment; (2^{nd}): during the experiment

Table 7. Summary of two 3-day 24-hour dietary records for subject #8

MS-8 (M)

	Calories (Kcal)	Protein (g)	CHO (g)	B1 (mg)	B2 (mg)	B3 (mg)	B6 (mg)	Zn (mg)
Day 1 (1^{st})	2329.17	102.02	432.94	1.68	2.09	25.83	1.79	8.29
(2^{nd})	1749.08	112.98	189.94	1.66	2.63	26.31	2.12	12.88
Day 2	2175.37	78.10	346.85	1.08	5.44	8.07	1.25	6.60
	2988.54	105.66	541.73	2.40	2.34	28.16	1.33	11.64
Day 3	2554.39	86.72	443.11	2.24	2.50	20.49	1.22	9.80
	1602.67	61.12	268.46	1.30	2.03	12.77	1.01	7.51
Ave.	2352.98	88.95	407.63	1.67	3.35	18.13	1.42	8.23
	2113.43	93.25	333.38	1.79	2.33	22.42	1.49	10.68

(1^{st}): before the experiment; (2^{nd}): during the experiment

Table 8. Individual fasting blood screening

	Hgb (g/L)	Hct (%)	Plasma glucose (mmol/L)	Plasma PLP (nmol/L)
YKK-1	158	45.8	4.33	41.3
JT-2	168	50.5	4.33	45.1
DM-4	153	45	3.88	54.7
SI-5	155	44	4.47	43.7
FM-6	135	47	4.22	20.2
JJ-7	160	41	4.88	76.3
MS-8	146	41.3	4.41	23.5

Table 9. Individual plasma glucose concentration (mmol/L) after water ingestion

H2O		T0	T1	T2	T3
JT-2	M	4.94	4.36	4.55	4.99
JJ-7	M	5.11	4.69	4.88	4.69
MS-8	M	4.91	4.80	4.83	4.97
YKK-1	F	4.33	4.22	4.52	4.77
DM-4	F	4.77	4.80	4.69	4.83
SI-5	F	4.94	4.88	4.94	4.97
FM-6	F	4.33	4.25	4.16	4.22

Table 10. Individual plasma glucose concentration (mmol/L) after 25g glucose ingestion

25g Glucose		T0	T1	T2	T3
JT-2	M	5.97	8.24	4.77	5.16
JJ-7	M	5.05	3.83	4.16	4.66
MS-8	M	4.88	5.63	4.02	4.41
YKK-1	F	4.22	4.47	3.77	4.16
DM-4	F	4.55	3.25	4.25	4.77
SI-5	F	4.50	4.94	3.88	4.57
FM-6	F	4.83	6.83	4.36	4.50

Table 11. Individual plasma glucose concentration (mmol/L) after 75g glucose ingestion

75g Glucose		T0	T1	T2	T3
JT-2	M	4.22	5.83	3.52	2.22
JJ-7	M	4.97	6.85	5.85	3.11
MS-8	M	4.91	5.72	5.77	4.69
YKK-1	F	4.66	5.33	3.88	2.19
DM-4	F	4.55	4.94	4.11	3.88
SI-5	F	4.97	5.83	5.24	4.55
FM-6	F	4.13	5.77	4.11	4.69

Table 12. Individual plasma insulin concentration (μU/ml) after water ingestion

H2O		T0	T1	T2	T3
JT-2	M	9.0	-	-	8.5
JJ-7	M	12	-	-	9.4
MS-8	M	9.7	-	-	8.0
YKK-1	F	6.8	-	-	5.0
DM-4	F	7.2	-	-	4.1
SI-5	F	5.8	-	-	5.0
FM-6	F	11.7	-	-	7.5

Table 13. Individual plasma insulin concentration (μU/ml) after 25g glucose ingestion

25g Glucose		T0	T1	T2	T3
JT-2	M	9.3	31	10.9	8.5
JJ-7	M	11.2	26	10.5	9.8
MS-8	M	8.8	37	6.8	7.7
YKK-1	F	7.5	13.3	4.0	4.0
DM-4	F	5.4	7.8	5.0	4.9
SI-5	F	5.4	24.8	5.0	4.6
FM-6	F	7.6	31.5	5.3	5.9

Table 14. Individual plasma insulin concentration (μU/ml) after 75g glucose ingestion

75g Glucose		T0	T1	T2	T3
JT-2	M	7.5	50	36	8.0
JJ-7	M	9.3	51	60	9.5
MS-8	M	9.0	38	64	29.8
YKK-1	F	10.5	44	27	5.8
DM-4	F	4.4	50	16	3.25
SI-5	F	5.4	38	35.5	17.5
FM-6	F	7.3	35	28	25.5

Table 15. Individual plasma alkaline phosphatase activity (U/L) after water ingestion

H2O		T0	T1	T2	T3
JT-2	M	26.7	25.4	25.4	25.4
JJ-7	M	15.2	16.0	15.0	15.4
MS-8	M	35.7	35.1	36.2	36.1
YKK-1	F	16.0	15.8	16.6	17.3
DM-4	F	19.7	18.5	18.5	19.5
SI-5	F	14.5	14.5	14.7	15.2
FM-6	F	22.7	22.7	22.7	22.4

Table 16. Individual plasma alkaline phosphatase activity (U/L) after 25g glucose ingestion

25g Glucose		T0	T1	T2	T3
JT-2	M	25.1	25.6	26.6	26.6
JJ-7	M	22.7	22.6	22.5	22.2
MS-8	M	37.6	35.1	40.8	37.4
YKK-1	F	17.9	17.1	16.5	17.3
DM-4	F	17.2	17.5	18.2	17.2
SI-5	F	16.4	15.5	15.5	15.8
FM-6	F	22.1	17.3	16.3	13.9

Table 17. Individual plasma alkaline phosphatase activity (U/L) after 75g glucose ingestion

75g Glucose		T0	T1	T2	T3
JT-2	M	25.9	24.9	24.4	24.9
JJ-7	M	16.7	16.7	16.9	16.9
MS-8	M	34.6	35.2	35.2	35.4
YKK-1	F	18.6	18.4	17.9	18.7
DM-4	F	16.9	15.7	16.2	18.2
SI-5	F	15.5	15.2	15.9	15.2
FM-6	F	28.7	26.7	27.2	25.2

Table 18. Individual plasma pyridoxal 5′-phosphate concentration (nmol/L) after water ingestion

H2O		T0	T1	T2	T3
JT-2	M	47.4	39.9	41.9	37.9
JJ-7	M	84.9	79.6	71.4	71.1
MS-8	M	27.1	25.6	23.0	19.9
YKK-1	F	34.0	28.3	25.5	29.6
DM-4	F	41.9	43.8	40.5	36.3
SI-5	F	42.2	35.0	33.2	32.4
FM-6	F	30.0	25.4	25.6	34.4

Table 19. Individual plasma pyridoxal 5′-phosphate concentration (nmol/L) after 25g glucose ingestion

25g Glucose		T0	T1	T2	T3
JT-2	M	30.5	27.7	26.1	25.6
JJ-7	M	66.8	55.9	51.3	59.9
MS-8	M	33.9	28.0	25.6	28.3
YKK-1	F	45.5	45.4	44.7	37.6
DM-4	F	31.1	27.9	27.9	26.9
SI-5	F	45.2	37.5	37.3	38.7
FM-6	F	22.5	20.7	19.2	16.8

Table 20. Individual plasma pyridoxal 5′-phosphate concentration (nmol/L) after 75g glucose ingestion

75g Glucose		T0	T1	T2	T3
JT-2	M	41.3	34.1	31.6	31.7
JJ-7	M	74.6	67.8	58.3	63.8
MS-8	M	29.9	27.4	23.7	22.1
YKK-1	F	34.5	31.6	27.5	34.9
DM-4	F	51.1	38.5	37.5	40.4
SI-5	F	64.3	61.1	57.1	55.2
FM-6	F	41.0	32.2	29.2	30.9

Table 21. Individual plasma pyridoxal concentration (nmol/L) after water ingestion

H2O		T0	T1	T2	T3
JT-2	M	7.11	11.08	12.75	12.64
JJ-7	M	14.65	26.90	14.64	14.11
MS-8	M	8.72	13.68	4.16	10.49
YKK-1	F	8.26	10.31	7.69	10.07
DM-4	F	12.34	17.58	11.44	12.17
SI-5	F	13.03	11.94	9.66	12.15
FM-6	F	7.36	14.33	10.14	15.94

Table 22. Individual plasma pyridoxal concentration (nmol/L) after 25g glucose ingestion

25g Glucose		T0	T1	T2	T3
JT-2	M	-	7.41	5.97	9.66
JJ-7	M	17.64	16.84	7.79	16.13
MS-8	M	-	8.20	-	-
YKK-1	F	11.55	9.19	12.90	12.50
DM-4	F	8.11	11.16	15.24	10.93
SI-5	F	11.16	9.09	7.45	8.97
FM-6	F	8.76	8.60	5.07	11.47

Table 23. Individual plasma pyridoxal concentration (nmol/L) after 75g glucose ingestion

75g Glucose		T0	T1	T2	T3
JT-2	M	8.85	4.98	9.25	4.85
JJ-7	M	13.72	20.06	15.02	10.10
MS-8	M	11.05	13.29	6.83	8.33
YKK-1	F	4.89	8.01	14.21	11.41
DM-4	F	8.85	9.58	12.79	6.58
SI-5	F	24.58	38.56	18.31	18.11
FM-6	F	14.10	18.65	15.03	14.76

Table 24. Individual plasma 4-pyridoxic acid concentration (nmol/L) after water ingestion

H2O		T0	T1	T2	T3
JT-2	M	14.72	14.24	15.66	12.78
J-7	M	45.87	34.74	34.96	36.41
MS-8	M	24.43	20.53	22.65	22.04
YKK-1	F	10.00	8.48	7.70	7.02
DM-4	F	14.32	12.41	9.69	11.33
SI-5	F	11.23	8.91	10.94	9.03
FM-6	F	17.01	16.20	12.66	12.77

Table 25. Individual plasma 4-pyridoxic acid concentration (nmol/L) after 25g glucose ingestion

25g Glucose		T0	T1	T2	T3
JT-2	M	10.51	10.24	12.64	12.39
JJ-7	M	30.30	22.46	22.04	25.67
MS-8	M	21.30	14.20	19.92	19.90
YKK-1	F	13.31	13.10	13.82	13.11
DM-4	F	17.22	9.76	10.36	9.07
SI-5	F	12.22	7.99	10.48	13.43
FM-6	F	12.76	10.37	11.49	9.45

Table 26. Individual plasma 4-pyridoxic acid concentration (nmol/L) after 75g glucose ingestion

75g Glucose		T0	T1	T2	T3
JT-2	M	15.03	14.88	13.99	14.53
JJ-7	M	20.20	17.67	17.68	16.58
MS-8	M	24.61	22.13	26.68	19.33
YKK-1	F	10.28	9.27	8.86	9.92
DM-4	F	12.88	9.18	8.16	11.34
SI-5	F	13.68	14.19	13.75	12.44
FM-6	F	18.46	15.27	17.40	17.25

Table 27. Individual plasma pyridoxine concentration (nmol/L) after water ingestion

H2O		T0	T1	T2	T3
JT-2	M	10.12	9.40	7.14	15.77
JJ-7	M	8.78	6.94	7.17	7.74
MS-8	M	8.40	10.01	13.91	12.58
YKK-1	F	10.41	7.83	5.78	7.07
DM-4	F	7.45	8.13	8.06	8.17
SI-5	F	10.15	9.32	10.87	11.52
FM-6	F	14.54	11.21	11.72	10.04

Table 28. Individual plasma pyridoxine concentration (nmol/L) after 25g glucose ingestion

25g Glucose		T0	T1	T2	T3
JT-2	M	7.95	8.16	7.48	9.82
JJ-7	M	8.72	6.79	8.27	8.82
MS-8	M	10.03	12.54	9.39	8.11
YKK-1	F	5.37	6.67	7.53	7.86
DM-4	F	6.15	18.54	16.54	8.50
SI-5	F	7.98	9.38	8.02	7.73
FM-6	F	6.11	11.51	14.66	12.41

Table 29. Individual plasma pyridoxine concentration (nmol/L) after 75g glucose ingestion

75g Glucose		T0	T1	T2	T3
JT-2	M	11.29	9.91	13.13	11.37
JJ-7	M	14.21	11.20	5.33	8.49
MS-8	M	6.91	5.92	8.11	11.07
YKK-1	F	8.68	14.63	8.07	8.08
DM-4	F	17.84	12.95	13.26	11.80
SI-5	F	12.23	8.40	9.37	7.36
FM-6	F	14.23	17.57	14.92	13.67

Table 30. Individual red blood cell PLP concentration (nmol/L) after water ingestion

H2O		T0	T1	T2	T3
JT-2	M	46.05	39.47	40.99	40.48
JJ-7	M	53.13	51.11	47.56	48.07
MS-8	M	27.83	31.88	30.36	29.85
YKK-1	F	26.82	35.42	36.43	40.99
DM-4	F	35.42	33.40	30.87	31.3
SI-5	F	57.68	54.14	50.60	56.67
FM-6	F	56.67	56.17	55.66	55.15

Table 31. Individual red blood cell PLP concentration (nmol/L) after 25g glucose ingestion

25g Glucose		T0	T1	T2	T3
JT-2	M	41.49	40.99	39.97	40.48
JJ-7	M	47.06	49.08	52.12	45.54
MS-8	M	29.85	32.38	30.36	32.38
YKK-1	F	35.42	36.43	42.50	43.01
DM-4	F	34.41	40.48	40.48	43.52
SI-5	F	51.61	54.14	56.17	50.60
FM-6	F	52.12	51.11	55.15	58.19

Table 32. Individual red blood cell PLP concentration (nmol/L) after 75g glucose ingestion

75g Glucose		T0	T1	T2	T3
JT-2	M	52.62	37.95	49.08	46.05
JJ-7	M	52.62	51.11	47.56	58.70
MS-8	M	27.32	34.91	32.38	37.44
YKK-1	F	40.99	36.43	43.52	38.96
DM-4	F	31.88	34.91	44.53	46.55
SI-5	F	55.15	59.20	61.23	64.26
FM-6	F	60.72	54.14	65.78	56.67

Table 33. Individual hemoglobin (g/L)

		Trial 1	Trial 3
JT-2	M	168	187
JJ-7	M	164	161
MS-8	M	158	156
YKK-1	F	144	137
DM-4	F	145	134
SI-5	F	148	139
FM-6	F	143	139

Table 34. Individual hematocrit (%)

		Trial 1	Trial 3
JT-2	M	49	52
JJ-7	M	47.5	45.8
MS-8	M	46	45
YKK-1	F	41	39.5
DM-4	F	42.3	39
SI-5	F	41.8	39.3
FM-6	F	41	40

Appendix B: Figures

Figure 1. Approval of the oral glucose tolerance test (OGTT) study

Report of Review

TO: James Leklem and Ying-hui Huang
Nutrition and Food Management

FROM: Warren E. Suzuki, Chair, IRB

RE: "Effects of Two Levels of Glucose Ingestion on Plasma Pyridoxal 5'-Phosphate Concentration"

The referenced project was reviewed under the guidelines of Oregon State University's Committee for the Protection of Human Subjects and the U.S. Department of Health and Human Services. The committee has **approved** your application. The informed consent form obtained from each subject should be retained in your files for three years beyond the end date of the project.

Any proposed change to the protocol or informed consent form that is not included in the approved application must be submitted to the IRB for review and approval before it can be implemented. The approval of this application expires upon the completion of the project or one year from the approval date, whichever is sooner.

Date: 07/22/98

Warren E. Suzuki, Chair
Committee for the Protection of Human Subjects
(Education, 737-6393; suzukiw@ccmail.orst.edu)

Figure 2. Form of informed consent

Informed Consent Form for Two Nutrient Study
Department of Nutrition and Food Management
Oregon State University

Informed Consent

The purpose of this study is to examine the change of vitamin B6 in blood levels after glucose ingestion. In this study, participants will be asked to maintain their usual diets.

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

1. At least ten days before the beginning of the project, I will complete a healthy history questionnaire form which will be kept confidential. I will also have 7ml of blood drawn (equivalent to about ½ tablespoon) to measure my fasting glucose level and vitamin B6 level as screening criteria.

2. At least ten days before the beginning of the project, I will complete a 3-day 24-hour dietary record to evaluate my nutrient intake.

3. I will not have taken any drugs, oral contraceptives, or vitamin supplements for at least 6 weeks before the beginning of the project.

4. If I have fasting glucose concentration >126 mg/100 ml or use of drugs known to interfere with glucose tolerance or vitamin B6 metabolism, I will be eliminated from the study.

5. I will not take any drugs, oral contraceptives, or vitamin supplements during the study.

6. If I take any medication I will report this to the principle investigators.

7. I do not smoke and I will not smoke during the study.

8. This is a 3-week study.

9. I will maintain my body weight as much as possible during the study.

10. I will eat my own food and maintain an adequate nutrient diet during the study.

11. I will consume at least 200g carbohydrate/day (more than 7 ounces) for at least 3 days before each experimental period to ensure that I utilize the glucose load properly.

12. I will have a glucose load test on three separate occasions over a period of 3 weeks. For each of the three occasions, I will fast overnight (10-12 hours). After a fasting blood sample is collected, I will ingest the assigned solutions (300ml water with artificial sweetner equivalent to 25g glucose, 25g glucose in 300ml water, or 75g glucose in 300ml water) in a randomized order.

13. I will drink no more than 250ml water (equivalent to about 1 cup) 2 hours before each experiment.

14. During the study, I will maintain my usual level of physical activity.

15. I will not exercise 24 hours before each experiment.

16. At regular intervals, a total of 3 times over a period of 3 weeks, a medical technologist will draw 14ml of blood at time 0, 1, 2, and 3 hours (a total of 56ml equivalent to about 4 tablespoons) from my forearm. I understand that this procedure may cause a slight bruise.

17. All information obtained from me will be confidential. My data will be identified by a code number. The only person who will have access to my data are the principal investigator, the medical technologist and the graduate student who are assisting this research.

18. My participation in this research is voluntary and I can withdraw at anytime without loss of benefits except to those I am entitled.

19. At the end of this investigation, I will receive $35.00 for each glucose load test I participated in. (Total amount is $105.)

20. I will incur no medical or health risk from participating in this research. I will receive some benefits: a meal free of charge each time after the glucose load test, and results of Glucose Tolerance Test and other laboratory analyses if I request.

21. I understand the University does not provide a research subject with compensation or medical treatment in the event a subject in 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 Dr. James E. Leklem at 737-0969.

Signed_____Date_____
Printed Name_____
Present Address_____Phone No._____
Principle Investigator_____

Figure 3. Health/diet history questionnaire

8/20/10

HEALTH / DIET HISTORY--Aug 1990 version
CONFIDENTIAL

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

Project Name _____
_____
Project Dates _____

_____

Code #: _____ Today's Date: _____
Age: _____ Date of Birth: _____ Place of Birth: _____
Sex: M / F Predominant Place of Residence: _____
Present Employment: _____

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

Marital Status (circle one): a. Single b. Married c. Divorced/Separated d. Widowed
How many people live in your household? _____
Do you have any children? __ Yes __ No If yes, give ages _____

Females : MENSTRUAL and REPRODUCTIVE HISTORY

When did your last menstrual period begin? _____
Do you have regular menstrual periods? __ Yes __ No
How long is your menstrual cycle? _____
Do you have problems with your menstruation? __ Yes __ No
If yes, please explain:

Are you pregnant? __ Yes __ No Breast Feeding? __ Yes __ No
Have you ever been pregnant? __ Yes __ No
If yes, how many times? _____
How many children have you carried? _____
Please check if you have had any of the following complications of pregnancy:

__ 1. hyperemesis gravidarum (morning sickness)
__ 2. pre-eclampsia or eclampsia (toxemia)
__ 3. high blood pressure
__ 4. severe edema (swelling of your legs and feet)
__ 5. numbness and tingling in your hands, wrists, or arms
__ 6. gestational diabetes
__ 7. premature birth(s) (please indicate gestational age of infant(s))
_____
__ 8. kidney or bladder infections
__ 9. premature rupture of membranes
__ 10. small for dates infant (less than 5 lbs or 2500 g at term)

(Health/diet questionnaire, continued)

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

DIETARY HISTORY

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

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

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

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

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

(Health/diet questionnaire, continued)

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

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

Type	Amount per day	How long have you taken

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

Type	Amount per day	How long have you taken

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

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

(Health/diet questionnaire, continued)

HABITS: A. Smoking:

1) Do you currently smoke? __ Yes __ No

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

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

At what age did you start smoking? _____

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

If yes, at what age did you start? _____

If yes, when did you quit? _____

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

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

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

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

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

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

B. Alcohol:

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

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

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

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

C. Caffeine:

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

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

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

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

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

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

D. Diet Soda Pop and other Sugarless Beverages

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

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

(Health/diet questionnaire, continued)

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

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

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

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

MEDICAL HISTORY:

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

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

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

(Health/diet questionnaire, continued)

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

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

Comments:

(Health/diet questionnaire, continued)

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

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

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

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

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

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

Operation	Age or Year

(Health/diet questionnaire, continued)

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

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

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

COMMENTS:

Checked by _____ Date _____

Figure 4. Form for the 24-hour dietary record

INSTRUCTIONS FOR RECORDING FOOD

1. Please record each food and beverage you consume (except water) on a separate line. Be sure to indicate all snacks.

2. Record them in reasonably exact amounts: liquids in cups, fluid ounces or milliliters; vegetables and fruits in cups or inches using the ruler on the record sheets; beans, grains and pasta in cups dry or cups cooked; bread in slices, indicate what kind of bread; meats, fish and cheeses in ounces (an average meat portion is 3 oz., a slice of American cheese is about 1 oz.) or measure your servings with the ruler.

   If it is impractical to measure foods at certain meals, measure a comparable food at least once to establish in your mind the measure of certain quantities. Remember: the more accurate you record the more accurate analysis will be.

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

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

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

6. Use brand names wherever possible, or mention comparable brand name products.

7. Specify if a food is fortified with vitamins and minerals, or if it is a diet product. Please include the brand names.

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

9. Provide any other information you feel might be helpful.

10. Indicate if milk is whole, skim, 2% or dry non-fat milk.

11. Be sure to include sauces, gravies, milk in coffee etc. Everything you eat or drink.

DIET RECORD SHEET

CODE NO. _____

DATE CONSUMED: _____

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

FOOD Specify each food or beverage on a separate line	SOURCE Canned, dried, fresh etc.	BRAND Be specific	PREPARATION Fried, baked, raw etc.	AMOUNT Measure in cups inches etc.	FOR OFFICE USE AMT. code	WT. code