AN ABSTRACT OF THE THESIS OF

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The purpose of this study was to determine the relationship between the overall changes in concentration of plasma pyridoxal 5'-phosphate (PLP), red blood cell PLP (rbc PLP) and plasma glucose during an oral glucose tolerance test (OGTT) in persons with diabetes mellitus (DM), and to test the hypothesis that the decrease in plasma PLP concentration that occurs with increasing plasma glucose would be explained by a subsequent increase in rbc PLP concentration. A second objective was to compare the distribution of PLP between the red blood cell and the plasma (as measured by the rbc PLP/ plasma PLP ratio) in persons with diabetes to the distribution in non-diabetic controls. The third objective was to measure fasting plasma alkaline phosphatase (AP) activity, and to compare it to fasting plasma PLP concentrations, fasting rbc PLP concentrations, and the rbc PLP/plasma PLP ratio. The purpose of this third objective was to test the hypothesis that an increased plasma AP activity in persons with DM would be associated with decreased plasma PLP and increased rbc PLP concentrations.

The study included 8 persons (3F; 5M) with insulin dependent diabetes mellitus (IDDM), 9 persons (5F; 4M) with non-insulin dependent diabetes mellitus (NIDDM) and 18 healthy control individuals (9F; 9M). All subjects were given a 75 gm oral D-glucose dose, and blood was drawn at 0 (fasting), 30, 60 and 120 minutes after the glucose load.

Plasma glucose, PLP, insulin, and rbc PLP concentrations were measured at all time points during the OGTT. Fasting plasma alkaline phosphatase (AP) activity, percent glycosylated hemoglobin (%GlyHb), and the ratio between fasting rbc PLP and fasting plasma PLP were also determined.

In general, females with DM were in poorer diabetic control as compared to males with DM. Mean fasting glucose levels, %GlyHb and body mass index (BMI) were highest in females with DM as compared to all other groups, and fasting insulin was nearly 2x higher in females with NIDDM as compared to males with NIDDM.

There was an overall decrease in plasma PLP during the OGTT with increasing plasma glucose, which agrees with results from other studies. The overall decrease in plasma PLP (as measured by the negative, cumulative area under the curve: -AUC plp) was significantly correlated with the overall increase in plasma glucose (as measured by the positive, cumulative area under the curve: +AUC glu) for all study groups. The relationship was stronger in all males, and control females as compared to females with diabetes ($p< 0.001$ vs. $p< 0.01$, respectively). This difference was in part explained by lower mean fasting PLP levels in females with DM (19.3 nmol/L), as compared to males with DM (47.2nmol/L) and male and female controls (35.4 nmol/L and 34.0 nmol/L, respectively).

The changes in rbc PLP during the OGTT were minimal, and did not significantly correlate with the increase in plasma glucose or the decrease in plasma PLP. Thus, the acute drop in plasma PLP concentration that occurred during the OGTT was not explained by a subsequent increase in rbc PLP concentration, as had been hypothesized. However, the higher than normal % glycosylated hemoglobin levels along with elevated rbc PLP concentrations in persons with diabetes as compared to controls suggests that chronically elevated blood glucose can contribute to increased rbc PLP concentrations.

This was the first study to date that has measured rbc PLP in persons with diabetes mellitus. Rbc PLP values for persons with DM were 20-40% greater than respective

control values at all time points during the OGTT. These differences between mean rbc PLP in persons with DM as compared to control groups were all statistically significant ($p < 0.05$) with the exception of the difference in the mean fasting rbc PLP value for females with NIDDM as compared to controls. The mean values ± standard deviations (SD) for fasting rbc PLP (nmol/L) were as follows: Females-IDDM, 49.5 ± 6.5; NIDDM, 39.3 ± 4.9; controls, 31.4 ± 9.0; Males-IDDM, 37.8 ± 10.9; NIDDM, 45.6 ± 12.3; controls, 28.3 ± 4.4. The ratio of fasting rbc PLP concentration to fasting plasma PLP concentration was 2-3x higher in females with DM as compared to control females and all male groups. Females with IDDM had a ratio of 3.2, and the ratio for females with NIDDM was 2.2. The ratios for all male groups, and control females were approximately 1:1, with a range of 0.8-1.2.

The mean fasting plasma AP activity was within the normal range for all study groups. However, females with DM had higher AP activity (0.543 μkat/L) as compared to female controls and males with DM (0.408 μkat/L, .425 μkat/L, respectively $p<0.05$). There were no significant differences in mean fasting plasma AP activity between any male group (range 0.390-0.465 μkat/L).

These results suggest that increased plasma glucose levels, increased AP activity, and overall poor glycemic control contribute to decreased plasma PLP concentrations, increased rbc PLP concentrations, and possibly to changes in the PLP distribution within the body.

Changes in Plasma Pyridoxal 5'-Phosphate and Red Blood Cell Pyridoxal 5'-Phosphate Concentration during an Oral Glucose Tolerance Test in Persons with Diabetes Mellitus.

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I. INTRODUCTION

Diabetes mellitus is a disease of the endocrine system that affects nearly 6 million persons in the U.S. (Harris, 1985). The disease is characterized by hyperglycemia that is the result of an absolute or relative deficiency of insulin (National Diabetes Data Group (NDDG), 1979). 5%-10% of the diabetic population have type-1 diabetes, or insulin dependent diabetes mellitus (IDDM) and 75-95% have type-2 diabetes, or non-insulin dependent diabetes mellitus (NIDDM) (Zeeman, 1983). Complications of diabetes include neuropathy, nephropathy, retinopathy, coronary artery disease, and decreased immunologial function (Anderson, 1988, Reaven, 1988).

Vitamin B6, a water soluble vitamin, refers to the six derivatives of 3-hydroxy-2-methylpyridine that are collectively known as vitamin B6 (IUPAC-IUB, 1973). These six forms and abbreviations are: pyridoxal (PL), pyridoxine (PN), pyridoxamine (PM) and their respective phosphorylated forms: pyridoxal-5'-phosphate (PLP), pyridoxine 5'-phosphate (PNP), and pyridoxamine 5'-phosphate (PMP). PLP, the active form of vitamin B6 (Leklem, 1988-b) is involved in over 100 enzymatic reactions in the human body (Sauberlich, 1985).

Sustained hyperglycemia, such as that which occurs in diabetes mellitus, leads to increased nonenzymatic glycosylation of proteins in blood and tissues (Miedema and Casparie, 1984). Increased levels of glycosylated proteins (such as glycosylated hemoglobin) in turn increases the risk for many diabetic complications such as peripheral neuropathy, nephropathy and retinopathy (Klein et al, 1988; Cahill, 1985). Alternatively, in vitro studies have shown PLP to inhibit the nonenzymatic glycosylation of proteins by sugar (Khatami, 1990; Shepard et al, 1985). Although this inhibition of nonenzymatic

glycosylation may have different characteristics in vivo, the possibility exists that a low level of PLP in persons with diabetes may result in a relative loss of inhibition of nonenzymatic glycosylation, further increasing the chance for complications.

Diabetes mellitus is associated with a low vitamin B6 status (as measured by plasma PLP concentration), despite adequate intake of vitamin B6 (Hollenbeck et al, 1983; Rogers et al, 1986; Manore et al, 1989, 1991). The reason for lower plasma PLP levels in persons with diabetes as compared to non-diabetic individuals may be related to higher than normal blood glucose levels. Studies have shown plasma PLP concentration to decrease with increasing plasma glucose concentrations after an oral glucose load (Leklem, 1984, 1990; Hollenbeck and Leklem, 1985).

The first objective of the current study was to conduct an oral glucose tolerance test (OGTT) in persons with and without diabetes, and to see if the overall change in plasma PLP concentration during the OGTT was significantly related to the overall change in plasma glucose concentration. Also, the study was designed to help answer the questions: why does plasma PLP decrease (during the OGTT), and where does it go?

Results from several studies suggest that glucose enhances the uptake of B6 vitamers into the red blood cell (Ink et al, 1982, 1984; Yamada et al, 1968). Thus, a second objective was to measure red blood cell (rbc) PLP values at all time points during the OGTT, and to determine whether the increase in plasma glucose concentration and the decrease in plasma PLP concentration that occurred during the OGTT correlated with a subsequent increase in rbc PLP concentration. Also, the ratio of fasting rbc PLP/plasma PLP was calculated in order to compare the distribution of PLP between the plasma and the red blood cell in persons with and without diabetes. This was the first study to date to document red blood cell PLP values in persons with diabetes.

The third objective of this study was to measure fasting plasma alkaline phosphatase (AP) activity, and to examine the relationship between fasting plasma AP, fasting rbc PLP concentration and plasma PLP concentration, and the fasting rbc PLP/plasma

PLP ratio. AP activity has been shown to be higher in persons with diabetes (Goldberg et al, 1977; Stephan et al, 1980; Maxwell et al, 1986). Studies have also shown an inverse relationship between AP activity and plasma PLP concentration (Whyte et al, 1985, 1988; Kant et al, 1988). AP dephosphorylates PLP, resulting in pyridoxal (PL). PL is readily taken up by the red blood cell, where as PLP is not. Once inside the red blood cell, PL can be re-phosphorylated to PLP. Thus, the hypothesis was that fasting AP activity would be increased in persons with DM, and that the increased activity would correlate with decreased fasting plasma PLP levels, increased fasting rbc PLP concentration and an increased rbc PLP/plasma PLP ratio.

In summary, the purpose of this study was to determine the relationship between the overall changes in plasma PLP concentration, red blood cell PLP concentration and plasma glucose concentration during an oral glucose tolerance test in persons with diabetes mellitus, in order to determine the effect of high blood glucose levels on PLP, the active form of vitamin B6 in the human body. Another objective was to examine the distribution of PLP between the plasma and red blood cell in persons with diabetes, and to compare the results to non-diabetic controls. Finally, fasting AP activity was measured and correlated with fasting plasma PLP concentrations, fasting rbc PLP concentrations, and the fasting rbc PLP/plasma PLP ratio in order to determine the relationship between plasma AP activity and the distribution of PLP between red blood cells and plasma.

II. LITERATURE REVIEW

VITAMIN B6

History and Chemistry

Vitamin B6, a water soluble vitamin was discovered (Gyorgy, 1934), isolated (Gyorgy, 1938), and synthesized (Gyorgy and Eckardt, 1939) by Paul Gyorgy a little over a half century ago. Many trivial and sometimes incorrect names for vitamin B6 and it's derivatives arose in the following decades (IUPAC-IUB, 1973). In 1973 the IUPAC-IUB Commission on Biochemical Nomenclature presented recomendations for names and abbreviations to be used to correctly describe the six derivatives of 3-hydroxy-2-methyl-pyridine that are collectively known as vitamin B6 (IUPAC-IUB, 1973). These six forms and abbreviations are: pyridoxal (PL), pyridoxine (PN), pyridoxamine (PM) and their respective phosphorylated forms; pyridoxal-5'-phosphate (PLP), pyridoxine 5'-phosphate (PNP), and pyridoxamine 5'-phosphate (PMP). The major excretory product of vitamin B6 is 4-pyridoxic acid (4-PA), an inactive metabolite that is formed from the irreversible oxidation of PL. Figure 1 illustrates the chemical structure of the above seven forms. Another form of vitamin B6, 5'-O-(B-D-glucopyranosyl) pyridoxine (PN-glucoside) was first isolated in rice bran (Yasumoto et al, 1977) and is found in many foods of plant origin.

Vitamin B6 is a light sensitive compound (Ang, 1979). Using various concentrations of PN.HCL, PL.HCL, and PM.HCL solutions, Ang demonstrated that the solutions were stable when exposed to yellow or golden flourescent light (94-108% retention), but that regular white light resulted in destruction to the vitamins, with increased destruction at increasing pH and longer exposure (Ang, 1979).

Food Sources

The six forms of vitamin B6 are found in both plant and animal foods (Orr, 1969). PL, PLP and PMP are the predominant forms in animal products, whereas PN, PM and their respective phosphorylated forms are the predominant forms in plant products. According to a 1980 survey, the contribution of major food groups to the United States food supply of vitamin B6 are as follows: meats 40.0%, vegetables 22.2%, dairy items 11.6%, cereals 10.2%, fruits 8.2%, legumes 5.4%, and eggs 2.1% (Sauberlich, 1981). Estimates of major dietary sources of vitamin B6 from the second National Health and Nutrition Examination Survey (NHANES II, 1976-1990) are: meats 28.0%, vegetables 14.5%, breads and cereals 13.2%, dairy 8.0%, fruits 7.0%, alcohol 6.0%, nuts and legumes 3.8%, eggs 2.0%. As mentioned earlier, many plant foods also contain a conjugated form of vitamin B6. The bioavailability of PN-glucoside (glycosylated B6) will be discussed in further detail in a later section.

Absorption

The majority of information on vitamin B6 absorption has come from studies utilizing rats as models (Middleton, 1977, 1982, 1985). Absorption of the non-phosphorylated forms of vitamin B6 occurs in the small intestine, decreasing in absorption from the proximal to the distal end. Most studies indicate that absorption at the mucosal level occurs via passive diffusion, (Middleton 1977, 1982) although recently there has been some indication that there may be a saturable component of intestinal vitamin B6 absorption that is linked to intracellular metabolism (Middleton, 1985). Middleton has suggested that intracellular phosphorylation of B6 compounds may create a larger mucosal transmembrane concentration gradient for absorption of non-phosphorylated forms than would be possible in the absence of intracellular phosphorylation (Middleton, 1985). Phosphorylated forms of vitamin B6 are dephosphorylated by alkaline phosphatase (Middleton, 1982) and the subsequent non phosphorylated forms are absorbed. Limited

absorption of phosphorylated forms does occur, but only at high levels (Henderson, 1985).

Bioavailability

The definition of bioavailability of a nutrient is the amount absorbed and available to cells compared to the total amount present. It is important to study the bioavailability of a nutrient for several reasons: 1) to better evaluate the nutritional status, 2) to identify potential nutritional deficiencies in a given population, 3) as an aid in setting nutrient requirements, and 4) to understand the nutritional qualities of food. The bioavailability of vitamin B6 has been shown to be affected by several factors, including fiber (bran) content of food, type of food (plant vs animal) and the amount of glycosylated B6 in food.

The bioavailabilty of B6 in different kinds of bread was studied in nine men (Leklem et al, 1980-a). For one week, men were fed 570-600 gm/day of either whole wheat bread (WHW), white bread fortified with B6 (WB6), or white bread (W) that provided 1.2 mg, 1.18 mg and 0.35 mg vitamin B6, respectively. A constant diet also provided 0.38 mg B6/day, and a daily oral dose of 0.8 mg PN.HCL was taken during the W phase to bring the total vitamin B6 intake up to 1.5 mg during each phase. Urinary 4-PA excretion was decreased and fecal B6 excretion was increased when men were fed the whole wheat bread, as compared to the fortified white bread and white bread along with the B6 solution. These results indicate that vitamin B6 from whole wheat bread is 5-10% less available than from white bread or crystalline B6.

Other studies have shown that when various types of bran are added to the diet, urinary 4PA excretion decreases, as compared to diets without added bran (Lindberg et al, 1983; Kies et al, 1984). As bran is a good source of vitamin B6, it is unclear whether the B6 in the bran is unavailable, or whether the bran itself is limiting the amount of B6 available from other foods by increased binding and/or decreased transit time (Leklem,

1991). In contrast, results from a study of vegetarian vs nonvegetarian women showed that although the fiber content of the vegetarian women's diet was significantly higher than that of the nonvegetarians diet, there was no significant difference in urinary 4PA excretion (Shultz and Leklem, 1987). However, there was an overall decrease in vitamin B6 status in vegetarians as compared to nonvegetarians.

Bioavailability of vitamin B6 from different types of food has been measured in several studies. Leklem et al demonstrated that vitamin B6 in beef is 6-7% more available than equal amounts of vitamin B6 in soybeans (Leklem et al, 1980-b). Kabir measured vitamin B6 bioavailabilty in tuna, peanut butter, and whole wheat bread, and found that compared to tuna (which was considered to be 100% available), the bioavailability from peanut butter and whole wheat bread was 65% and 75%, respectively (Kabir et al, 1983). These results indicate that the bioavailabilty of vitamin B6 may be greater in animal foods as compared to vegetable foods.

Animal products do not contain measurable glycosylated B6, whereas foods of vegetable origin contain varying amounts (Leklem, 1988-a). The amount of glycosylated B6 has been shown to be inversely correlated with bioavailability as measured by urinary B6 and 4PA excretion (Kabir et al, 1983; Bills et al, 1987). Although there are other factors that may contribute to the bioavailabilty of vitamin B6 in a specific food, such as the binding of vitamin B6 to fiber (Lindberg et al, 1983) or exposure to elevated temperatures (Harding et al, 1959), percent glycosylated B6 is considered a relatively good indicator of B6 bioavailability (Bills et al, 1987).

Metabolism

Upon reaching the circulation, PN, PL and PM are rapidly transported into organs and tissues (Ink and Henderson, 1984). The liver is the primary organ for the interconversion and metabolism of vitamin B6 vitamers (Lumeng, 1985), and is thought to be responsible for forming the majority of PLP found in plasma (Lumeng et al, 1974). Work

by Merrill et al (1984, 1990) demonstrated the existence and activity of the enzymes involved in B6 metabolism in the human liver (figure 2). Pyridoxal kinase, located in the cytosol of liver cells, adds a phosphate to PN, PL, and PM to form their respective phosphorylated forms. Both zinc and ATP are required for kinase activity. Magnesium may also serve as a cation for kinase activity, but enzyme activity yield is lower with magnesium, as compared to zinc (Merrill et al, 1984).

Pyridoxine (pyridoxamine) 5'-phosphate oxidase, also located in the cytosol, oxidizes PNP and PMP to form PLP (Kazarinoff and McCormick, 1975). Oxidase is highly sensitive to product inhibition, thus activity may be controlled by cytosolic PLP concentration (Merrill et al, 1984, 1990). Riboflavin, in the form of flavin mononucleotide (FMN) is required for maximal oxidase activity (Wada and Snell, 1961).

Pyridoxal-5'-phosphatase removes a phosphate from PLP, forming PL. Although the maximal activity of PL-5'-phosphatase is over 20 times greater than PL-kinase, the actual phosphatase activity at the physiological pH of 7.4 is only 0.3% of maximum; thus, the kinase reaction is favored, preventing immediate conversion of all PLP to PL (Merrill et al, 1984, 1990). Tissue non-specific alkaline phosphatase appears to be the phosphatase enzyme responsible for the majority of PLP hydrolysis (Lumeng et al, 1974, Coburn and Whyte, 1988), although Fonda has recently isolated a neutral phosphatase in human erythrocytes, which hydrolyze both PLP and PNP (Fonda, 1987).

Pyridoxal (aldehyde) oxidase and dehydrogenase convert PL to 4PA in an irreversible reaction. In humans, only the oxidase has been detected, as opposed to other species in which the dehydrogenase predominates (Merrill et al, 1984). Under physiological concentrations, pyridoxal oxidase has the highest activity of all the vitamin B6 enzymes involved in B6 metabolism in humans (Merrill et al, 1984).

PLP is highly reactive to proteins, and readily forms a Schiff-base (Li et al, 1974). Li et al demonstrated that at least 50% of the PLP in rat hepatocyte is bound to cytosolic proteins, with the rest existing as free (unbound) PLP in the cytosolic fraction of hepato-

cytes (Li et al, 1974). The binding markedly decreases the susceptibility of PLP degradation by phosphatase to PL and thus 4PA. This metabolic trapping of phosphorylated compounds, along with the favored kinase reaction helps offset the high activity of PL (aldehyde) oxidase, assuring adequate supplies of PLP.

Lumeng et al (1974) demonstrated that most of the PLP in human plasma is protein bound through formation of a Schiff-base, with preferential binding to albumin. Hydrolysis of protein bound PLP by alkaline phosphatase still occurs, but at a much slower rate than hydrolysis of unbound PLP (Lumeng et al, 1974). Albumin also serves as a transport molecule for PLP, delivering it from the liver to other tissues and organs, where membrane bound alkaline phosphatase can hydrolyze PLP to PL, thus allowing uptake of PL by passive diffussion (Mehansho et al, 1979).

Under fasting conditions, PLP, PMP, PL, PM and PN have been detected in human plasma (Lumeng et al, 1985; Coburn and Mahuren, 1983). PLP and PL are the predominant forms, making up 70-90% of total B6 in plasma, with PLP comprising 50-75% (Leklem, 1991). After an oral dose of 10-25 mg PN.HCL, PLP, PL and 4-PA rapidly increase in plasma, while PN is quickly eliminated from plasma (Ubbink and Serfontein, 1988; Lumeng et al, 1985; Henderson et al, 1986). Most organs and tissues contain pyridoxal kinase (McCormick et al, 1961), but many lack the oxidase that is required to convert PNP and PMP to PLP, and thus PL and 4-PA. This again points to the liver as being the primary organ for metabolism of vitamin B6.

Red Blood Cell Metabolism

The erythrocyte is another cell type that can actively metabolize vitamin B6 to the various vitamer forms. Red blood cells contain kinase, oxidase and phosphatase enzymes, thus allowing interconversion of all forms of vitamin B6 (Anderson et al, 1971; Lumeng and Li, 1974). Once considered to play only a minor role in total B6 metabolism in comparison to that of the liver, it now appears that erythrocytes may contribute to

overall vitamin B6 metabolism. The significance of the contribution, and the exact nature of the role that red blood cells play has not been fully elucidated.

Rapid uptake of PN and PL by red blood cells has been demonstrated by several researchers (Anderson et al, 1971, 1989; Fonda and Harker, 1982, Mehansho and Henderson, 1980; Ink et al, 1982, 1984). Both B6 vitamers enter the red blood cell by passive diffusion (Menshano and Henderson, 1980; Ink et al, 1982). PL binds to both albumin and hemoglobin (Mehansho and Henderson, 1980; Fonda and Harker, 1982; Ink et al, 1982, 1984). Whereas less than 50% of plasma PL is bound to albumin, (Anderson, 1980), nearly all of erythrocyte PL is hemoglobin bound (Mehansho and Henderson, 1980; Ink et al, 1982). This suggests that PL binds more tightly to hemoglobin than to albumin. In whole blood the ratio of PL in erythrocytes vs. plasma has been shown to be 4-5:1 (Ink and Henderson, 1984). This concentrative ablity of RBC's to store PL may play a key role in red blood cell metabolism of vitamin B6. By decreasing erythrocyte concentration of whole blood in rats, Ink and coworkers demonstrated that 4-PA excretion increased compared to rats with normal hemoglobin levels (Ink and Henderson, 1984). This suggests that red blood cells may have a protective role in B6 metabolism, by preventing quick breakdown of B6 vitamers to the end product, 4-PA.

The strong binding of PL to hemoglobin also suggests that the erythrocyte could be a major source of circulating B6 (Mehansho and Henderson, 1980). On the other hand, Fonda suggests that there is an equilibrium between hemoglobin-bound PL, and PL free in the cytosol and that it is this free PL that is relased to plasma, and available to other tissues (Fonda and Harker, 1982). The extent and significance of this availability is unknown. Furthermore, as mentioned earlier, after an oral dose of 10 mg PN (12.2 mg PN.HCL), PLP, PL and 4-PA increased rapidly in plasma, while PN quickly declined (Ubbink and Serfontein, 1988). Ubbink suggests that because the rate of appearance of PL was significantly higher than that of PLP, this indicates that plasma PL is derived

from more than one source (ie, liver and erythrocytes), whereas plasma PLP is solely derived from liver (Ubbink and Serfontein, 1988).

PN, the most common form of vitamin B6 in both food and therapeutic sources does not bind to protein (Anderson 1980; Fonda and Harker, 1982). After uptake by the erythrocyte, PN is "trapped" by phosphorylation to PNP, and then can be converted to PLP and PL. Fonda and coworkers demonstrated this conversion of PN to PLP in human erythrocytes (Fonda and Harker, 1982). Their results also showed that much of the newly synthesized PLP was bound to hemoglobin. Anderson recently verified (in vivo) the rapid uptake of PN into red blood cells, and it's conversion to PL and PLP (Anderson et al, 1989). However, the subsequent rapid disapearence of PN was not fully accounted for by conversion to PL and PLP, suggesting that erythrocytes may deliver PN to other tissues (liver) for conversion to PL and PLP.

PLP has been shown to exist in a 1:1 to 1:1.8 ratio between plasma and red blood cells (Bhagavan et al, 1975; Kant et al, 1988). Plasma PLP does not readily cross the erythrocyte membrane (Anderson et al, 1971; Lumeng and Li, 1974; Coburn et al, 1992). The strong binding of PLP to albumin prevents uptake of PLP by the erythrocyte (Anderson et al, 1971; Lumeng and Li, 1974). PLP that is formed inside the red blood cell (from other B6 vitamers) is bound to both hemoglobin and aminotransferases (Fonda and Harker, 1982; Yagi et al, 1993). Yagi showed that 13% of erythrocyte PLP was bound to aminotransferases, and the remaining 87% was bound to hemoglobin. Red blood cell PLP is also not released in the phosphorylated form, but can be dephosphorylated to PL and is available to other tissues indirectly, in the form of PL (Anderson et al, 1971). The extent of this availability is unknown.

PLP and PL bind to separate sites on the hemoglobin molecule, and thus do not compete for binding sites (Benesch et al, 1972; Ink et al, 1982). PL binds predominantly to the NH2-terminal of the alpha chain , whereas preferential binding of PLP to hemoglo bin occurs on the beta chain. The binding of PLP to hemoglobin is tighter than that of PL

(Benesch et al, 1973). Glucose also binds to the NH2 terminus of the alpha chain of hemoglobin, but does not signifcantly affect binding of PL (Ink et al, 1982). In fact, uptake of H3-PL by human erythrocytes in vitro was increased when increasing concentrations of glucose were added to the incubating medium (Ink et al, 1982, 1984). Although the statistical significance of the data were not given, these results provide some evidence that PL uptake by red blood cells may be enhanced by glucose. Yamada et al (1968) reported glucose enhancement of B6 vitamers uptake into tumor cells.

Methods

Enzymatic, microbiological and chemical assays are three types of methods that have been used by most researchers for measurement of B6 vitamers in red blood cells. The most sensitive assay for PLP appears to be an enzymatic method that utilizes tyrosine decarboxlyase to convert L-tyrosine-14C to tyramine and 14CO2 (Reynolds and Leklem, 1981), with PLP acting as a coenzyme in the conversion. Unfortunately, measurement of other B6 vitamers is not possible with this method. Also, the tight binding of PLP to hemoglobin makes deproteinization of the PLP-hemoglobin molecule difficult, and thus recoveries variable. Preliminary results from the Foods and Nutrition lab at Oregon State University have shown that varying the concentration of trichloroacetic acid (TCA) in the deproteinization step significantly changes the amount of PLP available for measurement in the tyrosine decarboxylase enzymatic assay. Anderson and coworkers have used the organism Lactobacillus casei in the microbiological measurement of B6 vitamers in red blood cells (Anderson et al, 1971, 1980, 1989). As the organism only responds to PL, measurement of other B6 vitamers can be done only after they are first converted to PL. This extra step may increase the chance of experimental error. More recently, researchers have been utilizing various chemical assays to measure B6 vitamers in red blood cells (Ink et al, 1982; Fonda et al, 1982, 1989; Ubbink and Schnell, 1988).

All six forms of vitamin B6 can be detected fluorometrically by HPLC methods of chemical analysis (Coburn and Mahuren, 1983; Fonda et al, 1989). Although fluorometric methods can be automated, and thus are useful for quick and numerous measurements, they are extremely sensitive to interfering compounds, making over or underestimation a likely possibility. Also, as the sample must first be deproteinized, with PLP there still is the problem of low recoveries due to the strong binding of PLP to hemoglobin in the erythrocyte (Fonda et al, 1989).

Microbiological assays using Saccharomyces uvarum are used for the determination of total B6 in foods and other biological materials (Gregory, 1988). Although use of the assay is generally accepted (Toukairin-Oda et al, 1989), it is difficult to execute properly. The extraction steps are cumbersome and the column separation of vitamers (if used) is time consuming (Toukairin-Oda et al, 1989; Reynolds and Leklem, 1981). Also, responsiveness of Saccharomyces uvarum to different B6 vitamers has varied between laboratories, for as yet unknown reasons (Reynolds and Leklem, 1981).

Function

PLP is the active form of vitamin B6, thus most of the vitamins functional properties are as PLP. PLP forms a Schiff base with amino acids and other nitrogen containing compounds, enabling the vitamer to carry out a number of enzymatic reactions. In fact, PLP is the necessary coenzyme for over 100 enzymatic reactions in the human body (Sauberlich, 1968).

Amino acid metabolism is largely dependent on PLP, as all of the known aminotransferases are PLP dependent (Sauberlich, 1985). For example, the transamination of aspartate and alpha-ketogluterate to oxalacetate and glutamate require aspartate aminotransferase as the enzyme, and PLP as the coenzyme and the transamination of alanine and alpha-ketogluterate to pyruvate and glutamate require the enzyme alanine aminotransferase, with PLP as the coenzyme. Amino acid metabolism is also dependent

upon PLP as the coenzyme in other reactions including decarboxylations, aldolizations, alpha-beta and beta-alpha eliminations, and desulfurations (Sauberlich 1985).

The direct involvement of PLP in amino acid metabolism indirectly involves vitamin B6 in many other cellular functions, including: lipid metabolism, nervous system function, immune function, niacin formation, red blood cell function, and gluconeogenesis (Leklem, 1988-b). Vitamin B6 is thought to be involved in lipid metabolism (Sauberlich, 1985; Cunnane et al, 1985), although the exact role remains unclear (Leklem, 1991). Possibilities include PLP's involvement in methionine metabolism (Loo and Smith,1986) and thus phospholipid and fatty acid metabolism, and PLP's role in carnitine synthesis (Cunnane et al, 1985; Cho and Leklem, 1990).

Vitamin B6 depletion in Rhesus monkeys resulted in arteriosclerosis and development of fatty, cirrohtic livers (Rinehart and Greenberg, 1956). Okada and coworkers showed that feeding of a high protein diet to B6 deficient rats caused accumulation of triglycerides and cholesterol in the liver (Okada et al, 1971, 1977). Supplementation of high protein diets with pyridoxine, or low protein, pyridoxine-free diets caused decreased lipid levels in rats (Suzuki et al, 1976; Okada et al, 1977). The results of vitamin B6 deficiency in rats on plasma cholesterol have been varied, with reports of an increase (Goswami and Sadhu, 1960), no change (Swell et al, 1961), and decrease (Williams et al, 1966; Suzuki and Okada, 1982). Lack of agreement between these results in studies of B6 deficiency and cholesterol levels in rats may be related to differences in feeding conditions (Okada et al, 1977). Loo and Smith (1986) demonstrated that pyridoxine deficiency led to decreased phospholipid methylation in rat liver microsomes. Cunane et al (1985) showed that essential fatty acids were proportionately increased in the triglyceride portion of liver in B6 deficient rats, suggesting that B6 has a role in fatty acid mobilization from liver triglycerides to plasma. B6 deficiency in rats also has been shown to have an inhibitory effect on arachidonic and linolenic acid metabolism (Cunane et al, 1985).

Vitamin B6 is also required for carnitine synthesis (Cho and Leklem, 1990). Carnitine is necessary for the oxidation of long chain fatty acids.

Impaired lipid metabolism is a possible contributer to atherogenesis (Conner and Conner, 1985). In humans, myocordial infarctions have been associated with a low vitamin B6 status in some studies (Kok et al, 1989; Serfontein and Ubbink, 1988), whereas others have demonstrated no association (Vermaak et al, 1987). Baysal et al (1966) reported that serum cholesterol was not signicantly changed in 6 normal males who consumed a vitamin B6 deficient diet for 50 days.

PLP is required for the synthesis of several neurotransmitters, including dopamine, norepinephrine and epinephrine. The neurohormone serotonin also requires PLP for synthesis (Sauberlich, 1985). This function of PLP, along with the direct and indirect involvement of PLP in cellular structure link vitamin B6 to nervous system function (Leklem, 1988-b). Animal studies have shown that B6 deficiency affects brain development, including a decrease in brain enzyme activity and low brain weights of offspring born to vitamin B6 deficient rats (Aycock and Kirksey, 1976).

PLP may also play a role in steroid hormone expression (Bender, 1987; Allgood and Cidlowski, 1992). Because of the reactive nature of PLP with protein, PLP can bind to some steroid receptor sites, inhibiting the binding of steroid-receptor complexes to DNA (Bender, 1987). A moderate B6 deficiency in experimental animals resulted in increased end-organ sensitivity to both estrogens and androgens (Holly et al, 1983; Symes et al, 1984). In-vitro studies have also demonstrated an increase in hormone induced gene expression with vitamin B6 deficiency, and reduced hormone induced gene expression with elevated intracellular vitamin B6, in multiple classes of steroid hormone receptors (Allgood and Cidlowski, 1992).

Vitamin B6 also plays an important role in tryptophan metabolism and thus niacin formation. PLP is a cofactor in four enzymatic steps in the metabolism of tryptophan, but is required for only one step in the direct formation of niacin from tryptophan. This step

is the conversion of 3-hydroxykynurenine to 3-hydroxyanthranilic acid. The enzyme kynureinase catalyzes this reaction, and PLP functions as a coenzyme (Brown, 1985). In women fed a vitamin B6 deficient diet for 28 days, some conversion of tryptophan to niacin still occured (Leklem et al, 1975), thus a vitamin B6 deficiency does not necessarily result in a complete inhibition of niacin formation from tryptophan.

Vitamin B6 plays an important role in maintance of normal immune function (Axelrod and Trakatellis, 1964). Vitamin B6 deficiency in rats results in compromised immune response (Axelrod and Trakatellis, 1964), and supplementation with B6 has been shown to both stimulate or produce no change in immunocompetance in elderly persons (Talbott et al, 1987; Goodwin and Garry, 1983). Differences may be due in part to variations in methdological procedures (Miller and Kerkvliet, 1990). The mechanism for vitamin B6's role in immune function appears to be related to PLP's participation in 1-carbon metabolism in the conversion of serine to glycine, and subsequent incorporation of these 1-carbon units into DNA and RNA (Axelrod and Trakatellis, 1964). Results from Trakatellis et al showed that pyridoxine-deficient rats possesed fewer cells, and decreased DNA and RNA synthesis (Trakatellis et al, 1964; Axelrod and Trakatellis, 1964). Decreased DNA and RNA can lead to decreased cell multiplication and decreased antibody production, both of which would adversly effect immune function.

Vitamin B6 is involved in both gluconeogenesis and glycogenolysis. PLP is a coenzyme required for glycogen phosphorylase, the rate limiting enzyme in glycogenolysis (Krebs and Fischer, 1964). PLP is involved in gluconeogenesis via its' role in amino acid metabolism and transamination, as discussed previously. The carbon chains of most amino acids can be used to form glucose (Stryer, 1981). Results from Rose and coworkers revealed that fasting blood glucose values were not significantly altered in women following a 28 day period of low vitamin B6 intake (0.19 mg) as compared to values after 28 days of adequate (1.8 mg) intake (Rose et al, 1975). Glucose tolerance was significantly decreased in oral contraceptive users, however, as a result of feeding the B6

deficient diet. Activities of liver alanine and aspartate aminotransferase were decreased, glycogen content of liver was increased, and glycogen phosphorylase activity in muscle and liver was decreased in vitamin B6 deficient rats (Angel, 1974).

Vitamin B6 also has a role in red blood cell function. PLP acts as a coenzyme for the red blood cell transaminases, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (Raica and Sauberlich, 1964; Solomon and Hillman, 1979; Toshiharu, 1993). Oxygen affinity of hemoglobin is also affected by vitamin B6. PL binds to the alpha chain of hemoglobin (Ink et al, 1982), increasing the oxygen affinity for hemoglobin (Benesch et al, 1973) whereas PLP binds to the beta chain of hemoglobin, causing a decrease in oxygen affinity (Benesch et al, 1973). Although the overall contribution of each vitamer is unknown, Solomon and Cohen (1989) demonstrated that supplementation for 6 weeks with 150 mg of PN.HCL in15 men with non-insulin-dependent diabetes mellitus resulted in an overall increase in the oxygen affinity for hemoglobin.

PLP also competes with glucose for binding sites on hemoglobin (Anderson et al, 1980; Ink et al, 1982; Solomon and Cohen, 1989) and may modify nonenzymatic glycosylation of hemoglobin (Solomon and Cohen, 1989). This later function of vitamin B6 may play an important role in red blood cell metabolism in persons with diabetes. Heme synthesis also requires vitamin B6. PLP is a required coenzyme for delta-aminolevulinate synthase, the enzyme that catalyzes the condensation of glycine and succinyle-coA, forming delta-aminolevulinic acid. (Sauberlich 1985). Delta-aminolevulinic acid is the precursor in heme synthesis.

Storage

Muscle contains the largest pool of vitamin B6 in the body (Li, 1981). In rats it was found that 75-80% of the vitamin B6 pool was located in muscle, and 5-19% in the liver (Coburn and Whyte, 1988). Most of the vitamin B6 in muscle is associated with glycogen phosphorylase (Krebs and Fischer, 1964). Muscle vitamin B6 content, as well

as muscle glycogen phosphorylase increased in rats fed diets high in vitamin B6 (Black et al, 1977). Conversly, where as a low vitamin B6 diet did not result in decreased muscle vitamin B6 as measured by glycogen phosphorylase, partial or total starvation did result in decreased glycogen phosphorylase content in rat muscle tissue (Black et al, 1978). Leklem et al demonstrated that in humans, plasma PLP increased with strenuous exercise (an acute form of starvation) and suggested that muscle vitamin B6 was the source (Leklem and Shultz, 1983; Manore et al, 1987). Increased levels of plasma PLP in obese women after gastric surgery also suggests that muscle PLP is mobilized as a result of an energy deficit (Turkki et al, 1989).

Coburn et al performed muscle biopsies on 12 humans in order to quantify the amount of vitamin B6 stored in total body pools (Coburn and Whyte, 1988). Their estimates of approximatly 1000 *m*mol total B6 in a 70 kg human adult is significantly higher than previous estimates of 107-725 *m*mol (Johansson et al, 1966; Coburn and Whyte, 1988). By use of oral and intravenous labled PN in humans, Johansson suggested that the body contains two pools of vitamin B6: a small pool with rapid turnover in equilibrium with a larger pool with slow turnover (Johansson et al, 1966). In females fed a vitamin B6 deficient diet, (0.19 mg for 4 weeks), plasma PLP concentration fell rapidly during the first week, and then continued to decline over the next three weeks, but at a slower rate (Brown et al, 1975), indicating more than one pool of vitamin B6 in the human body. More recent work by Coburn (1990) suggests that there may be multiple pools of vitamin B6 in the body.

Status

The requirement for vitamin B6 is related to protein intake. Vitamin B6 requirement increases as protein intake increases (Miller and Linkswiler, 1967; Miller et al, 1985). The recommended dietary allowance (RDA) for adults for vitamin B6 is 0.016mg/gm protein (National Research Council (NRC), 1989). This level appears to ensure

acceptable values for most indices of vitamin B6 status and is based on two times the RDA for protein. The resulting vitamin B6 allowance of 2.0 mg/day for males, and 1.6 mg/day for females is lower than the RDA for vitamin B6 set in 1980. As vitamin B6 and protein tend to naturally occur in the same types of food, vitamin B6 is likely to be adequate with increased protein intake (NRC, 1989). Recently, Leklem has recommeded that the vitamin B6:protein ratio be > 0.020 for adequate B6 status (Leklem, 1990-a).

In 1985, the average intake of vitamin B6 for adult males was 1.87 mg (0.019 mg/ gm protein), and was 1.16 mg ((0.019 mg/gm protein) for adult females (NRC, 1989). Studies have shown persons to have acceptable vitamin B6 status with intakes below the RDA (Miller and Linkswiler, 1967; Schultz and Leklem, 1981; Driskell et al, 1988). A recommedation for adequate status is > 1.2-1.5 mg/day (Leklem, 1990-a).

Currently, the RDA for vitamin B6 is the same for adults of all ages. Some studies suggest that age may affect vitamin B6 status, however. Lee and Leklem (1985) showed that middle aged women (mean age 53) had significantly lower plasma PLP, plasma and urinary total B6, and slightly higher 4PA excretion than younger women (mean age 24). Both groups of women were fed a constant diet with 2.3 mg vitamin B6/ day. Kant et al (1988) demonstrated decreased plasma PLP in men aged 45-75 as compared to men aged 25-35. Dietary intake of vitamin B6 among the two groups was not significantly different.

Assessment of vitamin B6 status may involve both direct and indirect measures. Direct measures include: plasma PLP, PL, total B6, urinary 4PA and total B6, and possibly erythrocyte PLP. Indirect measures include: erythrocyte transaminases stimulation tests (AST and ALT), urine xanthurenic acid (XA) excretion after a 2 gm tryptophan load, dietary vitamin B6 intake, and dietary vitamin B6:protein ratio (Leklem, 1990-a).

Although it is recognized that there is no one "best" method for status assessment (Leklem and Reynolds, 1981), plasma PLP is considered one of the better indicators of vitamin B6 status (Leklem, 1991). The biochemical basis for this is that plasma PLP

levels seem to be indicative of muscle tissue PLP levels, as demonstrated in rats (Lumeng et al, 1978). The suggested value of plasma PLP for adequate vitamin B6 status is greater than 30 nmol/L (Leklem, 1990-a). Reported mean values for plasma PLP in men range from 27-75 nmol/L, and from 26-93 nmol/L for women (Leklem, 1990-a). The mean values for the above ranges do not all come from studies in which dietary intake of vitamin B6 was controlled, thus they do not necessarily represent normal values (Leklem, 1990-a).

Plasma PL may represent 8-30% of total vitamin B6 concentration in plasma (Coburn and Mahuren, 1983; Lumeng et al, 1985; Hollins and Hendersons, 1986). Data on plasma PL is limited, however and a normal value has not been set (Leklem, 1990-a). Leklem has suggested that each laboratory establish its own reference value of plasma PL with an appropriate healthy control population (Leklem, 1991). The normal value for plasma total vitamin B6 is greater that 40 nmol/L. As PLP and PL comprise approximately 90% of total B6 in plasma, measurement of total B6 along with plasma PLP can help to estimate plasma PL (Leklem, 1990-a).

4-PA is the major metabolite of vitamin B6 metabolism. Urinary excretion of 4-PA changes rapidly with a change in vitamin B6 intake, and is thus considered to be a short term indicator of vitamin B6 status (Brown et al, 1975; Leklem, 1990-a). The suggested value of 4-PA for adequate vitamin B6 status is >3.0μmol/day (Leklem, 1990-a). With increased levels of protein intake, or decreased vitamin B6 intake, urinary 4-PA excretion decreases (Brown et al, 1975; Miller et al, 1985).

Measurement of urinary xanthurenic acid (XA) excretion after a 2 gm tryptophan load gives an indirect measure of vitamin B6 status. The tryptophan load "stresses" the tryptophan to niacin pathway, resulting in increased levels of XA excretion, with decreased vitamin B6 status (Leklem, 1971; Brown, 1985). The enzyme kynureninase requires vitamin B6 in conversion of 3-hydroxykynurenine to 3-hydroxyanthranilic acid. With vitamin B6 deficiency, more 3-hydroxykynurenine is converted to XA in a non-

vitamin B6 dependent reaction. The average excretion of XA after a 2 gm tryptophan load is 30-40 μmol/d, and the recommeded value for adequate status is <65 μmol/day (Leklem, 1990-a).

Erythrocyte amino transaminase activity may be an indicator of long term vitamin B6 status (Leklem, 1990-a). The two aminotransferase enzymes whose activities are most commonly determined as a measure of vitamin B6 status are erythrocyte alanine aminotransferase (EALT - formerly EGPT) and erythrocyte aspartate aminotransferase (EAST - formerly EGOT). EALT activity is thought to be a more accurate indicator of vitamin B6 status, as compared to EAST. A diet deficient in vitamin B6 results in decreased EALT activity, and an increase in in vitro EALT stimulation, while vitamin B6 supplementation increases EALT activity, and decreases in vitro stimulation by PLP (Baysal et al, 1966; Sauberlich et al, 1972; Brown et al, 1975; Solomon and Hillman, 1979). The suggested value for adequate status for EALT and EAST index (percent stimulation) is <1.25, and <1.80, respectively (Leklem, 1990-a). As mentioned earlier, the erythrocyte may play a role in overall vitamin B6 metabolism (Anderson et al, 1980). Data on normal values for erythrocyte PLP is limited, however, and no value for adequate status has been set (Leklem, 1990-a). For the most complete picture of vitamin B6 status, Leklem has recommended that PLP, 4-PA, one indirect method, and dietary assessment of vitamin B6 and protein intake be measured (Leklem, 1990-a).

Alkaline Phosphatase

PLP is dephosphorylated to PL by alkaline phosphatase (AP) (Lumeng and Li, 1974). At least three different isoenzymes of alkaline phosphatase are known to exist in humans: placental, intestinal and tissue non specific (bone, liver, kidney) (Coburn et al, 1988). AP activity in serum of normal adults is composed primarily of equal amounts of the bone and liver enzymes (Coburn, 1988). Whyte suggests that the tissue non specific AP enzyme regulates extracellular rather than intracellular concentration of PLP (Whyte,

1988), and it has further been suggested that plasma AP is responsible for only a small fraction of PLP metabolism (Whyte et al, 1984; Leibman et al, 1990).

The normal range for total plasma AP is 30-85 IU/L (Zeeman, 1983). Increased levels of plasma AP are found in certain disease states, including liver disease, bone disorders (Paget's disease) and diabetes (Lumeng and Li, 1974; Anderson et al, 1980; Cole et al, 1986; Stepan et al, 1980; Goldberg et al, 1977; Maxwell et al, 1986). Plasma PLP has been shown to be inversely correlated with plasma alkaline phosphatase in liver and bone disease (Anderson et al, 1980; Henderson et al, 1986; Cole et al, 1986; Coburn et al, 1988). Whether or not increased AP activity in diabetes is in part responsible for decreased plasma PLP is yet undetermined. Increased levels of circulating plasma PLP is associated with hypophosphatemia, an inborn error of metabolism characterized by deficient activity of the tissue non specific isoenzyme of AP (Whyte et al, 1985, 1988). Erythrocytes contain a phosphatase enzyme that hydrolyzes phosphorylated B6 compounds, and determines in part the concentration of erythrocyte PLP (Lumeng and Li, 1974). This enzyme is thought to be membrane bound (Yamada et al, 1968; Lumeng and Li, 1974). Lumeng and Li (1974) found the optimum pH for membrane bound phosphatase hydrolysis of erythrocyte PLP to be 7.1, thus concluding that the phosphatase was a neutral phosphatase, not an alkaline phosphatase (Lumeng and Li, 1974). Evidence for a non-membrane bound erythrocyte phosphatase comes from work by Fonda, who purified two phosphatases from human erythrocytes- one from the hemolysate fraction, which had an optimum pH of approximately 7.0, and another from the stromal fraction with an optimum pH of approximately 9.0 (Fonda, 1987).

DIABETES MELLITUS

Definition and Classification

Diabetes mellitus is a disease of the endocrine system that affects nearly 6 million persons in the U.S. (Harris, 1985). The disease is characterized by hyperglycemia that is the result of an absolute or relative deficiency of insulin (National Diabetes Data Group (NDDG), 1979). 5%-10% of the diabetic population have type-1 diabetes, or insulin dependent diabetes mellitus (IDDM) and 75-95% have type-2 diabetes, or non-insulin dependent diabetes mellitus (NIDDM) (Zeeman, 1983). Of those with IDDM, the disease is usually juvenile onset, and there is little or no insulin produced (NDDG, 1979). NIDDM is adult onset, and the ability of the pancreas to produce and secrete insulin is decreased or delayed, but is not absent. Also, with NIDDM many tissues are insulin insensitive (Waldhaust et al, 1982; Anderson, 1988).

Diagnosis

Diagnosis of diabetes mellitus should be based on one of the following criteria: 1. Unequivocal elevation of plasma glucose (>200mg/dL in children) along with the classic symptoms of diabetes mellitus such as polydipsia, polyuria, and rapid weight loss. 2. Fasting plasma glucose of ≥140 mg/dL (7.8 mmol/L) on more than one occassion 3. Elevated plasma glucose after an oral glucose challenge, on more than one occasion (NDDG, 1979). The oral glucose challenge, or oral glucose tolerance test (OGTT), consists of a 75 gm dose of glucose given after a 10-16 hr fast (NDDG, 1979). It is also recommended that persons should consume at least 150 g of carbohydrate as part of an isocaloric diet for at least three days before the test (Anderson, 1988). Blood is taken before glucose administration (fasting), and one-half, one, and two hours later for glucose measurements. A diagnosis of diabetes mellitus can be made if on more that one occa-

sion, both the two hour sample, and either the one-half or the one hour sample meets the following criteria (NDDG, 1979):

venous plasma glucose concentration > 200 mg/dL (11.1 mmol/L)

venous whole blood glucose concentration > 180 mg/dL (10.0 mmol/L)

capillary whole blood glucose concentration > 200 mg/dL (11.1 mmol/L)

Development of Diabetes

There are several factors associated with the development of diabetes mellitus. Onset of IDDM usually occurs in childhood, although occurrence has been noted in middle age and elderly persons (NDDG, 1979). Genetic determinants are thought to be important in thedevelopment of IDDM, as expressed by the associated increased or decreased frequency of certain histocompatibility antigens (HLA) on chromosome 6 (NDDG, 1979). Also, viral infection may trigger an autoimmune insult to the pancreatic beta cells in a genetically susceptible individual, resulting in an sudden cessation of insulin secretion (Cahill, 1981; Kahn, 1985; Anderson, 1988). More recently, ingestion of cows' milk protein before the age of one year has been associated with an increased risk of IDDM in susceptible individuals (Kostraba et al, 1993; Karjalainen et al, 1992; Mayer et al, 1988).

NIDDM usually develops after the age of 40 (Anderson, 1988). A normal process of aging is a decline in glucose tolerance, which is associated with increased insulin resistance, and diminished B-cell sensitivity to glucose (DeFronzo, 1979; Chen et al, 1988). Obesity is the major contributor to development of this type of diabetes in adults (Anderson, 1988), and it's been estimated that approximately 60% to 80% of all persons in westerns societies with NIDDM are obese (NDDG, 1979). Obesity and inactivity are associated with increased insulin resistance (Cahill, 1985). Development of NIDDM is also genetically influenced, although it is not associated with any specific HLA antigen, as with IDDM (Kahn, 1985).

Treatment and Control

For persons with NIDDM, weight loss through diet and exercise is the first step for treatment of NIDDM (Pemberton et al, 1988). As mentioned earlier, obesity is associated with increased insulin resistance and decreased glucose tolerance. Accordingly, weight loss has been shown to improve glucose tolerance by decreasing hepatic glucose output, decreasing insulin resistance, and increasing glucose uptake (Jallut, 1990; Henry, 1986). Exercise not only improves insulin sensitivity (Jensen, 1986) but can help with weight management, and thus better glycemic control. For persons with IDDM, caution must be taken with an exercise program, as the drastic changes in fuel metabolism that occur with exercise can complicate insulin therapy. Frequent monitoring of blood glucose concentration before, during and after exercise is the best way for persons with IDDM to manage blood glucose levels with exercise (Jensen, 1986).

An important part of treatment and control of diabetes mellitus is dietary modification. The goals of dietary management for both IDDM and NIDDM are (1) a nutritionally adequate intake with a caloric intake that is appropriate for the achievement and/or the maintenance of a desirable weight, (2) the prevention of hyperglycemia and of hypoglycemia, and (3) the reduction of the risk of atherosclerosis (Pemberton et al, 1988). Current recommendations for macronutrients are 50%-60% of calories from carbohydrate, with 70% being well tolerated, and an even division of carbohydrates from meal to meal. Protein should comprise 12-20% of calories, and less than 35% should come from fat, with 20%-30% desirable (U.S. Surgeon General's Report, 1988). Also, high fiber diets have been shown to improve glycemic control as well as lower plasma lipid levels (Anderson et al, 1979, 1987). There are no specific recommendations for fiber although 30-50 gm/day is regarded as safe, well tolerated, and effective (Anderson, 1988).

Oral hypoglycemic agents (OHGA) are useful as an adjunct to diet and exercise, in the treatment of hyperglycemia in persons with NIDDM. OHGA work to lower blood

glucose concentration by increasing insulin secretion and enhancing peripheral sensitivity to insulin (Cahill, 1985). For persons with IDDM, insulin therapy is a life sustaining treatment. Furthermore, many persons with NIDDM have a limited supply of endogenous insulin, and also require supplemental insulin injections (Anderson, 1988). Insulin is available in fast (regular, semilente), intermediate (NPH, lente), and long (ultralente, protamine zinc) acting forms (Campbell, 1988). A single injection of either one or two types of insulin may be sufficient for persons with type II diabetes mellitus, whereas most persons with type I diabetes mellitus require at least two insulin injections per day, often combining both short and intermediate acting forms of insulin for the best glycemic control (Anderson, 1988). A recent study has suggested even more frequent injections of insulin, (up to 7 times per day) in order to maintain very good diabetic control, and thus significantly reduce the complications associated with diabetes (Diabetes Control and Complications Trial Reasearch Group, 1993).

Long term glycemic control (2- 10 weeks) can be determined by measurement of glycosylated hemoglobin (Borsey et al, 1982; Miedema and Casparie, 1984; Nathan, 1990). Glycosylated hemoglobin is formed by a non enzymatic reaction between glucose and hemoglobin (Miedema and Casparie, 1984). A normal, non-diabetic value is considered to be less than 8% (Anderson, 1988; Cahill, 1985) and glycosylated hemoglobin values larger than 8% indicate poor glycemic control.

Energy Metabolism

Insulin, an anabolic hormone is the major regulator of fuel storage and release (Anderson, 1988). In healthy, non-diabetic persons, high levels of insulin during the fed state allow for the uptake (disposal) of blood glucose into liver, muscle and fat. Insulin also signals adipose tissue uptake of chylomicrons and very low density lipoproteins (VLDL), fat synthesis in adipose tissue, and amino acid uptake by muscle tissue (Cahill, 1985). Conversely, in the fasted state, insulin levels are low, creating mobilization of fuel

stores. Glucagon, a catabolic hormone, is also important in facilitating release of fuel when insulin levels are low (Anderson, 1988).

The diabetic state resembles the fasting state of non-diabetic persons, especially with regard to the release of fuel from the liver, muscle and adipose tissue (Bogardus, 1989; Anderson, 1988). The low ratio of insulin to glucagon seen in IDDM and NIDDM and insulin resistance associated with NIDDM leads to decreased tissue uptake of glucose and promotes increased gluconeogenesis by the liver resulting in increased levels of blood glucose (Anderson, 1988; Bogardus, 1989; Chen et al, 1988; Reaven, 1988). High levels of plasma free fatty acids also help sustain hepatic glucose production (Anderson, 1988). The low insulin/glucagon ratio also affects muscle and adipose tissue (Anderson, 1988; Bogardus, 1989; Reaven, 1988). Amino acid uptake into muscle tissue is decreased, muscle glycogen stores are depleted, and muscle tissue is broken down to support gluconeogenesis. Fat cells actively release fatty acids depleting their fat stores, while the liver secretes large amounts of VLDL. Liver, fat and muscle cells begin to utilize fatty acids as a source of fuel, producing the keto acids acetone, aceto acetate, and betahydroxy butyrate (Anderson, 1974, 1988). In prolonged states of insulin deficiency, diabetic ketoacidosis may result in persons with IDDM (Cahill, 1985).

Complications Associated with Diabetes

Sustained hyperglycemia in persons with diabetes contributes to many of the complications associated with the disease. These complications include neuropathy, nephropathy, retinopathy, coronary artery disease, and decreased immunological function (Reaven, 1988; Anderson, 1988). Macrovascular disease is the most common complication of diabetes mellitus (Bagdade and Subbaiah, 1989; Anderson, 1988). For example, people with diabetes have a two to three times greater risk of death from atherosclerosis as compared to persons without diabetes (Anderson et al, 1987). Although other factors

such as genetics and smoking can increase risk for atherosclerosis, risk factors that are influenced by diet (hyperglycemia, hyperlipidemia, hyperinsulinemia) play a significant role in the development of artherosclerosis in diabetes (Reaven, 1988).

As mentioned earlier, nonenzymatic glycosylation in blood and tissues occurs with hyperglycemia (Miedema and Casparie, 1984). The accumulation of glycosylated proteins may contribute to basement membrane thickening, vascular permeability, micro-circulation deficits, and functional abnormalities of erythocytes, leucocytes, and platelets, (Anderson, 1988), thus increasing susceptibility to the above mentioned risk factors (Klein et al, 1988; Cahill, 1985).

VITAMIN B6 and DIABETES

Vitamin B6 Status and Diabetes

There are several reports indicating that diabetes mellitus is associated with a low vitamin B6 status. Plasma PLP concentration has been shown to be lower in persons with diabetes when compared to non-diabetic controls (Davis et al, 1976; Wilson and Davis, 1977; Hollenbeck et al, 1983; Hollenbeck and Leklem, 1985; Leklem et al, 1985; Manore et al, 1989, 1991). In the studies that reported dietary intake of vitamin B6 (Hollenbeck et al, 1983; Manore et al, 1989, 1991), plasma PLP levels in persons with diabetes was found to be either lower than the expected norm for adequate B6 status (35 nmol/L) and/or lower than respective control values, despite similar and adequate intake for all subjects. Hollenbeck et al (1983) measured plasma PLP levels in 6 young women (age 26± 4 yrs) who were fed both a control diet (C) and a high fiber, high carbohydrate experimental diet (E). Diets were eucaloric to maintain current weight and met the 1980 recommended dietary allowances for all nutrients, including vitamin B6. All subjects completed both 4-week test periods, in a cross-over experimental design. The dietary vitamin B6 intake during the C and E phase was established to be 1.8 mg (B6:protein ratio = 0.020) and 2.3 mg

(B6:protein ratio = 0.028), respectively. Mean plasma PLP levels during the C phase was 30± 15nmol/L, and was 30± 17 nmol/L during the E phase. In both phases, 4 out of the 6 women with plasma PLP levels below the lower lmit of the normal range (35 nmol/L), and 2 women also had plasma PLP levels below the suggested lower boundrey of marginal deficiency (22 nmol/L) for plasma PLP (Shultz and Leklem, 1981) .

A more recent study by Manore et al (1991) followed the changes in plasma PLP in 4 normotensive persons with type II diabetes (D), 8 hypertensive persons with type II diabetes (HD), 13 hypertensive persons without diabetes (H), and 9 controls (C). All subjects were fed a metabolic diet with 2.1-2.2 mg B6/day, and 100 gm protein/day for 6 weeks. The authors reported that by the end of the study, all D subjects had low plasma PLP (<30nmol/L), indicative of low vitamin B6 status, while 54% of H and 63% of HD had low plasma PLP, and 33% of C had low plasma PLP, with one C subject starting with low plasma PLP. The mean beginning and end plasma PLP levels (nmol/L) of the 4 different groups were as follows: D 38.8± 15.2, and 20.3± 8.7; HD 36.1± 23.0, and 27.7± 7.1; H 45.8± 22.3, and 33.8 ± 19.8; C 45.1± 21.3, and 35.6± 15.2.

In studies where plasma PLP was measured in persons with diabetes, but dietary vitamin B6 intake was not reported, plasma PLP levels were also found to be either lower than control values and/or below the lower limit of the normal range (<35 nmol/L). The largest study was conducted by Davis et al (1976) and included 518 persons with diabetes (type not stated). The mean plasma PLP value for females was 26.6 nM and was 32.3 nM for males. The values for female and male controls were 42.4 nM and 50.1 nM, respectively. The suggested plasma PLP value for adequate vitamin B6 status in adults is > 30 nM (Leklem, 1991). Although dietary intake was not reported, serum folate was determined and reported to be adequate in all but 20 subjects. The authors suggested that in light of the normal folate levels, neither inadequate dietary intake nor malabsorption were reasons for low PLP levels, as poor nutrition and malabsortion would present with both low folate and pyridoxal levels. Wilson and Davis (1977) also showed that in 63

children with diabetes (age range 4.5-17.5 yrs), mean serum total aldehyde levels (PLP+PL) were lower than values for non-diabetic healthy children of the same age (44± 20 nmol/L vs. 65± 20 nmol/L, respectively). Serum folate levels were normal for all but one subject.

Leklem et al (1985) measured plasma PLP levels in 42 individuals with NIDDM (mean age 70± 6 yrs) and compared results to mean plasma PLP levels of 101 age matched controls. Dietary intake was not reported. However, the authors noted that both NIDDM and control subjects were non-vitamin users. The mean plasma PLP levels were 25± 14nmol/L and 33± 20 nmol/L for NIDDM and control groups, respectively (Leklem et al, 1985; Leklem, unpublished observations). Hollenbeck et al (1985) also measured plasma PLP concentrations in 6 subjects with NIDDM (mean age 56± 12 yrs) as well as 6 healthy controls (mean age 41± 14 yrs). Again, no dietary vitamin B6 intake was reported, but all subjects were non-vitamin users. Mean fasting plasma PLP levels were lower for persons with NIDDM as compared to controls (44± 8 nmol/L vs. 60± 5 nmol/L, respectively; $p < 0.8$).

Several earlier studies have measured urinary XA excretion in persons with diabetes as a way of evaluating vitamin B6 status relative to non-diabetic controls. It has been shown that basal excretion of XA is increased (Kotake and Tani, 1953; Rosen et al, 1955) and that the excretion of XA is significantly greater after a 10 gm load of DL-tryptophan in persons with diabetes as compared to non-diabetic controls (Rosen, 1955), suggesting a lower vitamin B6 status in persons with diabetes. Furthermore, a 300 mg intramuscular injection of PN reversed the excessive excretion of XA after the tryptophan load in six persons with diabetes (Rosen, 1955).

Glucose Tolerance and Vitamin B6

Studies by Leklem and co-workers have demonstrated that plasma PLP increases with exercise, a situation where there is an increased caloric need (Leklem and Shultz,

lowing an oral glucose load; ie, a metabolic situation opposite that of exercise. In a study in which 8 healthy subjects were orally administered 1 g/kg of either glucose, frutose or high fructose corn syrup, plasma PLP was observed to decrease 28%, 21% and 25% in response to the respective carbohydrate load (Leklem, 1984). Also, urinary 4PA excretion increased on the day of the carbohydrate load as compared to the previous day, despite similar intakes of vitamin B6. A similar study also showed that plasma PLP and plasma TB6 decreased by 18-21% from fasting values, after a 5 hour OGTT in which an oral 1g/kg dose of glucose was administered to nine healthy subjects (4F, 5M) (Leklem, 1990-b). This percent decrease corresponded to an overall 8-10 nmol/L decrease in plasma PLP, and a 8-12 nmol/L decrease in TB6. The mean fasting plasma PLP value was 39.7 ± 13.5 nmol/L for females, and 55.7 ± 5.7 nmol/L for males, with both values exceeding the lower limit for adequate B6 status (>30nmol/L, Leklem, 1990-a).

Hollenbeck and Leklem (1985) observed the change in plasma PLP in response to an OGTT in 10 persons with NIDDM and 10 normal controls. They found that plasma PLP decreased significantly following the glucose load, but also observed that the decrease was of significantly greater magnitude for the control subjects as compared to subjects with NIDDM. Unfortunately some of the subjects with NIDDM were vitamin users, and thus the nmber of non-vitamin users with NIDDM tested was small. Furthermore, it is important to note that the subjects with the highest initial plasma PLP values had the largest decrease in plasma PLP. Fasting plasma PLP values were higher for control subjectrs as compared to subjects with NIDDM- both vitamin users and non-vitamin users. Persons with diabetes have higher than normal levels of circulating glucose at all times, however. Thus a relatively small decrease in plasma PLP in response to high plasma glucose could potentially, over a period of time, lead to a decrease in vitamin B6 status, desite adequate intakes of vitamin B6.

Results from studies on vitamin B6 status and the effects on glucose tolerance have been conflicting. Ribaya-Mercado et al (1990) found that a vitamin B6 defiecient

diet did not result in any significant changes in glucose tolerance in elderly persons. The authors did note, however, that the B6 deficient diet appeared to induce a state of insulin resistance, especially in males (Ribaya-Mercado et al, 1990). Rose et al (1975) found that a vitamin B6 deficient diet resulted in a decreased glucose tolerance in oral contraceptive users, but did not affect glucose tolerance in control women who were not using oral contraceptives. Conversely, a study by Rao (1983) showed that in 16 healthy, non-diabetic persons, subclinical pyridoxine deficiency was associated with an increase in insulin sensitivity and improved glucose tolerance. In rats, studies have shown vitamin B6 deficiency results in lowered levels of both fasting and nonfasting blood glucose (Huber et al, 1964), increased blood glucose (Tezuka and Makino, 1959), decreased glucose tolerance (Huber et al, 1964; Tezuka and Makino, 1959), decreased insulin activity (Huber et al, 1964), and increased insulin sensitivity (Tezuka and Makino, 1959; Huber et al, 1964; Makris and Gershoff, 1974).

Supplementation with Vitamin B6

Several studies have evaluated the results of vitamin B6 supplementation on glucose tolerance and plasma glucose concentration. In a double blind controlled investigation, 10 subjects with diabetes received 150 mg PN for 6 weeks. Although glycosylated hemoglobin decreased slightly and fasting blood sugar increased slightly, neither change was significant (Cohen et al, 1984). Unfortunately, the study was not diet controlled. In a study by Rao (1980), 13 persons with adult onset diabetes received 80 mg PN for three weeks. Glucose tolerance, as measured by the positive area under the curve for plasma glucose, improved slightly after B6 supplementation in both non-deficient and deficient subjects. The improvement was not significant, however, and again the study was not diet controlled. Supplementation of 100 mg PN.HCL for two weeks has been shown to improve oral glucose tolerance in persons with gestational diabetes (Coelingh-Bennink and Schreurs, 1975; Spellacy it al, 1977). Rose et al (1975) demonstrated an improvement

in glucose tolerance with PN.HCL supplementation, in 9 oral contraceptive users who had been on a vitamin B6 deficient diet for 4 weeks.

Observations of 1 subject in a study by Hollenbeck et al (1983) suggest that a normal vitamin B6 status can be obtained in persons with diabetes when B6 supplements are taken, whereas maintenance of a normal vitamin B6 status (as measured by plasma PLP) is not possible from the RDA of vitamin B6 alone (Hollenbeck et al, 1983). The plasma PLP concentration of the above mentioned subject was 121 nmol/L while taking 10 mg PN.HCL/day, and fell to 15 nmol/L nine weeks after cessation of the vitamin, but while consuming a diet that met the 1980 RDA for vitamin B6. The normal lab range for plasma PLP for persons not using vitamin supplements is 35-100nmol/L (Leklem, 1994).

Diabetic Complications and Vitamin B6

As mentioned earlier, sustained hyperglycemia leads to increased nonenzymatic glycosylatin of proteins in blood and tissues (Miedema and Casparie, 1984), increasing the risk for diabetic complications such as peripheral neuropathy, nephropathy and retinopathy (Klein, 1988; Cahill, 1985). Alternatively, PLP has been shown to inhibit the nonenzymatic glycosylation of proteins by sugar (Khatami, 1990; Shepard et al, 1985). Shepard et al demonstrated that in vitro, PLP competes with glucose for binding on human serum albumin. Although this inhibition of nonenzymatic glycosylation may have different characteristics in vivo, the possibility exists that a low level of PLP in persons with diabetes may result in a relative loss of inhibition of nonenzymatic glycosylation, further increasing the chance for complications.

Studies that have evaluated the relationship of vitamin B6 deficiency and complications of diabetes have had varied results. In diabetic patients with peripheral neuropathy, vitamin B6 therapy of 150 mg PN.HCL/day for 6 weeks resulted in a slight but non-significant decrease in percent glycosylated hemoglobin (Cohen et al, 1984). Also, there was no objective improvement in peripheral neuropathy. Solomon and Cohen (1989) also

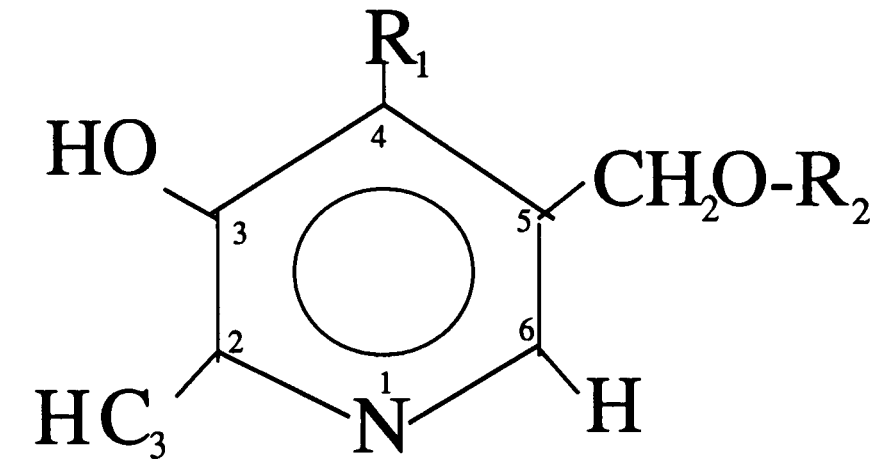
demonstrated that vitamin B6 therapy of 150 mg of PN.HCL per day for 6 weeks resulted in a decrease (non-significant) in glycosylated hemoglobin. This decrease was not explained by a decrease in fasting blood glucose.

Kompf concluded that in 94 patients with IDDM, there was no significant relationship between vitamin B6 deficiency as measured by red blood cell enzyme activation (enzyme type not specified), and peripheral neuropathy (Kompf, 1985). Conversely, in 10 patients with IDDM, peripheral neuropathy and low vitamin B6 status (based on increased excretion of XA and KA), symptoms of peripheral neuropathy decreased or disappeared after 6 weeks of supplementation with 150 mg PN.HCL/day (Jones and Gonzalez, 1978). Another study showed that in 50 patients with diabetes and significant peripheral neuropathy, mean serum pyridoxal concentration was significantly lower as compared to 50 age and sex matched patients with diabetes and no symptoms of peripheral neuropathy (McCann and Davis, 1978). Differences in study results may be due in part by differences in indices used to assess vitamin B6 deficiency, and whether or not pyridoxine supplementation is indicated for persons with diabetes and peripheral neuropathy is still unclear (McCann and Davis, 1983). In a study by Roy, 15 persons with diabetic retinopathy had a higher, but nonsignificant level of percent glycosylated hemoglobin when compared with 19 persons with diabetes and no retinopathy (Roy et al, 1989). Indicators of vitamin B6 status were not measured.

The abnormal tryptophan metabolism and subsequent increase in urinary XA that is seen with vitamin B6 deficiency and low plasma PLP may also be related to complications of diabetes. Persons with diabetes have been shown to have an increased level of urinary XA excretion (Kotake and Tani, 1953; Rosen et al, 1955). Furthermore, XA has been shown to combine with insulin in vitro, forming a XA-insulin complex that has lower insulin sensitivity than insulin alone (Hattoiri et al, 1984).

Decreased immunological function is associated with both diabetes (Anderson, 1988) and low vitamin B6 status (Miller and Kerkvliet, 1990; Talbot et al, 1987). Finally,

a low vitamin B6 status as measured by plasma PLP has been associated with abnormal methionine metabolism (Linkswiler, 1981). An abnormality in methionine metabolism may have a role in increased susceptibility to vascular and cardiovascular damage (Shultz et al, 1988), both of which are complication of diabetes mellitus (Anderson, 1988).



R_1	R_2= H	R_2= PO_3^{2-}
CH_2OH	Pyridoxine (PN)	Pyridoxine 5'-phosphate (PNP)
CH_2NH_2	Pyridoxamine (PM)	Pyridoxamine 5'-phosphate (PMP)
CHO	Pyridoxal (PL)	Pyridoxal 5'-phosphate (PLP)

Figure I. Structures and IUPAC-IUB nomenclature for the 6 forms of vitamin B6.

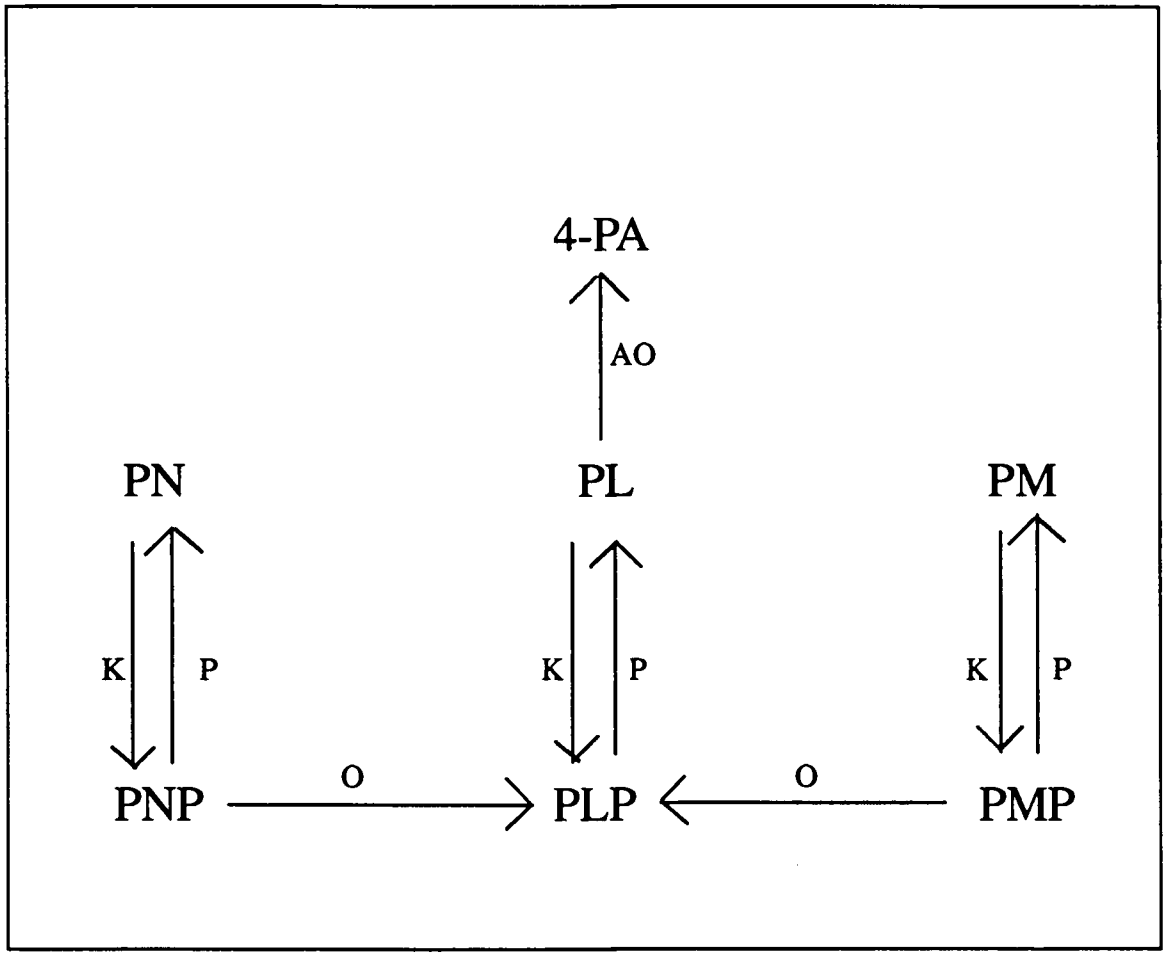


Figure 2. Metabolism of vitamin B6. K, pyridoxal kinase; O, pyridoxine (pyridoxamine) 5'-phosphate oxidase; P, phosphatase; AO, aldehyde oxidase.

III. CHANGES IN PLASMA PYRIDOXAL 5'-PHOSPHATE AND RED BLOOD CELL PYRIDOXAL 5'-PHOSPHATE CONCENTRATION DURING AN ORAL GLUCOSE TOLERANCE TEST IN PERSONS WITH DIABETES MELLITUS

METHODS

Study Overview

This study was part of a larger study conducted along with another coworker. There were three separate phases: A, B and C. Subjects were screened and selected during phase A. Phases B and C were test phases and were approximately 2 months apart. All data for the current study comes from phase C, during which the Oral Glucose Tolerance Test (OGTT) was conducted. Fasting data from phases A, B and C were used by the other coworker (Smith, 1991).

Subject Recruitment and Screening

This study was approved by the Oregon State University Committee for the Protection of Human Subjects. Recruitment of subjects with diabetes included a mailing to persons who had attended local diabetes education classes, announcements in the University newsletter and at a local senior center, and by presentation at a meeting of the local chapter of the Oregon Diabetes Association. Control subjects were recruited through an announcement in the University newsletter and by word of mouth within several University departments.

During the initial visit, all subjects filled out a volutary medical history form and an informed consent. A 10-12 hr fasting blood sample was taken and subsequently tested for plasma PLP, glucose and c-peptide concentration. The initial plasma PLP concentration was used to assess whether any potential subject had an extremely high value (> 75

nmol/L), indicative of vitamin use or a possible disease state such as hypophosphatasia (Leklem, 1991). A fasting glucose value of <6.5 mmol/L (NDDG, 1979) was required for control subjects, and a c-peptide concetration of <200 pmol/L was used to validate the diagnosis of insulin dependant diabetes (IDDM) as opposed to non-insulin diabetes (NIDDM) in subjects with diabetes (Rubenstein, 1977). Other requirements were that persons with diabetes had to provide a note from their physician verifying a diagnosis of diabetes for at least one year prior to the study, that control subjects had no history of abnormal carbohydrate metabolism and were in overall good health, and that all subjects were non smokers, were taking no medications known to interfere with vitamin B6 metabolism, were not pregnant or taking oral contraceptives, and were not taking vitamins or were willing to stop taking vitamins for one month prior to and throughout the study.

Subjects were separated into six different groups based on type of diabetes (or controls), and sex. There were 3 IDDM females, 5 NIDDM females, 5 IDDM males, 4 NIDDM males, 9 control females and 9 control males for a total of 35 subjects (8 females with diabetes, 9 female controls, 9 males with diabetes and 9 male controls). Originally there were 4 females with IDDM, but one had to be dropped from data analysis as her insulin values were extremely high prior to the OGTT (approximately 100 fold above values for other females with IDDM), indicating she had not omitted her usual insulin dose as requested .

Experimental Procedure

The study consisted of two phases, B and C. All data for this study comes from phase C, during which the OGTT was conducted. Results for dietary intake of vitamin B6, protein and energy is an average from food records recorded for 3 days prior to the OGTT.

Oral Glucose Tolerance Test

Persons with diabetes had been asked to withhold any insulin, or oral hypoglycemic agent that they would otherwise take, during the fasting period. Subjects arrived at the lab on the morning of the oral glucose tolerance test, after a 10-12 hour fast. They were seated for at least 5 minutes prior to the fasting blood draw. Two 10 ml samples of fasting blood were obtained from each subject prior to the OGTT. Subjects were then given a 75 gm dose of D-glucose in 300 ml of distilled water, with approximately 20 ml of lemon juice added for flavoring. Subjects were required to drink the solution within 5 minutes, and used squirt bottles of 50 ml distilled water to rinse the glass. Two 10 ml samples of blood were obtained at 30, 60 and 120 minutes after the solution was finished.

Food Records

For three days prior to the OGTT, subjects weighed and recorded food intake for all of their meals and snacks. Subjects were provided with the necessary recording forms, and a Hanson® brand dietetic scale that measured weight to the nearest 1 gram. Subjects were given individual instructions on how to accurately weigh and measure food, and were also individually interviewed after forms were completed. Food models were used in order to help subjects best estimate portions sizes of any foods that did not get weighed or measured (ie, restaurant meals). Approximately 90% of all food was weighed, the other 10% estimated. Also, subjects were asked to bring in food labels and/or recipes of combination-type foods whenever possible, to help with nutrient determination.

Analytical Prodecures

Blood was collected in heparin coated Vacutainer® tubes, and immediately stored on ice. Appropriate volumes of whole blood were removed, and the remaining blood was separated by centrifugation into plasma and red blood cells within 30 minutes of the blood draw. Red blood cells were then washed three times with 0.9% saline and centri-

fuged after each washing. Appropriate aliquots of plasma and red blood cells were frozen at -40 C until analysis. All laboratory samples were assayed in duplicate, except microhematocrit, hemoglobin and c-peptide, which were done in triplicate.

Plasma

Plasma glucose was analyzed using a glucose oxidase-peroxidase method (Trinder, 1969) on the Alpkem Autoanalyzer II® System (Alpkem Corporation, 1983). Each assay included a standard curve consisting of glucose concentrations of 5, 10, 15, 20 and 30 mg/ml. One to three plasma control samples were run with each batch to determine the interassay coefficient of variation (CV). The mean, CV and n of the three control samples were: 83.4 mg/dL, CV= 1.4%, n=4; 175 mg/dL, CV= 2.3%, n=4; 95.8 mg/dL, CV=2.4%, n=4.

Plasma insulin and c-peptide were measured using double antibody radioimmunoassay (RIA) kits (Coat-A-Count®, Diagnostic Products Corporation, 1988). Each assay also included a standard curve, and the plasma control sample for the insulin assay had a mean insulin concentration of 17.2μU/ml with a CV=12.9%, n=10.

Alkaline phosphatase was analyzed by a colorimetric method using thymolphthalein monophosphate as the substrate (Roy, 1970). Each assay included a standard curve and at least one control sample. The mean and coefficient of variation (CV) for the two control samples were 0.319μkat/L, CV=7.1%, n=4 and 0.776μkat/L, CV=4.1%, n=5.

Plasma pyridoxal 5'-phosphate (PLP) was measured using a modified version of Chabner and Livingston's radioenzymatic method (Chabner, 1970). The CV of the control pool was 6.2% (n=41), and the average recovery of added PLP was 91%. Recoveries were performed on approximately 20% of all samples.

Red Blood Cells

Red blood cell PLP (rbc PLP) was determined by a further modified version of Chabner and Livingston's tyrosine decarboxylase enzymatic assay (Chabner, 1970). A different extraction process was used for rbc PLP as compared to plasma PLP, due to the tight binding of PLP to hemoglobin in the erythrocyte. 0.25 ml samples of red blood cells were mixed with 1.5 ml water and vortexed. 0.45 ml trichloroacetic acid (TCA) was then added in two steps of 0.225 ml, with vortexing after each addition. The mixture was covered and allowed to stand for 1 hour. After 1 hour, it was centrifuged for 15 minutes at 13,500 g. and the supernate was decanted into glass stoppered tubes. The PLP was then extraced from the TCA with 8 ml of water saturated ether. This extraction was performed 3 times. 0.1 ml of the remaining solution was analyzed for rbc PLP by the enzymatic assay as described by Chabner and Livingston (Chabner, 1970).

Preliminary results from the Foods and Nutrition laboratory at Oregon State University have shown that varying the amount of TCA affects the concentration of rbc PLP. Using TCA volumes of 0.2, 0.3, 0.4, 0.5 and 0.6ml and 0.25 ml of the same pool of red blood cells, it was found that 0.4 and 0.5 ml of TCA gave slightly higher values for rbc PLP (52.5 nmol/L, n=4 and 55.0 nmol/L, n=1 respectivly) as compared to 0.2, 0.3 and 0.6 ml of TCA (46.8 nmol/L, n=4; 52.0 nmol/L, n=1 and 40.0 nmol/L, n=1, respectivly). The red blood cell precipitate after adding the TCA was successively "stickier" with increasing additions of TCA; ie, 0.6 ml TCA > 0.4 ml TCA > 0.2 ml TCA in regards to stickiness, in a noticably linear fashion. Thus it appears that for a red blood cell sample of 0.25 ml, 0.2-0.3 ml of TCA is not enough acid to completely separate the PLP from the hemoglobin, and 0.6 ml is too much, such that the rbc PLP precipitate becomes so sticky that complete extraction is also not possible. Based on these preliminary results, the volume 0.45ml TCA was chosen as the best volume of acid for rbc PLP extraction in the modified version of Chabner and Livingstons' enzymatic assay for rbc PLP analysis.

Despite the above tests for the best amount of TCA to be used in the red blood cell assay for PLP in order to maximize rbc PLP recovery, the actual recoveries of rbc PLP in study samples were low, as compared to plasma PLP samples. The average recovery was 51.3% ± 18.4% (n=37), with a range of 0-122%, and a median of 47%. Rbc PLP values obtained for this study were not corrected for percent recovery. There were 3 different pools of red blood cells during the course of rbc PLP data analysis with the following means and CV: 293 nmol/L, n=5, CV= 14.3%; 70.4 nmol/L, n=16, CV= 15.4%, and 102.5 nmol/L, n=11, CV= 11.1%. One or more pool samples were run with each assay.

Whole Blood

Microhematocrit and hemoglobin were determined on whole blood samples (Natl. Acad. Sci., 1958). Percent glycosylated hemoglobin was also determined in whole blood, using an affinity chromatography kit (Glyc-Affin™ GHb, Isolab, Inc).

Dietary Analysis

Food records were analyzed for vitamin B6, protein and energy content. The majority of information came from the nutrient data base Food Processor II®, and the USDA handbook #8 (Ag. Res. Serv., 1976-1989). A small percentage (2-3%) of nutrient information came from other sources (Ohio St. Univ., 1984; Carper, 1985; Netzer, 1988). Information was unavailable on vitamin B6 content for approximately 20% of all foods (specific brand-name frozen meals, cereals, soup mixes, etc). In order to determine the vitamin B6 content of these foods, the exact items were purchased at the grocery store and analyzed in the Oregon State University (O.S.U.) Foods and Nutrition Lab. The analysis was done by Karin Hardin, B.S., R.M.T., a laboratory technician in the O.S.U. Foods and Nutrition Lab., using the Saccharomyces uvarum microbiological assay (Horwitz, 1980; Polansky, 1981).

Statistical Analysis

Descriptive statistics (mean ± standard deviation) were performed on all data. The positive, negative and cumulative areas under the curve (AUC) were calculated for changes in plasma PLP, plasma insulin, plasma glucose and red blood cell PLP concentrations that occured during the OGTT. A pooled t-test (2 tailed) was used to determine the significance of difference between group means. A paired t-test (2 tailed) was used to determine the difference within a group mean (for example, the difference between the -AUC and +AUC in rbc PLP at t=0, for females with IDDM). Simple linear regression analysis was used to calculate correlation coefficients. Statistical significance was set at $p<0.05$) (Neter and Wasserman, 1974; Devore and Peck, 1986).

RESULTS

Subject Description

The mean values for the subjects' age, height, weight, body mass index (BMI) and percent glycosylated hemoglobin (%GlyHb) are listed in Table 1. Subjects with NIDDM were significantly older (F 61± 11 yrs; M 55± 9 yrs) than those with IDDM (F 37± 8 yrs; M 38± 9 yrs) or controls (F 42± 13 yrs; M 41± 11 yrs). Subjects with NIDDM also tended to be heavier and shorter, with a resultant larger BMI. The mean weight and BMI for females with NIDDM were significantly greater than respective values for female controls ($p<0.001$). The differences between females with IDDM and NIDDM for height, weight and BMI were not significant. The mean % GlyHg for both male and female subjects with NIDDM and IDDM were significantly greater than respective control values.

Dietary Intake

Results for dietary intake of vitamin B6, protein and energy intake are an average determined from food records recorded for 3 days prior to the OGTT. Mean vitamin B6 intake, and the B6:protein ratio was lower in females with IDDM as compared to other groups; however the differences were not significant (Table 2). Mean values for dietary vitamin B6 and the B6:protein ratio met the current recommended dietary allowances for all study groups (NRC, 1989). The mean energy intake (Kcal) for females was: IDDM, 2005± 92; NIDDM, 1630± 311; control, 1799± 575 and for males was: IDDM, 2754± 712; NIDDM, 1963± 352; control, 2694± 690.

Plasma Glucose during the OGTT.

Table 3 contains the mean values for plasma glucose concentrations at each time point during the OGTT. Mean values for both male and female IDDM, NIDDM and

combined diabetes (DM) groups were significantly greater than respective control group values at all time points during the OGTT ($P<0.05$). There were no significant differences at any time point, between mean values for respective male and female IDDM and NIDDM groups. The mean fasting glucose concentration in females with IDDM was 3x greater than female controls, and the mean fasting glucose concentration in females with NIDDM was 2x greater than the mean value for female controls. The mean fasting glucose concentrations in males with both IDDM and NIDDM were similar, and were approximately 2x greater than the mean value for male controls. As would be expected, there was an overall rise in plasma glucose during the OGTT for all study participants. The mean peak value was at 30 minutes for controls, and the mean peak value for glucose was still increasing at 120 minutes for all diabetes groups.

Area Under the Curve for Glucose during the OGTT

Table 4 contains the mean values for cumulative, positive areas under the curve for glucose during the OGTT (+AUC glu). Mean values for both male and female IDDM and NIDDM groups were significantly greater than respective control group values at all time points ($p< 0.05$). Within the diabetic population, mean +AUC glu values for both males and females with IDDM tended to run higher than values for those with NIDDM, but because of large standard deviations and small sample sizes, the majority of the differences were not significant. The overall mean increase in plasma glucose (as measured by the +AUC glu at 120 minutes) for all study members ($n=35$) was +653 mmol/L x min. (There also was an overall mean decrease of -81 mmol/L x min. due to plasma glucose levels at 120 minutes that were lower than fasting levels in several control individuals).

Plasma Insulin during the OGTT

The mean values for plasma insulin concentrations at each time point during the OGTT are listed in table 5. Fasting insulin values in females with NIDDM were 3x greater than female controls ($p<0.01$). Fasting insulin values in males with NIDDM were 1.5x greater than the values for control males, but this difference was not significant. Plasma insulin was measured in persons with IDDM, but the values are simply a reflection of the amount of exogenous insulin present in the plasma during the time of the OGTT. There was an overall rise in plasma insulin during the OGTT for all control and NIDDM study participants. The mean peak value was at 60 minutes for control subjects and was still increasing at 120 minutes for persons with NIDDM. The mean insulin values at zero and 60 minutes for females with NIDDM were significantly higher than the respective control values ($p<0.01$; $p<0.05$, respectively). At 30 minutes, the mean plasma insulin value for NIDDM males was significantly greater than the mean control value ($p<0.05$).

In the male population, mean insulin values were highest in persons with IDDM. The high values (and subsequent large standard deviations) reflect the fact that one of the subjects was on an insulin pump and was not able to withhold his usual dose of insulin during the 10-12 hour fast prior to the OGTT, as other subjects had. Although his plasma insulin values were significantly greater than all other males with IDDM, his glucose tolerance curve and other study data were similar to the four other subjects, and so it was decided not to discard him from data analysis.

Area Under the Curve for Insulin during the OGTT

Mean values for cumulative, positive areas under the curve for insulin (+AUC ins.) are listed in table 6. Mean values for males with NIDDM at 30 and 60 minutes were significantly lower than respective values for control males. Mean values for females with NIDDM were not significantly different from female controls. There were no

significant changes over time for mean values in persons with IDDM. Any absolute changes within individuals with IDDM were indicative of changes in exogenous insulin, relative to when they took their last insulin dose, and were not reflective of changes due to the oral glucose dose.

Plasma PLP during the OGTT

There were no significant differences between any same sex group, at any time point for mean PLP values during the OGTT (Table 7). In the female population, mean values were lowest in the IDDM group and highest in the control group, but differences were not statistically significant due to large standard deviations and small population size. In the male population, the opposite seemed to be true. Mean PLP values were lowest in the control population and highest in persons with IDDM, but again differences were not significant. However, the mean plasma PLP values for DM females were significantly lower at all time points than the respective values for DM males, ($p<0.001$).

Table 8 contains the overall change in mean plasma PLP from zero to 120 minutes (Δ PLP). There was an overall decrease in mean plasma PLP values during the OGTT for all groups. Using a value of at least 10% different than the fasting value as indicative of a significant change (10% is based on a conservative value for the error of method in the PLP assay), values at 120 minutes were significantly less than fasting values in 8 out of 9 female controls, 6 out of 9 male controls, all NIDDM subjects, 1 out of 3 females with IDDM, and all males with IDDM. In the female population, the range of decrease in plasma PLP was from -1.1 to -5.9 nmol/L. Females with IDDM had the lowest mean fasting PLP (16.1 nmol/L) and the smallest decrease (-1.1 nmol/L). In contrast, control females had the highest fasting plasma PLP (34.0 nmol/L) and the largest decrease (-5.9 nmol/L). Results from the male population show that in general, the males with diabetes had both the highest fasting plasma PLP (47.2 nmol/L) and the largest decrease in plasma PLP during the OGTT (-7.6 nmol/L), whereas the control

males had a relatively smaller fasting plasma PLP (35.9 nmol/L) as well as a smaller decrease (-5.7 nmol/L).

Also listed in table 8 are the correlation coefficients between the Δ PLP and fasting plasma PLP. Correlation coefficients between the Δ PLP and fasting plasma PLP concentration (fast PLP), were determined to see if the change in plasma PLP during the OGTT was related to fasting plasma PLP. The relationship was statistically significant for females with NIDDM ($r=.976$, $p<0.01$), control females ($r=.957$, $p<0.01$) and combined female and combined male groups of persons with diabetes ($r=.877$, $p<0.01$; $r=.718$, $p<0.05$, respectively).

Area Under the Curve for Plasma PLP during the OGTT

As with the absolute plasma PLP values, there were no significant differences between any group for mean cumulative negative areas under the curve for PLP (-AUC plp) at any time point during the OGTT (Table 9). Values ran lower in persons with IDDM, and higher in controls for the female population, but differences were not statistically significant due to large standard variations and small population size. In the male population, results were the opposite. Cumulative negative areas under the curve for PLP were generally lowest in the control population, and highest in persons with IDDM, but again the differences were not statistically significant. The overall mean decrease in plasma PLP concentration (as measured by the -AUC plp at 120 minutes) for all subjects (n=35) was -420 nmol/Lx min. Comparatively, the overall mean increase in plasma PLP concentration (+AUC plp) was only +7 nmol/Lx min..

Relationship Between the Decrease in Plasma PLP Concentration, Increase in Plasma Glucose Concentration, and Increase in Plasma Insulin Concentration during the OGTT.

Correlation coefficients reflecting the strengths of the relationships between the decrease in plasma PLP concentration (-AUC plp), increase in plasma glucose concentra-

tion (+AUC glu), and increase in plasma insulin concentration (+AUC ins) during the oral glucose tolerance test are listed in table 10. While all correlations between -AUC plp and +AUC glu were statistically significant for all study groups, the association was stronger in males and control females as compared to diabetic females ($p<0.001$ vs. $p<0.01$, respectively). The relationship between -AUC plp and +AUC ins was statistically significant for both male and female control groups ($r=.686$; $r=.879$, $p<0.001$, respectively) but not for male and female IDDM and NIDDM groups.

Red Blood Cell PLP during the OGTT

Mean red blood cell PLP (rbc PLP) concentrations during the OGTT are listed in table 11. Mean values for all persons with diabetes ranged from 38-51 nmol/L and were 20-40% greater at all time points, than respective control values (range 27-31 nmol/L). These differences in mean rbc PLP concentrations between DM and control groups were all significant ($p<0.05$) except the difference at t=0 (fasting) in females with NIDDM vs. controls. The mean fasting value for females with IDDM was significantly greater than the mean value for females with NIDDM ($p<0.05$). Otherwise, there were no significant differences between male or female IDDM and NIDDM groups at any time point during the OGTT. (Because of this one statistically significant difference between females with IDDM and NIDDM, rbc PLP values were not combined for all persons with diabetes (DM) as had been done for plasma glucose, insulin and plasma PLP).

Fasting Red Blood Cell PLP and Plasma PLP Ratios

Table 12 contains the values of the ratios of fasting rbc PLP to fasting plasma PLP. The ratios for females with IDDM and NIDDM were 3.2 and 2.2, respectively; however, this difference was not statistically significant. The rbc PLP/plasma PLP ratio for control females was 1.2, which was significantly smaller than the ratios for female IDDM and NIDDM groups ($p< 0.01$, $p<0.05$, respectively). The ratio for all females

with diabetes (ratio =2.5) was also significantly greater than the ratio for control females ($p<0.01$). There were no statistically significant differences between rbc PLP/plasma PLP ratios for any group in the male population. All groups had an approximate 1:1 ratio between RBC PLP and plasma PLP. The ratio for males with DM was significantly smaller as compared to the ratio for females with DM ($p< 0.001$). The rbc PLP/plasma PLP ratio was not significantly different between male and female controls. The rbc PLP/plasma PLP ratio was not significantly related to glucose intolerance (as measured by total positive area under the curve for glucose), fasting plasma glucose, %GlyHb or to fasting insulin. The correlation coefficient between glucose intolerance and rbc PLP/ plasma PLP was 0.959 for females with IDDM. This apparent strong relationship was not statistically significant, however, due to the small sample size. Table 13 provides a comparison of fasting red blood cell PLP and the fasting rbc PLP/plasma PLP ratios in the current study, and four previous studies.

Areas Under the Curve for Red Blood Cell PLP during the OGTT

The results for both positive and negative cumulative areas under the curve for rbc PLP concentrations are listed in table 14. In persons with diabetes, mean values for positive, cumulative areas under the curve (+AUC rbc) were consistently greater than mean negative areas under the curve (-AUC rbc) at all time points. Conversely, values for mean +AUC rbc were consistently lower than mean -AUC rbc at all time points in control subjects. Because of large standard deviations and small sample sizes, none of these differences were statistically significant with the exception of the difference between mean +AUC rbc and mean -AUC rbc at 60 minutes for males with IDDM ($p<0.05$). Also, there were no statistically significant differences between groups at any time point during the OGTT for either mean values for +AUC rbc or mean values for -AUC rbc. The overall mean increase and decrease in rbc PLP during the OGTT (as

measured by the +AUC rbc and -AUC rbc at 120 minutes) for all subjects (n=35) was +275 nmol/L x min. and -195 nmol/L x min., respectively.

Alkaline Phosphatase

The results for mean fasting plasma alkaline phosphatase activity (AP) are listed in table 15. The mean AP values for females with NIDDM and all females with diabetes (DM) were significantly greater than the mean value for female controls ($p<0.05$). Also, the values for mean plasma AP activity for all females with DM was significantly greater than the values for mean AP activity for all males with DM ($p<0.05$). The difference in mean plasma AP activity values between females with IDDM and NIDDM was not statistically significant. There were no statistically significant differences in AP activity between groups within the male population. Plasma AP activity was not significantly correlated with fasting plasma PLP, fasting rbc PLP, the rbc PLP/plasma PLP ratio, or the change in plasma PLP (Δ PLP) in any of the study groups.

TABLE 1
Subject description: Mean age, weight (Wt), height (Ht), body mass index (BMI) and glycoslyated hemoglobin (GlyHg).[a]

Group	n	Age	Wt	Ht	BMI	GlyHg
		(Y)	(kg)	(M)	(kg/M^2)	(%)
Females						
IDDM[f]	3	37[b]	78.8	1.67	27.7	9.01[e]
		±8	±22.0	±0.10	±4.62	±1.39
NIDDM[g]	5	61[c]	94.2[e]	1.60	36.2[e]	10.0[e]
		±11	±16.9	±0.08	±5.45	±2.61
Control	9	42	63.2	1.66	22.8	5.59
		±13	±7.22	±0.07	±3.00	±0.81
Males						
IDDM	5	38[b]	85.0	1.83	25.2	8.46[d]
		±9	±11.9	±0.04	±2.88	±1.78
NIDDM	4	55[c]	88.5	1.78	26.5	8.74[e]
		±9	±15.9	±0.06	±4.55	±1.47
Control	9	41	81.4	1.80	25.3	5.36
		±11	±14.6	±0.03	±4.96	±0.97

a. Mean ± SD.
b. Significantly different from mean of same sex NIDDM group, ($p<0.05$).
c, d, e. Significantly different from mean of same sex control group, ($p<0.05$), ($p<0.01$), ($p<0.001$), respectively.
f. Insulin dependent diabetes mellitus.
g. Non-insulin dependent diabetes mellitus.

TABLE 2
Mean intake of dietary vitamin B6 and protein, and the B6:protein ratio for all subjects.[a,b]

Group	n	Vitamin B6	Protein	B6:protein
		(μmol/d)	(g/d)	(mg/g)
Females				
IDDM	3	9.33 ±3.38	99.3 ±41.0	0.017 ±0.004
NIDDM	5	12.40 ±3.19	69.2 ±15.1	0.030 ±0.011
DM[c]	8	10.88 ±3.43	80.5 ±29.9	0.029 ±0.011
Control	9	11.65 ±4.55	80.1 ±29.2	0.024 ±0.010
Males				
IDDM	5	15.77 ±5.68	101.3 ±22.1	0.026 ±0.005
NIDDM	4	17.81 ±3.56	130.5 ±37.3	0.024 ±0.003
DM	9	16.68 ±4.69	114.2 ±31.6	0.025 ±0.004
Control	9	13.70 ±6.85	94.9 ±27.5	0.024 ±0.003

a. Mean ± SD.
b. Vitamin B6 expressed as mg/0.1691
c. Combined IDDM and NIDDM.

TABLE 3
Mean plasma glucose concentrations during the oral glucose tolerance test.[a]

Group	n	Time, minutes 0	30	60	120
Females			(mmol/L)		
IDDM	3	16.0[c] ±10.3	21.0[c] ±10.7	23.5[d] ±10.7	28.1[d] ±8.7
NIDDM	5	11.6[d] ±4.0	16.4[d] ±4.5	19.2[d] ±4.6	20.5[d] ±3.5
DM	8	13.3[c] ±6.7	18.1[d] ±7.0	20.8[d] ±7.1	23.4[d] ±6.6
Control	9	4.9 ±0.5	7.8 ±1.3	7.2 ±1.6	6.0 ±1.7
Males					
IDDM	5	10.0[b] ±5.4	15.7[c] ±5.5	21.1[d] ±5.6	25.3[d] ±4.9
NIDDM	4	9.2[d] ±2.2	14.0[d] ±2.2	17.0[d] ±1.8	18.8[d] ±2.8
DM	9	9.6[c] ±4.1	15.0[d] ±4.2	19.3[d] ±4.6	22.4[d] ±5.2
Control	9	5.3 ±0.6	7.7 ±2.4	6.9 ±3.2	5.5 ±1.6

a. Mean ± SD.
b, c, d. Significantly different from mean of same sex control group, for each respective time point ($p<0.05$), ($p<0.01$), ($p<0.001$), respectively.

TABLE 4

Mean cumulative, positive areas under the curve for changes in glucose concentrations during the oral glucose tolerance test.[a]

Group	n	Time, minutes 30	60	120
Females			(mmol/L x min)	
IDDM	3	75[c] ±28	262[d] ±84	847[e] ±193
NIDDM	5	72[c] ±25	258[d] ±65	753[e] ±171
Control	9	43 ±18	120 ±54	225 ±120
Males				
IDDM	5	86[d] ±23	339[e] ±79	1132[b,e] ±22
NIDDM	4	72[c] ±5.6	262[d] ±11	781[e] ±66
Control	9	37 ±28	103 ±87	178 ±167

a. Mean ± SD.

b. Significantly different from mean of same sex NIDDM group, for each respective time point ($p<0.05$).

c, d, e. Significantly different from mean of same sex control group, for each respective time point ($p<0.05$), ($p<0.01$), ($p<0.001$), respectively.

TABLE 5
Mean plasma insulin concentrations during the oral glucose tolerance test.[a]*

Group	n	Time, minutes 0	30	60	120
Females			(pmol/L)		
IDDM	3	194[d] ±109	208 ±105	186 ±91	159[b] ±68
NIDDM	5	235[d] ±110	401 ±139	435[c] ±162	518 ±222
Control	9	73 ±30	296 ±85	371 ±123	292 ±214
Males					
IDDM	5	890 ±1320	832 ±1148	825 ±1205	882[c] ±623
NIDDM	4	129 ±12	187[c] ±63	256 ±90	314 ±123
Control	9	88 ±36	431 ±209	437 ±270	292 ±214

a. Mean ± SD.
b. Significantly different from mean of same sex NIDDM group, for each respective time point ($p<0.05$).
c, d. Significantly different from mean of same sex control group, for each respective time point ($p<0.05$), ($p<0.01$) respectively.

*The insulin values in subjects with IDDM reflect the amount of exogenous insulin left over from from the last insulin injection prior to the OGTT. All subjects withheld their usual dose of insulin during the 10-12 hour fast prior to the OGTT, with the exception of one male subject with IDDM who was on an insulin pump. The large means and standard deviations in the male IDDM group are a reflection of this one subject's high plasma insulin concentrations.

TABLE 6

Mean cumulative, positive areas under the curve for changes in plasma insulin concentrations during the oral glucose tolerance test.[a]

Group	n	Time, minutes 30	60	120
Females			(pmol/L x min)	
IDDM	3	—	—	—
NIDDM	5	2488 ±1599	7959 ±4962	22406 ±14806
Control	9	3334 ±939	11135 ±3191	26644 ±11013
Males				
IDDM	5	—	—	—
NIDDM	4	975[b] ±882	3850[b] ±3126	13193 ±9766
Control	9	5148 ±2796	15523 ±8016	32337 ±18735

a. Mean ± SD.

b. Significantly different from mean of same sex control group, for each respective time point ($p<0.05$).

TABLE 7
Mean plasma pyridoxal 5'-phosphate concentrations during the oral glucose tolerance test.[a]

Group	n	Time, minutes 0	30	60	120
Females			(nmol/L)		
IDDM	3	16.1 ±3.2	15.7 ±4.3	15.5 ±4.5	15.0 ±5.0
NIDDM	5	21.2 ±10.1	19.0 ±7.8	18.2 ±7.6	16.9 ±6.7
DM	8	19.3[b] ±8.2	17.7[b] ±6.6	17.2[b] ±6.4	16.2[b] ±5.8
Control	9	34.0 ±23.4	32.7 ±22.5	29.9 ±21.0	28.1 ±28.1
Males					
IDDM	5	48.9 ±22.0	43.5 ±17.5	42.1 ±17.1	41.3 ±19.3
NIDDM	4	45.2 ±10.0	43.2 ±9.3	39.7 ±9.8	37.5 ±9.0
DM	9	47.2 ±16.8	43.3 ±13.6	41.0 ±13.6	39.6 ±14.8
Control	9	35.9 ±14.7	33.3 ±13.4	31.7 ±13.4	30.2 ±12.6

a. Mean ± SD.
b. Significantly different from mean of male DM group for each respective time point ($p<0.001$).

TABLE 8
Overall change in mean plasma PLP from 0 to 120 minutes (Δ PLP), and correlation coefficients for the relationship between Δ PLP and fasting plasma PLP concentration (fast. PLP).

Group	n	Δ PLP	Δ PLP vs. fast. PLP
Females		(nmol/L)	(r)
IDDM	3	-1.1	-0.148
NIDDM	5	-4.3	0.976[b]
DM	8	-3.1	0.877[b]
Control	9	-5.9	0.957[c]
Males			
IDDM	5	-7.6	-0.207
NIDDM	4	-7.7	0.482
DM	9	-7.6	0.718[a]
Control	9	-5.7	0.577

a. $p < 0.05$
b. $p < 0.01$
c. $p < 0.001$

TABLE 9

Mean cumulative, negative areas under the curve for changes in plasma pyridoxal 5'-phosphate concentrations during the oral glucose tolerance test.[a]

Group	n	Time, minutes 30	60	120
Females		(nmol/L x min)		
IDDM	3	-12 ±10	-35 ±33	-110 ±96
NIDDM	5	-34 ±38	-112 ±122	-332 ±307
Control	9	-22 ±24	-104 ±79	-402 ±282
Males				
IDDM	5	-81 ±69	-266 ±210	-699 ±440
NIDDM	4	-32 ±26	-146 ±67	-542 ±189
Control	9	-39 ±34	-141 ±115	-437 ±348

a. Mean ± SD.

TABLE 10

Correlation coefficients for the relationships between the cumulative, positive areas under the curve for glucose (+AUC glu), cumulative, negative areas under the curve for plasma pyridoxal 5'-phosphate (-AUC plp), and the cumulative, positive areas under the curve for insulin (+AUC ins).

Group	n	-AUC plp vs. +AUC glu	-AUC plp vs. +AUC ins
Females		(r)	(r)
IDDM	3	0.832[a]	——
NIDDM	5	0.700[a]	0.391
Control	9	0.678[b]	0.686[b]
Males			
IDDM	5	0.867[b]	——
NIDDM	4	0.948[b]	0.534
Control	9	0.874[b]	0.879[b]

a. $p < 0.01$
b. $p < 0.001$

TABLE 11
Mean red blood cell pyridoxal 5'-phosphate concentrations during the oral glucose tolerance test.[a]

Group	n	Time, minutes 0	30	60	120
Females			(nmol/L)		
IDDM	3	49.5 [b,d] ±6.5	51.2 [e] ±6.8	49.8[d] ±4.2	51.2 [e] ±5.9
NIDDM	5	39.3 ±4.9	41.5[c] ±8.6	39.7[c] ±9.0	41.5[c] ±9.8
Control	9	31.4 ±9.0	30.7 ±6.5	30.2 ±7.3	30.6 ±6.3
Males					
IDDM	5	37.8 c ±10.9	39.2[d] ±9.7	39.4[c] ±10.2	42.5[e] ±4.8
NIDDM	4	45.6[d] ±12.3	47.0 e ±11.1	46.6[d] ±11.5	43.8[d] ±12.8
Control	9	28.3 ±4.4	26.8 ±3.9	29.0 ±6.9	28.8 ±3.3

a. Mean ± SD.
b. Significantly different from mean of same sex NIDDM group, for each respective time point ($p<0.05$).
c,d,e. Significantly different from mean of same sex control group, for each respective time point ($p<0.05$), ($p<0.01$), ($p<0.001$), respectively.

TABLE 12
Mean ratios of fasting red blood cell pyridoxal 5'-phosphate concentration (rbc PLP) to fasting plasma pyridoxal 5'-phosphate concentration (PLP).[a]

Group	n	rbc PLP/PLP
Females		
IDDM	3	3.2 ± 0.7 [c]
NIDDM	5	2.2 ± 0.9 [b]
DM	8	2.5 ± 0.9 [c,d]
Control	9	1.2 ± 0.7
Males		
IDDM	5	0.8 ± 0.2
NIDDM	4	1.1 ± 0.4
DM	9	0.9 ± 0.3
Control	9	1.0 ± 0.6

a. Mean ± SD.
b,c. Significantly different from mean of same sex control group ($p<0.05$), ($p<0.01$), respectively.
d. Significantly different from mean of male DM group ($p<0.001$).

TABLE 13
Comparison of fasting red blood cell PLP (rbc PLP) and rbc PLP/plasma PLP (PLP) ratios in the current study, and four previous studies.

Reference	n	age (years)	B6 intake (µmol/d)	rbc PLP (nmol/L)	PLP (nmol/L)	rbc PLP/PLP (ratio)	method of B6 analysis
Bhagavan (1975)	9(M,F)	2-19	N/A	61.1	56.1	1.1	enzymatic
Kant (1988)	12M	29±1	12.56	85 ± 6	76 ± 6	1.1	enzymatic
	12M	50±1	11.79	72 ± 5	48 ± 6	1.5	
	12M	71±1	10.72	76 ± 5	42 ± 6	1.8	
Fonda (1989)	10M	31±5	N/A	56 ± 6	59 ± 8	1.0	HPLC
Hansen (1993)	6F	20-40	6.74	46 ± 10	29 ± 12	1.6	enzymatic
	6F	20-40	13.80	47 ± 14	59 ± 22	0.8	
Current study (1994)							
controls	9F	42±13	11.65	31 ± 9	34 ± 23	1.2	enzymatic
	9M	42±11	13.70	28 ± 4	36 ± 16	1.0	
IDDM	3F	37±8	9.33	50 ± 6	16 ± 3	3.2	
	5M	38±9	15.77	38 ± 11	49 ± 22	0.8	
NIDDM	5F	61±11	12.40	39 ± 5	21 ± 10	2.2	
	4M	55±9	17.81	46 ± 12	45 ± 10	1.1	

a. rbc PLP values determined by subtracting plasma PLP (enzymatic method) from whole blood PLP (enzymatic method).
b. rbc PLP/PLP ratios from current study are mean values from individual ratios, and do not necessarily equal ratios that would be obtained by dividing group mean rbc PLP by group mean plasma PLP.
c. Recommended Dietary Allowance (RDA) for vitamin B6 = 9.46 µmol/d (1.6 mg/day) and 11.83 µmol/day (2.0 mg/day) for adult females and males, respectively.

TABLE 14

Mean cumulative positive (pos) and negative (neg) areas under the curve for changes in red blood cell pyridoxal 5'-phosphate concentrations during the OGTT.[a]

Group	Time, minutes 30		60		120	
	pos	neg	pos	neg	pos	neg
Females			(nmol/L x min)			
IDDM	60 ±104	-34 ±30	171 ±296	-114 ±116	405 ±702	-288 ±384
NIDDM	49 ±80	-16 ±23	149 ±233	-78 ±95	368 ±552	-218 ±216
Control	24 ±33	-34 ±67	75 ±100	-112 ±229	194 ±247	-290 ±577
Males						
IDDM	22 ±19	-0.6 ±1.3	77 ±55	-2.4 [b] ±5.4	332 ±297	-53 ±118
NIDDM	59 ±96	-38 ±74	148 ±245	-91 ±179	198 ±315	-167 ±252
Control	6.7 ±14	-30 ±45	39 ±44	-75 ±127	151 ±153	-154 ±264

a. Mean ± SD.

b. Significantly different from value for +AUC for same time point, same group ($p<0.05$).

TABLE 15
Mean fasting plasma alkaline phosphatase concentrations.[a]

Group	n	μkat/L
Females		
IDDM	3	0.531 ±0.197
NIDDM	5	0.549[b] ±0.089
DM	8	0.543[b,c] ±0.127
Control	9	0.408 ±0.088
Males		
IDDM	5	0.390 ±0.037
NIDDM	4	0.465 ±0.145
DM	9	0.424 ±0.100
Control	9	0.398 ±0.135

a. Mean ± SD.
b. Significantly different from mean of same sex control group, ($p<0.05$).
c. Significantly different from mean of male DM group ($p<0.05$).

DISCUSSION

Population Characteristics Overview-Diabetic Control

In general, the females with diabetes in this study were in poorer diabetic control as compared to diabetic males. Mean fasting plasma glucose levels, percent glycosylated hemoglobin and BMI were highest in females with diabetes as compared to males. Fasting insulin was nearly 2x higher in females with NIDDM as compared to males with NIDDM. These differences between males and females with diabetes, as well as the differences between the diabetic population vs. controls become important when discussing the relationship between plasma glucose levels and PLP in the plasma and red blood cell.

Plasma PLP during the OGTT

The results from this study show that plasma PLP concentration decreases with increasing plasma glucose after an oral glucose load. The overall percent decrease in plasma PLP concentration from fasting values was 16-17% for both male and female diabetic and control groups. This is comparable to previous results from Leklem et al (1984, 1990), which showed an 18-25% decrease in plasma PLP concentration after a 5 hour OGTT in normal healthy controls. The slightly larger decrease observed by Leklem et al, as compared to the current study, may be due to the longer test period (5 hrs. vs. 2 hrs.). The overall change (decrease) in plasma PLP levels during the OGTT positively correlated with the overall change (increase) in plasma glucose levels. The negative, cumulative areas under the curve for PLP (-AUC plp) following the oral glucose load was used to describe the overall decrease in plasma PLP concentration, and the positive cumulative areas under the curve for glucose (+AUC glu) following the oral glucose load was used to describe the overall increase in plasma glucose concentration. A statistically significant relationship was seen between +AUC glu and -AUC plp for all study groups.

This relationship between -AUC plp and +AUC glu was stronger in all males and control females as compared to females with diabetes ($p<0.001$ vs. $p<0.01$, respectively). A possible explanation for this finding is that mean fasting plasma PLP levels were so low in females with diabetes (19.3 nmol/L) as compared to diabetic males (47.2 nmol/L), that much of a further decrease in plasma PLP in the diabetic female population may not have been possible. Support for this theory comes from previous research which has suggested that there are at least two pools of PLP in humans (Johansson et al, 1966; Shane 1978; Coburn, 1990; Leklem, 1991) and that depletion of the different pools occurs at different rates. Turnover of one pool occurs rapidly, in an estimated 0.5-7 days, and turnover of a second pool is thought to be slower, taking about 25-35 days (Shane, 1978; Leklem, 1991). Perhaps what is being observed in the current study is that the acute dose of glucose caused depletion of PLP in the pool with a rapid turnover rate, but did not have much of an affect (if any) on the pool with the slower turnover rate. Thus, assuming that a low plasma PLP level reflects depletion of at least the PLP pool with the rapid turnover rate, only minimal further decreases in plasma PLP concentration during a two hour glucose tolerance test would be expected in persons with a low fasting plasma PLP concentration. Therefore, subjects with the highest fasting plasma PLP concentration had the greatest potential for a large drop in plasma PLP during the OGTT.

This effect of the largest drop in plasma PLP occurring in persons with the highest fasting levels following an oral glucose load has also been observed in a study by Hollenbeck and Leklem (1985), and is further verified in the current study by the significant positive correlation between change in plasma PLP (Δ PLP) during the OGTT and fasting plasma PLP. The relationship between Δ PLP and fasting PLP was significant for both males and females with diabetes, but was statistically stronger for females, indicating the response of plasma PLP concentration during the OGTT was more related to fasting plasma PLP levels in females with diabetes as compared to males with diabetes. Or, to put it another way, even though the increase in plasma glucose was similar in both males

and females with diabetes, the decrease in plasma PLP in response to the given glucose load was limited in females due to their low fasting plasma PLP levels. Again, this helps to explain why the relationship between +AUC glu and -AUC plp was stronger in males with diabetes as compared to females.

A strong, positive statistically significant relationship was seen between the rate of change in plasma PLP (-AUC plp) and insulin production (+AUC ins) for both female and male control subjects. The relationship was not significant for any individual group of persons with diabetes. This makes sense, as the nature of diabetes is a lack of insulin production, or insulin insensitivity to a given glucose load. Therefore, as plasma PLP decreased in response to the glucose load, insulin production in persons with diabetes did not respond to the same degree as compared to the response in persons without diabetes.

Red Blood Cell PLP during the OGTT

The primary purpose of the OGTT in the current study was to provide an in vivo situation in which plasma glucose was increased over time above fasting levels, in order to determine the relative effects of increasing plasma glucose on red blood cell and plasma PLP concentrations. Three studies have shown that in vitro, the uptake of PL or other vitamin B6 vitamers into the red blood cell increases with increased glucose concentrations (Ink et al, 1982, 1984; Yamada et al, 1968). After uptake by the erythrocyte, PN or PL is "trapped" by phosphorylation to PNP or PLP (Fonda and Harker, 1982). The hypothesis was that rbc PLP concentrations would increase in a direct relationship with the decrease in plasma PLP concentration, indicating a sequestering of plasma PLP in the red blood cell in response to the glucose load, and answering the question of why plasma PLP decreases during the OGTT (glucose causes increased uptake into the red blood cell), and where does it go (into the red blood cell). Also, increased rbc PLP concentrations along with a concurrent decrease in plasma PLP and increase in plasma glucose would help to explain the lower plasma PLP levels normally seen in persons with diabe-

tes mellitus, a metabolic disease that is characterized by higher than normal blood glucose levels. However, the direction of change in rbc PLP concentration was not uniformly consistent, as it had been with plasma PLP (overall decrease) and plasma glucose (overall increase). Also, the magnitude of change in rbc PLP concentrations were small as compared to changes in plasma PLP and glucose. Thus the minimal changes in rbc PLP concentrations that occurred during the OGTT could not account for the significant decreases in plasma PLP.

This nonsignificant change in rbc PLP concentration with increased plasma glucose during the OGTT does not necessarily mean that high glucose levels do not contribute to increased levels of PLP in the red blood cell. What needs to be considered when analyzing these results is that the acute increase in plasma glucose concentrations that occurred during the two hour OGTT may or may not reflect the changes in rbc PLP levels that could occur with the chronic state of high blood glucose that is inherent in diabetes (Zeeman, 1983). In fact, subjects with diabetes in this study had both the highest mean percent glycosylated hemoglobin and the highest mean fasting rbc PLP concentrations as compared to controls. Although the relationship between %GlyHb and rbc PLP concentration was not statistically significant in any group, the elevated %GlyHb along with elevated rbc PLP supports the theory that chronic elevated blood glucose contributes to increased levels of rbc PLP.

Results from areas under the curve for red blood cells show a trend of a net increase in rbc PLP in persons with diabetes, and a net decrease in rbc PLP in persons without diabetes during the OGTT (Table 14). The mean positive areas under the curve were consistently greater than mean negative areas under the curve at all time points for each of the groups of persons with diabetes. Conversely, the mean negative areas under the curve were consistently greater than mean positive areas under the curve for male and female control groups. While not statistically significant, these results also suggest an

overall increase in rbc PLP concentration due to increasing plasma glucose, in persons with diabetes.

Error in methodology also needs to be considered when interpreting the changes in rbc PLP concentrations. The enzymatic technique that was used to measure rbc PLP in the current study was not without problems, and may have been a contributor to the small changes in mean rbc PLP during the OGTT. The tight binding of PLP to hemoglobin makes deproteinization of the PLP-hemoglobin molecule difficult, and thus recoveries were variable. In the current study, the average recovery of rbc PLP was only 51.3% and the coefficients of variation for three different rbc PLP pools were 11.6%, 14.2% and 15.5%. Rbc PLP values were not corrected for percent recovery. The impact that the low recovery of rbc PLP has on determining the relationship between glucose intolerance in diabetes, rbc PLP and overall vitamin B6 metabolism in unknown. More precise methods of rbc PLP analysis would be helpful in answering this question.

This is the first study to date that has measured rbc PLP concentration in persons with diabetes. For all time points during the OGTT, mean rbc PLP values were anywhere from 20-40% higher in persons with diabetes as compared to controls. Previous studies on normal, healthy humans have shown rbc PLP concentrations to range from 46-88 nmol/L (Table 12). These values are 1.5-3.0 fold higher than the mean values for control subjects in the current study (range 28-31 nmol/L), and up to 1.75 fold higher than mean values for subjects with diabetes (range 38-50 nmol/L). Part of this discrepancy may be explained by the different methods used to measure PLP in the red blood cells. HPLC was used by Fonda et al (1989), Bhagavan et al used an enzymatic method (1975), and Kant et al (1988) determined rbc PLP by enzymatically measuring whole blood PLP and plasma PLP, and subtracting to get rbc PLP. Also, the studies by Bhagavan and Fonda were not diet controlled. The enzymatic method used for rbc PLP analysis in the study by Hansen et al (1993) was identical to the one used in the current study. Mean rbc PLP values obtained by Hansen for non-diabetic females (46-47 nmol/L) were 1.5 times

higher than values for control females (31 nmol/L) in the current study, however, and did not seem to be explained by any differences in vitamin B6 intake. The females in the study by Hansèn were 10-15 yrs. younger than the control females in the current study, however. Thus this discrepancy in rbc PLP values may be related to age differences, or to the error of method as discussed above, or simply reflect a normal range of rbc PLP values for healthy, non-diabetic females.

The fasting red blood cell PLP/plasma PLP ratio was calculated to help determine the distribution of PLP between the plasma and the red blood cell in both diabetic and non diabetic healthy controls. A ratio significantly greater than 1:1 would indicate a sequestering of PLP in the red blood cell. Further, a ratio greater than 1:1 would also support the theory that red blood cell PLP and plasma PLP are two distinct circulating pools of PLP in the body (Kant et al, 1988). The ratios for all male groups (IDDM, NIDDM, controls), and control females were in an approximate 1:1 ratio, with the range being 0.8-1.2. The ratios for females with IDDM (3.2), NIDDM (2.2) and combined DM females (2.5) were significantly higher. Since this was the first study to determine rbc PLP in persons with diabetes, it was also the first study to determine rbc PLP/plasma PLP ratios in persons with diabetes, as well as to compare the rbc PLP/plasma PLP ratios in persons with diabetes to ratios in healthy normal controls. The reason for the differences in the ratios was because of a significantly lower mean plasma PLP concentration in females with diabetes as compared to males with diabetes, and higher rbc PLP values in all persons with diabetes as compared to controls. In the previous studies, the ratio between rbc PLP and plasma PLP concentrations ranged between 1-1.8 (Bhagavan et al, 1975; Fonda et al, 1989 Kant et al, 1988). In the study by Kant, the ratio increased with increasing age of subjects, and may have been related to the significant increase in fasting blood glucose that they observed, and which is normally associated with aging (Chernoff and Lipschitz, 1988).

The results from this study show that the distribution of PLP is different in females with diabetes as compared to males with diabetes and both male and female controls, even though vitamin B6 intake and B6:protein ratios met the current RDA for all study groups. However, the females with diabetes were also in worse diabetic control with increased fasting blood glucose levels, and increased %GlyHb as compared to all other groups. Although there were no significant correlations in the current study between fasting plasma glucose, %GlyHb or glucose intolerance (total positive area under the curve for glucose) and the rbc PLP/plasma PLP ratio, it is possible that future studies using larger populations of diabetics would show elevated rbc PLP/plasma PLP ratios to be related to elevated plasma glucose levels persons with diabetes, and that the poorer the diabetic control, the higher the rbc PLP concentration and the lower the plasma PLP concentration. In the study by Smith (1991) that utilized the same population as in the current study, it was reported that the B6:protein ratio was significantly related to plasma PLP concentration in control males and females and diabetic males, but not in females with diabetes. Smith reported that this lack of correlation between the dietary B6:protein ratio and plasma PLP levels in females with diabetes suggests the existance of another factor that is associated with plasma PLP in females with diabetes. Perhaps this "other factor" is in part elevated blood glucose concentrations, and overall poor glycemic control.

The impact of a relatively larger rbc PLP pool as compared to plasma PLP and overall PLP availability to tissues and thus vitamin B6 status is unknown. Diabetes mellitus has been associated with a low vitamin B6 status (as measured by plasma PLP), despite adequate intake (Hollenbeck et al, 1983; Manore et al, 1989, 1991). This relative "sequestering" or concentration of PLP in the red blood cell could be one reason plasma PLP levels in persons with diabetes are often lower than persons without diabetes. PLP binds tightly to hemoglobin in the red blood cell (Benesch et al, 1972; Fonda and Harker, 1982) and the extent of the availability of rbc PLP to other tissues is unknown (Leklem,

1988). Based on multiple indicators, however, the actual vitamin B6 status of all subjects with diabetes in this study was judged to be adequate, despite the low mean plasma PLP levels of females with diabetes (Smith, 1991). Perhaps this means that rbc PLP is available to tissues, and provide an additional source of vitamin B6 when plasma PLP levels are low. Further studies would be necessary to substantiate this theory.

Alkaline Phosphatase and PLP in Blood

Alkaline phosphatase (AP) is an enzyme that catalyzes the hydrolysis of the phosphorylated forms of vitamin B6 to the non-phosphorylated forms (Lumeng et al, 1974). AP activity has been shown to be elevated in persons with diabetes mellitus (Goldberg et al, 1977; Stephan et al, 1980; Maxwell et al, 1986). In non-diabetic healthy persons a high AP activity was associated with decreased plasma PLP (Kant et al, 1988). Further, it has been shown that plasma PLP concentration is significantly elevated in metabolic situations where AP activity is very low such as hypophosphatasia (Whyte et al, 1985, 1989).

The mean AP values for all study groups were within the normal range for AP activity (Roy, 1970). However, the mean plasma AP activities of all females diabetes was significantly greater than that of females controls. Also, the mean value for all females with diabetes was significantly greater than that for all males with diabetes. Smith suggested that in the current study this increased AP activity contributed in part to the lower levels of plasma PLP in females with diabetes (Smith, 1991). It also follows that this higher level of AP in females with diabetes as compared to males may in part be an explanation for the differences in rbc PLP/plasma PLP ratios observed in this study: In theory, increased levels of AP result in increased dephosphorylation of PLP, which leads to decreased plasma PLP, and increased plasma PL. PL can readily cross red blood cell membranes (Menhansho and Henderson, 1980; Fonda and Harker, 1982; Ink et al, 1982), thus resulting in increased rbc PLP concentrations. While females with diabetes had the

highest mean plasma AP activity levels and the largest rbc PLP/plasma PLP ratios (as compared to males with diabetes and both male and female controls), the correlation coefficients between AP activity and the rbc PLP/plasma PLP ratio were not statistically significant for any group. There were also no significant correlations between plasma AP activity and fasting plasma PLP, fasting rbc PLP, or overall change in plasma PLP (Δ PLP) during the oral glucose tolerance test. It is possible that a significant relationship could be demonstrated with a larger population size. It should also be noted that plasma AP activity per se may not be soley responsible for conversion of plasma PLP to PL. Results form Whyte have shown that membrane bound AP acts as a PLP ectophosphatase (Whyte et al, 1990). Thus plasma AP activity alone (as measured in ths current study) may or may not be expected to correlate with decreased plasma PLP and/or increased rbc PLP concentrations.

SUMMARY AND CONCLUSION

Summary

In this study we measured the effects of increased plasma glucose concentrations on plasma and red blood cell PLP levels in persons with diabetes mellitus and non-diabetic controls. The study population consisted of 3 females with IDDM, 5 IDDM males, 5 females with NIDDM, 4 NIDDM males, and 9 male and 9 female control subjects. All subjects were given a 75 gm oral dose of D-glucose. Plasma glucose, insulin, PLP and rbc PLP concentrations were measured at 0 (fasting), 30, 60 and 120 minutes after the glucose load. The rbc PLP/plasma PLP ratio was calculated on fasting samples in order to determine the relative distribution of PLP between the plasma and the red blood cell. Fasting AP activity and the %GlyHb were also measured. Dietary vitamin B6, protein and energy intake were calculated from food intake records that were kept for 3 days prior to the OGTT.

Females with diabetes had the highest fasting plasma glucose, the largest BMI, and the lowest fasting plasma PLP concentrations as compared to other study groups. Percent glycoslyated hemoglobin was also highest in females with diabetes, indicating poorest diabetic control. Vitamin B6 intake and the B6:protein ratio were lowest in females with diabetes, but mean values for all study groups met the current guidelines for recommended vitamin B6 and B6:protein ratio intakes (NRC, 1989).

A significant relationship was seen between the change in plasma PLP concentration and the change in plasma glucose concentration during an OGTT in all study groups. As plasma glucose increased, plasma PLP decreased in a direct and significant manner. The relationship was stronger in all males, and control females as compared to females with diabetes ($p< 0.001$ vs. $p<0.01$, respectively). This difference was in part explained by the lower fasting PLP levels in females with diabetes as compared to all other study groups.

A subsequent increase in rbc PLP concentration was not observed as had been hypothesized, however. Although mean rbc PLP values were 20-40% greater in persons with diabetes as compared to control subjects at all time points during the OGTT, the changes in mean rbc PLP concentrations during the OGTT were minimal, and did not support the theory that increased plasma glucose concentrations result in a sequestering of PLP in the red blood cell in persons with diabetes. However, the elevated % GlyHb that was seen in persons with diabetes suggests that increased plasma glucose over an extended period of time could contribute to increased levels of rbc PLP.

The distribution of PLP between the red blood cell and plasma was different in females with diabetes as compared to all other groups. The rbc PLP/plasma PLP ratio was 2:1 in females with NIDDM, 3:1 in females with IDDM, and approximately 1:1 in all other groups. The higher ratios in females with diabetes could in part be related to elevated glucose levels. Also, the fact that the ratios in diabetic females were significantly greater than 1 suggest that plasma PLP and rbc PLP are two separate pools of PLP.

The impact on vitamin B6 status of this apparent sequestering of PLP in the red blood cells of diabetic females is uncertain. However, based on results of a coworker the B6 status in females with diabetes was judged to be adequate despite the low plasma PLP levels. This suggests that the red blood cell pool of PLP may be available to other tissues for use in metabolic situations that require PLP.

Mean fasting plasma alkaline phosphatase activity was significantly higher in all females with diabetes as compared to other study groups. Females with diabetes also had the lowest mean fasting plasma PLP, and the highest mean fasting rbc PLP/plasma PLP ratio. Although there were no significant correlations between mean plasma AP activity and mean fasting plasma PLP or mean fasting rbc PLP, the direction of these results suggest that an elevated AP activity may also contribute to the difference in PLP distribution between red blood cells and plasma.

Conclusion

Plasma PLP decreased while plasma glucose increased during an oral glucose tolerance test for both diabetic and non diabetic controls. The relationship between the overall decrease in plasma PLP and the overall increase in plasma glucose was statistically significant for all study groups. A subsequent increase in red blood cell PLP during the OGTT did not occur as had been hypothesized . However, mean rbc PLP levels were 20-40% higher in persons with diabetes for all time points during the OGTT. This significant difference in rbc PLP concentration in persons with diabetes as compared to controls may be related to chronically elevated levels of plasma glucose that characterizes the disease of diabetes mellitus. The ratio of rbc PLP to plasma PLP was 2-3X higher in females with diabetes as compared to all other study groups. Females with diabetes also had the lowest plasma PLP, the highest plasma AP activity, the highest fasting blood glucose, the highest %GlyHb, and the largest BMI. These results suggest that increased plasma glucose levels, increased plasma AP activity, and overall poor glycemic control contribute to decreased plasma PLP concentrations, increased rbc PLP concentrations, and changes in the PLP distribution within the body.

Future Studies

The implications of these changes in plasma PLP, rbc PLP and the distribution of PLP between the plasma and red blood cell on vitamin B6 status are uncertain. Future studies that are designed to more fully elucidate this relationship are suggested by this author. Study designs should include a larger diabetic population, as many of the relationships in the current study appeared strong, but were not statistically significant due to small sample sizes. Also, a longer OGTT (5 hrs. vs. 2 hrs, for example) and/or an in vitro study in which red blood cells could be incubated with increasing levels of glucose for a period of time longer than 2 hours might help to validate the theory that chronically

elevated glucose contributes to increased rbc PLP concentrations. Specifically, the questions to be answered in future studies include:

- Does the decrease in plasma PLP and the increase in the rbc PLP/plasma PLP ratio as seen in females with diabetes, and the increase in red blood cell PLP as seen in both males and females with diabetes adversely affect vitamin B6 status?
- Are these changes in plasma PLP, rbc PLP and the rbc PLP/plasma PLP ratio magnified by poor diabetic control?
- If so, can improvement in diabetic control reverse the changes in PLP levels in the plasma and red blood cell?
- Is PLP sequestered in the red blood cell, or are the red blood cell a storage sight for PLP, and thus available to other tissues?
- Can vitamin B6 supplementation reverse the decrease in plasma PLP that occurs in persons with diabetes, and if so, what dose is needed?
- Does increased plasma AP activity in persons with diabetes also contribute to increased rbc PLP levels, and decreased plasma PLP levels? The assumption here is that plasma AP dephosphorylates plasma PLP to PL, and then PL is take up by the red blood cell and converted back to PLP. Thus, studies that included measurement of plasma PL along with plasma PLP and rbc PLP would be helpful to answer this question.

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APPENDIX

Table A1
Individual values of anthropometric and other descriptive indices of individuals with insulin dependent diabetes mellitus.

Subject	Age	Ht	Wt	BMI	%Gly Hb	Hct	Hb
	(y)	(M)	(kg)	(kg/M^2)	(%)	(l)	(g/dL)
Females							
CP-4	40	1.79	103.0	32.1	10.30	0.435	13.7
LN-11	28	1.62	60.0	22.9	7.53	0.402	13.7
KM-20	42	1.61	73.4	28.2	9.20	0.410	13.8
Mean	37	1.67	78.8	27.7	9.01	0.416	13.7
SD	8	0.10	22.0	4.6	1.39	0.017	0.1
Males							
JW-6	43	1.78	73.6	23.3	9.93	0.466	16.0
KR-10	35	1.89	96.6	27.2	7.88	0.463	15.8
AL-13	50	1.82	94.5	28.6	9.18	0.478	15.8
DL-15	26	1.87	89.1	25.6	9.69	0.484	16.1
DW-21	34	1.82	70.9	21.5	5.61	0.457	15.3
Mean	38	1.83	85.0	25.2	8.46	0.470	15.8
SD	9	0.04	11.9	2.9	1.78	0.011	0.3

Table A2
Individual values of anthropometric and other descriptive indices of individuals with non-insulin dependent diabetes mellitus.

Subject	Age	Ht	Wt	BMI	%GlyHb	Hct	Hb
	(y)	(M)	(kg)	(kg/M^2)	(%)	(l)	(g/dL)
Females							
FA-2	67	1.46	82.3	38.5	8.60	0.454	15.7
MR-8	70	1.61	80.2	30.9	9.00	0.445	14.5
MC-16	69	1.61	78.8	30.3	11.35	0.452	15.4
NU-24	50	1.63	114.5	43.0	7.30	0.441	14.1
MP-43	47	1.69	110.0	38.6	13.89	0.507	15.7
Mean	61	1.60	94.2	36.2	10.00	0.460	15.1
SD	11	0.08	16.9	5.4	2.61	0.027	0.7
Males							
TS-3	65	1.81	70.2	21.4	8.67	0.485	15.9
AP-9	52	1.81	99.1	30.2	10.82	0.483	16.6
GI-17	44	1.82	104.2	31.6	7.50	0.476	16.2
JL-22	59	1.70	80.4	27.8	7.95	0.419	15.2
Mean	55	1.78	88.5	26.5	8.74	0.466	16.0
	9	0.06	15.9	4.6	1.47	0.031	0.6

Table A3
Individual values of anthropometric and other descriptive indices of healthy control individuals.

Subject	Age	Ht	Wt	BMI	%GlyHb	Hct	Hb
	(y)	(M)	(kg)	(kg/M^2)	(%)	(l)	(g/dL)
Females							
HM-28	30	1.68	68.6	24.4	5.34	0.435	14.6
JS-29	39	1.72	67.5	22.8	6.86	0.415	12.8
CH-32	41	1.61	64.3	24.9	5.31	0.414	13.6
SR-34	44	1.73	58.6	19.6	5.42	0.371	11.2
BP-36	39	1.66	55.0	19.9	5.05	0.433	13.5
DM-37	29	1.52	55.4	23.8	7.03	0.443	13.7
SK-38	28	1.73	63.2	21.2	5.54	0.415	13.7
PC-47	59	1.64	77.3	28.8	4.94	0.426	14.1
MS-48	66	1.70	58.6	20.2	4.79	0.426	14.1
Mean	42	1.66	63.2	22.8	5.59	0.420	13.5
SD	13	0.07	7.2	3.0	0.81	0.021	1.0
Males							
DU-25	56	1.83	75.0	22.4	7.40	0.440	14.8
MC-27	53	1.75	86.8	28.2	5.32	0.449	16.0
KT-30	23	1.77	67.5	21.5	4.30	0.441	15.6
DR-31	47	1.76	114.1	36.6	6.40	0.488	16.3
DP-33	28	1.82	84.5	25.6	5.28	0.473	16.2
JR-35	44	1.78	70.4	22.1	5.12	0.442	15.1
CC-39	40	1.78	69.0	21.8	5.06	0.440	15.1
BA-40	42	1.80	89.1	27.4	4.71	0.487	16.8
GB-41	37	1.85	75.9	22.1	4.62	0.439	14.8
Mean	41	1.80	81.4	25.3	5.36	0.455	15.6
SD	11	0.03	14.6	5.0	0.97	0.021	0.7

Table A4
Individual values of plasma glucose during the OGTT of individuals with insulin dependent diabetes mellitus.

Subject	Time, minutes 0	30	60	120
		(mmol/L)		
Females				
CP-4	23.6	27.2	29.7	32.8
LN-11	4.3	8.7	11.1	18.1
KM-20	20.1	27.2	29.7	33.4
Mean	16.0	21.0	23.5	28.1
SD	10.3	10.7	10.7	8.6
Males				
JW-6	7.2	15.0	21.1	24.0
KR-10	19.5	25.0	30.1	32.5
AL-13	9.0	14.4	19.2	25.7
DL-15	7.3	13.6	20.1	25.4
DW-21	6.8	10.4	14.8	18.8
Mean	10.0	15.7	21.1	25.3
SD	5.4	5.5	5.6	4.9

Table A5
Individual values of plasma glucose during the OGTT of individuals with non insulin dependent diabetes mellitus.

Subject	Time, minutes 0	30	60	120
		(mmol/L)		
Females				
FA-2	13.2	15.9	19.4	19.6
MR-8	9.1	14.4	19.4	21.9
MC-16	11.2	18.2	18.0	20.0
NU-24	7.0	10.8	13.2	15.8
MP-43	17.5	22.9	26.0	25.3
Mean	11.6	16.4	19.2	20.5
SD	4.0	4.5	4.6	3.5
Males				
TS-3	7.7	13.0	15.4	20.0
AP-9	12.2	16.9	19.4	21.4
GI-17	7.4	11.9	16.0	14.9
JL-22	9.7	14.5	17.2	18.9
Mean	9.2	14.0	17.0	18.8
SD	2.2	2.2	1.8	2.8

Table A6
Individual values of plasma glucose during the OGTT of healthy control individuals.

Subject	Time, minutes 0	30	60	120
		(mmol/L)		
Females				
HM-28	4.9	7.8	6.5	5.2
JS-29	5.4	9.1	8.1	8.8
CH-32	5.1	6.6	7.0	6.6
SR-34	4.6	6.7	5.3	4.3
BP-36	4.2	8.4	7.3	6.3
DM-37	4.8	8.6	8.3	5.7
SK-38	4.6	5.4	4.6	3.8
PC-47	5.7	9.7	9.9	8.2
MS-48	5.1	7.9	8.1	5.4
Mean	4.9	7.8	7.2	6.0
SD	0.5	1.3	1.6	1.7
Males				
DU-25	4.5	5.7	6.0	4.5
MC-27	5.4	7.3	8.3	7.3
KT-30	5.8	11.7	11.1	7.3
DR-31	6.3	10.4	12.3	7.3
DP-33	5.3	9.3	6.6	5.0
JR-35	4.9	4.6	3.2	6.2
CC-39	4.7	6.3	3.8	3.1
BA-40	5.7	8.3	7.0	4.9
GB-41	5.0	6.2	3.9	4.0
Mean	5.3	7.7	6.9	5.5
SD	0.6	2.4	3.2	1.6

Table A7
Individual values of positive, cumulative areas under the curve for plasma glucose during the OGTT for persons with insulin dependent diabetes mellitus.

Subject	Time, minutes 30	60	120
		(mmol/L x min)	
Females			
CP-4	960.0	3555.0	11775.0
LN-11	1173.0	4179.0	15291.0
KM-20	1920.0	6420.0	18720.0
Mean	1351.0	4718.0	15262.0
SD	504.0	1507.0	3473.0
Males			
JW-6	2100.0	7950.0	24540.0
KR-10	1500.0	5880.0	18660.0
AL-13	1455.0	5685.0	20265.0
DL-15	1710.0	6885.0	23565.0
DW-21	975.0	4125.0	14955.0
Mean	1548.0	6105.0	20397.0
SD	409.0	1428.0	3868.2

Table A8
Individual values of positive, cumulative areas under the curve for plasma glucose during the OGTT for persons with non-insulin dependent diabetes mellitus.

Subject	Time, minutes 30	60	120
		(mmol/L x min)	
Females			
FA-2	735.0	3150.0	9990.0
MR-8	1425.0	5640.0	18150.0
MC-16	1890.0	5625.0	14085.0
NU-24	1005.0	3660.0	11670.0
MP-43	1440.0	5175.0	13965.0
Mean	1299.0	4650.0	13572.0
SD	444.0	1166.0	3075.1
Males			
TS-3	1440.0	4980.0	15870.0
AP-9	1275.0	4515.0	13455.0
GI-17	1200.0	4710.0	13350.0
JL-22	1305.0	4665.0	13755.0
Mean	1305.0	4718.0	14108.0
SD	100.0	194.0	1188.0

Table A9
Individual values of positive, cumulative areas under the curve for plasma glucose during the OGTT for healthy control individuals.

Subject	Time, minutes 30	60	120
		(mmol/L x min)	
Females			
HM-28	763.5	1945.5	2938.5
JS-29	996.0	2718.0	6012.0
CH-32	414.0	1347.0	3213.0
SR-34	582.0	1372.5	1687.5
BP-36	1132.5	3097.5	5887.5
DM-37	1006.5	2959.5	5305.5
SK-38	211.5	432.0	432.8
PC-47	1080.0	3300.0	6930.0
MS-48	739.5	2278.5	4039.5
Mean	769.5	2161.0	4049.6
SD	318.1	965.0	2170.8
Males			
DU-25	304.5	1003.5	1780.7
MC-27	508.5	1780.5	4294.5
KT-30	1605.0	4650.0	8370.0
DR-31	1110.0	3840.0	7650.0
DP-33	1089.0	2517.0	3072.2
JR-35	0.0	0.0	281.5
CC-39	423.0	692.9	692.9
BA-40	690.0	1740.0	2188.8
GB-41	324.0	499.4	499.4
Mean	672.7	1858.0	3202.3
SD	504.0	1564.0	3018.3

Table A10
Individual values of plasma insulin during the OGTT of individuals with insulin dependent diabetes mellitus.

	Time, minutes			
Subject	0	30	60	120
		(pmol/L)		
Females				
CP-4	161	200	128	149
LN-11	316	317	291	232
KM-20	105	106	138	96
Mean	194	208	186	159
SD	109	105	91	68
Males				
JW-6	362	330	312	366
KR-10	377	474	376	403
AL-13	3240	2871	2973	3120
DL-15	343	339	312	306
DW-21	126	138	159	205
Mean	890	832	825	882
SD	1320	1148	1205	623

Table A11
Individual values of plasma insulin during the OGTT of individuals with non insulin dependent diabetes mellitus.

	Time, minutes			
Subject	0	30	60	120
		(pmol/L)		
Females				
FA-2	309	495	496	524
MR-8	173	306	344	328
MC-16	70	311	317	545
NU-24	331	600	693	868
MP-43	293	291	324	324
Mean	235	401	435	518
SD	110	139	162	222
Males				
TS-3	130	104	130	156
AP-9	141	208	258	286
GI-17	113	255	339	440
JL-22	132	182	296	373
Mean	129	187	256	314
SD	12	63	90	123

Table A12
Individual values of plasma insulin during the OGTT of healthy control individuals.

	Time, minutes			
Subject	0	30	60	120
		(pmol/L)		
Females				
HM-28	46	176	194	118
JS-29	53	311	342	146
CH-32	136	398	446	500
SR-34	64	272	280	104
BP-36	54	268	255	192
DM-37	90	410	578	432
SK-38	57	202	392	243
PC-47	102	378	503	726
MS-48	54	243	348	168
Mean	73	296	371	292
SD	30	85	123	214
Males				
DU-25	81	386	170	189
MC-27	88	331	733	427
KT-30	67	455	638	537
DR-31	148	499	672	471
DP-33	143	951	772	496
JR-35	43	386	63.8	142
CC-39	65	301	388	78.9
BA-40	90	275	287	148
GB-41	66	297	212	204
Mean	88	431	437	292
SD	36	209	270	214

Table A13
Individual values of positive, cumulative areas under the curve for plasma insulin during the OGTT for persons with insulin dependent diabetes mellitus.

Subject	Time, minutes 30	60	120
	(nmol/L x min)		
Females			
CP-4	81.0	124.3	124.3
LN-11	1.5	1.5	1.5
KM-20	1.5	72.0	179.6
Mean	28.0	66.0	102.0
SD	46.0	61.0	91.0
Males			
JW-6	0.0	0.0	1.0
KR-10	204.0	406.5	514.6
AL-13	0.0	0.0	0.0
DL-15	0.0	0.0	0.0
DW-21	27.0	124.5	598.5
Mean	46.2	106.2	222.8
SD	89.0	176.0	306.0

Table A14
Individual values of positive, cumulative areas under the curve for plasma insulin during the OGTT for persons with non-insulin dependent diabetes mellitus.

Subject	Time, minutes 30	60	120
	(nmol/L x min)		
Females			
FA-2	388.5	1167.0	2847.0
MR-8	279.0	915.0	2277.0
MC-16	504.0	1524.0	4539.0
NU-24	564.0	1885.5	5647.5
MP-43	0.0	60.3	315.3
Mean	347.1	1110.0	3125.0
SD	223.0	692.0	2065.0
Males			
TS-3	0.0	0.0	111.0
AP-9	141.0	528.0	1626.0
GI-17	297.0	1066.5	3376.5
JL-22	105.0	553.5	2248.5
Mean	135.8	537.0	1840.0
SD	123.0	436.0	1362.0

Table A15
Individual values of positive, cumulative areas under the curve for plasma insulin during the OGTT for healthy control individuals.

Subject	Time, minutes 30	60	120
	(nmol/L x min)		
Females			
HM-28	271.5	853.5	1777.5
JS-29	540.0	1684.5	3283.5
CH-32	549.0	1746.0	4566.0
SR-34	435.0	1321.5	2392.5
BP-36	447.0	1314.0	2730.0
DM-37	669.0	2359.5	5833.5
SK-38	301.5	1302.0	3477.0
PC-47	577.5	1993.5	6280.5
MS-48	394.5	1402.5	3103.5
Mean	465.0	1553.0	3716.0
SD	131.0	445.0	1536.0
Males			
DU-25	637.5	1461.0	2286.0
MC-27	507.0	2361.0	6471.0
KT-30	811.5	2817.0	7173.0
DR-31	732.0	2557.5	6094.5
DP-33	1689.0	4693.5	8803.5
JR-35	717.0	1477.5	1978.5
CC-39	495.0	1666.5	3079.5
BA-40	385.5	1182.0	2244.0
GB-41	483.0	1272.0	2460.0
Mean	717.5	2165.0	4510.0
SD	390.0	1118.0	2613.0

Table A16
Individual values of plasma pyridoxal 5'-phosphate (PLP) during the OGTT of individuals with insulin dependent diabetes mellitus.

	Time, minutes			
Subject	0	30	60	120
		(nmol/L)		
Females				
CP-4	19.0	20.1	19.5	20.8
LN-11	16.7	15.6	16.3	12.4
KM-20	12.6	11.4	10.6	11.8
Mean	16.1	15.7	15.5	15.0
SD	3.2	4.4	4.5	5.0
Males				
JW-6	59.6	50.8	51.9	51.2
KR-10	38.4	35.6	33.6	33.5
AL-13	38.6	36.6	34.7	29.0
DL-15	81.8	70.0	66.6	70.2
DW-21	26.2	24.5	23.5	22.8
Mean	48.9	43.5	42.1	41.3
SD	22.0	17.5	17.1	19.3

Table A17
Individual values of plasma pyridoxal 5'-phosphate during the OGTT of individuals with non insulin dependent diabetes mellitus.

	Time, minutes			
Subject	0	30	60	120
		(nmol/L)		
Females				
FA-2	31.0	27.8	27.0	22.4
MR-8	32.6	26.3	24.7	24.9
MC-16	16.0	15.1	14.7	14.0
NU-24	9.6	9.5	8.3	8.5
MP-43	16.8	16.1	16.2	14.6
Mean	21.2	19.0	18.2	16.9
SD	10.1	7.8	7.6	6.7
Males				
TS-3	58.6	54.4	52.1	47.6
AP-9	40.5	38.4	37.3	34.8
GI-17	46.2	46.6	41.0	41.0
JL-22	35.5	33.2	28.4	26.6
Mean	45.2	43.2	39.7	37.5
SD	10.0	9.3	9.8	9.0

Table A18
Individual values of plasma pyridoxal 5'-phosphate (PLP) during the OGTT of healthy control individuals.

Subject	Time, minutes 0	30	60	120
		(nmol/L)		
Females				
HM-28	37.0	33.0	31.7	30.6
JS-29	11.0	10.7	9.9	9.8
CH-32	18.8	20.4	18.9	18.5
SR-34	18.6	17.4	15.0	14.1
BP-36	21.3	19.3	17.8	17.1
DM-37	29.3	28.8	26.1	23.3
SK-38	24.7	25.2	20.5	20.2
PC-47	64.7	60.6	57.4	52.2
MS-48	80.3	79.1	72.1	66.9
Mean	39.0	32.7	29.9	28.1
SD	23.4	22.5	21.0	28.1
Males				
DU-25	14.1	13.9	13.2	14.5
MC-27	32.9	29.8	29.7	25.4
KT-30	41.8	34.7	32.1	26.2
DR-31	37.4	33.6	30.3	31.3
DP-33	58.5	54.3	50.1	49.3
JR-35	38.2	36.7	36.0	33.9
CC-39	52.8	50.2	52.4	48.6
BA-40	31.4	30.4	26.8	28.8
GB-41	16.1	16.1	15.0	14.2
Mean	35.9	33.3	31.7	30.2
SD	14.7	13.4	13.4	12.6

Table A19
Individual values of negative, cumulative areas under the curve for plasma PLP during the OGTT for persons with insulin dependent diabetes mellitus.

	Time, minutes		
Subject	30	60	120
	(nmol/L x min)		
Females			
CP-4	0.0	0.0	0.0
LN-11	16.5	39.0	180.0
KM-20	18.0	66.0	150.0
Mean	11.5	35.0	110.0
SD	10.0	33.0	96.0
Males			
JW-6	132.0	379.5	862.5
KR-10	42.0	156.0	447.0
AL-13	30.0	118.5	523.5
DL-15	177.0	582.0	1386.0
DW-21	25.5	91.5	274.5
Mean	81.3	265.5	698.7
SD	69.0	210.0	439.7

Table A20
Individual values of negative, cumulative areas under the curve for plasma PLP during the OGTT for persons with non-insulin dependent diabetes mellitus.

	Time, minutes		
Subject	30	60	120
	(nmol/L x min)		
Females			
FA-2	48.0	156.0	534.0
MR-8	94.5	307.5	775.5
MC-16	13.5	46.5	145.5
NU-24	1.5	21.8	93.2
MP-43	10.5	30.0	114.0
Mean	33.6	112.4	332.4
SD	38.4	121.9	306.9
Males			
TS-3	63.0	223.5	748.5
AP-9	31.5	111.0	378.0
GI-17	0.0	72.4	384.4
JL-22	34.5	175.5	655.5
Mean	32.3	145.6	541.6
SD	25.8	67.0	189.1

Table A21
Individual values of negative, cumulative areas under the curve for plasma PLP during the OGTT for healthy control individuals.

	Time, minutes		
Subject	30	60	120
	(nmol/L x min)		
Females			
HM-28	60.0	199.5	550.5
JS-29	4.5	25.8	96.9
CH-32	0.0	0.0	6.8
SR-34	18.0	90.0	333.0
BP-36	30.0	112.5	343.5
DM-37	7.5	63.0	339.0
SK-38	0.0	56.3	317.3
PC-47	61.5	232.5	826.5
MS-48	18.0	159.0	807.0
Mean	22.2	104.3	402.3
SD	24.0	79.0	281.7
Males			
DU-25	3.0	19.5	38.2
MC-27	46.5	141.0	462.0
KT-30	106.5	358.5	1117.5
DR-31	57.0	220.5	616.5
DP-33	63.0	252.0	780.0
JR-35	22.5	78.0	273.0
CC-39	39.0	84.0	222.0
BA-40	15.0	99.0	315.0
GB-41	0.0	16.5	106.5
Mean	39.2	141.0	436.7
SD	33.9	115.5	348.0

Table A22
Individual values of red blood cell pyridoxal 5'-phosphate during the OGTT of individuals with insulin dependent diabetes mellitus.

	Time, minutes			
Subject	0	30	60	120
		(nmol/L)		
Females				
CP-4	44.5	56.5	54.7	57.7
LN-11	56.9	53.7	47.8	49.6
KM-20	47.1	43.5	47.0	46.2
Mean	49.5	51.2	49.8	51.2
SD	6.5	6.8	4.2	5.9
Males				
JW-6	43.1	44.0	44.0	44.4
KR-10	36.6	37.7	42.0	40.0
AL-13	25.6	28.7	25.9	47.4
DL-15	53.3	53.1	52.9	45.3
DW-21	30.2	32.5	34.4	35.4
Mean	37.8	39.2	39.4	42.5
SD	10.9	9.7	10.2	4.8

Table A23
Individual values of red blood cell pyridoxal 5'-phosphate during the OGTT of individuals with non insulin dependent diabetes mellitus.

	Time, minutes			
Subject	0	30	60	120
		(nmol/L)		
Females				
FA-2	43.6	56.1	54.6	56.2
MR-8	45.3	42.0	37.7	45.5
MC-16	36.9	37.6	34.1	33.2
NU-24	33.7	36.9	40.2	40.1
MP-43	36.9	34.8	31.7	32.5
Mean	39.3	41.5	39.7	41.5
SD	4.9	8.6	9.0	9.8
Males				
TS-3	43.1	42.9	43.8	43.8
AP-9	61.5	51.6	57.3	59.7
GI-17	31.6	34.0	31.6	28.4
JL-22	46.3	59.7	53.7	43.1
Mean	45.6	47.0	46.6	43.8
SD	12.3	11.1	11.5	12.8

Table A24
Individual values of red blood cell pyridoxal 5'-phosphate during the OGTT of healthy control individuals.

Subject	Time, minutes 0	30	60	120
		(mmol/L)		
Females				
HM-28	28.8	27.5	30.7	29.5
JS-29	25.4	27.4	34.2	31.4
CH-32	28.9	29.2	27.7	32.2
SR-34	44.6	31.1	25.1	28.5
BP-36	24.7	30.7	27.3	29.0
DM-37	28.4	32.9	33.4	32.2
SK-38	20.0	21.7	18.6	19.0
PC-47	34.6	30.2	29.4	30.0
MS-48	46.8	45.9	45.3	43.5
Mean	31.4	30.7	30.2	30.6
SD	9.0	6.5	7.3	6.3
Males				
DU-25	32.3	23.2	23.6	26.3
MC-27	26.7	25.1	25.4	25.2
KT-30	33.8	29.6	40.4	32.1
DR-31	20.0	19.8	16.6	24.2
DP-33	32.0	32.1	33.8	26.8
JR-35	24.0	26.5	27.9	29.9
CC-39	30.0	29.0	32.4	30.0
BA-40	26.9	25.1	32.9	30.4
GB-41	29.2	30.6	27.8	33.8
Mean	28.3	26.8	29.0	28.8
SD	4.4	3.9	6.9	3.3

Table A25
Individual values of negative and positive cumulative areas under the curve for red blood cell PLP during the OGTT for persons with insulin dependent diabetes mellitus.

Subject	Time, minutes 30		60		120	
	(nmol/L x min)					
	pos.	neg	pos	neg	pos	neg
Females						
CP-4	180.0	- 0.0	513.0	-0.0	1215.0	-0.0
LN-11	0.0	-48.0	0.0	-232.5	0.0	-724.5
KM-20	0.0	- 54.0	0.0	-109.5	0.0	-139.5
Mean	60.0	-34.0	171.0	-114.0	405.0	-288.0
SD	104.0	-30.0	296.0	-116.0	702.0	-384.0
Males						
JW-6	13.5	-0.0	40.5	-0.0	106.5	-0.0
KR-10	16.5	-0.0	114.0	-0.0	378.0	-0.0
AL-13	46.5	-0.0	97.5	-0.0	760.5	-0.0
DL-15	0.0	-3.0	0.0	-12.0	0.0	-264.0
DW-21	34.5	-0.0	132.0	-0.0	414.0	-0.0
Mean	22.2	-0.6	77.0	-2.4	332.0	-53.0
SD	18.3	-1.3	55.0	-5.4	297.0	-118.0

Table A26
Individual values of negative and positive cumulative areas under the curve for red blood cell PLP during the OGTT for persons with non-insulin dependent diabetes mellitus.

Subject	Time, minutes 30		60		120	
	(nmol/L x min)					
	pos	neg	pos	neg	pos	neg
Females						
FA-2	187.5	- 0.0	540.0	- 0.0	1248.0	-0.0
MR-8	0.0	-49.5	0.0	-213.0	0.2	-435.2
MC-16	10.5	-0.0	12.6	-33.6	12.6	-228.6
NU-24	48.0	-0.0	193.5	-0.0	580.5	- 0.0
MP-43	0.0	-31.5	0.0	-141.0	0.0	-429.0
Mean	49.2	-16.2	149.2	-78.0	368.0	-218.0
SD	79.8	-23.1	233.0	- 95.6	552.0	-216.0
Males						
TS-3	0.0	-3.0	8.2	-3.7	50.2	-3.7
AP-9	0.0	-148.5	0.0	-360.0	0.0	- 540.0
GI-17	36.0	-0.0	72.0	-0.0	72.0	-96.0
JL-22	201.0	-0.0	312.0	-0.0	668.0	-29.0
Mean	59.2	-37.9	148.2	-90.9	198.0	-167.0
SD	96.0	-73.8	245.4	-179.3	315.0	-252.0

Table A27
Individual values of negative and positive cumulative areas under the curve for red blood cell PLP during the OGTT for healthy control individuals.

Subject	Time, minutes 30		60		120	
	(nmol/L x min)					
	pos	neg	pos	neg	pos	neg
Females						
HM-28	0.0	-19.5	16.9	-27.4	94.9	-27.4
JS-29	30.0	0.0	192.0	0.0	636.0	0.0
CH-32	4.5	0.0	5.4	-14.4	78.0	-24.0
SR-34	0.0	- 202.5	0.0	-697.5	0.0	-1765.5
BP-36	90.0	0.0	219.0	0.0	426.0	0.0
DM-37	67.5	0.0	210.0	0.0	474.0	0.0
SK-38	22.5	0.0	33.4	-12.4	33.4	-96.4
PC-47	0.0	-66.0	0.0	-210.0	0.0	-504.0
MS-48	0.0	-13.5	0.0	-49.5	0.0	-193.5
Mean	23.8	-33.5	75.2	-112.4	193.6	-290.1
SD	33.5	-66.9	99.7	-229.3	247.4	-576.8
Males						
DU-25	0.0	-136.5	0.0	-403.5	0.0	-844.5
MC-27	0.0	-24.0	0.0	- 67.5	0.0	-151.5
KT-30	0.0	-63.0	60.5	-87.5	218.0	-98.0
DR-31	0.0	-3.0	0.0	-57.0	69.6	-102.6
DP-33	1.5	0.0	30.0	0.0	43.9	-115.9
JR-35	37.5	0.0	133.5	0.0	427.5	0.0
CC-39	0.0	-15.0	25.4	-19.4	106.4	-19.4
BA-40	0.0	-27.0	69.2	-33.2	354.2	-33.2
GB-41	21.0	0.0	31.5	-10.5	137.3	-20.3
Mean	6.7	-29.8	38.9	-75.4	151.0	-154.0
SD	13.5	-44.9	44.0	-127.0	153.0	-264.0

TABLE A28
Plasma alkaline phosphatase activity of all subjects

(μkat/L)

IDDM		NIDDM		Control	
			Females		
CP-4	0.547	FA-2	0.485	HM-28	0.277
LN-11	0.327	MR-8	0.685	JS-29	0.547
KM-20	0.720	MC-16	0.458	CH-32	0.393
		NU-24	0.573	SR-34	0.323
		MP-43	0.542	BP-36	0.412
				DM-37	0.448
				SK-38	0.360
				PC-47	0.523
				MS-48	0.392
Mean	0.531		0.549		0.408
SD	0.197		0.089		0.088
			Males		
JW-6	0.382	TS-3	0.443	DU-25	0.600
KR-10	0.407	AP-9	0.407	MC-27	0.282
AL-13	0.433	GI-17	0.338	KT-30	0.287
DL-15	0.417	JL-22	0.672	DR-31	0.407
DW-21	0.333			DP-33	0.573
				JR-35	0.248
				CC-39	0.302
				BA-40	0.363
				GB-41	0.523
Mean	0.390		0.465		0.398
SD	0.037		0.145		0.135

TABLE A29
Fasting plasma C-peptide of all subjects

(pmol/L)

IDDM		NIDDM		Control	
			Females		
CP-4	0.547	FA-2	0.485	HM-28	0.277
LN-11	0.327	MR-8	0.685	JS-29	0.547
KM-20	0.720	MC-16	0.458	CH-32	0.393
		NU-24	0.573	SR-34	0.323
		MP-43	0.542	BP-36	0.412
				DM-37	0.448
				SK-38	0.360
				PC-47	0.523
				MS-48	0.392
Mean	0.531		0.549		0.408
SD	0.197		0.089		0.088
			Males		
JW-6	0.382	TS-3	0.443	DU-25	0.600
KR-10	0.407	AP-9	0.407	MC-27	0.282
AL-13	0.433	GI-17	0.338	KT-30	0.287
DL-15	0.417	JL-22	0.672	DR-31	0.407
DW-21	0.333			DP-33	0.573
				JR-35	0.248
				CC-39	0.302
				BA-40	0.363
				GB-41	0.523
Mean	0.390		0.465		0.398
SD	0.037		0.145		0.135

Table A30
Dietary vitamin B6 intake of individuals with insulin dependent diabetes mellitus.

Subject	Day 1	Day 2 (μmol/day)	Day 3	Mean
Females				
CP-4	19.16	12.12	8.40	13.23
LN-11	11.18	7.69	2.91	7.26
KM-20	7.75	9.34	5.41	7.50
Mean	12.70	9.72	5.57	9.33
SD	5.85	2.24	2.75	3.38
Males				
JW-6	15.38	16.68	16.85	16.30
KR-10	6.98	9.52	11.53	9.34
AL-13	12.54	6.98	14.90	11.47
DL-15	26.02	23.18	22.12	23.77
DW-21	19.10	17.98	16.85	17.98
Mean	16.00	14.87	16.45	18.20
SD	7.14	6.57	3.84	3.02

Table A31
Dietary vitamin B6 intake of individuals with non-insulin dependent diabetes mellitus.

Subject	Day 1	Day 2 (μmol/day)	Day 3	Mean
Females				
FA-2	15.20	16.79	8.69	13.56
MR-8	17.56	14.61	18.98	17.05
MC-16	10.29	5.74	10.17	8.73
NU-24	11.41	13.48	11.95	9.40
MP-43	9.58	8.57	12.89	10.35
Mean	12.81	11.84	12.54	12.40
SD	3.43	4.55	3.95	3.19
Males				
TS-3	23.54	9.88	17.62	17.01
AP-9	19.22	22.41	27.56	23.06
GI-17	11.24	17.09	18.63	15.65
JL-22	19.40	12.12	15.08	15.53
Mean	18.35	15.38	19.72	17.81
SD	5.14	5.58	5.43	3.56

Table A32
Dietary vitamin B6 intake of control individuals.

Subject	Day 1	Day 2 (μmol/day)	Day 3	Mean
Females				
HM-28	5.36	14.73	2.99	7.69
JS-29	12.54	9.58	15.49	12.54
CH-32	9.40	7.69	6.02	7.96
SR-34	7.39	13.78	7.69	9.62
BP-36	8.75	12.83	14.25	9.76
DM-37	4.19	4.20	5.84	4.74
SK-38	11.65	24.13	14.19	16.66
PC-47	15.61	18.98	16.09	16.89
MS-48	17.50	18.86	14.84	17.07
Mean	10.27	13.86	10.82	11.65
SD	4.48	6.21	5.10	4.55
Males				
DU-25	8.57	8.34	12.18	9.70
MC-27	22.06	15.79	12.42	16.76
KT-30	10.35	10.17	5.20	8.57
DR-31	3.96	14.61	17.62	12.06
DP-33	9.52	14.73	21.35	15.20
JR-35	6.98	12.54	6.56	8.69
CC-39	20.05	25.84	30.51	25.47
BA-40	22.00	19.52	25.13	22.22
GB-41	6.03	2.28	5.51	4.61
Mean	12.17	13.76	15.17	14.04
SD	7.17	6.71	9.10	6.85

Table A33
Dietary protein intake of individuals with insulin dependent diabetes mellitus.

Subject	Day 1	Day 2 (gm/day)	Day 3	Mean
Females				
CP-4	194.0	127.0	98.4	139.8
LN-11	70.8	56.4	46.4	57.9
KM-20	97.7	119.0	84.2	100.3
Mean	120.8	100.8	76.3	99.3
SD	64.8	38.6	26.9	41.0
Males				
JW-6	116.0	106.0	116.0	112.7
KR-10	59.6	87.1	81.8	76.2
AL-13	83.3	65.5	87.6	78.8
DL-15	180.0	89.9	76.0	115.3
DW-21	124.0	132.0	114.7	123.6
Mean	112.6	96.1	95.2	101.3
SD	45.7	24.7	18.8	22.1

Table A34
Dietary protein intake of individuals with non-insulin dependent diabetes mellitus.

Subject	Day 1	Day 2	Day 3	Mean
		(gm/day)		
Females				
FA-2	74.6	128.0	68.6	90.4
MR-8	55.2	59.0	59.7	58.0
MC-16	85.8	48.6	27.8	54.1
NU-24	80.2	67.9	88.6	78.9
MP-43	59.4	44.7	90.6	64.9
Mean	71.0	69.6	67.1	69.2
SD	13.2	33.9	25.6	15.1
Males				
TS-3	123.0	108.0	109.0	113.3
AP-9	195.0	159.0	193.0	182.3
GI-17	118.0	175.0	97.4	130.1
JL-22	106.0	97.3	84.7	96.0
Mean	135.5	134.8	121.0	130.5
SD	40.3	38.0	49.0	37.3

Table A35
Dietary protein intake of control individuals.

Subject	Day 1	Day 2	Day 3	Mean
		(gm/day)		
Females				
HM-28	53.7	121.0	43.6	72.8
JS-29	80.3	110.4	81.8	90.8
CH-32	65.6	69.1	40.0	58.2
SR-34	79.6	91.2	87.1	86.0
BP-36	67.3	97.0	114.0	92.8
DM-37	75.6	60.7	59.3	65.2
SK-38	116.5	106.3	100.6	107.8
PC-47	83.0	83.0	87.2	84.4
MS-48	57.6	67.5	62.8	62.6
Mean	75.5	89.6	75.2	80.1
SD	18.5	21.0	25.3	16.4
Males				
DU-25	60.2	110.4	164.9	111.8
MC-27	69.4	107.9	98.1	91.8
KT-30	76.4	116.8	95.1	96.1
DR-31	40.0	57.0	76.0	57.7
DP-33	85.7	56.9	92.6	78.4
JR-35	72.7	78.9	66.5	72.7
CC-39	146.0	167.0	123.8	145.6
BA-40	88.7	155.0	123.1	122.3
GB-41	76.6	50.2	106.0	77.6
Mean	79.5	100.0	105.1	94.9
SD	28.8	42.7	29.4	27.5

Table A36
Energy intake of individuals with insulin dependent diabetes mellitus.

Subject	Day 1	Day 2	Day 3	Mean
		(kcal/day)		
Females				
CP-4	2777	1950	1477	2068
LN-11	2281	2135	1270	1895
KM-20	2748	1772	1634	2051
Mean	2602	2042	1460	2005
SD	278	131	183	95
Males				
JW-6	2990	2831	2135	2652
KR-10	1219	1736	2750	1902
AL-13	2081	2497	2224	2267
DL-15	3520	3752	2932	3401
DW-21	2770	3383	4496	3550
Mean	2516	2840	2907	2754
SD	890	785	950	712

Table A37
Energy intake of individuals with non-insulin dependent diabetes mellitus.

Subject	Day 1	Day 2	Day 3	Mean
		(kcal/day)		
Females				
FA-2	1458	1701	1092	1417
MR-8	1218	1152	1971	1447
MC-16	1066	933	813	937
NU-24	1559	2036	1839	1811
MP-43	1181	1170	1935	1429
Mean	1296	1398	1530	1630
SD	205	455	538	311
Males				
TS-3	2176	1684	2057	1972
AP-9	2192	2681	2408	2427
GI-17	1507	2552	1565	1875
JL-22	1617	1503	1613	1578
Mean	1873	2195	1911	1963
SD	362	598	399	352

Table A38
Energy intake of control individuals.

Subject	Day 1	Day 2	Day 3	Mean
		(kcal/day)		
Females				
HM-28	1357	2265	1245	1622
JS-29	2099	2690	2251	2347
CH-32	1452	1297	1328	1359
SR-34	1750	1758	2286	1931
BP-36	1612	1899	2426	1979
DM-37	1051	1026	1005	1027
SK-38	3044	2588	3022	2885
PC-47	1900	1785	1665	1783
MS-48	1200	1362	1205	1256
Mean	1718	1852	1826	1799
SD	598	577	694	575
Males				
DU-25	1847	2643	4211	2900
MC-27	2547	2893	2643	2694
KT-30	1878	2868	2206	2317
DR-31	610	1509	1802	1307
DP-33	2051	2316	2640	2478
JR-35	1606	3386	2766	2586
CC-39	3562	3722	3162	3482
BA-40	3025	4354	3729	3703
GB-41	2947	2083	3293	2774
Mean	2230	2864	2939	2694
SD	890	868	746	690

FORM A-I
Informed Consent

OREGON STATE UNIVERSITY
CONSENT FORM

I _____, volunteer to participate in the research study on nutrition and diabetes. In volunteering for this experiment, I understand, and give my consent to the following:

1. The research which is being conducted will evaluate my nutritional status of a specific vitamin, vitamin B-6. The purpose is to better understand the relationship between diabetes mellitus and vitamin B-6 nutrition. As there are two experiments in which I may participate, I understand that my acceptance into the first does not obligate me to participation in the second. People of ages 21-65 years will be studied.

Prior to the first experiment, and if I am a person with diabetes mellitus, I will provide a physician's evaluation of the type of diabetes mellitus I have. I will also complete a health/nutrition questionnaire prior to the experiment. Also,, prior to this experiment a fasting blood sample (10 ml) will be drawn for analyses of C-peptide as a further assessment of the type of diabetes. If I am a person with diabetes mellitus I will consult with my physician concerning participation in the study,, especially with respect to participation in the glucose tolerance test. The principle investigator requires a signed statement from your physician before participation in this specific test. The physician's advice on any adjustment of insulin dosage will be obtained.

For the experiment itself , I will do the following:

A. On each of <u>two</u> occasions, at least one month apart, collect complete urines for two consecutive days. Collection bottles will be provided. On the second day, I will consume 2 grams of L-tryptophan with my breakfast meal. L-tryptophan is a naturally occurring amino acid and the amount I am consuming represents about twice what is normally present in a person's daily diet.

B. The morning after the second day's urine collection I will report in a fasting (10-12 hours) condition and 20 ml of blood will be drawn from an arm vein by a medical technologist.

C. For each of three days prior to the blood collection (i.e., one day prior to the urine collection and the two days of the urine collection) I will weigh and keep a record of all food I consume. A dietetic balance will be provided for me on which to weigh my food.

FORM A-I (continued)

2. As in any blood test, there will be a minimal risk of infection. Sterile procedures will be used during the blood draw, however, I must ensure that the wound is kept clean and free of contamination after I leave the test.

3. The benefits I expect to receive include the monetary amount indicated above, the results of the tests, including the glucose tolerance data, and a meal following each test. I do acknowledge the potential benefit to myself and others as this will enhance understanding of diabetes mellitus and the relationship of vitamin B-6 nutrition.

4. I understand that records will be maintained with utmost confidentiality. My questionnaires, food records, menstrual cycle records, and test results will all be identified with a code that is known only to the researchers involved in this study. My records will be kept locked in the principle investigator's office.

5. If I have any questions or concerns about this research and/or my rights as a subject, I may contact Dr. James Leklem, at the Department of Foods and Nutrition, 754-3561.

6. I understand that my participation in this study is purely voluntary, and I may decline to participate or withdraw at any time.

7. I understand that this experiment will involve the withdrawal of blood. A person who is at increased risk for Hepatitis B or HTLV III (AIDS) infections should not donate blood for this experiment. If I feel that I am at increased risk for these infections for any reason, I will withdraw from the study.

I have read and understand the provisions of this consent form. I hereby give my consent to participate in this study and to have the above-described tests performed on myself.

_____ _____

Signature of Participant Date

_____ _____

Signature of Witness Date

FORM A-II
Instructions to subjects

INSTRUCTIONS FOR 24 HOUR FOOD RECORD

The 24 Hour Food Record is a written record of everything you eat and drink (except water) from the time you get up one morning until the time you get up the next morning. As in the previous phase of the study the diet record is kept for three consecutive days. In this case, though, you begin just two days prior to the day of your lab visit. The third day of the diet record is the day of your lab appointment. Keep a record for this entire day as well.

Begin diet record with the first food you eat on
end with the last food ( ) on
You should fast for 12-hours before your lab appointment, but do drink water during the fast (including at least 1/2 cup morning between).

Weigh and record all foods before you eat them. If you do not finish a certain food item, reweigh the portion that is not consumed, and subtract it from the original weight to get the weight of the actual amount eaten. Be sure to include everything such as snacks, beverages (coffee, diet drinks, etc.) and condiments such as gravies and sauces. Half a cracker or two bites of an apple do count! Remember to bring recipes (with total yield indicated in number of portions of the size you consumed), and labels from any packaged foods.

If you eat a meal or a snack away from home and are not able to weigh it, you still need to record every item of food and beverage that you consume, and make the best possible estimate of the amount eaten. Bring the food record form with you and record each food as you eat them.

Be sure and put your name and date on each form. The subject number will be filled in by us. Instructions on how to correctly fill out each part of the food record are as follows:

TIME: The first item on the left is time. Write down the time you start eating the food item. If several foods are eaten at the same time, simply draw an arrow down the time column or put quote marks to indicate it is the same time.

(please continue on reverse)

FORM A-II (continued)

LOCATION: Record if the food was eaten at home, at a friend's, at work, at a restaurant, etc.

FOOD ITEM: Describe the food you are eating. It is very important that you be as specific as possible. For example:

don't write -- meat sandwich

do write -- meat sandwich: whole wheat bread
roast beef
lettuce
mayonnaise

Use a separate line for each item. For casseroles and other homemade foods, include the recipe on a separate piece of paper.

BRAND: If the food was canned, frozen or came in a box indicate the brand name. If it is a generic brand or homemade write that down. There is no need to indicate brands on fresh fruits or vegetables. If the brand is unknown, leave this column blank or put a question mark down.

PREPARATION: How was the food prepared? Was it eaten raw? Fried? Baked? Roasted? Sauteed? Microwaved? Steamed? etc.

AMOUNT: WEIGH ALL FOOD AND BEVERAGES THAT YOU EAT OR DRINK! If it is absolutely impossible to weigh your food (for example, if you are dining out at a restaurant) estimate portion sizes using common household measures. (See abbreviations and measures listed at the bottom of the food record.)

This information is completely confidential. The food records will be computer analyzed by the investigators to determine dietary intake of certain nutrients.

FORM A-III
Medical history

Dr. Lekler
Foods and Nutrition Dept.
Oregon State University

Project Name _____
Date of Project _____

CONFIDENTIAL
Nutrition Project
Health/Diet/History

Code #: _____ Date: _____
Age: Birth Date: State or County of Birth: _____
State of Residence: City: No. of Yrs. _____
Present employment _____
Race (Circle one): a. American Indian e. Chinese
b. Black f. Japanese
c. Caucasian g. Other Oriental (specify) _____
d. Latin American h. Other (specify) _____

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

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

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

___ a. diabetes
___ b. hypothyroidism
___ c. hyperthyroidism
___ d. goiter
___ e. hypoadrenalism (Addison's dis.)
___ f. osteoporosis
___ g. hepatitis
___ h. cirrhosis
___ j. kidney stones
___ y. asthma
___ k. nephritis
___ l. cysticis
___ m. high bl. pressure
___ n. angina
___ o. mental depression req. medication
___ p. insomnia requiring freq. medication
___ q. ulcer
___ r. pancreatitis
___ z. carpal tunnel
___ s. ulcer. colitis
___ t. spastic colon/ diverticulitis
___ u. recurring gastritis
___ v. allergies
___ w. heart problems (specify) _____
___ x. cancer (specify type) _____

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

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

FORM A-III (continued)

MEDICATION HISTORY (Check any which you take on a regular basis):

___ a. sleeping tablets
___ b. barbiturates
___ c. tranquilizers
___ d. blood pressure tablets
___ e. diuretics
___ f. antibiotics
___ g. oral contraceptives
___ h. estrogens (femal hormones)
___ i. thyroid (thyroxin)
___ j. insulin
___ k. cortisone
___ l. isoniazid
___ m. other steroids (specify) _____

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

Surgery	Date	Age
_____	_____	_____
_____	_____	_____
_____	_____	_____

DIETARY HISTORY:

Are you a vegetarian: ___ yes ___ no
If yes, circle the type of vegetarian diet that you follow:
a. ovo-lacto b. ovo c. lacto d. vegan

Do you take vitamins: (Circle one)
a. yes, daily b. yes, frequently c. never
If yes, what type, amount and how long have you taken them?
Type _____ Amount _____ How long? _____
Type _____ Amount _____ How long? _____
Type _____ Amount _____ How long? _____

Do you take other nutritional supplements:
Type _____ Amount _____

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

_____
_____
_____

Do you drink alcohol: (beer, wine, liquor) (Circle one)
Yes No
If yes, give frequency and amount consumed:
Frequency (how often?) _____ How much (in ounces per time) ____

FORM A-III (continued)

Do you smoke: (Circle one)
Yes No
If yes, circle what you smoke:
Cigarettes Cigars
How many per day _____ Packs per day _____

Do you drink coffee or tea? (Circle one)
Yes No
Do you drink decaffeinated coffee (Circle one)
Yes No
If yes give number of cups per day _____
(Circle one) coffee tea

Do you drink alcoholic beverages?
Yes No
Type: _____ Amount per week: _____

EXERCISE LEVEL:

Do you have a daily fitness program?
If yes, describe:

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

Example Only

DIET RECORD SHEET

STUDY:

SUBJECT NO

DAY OF WEEK: Tuesday DATE 10/16/88 NAME: Jane Doe

Please leave a blank space between each meal.
Continue diet record on back if necessary.
Use a separate sheet for each day.

NOTES:

Time	Location	Food Item	Brand	Preparation	Amount	Office Use Only	
7:30 am	Home	Egg, Chicken	Lucerne	poached	50g (1 large)		
		Orange juice	Rexmore	frozen concentrate diluted with water	120g		
		bread, whole wheat	homemade	toasted	35g		
		butter	Marigold	on toast	5g		
12:15pm	cafeteria	Meat sandwich:					
		roast beef	?	then sliced	70g		
		lettuce	iceberg	on sandwich	8g		
		mayonaise	?	" "	10g		
		whole wheat bread	Franz	" "	70g		
		milk, 2%	Lucerne	—	300g		

Any questions call:
Department of
Foods & Nutrition
754-3561

Useful abbreviations:
cup.....C
ounce.....oz
teaspoon.....Tsp, Ts
tablespoon.....Tbsp, Tb

Useful Measurements:
1 cup=8 fl.oz.=16 Tbsp
1 lb.=454 gram=16 oz.
3 tsp.=1 Tbsp.=1/16 cup
1 tsp = approx 5gr solid