AN ABSTRACT OF THE THESIS OF

MIN S. KIM for the degree of Master of Science in Nutrition and Food Management presented on May 8, 1997. Title: Effect of Vitamin B-6 Status on Fatty Acid and Lipid Metabolism in Women.

Abstract approved by : _____

James E. Leklem

The effect of vitamin B-6 (B-6) status on plasma fatty acids (FA) levels and lipid metabolism was investigated in this metabolic study. Eight female subjects were fed for 28 days. For the first 7 days, they were fed a constant diet containing 2.10 mg of B-6. For the rest of the period (21 days), they were differentiated in terms of B-6 intake; 4 of them were fed a low (0.93 mg/day) and 4 a high (2.60 mg/day) B-6 diet. B-6 status indices, plasma FA concentration and lipid profile were determined.

Plasma pyridoxal 5'-phosphate and total B-6 concentration ($P<0.01$), urinary 4-pyridoxic acid and total B-6 concentration ($P<0.001$) showed a significant difference between the two groups at the end of the study. Erythrocyte PLP failed to show any significant difference between the two groups throughout the diet study.

There was no significant difference in the plasma FA or lipid profile between the two groups. Plasma total cholesterol (TC) of the low B-6 group decreased slightly (7 %), but was not statistically significant. When comparing day 7 and day 28 values, plasma triglycerides increased (9 %) for the high and decreased for the low B-6 group. LDL-C

decreased (5 %) for the high B-6 group but did not change in the low B-6 group. HDL-C decreased slightly in both groups (~8 %).

There was no clear evidence that a low intake of vitamin B-6 affects the fatty acid and lipid metabolism. Further studies are required to identify the relationship between vitamin B-6 and fatty acid and lipid metabolism in humans.

Effect of Vitamin B-6 Status on Fatty Acid and Lipid Metabolism in Women

by

Min Sun Kim

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirement for the
degree of

Master of Science

Presented May 8, 1997
Commencement June 1997

Master of Science thesis of Min S. Kim presented on May 8, 1997.

APPROVED:

Major Professor, representing Nutrition and Food Management

Head of Department of Nutrition and Food Management

Dean of Graduate School

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

Min S. Kim, Author

ACKNOWLEDGEMENTS

I would like to express my grateful mind from the deepest heart to the following people;

My major professor, Dr. James E. Leklem for all the supports he did for my graduate study, tough instructions and kind considerations he has given to me.

The Committee Members of my Master's degree, for their help and time on my work.

Dr. Jim Ridlington and Karin Hardin, for their technical help and friendship.

Dr. Rosemary Wander for her kind advice and help.

My fellow graduate students, especially Wen Yen Juan, Pascaline Ruhumba, Andrea Boli, and Scott Leonard for their kind help and support on my study and friendship.

I would like to say the last "Thank you" to my sister for everything she has done for me and I wish the best luck and happiness in her life.

TABLE OF CONTENTS

Page

INTRODUCTION....1

LITERATURE REVIEW....3

Vitamin B-6....3

History....3
Structure and chemistry....3
Metabolism and transport....5
Biochemical functions....8
Status assessment....21
Deficiency and effects....27
Toxicity....28
Recommended dietary allowances....29

Fatty Acid Metabolism....31

Oxidation of fatty acids....31
Synthesis....34
Desaturation and chain elongation....35
Essential fatty acid deficiency....36
Relationship between essential fatty acids and vitamin B-6 deficiency....39
Interrelationship between vitamin B-6, fatty acid, and phospholipid metabolism....44

Cholesterol Metabolism....46

Absorption....46
Cholesterol transport....48
Cholesterol synthesis....48
Cholesterol degradation and excretion....49
Relationship between vitamin B-6 and cholesterol metabolism....50

MATERIALS AND METHODS....55

Subjects....55

Experimental Design....56

TABLE OF CONTENTS (Continued)

Page

Sample Collection and Analysis....58

Statistical Analysis....61

RESULTS....63

Characteristics of Subjects....63

Vitamin B-6 Status....64

Plasma Fatty Acid Profile....67

Plasma Phospholipid....68

Plasma Lipid....80

DISCUSSION....87

SUMMARY & CONCLUSION....99

REFERENCES....102

APPENDICES....121

Appendix A: Tables....122

Appendix B: Figures....135

LIST OF FIGURES

Figure Page

1. Major naturally occurring forms of vitamin B-6 4
2. *Schiff* base formation between pyridoxal 5'-phosphate and an amino acid..5
3. Metabolic interconversion of the B-6 vitamers in human liver.....9
4. Overview of vitamin B-6 transport, metabolism and excretion.....10
5. Metabolism and biochemical pathways in which vitamin B-6 is involved...12
6. Cori-alanine cycle.....13
7. Involvement of pyridoxine in the central nervous system.....16
8. The β-oxidation cycle.....32
9. Carnitine-dependent uptake of fatty acid by mitochondria.....33
10. Plasma PLP concentration during the diet study 70
11. RBC PLP concentration during the diet study 70
12. Comparison of plasma linoleic acid (18:2n-6).....73
13. Comparison of plasma arachidonic acid (20:4n-6).....74
14. Comparison of 20:4n-6/18:2n-6 ratio 75
15. Comparison of plasma fatty acid 20:1n-9.....76
16. Comparison of plasma total n-6 fatty acids.....77
17. Comparison of plasma total n-3 fatty acids.....78
18. Comparison of plasma phosphatidylcholine.....79
19. Comparison of plasma total cholesterol.....82

LIST OF FIGURES (Continued)

Figure Page

20. Comparison of plasma HDL-cholesterol.....83

21. Comparison of plasma LDL-cholesterol.....84

22. Comparison of plasma triglycerides.....85

23. Comparison of TC/HDL-cholesterol ratio 86

LIST OF TABLES

Table Page

1. Alterations of desaturation activities....37
2. The basal diet....62
3. Characteristics of subjects and the corresponding experimental group....64
4. Mean intakes of nutrients from 3-day diet records....65
5. Mean plasma and red blood cell values of vitamin B-6 status indicators....69
6. Mean urinary 4-pyridoxic acid (4PA) and total vitamin B-6 (UB6) excretion....71
7. Plasma fatty acid profile (Area %)....72
8. Plasma phosphatidylcholine (mg/100 ml)....72
9. Plasma lipid profile....81

LIST OF APPENDIX FIGURES

Figure Page

1. Recipe for salad dressing....136
2. Recipe for pumpkin bars....137
3. Form of informed consent for screening....138
4. Form of informed consent....139
5. Form of nutrition screening data....141
6. Form of daily activity sheet....142
7. Menstrual chart....143
8. Standard for fatty acid profile....144
9. Area percent of standard fatty acid....145
10. Approval of the human diet study....146
11. Health/diet questionnaire....147

LIST OF APPENDIX TABLES

Table Page

1. Subjects' weight (lbs) during the diet study....123
2. Subjects' 24-hour urinary creatinine (g/day)....124
3. Intake of hard candy of subjects (as a number of regular size candies)....125
4. Intake of fruit jelly spread....126
5. Intake of carbonated drinks....127
6. Subjects' hematocrit (%)....128
7. Subjects' hemoglobin (g/ml)....128
8. Plasma pyridoxal 5'-phosphate (nmol/L)....129
9. Plasma total vitamin B-6 (nmol/L)....129
10. Red blood cell pyridoxal 5'-phosphate (nmol/L)....129
11. Urinary 4-pyridoxic acid (umol/day)....130
12. Urinary total vitamin B-6 (umol/day)....131
13. Plasma phosphatidylcholine (PC) concentration (mg/100 ml)....131
14. Plasma fatty acid profile (area %)....132
15. Plasma lipid profile (mg/100ml)....133
16. Plasma alkaline phosphatase (Units/L)....133
17. Mean nutrient intakes (from 3-day diet records....134

LIST OF ABBREVIATIONS

4PA	4-pyridoxic acid
B-6	Vitamin B6
FA	Fatty acid
HDL	High-density Lipoprotein
HDL-C	High-density Lipoprotein Cholesterol
LDL	Low-density Lipoprotein
NRC	National Research Council
PC	Phosphatidyl choline
PE	Phosphatidyl ethanolamine
PL	Pyridoxal
PLP	Pyridoxal 5'-phosphate
PM	Pyridoxamine
PMP	Pyridoxamine 5'-phosphate
PN	Pyridoxine
PNP	Pyridoxine 5'-phosphate
PNG	Pyridoxamine glucoside
RBC	Red Blood Cell
RDA	Recommended Dietary Allowance
SAH	S-Adenosyl Homocysteine
SAM	S-Adenosyl Methionine

TB6	Total Plasma Vitamin B-6
TG	Triglycerides
TC	Total Cholesterol
UB6	Urinary total vitamin B-6

DEDICATION

This thesis is dedicated to my parents who got over

all the difficulties for their children.

EFFECT OF VITAMIN B-6 STATUS ON FATTY ACID AND LIPID METABOLISM IN WOMEN

INTRODUCTION

Vitamin B-6 deficiency has been associated with an increased concentration of linoleic acid and decreased concentration of arachidonic acid in the plasma and liver in rat studies. Vitamin B-6 deficiency was speculated to affect or impair the conversion of linoleic to arachidonic acid in a manner not specifically identified.

Studies have shown that the content of phosphatidylcholine in the plasma and in tissues declined in vitamin B-6 deficient rats (She et al, 1994; She et al, 1995) and that decreased phosphatidylcholine content indirectly inhibits the conversion of linoleic acid to arachidonic acid. In vitamin B-6 deficiency the amount of arachidonic acid is decreased and that of linoleic acid is increased in the phospholipid fraction.

Most of the studies that have investigated the relationship between vitamin B-6 and fatty acid metabolism have been done with animals, mainly rats. Since little research has been conducted with human beings, the question still remains whether this relationship exists in man. In 1963, Mueller et al (1963) reported the effect of desoxypyridoxine-induced vitamin B-6 deficiency on polyunsaturated fatty acid metabolism in human beings. Although they observed relatively minor changes in the plasma polyunsaturated fatty acids, the results were consistent with those from animal experiments, in terms of the direction of changes in the plasma linoleic and arachidonic acid. They suggested that vitamin B-6 indeed is required for normal fatty acid metabolism in man. This study,

however, did not control the level of vitamin B-6 in the diet and the fatty acids they analyzed were not specific enough to indicate the specific change in fatty acid metabolism in humans. Thanks to the development of gas chromatography techniques, more advanced study can be performed to define the effect of vitamin B-6 deficiency on fatty acid metabolism in humans.

Despite the limitations of experiments with humans, this study was designed to investigate the effect of a change in vitamin B-6 status on the polyunsaturated fatty acid metabolism. Altered concentration of arachidonic acid in tissues due to vitamin B-6 deficiency would affect the prostaglandin biosynthesis and metabolism. Thus, if there is a reduced conversion of linoleic acid to arachidonic acid with a low intake of vitamin B-6, then vitamin B-6 can be considered a significant factor in modulating prostaglandin synthesis.

The objectives of this study were

1) To investigate the effect of vitamin B-6 intake on the plasma fatty acid profile.
2) To determine the relationship between essential fatty acid metabolism and vitamin B-6 intake by measuring the ratio of arachidonic acid (20:4n-6) to linoleic acid (18:2n-6) in plasma.
3) To determine the effect of vitamin B-6 intake on the concentration of cholesterol, HDL-, LDL-cholesterol, triglycerides in plasma.

LITERATURE REVIEW

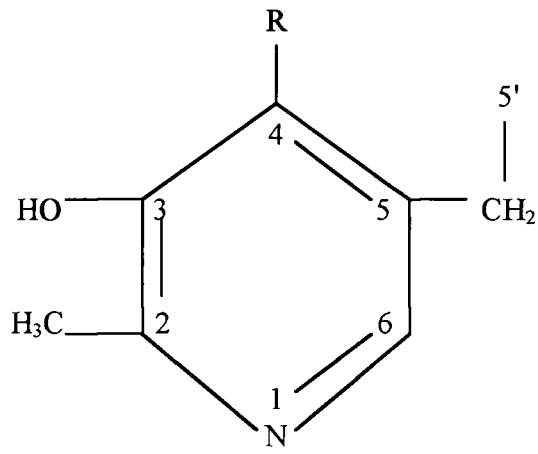
Vitamin B-6

History

Vitamin B-6 was first discovered and the name termed by Győrgy (1934) when a specific vitamin prevented acrodynia in rats. In subsequent years, pure crystalline vitamin B-6 was isolated by Gyorgy (1938) and Lepkovsky (1938). The chemical structure of vitamin B-6 was determined by Harris and Folkers (1939) to be 3-hydroxy-4,5-dihydroxymethyl -2-methylpyridine. Győrgy used the term pyridoxine first in 1939. The other forms of pyridoxine which are pyridoxamine and pyridoxal, were measured through the use of microorganisms (Snell, 1939; Snell et al, 1942).

Structure and chemistry

The term vitamin B-6 is the generic descriptor for all 3-hydroxy-2-methylpyridine derivatives that exhibit vitamin B-6 activity. Vitamin B-6 exists in three forms; PN: pyridoxine, PM: pyridoxamine, PL: pyridoxal, and each of these also exists as a phosphorylated form; PNP: pyridoxine 5'-phosphate, PMP: pyridoxamine 5'-phosphate, and PLP: pyridoxal 5'-phosphate. These forms are illustrated in Figure 1.



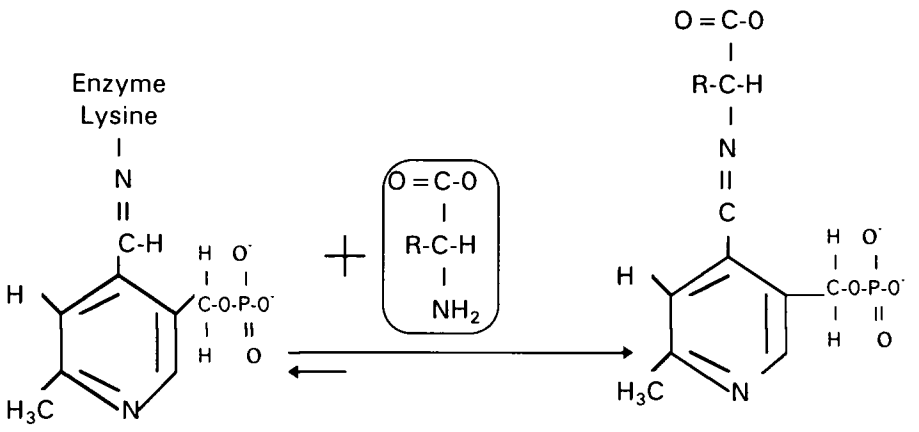
3-hydroxy-2-methylpyridine derivatives

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

Figure 1. Major naturally occurring forms of vitamin B-6 (Adapted from: Leklem, 1996)

4-Pyridoxic acid (4PA), an irreversible metabolite of vitamin B-6, has a -COOH group in the R position. In tissues PN, PM, and PL exist primarily as phosphorylated forms, and only PLP and PMP function as coenzymes (Leklem, 1991).

In solution these six forms are light sensitive (Ang, 1979; Schaltenbrand et al, 1987) and this sensitivity is affected by pH. Generally, B6 vitamers are quite labile, but the



PLP Bound to an enzyme as a *Schiff* base

Schiff base with an amino acid

Figure 2. *Schiff* base formation between pyridoxal 5'-phosphate and an amino acid. (Adapted from: Leklem, 1991)

degree to which each is degraded varies. Pyridoxine, pyridoxal and pyridoxamine are relatively heat-stable in an acid medium, but are not in an alkaline medium (Leklem, 1991).

PLP, the coenzyme form of vitamin B-6, is found covalently bound to enzymes via a *Schiff* base with an ε-amino group of lysine. In enzymatic reactions, the amino group of the substrate for the given enzyme forms a *Schiff* base via a transamination reaction. The formation of a *Schiff* base with PLP and an amino acid is shown in Figure 2.

Metabolism and transport

The primary forms of vitamin B-6 are absorbed by a non-saturable, passive process (Henderson, 1985). Phosphorylated forms are first dephosphorylated by hydrolysis of

nonspecific phosphatases in the gastrointestinal tract (Mehansho et al, 1979). Each of the unphosphorylated forms can be phosphorylated after absorption for retention in the tissues. Also, Middleton (1985) reported the possible existence of a saturable uptake process in the duodenal section. The intestinal absorption of vitamin B-6 in the rat has been found to decrease from the proximal to the distal section of the intestine.

Vitamin B-6 is transported and distributed in the body via the plasma and red blood cells (RBC). In the plasma, PLP and PL are bound to albumin (Dempsey and Christensen, 1962). Using a gel filtration technique, PLP was found to have stronger affinity for albumin than PL does (Anderson et al, 1974). RBC rapidly take up PL and PN and convert these to PLP. PLP is subsequently bound to hemoglobin (Ink et al, 1984).

Liver is the major organ which metabolizes the three forms of vitamin B-6 (Ink et al, 1984; Lumeng et al, 1985). PLP is taken up as PL after hydrolysis by plasma membrane alkaline phosphatase (Lumeng et al, 1985). The metabolic interconversion of B-6 vitamers in human liver is illustrated in Figure 3. All the three nonphosphorylated forms are converted to the respective phosphorylated forms by pyridoxine kinase in the presence of zinc and adenosine triphosphate (ATP) (Merrill et al, 1984). PLP can be formed from PMP and PNP by a flavin mononucleotide (FMN)-dependent oxidase (Wada et al, 1961). Each of the 5'-phosphate forms can be converted to their free forms after hydrolysis by alkaline phosphatase (Merrill et al, 1984; Lumeng et al, 1980). PL is irreversibly oxidized to 4-pyridoxic acid (4PA) by a flavin adenine dinucleotide (FAD)-dependent aldehyde oxidase. The formed 4PA is excreted in the urine. Wozenski et al (1980) reported that 40-60 % of the daily intake of vitamin B-6 is excreted as 4PA. The activity of the FAD

oxidase is sufficient to convert excess PL to 4PA (Merrill et al, 1984), which appears to be an important regulatory step in vitamin B-6 metabolism (Reynolds, 1995). Another means of regulating vitamin B-6 metabolism is achieved by product inhibition by PLP of the conversion of PMP and PNP to PLP (Pogell, 1958). Since PLP is highly reactive with proteins, limiting the amount of PLP circulating may prevent adverse effects of excess PLP (Merrill et al, 1979). PLP comprises about 50-75 % of the total vitamin B-6 in the plasma (Shultz and Leklem, 1983; Lumeng et al, 1985).

The total body pool of vitamin B-6 is estimated to be 1000 umol, of which 80-90 % is present in muscle (Coburn et al, 1988; Coburn, 1990). The idea that there is a repository role for muscle for vitamin B-6 was originated first by Krebs and Fischer (1964). They hypothesized that muscle phosphorylase acts as repository for vitamin B-6 in the sense that continued accumulation of enzyme is tantamount to a storing of the vitamin during a time of excess vitamin B-6. However, vitamin B-6 deficiency was ineffective in reducing total phosphorylase in gastrocnemius muscle in young rats over a period of at least 8 weeks (Black et al, 1978). Prolonged deficiency of vitamin B-6 did cause enzyme depletion but this was after anorexia had developed and weight loss had occured. When rats were partially starved for 1 to 4 days (fed 10 % of normal energy intake) they lost muscle phosphorylase. When totally starved, the rats lost more phosphorylase than during partial starvation. Starvation, not vitamin B-6 deficiency per se, caused depletion of muscle phosphorylase.

An age-related change in vitamin B-6 distribution was reported by Bode et al (1991a). Male and female rats were fed a purified diet (containing 250 g of casein and 6

mg of pyridoxine hydrochloride per kg) from weaning until 31 months of age. The plasma PLP concentration decreased with increasing age, with the largest decrease occuring in the first 1 year of life. B-6 vitamer content increased in heart and brain, whereas PLP content decreased in the gastrocnemius muscle, kidney, and liver. The decrease in muscle PLP content occurred in concert with a decrease in the muscle glycogen phosphorylase activity. This result may suggest that the muscle reservoir of vitamin B-6, as PLP, decreases with age. Bode et al (1991b) investigated the difference in retention of ^{14}C-labelled PN ·HCl in old and young Wistar rats. Ten young (6 month-old) and ten old (31 month-old) rats were fed a purified diet same as in the previous study from weaning and killed after given an oral dose of ^{14}C-labelled PN ·HCl. No difference was found in the absorption and distribution of ^{14}C among the various tissues and the various B-6 vitamers. Total body retention of ^{14}C was significantly lower in old rats.

Figure 4 summarizes vitamin B-6 transport, metabolism, and excretion and the involvement of different organs.

Biochemical functions

PLP is involved in over 100 enzymatic reactions and this capacity is mainly due to PLP readily forming a *Schiff* base with amino acids and other N-containing compounds. The types of reaction catalyzed by PLP includes transamination, decarboxylation, side chain cleavage, dehydratase and racemization. Figure 5 depicts the metabolism and biochemical pathways in which vitamin B-6 as PLP is involved. Some of the critical functions that PLP serves are reviewed in the following pages.

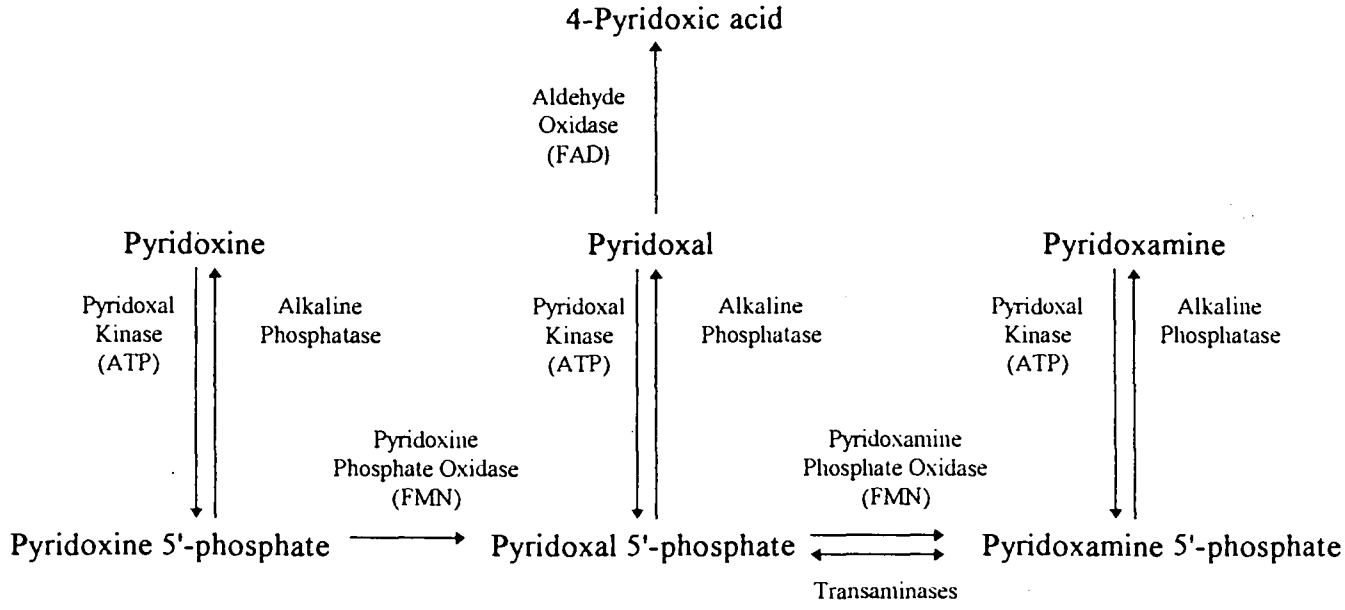


Figure 3. Metabolic interconversion of the B-6 vitamers in human liver.
(Adapted from: Leklem, 1996)

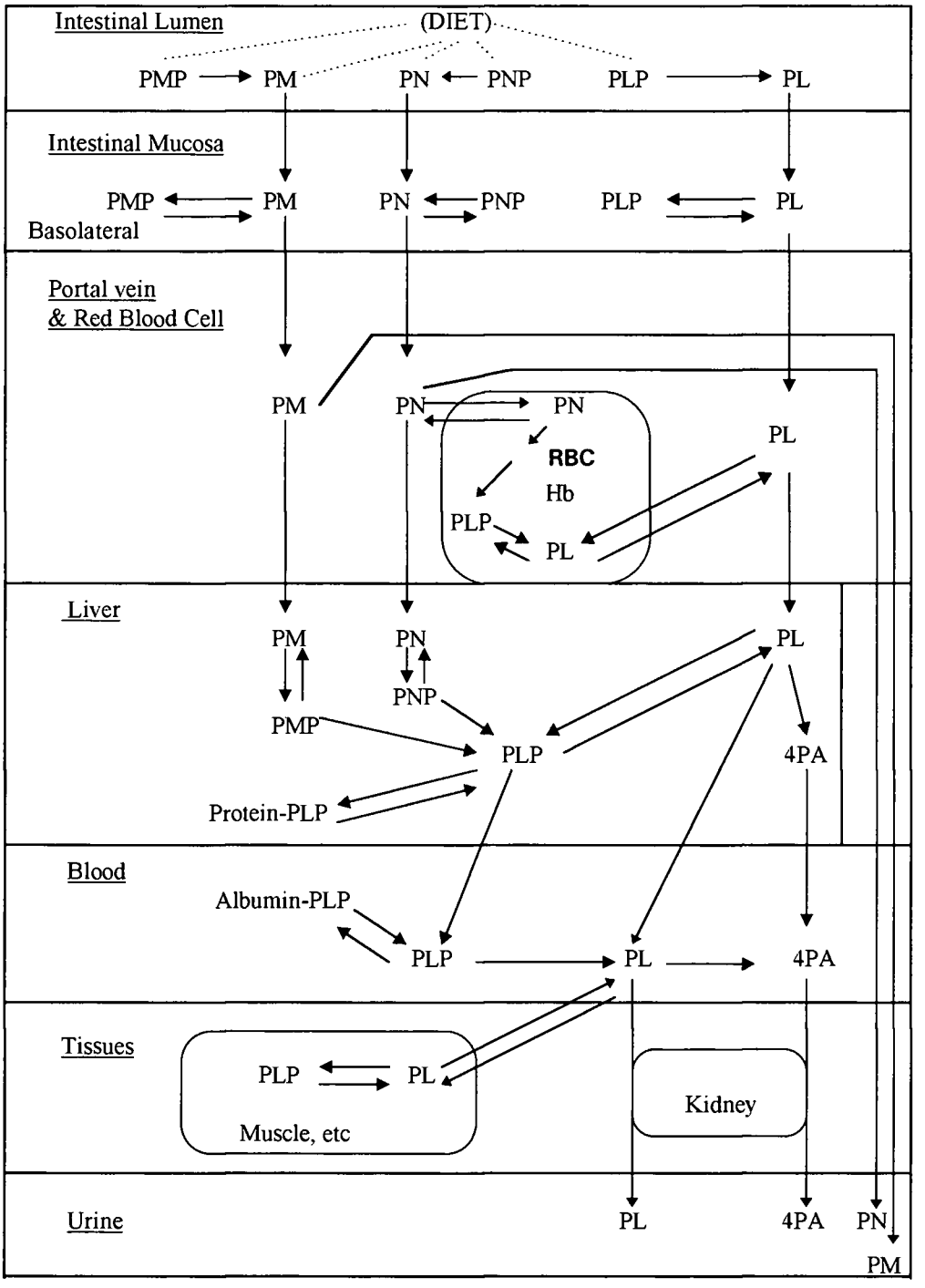


Figure 4. Overview of vitamin B-6 transport, metabolism and excretion. (Adapted from Leklem, 1996)

Gluconeogenesis and glycogen metabolism: As mentioned above, transamination is one of the main reactions in which vitamin B-6 is involved. In the liver, gluconeogenic amino acids undergo this reaction and following several steps, result in the formation ofoxaloacetate, the starting material for gluconeogenesis. This process involves the breakdown of amino acids (except leucine and lysine) and the conversion of lactic acid to glucose (Ahlborg et al, 1982). This overall process is called the Cori-alanine cycle (Cori, 1931; Felig et al, 1970) and depicted in Figure 6.

Maintaining an optimal level of blood glucose is critical during physical activity. Both the liver and extrahepatic tissues, especially muscle cooperate for this purpose. In addition to gluconeogenesis, the release of glucose from glycogen storage is accomplished by glycogen phosphorylase enzyme which is PLP-dependent. Helmreich (1992) proposed a mechanism for the phosphorylase reaction, showing the essential role of PLP in the glycogen phosphorylase reaction. The phosphate of the cofactor PLP acts as a general acid. It provides the substrate phosphate with a proton functioning as a proton shuttle.

Since 80-90 % of the total vitamin B-6 is present in muscle as PLP bound to glycogen phosphorylase, the breakdown of muscle glycogen to supply energy may release PLP into the circulation (Coburn, 1990). During a caloric deficit the amount of glycogen phosphorylase decreased and the stored PLP seemed to be mobilized (Black et al. 1978). Oka et al (1994) and Okada et al (1991) studied the effect of vitamin B-6 status on glycogen metabolism. The glycogen phosphorylase activity in the gastrocnemius muscle and heart but not in the liver of vitamin B-6 deficient rats was significantly decreased when compared with those of controls (Okada et al, 1991). Also, the glycogen content of the

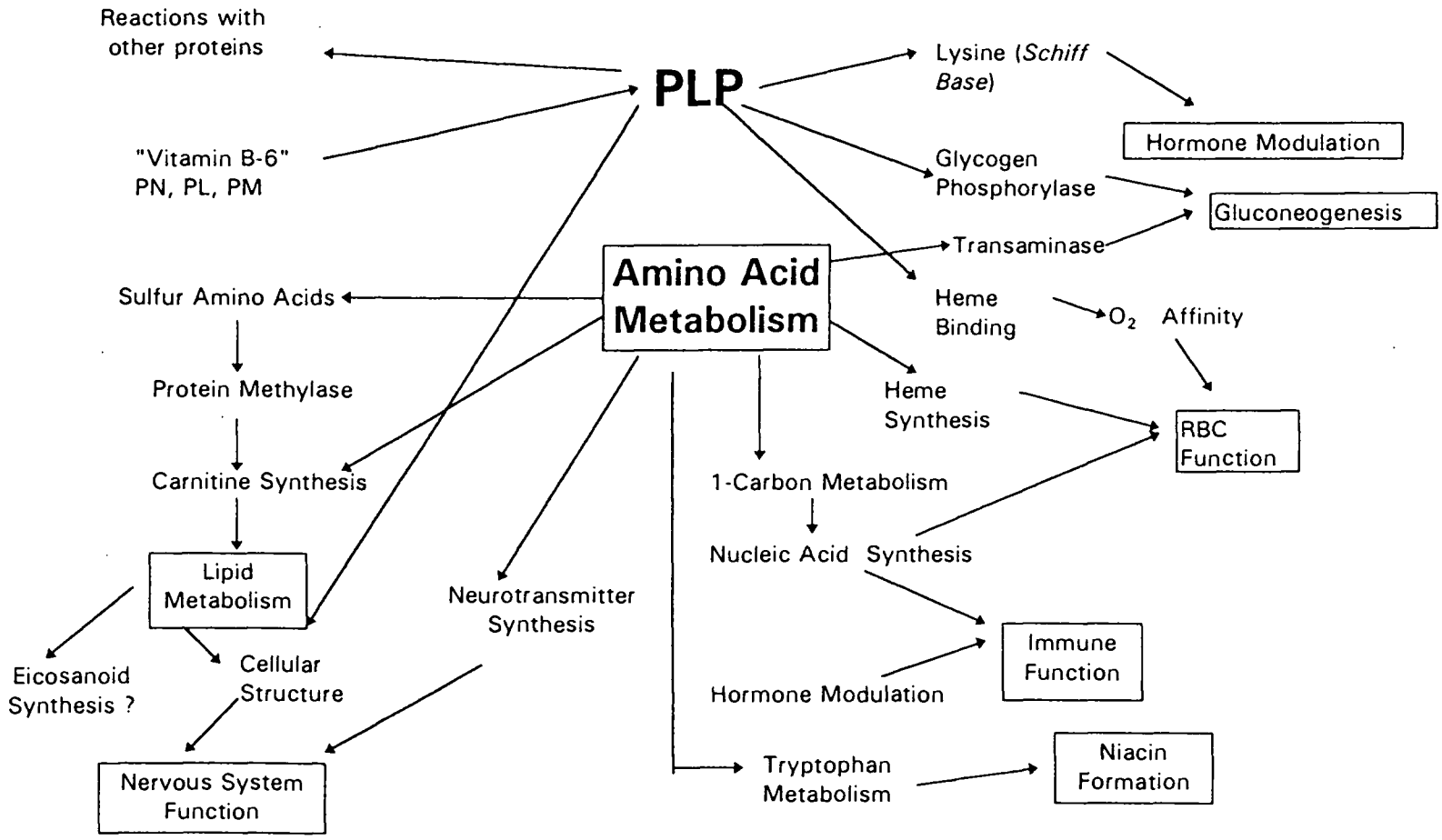


Figure 5. Metabolism and biochemical pathways in which vitamin B-6 is involved.
(Adapted from: Leklem, 1996)

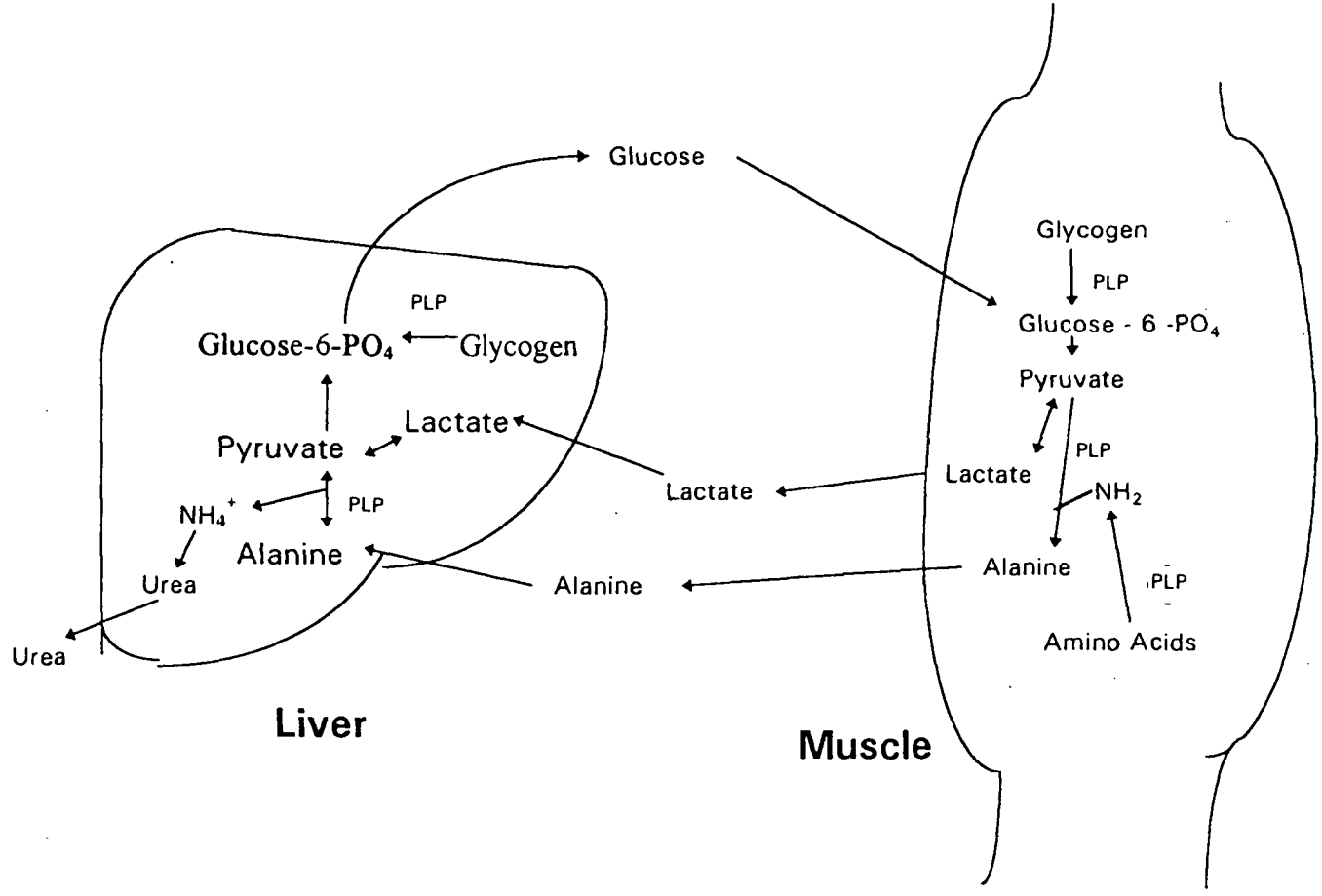


Figure 6. Cori-alanine cycle; the involvement of pyridoxal 5'-phosphate (PLP) in glucose and alanine metabolism. (Adapted from Leklem, 1985)

muscle of B6-deficient rats was higher than that of controls, but no difference was observed in liver glycogen content. These observations indicate that glycogen degradation was impaired in the muscle but not in the liver of vitamin B-6 deficient rats. PLP appears to affect the genetic expression of glycogen phosphorylase, thus regulating the enzyme activity (Oka et al, 1994). The level of phosphorylase mRNA in the muscle of vitamin B-6 deficient rats was reduced to 40 % of that in the control rats. By contrast, the phosphorylase mRNA level was increased five fold in the liver of vitamin B-6 deficient rats. The results from this study suggest that vitamin B-6 modulates the transcriptional activation of the phosphorylase gene in a tissue-specific manner. However, excess vitamin B-6 does not appear to affect the muscle glycogen phosphorylase (Schaeffer et al, 1995). Rats were fed a diet containing 1, 10, 100, 175 or 250 times the NRC recommended level of PN ·HCl (7 mg/kg). Neither plasma PLP concentration nor muscle glycogen phosphorylase reflected dietary intake of pyridoxine. The modulatory role of vitamin B-6 in the gluconeogenesis process and glycogen metabolism needs further investigations.

Nervous system: PLP is involved as a cofactor in the formation of several neurotransmitters including taurine, dopamine, norepinephrine, histamine and γ-aminobutyric acid. Neurological abnormalities observed in human infants (Coursin, 1954) and animals (Dakshinamurti, 1982) deficient in vitamin B-6 support the fact that vitamin B-6 plays a role in nervous system function. The general view of the involvement of PLP in the central nervous system (CNS) is illustrated in Figure 7.

Niacin formation: Vitamin B-6 functions in the conversion of tryptophan to niacin (Brown, 1985). PLP is required in the conversion of 3-hydroxykynurenine to 3-hydroxyanthranilic acid. Kynureninase catalyzes this conversion and is very sensitive to vitamin B-6 status. Vitamin B-6 deficiency impairs the activity of this enzyme, resulting in abnormal accumulation of tryptophan metabolites. Xanthurenic acid accumulates during the vitamin B-6 deficiency due to impaired conversion of 3-hydroxykynurenine to 3-hydroxyanthranilic acid. Leklem et al (1975) investigated the effect of vitamin B-6 deficiency on the conversion of tryptophan to niacin. They found a decrease in the excretion of niacin metabolites, N'-methylnicotinamide and N'-methyl-2-pyridone-5-carboxamide, after 4 weeks of a low vitamin B-6 diet in women. The excretion of tryptophan metabolites following a tryptophan load is used to determine vitamin B-6 status. In a recent study by Hansen et al (1996a), low vitamin B-6 status induced by high protein intake (2.0 mg/kg body weight) was associated with an increase of urinary post-tryptophan load excretion of xanthurenic acid and kynurenic acid. Low vitamin B-6 status appears to have a moderate negative effect on niacin formation from tryptophan.

Immune system: The involvement of PLP in 1-carbon metabolism as a coenzyme for serine transhydroxymethylase (Schirch and Jenkins, 1964) suggests that vitamin B-6 status affects nucleic acid synthesis. The changes in nucleic acid synthesis via abnormal 1-carbon metabolism due to vitamin B-6 deficiency could affect immune function. (Axelrod et al, 1964; Chandra and Puri, 1985). This is supported by animal studies in which a vitamin B-6 deficiency has been found to adversely affect lymphocyte production (van den

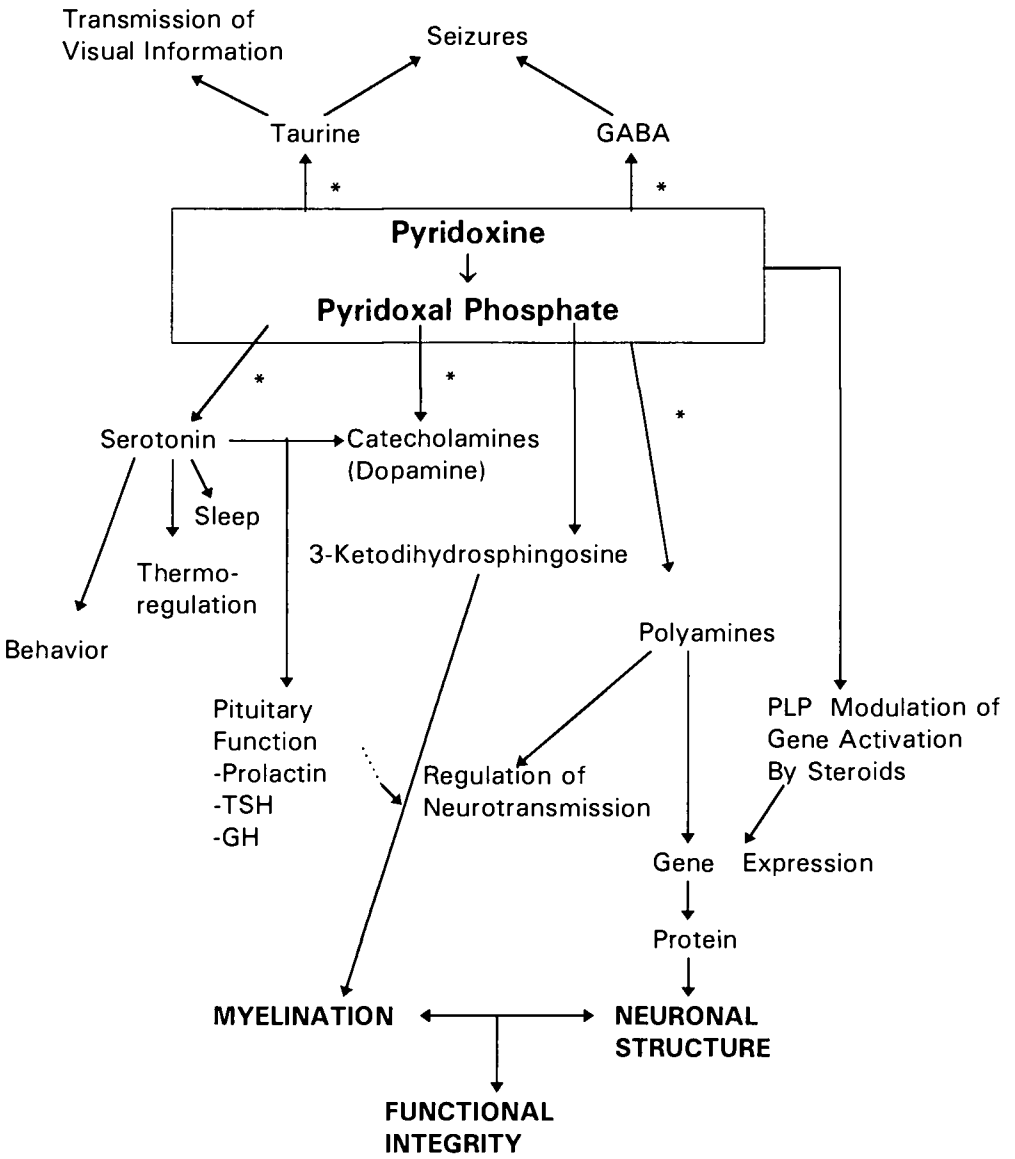


Figure 7. Involvement of pyridoxine (*) in the central nervous system (CNS)
(Adapted from: Dakshinamurti et al, 1985)

Berg et al, 1988) and antibody response to antigens (Cheslock and McCully, 1960). Miller and coworkers (1987) found impairment in the immune system of 11 elderly females and saw improvement in the lymphocyte response after treatment with 50 mg pyridoxine per day for two months. Vitamin B-6 deficient rats showed a 25 % reduction in the total number of peripheral white blood cells and a 50 % decrease in lymphocyte numbers as compared to controls (Robson and Schwarz, 1975). Deficiency of vitamin B-6, through its adverse effect on nucleic acid production, impairs both DNA and mRNA synthesis. A recent study (Trakatellis et al, 1992) confirms the importance of vitamin B-6 in serine hydroxymethyl transferase. Addition of 4-deoxypyridoxine inhibited the induction of serine hydroxymethyl transferase and synchronous addition of pyridoxine with deoxypyridoxine reversed the inhibitory effect. The effect of phytohaemagglutinin or concanavalin A on the production of interleukin-1b, interleukin-2 and interleukin-2 receptors was profoundly affected by deoxypyridoxine. This study suggested that the enzyme serine hydroxymethyltransferase is a key element in the processes of immune responses and cell proliferation. The critical role of vitamin B-6 in the human immune system was demonstrated by Meydani et al (1991). Their *in vivo* study showed that vitamin B-6 is an independent factor affecting immune status. Vitamin B-6 depletion for 20 days in eight healthy elderly adults significantly decreased the percentage and total number of lymphocytes, mitogenic responses of peripheral blood lymphocytes to T- and B-cell mitogens and interleukin 2 production. These indices returned to baseline values after about 60 days of vitamin B-6 repletion period, when the average vitamin B-6 intakes were 1.9 mg /day for women and 2.88 mg/day for men. They have suggested that for

maintenance of normal immune function, older adults may require amounts of vitamin B-6 higher than those currently recommended.

Currently researchers are concerned with the potential protective role of vitamin B-6, in the use of caramel color III, and in the process of human acquired immunodeficiency virus (HIV). Caramel color III is commonly used in food products such as bakery products, soup aromas, and soya-bean sauces. Administration of caramel color III to rats has been associated with decreased numbers of lymphocytes and several other changes in the immune system, such as altered responses of spleen cells to B- and T-cell mitogens and reduced hypersensitivity responses (Thuvander and Oskarsson, 1994; Reeve et al, 1995; Houben et al, 1993; Houben et al, 1992 b). The component of caramel color III that is responsible for the immunological effects has been found to be 2-acetyl-4-tetrahydroxybutylimidazole (THI) (Houben et al, 1993; Houben et al, 1992b). THI appears to interfere with the lymphoid system in mice with an adequate vitamin B-6 status even when THI is administered at a level of less than 25 mg/kg caramel color III, which is the specified limit of usage as a food additive (Thuvander et al, 1994). A protective role of pyridoxine supplementation in mice administered with THI was reported (Reeve et al, 1995). When rats were supplemented with pyridoxine (30 mg/kg diet), the THI-induced suppression of contact hypersensitivity and the lymphopenia were strongly inhibited in comparison with rats fed a normal diet containing 7 mg pyridoxine/kg diet. However, the effect of THI on the human immune system is not clear. Houben et al (1992a) did not find any changes in the immune system when elderly male volunteers with a marginal deficit in

vitamin B-6 were administered caramel color III containing THI at the level of the current acceptable daily intake of 200 mg /kg body weight for 7 days.

Protein-energy malnutrition is common in HIV-infected patients, and simultaneous deficiencies of micronutrients such as vitamin B-6 and B-12, folate, selenium and zinc have been described (Hecker and Kotler, 1990). Impairment in the micronutrient status could contribute in part to the clinical immunodeficiency in HIV-infected individuals. Recently, Baum et al (1992) identified an apparent vitamin B-6 deficiency among CDC stage III (HIV-1 seropositive, with persistent generalized lymphademopathy only) HIV-infected individuals, despite adequate dietary vitamin B-6 intake. Vitamin B-6 status in HIV-1 infected subjects was significantly associated with functional parameters of immunity. Analyses indicated that overtly B-6 deficient individuals exhibited significantly decreased lymphocyte responsiveness to the mitogens phytohemagglutinin and pokeweed, and reduced natural killer cell cytotoxicity, compared to subjects with clearly adequate vitamin B-6 status. Although vitamin B-6 is not a primary etiological factor in HIV-1-related immunological dysregulation, it appears to be an important cofactor in immune function.

Hormone modulation: During the last 15 years, PLP has been identified as a modulator of steroid action (Litwack et al, 1985; Cidlowski et al, 1981). PLP forms a *Schiff* base with a lysine residue on the steroid receptor. Reversible reactions have been known to occur between PLP and receptors for estrogen (Muldoon and Cidlowski, 1980), androgen (Hiipakka and Liao, 1980), progesterone (Nishigori et al, 1978) and

glucocorticoids (Disorbo et al,1980). These reactions inhibit the binding of the steroid-receptor complex to DNA (Litwack et al, 1985), resulting in a decreased action of the steroid. Holley et al (1983) found that vitamin B-6 deficient rats accumulated a greater amount of the isotope in the uterine tissues when injected with ^{3}H-estradiol. This result suggests that there is an increased sensitivity of uterus or other target tissues to steroids when vitamin B-6 status is subnormal. Majumder et al (1983) has shown that increased concentrations of PLP inhibit in a dose-dependent manner glucocorticoid-induced casein mRNA accumulation in mouse mammary gland. The inhibitory action of PLP is reversible because its withdrawal from the medium restores nuclear binding of the receptor complex with concurrent increase of casein mRNA accumulation in the glands in medium with insulin, prolactin, and hydrocortisone (Majumder et al, 1983).

Several studies (Symes et al, 1984; Bunce and Vessal, 1987; Holley et al, 1983; Silva et al, 1987) investigated alterations in the physical properties of steroid hormone receptors. Translocation of receptors from the cytoplasmic to the nuclear compartment of cells appears to be increased under conditions of vitamin B-6 deficiency (Symes et al, 1984; Bunce and Vessal, 1987) and decreased under the opposing condition of elevated B-6 concentration (Holley et al, 1983). Steroid receptors were also observed to have decreased affinity for DNA following treatment with PLP in vitro (Silva et al, 1987). Allgood and coworkers (1990) have demonstrated that vitamin B-6 selectively influences glucocorticoid receptor-dependent gene expression through a novel mechanism that does not involve alterations in glucocorticoid receptor concentration or ligand binding capacity. Their subsequent work (Allgood et al, 1992) showed that PLP concentration affects the

level of glucocorticoid receptor-induced gene expression without dependence on receptor species or cell type. In 1993, Allgood et al reported that vitamin B-6 modulates steroid hormone-mediated gene expression through its influence on a functional or cooperative interaction between steroid hormone receptors and the transcription factor NF1 (Nuclear Factor 1). The level of glucocorticoid-induced gene expression from simple promoters, containing only hormone response elements and a TATA sequence, was not affected by alterations in intracellular vitamin B-6 concentrations. However, modulation of hormone-induced gene expression was restored with the inclusion of a binding site for NF1. These findings suggest that the intracellular nutritional status of vitamin B-6 modulates the capacity of cells to respond to steroid hormones. Further studies are needed to determine the effect of vitamin B-6 on the endocrine-mediated diseases.

Status assessment

Considering all the biochemical and physiological roles that vitamin B-6 plays (Figure 5), appropriate methods of status assessment is important. There are numerous methods available and those are divided into biochemical and functional indices (Reynolds, 1995). The biochemical indices include direct and indirect methods for status determination. The direct methods involve the measurement of one or more B6 vitamer concentrations. The indirect methods involve the measurement of compounds other than the B6 vitamers in pathways which are responsive to the vitamin B-6, such as plasma PLP concentration. Functional indices that reflect the vitamin B-6 status are not sensitive or specific enough to be consistently useful methods (Reynolds, 1995). These are limited to

measurements of immunological and neurological function. A few indices commonly used for determination of vitamin B-6 status are discussed below.

Dietary intake of vitamin B-6 and protein: The dietary intake of vitamin B-6 provides reliable information in terms of possible vitamin status in a given population. Most studies that used dietary intake as a main or sole source of nutritional status are concerned with multiple nutrients. Johnson et al (1994) used monthly 24-hour food recall to correlate pregnancy outcomes with the intake of several nutrients including vitamin B-6. The average intake of vitamin B-6 in pregnant women was 1.7 mg per day (RDA 2.2 mg) and was not significantly correlated with pregnancy outcomes. A detailed 24-hour dietary recall was used to measure nutritional status in a multiethnic homeless population (Wolgemuth et al, 1992). The average intake of vitamin B-6 was 68 % of the RDA. Bioavailability is an additional factor when vitamin B-6 status assessment is based on the food intake. Hansen et al (1996b) investigated the effect of the pyridoxine glucoside (PNG) form of vitamin B-6 on vitamin status. Women consuming a diet containing a higher percentage of the total vitamin B-6 as PNG exhibited a decrease in vitamin B-6 status indicators, agreeing with the reduced bioavailability of PNG demonstrated in other studies (Bills, 1991; Gregory et al, 1991; Kabir et al, 1983).

Another consideration that should be taken into account when assessing vitamin B-6 status is dietary protein. Early work by Baker et al (1984) showed that as compared to a lower protein intake (30 g/day), a higher protein intake (100 g per day) increased the amount of vitamin B-6 required to normalize urinary excretion of tryptophan metabolite.

Miller et al (1985) found that excretion of urinary 4PA and concentration of plasma vitamin B-6 compounds were negatively correlated with protein intake. Recently, Hansen et al (1996a) reported an inverse correlationship between protein intake and vitamin B-6 status when nine women were fed diets providing daily intakes of 1.25 mg vitamin B-6 and 0.5, 1.0, and 2.0 g protein per kg body weight.

Dietary intake is not recommended as the only method to assess vitamin status. However, the use of data from dietary intake as information in addition to biochemical indices could be helpful in understanding and determining vitamin B-6 status.

Plasma pyridoxal 5'-phosphate(PLP): This index is regarded as a direct measurement of vitamin B-6 status and presumably reflective of tissue levels (Lumeng et al., 1978). Weaning rats were fed ad lib, for 9 weeks, purified liquid diets containing 0, 4, 12, 24, and 100 ug of PN daily. Growth reached a maximum at a level of 24 ug per day. The activities of erythrocyte aspartate aminotransferase and of hepatic alanine, aspartate, and tyrosine aminotransferase reached maximal values at 24 ug and 4 ug of PN, respectively. Liver and brain PLP increased with increasing PN intakes and reached maximum at a level of 12 ug. By contrast, muscle and plasma PLP did not saturate when PN intake was increased up to 100 ug. It was concluded that plasma PLP is a sensitive and reliable indicator of vitamin B-6 status. Following this report, plasma PLP became the most frequently used index to determine vitamin B-6 status of individuals and various segments of the population. Plasma PLP is the major form of circulating vitamin B-6 and comprises 70-90 % of the total vitamin B-6 in the plasma (Leklem, 1991). Metabolic

studies (Brown et al, 1975; Leklem, 1988) have shown that plasma PLP reached a plateau 7-10 days after a change in intake within the range of 0.5-10 mg vitamin B-6. The suggested plasma PLP value for adequate status is ≥30 nmol /L (Leklem, 1990).

Plasma PLP is affected by alkaline phosphatase activity in rats (Wan et al, 1993). The activity of alkaline phosphatase, the zinc metalloenzyme, was decreased in low zinc status when rats were fed a liquid diet containing either adequate or moderately low zinc during gestation and lactation. Plasma PLP increased significantly in rats fed a low zinc diet and was negatively correlated with plasma alkaline phosphatase activity. Under normal alkaline phosphatase activity with adequate zinc status, plasma PLP is still a reliable and useful direct measurement of vitamin B-6 status.

Erythrocyte PLP: Although plasma PLP is the measure most commonly used for status measurement of vitamin B-6, erythrocyte PLP may be a sensitive indicator. Unlike plasma PLP which could be affected by abnormal alkaline phosphatase activity, erythrocyte PLP may reflect the vitamin status of the cells (Coburn and Whyte, 1988). This difference in the cellular location of plasma PLP may make erythrocyte PLP a useful vitamin B-6 status indicator. However, Hansen et al (1993) did not observe a significant change of RBC concentration when women were fed 0.8 to 2.4 mg of vitamin B-6. In another study Hansen et al (1996b) showed that there was a 17 % reduction in RBC PLP concentration when subjects were fed a high PNG diet. They postulated that the reduction in RBC PLP with the high PNG diet might be explained, in part, by the competition for uptake of PN or PL into tissues. Human erythrocytes contain both PN kinase and PNP

(PMP) oxidase which are necessary for the conversion of PN or PL to PLP (Lumeng et al, 1985). Oral ingestion of 100 mg pyridoxine (PN) was found to increase erythrocyte PLP rapidly within 40 minutes with a peak level of approximately 4000 nmol/L from a baseline of 100 nmol/L, followed by a rapid decrease over the next 4 hours (Reynolds, 1995). Also, Anderson et al (1989) reported a rapid uptake and clearance of pyridoxine by RBC *in vivo*. A healthy female subject was given the equivalent of 8.6 and 118 umol PN intravenously. Vitamin B-6 compounds in blood were measured 1-60 minutes after injection. There was a considerable amount of PN in RBC at 1 min but by 3 min a large amount of that PN had disappeared after either injection. These results imply that even though the RBC is able to convert PN to PLP, the RBC tends to transport PN to other sites of metabolism. It is possible that PN in the RBC is not useful other than for transport to other cells. Only after all the tissues are saturated with PN, would extra injestion of PN result in an increase in RBC PLP. Unfortunately, there is not data which relates the level of RBC PLP in response to the long-term supplementation of vitamin B-6. Because there is a rapid and dramatic increase in the concentration of erythrocyte PLP following oral ingestion of PN, only fasting samples of blood should be analyzed if erythrocyte PLP is to be used as a valid status index (Reynolds, 1995). The reliability of RBC PLP as a vitamin B-6 status indicator for short and long term needs further study.

4-Pyridoxic acid (4PA): 4PA is the major end-product of vitamin B-6 metabolism and is excreted in the urine. Since 4PA is produced by oxidation of PL, PN and PMP must first be converted to PLP and then to PL before they contribute to urinary 4PA (Leklem,

1990). Thus, the turnover of PLP via conversion to PL is the main control point determining the amount of 4PA produced. The determination of the output of urinary 4PA represents a measure of the vitamin B-6 flux through PLP (Merrill et al, 1984). Studies (Leklem, 1991; Leklem, 1988) have shown that more 4PA is excreted when PL is consumed compared to other forms. Urinary 4PA is considered to reflect short-term vitamin B-6 status. The excretion of 4PA changes rapidly in response to a change in vitamin B-6 intake (Leklem, 1988; Brown et al, 1975). Urinary 4PA can provide useful information about vitamin B-6 status if dietary vitamin B-6 intake is considered to be representative of a person's usual diet. Excretion of urinary 4PA of more than 3 umol per day has been suggested as indicative of adequate vitamin B-6 status (Leklem, 1990; Sauberlich et al, 1974).

Other useful methods for determination of vitamin B-6 status include the measurements of plasma and urinary total vitamin B-6 (Leklem, 1991; Leklem, 1988), erythrocyte aminotransferase (Sauberlich et al, 1974; Sauberlich , 1981; Heiskanen et al, 1994), and tryptophan metabolites in the urine after a tryptophan load test (Brown, 1985 and 1988; Leklem, 1971). Since there is no one best way for vitamin B-6 status assessment currently, combination of more than one indices will provide better information for determining status.

Deficiency and effects

The occurrence of a deficiency of vitamin B-6 is not common due to the wide distribution of this vitamin in foods (Leklem, 1991). The lack of this vitamin in the diet does not occur alone but usually accompanies other water-soluble vitamin insufficiency. However, the use of certain kinds of drugs which act as an antagonist of vitamin B-6 metabolism can result in impaired vitamin B-6 status. These drugs include theophylline, cycloserine, hydrazine derivatives, L-DOPA, penicillamine, and oral contraceptives (Bhagavan, 1985). Patients treated with drugs mentioned here are often supplemented with vitamin B-6.

Snyderman et al (1950) reported a deficiency of vitamin B-6 in infants fed a therapeutic diet. Initial changes that occurred in these infants were the disappearance of 4PA from the urine and an extremely low level of pyridoxine in the urine, as low as 0.2-2 ug per day. Subsequently, there was an impaired conversion of tryptophan to niacin. Convulsions and hypochromic anemia were observed after 11 and 19 weeks of this diet, respectively. Also, Mueller and Vilter (1950) reported development of a vitamin B-6 deficiency in adults administered deoxypyridoxine for a period 55 days. Deoxypyridoxine, an analog of pyridoxine, antagonizes pyridoxine metabolism. They observed that seborrhea-like skin lesions developed around the eyes, nose, and mouth within 2-3 weeks in most of the patients. No anemia was observed . Turnlund et al (1992) observed an altered calcium and magnesium metabolism in young women fed a vitamin B-6 deficient diet. Calcium balance was negative during the vitamin B-6 depletion period. Magnesium balance was negative during the vitamin B-6 depletion period due to increased urinary

magnesium excretion. Urinary calcium decreased during the vitamin B-6 depletion period and increased as dietary vitamin B-6 increased. Miller et al (1992) investigated the effect of vitamin B-6 deficiency on fasting plasma homocysteine concentrations. Total fasting plasma homocysteine concentrations were measured in 11 elderly subjects with a mean age of 64 who consumed a vitamin B-6 deficient diet for less than or equal to 20 days. Although the catabolism of homocysteine through cystathione synthesis requires PLP, only 1 of the 11 subjects was found to have elevated plasma homocysteine concentrations. In contrast, all subjects exhibited increased urinary xanthurenic acid concentrations after a tryptophan load. This result indicates that fasting plasma homocysteine concentration are not sensitive to vitamin B-6 status.

There are studies in rats which reported the effects of vitamin B-6 deficiency. Dubick et al (1995) found that obvious vitamin B-6 deficiency decreases rat pancreatic digestive enzyme and glutathione reductase activities. Also, plasma lipid peroxidation was stimulated in rats fed a vitamin B-6 deficient diet for 12 weeks (Ravichandran and Selvam, 1991). Plasma malondialdehyde level, conjugated dienes and lipofuscin like pigments were also increased in vitamin B-6 deficiency.

The signs of severe and chronic deficiency of vitamin B-6 are as follows: stomatitis, cheilosis, glossitis, irritability, depression and confusion.

Toxicity

Although the metabolism of vitamin B-6 is well-regulated, toxic effects of excess intakes have been reported. The proposed beneficial effect of pyridoxine supplementation

attracted those who suffer from Premenstrual Syndrome (PMS) and other disorders. Neurotoxicity and photosensitivity were associated with chronic high-dose of PN (Schaumburg et al., 1983; Bersteine and Lobitz, 1988). Daily doses more than 500 mg of PN on a chronic basis are considered to be hazardous, and may cause neurologic symptoms. Supplements of less than 250 mg per day are safe for most individuals.

Recommended dietary allowances

The Recommended Dietary Allowances (RDA) for vitamin B-6 is based on protein intake; the requirement for vitamin B-6 increases as the intake of protein increases (Baker et al., 1964; Linkswiler, 1978; Shultz and Leklem, 1981; Hansen et al, 1996a). This relationship is associated with the major role vitamin B-6 plays in amino acid metabolism. Under conditions of a constant intake of vitamin B-6, the level of dietary protein affects vitamin status, i.e., a higher protein intake decreases the urinary excretion of 4PA and vitamin B-6 (Hansen et al, 1996a; Miller et al, 1985). It was postulated that higher protein diets increase the physiologic and biochemical need for vitamin B-6 in that more vitamin B-6 is retained in the tissues to provide PLP for the amino acid metabolism (Hansen et al, 1996a). Compared with men consuming diets with similar B-6 to protein ratios (Miller et al, 1985), the women excreted a greater percentage of the B-6 intake as 4PA, had lower plasma PLP concentrations and excreted greater amounts of postload urinary tryptophan metabolites at all three protein levels (Hansen et al, 1996a). Hansen et al (1996a) suggested that a vitamin B-6 to protein ratio of greater than 0.020 mg is required for adequate vitamin B-6 status in women.

An age-associated difference in the vitamin B-6 and protein relationship was reported by Pannemanns et al (1994). They found a lower urinary 4PA excretion in young (mean age = 29) adults fed a higher protein diet while a higher urinary excretion of 4PA was observed in older (mean age = 70) subjects fed a higher protein diet when compared with younger subjects. A higher vitamin B-6 requirement has been suggested for the elderly. Ribaya-Mercado et al (1991) investigated vitamin B-6 requirements of 12 men and women over 60-years of age. The subjects successively received 0.003, 0.015, 0.0225, and 0.03375 mg of vitamin B-6 /kg body weight per day after a 5-day baseline period. Dietary protein was 1.2 or 0.8 g per kg body weight per day. The indices for the determination of vitamin B-6 status included xanthurenic acid excretion after a 5 g L-tryptophan load and urinary 4PA excretion, erythrocyte aspartate transaminase activity coefficient, and plasma PLP. These measurements were abnormal during the depletion period. The amounts of vitamin B-6 (as PN·HCl) required to normalize these measurements were 1.90 mg and 1.96 mg for women and men, respectively. Since normal dietary sources of vitamin B-6 contain a significant amount of B-6 that is not as bioavailable as PN·HCl (Andon et al, 1989; Hansen et al, 1996a), Ribaya-Mercado et al (1991) suggested that current RDA of vitamin B-6 for elderly men and women are probably insufficient and should be reevaluated.

For adults, the ratio of 0.016 mg vitamin B-6 per g protein is used in the current RDA. The reported average intake of protein is approximately 100 g per day for men (USDA, 1986) and 60 g per day for women (USDA, 1987). Assuming a protein intake of twice the RDA (NRC, 1989), the RDA of vitamin B-6 is 2.0 mg per day for men and 1.6

mg for women. This level is considered to be enough to ensure a normal vitamin B-6 status for most populations.

Fatty Acid Metabolism

Fatty acids are a class of compounds containing a long hydrocarbon chain and a terminal carboxyl group. Fatty acids are a main source of fuel in man and animals. They are stored as triacylglycerols which are uncharged esters of glycerol. Fatty acid metabolism, including oxidation, synthesis, desaturation and chain elongation will be reviewed below.

Oxidation of fatty acids

In 1904, Knoop, as cited by Schulz (1985), conducted a classical study from which he derived the concept of 'β-oxidation'. He fed dogs straight-chain fatty acids that contained a phenyl residue in place of the terminal methyl ($-CH_3$) group. The urine of these dogs contained a derivative of phenylacetic acid when they were fed phenylbutyrate whereas a derivative of benzoic acid was excreted when they were fed phenylpropionate. Through several similar experiments, he found that phenylacetic acid and benzoic acid are the products of fatty acid oxidation with even and odd number carbon chains, respectively. Knoop proposed that the oxidation of fatty acids begins at C-3, the β-carbon, and that the resulting β-keto acids are cleaved between the α- and β-carbon to yield fatty acids shortened by 2 carbons. Figure 8 depicts the β-oxidation cycle of fatty acids.

Fatty acids are activated on the outer mitochondrial membrane via linkage to coenzyme A (CoA). Oxidation of activated fatty acids occurs in the mitochondrial matrix (Groot et al, 1976). Since long-chain acyl CoA molecules do not readily cross the inner mitochondrial membrane, a special transport system, mediated by carnitine, plays an important role in β-oxidation (Pande, 1975). Carnitine was found to be unnecessary for the transfer of medium chain acyl CoAs into the mitochondrial matrix. Carnitine-dependent uptake of fatty acids by mitochondria is illustrated in Figure 9.

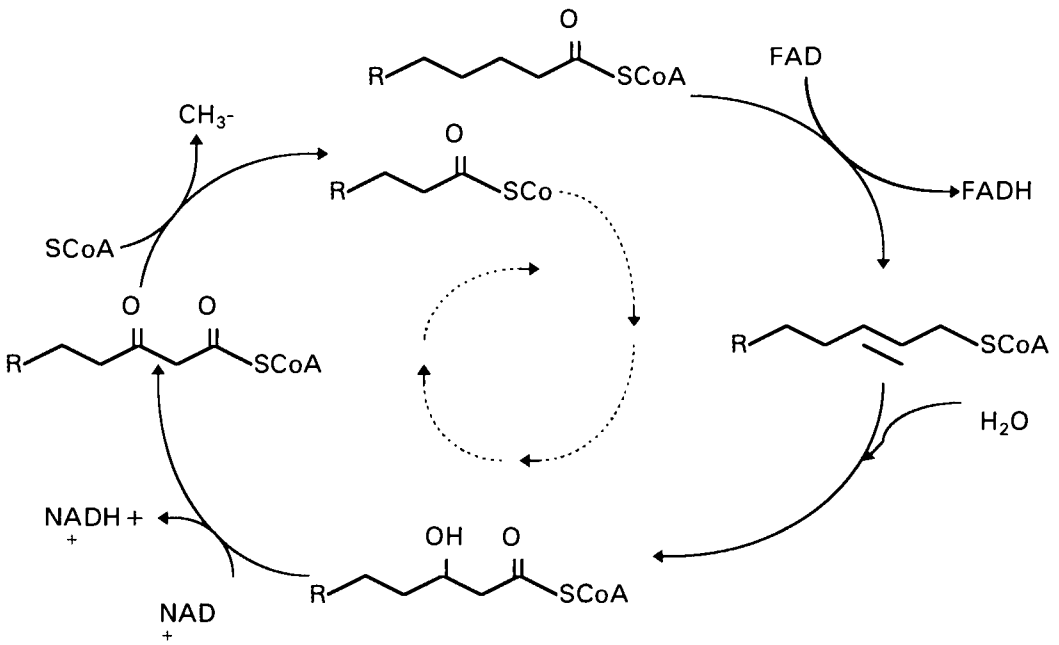


Figure 8. The β-oxidation cycle (From: Schultz, 1985)

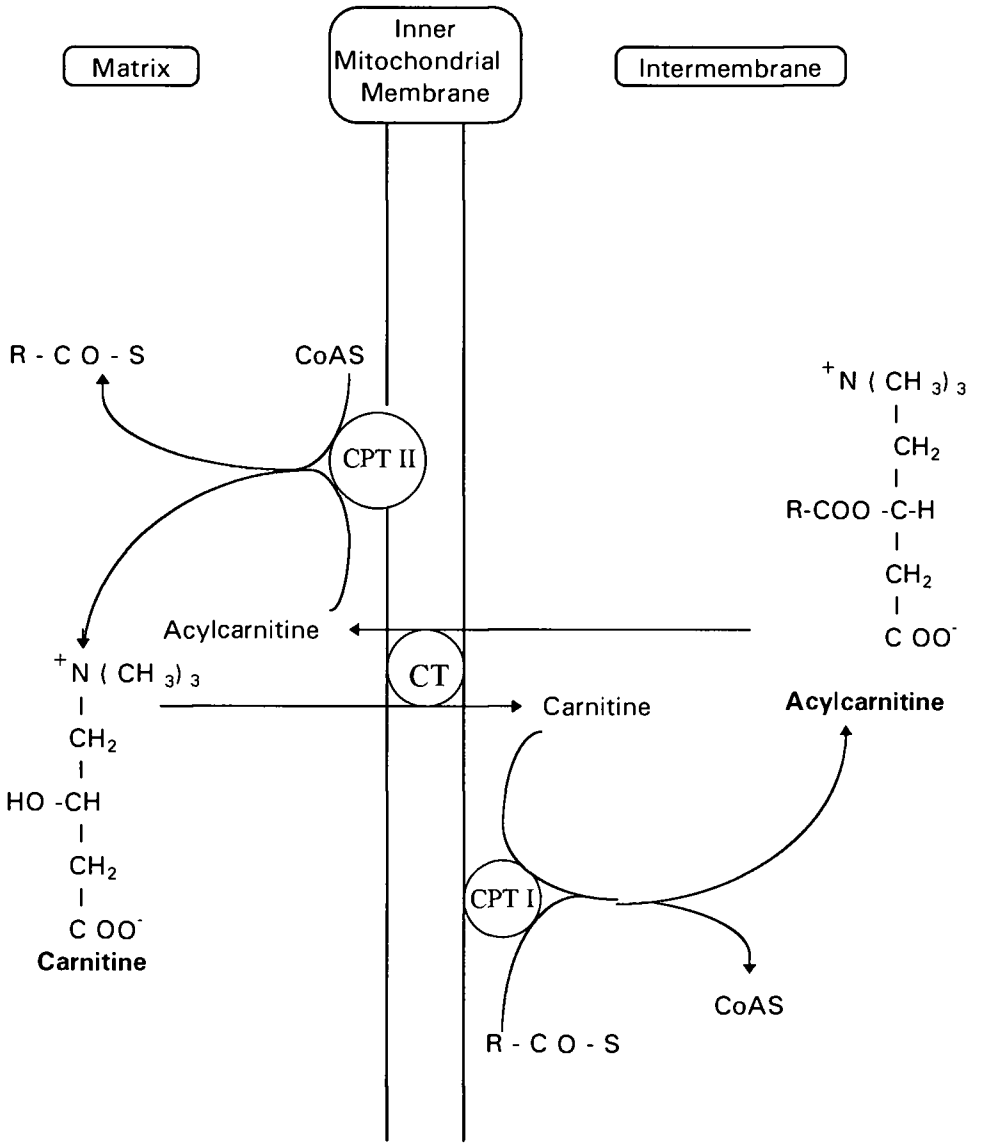


Figure 9. Carnitine-dependent uptake of fatty acids by mitochondria. CPT I, carnitine palmitoyltransferase I; CPT II, carnitine palmitoyltransferase II; CT, carnitine: acylcarnitine translocase. (From: Schultz, 1985)

Most of the oxidation reactions are the same for the unsaturated and saturated fatty acids. However, two additional enzymes, isomerase and epimerase are required for oxidation of unsaturated fatty acids (Kunau and Dommes, 1978).

The rate of β-oxidation is determined by the availability of fatty acids. McGarry and Foster (1980) suggested that the concentration of malonyl-CoA, the first committed intermediate in fatty acid synthesis, determines the rate of fatty acid oxidation. This regulation is associated with carnitine-mediated fatty acid transport in β-oxidation. The detailed mechanism will not be discussed here.

Synthesis

Fatty acid synthesis is not just a reverse of β-oxidation. The differences in these two pathways include the following: First, synthesis occurs in the cytosol and β-oxidation occurs in the mitochondria. Second, intermediates in fatty acid synthesis are covalently linked to the -SH group of an acyl carrier protein (ACP), not coenzyme A. Third, malonyl -CoA is the two-carbon donor for elongation in the synthesis process. Fourth, fatty acid synthesis requires NADPH as a reducing agent.

The synthesis of long chain fatty acids from two carbon units involves a sequence of six reactions for each two-carbon elongation. These six reactions are facilitated by fatty acid synthetase, a multifunctional peptide, that is encoded by a single gene (Tsukamoto et al, 1983; Volpe and Vagelos, 1973; Wakil et al, 1983). The rate-limiting step in fatty acid synthesis is the formation of malonyl -CoA from acetyl -CoA, a reaction that is catalyzed

by acetyl-CoA carboxylase. This enzyme is modulated by citrate, insulin, palmitoyl-CoA, and glucagon (Lane et al, 1974; Lane et al, 1979)

Desaturation and chain elongation

As major membrane components, fatty acyl chains influence various membrane properties, such as transport, endocytosis, and exocytosis, and the activity of membrane - associated enzymes. Also, polyunsaturated fatty acids (PUFA) derived from essential fatty acids are precursors of prostaglandins, leukotrienes, and hydroxy-fatty acids. *De novo* fatty acid synthesis mainly produces palmitate. The modification of synthesized fatty acids by desaturation and chain elongation is essential to widen the use of fatty acids for the modification of membrane properties and other biological needs. Elongation occurs in both microsomes and the mitochondria. The microsomal elongation system is more active than the mitochondrial system and also is able to introduce double bonds into long-chain acyl CoAs while the mitochondrial system is not. Unsaturated fatty acids must be provided by oxidative desaturation of saturated chains and by modification of dietary acids from plant and animal origin. Unsaturated fatty acids in mammals are derived from either palmitoleate (16:1), oleate (18:1), linoleate (18:2) or linolenate (18:3). Mammals can not introduce double bonds at carbon atoms beyond C-9 in the fatty acid chain, thus they are unable to synthesize linoleate and linolenate. These two are referred to as essential fatty acids (EFAs). The essentiality of these fatty acids was first suggested by Burr and Burr in 1929 and will be reviewed in detail later.

Three desaturation enzymes, Δ^9 , Δ^6, and Δ^5-desaturase, are found in animals (Cook, 1985). The Δ^9 -desaturase is usually the predominant desaturation enzyme for saturated acids in the endoplasmic reticulum. Okayasu et al (1981) isolated the terminal Δ^6 -desaturase from rat liver microsomes and found that the enzyme is a single polypeptide chain. The terminal Δ^5 -desaturase has not been purified.

The overall Δ^9 -desaturation system requires four components for activity: NADH-cytochrome b_5 reductase, cytochrome b_5, terminal desaturase and lipid. The desaturation system was found to be affected or regulated by dietary and hormonal factors (Jeffcoat, 1980). When excess carbohydrate was ingested, animals responded by increasing Δ^9 -desaturase content. Both Δ^5- and Δ^6 -desaturase responded much less dramatically to the dietary carbohydrate than does the Δ^9 -desaturase (Brenner, 1981), but animals fed diets deficient in essential fatty acids have increased Δ^9 -desaturation (4-fold) (Brenner, 1977) and decreased Δ^5 -desaturation (Brenner, 1981). These saturation systems are also controlled by insulin, epinephrine, and other hormones. Insulin increases all three desaturation enzymes whereas epinephrine decreases the Δ^6 -desaturase activity (Joshi, 1979). A summary of the effects of dietary, hormonal, and other manipulations on these desaturation activity is shown in Table 1.

Essential fatty acid deficiency

The essentiality of fat in the diet was first studied by Burr and Burr in 1929 as part of their initial work on the effects of fat in the diet. They excluded fat from the diet of rats.

Table 1. Alterations of desaturation activities: Effects of dietary, hormonal and other manipulations on Δ^9, Δ^6, and Δ^5-desaturation activities in experimental animals. (From: Cook, 1985)

	Effect on desaturation		
Treatment	Δ^9	Δ^6	Δ^5
Dietary:			
High Glucose-short term	↑	↑	↑
long term	↑	↓	
High protein	↑	↑↑	↑
Fasting	↓↓	↓	↓
Refeeding	↑↑	↑	↑
Hormonal:			
Insulin	↑	↑	↑
Glucagon		↓	↓
Epinephrine	↑	↓	↓
cAMP		↓	↓
Glucocorticoids		↓	↓
Thyroxine	↑	↓	
Hypothyroidism	↓↓	↓	
Others			
Sterculic Acid	↓		
Cytosolic Proteins	↑	↑↑	↑
Retinoic Acid	↓	↑	

Those rats fed a fat-deficient diet developed symptoms which were characterized by inflamed and swollen tails in mild cases and scaled and ridged tails in severe cases. Feet and skin showed similar symptoms as seen for the tail. They also reported poor ovulation and prolapsed penis for female and male rats, respectively. Growth was also affected by the absence of fat in the diet. Those rats fed a diet without fat stopped growing and began to die after 220 days. The minimum amount of fat (lard) for the normal growth of rats was determined to be 2 % lard and this amount was as beneficial as 20 % lard. These researchers reported that only the saponifiable fraction of fat was effective in curing the

symptoms observed with fat deficiency. In subsequent experiments (Burr and Burr, 1930; Burr and Burr, 1932), they found that rats fed a diet totally devoid of fat suffered from a deficiency disease which was curable by administration of fats containing highly unsaturated fatty acids or by pure methyl linoleate.

The second study (Burr and Burr, 1930) from this group identified the symptoms of fat deficiency. Fat deficiency was characterized by the scaly condition of skin while growth continued at an approximately normal rate. Later, kidneys degenerated and blood appeared into the urine. They speculated that this kidney degeneration and urinary excretion of blood caused early death of rats fed a fat-deficient diet. Also, this is the first study which demonstrated the effect of protein intake on the severity of symptoms observed in fat-deficiency. High protein intake aggravated the kidney degeneration. When they compared the efficiency of saturated and unsaturated fatty acid in alleviating the symptoms, linoleic acid was effective and defined to be an essential fatty acid. Other fatty acids, such as oleic acid and α-eleostearic acid, were not effective in relieving a fat-deficiency. A mixture of fatty acids had no more of an effect than a single fatty acid. Unexpectedly, addition of arachidonic acid to each kind of fatty acid had slight depressing effect on alleviation of the fat-deficiency.

These studies by Burr and Burr became the ground work in the research of essential fatty acid metabolism and were confirmed subsequently by other researchers (Birch et al, 1931; Hogan and Richardson, 1935; Turpeinen, 1938; Quackenbush et al, 1939). There are several studies (Turpeinen, 1938; Quackenbush et al, 1939; Quackenbush et al, 1942) which attempted to identify the fraction of fatty acids which

alleviated the fat-deficiency symptoms. Turpeinen (1938) supplemented different kinds of fatty acid to rats with fat-deficiency. He found that those fatty acids which were effective in alleviating the symptoms were linoleic acid, linoleyl alcohol, linolenic acid and arachidonic acid. Among these, arachidonic acid was a powerful curative agent manifesting maximal growth response with 33 mg methyl arachidonate per day. In contrast, maximal growth response plateaued in fat-deficient female rats with 100 mg methyl linoleate. However, this result is not completely consistent with the study by Quackenbush et al (1942) which found that arachidonic and linoleic acids were similar to each other in their effect on alleviating deficiency symptoms. Rats supplemented with ethyl linolenate could not produce normal young rats and the dermal symptoms were not cured in these rats.

Some of these studies (Turpeinen, 1938; Quackenbush et al, 1939; Witten and Holman, 1952) suggested that the principal unsaturated fatty acid required by the body is arachidonic acid and that linoleic acid is converted into arachidoinc acid. This was also supported by the work of Greenberg et al (1951). Linoleate, linolenate and arachidonate were tested for their effect on the efficiency of growth and weight gain of rats after a fat-depletion diet. Arachidonate was most effective and the efficiency rate of arachidonate when compared with linoleate was 3.5:1.

Relationship between essential fatty acids and vitamin B-6 deficiency

After the symptoms of fat deficiency were identified by Burr and Burr in 1929, the similarity of these symptoms to those seen with a vitamin B-6 deficiency was observed by

Birch et al (1938). Rats fed a vitamin B-6 deficient diet often developed a scaliness of the tail and skin in some rats, while swollen, red paws were always observed. Also, albumin and blood appeared in the urine, followed by death. They found that certain fats had a sparing action on vitamin B-6 and suggested that this action was due to the linoleic acid present in the fat. When the amount of fat in the diet was increased from 5 to 75 % of total diet, the onset of vitamin B-6 deficiency symptoms was delayed.

Hogan and Richardson (1935) observed dermatitis in rats caused by feeding a diet low in fat supplemented with yeast which was irradiated by ultraviolet light. The characteristics of this dermatitis corresponded to the acrodynia-like dermatitis of vitamin B-6 deficiency. They found that the symptoms could be cured by certain vegetable oils as well as by water extracts of yeast. This report is similar to the one by Birch (1938) in terms of the role of vitamin B-6 in the fat-deficiency. The latter study suggested that the amount of vitamin B-6 determines the severity and kinds of symptoms of rats fed a fat-free diet.

Schneider et al (1940) suggested there were two factors that cured rat acrodynia. One, an essential fatty acid factor independent of vitamin B-6 and the other, a rice bran concentrate. The latter factor is dependent on vitamin B-6 and independent of fatty acid. However, this factor required an accessory factor. This factor was in the filtrate from the fuller's earth treatment of rice bran concentrate which was believed to contain soluble vitamins, including vitamin B-6. In the study of Witten and Holman (1952), rats were fed either a fat-deficient diet or a pyridoxine-deficient diet and then supplemented with one of the followings: 1 mg pyridoxine , 100 mg ethyl linoleate, 100 mg ethlyl linolenate, 100 mg

ethyl linoleate plus 1 mg pyridoxine, or 100 mg ethyl linolenate plus 1 mg pyridoxine. Rats were supplemented after they showed acrodynia symptoms. Linolenate stimulated growth and fat deposition but failed to relieve the symptoms. Linoleate alone relieved symptoms and the combination of PN and linoleate resulted in a rapid alleviation of these symptoms. This research also showed that the combined supplementation of pyridoxine and linoleate resulted in the formation of a very high amount of arachidonic acid.

Several investigators (Swell et al, 1961; Medes and Keller, 1947; Zehaluk and Walker, 1973; Kirschman and Coniglio, 1961; Cunnane et al, 1984; Dussault and Lepage, 1975) have studied the fatty acid composition of various tissues in rats fed a pyridoxine-deficient diet. Swell et al (1961) fed rats a pyridoxine-depleted diet for 40 days and found decreased levels of arachidonic acid and increased levels of linoleic acid in the TG fraction of serum, liver, and adrenal lipid. Liver phospholipid and free cholesterol levels were also decreased. Based on the magnitude and the tissue localization of the effect of vitamin B-6 deficiency on the fatty acid profile, this study suggested that vitamin B-6 may not act directly in the biosynthetic scheme from linoleate to arachidonate, but perhaps in earlier metabolic reactions.

Medes and Keller (1947) analyzed the total body fat content in rats given supplements of pyridoxine, pyridoxine plus tryptophan, or ethyl linoleate after rats were fed a fat- and pyridoxine-deficient diet. The total body fat increased with supplementation. The magnitude of increase was approximately the same whether the supplement was pyridoxine, pyridoxine and tryptophan, or ethyl linoleate. All fractions of the fat in rats were increased, including the highly unsaturated fatty acids. When linoleate and pyridoxine

were administered together, the increase in the body fat was greater than when fed either of these alone. With PN supplementation the increase in the body fat was distributed almost equally among the di-, tri-, and tetraenoic fatty acid fractions. When linoleate was fed, there was a greater elevation in the dienoic fraction. The dermal symptoms were alleviated by either linoleate plus PN, or PN alone. There also was resumption of growth. In another study Zehaluk and Walker (1973) investigated the effect of different levels of PN intake on red blood cell fatty acid composition in mature male Wistar rats fed an EFA-deficient diet (10 % corn oil) for 12 weeks. The rats were either supplemented with PN or fed a diet deficient in PN for 20 weeks. At the end of the experiment, there was less arachidonic acid and more oleic (18:1n-9) and eicosatrienoic acid (20:3n-6) in erythrocyte lipids in rats of PN-deficient group than the other group. This study found that the lack of vitamin B-6 during feeding of the EFA-deficient diet was more critical than the EFA-deficient diet alone in the short-term, especially on the erythrocyte concentration of arachidonic acid. Zehaluk and Walker (1973) suggested that PN might be involved in the metabolism of unsaturated fatty acid and that there was a greater turnover of arachidonate in vitamin B-6 deficient rats, leading to the loss of linoleate. This agrees with the results of the study by Kirshman and Coniglio (1961). They suggested that the conversion of linoleic to arachidonic acid is not affected by vitamin B-6 intake, but PLP may affect the overall fatty acid metabolism by a mechanism other than specifically influencing the specific conversion of linoleic to arachidonic acid.

Dussault and Lepage (1975) studied the effect of PN deficiency on the fatty acid content of liver and plasma in rats fed diets containing 1, 10, or 20 % fat. With a

deficiency of PN, the level of arachidonic acid decreased while the concentration of linoleic acid increased in the plasma and the liver. However, incubation of liver slices with [1-^{14}C] acetate showed no significant alteration of fatty acid synthesis in PN-deficient rats fed 1 or 10 % fat as compared to ad libitum fed rats. Also, there was a marked increase in cholesterogenesis of the deficient group as compared to the cholesterol synthesis of either control group. When [1-^{14}C] linoleate was incubated with the liver slices there was no difference in the synthesis of arachidonate between the experimental deficient and the control groups. Therefore, this study supports the theory that the diminution of arachidonic acid in the liver lipid fraction from PN-deficient rats is not due to a lower conversion of linoleic acid to arachidonic acid.

However, Cunnane et al (1984) suggested that both linoleic desaturation and γ-linolenic acid elongation may be impaired in PN-deficient rats. They found that in PN-deficient rats phospholipid levels of linoleic and γ-linolenic acids were increased in plasma, liver, thymus and skin while arachidonic acid levels in these tissues were decreased compared to controls. In addition, they found that the accumulation of essential fatty acids in the liver TG of PN-deficient rats might indicate an influence of PN on the EFA exchange between TG and phospholipid. With all the interest in the relationship between PN and the metabolism of EFA, Coniglio et al (1967) studied the metabolism of 1-^{14}C - arachidonic acid in PN-deficient and pair-fed control rats. In both groups, liver had the largest percentage of 1-^{14}C -arachidonic acid compared to other tissues such as stomach, kidney, and brain. Generally, there was little or no alteration of the metabolism of

arachidonic acid 6 and 12 hours after oral administration of 1-^{14}C -arachidonic acid in PN-deficient rats.

Interrelationship between vitamin B-6, fatty acid, and phospholipid metabolism

Delorme and Lupien (1976a, 1976b) were the first researchers to suggest a relationship between vitamin B-6 and phospholipid metabolism. In vitamin B-6-deficient rats, they observed a decrease in the proportion of arachidonic acid in the phospholipid fractions and an increase in the proportion of linoleic acid in plasma and the liver (Delorme and Lupien, 1976a). They explained these changes on the basis of a decrease in the synthesis of phosphatidylcholine (PC) via methylation of phosphatidylethanolamine (PE), a pathway which leads mainly to the production of arachidonoyl-PC. The alternative synthetic pathway of PC is accomplished via CDP-choline and this pathway produces mainly linoleoyl-PC. Thus, the decrease of arachidonic acid may decrease the importance of the *de novo* pathway via CDP-choline. When B-6-deficient rats were supplemented with PN (3 mg per kg of diet), the proportion of arachidonic acid increased with time in the phospholipids while the proportion of linoleic acid decreased. Therefore, the decrease in the proportion of arachidonic acid in the phospholipids of vitamin B-6 -deficient rats might reflect a decrease in the formation of PC via methylation of PE. This suggestion was supported by Suzuki and Okada (1982). In their study, despite feeding a choline-sufficient diet, the level of phosphatidylcholine was decreased in the liver of rats fed a pyridoxine-deficient, 70 % casein diet.

Recently, She et al (1994) studied the effect of vitamin B-6 deficiency on the conversion of linoleic acid to arachidonic acid by measuring the activity of Δ^6-desaturase in rat liver microsomes. The activity of terminal Δ^6-desaturase in the linoleic acid (18:2n-6) desaturation system was strikingly decreased in rats fed a vitamin B-6 deficient, 70 % casein diet for 5 weeks. Also, they observed a significant decrease in the activity of the electron-transport enzyme, NADH-cytochrome b_5 reductase. In this study there was a highly positive correlation between PC content and Δ^6-desaturase activity in liver microsomes, suggesting that the altered PC content in microsomes induced by vitamin B-6 deficiency *in vivo* may affect linoleic acid (18:2n-6) desaturation and thus decrease the biosynthesis of arachidonic acid (20:4n-6). In a further study She et al (1995) confirmed these findings by demonstrating a decreased molar ratio of SAM (S-adenosyl methionine) to SAH (S-adenosyl homocysteine) in vitamin B-6 deficient rats. SAH has been shown to be a potent and competitive inhibitor of PE methyltransferase, the rate limiting enzyme in the methylation step (Hoffman et al, 1981). Loo and Smith (1986) reported altered methionine metabolism in rats fed a vitamin B-6 -deficient diet, as indicated by an accumulation of SAH in liver accompanied by a reduction in the methylation of PE into PC.

The alterations in the vitamin B-6 deficiency may indirectly affect essential fatty acid metabolism. The combined action of abnormal methionine metabolism and altered activity of relevant enzymes in vitamin B-6 deficiency should be considered in explaining disrupted essential fatty acid metabolism.

Arachidonic acid is the precursor of prostaglandin biosynthesis. Fujimoto et al (1987) showed that pyridoxine stimulated the generation of prostaglandin E_2 in rabbit kidney medulla slices. Moreover, pyridoxine in the presence of aspirin reduced the release of oleic acid and linoleic acid, but enhanced (1.4 fold) the release of arachidonic acid from the medulla slices, as compared to aspirin alone. This finding suggests that the enhancement of prostaglandin E_2 formation elicited by pyridoxine may be ascribed to an increased conversion of linoleic acid to arachidonic acid.

Cholesterol Metabolism

Absorption

Intestinal cholesterol has two different sources. One is dietary (exogenous) and the other is endogenous. Dietary cholesterol is absorbed in the small intestine by passive diffusion (Dietschy and Wilson, 1970b). In the lumen, exogenous cholesterol is mixed with endogenous cholesterol. This mixed cholesterol may exist in either the free form or esterified with a long-chain fatty acid. Pancreatic juice contains cholesterol esterase which hydrolyze the cholesterol esters (CE) to free form. The resulting free form can be solubilized in mixed micelles. In the mucosal cells most free cholesterol is reesterified into a large lipoprotein, termed a chylomicron, and released into the lymph.

The absorption of cholesterol in humans was investigated by Bosner et al (1993) using stable isotopes. [26, 26, 26, 27, 27, 27-2H]cholesterol (30 mg) was administered orally and [23, 24, 25, 26, 27-2H]cholesterol (15 mg) was administered intravenously on

day 0 and the percent of cholesterol absorbed was calculated as the plasma ratio of oral/intravenous isotopic tracer on day 3 as determined by gas chromatography-mass spectrometry with selected ion monitoring. Tracer cholesterol given orally peaked in plasma on day 2 and then slowly declined in parallel with the intravenous tracer. The mean cholesterol absorption in 16 healthy subjects consuming a Step One Diet was 53.5 %. Cholesterol absorption was found to be a dose- and time-dependent process (Safonova et al, 1993).

Mattson and Grundy (1985), who worked with hypercholesterolemic patients, compared the effects of liquid diets in which 40 % of the calories were supplied by either saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), or polyunsaturated fatty acids (PUFA), on circulating cholesterol levels. LDL-cholesterol and total cholesterol were significantly reduced in patients receiving either the PUFA or MUFA diets, compared with those receiving the SFA diet. Reiser et al (1985) found that beef tallow, a source of SFA, had no effect on plasma cholesterol levels or LDL-cholesterol levels in normolipidemic men. By contrast, O'Dea et al (1990) have demonstrated that the addition of beef fat to the diet results in increased levels of serum cholesterol. This contradictory effect of beef fat on serum cholesterol is explained by the quality of the beef fat used in these two studies in terms of fatty acid composition. The beef fat used in the study by Reiser et al (1985) contained unusually high amount of stearic acid, which is known to be as effective as oleic acid in lowering plasma cholesterol levels when either replaces palmitic acid in the diet (Bonanome and Grundy, 1988). More work remains to be done on determining the precise effect of various types of fats on circulating cholesterol levels.

Cholesterol transport

The low solubility of lipids in the aqueous phase gives complexity to transport of these materials within the body through circulation. Development of the lipoprotein-apolipoprotein system facilitates this transport (Halliday et al, 1993).

The center of lipoprotein is composed of apolar lipids, such as cholesteryl esters (CE) and triglycerides (TG). The outer surface is made up of protein, phospholipids, and free cholesterol (Levy, 1980). Lipoproteins are secreted from the intestine and liver and contain specific apolipoproteins. Apolipoproteins (apo) control the production and secretion of the lipoproteins, regulate specific key enzyme activities, and modulate the clearance of plasma lipoproteins through a receptor-mediated process (Halliday et al, 1993). Efficiency of lipid transport is heavily dependent on the ordered synthesis and degradation of these apolipoproteins.

Cholesterol synthesis

Cholesterol metabolism plays a pivotal role in atherosclerosis. This is mainly due to the pathological deposition of lipids, especially cholesterol and its esters, in the vascular bed. Although all nucleated mammalian cells can synthesize cholesterol to a certain degree, many cells take up cholesterol from the plasma lipoproteins rather than carrying out *de novo* synthesis to meet their requirement (Fielding and Fielding, 1982). In primates, the liver is the major organ of cholesterol biosynthesis, providing up to 82 % of total cholesterol synthesis in man (Dietschy and Wilson, 1970a).

The synthetic pathway is roughly divided into four sections: the synthesis of mevalonic acid from acetyl-CoA, the synthesis of squalene from mevalonic acid by the sequential condensation of isoprene units, the cyclization of squalene to lanosterol and a series of demethylation and isomerization reactions that convert lanosterol to cholesterol. The source of the precursor for cholesterol synthesis in mammalian cells is acetyl-CoA present in the cytosol. These acetate units are provided, for example, from catabolism of long-chain fatty acids in the mitochondria.

Dietary fat has been known to affect serum lipid profiles, probably by modulating cholesterol and lipoprotein synthesis. Spady and Dietschy (1988) observed increased rates of hepatic cholesterol synthesis following ingestion of safflower oil when compared with either olive oil or coconut oil in hamster rats. By contrast, no difference in hepatic cholesterol synthesis was found in rats following consumption of saturated (18:0), polyunsaturated (18:2) or monounsaturated (18:1) fatty acids (Triscari et al, 1978).

Cholesterol degradation and excretion

Cholesterol is excreted from the body by two main pathways: excretion of cholesterol in the feces and the conversion of cholesterol to bile acids which are then partially excreted. Also, cholesterol is the precursor of several steroid hormones, such as glucocorticoids, androgens, and estrogens. Cholesterol in the feces is degraded by bacteria in the large intestine to the neutral steroids, coprastanol and coprostanone.

Conversion of cholesterol to bile acids is the major mechanism for elimination of cholesterol from the body. The formation of primary bile acids occurs in the liver (Gibbons

et al, 1982). The first step in bile acid synthesis is the introduction of a hydroxyl group at the 7 position of cholesterol, producing cholest-5-ene-3β, 7α-diol. Early studies with rodents showed that newly synthesized cholesterol provided the major substrate for bile acid synthesis. However, a whole-body turnover study with human subjects indicated that the plasma cholesterol pool provides about two-thirds of cholesterol substrate, mainly as free cholesterol of lipoproteins for 7-α-hydroxylase activity (Schwartz et al, 1982).

Some of the lipid-lowering agents are used to increase or stimulate the catabolism of lipid materials (Sirtori et al, 1991). Nicotinic acid and acipinox interfere with the synthesis of LDL and can also improve the clearance of VLDL/LDL. Nonabsorbable agents (anion-exchange resins, neomycin, and beta-sitosterol) interrupt the recirculation of bile acids and/or reduce the absorption of cholesterol within the gut (Sirtori et al, 1991)

Relationship between vitamin B-6 and cholesterol metabolism

The possibility of a relationship between vitamin B-6 and cholesterol metabolism was derived from an animal study (Rinehart and Greenberg, 1949) in which Rhesus monkeys were fed a pyridoxine-deficient diet and developed degenerative changes in the arterial system. This finding was confirmed by other animal studies by Rinehart and Greenberg (1951), Dam et al (1958), Shah et al (1960), and Okada et al (1981).

There are few studies that have examined the relationship between vitamin B-6 and cholesterol in humans. Brattstrom et al (1990) investigated the effect of pyridoxine supplementation (120 mg per day) on the cholesterol metabolism for 8 weeks in 80-year-old men. The subjects were atherosclerotic patients with subnormal plasma PLP levels (2-

11.9 nmol/L). They found that after vitamin B-6 supplementation TC and LDL-cholesterol were decreased by 10 % and 17 %, respectively. No change was observed in HDL-cholesterol. They mentioned that they did not control the changes in body weight, caloric intake, caloric intake from fat or changes in physical activity. There was no clear mechanism identified to explain these results. They suggested that the decrease of TC and LDL-cholesterol might not be due to the initially low plasma PLP level since they observed a positive correlation between plasma PLP and TC level in 122 males from which the seven subjects of this study were selected. They also observed a decrease in TC and LDL-cholesterol in 16 subjects with normal plasma PLP concentrations by pyridoxine treatment. They hypothesized that PN-derived PLP may enhance the catabolism of LDL and the activity of antithrombin III (AT III) by inhibiting glycosylation of AT III. The results of this study agree with the study by Frolova et al (1975) who demonstrated a cholesterol-lowering effect of pyridoxine treatment in obese patients.

There have been theories concerning the cholesterol-lowering effect of pyridoxine supplement. Shah et al (1960) investigated the incorporation of labeled acetate and mevalonic acid into liver cholesterol in rats fed either a PN-deficient or a PN-supplemented diet. The incorporation of mevalonic acid-2-^{14}C into liver cholesterol was not affected in PN-deficiency whereas the incorporation of sodium acetate-2-C^{14} into liver cholesterol in PN-deficient rats was markedly increased. However, there was not a concomitant increase in the serum cholesterol concentration in the PN-deficient group. In fact, there was no difference in serum cholesterol concentration between the two groups. These results do not mean that there was a greater degradation of cholesterol in PN-

deficient group since no change in the degradation rate was observed in PN-deficient rats. They postulated that in PN-deficient rats, the growth rate was retarded significantly. Therefore, the energy and the amount of two carbon fragments which are otherwise used for tissue growth became available for the cholesterol synthesis. It is not clear why there was no increase in serum cholesterol in spite of the marked increase of cholesterol synthesis in the liver. In contrast, Iwami and Okada (1982) found a change in cholesterol catabolism in pyridoxine-deficient rats. Rats were fed either a PN-deficient *ad lib* diet or a pyridoxine-adequate pair-fed diet for five weeks. The concentration of bile lipid components was higher in PN-deficient rats than in controls. The decreased taurine conjugates in bile salts and the increase in the fecal bile acid content were observed in PN-deficient rats. These results suggested that PN-deficiency increases cholesterol catabolism or the overall turnover of the cholesterol, as indicated by the shorter half-life of the [^{14}C] cholesterol injected into vitamin B-6 deficient rats.

Bile salts have been postulated to be the primary regulatory mechanism of intestinal cholesterol synthesis (Avery and Lupien, 1969). Lupien and Avery (1970) investigated the *in vitro* conjugation of labeled free cholic acid with glycine and taurine in the liver of vitamin B-6 deficient and pair-fed rats. The results showed that the capacity to conjugate cholic acid to taurine and glycine in the homogenates of liver from PN-deficient rats was approximately three fold greater than in those from the pair-fed control rats. It was postulated that in PN-deficiency the higher percentage of conjugation of bile results in diminution of free bile acids. These are more potent inhibitors of cholesterogenesis than

conjugated bile acids. PLP may lower the conjugation of bile acids by reacting with an ε-lysine group at the active site of the apoenzyme.

Gomikawa and Okada (1980) and Lupien et al (1969) investigated the effect of pyridoxine deficiency on the activity of HMG-CoA reductase, a rate-limiting enzyme of cholesterol synthesis in rats. They observed an increase in the enzyme activity in rats with PN-deficiency. After 8 weeks of pyridoxine deficiency, acetate- ^{14}C incorporation into rat liver cholesterol increased by a factor of approximately 10. However, the change in the activity of this enzyme was not enough to explain the effect on cholesterol metabolism in terms of the increase of ^{14}C-acetate incorporation (Lupien et al, 1969). In the study of Gomikawa and Okada (1979), the activity of HMG-CoA reductase was twice as high in PN-deficient rats as compared to that of controls. They suggested that the increased activity in PN-deficient rats was due to increased enzyme synthesis or increased specific activity of the enzyme induced by some unknown mechanism.

PN and PLP are known to inhibit the glucose-induced nonenzymatic glycosylation by forming a *Schiff* base with proteins (Kahtami et al, 1988). PN supplementation increases the catabolism of LDL partly due to the inhibition of glycosylation of LDL, thereby increasing its uptake by LDL receptor (Arnatottir et al, 1993).

In men depleted of vitamin B-6, serum cholesterol concentration was not affected by vitamin B-6 intake (Baysal et al, 1966). A pharmaceutical dose (300 mg per day) significantly decreased serum cholesterol concentration in dialysis patients when compared to those values before the supplementation (Arnadottir et al, 1993). The amount of

vitamin B-6 supplementation seems to be important with respect to the effect on cholesterol metabolism.

MATERIALS AND METHODS

Subjects

Fifteen potential female subjects were recruited from the community by advertisements on bulletin boards on campus and eight of them were selected based on the following selection criteria; (a) non-pregnant women; (b) no smoking for at least 6 months before the study; (c) ages of 20 to 40; (d) type and amount of physical activity (<3 hours exercise per week); (e) no history of intestinal, renal or metabolic disorders which would affect absorption, metabolism or excretion of vitamin B-6 and dietary fat (e.g., bowel surgery, colitis, diabetes, and asthma); (f) no illness or medical condition requiring constant supervision of a physician; (g) no use of vitamin or other nutritional supplements; (h) no food allergies; (i) no alcohol consumption greater than 2 oz pure alcohol per week; (j) no use of hormones (e.g., oral contraceptive steroids, conjugated estrogen, corticosteroids, thyroxin) or other drugs which influence vitamin B-6 or fat metabolism (e.g., isoniazid, penicillamine) or determination (e.g., antibiotics); (k) normal blood chemistry including hemoglobin and hematocrit; and (l) normal menstrual cycle (26-32 days) with no exceptional variation in the last 6 months. Those who did not meet one or more of criteria described above were not included in the diet study.

Selection criteria of subjects was determined in part by a questionnaire which included a health history (refer Appendix Figure 11), and 3-day food record. Based on self-reported information, the subjects were non-pregnant, non-smoking, and did not take oral contraceptives. Also, no subject with severe exercise (more than 2 hours a day) was

selected. To determine the suitability for participation in this diet study, potential subjects signed an informed consent form for screening (refer Appendix Figure 3), completed a health questionnaire and provided a blood specimen for blood chemistry, assessment of vitamin B-6 and lipid profiles. After screening, informed consent (refer Appendix Figure 4) was obtained from each subject before the study began. Three of the subjects were Asian, and five were Caucasians.

Experimental Design

Each subject filled out a daily log in the morning. This log provided information on physical activity, the amount of the free food intake consumed, body weight (measured before breakfast), and their general feeling of well-being. Also, a record of their menstrual history during the 28 days was obtained.

This metabolic study lasted 28 days. During the first 7 days, everybody was fed the same diet with a sufficient amount of vitamin B-6 (2.10 mg; 12.4 umol/day). Four of subjects were randomly assigned to the vitamin B-6 adequate group (high vitamin B-6) and the other 4 to the low vitamin B-6 group. The experimental group was fed a low vitamin B-6 diet (0.93 mg; 5.5 umol/day) for the following 21 days while the vitamin B-6 adequate group (High) was fed 2.60 mg (15.4 umol) of vitamin B-6 per day. The diet is shown in Table 2. The diet provided 0.93 mg of vitamin B-6 and the additional vitamin B-6 (1.63 mg for the high B-6 group and 1.13 mg during the adjustment period) came from a crystalline pyridoxine-hydrochloride solution. Vials containing pyridoxine were stored frozen for the adjustment period (~150 vials) and for the experimental period (~ 200 vials

for both vitamin B-6 and placebo). The vials were color coded to avoid confusion among subjects. This supplement was given in evenly divided doses at breakfast and dinner and was taken with appropriate water rinses to ensure complete transfer. The low vitamin B-6 group was given a placebo. The diet was exactly the same for both groups except for the vitamin B-6 level. Throughout the study, less than 100 mg per day of arachidonic acid was provided by the diet. The fat content in the diet was maintained around 30 % (60.5 g/day) of total calorie consumption with 10 % of the calories coming from polyunsaturated fatty acids. Protein intake was 83.4 g per day. Adequate energy was supplied by addition of carbohydrate foods free of vitamin B-6 and protein, such as various hard candies, and soft drinks. Essential fatty acids were supplied mainly with salad dressing which was made with canola oil. The subjects were instructed to consume all the foods provided. After each meal, all the dishes used were checked by the researchers to make sure there were no leftover or forgotten food by the subjects. The subjects were instructed not to consume any other foods which might affect the study. Free foods such as pop, coffee without cream, fruit jelly spread, and hard candies were allowed (refer Appendix Table 3, 4, and 5 for the consumption). The diet contained all nutrients in adequate amounts except vitamin B-6. Iron status was assessed by measuring hematocrit (Hct) and hemoglobin (Hgb) when blood was obtained. Two subjects who had borderline-iron deficiency (Hgb <12 g/100ml, Hct <38 %) (Herbert, 1992, Johnson, 1990) were provided with an iron tablet (as ferrous sulfate) at breakfast (32.5 mg Fe) and dinner (32.5 mg Fe) (refer Appendix Table 6 & 7 for hematocrit and hemoglobin).

Sample Collection and Analysis

Weekly food composites were made, aliquots frozen and analyzed later. The mean content of vitamin B-6 in the basal diet was 0.93 mg per day (SD= 0.02).

Blood was collected from fasting subjects at the beginning of the study (day 1) and at the end of every 7 days (day 7, 14, 21 and 28) to assess the changes in plasma fatty acid and lipid profiles and vitamin B-6 status. Blood was drawn into tubes containing heparin (3 tubes) and EDTA (1 tube) as a coagulant. Blood in the heparin tube was used for the assay of B-6 vitamers and blood in the EDTA tube was used for the assay of fatty acid and lipid profiles. After blood was drawn, the tube was stored on ice until centrifuged to separate plasma from the erythrocytes and aliquot stored at -40 °C. The erythrocytes were washed three times with saline and stored at -80 °C. All the samples were assayed within 4 months after storage.

Each subject collected 24-hour urine samples during the entire period of the diet study. Ten ml of toluene was added to urine collection bottles as a preservative. Urine was processed each day and aliquots were frozen at -20 °C for analysis of 4-PA and total vitamin B-6.

Plasma and RBC pyridoxal 5'-phoshate (PLP) was analyzed by measurement of $^{14}CO_2$ evolved during the PLP-dependent decarboxylation of L-tyrosine-1-^{14}C using tyrosine decarboxylase apoenzyme which was derived from *Streptococcus faecalis* (Chabner and Livingston, 1970). Plasma and urinary total vitamin B-6 was assayed by microbiological turbidity method using *Saccharomyces uvarum* (Miller and Edwards, 1981). A high performance liquid chromatographic (HPLC) method was used to

determine the amount of urinary 4-pyridoxic acid (Gregory III, 1979). The Jaffe reaction was used to quantitate 24-hour urinary creatinine excretion using an automated method (Pino et al, 1965).

Plasma fatty acid profiles were analyzed using gas liquid chromatography (GLC) after extraction with a methanol and chloroform mixture (Bligh and Dyer, 1959). Fifteen ul of margaric acid (17:0) was used as an internal standard and added to each tube. A 0.3 ml aliquot of plasma was combined with 0.7 ml saline before the extraction procedure. After the sample and the 2:1 methanol chloroform mixture were mixed, the tube was centrifuged for ten minutes at 1,500 rpm and the supernatant was saved. The residue was extracted again with 1 ml of distilled water and 3.75 ml of the methanol chloroform mixture and the supernatant was combined with the former supernatant. The combined supernatant was extracted with water, methanol and chloroform mixture (1.8:2:2) and centrifuged. The upper white portion was removed and discarded. The lower portion was dried with nitrogen gas and methylated with 0.2 ml of benzene and 1 ml of boron trichloride. The tube containing this mixture was heated on a 95 °C heat block. After 90 minutes, the tube was removed from the heat block and cooled to room temperature. The contents in the tube was mixed with 5 ml of distilled water and 5 ml of hexane, and vortexed for 2 minutes and then centrifuged. The top hexane layer was transferred to a test tube. The residue was mixed with 5 ml of hexane on a vortex mixer for 2 minutes. After centrifuging, the hexane layer was combined with the former one.The extracts were mixed with 0.3 g of Na_2SO_4 on a vortex mixer for 45 seconds and was evaporated under nitrogen gas. The sample was reconstitued in 75 ul of iso-octane and 2 ul of this was

injected into the gas-chromatograph. The data was reported as area %. The standard fatty acid profile diagram is illustrated in Appendix Figure 8 (For the area % of standard fatty acids, refer to Appendix Figure 9). The conditions for gas chromatography of the fatty acids were as follows:

Injection Port Setpoint

Injection Port A : Split/Splitless
Injector A Temp: 235
Injection mode : Split mode
Zone is :On

Oven Temperature Program

Oven Temperature: 170 F	
Initial " : 170 "	Initial Time : 4.00
Rate: 3	
Final Temperature : 188 "	Final Time: 0.00
Rate A: 2	
Final Temperature A: 200	Final Time A: 0.00
Rate B: 3	
Final Temperature B: 220	Final Time B: 2.00

HP 5890A Detector Setpoints

Detector A: FID
Detector A Temperature : 240
Detector B: FID
Detector B Temperature : 250

Plasma phosphatidylcholine concentration was determined with a kit for determining phospholipids from Wako (Richmond, VA). This is an enzymatic colorimetric method (Takayama et al, 1977). Plasma phosphatidylcholine is hydrolyzed to free choline by phospholipase D. The liberated choline is subsequently oxidized to betaine by choline

oxidase with the simultaneous production of hydrogen peroxide. The hydrogen peroxide produced was oxidatively coupled with 4-aminoantipyrine and phenol to yield a chromogen with a maximum absorption at λ=505 nm.

The plasma lipid profile, including total cholesterol (TC), HDL-cholesterol (HDL-C), and triglycerides (TG), was quantitated by a colorimetric method (Allain et al, 1974; Burnstein et al, 1970) using a kit from Sigma Chemical Company (St. Louis, MS). LDL-cholesterol (LDL-C) was calculated from the result of the analysis based on the following equation: LDL-C = TC- (HDL-C)- (TG/5)

Statistical Analysis

Statistical analyses were conducted with Excel computer software program (Microsoft, Seattle, WA). Means for vitamin B-6 status indicators at each time point were compared between the two experimental groups using single factor ANOVA test. For mean values of plasma fatty acid and lipid profiles, and PC, the single factor ANOVA test was used for the comparison of these numbers between the two groups before and after the experimental diet. The statistically significant difference was determined with P value less than 0.05. Correlation coefficient was calculated between plasma alkaline phosphatase activity and plasma PLP concentration.

Table 2. The basal diet

Food Item	Amount
Breakfast	
Orange juice, frozen, reconstituted	160 g
Puffed wheat cereal	20 g
Milk, 2 % milkfat	160 g
Grapefruit section, canned, light syrup	60 g
Sweet cherries, canned, heavy syrup	40 g
White bread, soft	60 g
Lunch	
Cheddar cheese, diced	45 g
Lettuce, iceberg	60 g
Red cabbage, raw	20 g
Carrot, raw	20 g
Celery, raw	20 g
Red kidney beans, canned, drained	30 g
Italian dressing[1]	20 g
Margarine	15 g
Apple, raw	100 g
White bread, soft	60 g
Egg white, cooked	60 g
Gelatin[2]	14 g[2]
Dinner	
Turkey, precooked frozen	40 g
Green beans, canned, drained	80 g
Yellow corn, canned, drained	80 g
Peaches, canned, light syrup	100 g
Milk, 2 % milkfat	160 g
White bread, soft	60 g
Snack	
Popcorn, air popped	15 g
Vanilla wafers cookies	15 g
Pumpkin bars[3]	60 g

[1] For recipe, refer to appendix figure 1.
[2] 14 g of gelatin plus 240 ml prepared fruit drink mix (Koolaid).
[3] For recipe, refer to appendix figure 2.

RESULTS

Characteristics of Subjects

The characteristics of the subjects and the corresponding experimental group are given in table 3. There was no significant difference in mean height, weight and age between the high and the low vitamin B-6 group. The average height of the high and the low vitamin B-6 group was 162.3 and 165.8 cm, respectively. The age of the subjects ranged from 23 to 39. The weight of each subject did not change significantly during the diet study (refer Appendix Table 1). One of the subjects (#1) had a fever at day 10, 11, and 12 and had diarrhea at day 10 during the diet study. No other illness or disease was reported during the diet study. All subjects had regular menstrual cycles during the study.

Nutrient intake based on 3-day diet records is shown in table 4. There was a significant difference in the mean intakes of kcalories ($P<0.005$), total fat ($P<0.005$), % calorie as PUFA ($P<0.05$), and protein ($P<0.05$) between the two experimental groups. The mean intake of kcalories was 2046 kcal and 1242 kcal for the high and for the low vitamin B-6 group, respectively. The average total fat intake of the low B-6 group (41 g) was half that of the high B-6 group (82 g). The % calorie as PUFA was 6 for the high and 3.5 for the low B-6 group. The mean intake of protein was 99 g for the high and 57.8 g for the low B-6 group. The mean intakes of all the nutrients concerned in this diet study were lower for the low vitamin B-6 group when compared with those for the high vitamin B-6 group. There was no significant difference in the consumption of free foods between two groups (Refer Appendix Table 3, 4, and 5).

Table 3. Characteristics of subjects and the corresponding experimental group

Subject	Age	Weight (kg)		Height	Vitamin B-6
	(yrs)	Before	Final	(cm)	
1	31	89.1	88.9	167	H[1]
2	39	84.2	84.7	160	H
3	23	57.9	58.4	165	H
4	23	71.8	71.3	157	H
Mean	29.0	75.7	75.8	162	
±SD	7.7	13.9	13.8	4.6	
5	39	86.6	84.2	172	L[2]
6	35	87.1	86.1	157	L
7	36	66.8	62.9	167	L
8	25	64.4	63.9	167	L
Mean	33.8	74.9	74.3	166	
±SD	6.1	11.0	12.6	6.3	

[1] High vitamin B-6 group during the experimental period
[2] Low vitamin B-6 group during the experimental period

Vitamin B-6 Status

The vitamin B-6 status of the high and the low vitamin B-6 group at each time point during the diet study is shown in Table 5 and 6. There was no significant difference between the two groups for all the B-6 indicators at day 1. At day 7, the mean values of plasma PLP, RBC PLP and TB6 was not significantly different between the two experimental groups. Seven days after the experimental diet began (day 14), there was a significant difference between the high and the low vitamin B-6 group for the mean plasma PLP and TB6 concentration ($P<0.05$). The concentration of plasma PLP continued to increase in the high vitamin B-6 group throughout the diet study while it began to

Table 4. Mean intakes of nutrients from 3-day diet records[1]

	Group	
Nutrient	High B-6	Low B-6
Calorie (kcal)*	2046±143	1242±238
Vitamin B-6 (mg)	1.54±0.60	1.10±0.59
Total Fat (g)*	82.1±8.31	41.0±12.2
% calorie as SFA[2]	11.3±2.40	7.37±2.59
% calorie as MUFA[3]	14.1±5.43	7.96±3.25
% calorie as PUFA[4]**	6.07±0.59	3.53±1.32
Cholesterol (mg)	289±115	198±82.0
Riboflavin (mg)	1.92±0.44	1.37±0.53
Protein (g)**	99.0±24.5	57.8±21.4

*Significant difference between the high and the low vitamin B-6 group ($P < 0.005$)
**Significant difference between the high and the low vitamin B-6 group ($P < 0.05$)
[1]One subject in low vitamin B-6 group has 2-day diet records.
[2]SFA: Saturated fatty acids
[3]MUFA: Monounsaturated fatty acids
[4]PUFA: Polyunsaturated fatty acids the low B-6 group.

decrease in the low vitamin B-6 group at day 7. Fourteen and twenty one days after the experimental diet (day 21 and day 28), the difference in the mean concentration of plasma PLP between the high and the low vitamin B-6 group became more significant ($P<0.01$). Through day 21, there was no significant difference in the ratio of plasma PLP/TB6 between the high and the low vitamin B-6 group. However, at day 28, the last day of the study, there was a significant difference ($P<0.05$) in the ratio between the two experimental groups. The high vitamin B-6 group had a higher (0.88) ratio compared to that (0.69) of the low vitamin B-6 group.

The activity of plasma alkaline phosphatase (Table 5) showed no significant difference between the two experimental groups during the diet study. There was no significant correlationship between the plasma PLP concentration and the activity of plasma alkaline phosphatase.

The mean plasma PLP and RBC PLP concentration over the 28 days are illustrated in Figures 10 and 11, respectively. The magnitude of the change from day 1 to day 7 of the mean plasma PLP concentration of the high vitamin B-6 group was similar to that of the low vitamin B-6 group. After day 7, the mean plasma PLP concentration of the high vitamin B-6 group increased until the end of the diet study while that of the low vitamin B-6 group decreased significantly.

The mean RBC PLP concentrations displayed a different trend from those of the mean plasma PLP concentrations (Figure 11). The mean RBC PLP concentrations responded relatively slowly to the dietary intake of vitamin B-6. Seven days after beginning the low vitamin B-6 intake, the mean RBC PLP was slightly higher compared to day 1. There was a slight decrease in the mean RBC PLP concentration of the low vitamin B-6 group after 14 days of the diet study. Also, the mean RBC PLP concentration of the high vitamin B-6 group increased slightly until day 21 and decreased very slightly at day 28. The mean RBC PLP concentrations did not change significantly in either the high or the low vitamin B-6 group. In addition, no significant difference was observed in the mean RBC PLP concentrations between the two experimental groups during the diet study.

Table 6 shows the mean urinary excretion of 4PA and vitamin B-6 (UB6). There was no significant difference in the mean urinary excretion of 4PA and UB6 between the

high and the low vitamin B-6 group at the start and at the end of week 1. The mean urinary 4PA and UB6 excretion was significantly different ($P < 0.001$) between the two groups at the end of week 2. The excretion of 4PA of the low vitamin B-6 group was similar for weeks 3 and 4. The mean values of urinary excretion of UB6 as well as 4PA during the study did not change significantly for the high vitamin B-6 group. However, the mean UB6 excretion of the low vitamin B-6 group decreased significantly ($P< 0.0001$) from 0.82 at the start to 0.42 umol per day at the end of week 4.

The percent of the daily vitamin B-6 intake excreted as 4PA and B6 (Table 6) was not significantly different between the two experimental groups and did not change significantly in either the high or the low vitamin B-6 group.

Plasma Fatty Acid Profile

The plasma fatty acid profiles before and after the experimental diet for the high and the low vitamin B-6 groups are given in Table 7. For all the fatty acids, there was no significant difference between the two groups either before or at the end of the experimental period. There was no change in linoleic (18:2n-6) and arachidonic (20:4n-6) acid. For arachidonic acid, the low B-6 group started with a slightly higher level than that of the high B-6 group though this difference was not significant. However, there was no significant change in both groups after the experimental diet as compared to the values before the start of the experimental diet. Figures 12 and 13 shows the mean area % of linoleic and arachidonic acid before and after the experimental diet for the high and the low vitamin B-6 groups, respectively. The ratio of 20:4n-6/18:2n-6 (Figure 14) also did

not show any significant difference between the two groups before and at the end of the experimental diet period. Among the fatty acids, 20:1 (n-9) showed contrasting changes in the high and the low B-6 group. This fatty acid decreased (23 %) in the high B-6 group and increased (35 %) in the low B-6 group although the difference was not significant due to the large variations within each group (Figure 15).

The effect of vitamin B-6 intake on the plasma polyunsaturated fatty acids (PUFA), n-6 and n-3 series fatty acid content as area % concentrations are illustrated in Figures 16 and 17, respectively. No statistical significance was found in the mean area % of plasma PUFA concentrations between the two groups before and at the end of the experimental diet period. However, there was a slight but non-significant increase (16 %) in the mean area % of plasma n-3 fatty acids for the high vitamin B-6 group when compared with those for the low vitamin B-6 group.

Plasma Phospholipid

The mean values of plasma phosphatidylcholine concentrations are given in Table 8. There was no significant difference between the two experimental groups before and at the end of the experimental diet period. Also, no significant change was observed at the end of the experimental diet period in both groups (Figure 18).

Table 5. Mean plasma and red blood cells values of vitamin B-6 status indicators

		Time point during the diet study				
		Day 1	Day 7[5]	Day 14	Day 21	Day 28
Plasma PLP[3] (nmol/L)						
	High[6]	34.9±15.9*	48.2±9.3	57.8±17.1	61.9±7.5	65.5±17.8
	Low[6]	28.8±11.6	41.6±19.8	25.1±12.8[1]	23.7±10.7[2]	20.3±6.4[2]
Plasma TB6[4] (nmol/L)						
	High	49.5±22.6	57.9±12.3	76.9±17.5	71.0±7.3	73.9±16.5
	Low	44.1±18.3	51.4±25.8	35.9±13.6[1]	34.5±11.1[2]	29.3±9.7[2]
RBC PLP (nmol/L)						
	High	35.6±6.4	41.9±6.3	42.1±5.7	47.0±8.0	43.6±7.6
	Low	36.6±3.1	39.8±5.0	42.3±4.6	39.8±8.8	36.3±8.0
Plasma PLP/TB6						
	High	0.71±0.0	0.84±0.0	0.76±0.2	0.88±0.1	0.88±0.1
	Low	0.66±0.1	0.82±0.1	0.67±0.1	0.68±0.2	0.69±0.0[1]
Alkaline Phosphatase (Units/L)						
	High	29.9±7.6	30.7±9.4	30.5±8.4	29.6±7.5	29.8±8.8
	Low	26.5±7.4	26.8±6.5	27.0±5.4	26.9±5.1	25.9±5.8

*Mean±SD

[1]Significant difference from the high vitamin B-6 group ($P<0.05$)

[2]Significant difference from the high vitamin B-6 group ($P<0.01$)

[3]Pyridoxal-5'-phosphate

[4]Total vitamin B-6

[5]All subjects were fed the same level of vitamin B-6 from day 1 to day 7; 2.1 mg/d (12.4 umol/d)

[6] High = high vitamin B-6 group (2.6 mg/d); n=4;
Low = low vitamin B-6 group (0.93 mg/d); n=4

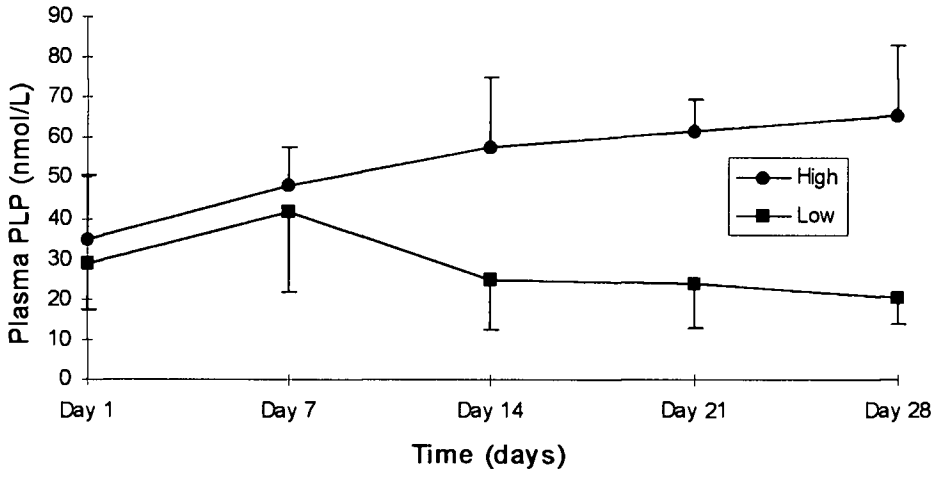


Fig. 10. Plasma PLP concentration during the diet study at each time point for the high (2.60 mg or 15.4 umol/day) and the low (0.93 mg or 5.5 umol/day) vitamin B-6 group.

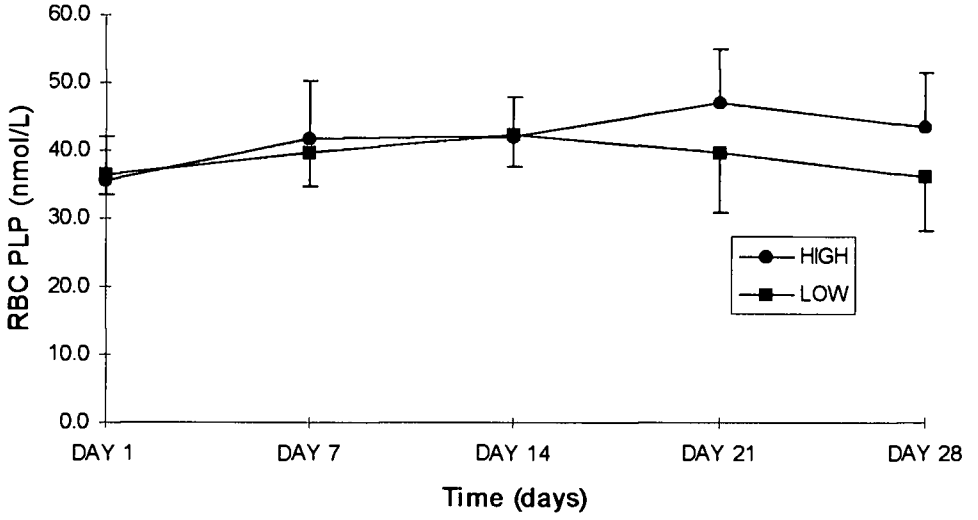


Fig. 11. RBC PLP concentration during the diet study at each time point for the high (2.60 mg or 15.4 umol/day) and the low (0.93 mg or 5.5 umol/day) vitamin B-6 group.

Table 6. Mean urinary 4-pyridoxic acid (4PA)*and total vitamin B-6 (UB6)* excretion

	Time point during the diet study				
	Start[4]	End of Week 1[5]	End of Week 2	End of Week 3	End of Week 4
4PA (umol/day)					
High[3]	7.26±1.8	7.63±1.3	8.14±1.4	9.53±0.9	9.24±1.0
Low[3]	6.17±1.5	7.00±0.3	3.41±0.6[1]	2.65±0.7[1]	2.67±0.6[1]
UB6 (umol/day)					
High	1.08±0.2	0.96±0.2	1.07±0.2	1.12±0.1	1.09±0.3
Low	0.82±0.2	0.79±0.2	0.53±0.1[1]	0.43±0.1[1]	0.42±0.0[1, 2]
4PA+UB6 as % of B-6 intake					
High	67.3±14.7	69.6±9.3	59.9±8.1	69.3±2.7	67.3±1.8
Low	56.3±11.5	62.8±1.4	71.7±9.0	56.0±12.4	56.2±11.4

*Mean ±SD

[1] Significantly different from the high vitamin B-6 group ($P<0.001$)

[2] Significant difference from the mean values at start ($P<0.00005$)

[3] High = high vitamin B-6 group (2.6 mg/d); n=4;
Low = low vitamin B-6 group (0.93 mg/d); n=4

[4]Data from day 1 and 2

[5]All subjects were fed the same level of vitamin B-6 from day 1 to day 7; 2.1 mg/d (12.4 umol/d)

Table 7. Plasma fatty acid profile (Area %)*

	Before[1]		After[1]	
Fatty Acid	High [2]	Low[2]	High	Low
16:0	18.9±1.4	20.7±1.5	19.8±0.8	20.3±0.8
16:1 (n-7)	6.92±9.3	2.19±0.4	2.36±0.1	1.95±0.2
18:0	6.02±0.5	6.69±0.7	6.13±0.7	6.57±0.8
18:1 (n-9, T[3])	1.06±0.2	0.96±0.4	1.04±0.2	0.94±0.4
18:1 (n-9, C[4])	17.9±1.6	18.7±0.9	19.5±0.6	18.8±1.5
18:1 (n-7)	1.91±0.4	1.73±0.4	1.92±0.2	1.88±0.2
18:2 (n-6, T)	0.25±0.0	0.27±0.0	0.25±0.2	0.09±0.2
18:2 (n-6, C)	28.5±3.9	28.1±2.4	30.6±1.6	29.5±2.6
18:3 (n-3, C)	1.13±0.1	0.88±0.1	1.19±0.2	0.84±0.1
20:1 (n-9)	0.26±0.1	0.23±0.0	0.20±0.1	0.31±0.4
20:2 (n-6)	0.28±0.0	0.29±0.0	0.23±0.2	0.25±0.2
20:3 (n-6)	1.51±0.2	1.73±0.1	1.59±0.1	1.70±0.1
20:4 (n-6)	5.76±1.3	7.20±0.6	5.93±1.3	7.44±0.7
20:5 (n-3)	0.46±0.2	0.60±0.3	0.53±0.2	0.52±0.1
22:5 (n-3)	0.51±0.1	0.63±0.1	0.58±0.1	0.65±0.1
22:6 (n-3)	1.51±0.4	1.82±0.4	1.90±0.9	2.06±0.5
20:4(n-6)/18:2 (n-6)	0.20±0.0	0.26±0.0	0.20±0.1	0.25±0.0
Total n-6	36.3±4.7	37.6±2.9	38.6±1.2	39.0±2.7
Total n-3	3.61±0.4	3.92±0.7	4.20±0.9	4.06±0.7

*Mean±SD
[1]Before (day 7) and after (day 28) the experimental diet
[2]High = high vitamin B-6 group (2.6 mg/d); n=4;
Low = low vitamin B-6 group (0.93 mg/d); n=4
[3]T: *trans* form
[4]C: *cis* form

Table 8. Plasma phosphatidylcholine (mg/100 ml)*

Time Point	Vitamin B-6 intake	
	High[1]	Low[1]
Start of the experimental diet	134±25.2	132±11.5
End of the experimental diet	126±23.4	124±6.24

*Mean±SD
[1] High = high vitamin B-6 group (2.6 mg/d): n=4; Low = low vitamin B-6 group (0.93 mg/d): n=4

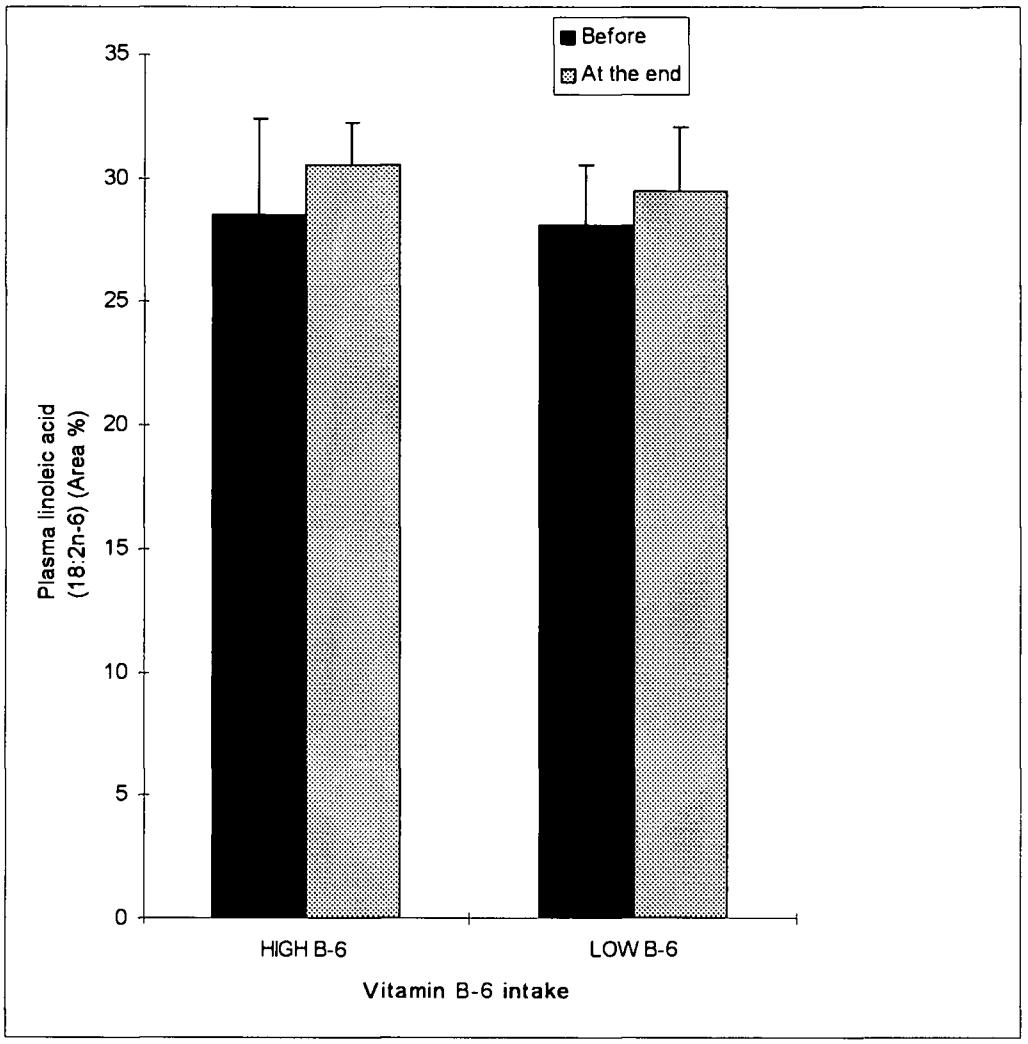


Figure 12. Comparison of plasma linoleic acid (18:2n-6) as area % in the high (2.60 mg/day) and the low (0.93 mg/day) vitamin B-6 group 'before' and 'at the end' of the experimental diet.

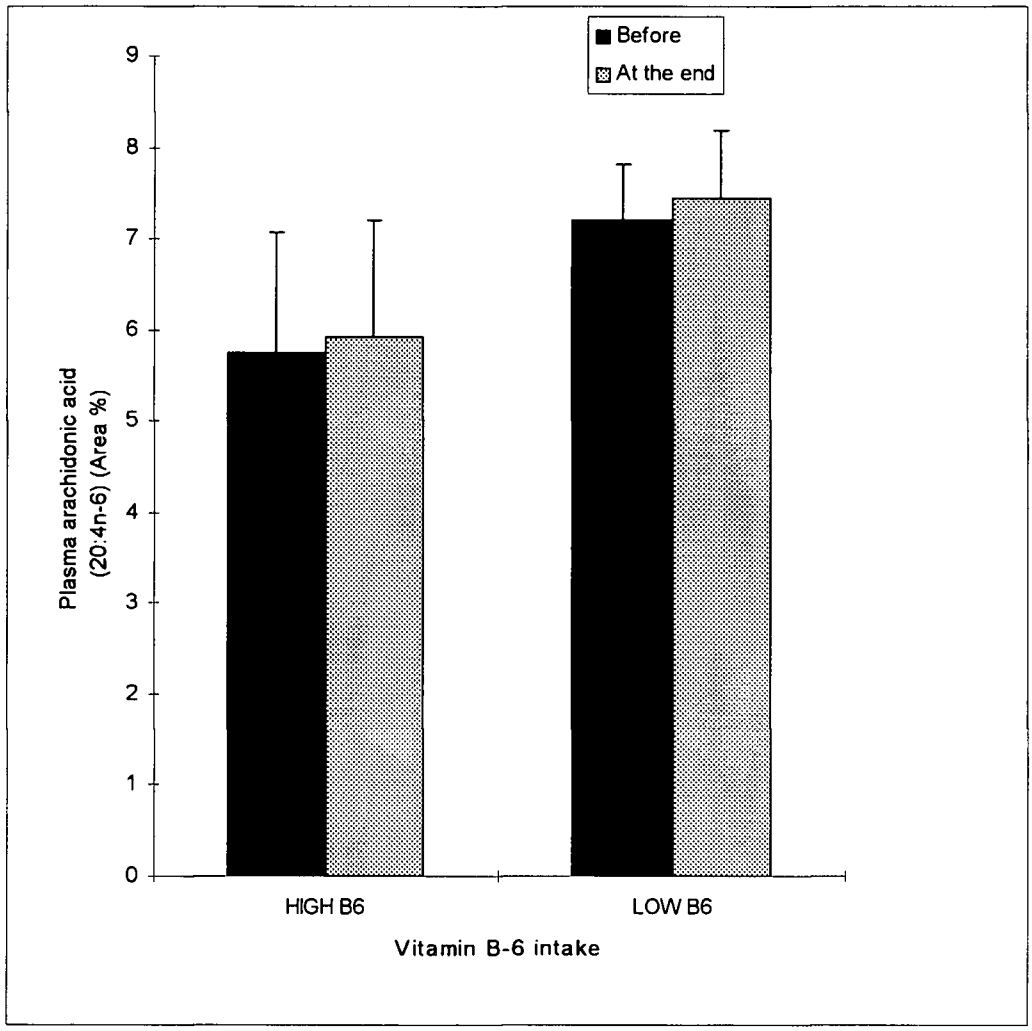


Figure 13. Comparison of plasma arachidonic acid (20:4n-6) as area % in the high (2.60 mg/day) and the low (0.93 mg/day) vitamin B-6 group 'before' and 'at the end' of the experimental diet.

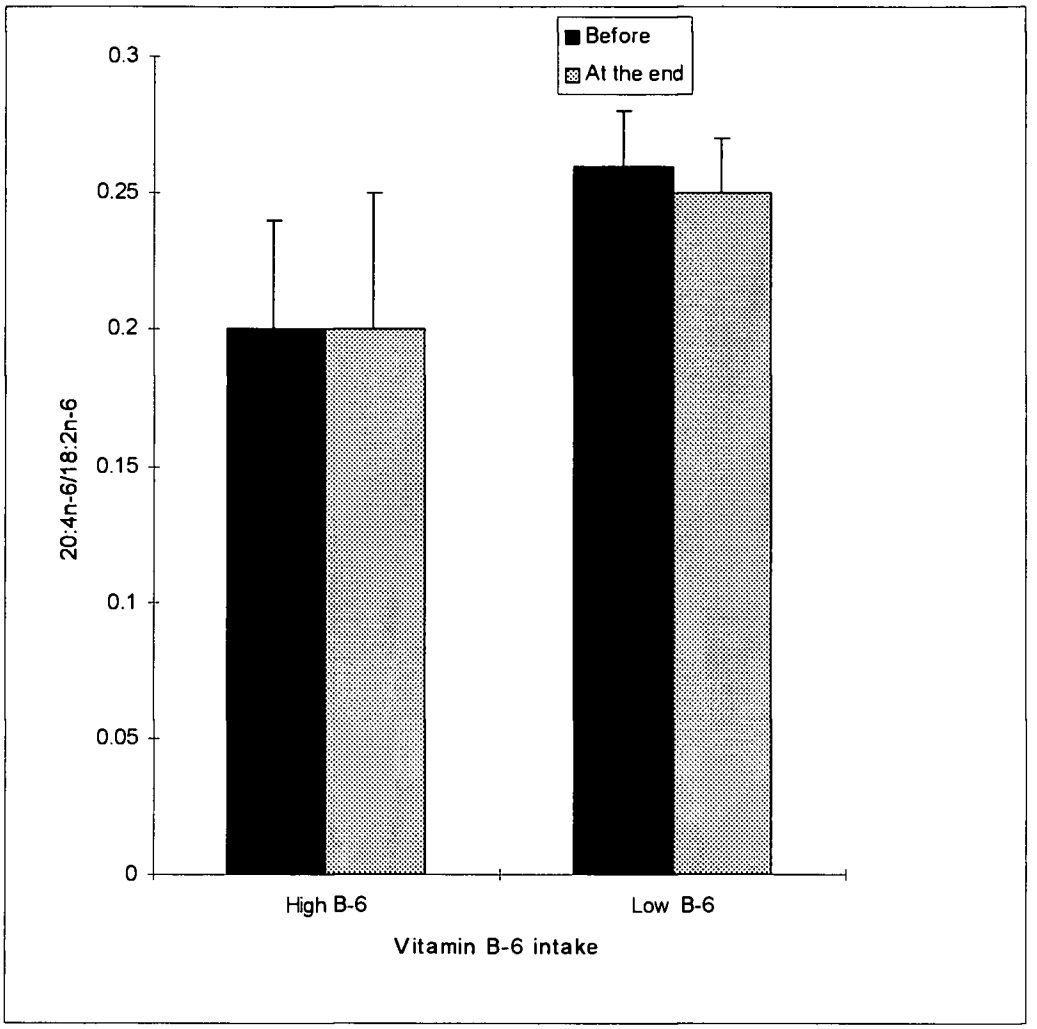


Figure 14. Comparison of 20:4n-6/18:2n-6 ratio in the high (2.60 mg/day) and the low (0.93 mg/day) vitamin B-6 group 'before' and 'at the end' of the experimental diet.

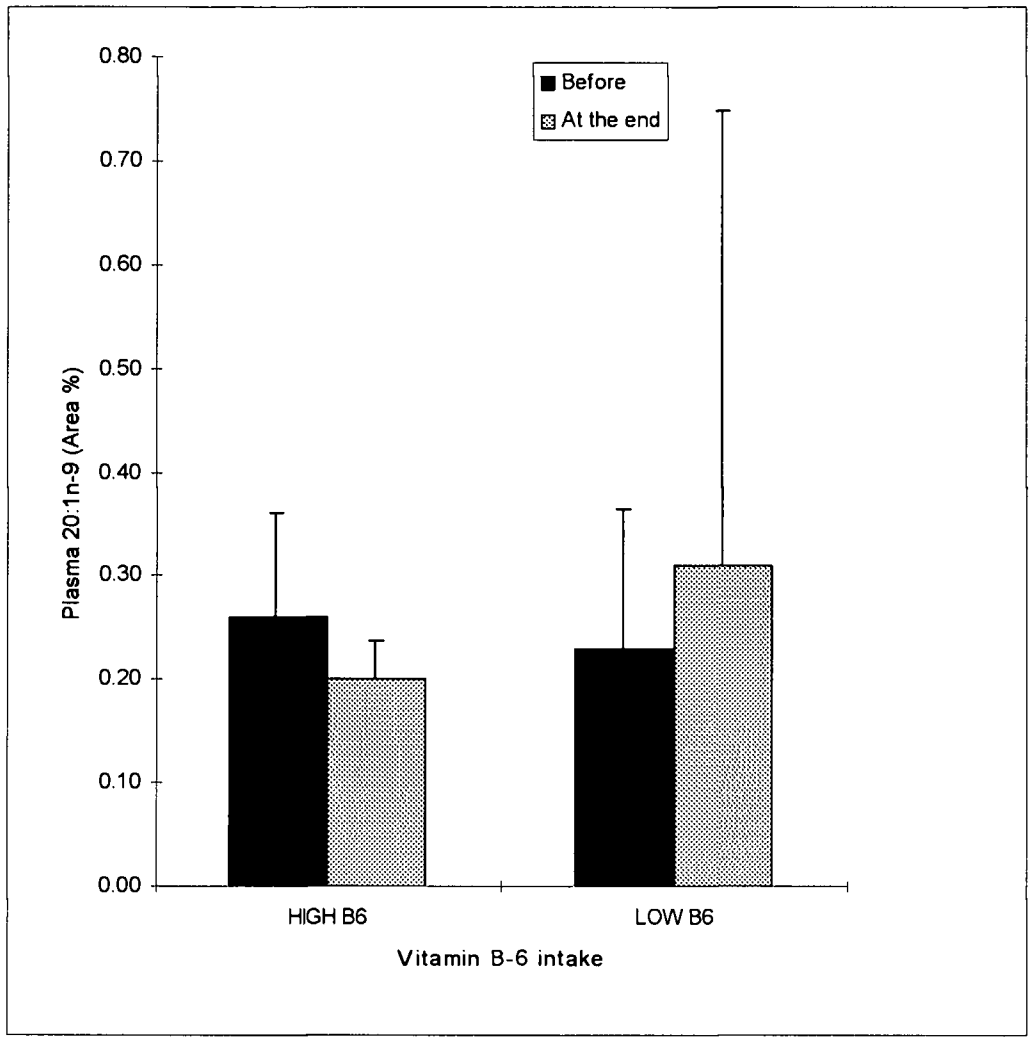


Figure 15. Comparison of plasma fatty acid 20:1(n-9) as area % in the high (2.60 mg/day) and the low (0.93 mg/day) vitamin B-6 group 'before' and 'at the end' of the experimental diet.

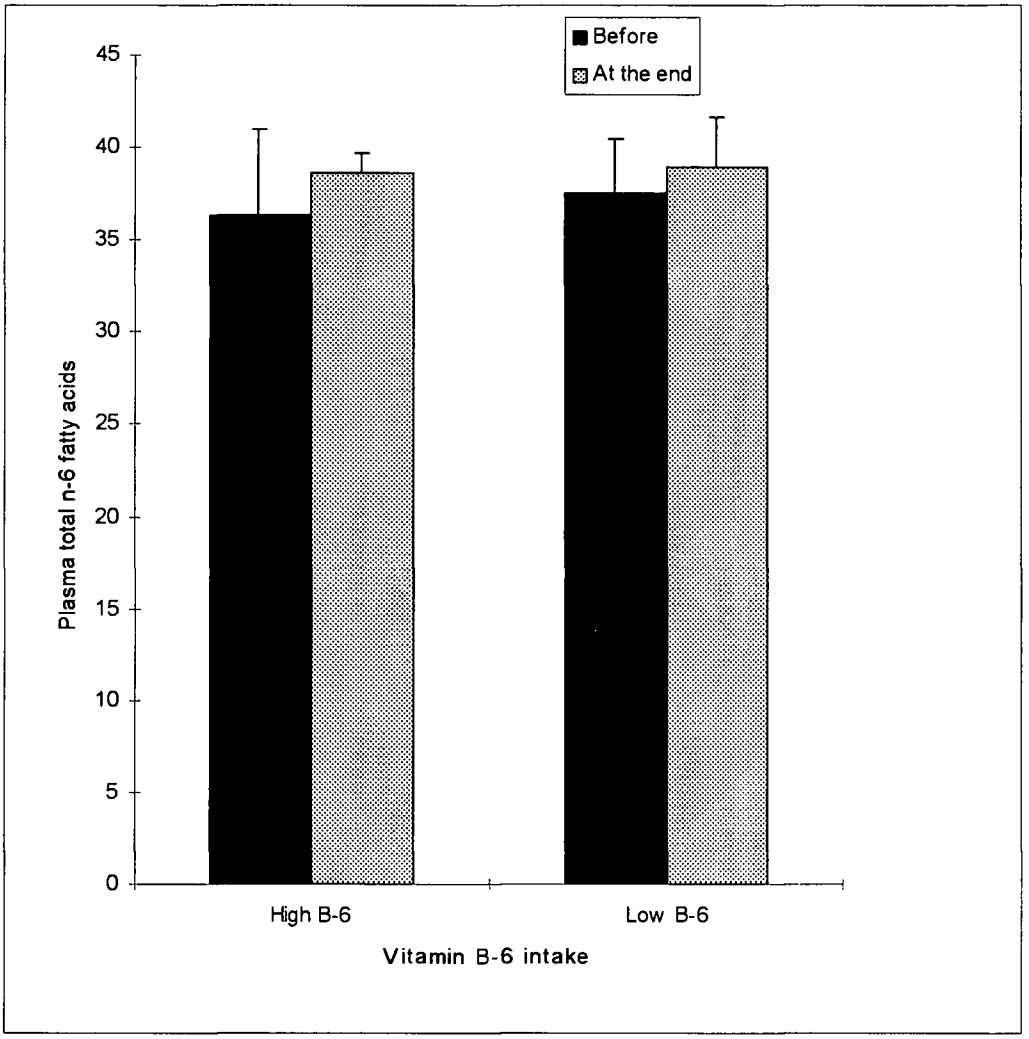


Figure 16. Comparison of plasma total n-6 fatty acids as area % in the high (2.60 mg/day) and the low (0.93 mg/day) vitamin B-6 group 'before' and 'at the end' of the experimental diet.

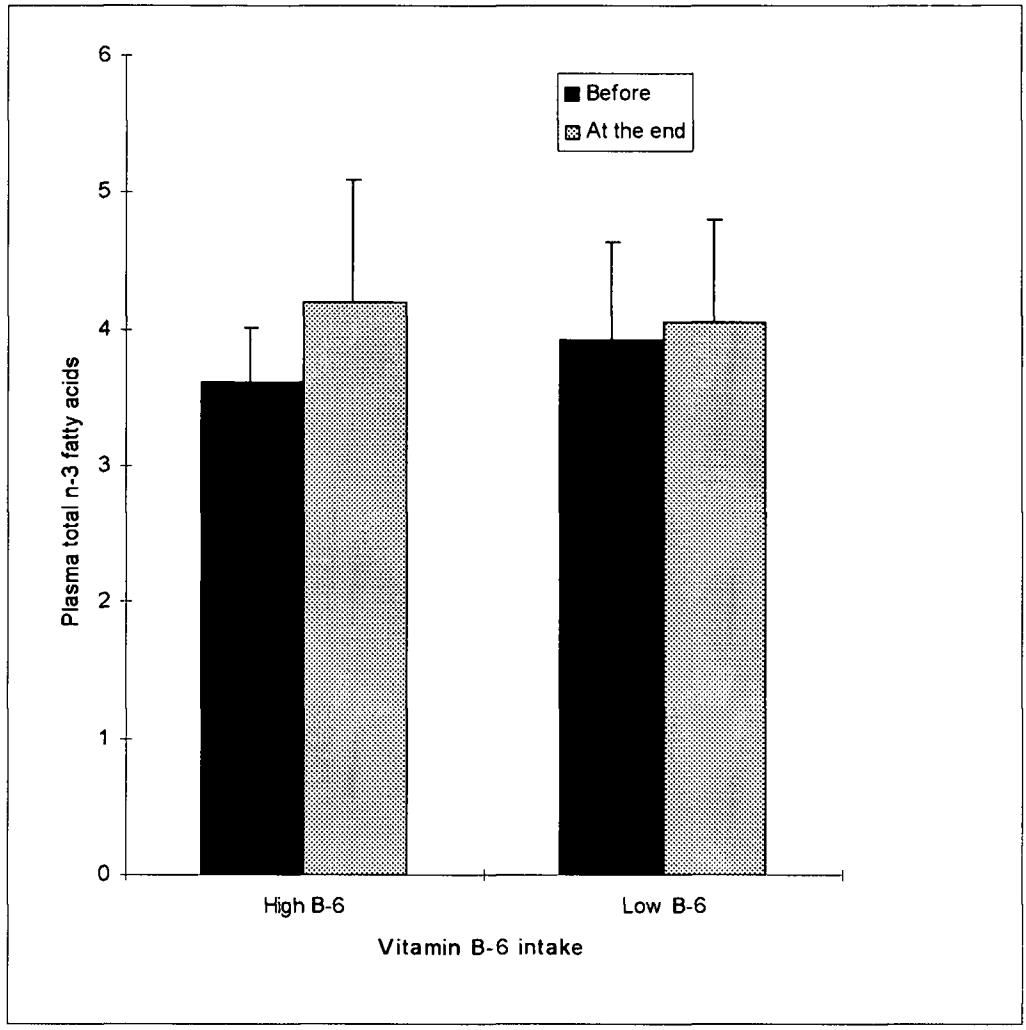


Figure 17. Comparison of plasma total n-3 fatty acids as area % in the high (2.60 mg/day) and the low (0.93 mg/day) vitamin B-6 group 'before' and 'at the end' of the experimental diet.

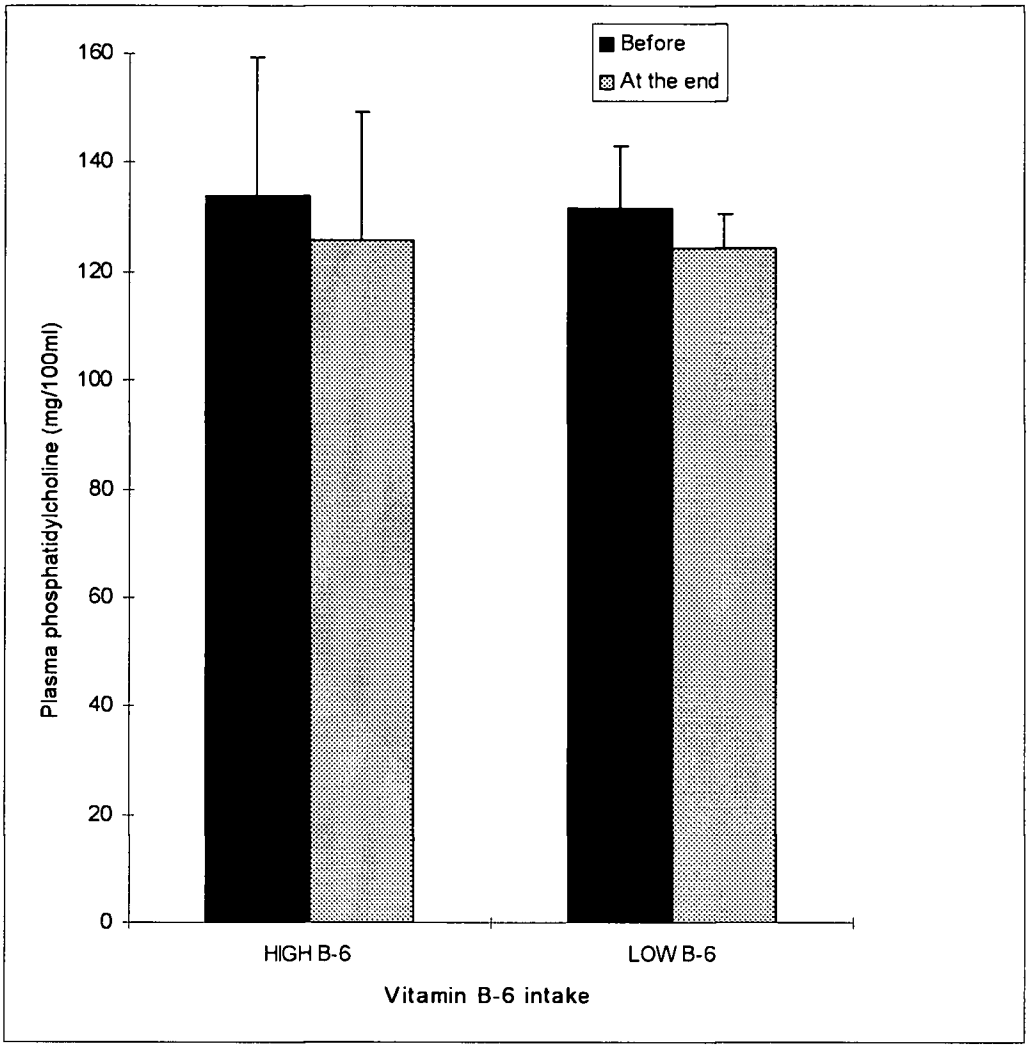


Figure 18. Comparison of plasma phosphatidylcholine (mg/100 ml) in the high (2.60 mg/day) and the low (0.93 mg/day) vitamin B-6 group 'before' and 'at the end' of the experimental diet.

Plasma Lipid

The mean plasma cholesterol concentrations contained in each type of plasma lipoprotein are shown in Table 9. The mean plasma total cholesterol (TC) concentration (Figure 19) did not change in the high vitamin B-6 group before and after the experimental diet, but decreased (7%) slightly in the low vitamin B-6 group. The mean plasma high-density lipoprotein-cholesterol (HDL-C) concentration (Figure 20) decreased in both groups and the degree of decrease was about same (~8%). The mean plasma low-density lipoprotein-cholesterol (LDL-C) concentration (Figure 21) decreased (5 %) for the high B-6 group but did not change in the low B-6 group. The mean plasma triglycerides (TG) concentrations (Figure 22) increased (9 %) for the high vitamin B-6 group while it decreased (7 %) for the low vitamin B-6 group. The ratio of plasma TC to HDL-C (Figure 23) increased (18 %) in the high vitamin B-6 group when compared with the low B-6 group (5 %). In general, the mean concentrations of plasma cholesterol in all fractions and plasma TG concentrations showed no statistically significant difference within and between the two experimental groups for their mean values at the end of the experimental diet period.

Table 9. Plasma lipid profile[1]

	Before[5]		At the end[5]	
Lipid	High[6]	Low[6]	High	Low
Total cholesterol (mM)	4.42±0.71	4.46±0.29	4.40±0.97	4.14±0.26
HDL[2] cholesterol (mM)	1.25±0.17	1.35±0.13	1.05±0.18	1.21±0.22
LDL[3] cholesterol (mM)	1.27±0.50	0.81±0.18	1.21±0.42	0.83±0.37
Triglycerides (mM)	2.50±0.73	2.69±0.38	2.72±0.77	2.50±0.41
TC/HDL-C[4]	3.64±1.03	3.34±0.52	4.29±1.22	3.52±0.64

[1] Mean±SD
[2] HDL: High density lipoprotein
[3] LDL: Low density lipoprotein
[4] Ratio of total cholesterol to HDL-cholesterol
[5] *Before* and *At the end* of the experimental diet
[6] *High* (2.60 mg or 15.4 umol/day) and *Low* (0.93 mg or 5.5 umol/day) vitamin B-6 group

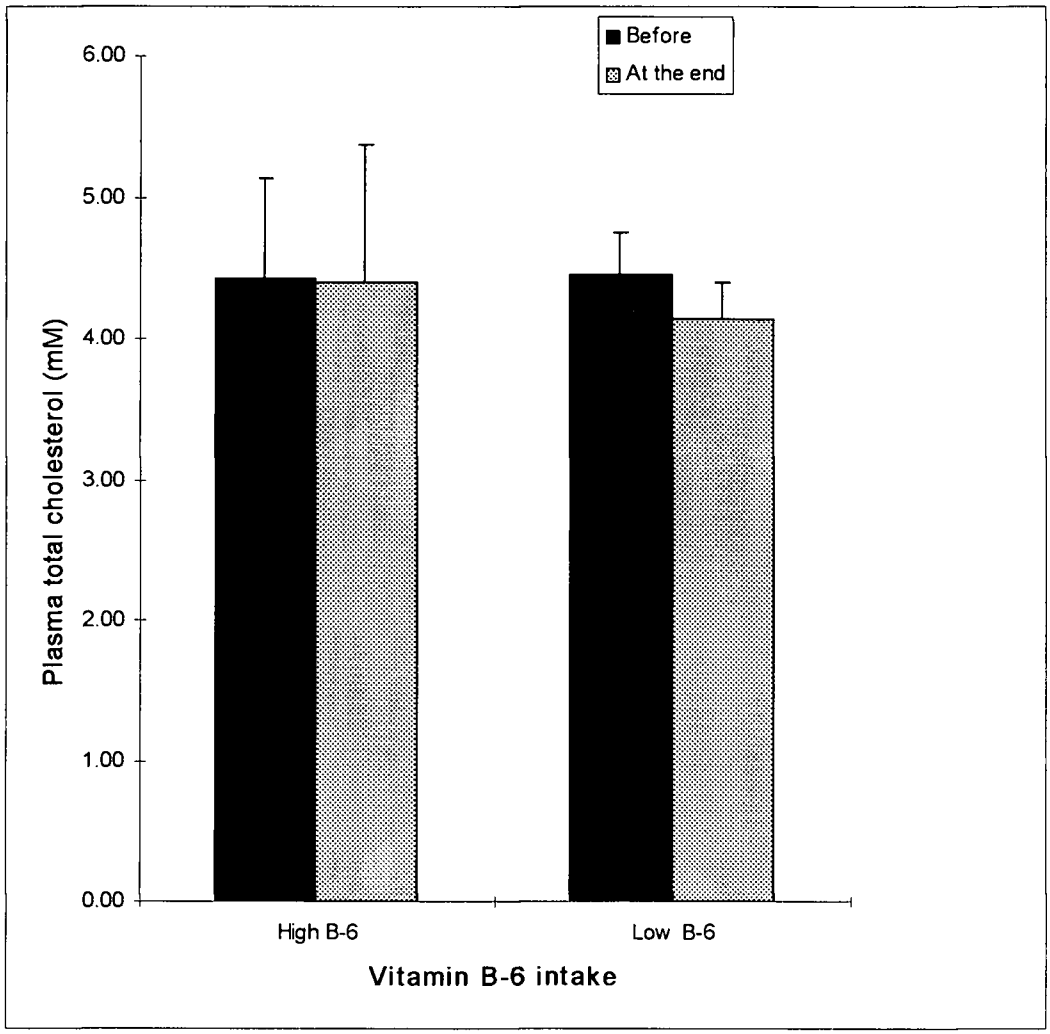


Figure 19. Comparison of plasma total cholesterol (TC) (mM) in the high (2.6 mg/day) and the low (0.93 mg/day) vitamin B-6 group 'before' and 'at the end' of the experimental diet period.

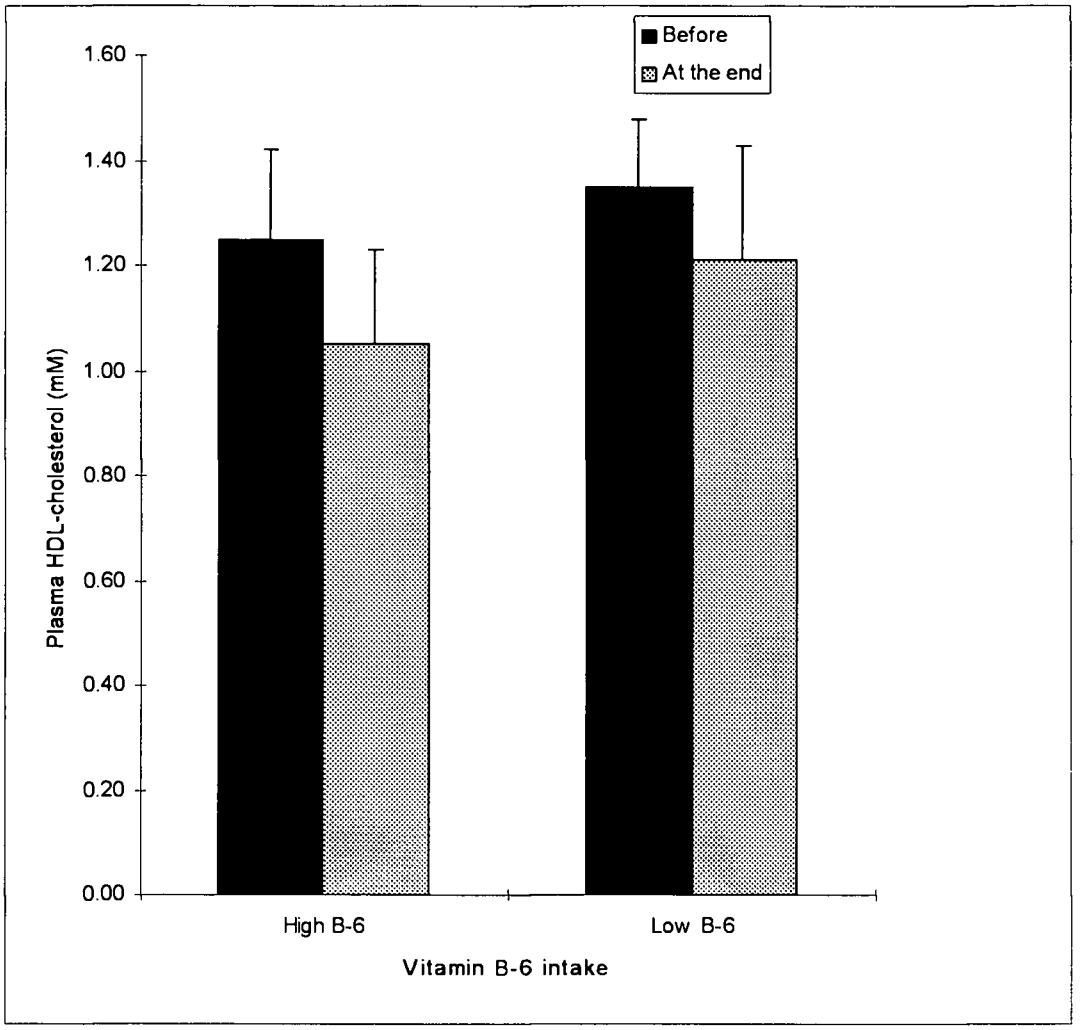


Figure 20. Comparison of plasma HDL-cholesterol (mM) in the high (2.6 mg/day) and the low (0.93 mg/day) vitamin B-6 group 'before' and 'at the end' of the experimental diet period.

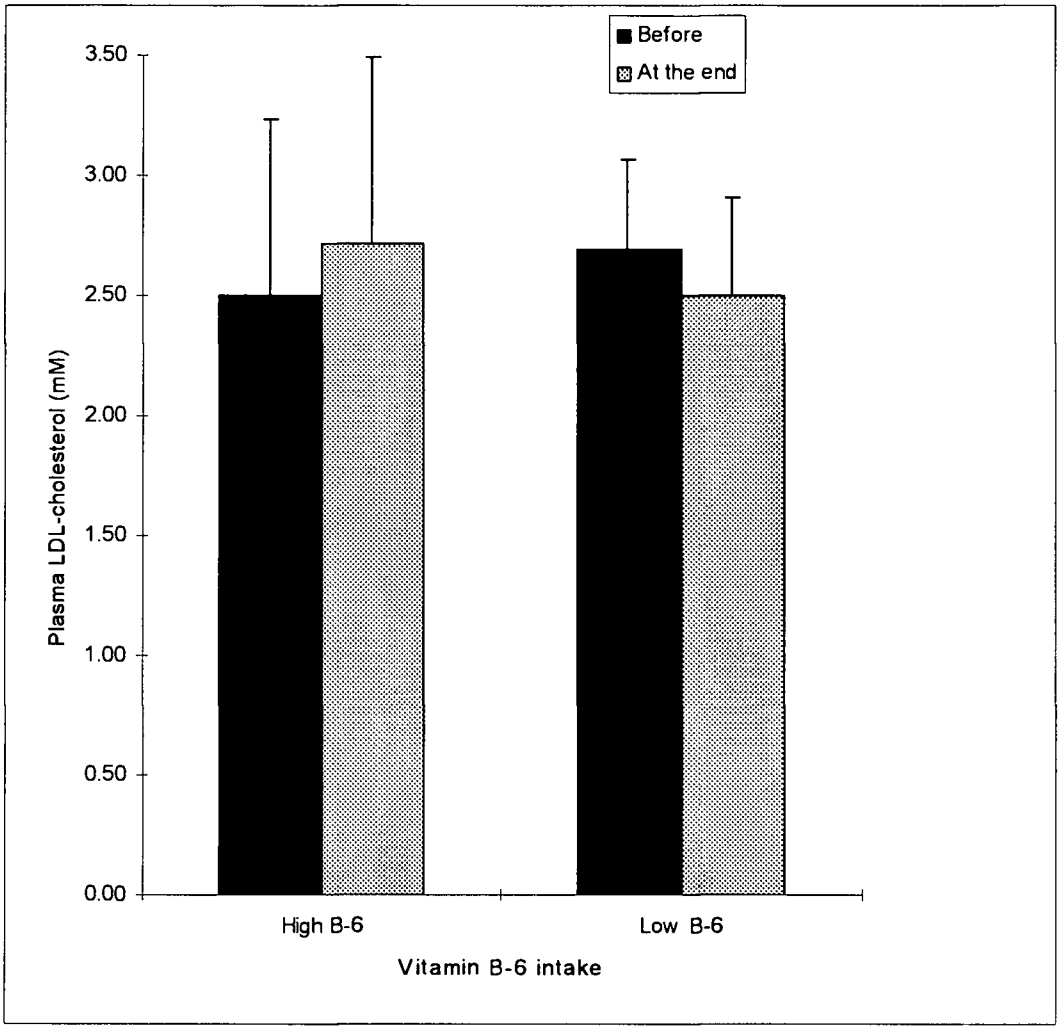


Figure 21. Comparison of plasma LDL-cholesterol (mM) in the high (2.6 mg/day) and the low (0.93 mg/day) vitamin B-6 group 'before' and 'at the end' of the experimental diet period.

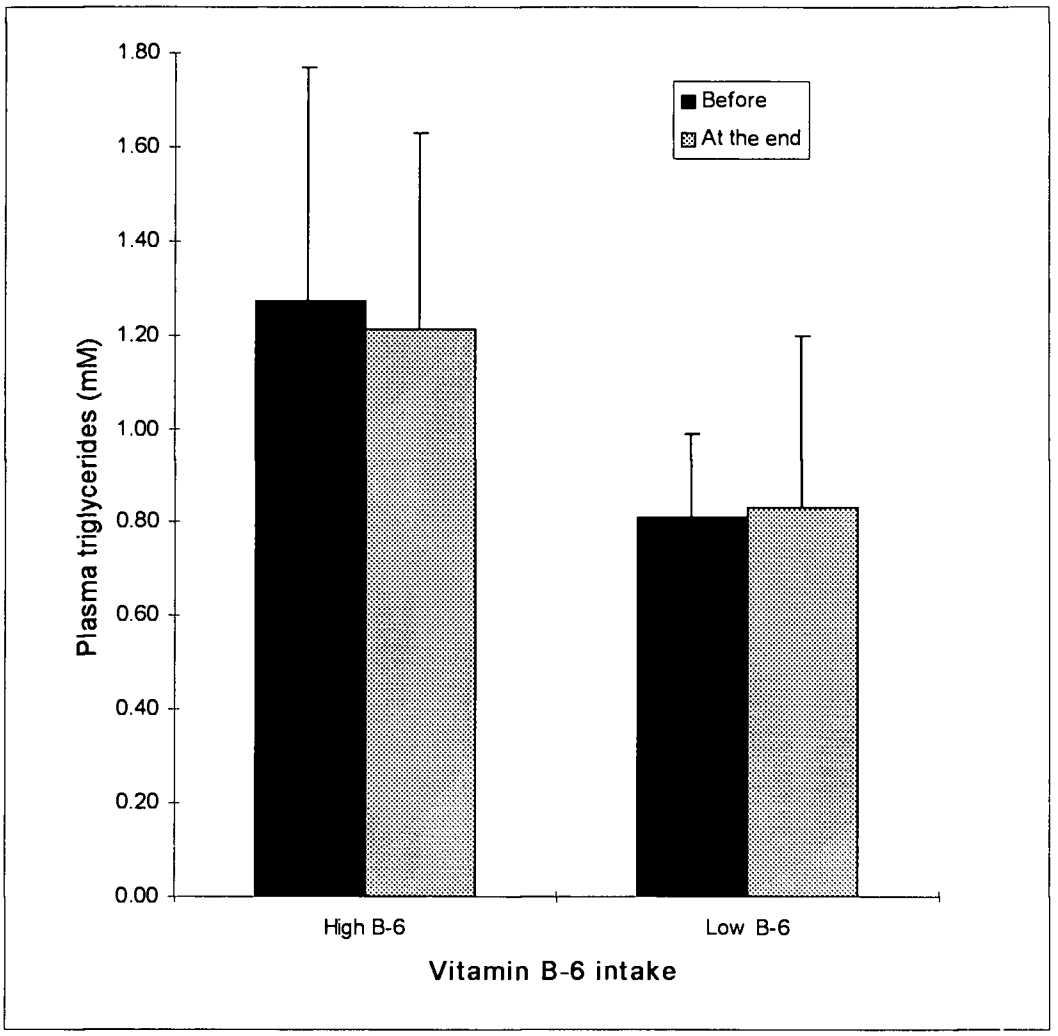


Figure 22. Comparison of plasma triglycerides (mM) in the high (2.6 mg/day) and the low (0.93 mg/day) vitamin B-6 group 'before' and 'at the end' of the experimental diet period.

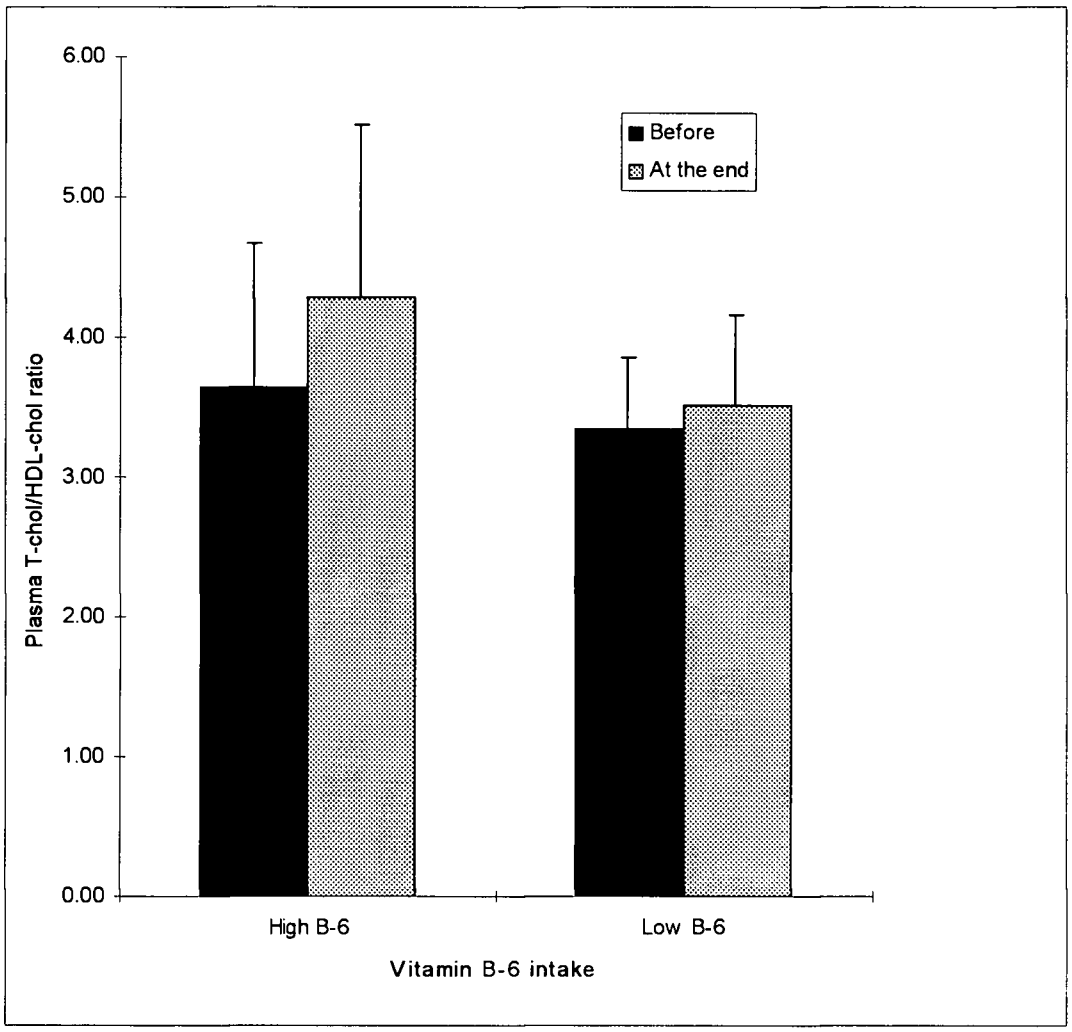


Figure 23. Comparison of TC/HDL-cholesterol ratio in the high (2.6 mg/day) and the low (0.93 mg/day) vitamin B-6 group 'before' and 'at the end' of the experimental diet period.

DISCUSSION

This diet study was conducted to investigate the effect of vitamin B-6 status on the plasma fatty acid metabolism and plasma lipid profiles. Eight female subjects were divided into two groups and randomly assigned to the high or the low vitamin B-6 group.

The mean calorie consumption as calculated from 3-day diet record of the high and the low vitamin B-6 group were 2046 and 1242 kcal, respectively. This statistically significant difference in the calorie consumption from 3-day diet records may be due to the underestimation of intake by subjects, especially, those in the low vitamin B-6 group. Also, the lack of training of subjects in keeping diet records probably contributed to this difference in the calorie intake and underestimation of nutrient consumption of the subjects.

There was no significant change in the mean value of subjects' weight during the diet study. For two subjects in the low B-6 group their weight decreased 2.4 (#5) and 3.9 kg (#7) by the end of the diet study as compared with the starting weight. It is possible that those who lost weight more than 2 kg during the diet study did not consume free foods enough to supply their energy requirement.

Most of the vitamin B-6 status indicators in the plasma and urine (Table 5 and 6) responded rapidly to the dietary level of vitamin B-6. The high vitamin B-6 group started with slightly higher mean values of plasma PLP than that of the low vitamin B-6 group at the beginning of the diet study though it was not significantly different. This slight difference may have been due to the intake of vitamin B-6 prior to day 1. After the 7 day

of adjustment period (2.10 mg vitamin B-6 per day), the mean plasma PLP concentration increased slightly in both groups. After the experimental diet began the mean plasma PLP concentration responded to the dietary intake of vitamin B-6 in both groups. Also, the mean plasma TB6 concentration started with a higher value for the high B-6 group than for the low B-6 group at the beginning of the diet study and manifested a plateau during the last 14 days in the high B-6 group. In the low vitamin B-6 group the mean plasma TB6 concentration increased during the first 7 days of the study and decreased by gradually thereafter until the end of the study (Δ=22 nmol/L).

The mean urinary 4PA and UB6 excretion also responded quickly to the dietary intake of vitamin B-6. Urinary excretion of 4PA is known to be a measure of the flux of the vitamin through PLP. Based on kinetic studies, PL is three times more likely to be oxidized to 4PA than to be rephosphorylated to PLP (Merrill et al, 1984). Twenty-four hour urinary excretion of 4PA has been reported to reflect the recent dietary intakes of vitamin B-6 (Leklem, 1988; Brown et al, 1975) and this was confirmed in the present study. The starting values (day 1 & 2) of 4PA in the two groups were not significantly different (Table 6). The mean urinary 4PA excretion did not change significantly at the end of week 1 as compared to the beginning value in both groups. The mean 4PA excretion increased in the high B-6 group until the end of week 3 and showed a plateau while it decreased (4.33 umol) in the low B-6 group from the end of week 1 until the end of week 4. The body was adjusting to the low intake of vitamin B-6 by decreasing the urinary excretion of 4PA. The mean values of urinary UB6 did not change significantly in the high B-6 group throughout the diet study, which suggests that the dietary intake of vitamin B-6

during the diet study was not significantly different from that of the subjects' usual diets. For the low vitamin B-6 group the mean UB6 excretion decreased (0.36 umol) until the end of week 3 and showed a plateau during the last seven days of the diet study. These results indicate that plasma PLP and TB6 as well as urinary excretion of 4PA and UB6 reflect the short-term dietary intake of vitamin B-6 and that these indicators are good B-6 status indicators of short-term B-6 status.

With respect to determination of vitamin B-6 status of the subjects in the present study, there are several cut-off points between adequate and marginal and/or deficient levels suggested. Shultz and Leklem (1981) conducted a study to investigate the vitamin B-6 status and dietary intake of vitamin B-6. In their study, vitamin B-6 status indicators were measured in male and female subjects. Results showed that females aged 25-79 consumed 1.6 (0.8-3.2) mg per day of dietary vitamin B-6 and the plasma PLP concentration ranged from 14 to 80 nmol/L with a mean value of 37 nmol/L. They suggested that plasma PLP concentration greater than 30 nmol/L is acceptible for normal vitamin B-6 status. Reynolds (1987) proposed that plasma PLP values less than 30 nM for females are generally considered to be borderline between marginal and adequate nutritional status for vitamin B-6. He also suggested that concentrations less than 15 nM, in absence of an underlying medical condition, are likely to represent a true deficiency of vitamin B-6. In the present study, the mean plasma PLP of the low vitamin B-6 group decreased down to about 20 nM while that of the high vitamin B-6 group increased up to ~60 nM at the end of the experimental diet period. The mean plasma PLP concentration of the low vitamin B-6 group at the end of the experimental diet is lower than the acceptible

range of plasma PLP of Shultz and Leklem (1981). It is concluded that the subjects in the low vitamin B-6 group had a subnormal vitamin B-6 status. There was therefore a drastic difference in the vitamin B-6 status between the two experimental groups.

Unlike other B-6 indicators, the RBC PLP concentrations responded slowly to the dietary intake of vitamin B-6. This slow response was reflected by the slight increase in RBC PLP concentration 1 week after the low vitamin B-6 diet in the low B-6 group. The mean values of RBC PLP were not significantly different between the two experimental groups at any time point during the diet study. Reynolds (1991) reported that erythrocyte PLP increased rapidly within 40 minutes following the oral ingestion of 100 mg PN and decreased quickly over the subsequent 4 hours. However, this phenomenon appears to be only an acute response to a supplement of vitamin B-6. In the present study, the dietary intake of vitamin B-6 in both groups were not as high as the report mentioned above. Since all the blood samples were collected after an overnight fast, there was no possible acute effect of dietary vitamin B-6 on RBC PLP levels. The result from this study in terms of RBC PLP concentrations is similar to that of the study by Hansen et al (1993). They did not observe a significant change in the RBC PLP concentrations when women were fed 0.8 to 2.4 mg of vitamin B-6. The slight difference in the mean values of RBC PLP between the two experimental groups might be explained by the slow response of RBC PLP to a long-term change of dietary vitamin B-6 intake and also by the changes in the recent dietary intakes of vitamin B-6. The results from this study suggest that the RBC PLP concentrations may not be a reliable measure of recent dietary intake of vitamin B-6.

The ratio of plasma PLP/TB6 (the percent of vitamin B-6 in the form of PLP) decreased significantly at the end of the experimental diet for the low vitamin B-6 group. If the change in the ratio indicates a change of vitamin B-6 metabolism in the body, the result would imply that under the condition of low vitamin intake, the body may be adjusting the distribution of dietary vitamin B-6 to minimize the amount of vitamin B-6 to be excreted. In other words, it is possible that more vitamin B-6 is present in the form of PL such that more is taken up by tissues. If tissues need similar amounts of vitamin B-6 for metabolism regardless of the quantity of dietary vitamin B-6, tissues may need a greater percent of dietary vitamin B-6 and less would be present in the circulation in the form of PLP.

The low intake of vitamin B-6 did not influence the percent of the intake excreted as urinary 4PA and UB6 in this diet study. This would indicate that even with a low B-6 intake, the body is excreting a constant percentage of dietary vitamin B-6 in the urine.

This study did not find evidence that vitamin B-6 status affects fatty acid metabolism, especially the conversion of linoleic (18:2n-6) to arachidonic acid (20:4n-6). There was no difference in the plasma fatty acid profile before and at the end of the experimental diet in both groups (Table 7). These findings are in contrast to the findings of Mueller et al (1963) who investigated the effect of desoxypyridoxine on the PUFA metabolism in human beings. They assessed vitamin B-6 status by measuring serum glutamic oxaloacetic transaminase (SGOT) activity and urinary excretion of xanthurenic acid after a tryptophan load test. During a deficiency period, urinary excretion of xanthurenic acid ranged from 72 to 329 mg/24hr (one subject has 16 mg/24hr) whereas it

ranged from 3 to 38 during control period. The activity of SGOT, which was measured in thirteen of the seventeen subjects, decreased significantly during the deficiency period as compared to that during the control period. They were able to duplicate the changes in the fatty acid patterns which were predicted from animal studies (Swell et al, 1961; Medes and Keller, 1947; Cunnane et al, 1984) even though the changes were minor. They found that plasma arachidonic acid was slightly decreased and that cholesterol transport was impaired with administration of desoxypyridoxine. They suggested that vitamin B-6 indeed is required for normal fatty acid metabolism in man. However, their subjects had chronic illnesses and they did not control vitamin B-6 intake. They created a vitamin B-6 deficiency by administrating desoxypyridoxine which is a metabolic antagonist of pyridoxine. In the present study, subjects in the low vitamin B-6 group were fed 0.93 mg of vitamin B-6 per day. The Third Report on Nutrition Monitoring in the United States (vol. 2, 1993) showed that the mean intake of vitamin B-6 was 1.5 mg per day for females aged 21-39. Block and Abrams (1993) examined the NHANES II data and reported that the mean dietary intake of vitamin B-6 in women of childbearing age and with below or near poverty was 0.96 mg per day. This indicates that there are certain populations vulnerable to the subnormal vitamin B-6 status. The low (0.93 mg/day) vitamin B-6 group in the present study shows what vitamin B-6 status would be in those populations. Also, the subjects in the high vitamin B-6 group were not fed an extremely high level of vitamin B-6. The amount of vitamin B-6 consumed (2.60 mg per day) was slightly more than the RDA. Most studies conducted to investigate the relationship between vitamin B-6 and fatty acid metabolism did not measure vitamin B-6 status and were done in rats depleted

of vitamin B-6 or supplemented with high doses of vitamin B-6 (Witten and Holman, 1952; Dam et al, 1958; Swell et al, 1961; Kirschman and Coniglio, 1961).

The hypothesis of this study was based on the interrelationship of vitamin B-6, dietary fatty acid, and amino acids, especially, methionine. Under conditions of vitamin B-6 deficiency, methionine metabolism was reported to be impaired in rats and humans, increasing plasma homocysteine and S-adenosyl homocysteine (SAH) (She et al, 1994; Ubbink et al, 1996). SAH inhibits the activity of the enzyme PE methyltransferase, resulting in a lower production of PC via the methylation pathway (She et al, 1995). Ubbink et al (1996) investigated the effect of subnormal vitamin B-6 status on homocysteine metabolism using a methionine loading test. Oral methionine load tests were performed with 22 asthma patients treated with theophylline, a vitamin B-6 antagonist, and with 24 age- and sex-matched controls with a normal vitamin B-6 status. They observed significantly higher plasma concentrations of homocysteine, cystathionine and a significantly lower plasma α-aminobutyrate concentration in the asthma patients as compared to those in the control group. The mean plasma PLP concentration was 16.7 and 52.4 nmol/L for the asthma patients and for the control group, respectively. However, results from a study by Selhub et al (1996) showed that plasma homocysteine concentration exhibited a strong inverse association with plasma folate and weaker associations with plasma vitamin B-12 and PLP concentrations when they analyzed plasma samples from the 20^{th} biannual examination of the Framingham Heart Study cohort. The result from this latter study is supported by Verhoef et al (1996) who suggested that folate is the most important determinant of plasma homocysteine. Assuming the folate status of

subjects in the present study was adequate when determined by dietary intake of folate (~320 ug per day), it is not clear whether the significantly low vitamin B-6 status affected methionine metabolism enough to influence fatty acid metabolism. Unfortunately, metabolites from methionine metabolism, i.e., SAM and homocysteine, were not measured in the present study. It is not clear whether vitamin B-6 status plays a major role in fatty acid metabolism even if methionine metabolism had been altered due to vitamin B-6 status of the subjects.

Phosphatidylcholine (PC) synthesis *in vivo* in human beings could be somewhat different from that in animals. PC synthesis in PN-deficient rats was impaired even with an adequate supply of choline (Delorme and Lupien, 1976). Choline is the substrate for the *de novo* synthesis of PC via CDP-choline pathway. The decrease in the synthesis of PC due to pyridoxine deficiency indirectly inhibits the conversion of linoleic to arachidonic acid (She et al, 1994). However, PC synthesis via PE methylation may not be the only pathway for production of PC in human beings. PC synthesis via CDP-choline probably is a good alternative for the PC synthesis. The mean plasma PC concentration was 134 (before) and 132 (at the end) for the high vitamin B-6 group and was 126 (before) and 124 (at the end) mg/100ml for the low vitamin B-6 group, respectively. All the subjects had normal plasma PC concentrations (refer Appendix Table 13) when compared with the range of 106-200 mg/100ml by Petersen (1950). There was no significant difference in the mean plasma PC concentration (Table 8 and Fig. 18) between the two experimental groups before and at the end of the experimental diet period. The mean plasma PC concentrations decreased slightly in both groups to a similar degree at the end of

experimental diet period. This slight decrease may have been due to the effect of the diet fed to the subjects. Also, there was no significant correlation between arachidonic acid and PC content in the plasma. The results from the present study are not in agreement with the hypothesis that vitamin B-6 is specifically involved in the conversion of linoleic acid into arachidonic acid and that decreased PC content in the plasma may indirectly affect the conversion pathway of these fatty acids.

The linoleic acid (18:2n-6) desaturation index, the ratio of 20:4n-6/18:2n-6 (Fig. 14) did not show any significant difference between the two groups before and at the end of the experimental diet. There was no change for the ratio before (0.20) and at the end (0.20) of the experimental diet for the high vitamin B-6 group. The ratio decreased (4 %, from 0.26 to 0.25) slightly in the low B-6 group after the experimental diet. The ratio from the low vitamin B-6 group is similar to those (0.25~0.26) of a study by Wander et al (1996). Although it was not statistically significant, this change suggests a possible relationship between vitamin B-6 status and fatty acid metabolism when one considers the small number of subjects in the present study. The plasma total n-6 fatty acid content showed no significant difference between the two experimental groups. For the plasma total n-3 fatty acid content, there was a slightly greater increase in the high vitamin B-6 group compared with the low B-6 group although it was not statistically significant, probably due to the small number of subjects. For this reason, further studies with a larger number of subjects may be required.

The present study found no significant difference or change in the serum concentration of cholesterol. The plasma concentration of cholesterol in HDL and LDL

did not change significantly before and at the end of the experimental period and there was no significant difference between the two experimental groups. There was a slight decrease (7 %) in the plasma total cholesterol (TC) concentrations in the low vitamin B-6 group. The mean values of plasma HDL-cholesterol concentrations decreased in both groups to a same degree (~8 %). The plasma triglycerides (TG) content increased (9 %) in the high B-6 group and decreased (7 %) in the low B-6 group.

Since there are few studies of the effect of vitamin B-6 status on serum cholesterol concentration in human beings, it is hard to compare the results of this study. There are two human studies that investigated the effect of vitamin B-6 supplementation on plasma lipid profiles (Brattstrom et al, 1990; Arnatottir et al, 1993). Atherosclerotic, 80-year-old men with subnormal plasma PLP levels were supplemented with 120 mg of pyridoxine per day for eight weeks. The plasma concentrations of TC and LDL-cholesterol were found to decrease by 10 and 17 %, respectively. No change was observed in the plasma HDL-cholesterol concentrations. In the other study, a pharmaceutical dose (300 mg of pyridoxine per day) of vitamin B-6 significantly decreased (7 %) plasma TC concentrations in dialysis patients. Although these results are not consistent with those from the present study, there are several differences in the design of the studies. First, the level of vitamin B-6 intake in the present study was much lower than that used in these studies. Second, the subjects in the present study were healthy and without any clinical illnesses and were young as compared to those in the study by Brattstrom et al (1990). Third, all the nutrients and the calorie intakes in the present study were controlled whereas those studies mentioned above controlled only vitamin B-6 intake by supplementation.

Increased catabolism of cholesterol in vitamin B-6 deficient rats has been observed (Iwami and Okada, 1982). The lipid content in bile was higher in PN-deficient rats as compared to that of control rats. Also, an increase in the bile acid content of feces was observed in PN-deficient rats. The increased catabolism of cholesterol in PN-deficient rats was also confirmed by the shorter half-life of the [^{14}C] cholesterol, indicating an increased turnover of the cholesterol. If this is true in human beings, the slight decrease in the mean plasma TC for the low vitamin B-6 group may be partially due to the increased catabolism of cholesterol in the body. Unlike atherosclerotic patients, the subjects in the present diet study had normal values of plasma cholesterol concentrations. Thus, dramatic changes in the plasma cholesterol concentrations might not be expected in subjects in the present study.

The current study was conducted to investigate the effect of vitamin B-6 status on the fatty acid and lipid metabolism in women. There was a highly significant difference in the vitamin B-6 status between the two experimental groups. Also, the vitamin B-6 status for the low vitamin B-6 group was subnormal when determined based on the level of plasma PLP. However, the low vitamin B-6 group did not show any significant difference in the plasma fatty acid, lipid profiles, and PC concentrations as compared to those of the high vitamin B-6 group. The inability to show any significant difference between the two groups in the present study, even with such a low vitamin B-6 status of the low B-6 group, suggests vitamin B-6 plays no significant role in the fatty acid and lipid metabolism. However the small number of subjects utilized in this study may be a reason for insignificant results observed. Although there were no observed significant differences that

would lead one to conclude that there is a significant relationship between vitamin B-6 and the metabolism of fatty acid and lipid, further studies may be required.

SUMMARY & CONCLUSION

The effect of vitamin B-6 (B-6) status on plasma fatty acids (FA) levels and lipid was investigated in this metabolic study. This study is based on the hypothesis that under the conditions of vitamin B-6 deficiency, methionine metabolism is impaired, leading to the increase of plasma homocysteine and S-adenosyl homocysteine (SAH). The increased SAH, in turn, inhibits the activity of the enzyme, PE methyltransferase, decreasing the production of phosphatidylcholine (PC) from phosphatidylethanolamine (PE). The decrease of PC would affect the conversion of linoleic acid to arachidonic acid by inhibiting the enzyme system involved in desaturation and elongation of polyunsaturated fatty acids. This theory is mainly based on animal studies and the present study was designed to see if there is such a relationship between vitamin B-6 and fatty acid metabolism in humans under controlled situation.

Eight female subjects were fed a constant diet for 28 days. For the first seven days, they were fed a diet containing 2.10 mg of B-6. For the rest of the period (21 days), they were differentiated in terms of B-6 intake; four women were fed a low (0.93 mg/day) and four a high (2.60 mg/day) B-6 diet. Vitamin B-6 status indices, plasma fatty acid profiles, PC concentration and lipid profile were determined.

There was a statistically significant difference ($P<0.01$) between the two groups for the plasma pyridoxal 5'-phosphate (65.5 vs. 20.3 nmol/L) and total B-6 concentration (73.9 vs. 29.3 nmol/L) at the end of the study. Also urinary 4-pyridoxic acid (9.24 vs. 2.67 umol/d) and total B-6 excretion (1.09 vs. 0.42 umol/d) showed a significant difference

($P<0.001$) between the high and the low vitamin B-6 groups at the end of the study. The concentration of erythrocyte PLP failed to show any significant difference between the two groups throughout the diet study.

There was no significant difference in the plasma FA or lipid profiles between the two groups. There was a slight but not significant increase in the mean plasma linoleic acid (area %) in both experimental groups. The mean of plasma arachidonic acid (area %) also increased slightly but not significantly in both groups. There was no change in the ratio of 20:4(n-6)/18:2(n-6) for the high vitamin B-6 group. The ratio decreased slightly but not significantly in the low vitamin B-6 group. The mean plasma phosphatidylcholine (PC) concentration decreased slightly in both groups at the end of the experimental diet.

Plasma total cholesterol (TC) of the low B-6 group decreased slightly (7 %), but was not statistically significant. When comparing day 7 and day 28 values, plasma triglycerides increased (9 %) for the high B-6 group and decreased (7 %) for the low B-6 group. LDL-C decreased (5 %) for the high B-6 group but did not change in the low B-6 group. HDL-C decreased slightly in both groups (~8 %).

It is not clear from the present study whether vitamin B-6 affects the metabolism of fatty acid and cholesterol. In spite of the subnormal vitamin B-6 status, the low vitamin B-6 group did not show any significant difference in the plasma fatty acid and lipid profiles as compared with those of the high vitamin B-6 group. This suggests that vitamin B-6 plays a minor role in polyunsaturated fatty acid metabolism, especially, the conversion of linoleic to arachidonic acid. This diet study was designed to control the dietary intake of nutrients and the physical activity of healthy subjects. Therefore, the results are still

valuable even though they were not consistent with animal studies. There are many things which are not fully understood about the relationship between vitamin B-6 and the metabolism of fatty acid and cholesterol. The dietary level of essential fatty acid and the beginning level of plasma cholesterol of subjects would be important factors to be considered in future research. Further studies are needed to determine the effect of vitamin B-6 status on the fatty acid and lipid metabolism.

REFERENCES

Ahlborg G, and Felly P. 1982. Lactate and glucose exchange across the forearm, legs, and splanchnic bed during and after prolonged leg exercise. J Clin Invest 69:45-54

Allain CC, Poon LS, Chan CSG, Richmond W, and Fu PC. 1974. Enzymatic determination of total serum cholesterol. Clin Chem 20:470-5

Allgood VE, and Cidlowski JA. 1992. Vitamin B6 modulates transcriptional activation by multiple members of the steroid hormone receptor superfamily. J Biol Chem 67:3819-24

Allgood VE, Oakley RH, and Cidlowski JA. 1993. Modulation by vitamin B6 of glucocorticoid receptor-mediated gene expression requires transcription factors in addition to the glucocorticoid receptor. J Biol Chem 268: 20870-6

Allgood VE, Powell-oliver FE, and Cidlowski JA. 1990. Vitamin B6 influences glucocorticoid receptor-dependent gene expression. J Biol Chem 12424-433

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

Andon MB, Reynolds RD, Moser-Veillon PB, and Howard MP. 1989. Dietary intake of total and glycosylated vitamin B-6 and the vitamin B-6 nutritional status of unsupplemented lactating women and their infants. Am J Clin Nutr 50: 1050-8

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

Arnadottir M, Brattstrom L, Simonsen O, Thysell H, Hultberg B, Anderson A, and Nilsson-Ehle P. 1993. The effect of high-dose pyridoxine and folic acid supplementation on serum lipid and plasma homocysteine concentrations in dialysis patients. Clin Nephrol 40:236-240

Avery MD, and Lupien PJ. 1969. Cholesterol metabolism and vitamin B-6. II. Intestinal cholesterogenesis in vitamin B-6-deficient rats. Lipids 5:109-113

Avery MD, and Lupien PJ. 1971. Cholesterol metabolism and vitamin B-6. IV. Synthesis of cholic acid conjugates in vitamin B-6 -deficient rats. Can J Biochem 49:1026-1030

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

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

Baum MK, Mantero-Atienza E, Shor-Posner G, et al. 1991. Association of vitamin B6 status with parameters of immune function in early HIV-1 infection. J Acq Immun Def Synd 4:1122-1132

Baysal A, Johnson BA, and Linkswiler H. 1966. Vitamin B-6 depletion in Man : Blood vitamin B-6, plasma pyridoxal phosphate, serum cholesterol, serum transaminases and urinary vitamin B_6 and 4-pyridoxic acid. J Nutr 89:19-23

Bernstein AL, and Lobitz CS. 1988. A clinical and elctro-physiologic study of the treatment of painful diabetic neurophatics with pyridoxine. In: Reynolds RD and Leklem JE, eds. Clinical and physiological applications of vitamin B-6. AR Liss, New York. Page 415-423

Bhagavan HN. 1985. Interaction between vitamin B-6 and drugs. In: Reynolds RD and Leklem JE, eds. Vitamin B-6: its role in health and disease. AR Liss, New York. Page 401-415

Bilheimer DW, Stone NJ, and Grundy SM. 1979. Metabolic studies in familial hypercholesterolemia :Evidence in gene-dosage effect in vivo. J Clin Invest 64:524-33

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

Birch TW. 1938. The relation between vitamin B-6 and the unsaturated fatty acid factor. J Biol Chem 124:775-793

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

Bligh EG, and Dyer WJ. A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37:911-917

Block G, and Abrams B. 1993. Vitamin and mineral status of women of childbearing potential. Ann NY Acad Sci 678:244-54

Bode W, Mocking JA, and van-den-Berg H. 1991. Influence of age and sex on vitamin B-6 vitamer distribution and on vitamin B-6 metabolizing enzymes in Wistar rats. J Nutr 121:318-29

Bode W, Mocking JA, and van-den-Berg H. 1991. Retention of [14 C] label is lower in old than in young Wistar rats after oral dosing with [14C] pyridoxine. J Nutr 122:1462-71

Bonanome A, and Grundy SM.1988. Effect of dietary stearic acid on plasma cholesterol and lipoprotein levels. New Engl J Med 318:1244-8

Borgstrom B. 1969. Quantification of cholesterol absorption in man by fecal analysis after the feeding of a single isotope-labeled meal. J Lipid Res 10:331-337

Bosner MS, Ostlund RE, Jr., Osofisan O, Grosklos J, and Fritschle C. 1993. Assessment of percent cholesterol absorption in humans with stable isotopes. J Lipid Res 34:1047-53

Brattstrom L, Stavenow L, Galvard H, Nilsson-Ehle P, Berntorp E, Jerntorp P, Elsmtahl S, and Pessah-Rasmussen H. 1990. Pyridoxine reduces cholesterol and low-density lipoprotein and increase antithrombin III activity in 80-year-old men with low plasma pyridoxal 5'-phosphate. Scand J Clin Lab Invest 50:873-877

Brenner RR. 1981. Nutritional and hormonal factors influencing desaturation of essential fatty acids. Prog Lipid Res 20:41-47

Brown M, Kovanen PT, and Goldstein JL. 1981. Regulation of plasma cholesterol by lipoprotein receptors. Science 212:628-634

Brown MS, and Goldstein JL. 1979. Familial hypercholesterolemia: Model for genetic receptor disease. Harvey Lect 73:163-201

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

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

Brown RR, Rose DP, Leklem JE, Linkswiler H, and Anand R. 1975. Urinary 4-pyridoxic acid, plasma pyridoxal phosphate, and erythrocyte aminotransferase levels in oral contraceptive users receiving controlled intake of vitamin B-6. Am J Clin Nutr 28:10-19

Brunzell JD, Albers JJ, Chait A, Grundy SM, Groszek E, and McDonald GB. 1983. Plasma lipoproteins in familial combined hyperlipidemia and monogenic familial hypertriglyceridemia. J Lipid Res 24:147-55

Brunzell JD, Hazzard W, Porte D, Jr., and Berman E. 1973. Evidence for a common, saturable TG removal mechanism for chylomicrons and VLDLs in man. J Clin Invest 52:1578-1585

Bunce GE and Vessal M. 1987. Effect of zinc and/or pyridoxine deficiency upon oestrogen retention and oestrogen receptor distribution in the rat uterus. J Steroid Biochem 26:303-8

Burnstein M, Scholnick HR, and Morfin R. 1970. Rapid method for isolation of lipoproteins from human serum by precipitation with polyanions. J Lipid Res 11:583

Burr GO, Burr MM, and Miller ES. 1932. On the fatty acids essential in nutrition. III J Biol Chem 97: 1-9

Burr GO, and Burr MM. 1929. A new deficiency disease produced by the rigid exclusion of fat from the diet. J Biol Chem 82: 345-367

Burr GO, and Burr MM. 1930. On the nature and role of the fatty acids essential in nutrition. J Biol Chem 86:587-620

Chabner B, and Livingston D. 1970. A simple enzymic assay for pyridoxal phosphate. Anal Biochem34:413-423

Chandra RK and Puri S. 1985. Vitamin B-6 modulation of immune responses and infection. In: Reynolds RD and Leklem JE, eds. Vitamin B-6: its role in health and disease. AR Liss, New York. Page 163-175

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

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

Coburn SP, and Whyte MP. 1988. Role of phosphatases in the regulation of vitamin B-6 metabolism in hypophosphatasia and other disorders. In: Reynolds RD and Leklem JE, eds. Clinical and physiological applications of vitamin B-6. AR Liss, New York. Page 65-93

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

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

Coniglio JG, Culp FB, and Goswami A. 1967. The metabolism of 1-C arachidonic acid in pyridoxine-deficient and pair-fed control rats. Lipids 2:484-488

Connor W, and Lin D. 1974. The intestinal absorption of dietary cholesterol by hypercholesterolemic (type II) and normocholesterolemic humans. J Clin Invest 53:1062-1070

Cook HW. 1985. Fatty acid desaturation and chain elongation in eukaryotes. In: Vance DE, and Vance JE, eds. Biochemistry of lipids and membranes. Benjamin Cummings.

Cori CF. 1931. Mammalian carbohydrate metabolism. Physiol Rev 11: 143-275

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

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

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

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

Dam H, Kristensen G, Nielsen GK and Sondergaard E. 1958. Effect of pyridoxine deficiency on cholesterol and polyenoic fatty acids in chicks. Acta Physiol Scand 44:67

Delorme CB, and Lupien PJ. 1976. The effect of a long-term excess of pyridoxine on the fatty acid composition of the major phospholipids in the rat. J Nutr 106:976-984

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

Dempsey WB and Christensen HN. 1962. The specific binding of pyridoxal 5'-phosphate to bovine plasma albumin. J Biol Chem 237:1113-1120

Dietschy JM and JD Wilson. 1970a. Regulation of cholesterol metabolism. Part I. New Eng J Med 282:1128-1138

Dietschy JM and JD Wilson. 1970b. Regulation of cholesterol metabolism. Part II. New Eng J Med 282:1179-1183

Dietschy JM and JD Wilson. 1970c. Regulation of cholesterol metabolism. Part III. New Eng J Med 282:1241-1249

Dietschy JM, and Brown M. 1974. Effect of alterations of the specific activity of the intracellular acetyl-CoA pool on apparent rates of cholesterogenesis. J Lipid Res 15:508-516

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

Dudman NP, Guo XW, Gordon RB, Dawson PA, and Wilcken DE. 1996. Human homocysteine catabolism: three major pathways and their relevance to development of arterial occlusive disease. J Nutr 126:1295S-1300S

Dussault PE, and Lepage M. 1975. Effects of pyridoxine deficiency on the composition of plasma and liver fatty acids in rats fed low and high fat diets. J Nutr 105:1371-1376

Edwards PA, Popjak G, Fogleman A, and Edmond J. 1977. Control of 3-hydroxy 3-methyl glutaryl-CoA reductase by endogenously synthesized sterols in vitro and in vivo. J Biol Chem 252:1057-1063

Eisenberg S, and Levy R. 1975. Lipoprotein metabolism. Adv Lipid Res 13:2-80
Felig P, Pozefsky T, Marliss E, Cahill GF, Jr. 1970. Alanine : key role in gluconeogenesis. Science 167:1003-1004

Fielding C, and Fielding P. 1981. Evidence for a lipoprotein carrier in human plsma catalyzing sterol efflux from cultured fibroblasts and its relationship to LCAT. Proc Natl Acad Sci USA 78:3911-3914

Fielding C, and Fielding P. 1982. Cholesterol transport between cells and body fluids " role of plasma lipoproteins and the plasma cholesterol esterification system. Medical Clinics of N. America. 66:363-373

Frolova IA, Oleneva LA, Sirota II, and Nikiforova GD. 1975. Effect of pyridoxine on patterns of lipid metabolism in patients with alimentary obesity. Vopr Med khim 21:299-306

Fujimoto Y, Nishioka K, Ohtani N, Aihara M, and Fujita T. 1987. Effect of pyridoxine on prostaglandin synthesis in rabbit kidney medulla slices. J Pharm Pharmacol 39:34-315

Gangl A, and Ockner R. 1975. Progress in Gastroenterology: Intestinal metabolism of lipids and lipoproteins. Gastroenterology. 68:167-186

Gibbons GF, Mitropolous KA, and Myant N. 1982. Biochemistry of cholesterol. Elsevier Biomedical Press B.V. The Netherlands. 9:155-167

Goldstein JL and Brown MS. 1977. The low density lipoprotein pathway and its regulation of atherosclerosis. Ann Rev Biochem 46:897-930

Gomikawa S, and Okada M. 1980. Liver 3-hydroxy-3-methylglutaryl coenzyme a reductase activity in pyridoxine-deficient rats. J Nutr Sci Vitaminol 26:1-14

Green P, and Glickman R. 1981. Intestinal lipoproteins metabolism. J Lipid Res 22:1153-1173

Greenberg LD and Rinehart JF. 1951. Plasma cholesterol levels of pyridoxine-fed controls and pyridoxine-deficient monkeys. Proc Soc Exp Biol Med 76:580

Greenberg SM, Calbert CE, Deuel HJ, and Brown JB. 1951. The effect of fat level of the diet on general nutrition. VII. Comparison of the potency of arachidonic and linoleic acids in furnishing the requirement for essential fatty acids in the rat. J Nutr 45:521-533

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

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

Groot PHE, Scholte HR, and Hulsmann WC. 1976. Fatty acid activation specificity localization, and function. In: Advances in lipid research. Paolett, R and Krichevsky D, eds. vol. 14. 75-126. New York, Academic press.

Grundy SM, Hoffman A, Davignon J, and Aherns EH, Jr. 1966. Human cholesterol synthesis is regulated by bile acids. J Clin Invest 45:1018-1019

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

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

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

Halliday D, Verkatesan S, and Pacy P. 1993. Apolipoprotein metabolism : a stable-isotope approach. Am J Clin Nutr 57: 726s-731s

Hansen CM, Leklem JE, and Miller LT. 1996a. Vitamin B-6 status of women with a constant intake of vitamin B-6 changes with three levels of dietary protein. J Nutr 126:1891-1901

Hansen CM, Leklem JE, and Miller LT. 1996b. Vitamin B-6 status indicators decrease in women consuming a diet high in pyridoxine glucoside. J Nutr 126:2512-18

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

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

Havel RJ. 1957. Early effects of fat ingestion on lipids and lipoproteins of serum in man. J Clin Invest 36:848

Hecker LM, and Kotler DP. 1990. Malnutrition in patients with AIDS. Nutr Rev 48:393-401

Heiskanen K, Siimes MA, Perheentupa J, and Salmenpera L. 1994. Reference ranges for erythrocyte aspartate transaminase stimulation test in lactating mothers and their infants. Am J Clin Nutr 59:1297-303

Helmreich EJ. 1992. How pyridoxal 5'-phosphate could function in glycogen phosphorylase catalysis. Biofactors 3:159-72

Henderson LM. 1985. Intestinal absorption of B-6 vitamers. In Reynolds RD, and Leklem JE, eds. Vitamin B-6; its role in health and disease. AR Liss, New York. Page 22-33

Herbert V. 1992. Everyone should be tested for iron disorders. J Am Diet Assoc 92:1502-1509

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

Hoffman DR, Haning JA, and Cornatzer WE. 1981. Microsomal phosphatidylethanolamine methyltransferase: inhibition by S-adenosylhomocysteine. Lipid 16:561-567

Hogan AG, and Richardson LR. 1935. The identity of the antidermatitis factor. J Biol Chem 109:43-44

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

Houben GF, Abma PM, van-den-Berg H, van-Dokkum W, and van-Loveren H. 1992a. Effects of the colour additive caramel colour III on the immune system : a study with human volunteers. Food Chem Toxicol 30:749-57

Houben GF, Penninks AH, Seinen W, Vos JG, and van-Loveren H. 1993. Immunotoxic effects of the color additive caramel color III: immune function studies in rat. Fund Appl Toxicol 20:30-7

Houben GF, van-den-Berg H, van-Dokkum W, van-Loveren H, Seinen W, and Penninks AH. 1992b. Effects of the color additives color III and 2-acetyl-4-tetrahydroxybutylimidazole (THI) on the immune system of rats. Toxicol Appl Pharmacol 113:43-54

Huber AM, Gershoff SN and Hegsted DM. 1964. Carbohydrate and metabolism and response to insulin in vitamin B-6 deficient rats. J Nutr 82:371-78

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

Ink SL, Mehansho H, and Henderson LM. 1982. The binding of pyridoxal to hemoglobin. J Biol Chem257:4753-4757

Iwami T, and Okada M. 1982. Stimulation of cholesterol metabolism in pyridoxine-deficient rats. J Nutr Sci Vitaminol 28:77-84

Jackson RL, Glueck CJ, Athur SN, and Spector A. 1980. Effects of diet and HDL subfractions on the removal of cellular cholesterol. Lipids 15: 230-235

Jackson RL, Morrisett J, and Gotto A. 1977. Lipoproteins and lipid transport: The lipid research clinics program prevalence study. Circulation 61:302-315

Jeffcoat R. 1980. The biosynthesis of unsaturated fatty acids and its control in mammalian liver. Essays Biochem 15:1-36

Johnson AA, Knight EM, Edwards CH, Oyemade UJ, Cole OJ, et al. 1994. Dietary intakes, anthropometric measurements and pregnancy outcomes. J Nutr 124:936s-942s.

Johnson MA. 1990. Iron: nutrition monitoring and nutrition status assessment. J Nutr 120:1486-1491

Joshi VC and Aranda LP. 1979. Hormonal regulation of the terminal enzyme of microsomal stearoyl coenzyme A desaturase in cultured avian liver explants. J Biol Chem 254:11779

Kabir H, Leklem JE, and Miller LT. 1983. Relationship of glycosylated vitamin B-6 content of foods to vitamin B-6 bioavailability in humans. Nutr Rep Int 28: 709-715

Kark JA, Bongiovanni R, Hicks CU, Tarassoff PG, Hannah JS, and Yoshida GY. 1982. Modification of intracellular hemoglobin with pyridoxal and pyridoxal 5'-phosphate. Blood Cells 8:299

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

Khatami M, Suldan Z, David I, Li W, and Rockey JH. 1988. Inhibitory effects of pyridoxal phosphate, ascorbate and aminoguanidine on nonenzymatic glycosylation. Life Sci 43:1725-1731

Kirschman JC, and Coniglio JG. 1961. The role of pyridoxine in the metabolism of polyunsaturated fatty acids in rats. J Biol Chem 236:2200-2203

Krebs EG, and Fisher EH. 1964. Phosphorylase and related enzymes of glycogen metabolism. Vitam Horm 22:399-410

Kudchodkar BJ, Sodhy HS, and Horlick L. 1973. Absorption of dietary cholesterol in man. Metabolism 22:155-163

Lane MD, Moss J, and Polakis SE. 1974. Acetyl coenzyme A carboxylase. Curr Top Cell Regul 8:139-95

Lane MD, Watkins PA, and Meredith MJ. 1979. Hormonal regulation of acetyl CoA carboxylase activity in the cell. CRC Crit Rev Biochem 7:121-41

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

Leklem JE, Brown RR, Rose DP, Linkswiler H, and Arend RA. 1975. Metabolism of tryptophan and niacin in oral contraceptive users receiving controlled intakes of vitamin B-6. Am J Clin Nutr 28:146-156

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

Leklem JE. 1985. Physical activity and vitamin B-6 metabolism in men and women: interrelationship with fuel needs. In : Reynolds RD, and Leklem JE, eds. Vitamin B-6: its role in health and disease. AR Liss, New York, NY. Page 221-241

Leklem JE. 1988. Vitamin B-6 metabolism and function in humans. In: Reynolds RD, and Leklem JE, eds. Clinical and physiological applications of vitamin B-6. Page 3-28 AR Liss, New York, NY.

Leklem JE. 1989. Changes in plasma pyridoxal 5'-phosphate (PLP) after a carbohydrate (CHO) load in CHO cravers and non-cravers. FASEB A455

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

Leklem JE. Vitamin B-6 . 1991. In: Machlin LJ,ed. Handbook of vitamins. 2nd ed. Page 343. Dekker, Inc. New York.

Leklem JE. Vitamin B-6. 1996. In: Ekhard E. Ziegler, and L. J. Filer, Jr., eds. ILSI Press, Washington D.C. Present knowledge in nutrition. 7th ed. Page 174-183

Levy RI. 1981. Cholesterol, lipoproteins, apoproteins, and heart disease: present status and future prospects. Clinical Chemistry. 27:653-662

Life Sciences Research Office, Federation of American Society for Experimental Biology. 1993. Third report on nutrition monitoring in the United States. Vol. 2. Interagency board for nutrition monitoring and related research. VA-106.

Lin DS, and Connor WE. 1980. The long term effects of dietary cholesterol upon the plasma lipids, lipoproteins, cholesterol absorption, and the sterol balance in man: the demonstration of feedback inhibition of cholesterol biosynthesis and increased bile acid excretion. J Lipid Res 21:1042-1052

Linkswiler HM. Vitamin B-6 requirement of men. In: Human vitamin B-6 requirements. National Academy of Sciences, Washington, D.C. Page 279-290

Litwack G, Miller-Diener A, Disorbo DM, and Schmidt TJ. 1985. Vitamin B-6 and the glucocorticoid receptor. In :Reynolds RD, and Leklem JE, eds. Vitamin B-6 in health and disease. Liss AR, New York. 177-191

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

Lumeng L, Brushear RE, and Li T-K. 1974. Pyridoxal 5'-phosphate in plasma ; source, protein-binding and cellular transport. J Lab Clin Med 84:334-44

Lumeng L, Li T-K, and Lui A. 1985. The interorgan transport and metabolism of vitamin B-6. In : Reynolds RD, and Leklem JE, eds. Vitamin B-6: its role in health and disease. AR Liss, New York. vol. 13. page 35-54

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

Lumeng L, Ryan MP, and Li T-K. 1978. Validation of the diagnostic value of plasma pyridoxal 5'- phosphate measurements in vitamin B-6 nutrition of the rat. J Nutr 108:545-553

Lupien PJ, Hinse CM, and Avery M. 1969. Cholesterol metabolism and vitamin B-6. I. Hepatic cholesterogenesis and pyridoxine deficiency. Can J Biochem 47:631-635

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

Majumder PK, Joshi JB, and Banerjee MR. 1983. Correlation between nuclear glucocorticoid receptor levels and casein gene expression in murine mammary gland in vitro. J Biol Chem 258-6793-8

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

Mattson FH, and Grundy SM. 1985. Comparison of effects of dietary saturated, monounsaturated, and polyunsaturated fatty acids on plasma lipids and lipoproteins in man. J Lipid Res 26:194-202

Medes G, and Keller DC. 1947. Essential fatty acid metabolism. I. Essential fatty acid content of rats on fat-free and pyridoxine-free diets. Arch Biochem Biophys 15:19-27

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

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

Merrill AH and Hendrson JM. 1990.Vitamin B-6 metabolism by human liver. In: Dakshinamurtri K, ed. Vitamin B-6. Ann N. Y. Acad Sci. 585:110-117

Merrill AH Jr, Horrike D, and McCormick DB. 1979. Evidence for the regulation of pyridoxal 5'-phosphate formation in the liver by pyridoxamine(pyridoxine) 5'-phosphate oxidase. Biophys Res Commun 83:984.

Merrill AH., Jr., Henderson JM, Wang E, McDonald BW, and Millikan WJ. 1984. Metabolism of vitamin B-6 by human liver. J Nutr 114:1664-1674

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

Middleton HM. 1985. Uptake of pyridoxine by in vivo perfused segments of rat small intestine: a possible role for intracellular vitamin metabolism. J Nutr 115:1079-1088

Miller JW, Ribaya-Mercado JD, Russel RM, Shepard DC, et al. 1992. Effect of vitamin B-6 deficiency on fasting plasma homocysteine concentrations. Am J Clin Nutr 55:1154-60

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

Miller LT, Leklem JE, and Shultz TD. 1985. The effect of dietary protein on the metabolism of vitamin B-6 in humans. J Nutr 115:1663-72

Mistry P, Miller NE, Lakes M, Hazzard W, and Lewis B. 1981. Individual variation in the effects of dietary cholesterol on plasma lipoproteins and cellular cholesterol homeostasis in man. J Clin Invest 67:493-503

Mitropolous KA, Balasubrmaniam S, Venkatsens S, and Reeves B. 1978. On the mechanism for the regulation of 3-hydroxy 3-methyl glutaryl-CoA reductase, of cholesterol 7-α-hydrolase and of acyl-CoA: cholesterol acyltransferase by free cholesterol. Biochim Biophys Acta 530:99-111

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

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

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

Nervi FO, and Dietschy JM. 1975. Ability of six different lipoprotein fractions to regulate the rate of hepatic cholesterogenesis in vivo. J Biol Chem 250:8704-8711

Nestel PJ, and Poyser A. 1976. Changes in cholesterol synthesis and excretion when cholesterol intake is increased. Metabolism 25:1591-1599

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

O'Dea K, Traianedes K, Chisholm K, Leyden H, and Sinclair AJ. 1990. Cholesterol-lowering effect of a low-fat diet containing lean beef is reversed by the addition of beef fat. Am J Clin Nutr 52:491-4

Oka T, Komori N, Kuwahata M, Suzuki I, Okada M, and Naton Y. 1994. Effect of vitamin B-6 deficiency on the expression of glycogen phosphorylase mRNA in rat liver and skeletal muscle. Experientia 50:127-9

Okada M, Ishikawa K, and Watanabe K. 1991. Effect of vitamin B-6 deficiency on glycogen metabolism in the skeletal muscle, heart, and liver of rats. J Nutr Sci Vitaminol Tokyo 37:349-57

Okayasu T, Ono T, Shinojima K, and Imai Y.1976. Involvement of cytochrome b_5 in the oxidative desaturation of linoleic acid in rat liver microsomes. Lipids 12:267-271

Pande SV. 1975A mitochondrial carnitine acylcarnitine translocase system. Proc Natl Acad Sci USA. 72:883-887

Pannemans DL, van-den-Berg H, and Westerterp KR. 1994. The influence of protein intake on vitamin B-6 metabolism differs in young and elderly humans. J Nutr 124: 1207-14

Petersen VP. 1950. Scand J Clin Lab Invest 2:44

Pino S, Benotti J, and Gardyna H. 1965. An automated method for urine creatinine which does not require a dialyzer module. Clin Chem 11:664-6

Pogell BM. 1958. enzymatic oxidation of pyridoxamine phosphate to pyridoxal phosphate in rabbit liver. J Biol Chem 232:761-766

Quackenbush FW, Kummerow FA and Steenbock H. 1942. The effectiveness of linoleic, arachidonic, and linolenic acids in reproduction and lactation. J Nutr 24:213-225

Quackenbush FW, Platz BR and Steenbock H. 1939. Rat acrodynia and the essential fatty acids. J Nutr 115-126

Quinto E, Grundy and Aherns EH Jr. 1971. Effects of dietary cholesterol on the regulation of total body cholesterol in man. J Lipid Res 12:233-247

Ravichandran V, and Selvam R. 1991. Increased plasma lipid peroxidation in vitamin B-6 deficient rats. Indian J Exp Biol 29:56-8

Recommended Dietary Allowances. 1989. 10th ed. Natl Acad Sci Natl Res Council, Washington D.C.

Reeve VE, Bosnic M, Boehm-Wilcox C, and Cope RB. 1995. Pyridoxine supplementation protects mice from suppression of contact hypersensitivity by 2-acetyl-4-tetrahydroxybutylimidazole (THI) , ultraviolet B radiation (280-320 nm) or cis-urocanic acid. Am J Clin Nutr 61:371-6

Reiser R, probstfield JL, Silvers A, et al. 1985. Plasma lipid and lipoprotein responses of humans to beef fat, coconut oil and safflower oil. Am J Clin Nutr 42:190-7

Reynolds RD, and Natta CL. 1985. Vitamin B-6 and sickle cell anemia. In:Reynolds RD, and Leklem JE, eds. Vitamin B-6: Its role in health and disease. Liss AR. New York page 301-306

Reynolds RD. Vitamin B-6. 1987. In: Pesce AJ, and Kaplan LA, eds. Methods in clinical chemistry. St. Louis:CV Mosby. p 558

Reynolds RD. 1995. IX. Erythrocyte PLP concentrations ; An alternative status index for humans. In: Vitamin B-6 metabolism in pregnancy, lactation, and infancy. Biochemical method for status assessment. Raiten DJ, eds. CRC Press, Dallas, Tx, Inc. page 48

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

Rinehart JF, and Greenberg LD. 1949. Arteriosclerotic lesions in pyridoxine-deficient monkeys. Am J Path 25:481-486

Robson LC, and Schwarz MR. 1975. Vitamin B-6 deficiency and the lymphoid system. I. Effect of cellular immunity and in vitro incorporation of ^{3}H-uridine by small lymphocytes. Cell Immunol 16:135-144

Rose DP, and Braidman IP. 1971. Excretion of tryptophan metabolites as affected by pregnancy, contraceptive steroids, and steroid hormones. Am J Clin Nutr 24:673-683

Safonova IG, Sviridov DD, Rampal' P, Nano ZhL, Pavlov Miu, and Repin VS. 1993. Regulation of cholesterol absorption in humans and rat small intestine epithelial cells. Biokhimiia 58:274-84

Sauberlich HE, Dowdy RP, and Skala JH. 1974. Laboratory tests for the assessment of nutritional status. CRC Press. Cleveland, OH. page 37-49

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

Scanu AM. 1978. Plasma lipoproteins and coronary heart disease. Ann Clin Lab Sci 8:79-83

Schaeffer MC, Gretz D, Mahuren JD, and Coburn SP. 1995. Tissue B-6 vitamer concentrations in rats fed excess vitamin B-6. J Nutr 125-2370-8

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

Schaumburg H, Kaplan J, Windebank A, et al. 1983. Sensory neurophathy from pyridoxine abuse: a new megavitamin syndrome. New Engl J Med 309:445-448

Schirch L and Jenkins WT. 1964. Serine transhydroxymethylase. J Biol Chem 239:2797-3800

Schneider H, Steenbock H, and Platz BR. 1940. Essential fatty acids, vitamin B-6, and other factors in the cure of rat acrodynia. J Biol Chem 132:539-551

Schulz H. 1985. Oxidation of fatty acid. In: Bioichemistry of lipids and membranes. Vance DE and Vance JE, eds. 1985. Benjamin/Cummings, Inc. page 116-142

Schwartz CC, Berman M, Halloran LG, Swell L, and Vlahcevic ZR. 1982. Cholesterol disposal in man: special role of HDL free cholesterol. In: Berman M, Grundy SM, and Howard BV, eds. Lipoprotein kinetics and modelling. New York, Academic Press. page 337-49

Shah SN, Johnston PB, and Kummerow FA. 1960. The effect of pyridoxine on cholesterol metabolism. J Nutr 72:81-86

She QB, Hayakawa T, and Tsuge H. 1994. Effect of vitamin B-6 deficiency on linoleic acid desaturation in the arachidonic acid biosynthesis of rat liver microsomes. Biosci Biotech Biochem 58:459-463

She QB, Hayakawa T, and Tsuge H. 1995. Alteration in the phosphatidylcholine biosynthesis of rat liver microsomes caused by vitamin B-6 deficiency. Biosci Biotech Biochem 59:163-167

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

Silva CM, Tully DB, Petch LA, Jewell CM, and Cidlowski JA. 1987. Proc Natl Acad Sci U.S.A. 84:1744-8

Sirtori CR, Manzoni C, and Lovati MR. 1991. Mechanisms of lipid-lowering agents. Cardiology. 78:226-35

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

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

Snyderman SE, Carretero R, and Holt LE, Jr. 1950. Pyridoxine deficiency in human being. Fed Proc 9:371-372

Spady DK, and Dietschy JM. 1988. Interaction of dietary cholesterol and triglycerides in the regulation of hepatic low density lipoprotein transport in the hamster. J Clin Invest 81:300-9

Suzuki K, and Okada M. 1982. Alterations of phospholipid and triglyceride metabolism in fatty liver caused by pyridoxine deficiency in rats. J Nutr Sci vitaminol 28:377-390

Swell L, Gustafsson J, Schwartz CC, Halloran LG, Danielson H, and Vlahcevic ZR. 1980. An in vivo evaluation of the quantitative significance of several potential pathways to cholic and chenodeoxycholic acids from cholesterol in man. J Lipid Res 21:455-66

Swell L, Law MD, Schools, Jr. PE, and Treadwell CR. 1961. Tissue lipid fatty acid composition in pyridoxine-deficient rats. J Nutr 74: 148-156

Symes EK, Bender DA, Bowden JF, and Coulson WF. 1984. Increased target tissue uptake of, and sensitivity to, testosterone in the vitamin B-6 deficient rat. J Steroid Biochem 20:1089-93

Takayama M, Itoh S, Nagasaki T, and Tanimizu I. 1977. A new enzymatic method for determination of serum choline containing phospholipids. Clin Chim Acta 79:93-98

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

Trakatellis A, Dimitriadou A, Exindari M, and Scountzou J. 1992. Effect of pyridoxine deficiency on immunological phenomena. Postgrad Med J 68: S70-77

Triscari J, Hamilton JG, and Sullivan AC. 1978. Comparative effects of saturated and unsaturated lipids on hepatic lipogenesis and cholesterogenesis in vivo in meal-fed rats. J Nutr 108:81-25

Tsukamoto Y, Wong H, Mattick JS, and Wakil SJ. 1983. The architecture of the animal fatty acid synthetase complex. IV. Mapping the active centers and model for the mechanism of action. J Biol Chem 258:15312-22

Turnlund JR, Botschart AA, Liebman M, Kretsch MJ, and Sauberlich HE. 1992. Vitamin B-6 depletion followed by repletion with animal- or plant-source diets and calcium and magnesium metabolism in young women. Am J Clin Nutr 56:905-10

Turpeinen O. 1938. Further studies on the unsaturated fatty acids essential in nutrition. J Nutr 15: 351-366

Ubbink JB, van-der-Merwe A, Delport R, Allen RH, and Stabler SP. 1996. The effect of a subnormal vitamin B-6 status on homocysteine metabolism. J Clin Invest 98:177-84

USDA (U.S. Department of Agriculture). 1986. Nationwide Food Consumption Survey. Continuing Survey of Food Intakes by Individuals: Men 19-50 Years, 1 Day, 1985. Report No. 85-3. Nutrition Monitoring Division, Human Nutrition Information Service. U.S. Department of Agriculture, Hyattsville, Md. 94 pp.

USDA (U.S. Department of Agriculture). 1987. Nationwide Food Consumption Survey. Continuing Survey of Food Intakes by Individuals: Women 19-50 Years and Their

Children 1-5 Years, 4 Day, 1985. Report No. 85-4. Nutrition Monitoring Division, Human Nutrition Information Service. U.S. Department of Agriculture, Hyattsville, Md. 182 pp.

van den Berg H, Mulder J, Spanhaak S, van Dokkum W and Ockhuizen T. 1988. The influence of marginal vitamin B-6 status on imunological indices. In: Reynolds RD and Leklem JE, eds. Clinical and physiological applications of vitamin B-6. AR Liss, New York. page 147-155

Venkatesan S, Pacy P, Mitropoulos KA, Watts GF, and Halliday D. 1992. Fractional and absolute synthetic rate of very low-density lipoprotein apo B-100 in lipoprotein lipase deficient patients. Biochem Soc Trans 20:103S

Venkatesan S, Pacy P, Wenham D, and Halliday D. 1990. Very-low-density lipoprotein apolipprotein turnover studies in normal subjects: a stable isotope study. Biochem Soc Trans 18:1192-3

Verhoef P, Stampfer MJ, Buring JE, Gaziano JM, Allen RH, Stabler SP, Reynolds RD, Kok FJ, Hennekens CH, and Willett WC.1996. Homocysteine metabolism and risk of myocardial infarction: relation with vitamin B-6, B12, and folate. Am J Epidemiol 143:845-59

Volpe JJ and Vagels PR. 1973. Saturated fatty acid biosynthesis and its regulation. Ann Rev Biochem 42:21-60

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

Wakil SJ, McLain LW. Jr., and Warshaw JB. 1960. Synthesis of fatty acids by mitochondria.. J Biol Chem 235:PC31-32

Wakil SJ, Stoops JK, and Joshi VC. 1983. Fatty acid synthesis and its regulation. Ann Rev Biochem 52:537-579

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

Wander RC, Du SH, Ketchum SO, and Rowe KE. 1996. α-Tocopherol influences in vivo indices of lipid peroxidation in postmenopausal women given fish oil. J Nutr 126:643-652

Williams KJ, Petrie KA, Brocia RW, and Swenson TL. 1991. Lipoprotein lipase modulates net secretory output of apolipoprotein B in vitro. A possible pathophysiological explanation for familial combined hyperlipidemia. J Clin Invest 88:1300-6

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

Wolgemuth JC, Myers-Williams C, Johnson P, and Henseler C.1992. Wasting malnutrition and inadequate nutrient intakes identified in a multiethnic homeless population. J Am Diet Assoc 92:834-9

Wozenski JR, Leklem JE, and Miller LT. 1980. The metabolism of small doses of vitamin B-6 in men. J Nutr 110:275-285

Wu J, and Bailey J. 1980. Lipid metabolism in cultured cells: studies on lipoprotein catalyzed reverse cholesterol transport in normal and homozygous familial hypercholesterolemic skin fibroblasts. Arch Biochem Biophys 202: 467-473

Zehaluk CM, and Walker BL. 1973. Effect of pyridoxine on red cell fatty acid composition in mature rats fed an essential fatty acid-deficient diet. J Nutr 103:1548-53

APPENDICES

Appendix A: Tables

Table1. Subjects' weight (lbs) during the diet study

Date	Subject							
	1	2	3	4	5	6	7	8
5/7/96	181.0	175.0	118.0	144.5	175.5	176.0	130.2	130.0
5/8/96	181.5	172.0	118.0	145.0	175.2	177.0	129.0	130.0
5/9/96	181.7	173.5	117.0	145.0	175.0	176.5	130.0	130.0
5/10/96	182.0	174.2	117.7	145.0	173.5	176.0	129.7	129.0
5/11/96	181.5	174.0	116.7	145.2	173.0	176.0	131.0	129.0
5/12/96	181.5	173.0	117.7	144.7	173.5	177.5	129.2	128.0
5/13/96	182.0	173.5	117.2	144.7	173.0	177.0	129.5	129.0
5/14/96	182.0	173.2	117.7	145.5	172.5	176.2	130.0	129.0
5/15/96	182.5	173.5	117.2	145.0	172.5	177.5	128.5	129.5
5/16/96	182.0	174.0	117.5	144.0	172.0	176.2	129.5	129.0
5/17/96	182.0	173.5	117.2	145.2	172.2	175.0	130.0	129.0
5/18/96	183.0	172.2	117.7	144.5	172.0	173.5	129.5	129.0
5/19/96	184.0	174.0	117.2	144.0	171.7	175.5	128.7	129.0
5/20/96	184.0	174.5	117.7	145.0	172.0	176.5	130.5	128.5
5/21/96	184.0	172.5	117.0	143.7	173.2	175.0	129.0	128.0
5/22/96	184.0	172.5	117.5	143.7	173.0	175.5	129.7	128.2
5/23/96	184.0	172.5	117.2	144.0	172.0	174.7	130.7	128.2
5/24/96	183.0	170.0	117.2	144.5	171.7	174.0	128.7	128.2
5/25/96	181.0	171.2	117.5	144.5	172.0	172.7	128.5	128.0
5/26/96	181.0	171.0	116.5	143.7	172.0	173.5	129.0	128.0
5/27/96	181.0	171.5	116.7	144.5	171.7	173.5	130.0	128.2
5/28/96	181.0	171.2	117.2	144.0	172.0	173.5	128.5	128.5
5/29/96	181.5	171.0	117.7	144.0	172.0	172.7	128.0	128.5
5/30/96	180.0	170.5	117.7	143.0	171.5	173.5	128.5	128.5
5/31/96	180.0	171.2	117.7	143.2	171.5	173.0	127.7	128.5
6/1/96	179.5	171.2	117.2	142.2	169.5	173.5	128.5	129.0
6/2/96	180.0	171.5	116.7	143.2	168.7	173.5	126.7	128.5
6/3/96	179.5	171.0	117.7	144.0	170.0	174.0	127.0	129.0

Table2. Subjects' 24-hour urinary creatinine (g/day)

	Subject							
Date	1	2	3	4	5	6	7	8
5/7/96	1.31	1.30	0.64	0.95	0.90	0.74	0.81	0.83
5/8	1.18	1.08	0.81	1.06	0.97	1.06	0.94	0.81
5/9	1.33	1.28	0.94	1.14	1.05	1.01	0.95	0.92
5/10	1.50	1.02	0.88	0.97	0.88	1.06	0.90	1.02
5/11	1.53	1.47	1.02	1.08	0.97	1.05	0.96	0.84
5/12	1.07	1.12	0.99	1.00	0.96	1.12	0.81	0.95
5/13	1.29	1.30	0.93	0.96	1.04	0.99	1.06	1.02
5/14	1.21	1.25	0.99	0.95	0.99	0.92	1.07	1.08
5/15	1.33	1.15	0.95	1.10	0.98	1.00	1.00	1.03
5/16	1.25	1.25	1.07	0.91	1.04	1.06	1.03	1.09
5/17	1.31	1.10	0.94	1.20	1.18	1.02	1.06	1.04
5/18	1.28	1.37	0.73	0.87	0.96	1.12	1.15	1.04
5/19	1.22	1.16	0.77	0.84	1.05	0.86	0.95	1.01
5/20	1.29	1.26	1.06	1.10	1.08	1.17	1.20	1.08
5/21	1.39	1.38	1.01	1.25	1.04	1.10	1.09	1.08
5/22	1.50	1.42	1.20	0.91	1.08	1.16	1.03	1.16
5/23	1.18	1.35	0.89	1.15	1.14	0.99	1.02	1.05
5/24	1.47	1.30	1.07	1.10	0.97	1.02	1.08	1.17
5/25	1.37	1.25	1.13	1.18	0.94	1.12	1.06	1.15
5/26	1.38	1.44	1.01	1.29	1.00	1.06	0.91	1.09
5/27	1.16	1.43	0.95	0.87	1.12	0.98	1.09	1.12
5/28	1.22	1.35	1.02	1.07	1.00	1.17	1.15	1.10
5/29	1.36	1.33	0.95	1.21	0.99	0.90	1.07	1.06
5/30	1.35	1.41	1.00	1.02	1.08	1.26	1.01	1.02
5/31	1.26	1.27	1.10	1.00	1.22	1.01	1.03	0.92
6/1	1.58	1.18	0.90	0.85	1.06	1.06	1.14	1.15
6/2	1.30	1.25	0.94	0.97	1.21	1.16	1.19	1.08
6/3	1.33	1.22	1.15	1.29	1.09	1.01	1.16	1.13

Table 3. Intake of hard candy of subjects (as number of regular size candies)

	Subject							
Date	1	2	3	4	5	6	7	8
5/7/96	5	-	-	4	-	3	19	1
5/8/96	8	-	-	6	1	1	25	4
5/9/96	6	12	-	7	-	3	26	1
5/10/96	8	-	-	3	-	11	25	-
5/11/96	5	6	-	3	-	8	17	10
5/12/96	6	1	-	6	1	1	20	10
5/13/96	13	15	-	6	-	4	14	3
5/14/96	11	1	-	6	-	5	27	1
5/15/96	8	1	-	8	1	1	25	3
5/16/96	3	5	-	2	-	6	28	-
5/17/96	16	5	-	1	1	7	11	-
5/18/96	6	5	-	1	1	1	10	-
5/19/96	4	5	-	3	1	3	9	-
5/20/96	5	-	1	1	-	5	10	-
5/21/96	8	-	-	13	1	5	15	-
5/22/96	6	-	-	10	-	3	12	1
5/23/96	24	-	-	9	-	1	14	-
5/24/96	15	35	-	9	-	1	17	-
5/25/96	20	15	1	3	-	1	18	1
5/26/96	24	-	5	3	-	1	25	2
5/27/96	17	15	-	2	-	1	6	-
5/28/96	15	-	-	7	-	3	15	-
5/29/96	27	-	-	12	-	4	15	-
5/30/96	17	5	-	5	-	2	12	-
5/31/96	28	7	1	5	1	5	15	-
6/1/96	20	-	-	7	-	1	6	-
6/2/96	5	-	1	2	-	1	8	-
6/3/96	10	-	-	1	-	5	5	-

In calculating carbohydrate intake, 1 regular size candy was considered to equal 1.

Table 4. Intake of fruit jelly spread[1]

	Subject							
Date	1[2]	2	3	4	5	6	7	8
5/7/96	3	2	2	2	-	-	3	3
5/8/96	4	2	2	1	-	2	3	3
5/9/96	4	2	2	-	-	2	3	3
5/10/96	4	2	2	-	-	2	3	3
5/11/96	5	-	2	-	-	2	3	3
5/12/96	3	2	2	-	-	2	3	3
5/13/96	4	1	2	-	-	2	3	3
5/14/96	2	1	3	-	-	2	3	3
5/15/96	2	1	3	-	-	2	3	5
5/16/96	3	1	3	-	-	2	3	4
5/17/96	3	1	2	-	-	1	3	4
5/18/96	2	1	2	-	-	1	3	6
5/19/96	2	1	2	-	-	1	3	6
5/20/96	2	1	1	-	-	-	3	5
5/21/96	3	1	1	-	-	-	3	4
5/22/96	3	1	-	-	-	-	3	3
5/23/96	-	1	-	-	-	1	3	3
5/24/96	3	-	-	-	-	1	3	5
5/25/96	3	1	-	-	-	1	3	5
5/26/96	4	1	1	-	-	1	3	4
5/27/96	5	-	1	-	-	-	3	4
5/28/96	-	1	-	-	-	-	3	3
5/29/96	3	1	-	-	-	-	3	2
5/30/96	4	1	-	-	-	-	3	3
5/31/96	4	1	-	-	2	-	3	3
6/1/96	5	1	-	-	2	-	3	3
6/2/96	5	1	1	-	2	1	3	1
6/3/96	5	1	-	-	2	-	3	1

[1]Smucker's jelly spread, weight =1/2 oz.

Intake was calculated as the number of one serving size packages

Table 5. Intake of carbonated drinks*

Date	Subject							
	1	2	3	4	5	6	7	8
5/7/96	2/3D	3D	1I	2D	1/2R	1I	1I	1R
5/8/96	-	2D	1I	1I, 1D	2R	2R	1I	2R
5/9/96	-	4D	1I	1D	2R	1R	1I	3R
5/10/96	-	-	1I, 1D	1I	2R	1I	1I	3R
5/11/96	-	3D	1I	1D	2R	4I	1I	3R
5/12/96	1D	3D	1D	2D	2R	3R	1I	1I, 1R
5/13/96	2D	3D	1S	-	2R	2R	1I	2R
5/14/96	2R	3D	1S, 1R	1D	1R	2S	1I	2R
5/15/96	-	4D	1S	2D	1R	1S, 1R	-	1R, 1I
5/16/96	-	3D	1S	1D	1R	1S	1I	3R
5/17/96	-	4D	1S	2D	1R	1R	1I	1S, 1R
5/18/96	-	4D	1S	1D	1R	1R	1I	1I, 1R
5/19/96	-	3D	1S	1D	-	2R	1I	1R, 1S
5/20/96	-	3D	1S	2D	1R	1I	1I	1S
5/21/96	-	3D	1S	1D	1R	-	1I	1S, 1R
5/22/96	-	5D	1S	1D	1R	1R	1I	1R, 1I
5/23/96	2D	3D	-	1/2D	1R	1I, 1R	1I	1R, 1I
5/24/96	2R	4D	1S	-	1R	1R	1I	1R, 1I
5/25/96	1R	4D	1S	1D	1R	2R	1I	1R, 1I
5/26/96	1R	3D	1S, 1R	1D	1R	2R	2I	1R, 1I
5/27/96	1R	3D	1S	-	1R	2R	1I	1R, 1I
5/28/96	1D	3D	1S	1/2S	1R	1S, 1R	1I	1R, 1I
5/29/96	-	4D	1S	1/2D	1R	2R	1I	1R, 1I
5/30/96	1R	4D	-	-	1R	1R	1I	-
5/31/96	1R	4D	1S	-	1R	1R	1I	1R, 1I
6/1/96	1R	3D	-	1D	1R	1R, 1S	1/2I	1R, 1I
6/2/96	3R	4D	-	1D, 1S	1R	-	1I	1R, 1I
6/3/96	3R	3D	-	-	1R	-	1I	1R, 1I

I: Italian soda
D: Diet cola
R: Regular cola
S: Sprite
*Size: 12 oz aluminum can

Table 6. Hematocrit (%)

Subject	Day 1	Day 7	Day 14	Day 21	Day 28
1	38.0	35.5	38.0	39.5	38.5
2	39.0	38.5	38.5	38.5	38.5
3	44.5	44.0	41.5	46.0	46.5
4	41.0	40.5	39.0	38.5	39.0
5	36.5	35.0	37.5	37.5	38.5
6	41.5	40.5	39.5	41.5	40.5
7	41.5	39.5	40.0	41.0	42.0
8	39.5	39.5	42.0	41.0	40.0
Mean	40.2	39.1	39.5	40.4	40.4
±SD	2.5	2.9	1.6	2.7	2.7

Table 7. Hemoglobin (g/100 ml)

Subject	Day 1	Day 7	Day 14	Day 21	Day 28
1	12.4	12.0	13.0	13.7	13.2
2	13.6	13.4	13.6	13.3	13.6
3	15.2	15.6	15.9	16.3	16.0
4	14.6	14.5	14.3	13.9	14.4
5	12.0	12.4	12.0	11.8	12.7
6	14.4	12.6	13.9	14.5	14.3
7	13.6	13.3	13.0	13.5	14.0
8	13.5	14.1	14.6	14.3	14.0
Mean	13.7	13.5	13.8	13.9	14.0
±SD	1.1	1.2	1.2	1.3	1.0

Table 8. Plasma pyridoxal 5'-phosphate (nmol/L)

Subject	Day 1	Day 7	Day 14	Day 21	Day 28
1	24.0	37.0	39.8	57.1	46.6
2	50.9	53.3	47.0	57.6	59.0
3	18.7	44.6	75.4	73.0	88.8
4	46.1	58.1	69.1	60.0	67.7
5	13.9	27.8	14.4	15.7	13.0
6	29.3	45.6	33.6	36.0	25.4
7	29.8	25.0	13.9	13.9	16.8
8	42.2	67.9	38.4	29.3	25.9

Table 9. Plasma total vitamin B-6 (nmol/L)

Subject	Day 1	Day 7	Day 14	Day 21	Day 28
1	34.1	45.9	51.2	60.8	55.0
2	69.8	60.6	88.4	70.6	67.6
3	26.1	51.2	80.6	76.4	93.6
4	67.9	73.9	87.3	76.2	79.5
5	24.5	37.6	24.8	22.6	17.9
6	39.3	50.3	40.4	40.9	35.1
7	44.0	29.7	25.1	27.9	24.9
8	68.6	87.9	53.1	46.4	39.2

Table 10. Red blood cell pyridoxal 5'-phosphate (nmol/L)

Subject	Day 1	Day 7	Day 14	Day 21	Day 28
1	36.69	40.88	39.92	45.93	39.97
2	28.84	32.89	35.93	36.43	35.93
3	32.89	40.99	43.01	55.15	54.14
4	43.92	52.98	49.44	50.55	44.33
5	35.77	38.69	49.13	47.82	47.11
6	40.99	47.06	39.97	46.93	37.34
7	33.55	37.49	40.13	33.29	29.50
8	36.13	35.98	39.87	31.12	31.17

Table 11. Urinary 4-pyridoxic acid (umol/day)

	Subject							
Date	1	2	3	4	5	6	7	8
5/7/96	5.71	10.03	4.56	7.39	4.84	5.44	8.38	4.23
5/8	7.14	9.25	6.63	7.88	5.76	7.04	8.21	5.42
5/9	7.55	9.50	6.87	7.42	7.04	6.68	7.64	5.98
5/10	6.52	8.72	7.67	7.70	7.22	7.42	6.60	6.01
5/11	8.36	9.61	6.82	7.76	7.08	6.10	7.20	6.40
5/12	6.12	7.75	5.93	7.23	7.06	7.36	7.06	6.50
5/13	6.86	10.61	6.55	7.92	7.33	7.51	6.99	6.83
5/14	7.45	8.36	8.02	8.19	5.70	5.45	5.52	4.56
5/15	8.82	9.06	8.02	10.23	4.43	5.09	4.15	3.55
5/16	9.01	9.77	8.83	7.70	4.40	4.55	3.31	3.36
5/17	8.03	9.42	7.75	9.59	4.11	4.08	3.10	2.92
5/18	7.84	9.75	5.87	7.58	3.80	4.45	3.24	2.65
5/19	8.93	9.10	7.52	6.82	3.90	3.12	3.20	2.96
5/20	9.50	10.30	8.46	8.53	3.63	3.93	3.36	2.67
5/21	8.66	9.02	9.28	9.65	3.63	3.57	3.16	2.69
5/22	10.04	9.60	10.14	7.39	3.51	3.74	3.24	2.52
5/23	8.42	8.23	8.63	9.80	3.71	3.91	3.02	2.88
5/24	8.96	9.77	9.74	9.79	3.35	3.80	3.08	2.80
5/25	8.98	8.56	8.84	10.25	3.31	3.98	2.21	2.00
5/26	9.59	10.48	8.71	10.94	2.91	3.21	2.65	1.51
5/27	8.75	10.21	9.89	8.15	3.17	2.75	2.65	1.69
5/28	9.51	10.66	9.16	9.67	3.00	2.90	2.82	1.70
5/29	10.39	10.12	10.52	9.35	2.45	3.23	2.68	1.68
5/30	9.65	10.48	10.21	9.14	2.41	2.75	2.71	2.01
5/31	8.15	10.52	10.77	9.00	3.28	3.17	2.72	1.98
6/1	11.00	9.18	8.93	7.62	3.21	2.97	2.59	1.80
6/2	10.17	8.95	8.87	7.92	3.74	3.14	2.67	1.76
6/3	9.71	9.43	10.00	10.96	2.30	1.91	2.71	1.80

Table 12. Urinary total vitamin B-6 (umol/day)

Subject	End of Week 1[1]	End of Week 2	End of Week 3	End of Week 4
1	0.83	0.82	0.95	0.82
2	1.14	1.14	1.11	1.03
3	1.08	1.17	1.19	1.21
4	1.29	1.15	1.24	1.32
5	0.88	0.56	0.43	0.40
6	0.63	0.53	0.46	0.39
7	0.72	0.47	0.37	0.40
8	1.04	0.58	0.48	0.47

[1]Based on last 3 days of the week

Table 13. Plasma phosphatidylcholine (PC) concentration (mg/100ml)

	Subject							
PC	1	2	3	4	5	6	7	8
Before[1]	101	151	156	128	135	146	120	125
After[1]	95	145	143	120	128	130	123	116

[1]*Before* and *After* the experimental diet

Table 14. Plasma fatty acid profile (area %)

Fatty acid	Time	Subject 1	2	3	4	5	6	7	8
16:0	Before	19.6	19.3	16.8	19.9	18.8	20.2	22.5	21.2
	After	21.0	19.4	19.4	19.5	19.3	20.3	21.2	20.3
16:1(n-7)	Before	2.6	2.0	20.9	2.2	1.8	2.0	2.4	2.6
	After	2.4	2.3	2.5	2.3	1.7	2.1	1.9	2.1
18:0	Before	6.4	6.0	5.4	6.3	5.7	7.1	7.1	7.0
	After	7.1	6.0	6.0	5.4	5.5	7.1	6.9	6.9
18:1(n-9,T)	Before	1.1	1.1	0.8	1.2	1.4	1.2	0.5	0.7
	After	0.8	1.2	1.1	1.2	1.3	1.2	0.9	0.4
18:1(n-9,C)	Before	18.3	19.6	15.8	18.0	17.5	19.7	18.6	19.0
	After	19.1	20.3	19.6	19.1	18.2	21.1	17.6	18.4
18:1(n-7,T)	Before	2.3	1.8	1.4	2.1	1.9	1.8	1.2	2.0
	After	1.8	1.9	1.9	2.2	2.1	1.9	1.8	1.7
18:2(n-6,T)	Before	0.2	0.3	0.2	0.3	0.3	0.3	0.3	0.2
	After	0.0	0.3	0.3	0.4	0.0	0.4	0.0	0.0
18:2(n-6,C)	Before	28.6	30.0	23.2	32.3	31.6	27.5	27.0	26.1
	After	30.3	29.2	29.9	32.9	32.9	26.7	29.3	29.1
18:3(n-3,C)	Before	1.0	1.2	1.2	1.2	0.9	0.8	0.8	1.1
	After	1.0	1.2	1.3	1.3	0.8	0.9	0.7	1.0
20:1(n-9)	Before	0.2	0.3	0.1	0.4	0.2	0.2	0.3	0.2
	After	0.0	0.3	0.3	0.3	0.9	0.3	0.0	0.0
20:2(n-6)	Before	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
	After	0.0	0.3	0.3	0.4	0.0	0.3	0.3	0.3
20:3(n-6)	Before	1.5	1.5	1.3	1.8	1.6	1.9	1.8	1.6
	After	1.6	1.5	1.5	1.7	1.6	1.8	1.8	1.6
20:4(n-6)	Before	7.4	6.2	4.4	5.0	7.6	7.2	7.7	6.3
	After	7.4	6.4	5.4	4.5	7.5	6.5	8.2	7.5
20:5(n-3)	Before	0.6	0.5	0.5	0.2	0.5	0.4	0.5	1.0
	After	0.6	0.6	0.6	0.2	0.5	0.4	0.5	0.7
22:5(n-3)	Before	0.6	0.6	0.5	0.4	0.6	0.6	0.5	0.8
	After	0.6	0.7	0.7	0.4	0.7	0.6	0.6	0.8
22:6(n-3)	Before	2.1	1.2	1.3	1.5	1.6	1.5	2.3	2.0
	After	3.3	1.3	1.7	1.4	1.9	1.4	2.3	2.6
Total n-6	Before	37.9	38.3	29.4	36.7	41.4	37.2	37.1	34.5
	After	39.2	37.7	37.4	39.9	42.1	35.6	39.7	38.5
Total n-3	Before	4.2	3.5	3.5	3.2	3.5	3.3	4.1	4.9
	After	5.4	3.7	4.3	3.4	3.8	3.3	4.1	5.1

Table 15. Plasma lipid profile (mg/100ml)

	Subject							
Lipid	1	2	3	4	5	6	7	8
Total Cholesterol								
Before	117.5	175.9	151.8	153.6	160.6	156.6	138.4	149.0
After	111.2	187.9	160.7	137.1	151.7	143.4	132.3	134.2
HDL-Cholesterol								
Before	46.2	35.7	48.2	39.0	43.2	41.1	50.8	47.8
After	31.6	30.8	44.2	35.2	38.3	37.9	51.8	35.7
LDL-Cholesterol								
Before	60.1	116.0	70.9	92.6	105.4	96.7	75.1	87.1
After	68.2	129.1	91.0	81.1	104.0	82.4	70.8	82.1
Triglycerides								
Before	56.3	121.4	163.4	110.1	59.9	94.3	62.4	70.8
After	56.7	139.9	127.7	103.9	47.3	115.6	48.8	81.9
TC/HDL-C								
Before	2.6	4.9	3.2	3.9	3.7	3.8	2.7	3.1
After	3.5	6.1	3.6	3.9	4.0	3.8	2.6	3.8

1 mg cholesterol = 0.00259 mmol

Table 16. Plasma alkaline phosphatase (Units/L)

Subject	Day 1	Day 7	Day 14	Day 21	Day 28
1	20.3	20.5	21.4	23.1	22.5
2	30.3	28.8	27.2	24.9	24.4
3	30.0	30.3	32.1	30.6	30.3
4	38.8	43.3	41.3	39.7	42.1
5	36.1	35.5	34.0	32.5	31.7
6	24.8	24.5	25.1	25.1	23.2
7	26.8	26.9	27.5	29.2	29.5
8	18.2	20.1	21.2	20.7	19.1

Table 17. Mean nutrient intakes (from 3-day diet records)

	Subject							
Nutrient	1	2	3	4	5	6	7	8
Calorie (kcal)	1863	2010	2117	2193	997	1144	1558	1268
Vitamin B-6 (mg)	1.37	1.62	2.30	0.86	0.57	0.67	1.35	1.81
Fat (g)	82.9	81.6	71.1	92.0	29.5	39.4	58.1	36.9
SFA %	14.9	10.3	10.0	10.0	3.67	9.50	9.70	7.60
MFA%	15.4	7.67	12.7	20.7	4.00	8.50	11.9	7.43
PUFA%	5.97	6.33	5.30	6.67	2.33	3.00	5.40	3.37
Cholesterol (mg)	235	200	456	263	164	161	148	321
Riboflavin (mg)	1.35	2.41	2.00	1.90	0.77	1.61	1.11	1.97
Protein (g)	84.2	84.9	135.4	91.6	31.1	58.6	58.0	83.6

Appendix B: Figures

Figure 1. Recipe for salad dressing

Use a cruet or jar with lid for mixing the ingredients.

Ingredients are added in the following order for proper blending.

Add 40 g Vinegar
140 g Water
30 g Good Season's fat free Italian dressing mix
(use 1.05 oz packet)

Shake vigorously until well-blended.

Add 163 g Canola oil (high oleic acid)

Shake again until well-blended.

Refrigerate.

Shake well before weighing out portions

Figure 2. Recipe for pumpkin bars

Combine the following ingredients and set aside.

300 g all-purpose white flour
5 g salt
10 g baking powder
5 g baking soda
1 g ginger

Combine and thoroughly mix in a Kitchenaid bowl.

200 g egg white
240 g canola oil
420 g sugar, white granulated
440 g pumpkin, canned

Add dry ingredients to the wet mixture and mix.

Do not overmix.

Turn into large sheet pan (17 ½" by 12 ½") which has been sprayed with a non-stick

spray and wiped down with a paper towel.

Bake at 300° in a convection oven for 25-30 minutes.

Figure 3. Form of informed consent for screening

Department of Nutrition and Food Management
Oregon State University

Informed Consent for Screening

I am interested in being considered as a possible participant in a human metabolic study on the effect of vitamin B-6 intake on the metabolism of fatty acids. To determine my eligibility for participating in this investigation, I understand that I will be asked to do the following:

1. Answer a health/nutrition questionnaire.

2. Come to 103 MLM on between 7 and 8 am without having eaten or drunk anything except water since 7 p.m. the night before. This includes vitamins or any other nutritional supplements. A registered medical technologist will draw 25 ml of blood from a vein in my forearm.

3. Complete 3-day records.

I also understand:

1. That I will receive no direct benefits except information on my vitamin B-6 status and results of the chemistry screen (glucose, electrolytes, enzymes, etc.).

2. That I will receive a slight bruise at the site of blood drawing. A sterile, disposable needle will be used to draw blood into evacuated tubes.

3. Any information obtained from me will be confidential. the only persons who have access to information obtained from me are the principal investigator, the medical technologist and the graduate students assisting in this research. All in formation will be filed in the investigator's office.

4. That I am not committed to participate in any other metabolic study even if I meet all of the criteria.

All of my questions have been answered to my satisfaction. Should I have any further questions I can contact Dr. James E. Leklem between 8 am to 5 p.m. weekdays at 737-0969.

Name _____
Date _____
Principal Investigator _____

Figure 4. Form of informed consent

Informed Consent Form for Vitamin B-6 - Fatty Acid Study

Department of Nutrition and Food Management
Oregon State University

Informed Consent

The purpose of this investigation is to examine the effect of different intakes of vitamin B-6 on the metabolism of essential fatty acids. In this study, vitamin B-6 intake will be varied while all the other nutrient intake are held constant.

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

1. I will not have taken any vitamin or other nutritional supplement for at least four weeks before the beginning of this project.

2. I am not to take any vitamin or other nutritional supplement except the vitamin B-6 that I will receive during this investigation.

3. If I take any medication I will report this to the principal investigators.

4. This is a 28-day study, ending the morning after day 28.

5. I will consume only those foods and beverages that are served to me or permitted.

6. During this study, I will not engage in any strenuous physical activity such as running more than one mile per day or bicycling more than 6 miles per day. As much as it is possible, I will maintain a uniform daily schedule of physical activity and sleep.

7. I will keep a record of my daily activities, including exercise and sleep, which I will turn in each day.

8. Daily throughout this investigation, I will collect complete 24-hour urine specimens in the containers that are provided for me. If I accidentally lose some urine I will immediately report this to a member of the research staff.

9. At regular intervals, a total of 5 times, a registered medical technologist will draw 25 ml of blood (equivalent to about two tablespoons) from my forearm. On except water since 7 p.m. the night before. I understand that this procedure may cause a slight bruise (Blood will be drawn on days 1, 7, 14, 21 and 28 of the study period).

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

11. My participation in this research is voluntary and I can withdraw at any time with out loss of benefits except to those I am entitled.

12. At the end of this investigation I will receive $ 4.00 for each day I participated and collected complete urine samples. (For successfully completing this study, the total amount is $ 180.)

13. I will incur no medical or health risks from participating in this research. I will receive some benefits: a nutritionally adequate diet furnished free of charge for 28 my vitamin B-6 status and plasma fatty acid profiles will be available to me if I request them.

14. I understand the University does not provide a research subject with compensation or medical treatment in the event a subject is injured as a result of participation in this research project.

15. This study will be conducted from May, 7 to June, 4, 1996.

All of my questions have been answered to my satisfaction. If I have any questions I will call Dr. James E. Leklem at 737-0969.

Signed _____ Date_____

Printed Name _____

Present Address _____ Phone No._____

Principal investigator _____

Witness _____

Figure 5. Form for Nutrition Screening Data

NUTRITION SCREENING DATA

Name _____ Date _____

Address _____

Phone (H) _____ (W) _____ Hrs: _____

Age _____ Ht. _____ Present Wt. _____ Usual Wt _____

Smoking Yes _____ No _____ Use O. C. Yes_____ No _____

VITAMINS/ERGOGENIC AIDS:

Yes No (if yes, how long & which ones)

willing to stop? Yes_____ No _____

Activity:

Type of exercise days/week min./day perceived intensity

How long have you maintained this level of fitness? _____

Are you willing to continue with this level of fitness during the 28 days of this study? _____

Diet :

Food Preferences Foods will not eat(or allergies)

Regular menstrual cycle? Yes___ No ___How many days? _____

Medications:

Are you currently on any medications? Yes____ No _____
please list if yes : _____

Other:

Able to attend 3 meals/day on campus, May 7- June 4 ? Yes____ No _____
Other helpful info? _____

(would this person be a good subject?)

Appointment schedule: date_____ time_____ with _____

Interviewer initials _____

Figure 6. Form for daily activity sheet

DIET STUDY. 1996, SPRING
DEPT. OF NUTRITION AND FOOD MANAGEMENT
OREGON STATE UNIVERSITY

Name _____

Date _____

DAILY ACTIVITY SHEET

1 Record all activities for the Previous day and length spent at each.

Activity	Length of time (fraction of hours)	Time of Day*
Sleep		
Sitting		
Walking		
Physical Work		
Other Activities		
Other Sports or Activities (indicate type)		

*M - morning; A - Afternoon; E - Evening; L - Late night/early morning

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

Coffee (cups) _____

Tea (cups) _____

Pop (diet/regular) _____ Candy/sugar _____

3. How do you feel today?

Excellent _____
Good _____
Fair _____
Poor _____

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

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

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

7 Did you weigh yourself today? _____ Your weight today _____

8 Other comments

SPRING '96 WOMEN'S DIET STUDY

SUBJECT: _____

NO. _____

Menstrual Chart

Yr	Month	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	No. of days from start of period to beginning next
'96	March																																
	April																																
	May																																

Type of Flow : [X] normal

[O] exceptionally light

[///] exceptionally heavy

Figure 7. Menstrual chart

Figure 8. Standard of fatty acid profile

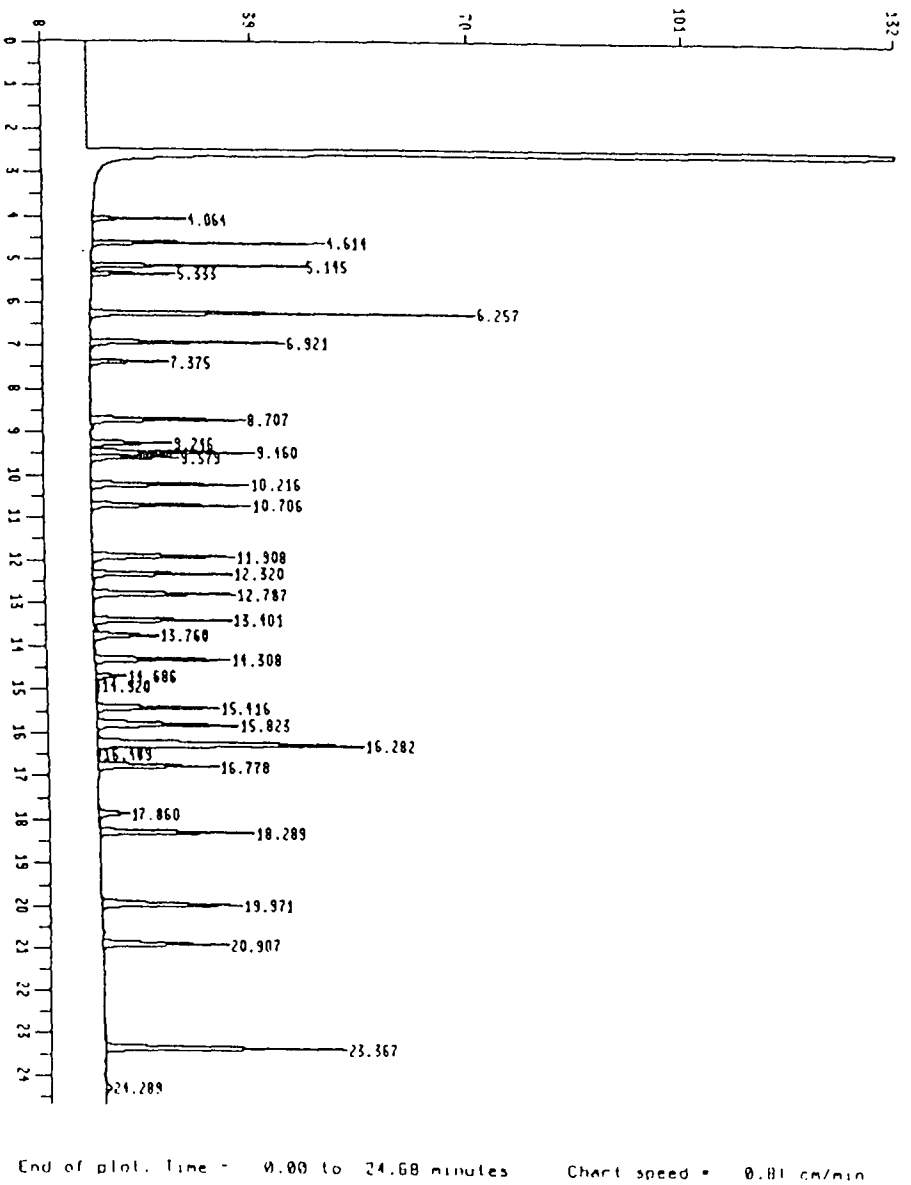


Figure 9. Area percent of standard fatty acid

Report by Signal

Operator: 24 Jun 96 2:05 pm
Method File Name : METH003.M
Sample Info :
Misc Info:
Integration File Name : V3:COMP30.I
consisting of channels : 1. GC Signal 1 of COMP30.D
Bottle Number : 0 Repetition Number: 1

GC Signal 1 of COMP30.D

Ret Time	Type	Area	Height	Area %	Ratio %
4.064	BV	390.08	13.8476	1.390	14.59
4.614	BV	1002.35	33.9408	3.571	37.49
5.145	BV	964.85	31.4652	3.438	36.08
5.333	VB	410.82	12.4501	1.464	15.36
6.257	BB	2029.59	56.0982	7.231	75.90
6.921	BV	1040.12	28.4931	3.706	38.90
7.375	PB	462.06	11.5632	1.646	17.28
8.707	BV	1008.67	22.9381	3.594	37.72
9.246	BV	519.51	12.0312	1.851	19.43
9.460	VV	1010.06	24.2476	3.599	37.77
9.579	VB	535.47	13.0110	1.908	20.03
10.216	BB	1034.23	23.3284	3.685	38.68
10.706	BB	968.38	23.4687	3.450	36.21
11.908	BV	1100.35	21.1303	3.920	41.15
12.320	VV	889.30	20.6598	3.168	33.26
12.787	VB	1018.82	21.0253	3.630	38.10
13.401	BV	909.93	20.4577	3.242	34.03
13.760	VB	523.98	9.5451	1.867	19.60
14.308	BV	955.18	19.9547	3.403	35.72
14.686	PV	229.57	4.4726	0.818	8.59
14.920	VV	99.4303	0.5796	0.354	3.72
15.416	VV	880.38	18.0875	3.137	32.92
15.823	VV	1175.04	21.0066	4.186	43.94
16.282	VV	2673.97	39.0442	9.527	100.00
16.489	VV	30.5363	0.4633	0.109	1.14
16.778	PV	941.72	17.8681	3.355	35.22
17.860	BB	251.59	4.4247	0.896	9.41
18.289	BB	1052.30	22.7881	3.749	39.35
19.971	BV	1217.11	20.8582	4.336	45.52
20.907	PV	969.02	18.7163	3.452	36.24
23.367	BB	1664.24	35.2307	5.929	62.24
24.289	BB	108.87	0.8707	0.388	4.07

Figure 10. Approval of the human diet study

OREGON STATE UNIVERSITY

COPY

Report of Review

TITLE: Effect of dietary level of vitamin B-6 on fatty acid metabolism.

PRINCIPAL INVESTIGATOR: James Leklem

DEPARTMENT: NFM

STUDENT: Min Sun Kim

COMMITTEE DECISION: Approved

COMMENTS:

1. The informed consent form obtained from each subject should be retained in program/project's files for three years beyond the end date of the project.

2. Any proposed change to the protocol or informed consent form that is not included in the approved application must be submitted to the IRB for review and must be approved by the committee before it can be implemented.

Date: April 12, 1996

Warren N. Suzuki, Chair
Committee for the Protection of Human Subjects
(Education, 7-6393, suzukiw@ccmail.orst.edu)

Figure 11. Health/diet questionnaire

8/20/90

HEALTH / DIET HISTORY--Aug 1990 version
CONFIDENTIAL

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

Project Name _____
_____
Project Dates _____

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

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

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

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

Are you pregnant? __ Yes __ No Breast Feeding? __ Yes __ No
Have you ever been pregnant? __ Yes __ No
If yes, how many times? _____
How many children have you carried? _____
Please check if you have had any of the following complications of pregnancy:
__ 1. hyperemesis gravidarum (morning sickness)
__ 2. pre-eclampsia or eclampsia (toxemia)
__ 3. high blood pressure
__ 4. severe edema (swelling of your legs and feet)
__ 5. numbness and tingling in your hands, wrists, or arms
__ 6. gestational diabetes
__ 7. premature birth(s) (please indicate gestational age of infant(s))
_____
__ 8. kidney or bladder infections
__ 9. premature rupture of membranes
__ 10. small for dates infant (less than 5 lbs or 2500 g at term)

(Health/diet questionnaire, continued)

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

DIETARY HISTORY

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

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

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

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

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

(Health/diet questionnaire, continued)

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

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

Type	Amount per day	How long have you taken

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

Type	Amount per day	How long have you taken

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

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

(Health/diet questionnaire, continued)

HABITS: A. Smoking:

1) Do you currently smoke? __ Yes __ No

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

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

At what age did you start smoking? _____

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

If yes, at what age did you start? _____
If yes, when did you quit? _____
Was this the only time you have quit? __ Yes __ No
If you quit, please check below what you did smoke, and how much per day:

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

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

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

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

B. Alcohol:

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

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

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

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

C. Caffeine:

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

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

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

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

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

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

D. Diet Soda Pop and other Sugarless Beverages

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

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

(Health/diet questionnaire, continued)

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

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

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

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

MEDICAL HISTORY:

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

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

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

(Health/diet questionnaire, continued)

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

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

Comments:

(Health/diet questionnaire, continued)

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

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

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

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

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

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

Operation	Age or Year

(Health/diet questionnaire, continued)

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

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

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

COMMENTS:

Checked by _____ Date _____