AN ABSTRACT OF THE THESIS OF

M. Carol Walter for the degree of Master of Science in Foods and Nutrition presented on August 10, 1984.

Title: Effect of Altered Carbohydrate Diet, Vitamin B-6 Supplementation, and Exercise on Vitamin B-6 Metabolism in Trained and Untrained Women

Abstract Approved: _____

James E. Leklem

This investigation was designed to add to present understanding of vitamin B-6 (B6) metabolism during exercise. Ten women, 5 aerobically trained and 5 untrained, were fed 4 controlled diets: a moderate carbohydrate (49%) (MCHO) for 2 weeks, a high carbohydrate (63%) (HCHO) for one week, MCHO+B6 for 2 weeks, and HCHO+B6 for 1 week. A one week MCHO diet separated the non-supplemented (2.3 mg B6) and supplemented (10.3 mg B6) diets.

The VO2 max of each subject was determined prior to the study. An exercise test was completed on day 5 or 6 of weeks 2, 3, 6, and 7. The test consisted of 20 minutes of cycle ergometer exercise at 80% VO2max, preceded by 10 minutes of warm-up and followed by a 5-10 minute active recovery. Blood samples were collected pre exercise (pre), 2-3 minutes post (post), 30 minutes post (p30), and 60 minutes post (p60) exercise. Samples were analyzed for plasma vitamin B-6 (PB6), hematocrit, and hemoglobin. Urine was collected

daily in 24-hr aliquots and samples were analyzed for 4-pyridoxic acid (4PA) and creatinine.

For all diets, exercise resulted in a significant increase in PB6 from pre to post and a significant decrease from post to p60, the magnitude of the change being greater with supplementation. PB6 fell below pre levels by p60 for all exercise sessions. 4PA increased significantly from the day before exercise to the day of exercise on all diets. There was no significant effect of dietary carbohydrate on levels of PB6 or excretion of 4PA. ANOVA showed no difference between the groups for PB6 or 4PA, though the trained group had lower PB6 and greater 4PA excretion throughout the study despite the controlled intake.

Tissue redistribution of B6 seems to occur with exercise. The increased magnitude of change in PB6 with exercise after supplementation suggests an increased storage of the vitamin, most likely associated with glycogen phosphorylase in the muscle. Trained women may have lower levels of PB6 and greater 4PA excretion as the result of a regular exercise program. However, supplementation with B6 cannot be recommended since the status of all subjects was adequate with the diet fed.

Effect of Altered Carbohydrate Diet, Vitamin B-6 Supplementation, and Exercise on Vitamin B-6 Metabolism in Trained and Untrained Women

by

M. Carol Walter

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

Completed August 10, 1984

Commencement June 1985

APPROVED:

Professor of Foods and Nutrition in charge of major

Head of department of Foods and Nutrition

Dean of Graduate School

Date thesis is presented August 10, 1984

ACKNOWLEDGEMENTS

Many people need to be thanked for my completion of this degree. In particular I would like to thank Dr. Jim Leklem for his responsibility in this research project and his continued patience with my progression. Dr. Margie Woodburn has been of help during times of need also. I thank Melinda Manore, my partner in this experience, for her friendship, enthusiasm, and support when I most needed them. I thank her also for kindling my desire to run!!! Dr. Don Campbell has been of immeasurable help to me with his knowlege of exercise physiology and his philosophies of Life. Linda Barstow, Karin Hardin, Hossein Kabir, and Carrol Lee were indispensible for their help with the diet study, their analysis of samples, and their friendship.

I must certainly thank the 18 wonderful women who participated in the study, for without their cooperation, none of this would have been possible! I have greatly appreciated all faculty and fellow graduate students of this department for their friendliness and help to me through trying times. I thank my parents, my aunt Dorothy and uncle Bob, for both spiritual and financial aid.

Finally, I thank my best friend, Ian Walsh, who has been just SUPER through all of this!!!!!!

TABLE OF CONTENTS

Chapter		Page
I.	Introduction	1
II.	Review of Literature	5
	Vitamin B-6	5
	History	5
	Metabolic Roles	5
	Chemistry	8
	Occurrence in Foods and Bioavailability	9
	Absorption	10
	Interconversion and Transport	12
	Tissue Distribution and Storage	14
	Levels of B-6 Vitamers in Blood	17
	Levels of B-6 Vitamers in Urine	19
	Recommended Intakes	21
	Exercise Metabolism	23
	Substrate Availability	23
	Substrate Utilization	25
	Muscle Glycogen	25
	Blood Glucose and Hepatic Glycogen	26
	Plasma Free Fatty Acids	28
	Training	30
	Diet and Exercise	32
	Sex	34
	Metabolic Roles of Vitamin B-6 in Exercise Metabolism	37
	Influence of Exercise on Vitamin B-6 Metabolism	39
III.	Methods and Materials	43
	Overview	43
	Subject Recruitment and Selection	43
	Baseline Subject Data Collection	44
	Experimental Design	46
	Diet	48
	Exercise	51
	Daily Procedure	53
	Sample Collection	53
	Sample Analyses	54
	Diet Composites	54
	Blood Analyses	54
	Urine Analyses	56
	Statistical Analyses	56
IV.	Results	58
	Blood Analyses	58
	Plasma Vitamin B-6	58
	Plasma Volume	65

Chapter | Page

Urine Analyses 67
Urinary Creatinine 67
Urinary 4-pyridoxic acid 67

V. Discussion 74

VI. Summary and Conclusions 84

References 88

Appendix 102

LIST OF FIGURES

Figure		Page
1.	Interconversions and structures of B-6 vitamers.	7
2.	Tissue interrelationships of pyridoxal and pyridoxal 5'-phosphate.	18
3.	Experimental design.	47
4.	Exercise test protocol and blood sampling times.	52
5.	Plasma vitamin B-6 response to exercise with the non-supplemented diets.	63
6.	Plasma vitamin B-6 response to exercise with the supplemented diets.	64
7.	Urinary 4-pyridoxic acid excretion response to exercise with the non-supplemented diets.	72
8.	Urinary 4-pyridoxic acid excretion response to exercise with the supplemented diets.	73

LIST OF TABLES

Table		Page
1.	Physical statistics for trained and untrained groups.	45
2.	Composition of moderate and high carbohydrate diets.	49
3.	Group mean plasma vitamin B-6 values for day 1 and day 10 of the study.	59
4.	Group mean plasma vitamin B-6 values and differences from pre to post, post to p60, and pre to p60 minutes of exercise for all the exercise tests.	60
5.	Mean % plasma volume changes and plasma vitamin B-6 values predicted from % hematocrit changes from pre to post and from pre to post 60 minutes of exercise for all the exercise tests.	66
6.	Group mean urinary 4-pyridoxic acid excretion values for day 1 and day 10 of the study.	69
7.	Group mean urinary 4-pyridoxic acid excretion values and differences from the day before to the day of exercise for all the exercise tests.	70

APPENDIX TABLES

Table		Page
1.	Individual blood chemistry screen values before and after the study.	102
2.	Individual hematocrit values and plasma volume changes from pre to post exercise for all the exercise tests.	103
3.	Individual hematocrit values and plasma volume changes from pre to post 60 minutes of exercise for all the exercise tests.	105
4.	Individual and mean plasma vitamin B-6 values for day 1, day 10, and all the exercise tests for the untrained group.	107
5.	Individual and mean plasma vitamin B-6 values for day 1, day 10, and all the exercise tests for the trained group.	108
6.	Individual and mean urinary 4-pyridoxic acid excretion values for day 1, day 10, and the day before and day after exercise for all the exercise tests for the untrained group.	109
7.	Individual and mean urinary 4-pyridoxic acid excretion values for day 1, day 10, and the day before and day after exercise for all the exercise tests for the trained group.	110

LIST OF ABBREVIATIONS

PL	Pyridoxal
PN	Pyridoxine
PM	Pyridoxamine
PLP	Pyridoxamine 5'-phosphate
PNP	Pyridoxine 5'-phosphate
PMP	Pyridoxamine 5'-phosphate
PB6	Plasma vitamin B-6
4PA	4-pyridoxic acid
PV	Plasma volume
MCHO	Moderate carbohydrate diet
HCHO	High carbohydrate diet
MCHO+B-6	Moderate carbohydrate diet with supplemental vitamin B-6
HCHO+B-6	High carbohydrate diet with supplemental vitamin B-6
RDA	Recommended Dietary Allowance
VO2 max	Maximal oxygen consumption
kcal	kilocalorie
g	gram
kg	kilogram
m	meter
ml	milliliter
nmol	nanomole, 10^{-9} moles
umol	micromole, 10^{-6} moles
S.D.	Standard Deviation
UB6	Urinary vitamin B-6
FFA	Free fatty acids
Hgb	Hemoglobin
Hct	Hematocrit

Effect of Altered Carbohydrate Diet, Vitamin B-6 Supplementation, and Exercise on Vitamin B-6 Metabolism in Trained and Untrained Women

I. INTRODUCTION

Exercise has been employed by researchers as a functional load to gain information about the capacity of the human organism to meet increased demands for energy above resting levels. Since food is the source of energy for muscular work, nutrient intake may effect work performance. In turn, the metabolism of nutrients may be altered in response to exercise. Vitamin B-6 plays an integral role in blood glucose homeostasis. Plasma pyridoxal 5'-phosphate (PLP), the active form of vitamin B-6, is the cofactor of glycogen phosphorylase, an enzyme which catalyzes the phosphorolytic cleavage of glucose-1-phosphate from glycogen. Additionally, PLP is the coenzyme for aminotransferases involved in gluconeogenesis. Vitamin B-6, as a member of the "B Complex" vitamins, is highly touted as a nutritional aid to physically active individuals. However, when members of a competetive swim team were supplemented with vitamin B-6, no improvement in endurance was noted (1). The general consensus of nutritionists is that the nutrient needs of an athlete can be met by a well-balanced diet (2). Recent observations do demonstrate an alteration of the metabolism of vitamin B-6 with exercise (3-6). Plasma levels of PLP and vitamin B-6 (PB6) are seen to increase with exercise to an extent previously seen only 0.5-1

hour after an oral 1-2 mg dose of vitamin B-6 (7). In addition, several investigators have suggested that the urinary excretion of 4-pyridoxic acid (4PA), the major metabolite of vitamin B-6, is increased after exercise (6,8,9).

The source of the increased plasma PLP seen with exercise is hypothesized to be the muscle. Black et al. (10,11) have shown muscle to be a storage depot of PLP in the rat, where it is associated with glycogen phosphorylase. They demonstrated a seemingly unlimited increase in glycogen phosphorylase with increased vitamin B-6 intake while the level of other PLP dependent enzymes remained stable. The decrease of the level of phosphorylase was seen to occur with caloric deprivation and starvation rather than a vitamin B-6 deficiency. Hatcher (6) manipulated muscle glycogen stores in men through a glycogen depletion-repletion diet and observed the PLP and PB6 response to cycle ergometer exercise. She also added a vitamin B-6 supplement to each subject's diet to hypothetically increase the storage of PLP in the muscle. Hatcher's results led her to the conclusion that the supplemental vitamin B-6 did increase the storage of PLP, and that muscle glycogen phosphorylase was the likely source of the increased PLP and PB6 seen with exercise. Hatcher also reported an increased excretion of 4PA with exercise, though this is difficult to state conclusively due to the nature of her study design. deVos (12) investigated the metabolism of fuels used for exercise in conjunction with the diet study of Hatcher. The combination of a high carbohydrate diet and a vitamin B-6 supplement resulted in increased blood lactate levels

with exercise as compared to exercise with the normal or low carbohydrate diets. deVos concluded that the high carbohydrate diet increased muscle glycogen stores and that the vitamin B-6 supplement increased the activity of glycogen phosphorylase, which then caused a more rapid utilization of the increased glycogen stores during exercise.

The present study was designed to further control diet and exercise variables to add to present knowledge of vitamin B-6 metabolism with exercise. The objectives are fourfold. First, the carbohydrate level of the diet will be altered to observe the effect of dietary carbohydrate on vitamin B-6 metabolism during exercise. In general, muscle glycogen synthesis increases in proportion to consumed dietary carbohydrate (13). In addition, as dietary carbohydrate is increased, carbohydrate becomes relatively more important as an energy source for the working muscle (14). Secondly, a vitamin B-6 supplement will be added to the diet to determine the effect of the suggested increased muscle storage of vitamin B-6 on vitamin B-6 metabolism during exercise. Plasma vitamin B-6, hematocrit, and hemoglobin will be analyzed with each exercise session. A third objective is to evaluate the effect of exercise on 4PA excretion under conditions of well-controlled vitamin B-6 intake. Vitamin B-6 and protein content of the diet will remain the same throughout the study. Sufficient time will be allowed for adaptation to each modification of the diet. Urine will be collected daily in 24-hr samples and analyzed for 4PA and creatinine. Finally, the metabolism of vitamin B-6 with exercise

will be compared in aerobically trained and untrained young women. Aerobic endurance training has been shown to increase muscle glycogen storage (15) and to increase the reliance upon free fatty acids as fuel during exercise (16). Training level will be assessed from reports of the subjects' exercise habits, and further confirmed by the determination of VO2 max.

II. REVIEW OF LITERATURE

Vitamin B-6

History

Discovery of vitamin B-6 as the rat antidermatitis factor by Paul György in 1934 came during a period when identification of the essential vitamins was occurring in laboratories around the world (17). Shortly after György reported his findings, vitamin B-6 was isolated and its structure characterized by five different groups (18-21). In 1939, the vitamin was synthesized by researchers in Germany and the United States and was given the name pyridoxine (22,23). Further investigations by Snell (24) revealed two additional forms of the vitamin. Today, pyridoxine, pyridoxal, and pyridoxamine are collectively referred to as vitamin B-6.

The essentiality of vitamin B-6 for humans was first reported by Spies (25,26) in 1939. Pyridoxine effectively alleviated the deficiency symptoms of subjects that had failed to respond to treatments of niacin, thiamin, and riboflavin. Further confirmation of its essentiality came with the 1952-1953 outbreak of pyridoxine-related convulsions in infants fed a formula in which the vitamin B-6 was accidentally destroyed by heat processing (27,28).

Metabolic Roles

Pyridoxine, pyridoxal, and pyridoxamine are converted to their 5' phosphate esters via the enzymatic pathways shown in Figure 1. The metabolic functions in which vitamin B-6 is involved necessitate a conversion to its most active coenzyme form, pyridoxal 5'-phosphate (PLP). PLP assumes a critical role in nearly all reactions involved in amino acid metabolism, predominantly aminotransferase reactions. Aminotransfer entails transfer of the αamino group of an amino acid to an αketo acid via a Schiff base mechanism. PLP dependent decarboxylations form biologically important amines from histidine, tryptophan, tryosine, arginine, and dihydroxyphenylalanine. Gamma aminolevulinic acid, a porphyrin intermediate which leads to heme synthesis, is formed by the PLP-dependent decarboxylation of a glycine derivative. The metabolism of tryptophan, resulting in the formation of niacin, is partially dependent upon PLP. This dependency of tryptophan metabolism for PLP is often used as a measurement of vitamin B-6 status. PLP is the cofactor for amino acid reactions involving dehydrases, desulfhydrases, racemaces, synthetases, and hydrolases. The involvement of vitamin B-6 in the production of C1 units, via conversion of serine to glycine for nucleic acid synthesis, has led investigators to identify a role for PLP in the maintenance and function of immune responses (29,30). PLP has also been implicated as a steroid hormone modulator (31,32).

The involvement of vitamin B-6 in fat metabolism has been appreciated, though not well understood, for many years (33). Wakil (34) identified, as a possible direct role for vitamin B-6, a PLP

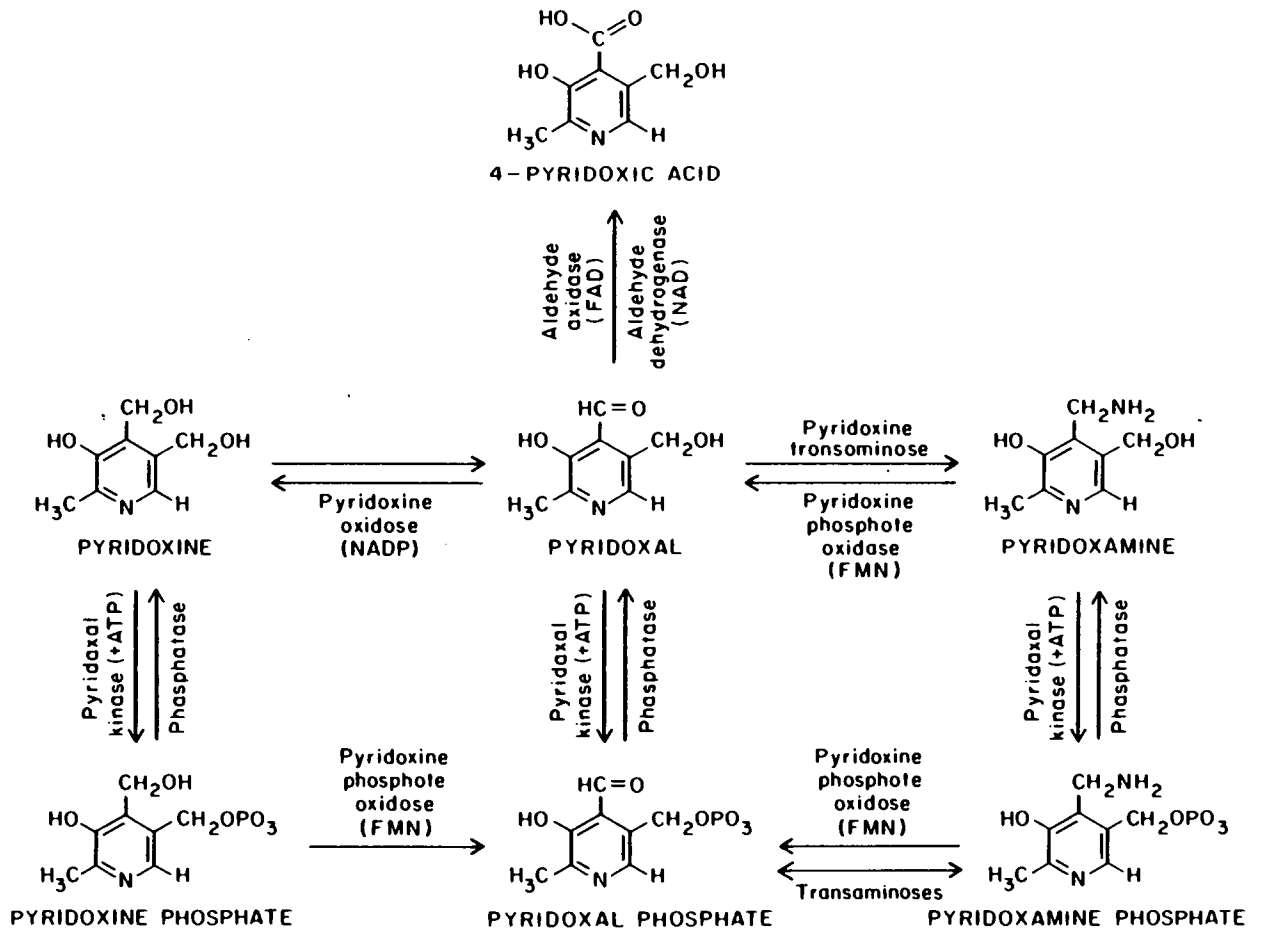

Figure 1 Interconversions and structures of B-6 vitamers.

dependent mitochondrial enzyme system responsible for the elongation of fatty acids. Acknowledging its necessity for protein synthesis, vitamin B-6 most assuredly plays a role in the metabolism of fats, though its role is considered by most to be indirect (35).

Additionally, PLP assumes a function in carbohydrate metabolism as the cofactor for glycogen phosphorylase (EC 2.4.1.1) (36). Release of energy stored in the form of glycogen requires the phosphorolytic cleavage of glucose units from glycogen by the phosphorylase enzyme. Each phosphorylase monomer contains one mole of PLP. In this enzyme system the mode of action of PLP is of a mechanism other than Schiff base formation (167).

Chemistry

Vitamin B-6 is the class name of all 3-hydroxy-2-methylpyridine derivatives showing biological activity in rats (38). Pyridoxine, the 4' alcohol form, has a molecular weight of 169.28. Pyridoxal is the 4' aldehyde form and pyridoxamine is the 4' amine form. Also included are the 5' phosphate esters pyridoxine phosphate (PNP), pyridoxal phosphate (PLP), and pyridoxamine phosphate (PMP). The primary excretory metabolite is the oxidized form, 4-pyridoxic acid (4PA). Structures and interconversions are shown in Fig. 1.

All forms of vitamin B-6 commonly occur as white crystals that are soluble in water. Vitamin B-6 undergoes rapid destruction in neutral or alkaline solution by white or UV light, but it is quite stable in acid. In aqueous solution the various forms of vitamin B-6

are subject to conversion to different ionic forms depending on changes in pH and temperature. The ionic changes, which can be observed as UV absorption spectral changes, lend information regarding the relationship of vitamin B-6 structure to its vitamin activities. The primary active form of vitamin B-6, PLP (and PMP as an intermediate form), functions as a coenzyme by virtue of the ability of its aldehyde group to react with the amino group of an amino acid substrate to yield a Schiff base between the enzyme bound PLP and the amino acid (38,39).

Occurrence in Foods and Bioavailability

The B-6 vitamers are widely distributed in nature. Pyridoxine and pyridoxine 5'-phosphate are found primarily in plant foods, while pyridoxal and pyridoxamine and their phosphorylated forms are found in greatest amounts in foods of animal origin (38). A major portion of the vitamin B-6 in foods occurs in a bound state (40). Gregory and Kirk (41) have identified the binding of PL and PLP to the εamino groups of lysyl residues during thermal processing and storage of foods. Plant foods contain variable amounts of a bound form of vitamin B-6 which appears to be a glycosylated form of the vitamin (42).

The vitamin B-6 content of various foods has been determined, yet research shows that all the vitamin may not be available. Bioavailability of vitamin B-6 refers to the portion of vitamin B-6 contained in the food that actually functions in vivo. (41). The

heat of processing decreases bioavailability as does the fiber content of the food (38,43). The bound forms of vitamin B-6 may also interfere with bioavailability. Bound pyridoxallysine was found to possess 50% vitamin B-6 activity as indicated by rat growth (44). The proportion of glycosylated vitamin B-6 in plant foods has been found to be inversely related to the vitamin B-6 bioavailability of foods as tested by Kabir et al. (42), suggesting a possible method for prediction of vitamin B-6 bioavailability from plant foods.

Absorption

Early studies of vitamin B-6 absorption relied upon measurement of urinary excretion of pyridoxine after large doses of pyridoxine (PN), given both orally and intravenously. Despite a lack of sophisticated laboratory procedures, results indicated that vitamin B-6 was rapidly and efficiently absorbed (45,46). The use of tritium labeled PN in animal studies allowed researchers to determine the site of vitamin B-6 absorption as primarily from the jejunum, and to a lesser extent the ileum (47). This was confirmed in humans when it was found that resection of the distal intestine did not affect the ability to absorb and excrete labeled PN given orally (48).

Urinary excretion of [3H] PN increased proportionally as increased doses of [3H] PN were administered to rabbits, with seemingly no limit to the absorption of PN (46). This suggested that passive diffusion is the mechanism of absorption. Experiments using everted sacs of rat small intestine to measure mucosal membrane

concentration and transport of [3H] PN have supported such observations (46).

Using everted rat small intestines, Yamada et al. (46) compared transport of [3H] PN to that of [3H] PL and [3H] PM. The rate of transport across the intestinal wall followed the order: PL>PM>PN, while the rate of accumulation in the intestinal tissue followed the order: PL>PN>PM. In addition, significant amounts of PL were found on the serosal side of the intestine after the transfer of PM, thus pyridoxal appeared to be the principal form to be released from the tissue. Using human subjects, Wozenski et al. (7) compared urinary vitamin B-6 and 4PA excretion after administering small doses of either PL, PN, or PM. Following the PM dose, the rise of vitamin B-6 compounds in the urine was slower than after the PL or PN doses. The different response to the PM dose was judged to be due to a slower absorption and/or metabolism of PM (7).

After absorption of vitamin B-6 into the mucosal cells of the jejunum, the vitamin is phosphorylated. The phosphorylated vitamin is then hydrolyzed prior to its release into the portal vein (49-51). The jejunum appears to protect itself from an excessive accumulation of phosphorylated vitamin B-6 during times of excess dietary vitamin B-6 by regulating its PLP content. This is accomplished by an increase in the activity of the brush border alkaline phosphatase (52). Middleton (53) has demonstrated a maintenance of uptake and phosphorylation of vitamin B-6 vitamers by rat jejunum despite a vitamin B-6 deficiency. It is important that the mucosa maintain its ability to utilize the absorbed vitamin for

its own purposes during periods of greater need, such as vitamin B-6 deficiency. The maintenance of necessary PLP levels in the jejunum may allow effective absorption of other nutrients, such as amino acids (54).

Interconversion and Transport

Following jejunal release, the free forms of vitamin B-6 are transported in the portal circulation to the liver (53). The liver serves as a primary site for interconversion of the vitamers to the coenzyme form, PLP (56). Present knowledge of interconversion pathways and the enzymes involved is shown in Figure 1 and explained below.

PL, PN, and PM are phosphorylated by pyridoxal kinase (EC 2.7.1.35). This enzyme has been detected in all tissues tested (57). The resultant PNP and PMP are converted to PLP by PNP oxidase (EC 1.3.4.5). PNP oxidase activity has been found to be highest in the liver, at lower levels in brain and kidney, and nearly non-existent in lung, heart, pancreas, and muscle (56,58). Interconversion of PNP/PMP occurs as a feature of aminotransfer. The hydrolysis of vitamin B-6 phosphates to their free vitamers is carried out by various phosphatases. Pyridoxal is oxidized in an irreversible reaction by aldehyde dehydrogenase and/or aldehyde oxidase to 4-pyridoxic acid, the main excretory form of vitamin B-6. Direct interconversion of free PN, PL, and PM does not occur in mammalian tissues; the preferred conversion pathway of PN to PLP is via

phosphorylation to PNP and subsequent oxidation to PLP (59).

PLP is the major form of vitamin B-6 found in plasma (60,61). Organ ablation studies using fasted, resting dogs indicated that the liver is the sole source of plasma PLP (62). The uptake of vitamin B-6 by the liver occurs by simple diffusion followed by rapid phosphorylation of the free vitamers. This has been interpreted by Mehansho et al. (56) as a "metabolic trapping". PLP over and above that able to bind with endogenous proteins and apoenzymes is released and found bound in the plasma to albumin, or is hydrolyzed to PL and released into plasma for circulation to other tissues. Though PLP is seen as the major form of vitamin B-6 in plasma, more PL than PLP is released from the liver during vitamin B-6 supplementation (61). This is probably an effect of rapid tissue uptake of the PL released. PLP is tightly bound to plasma albumin through Schiff base formation, while PL is less tightly bound to albumin (62,63). The protein bound PLP is not able to directly enter cells but can be slowly hydrolyzed by membrane bound alkaline phosphatases (62). A small amount of free PLP exists in equilibrium with albumin-PLP.

PL, PN, and PM are taken up by the red cell, converted to PLP and trapped by Schiff base binding to hemoglobin and vitamin B-6 dependent red cell enzymes. Anderson et al. (64) suggest that the red cell serves as an important supply of PL for other tissues. Erythrocyte metabolism of vitamin B-6 is more likely related to the cells' own requirement for vitamin B-6 since there is some evidence that mammalian tissues are able to take up all free B-6 vitamers and perhaps the phosphorylated forms (65).

Tissue Distribution and Storage

PMP and PLP are the prevailing forms of vitamin B-6 found in mammalian tissues. Very small amounts of the unphosphorylated vitamers have been found in liver, brain, and skeletal muscle of rats (66). Sample treatment is very important when making such determinations, for interconversion can occur rapidly. Using rats, Thiele and Brin (67) found the highest concentration of vitamin B-6 in liver, which was followed, in decreasing order of concentration, by kidney, brain, muscle, and heart. Vanderslice et al. (68) report PLP/PMP ratios, from a variety of extraction methods, to range from 0.41-0.67 in the kidney, 0.58-1.04 in the brain, 0.77-2.84 in the liver, and 2.53-5.10 in the muscle.

Vitamin B-6 depletion experiments lend information regarding loss of the vitamin from tissues. In one study, rats were fed diets containing 1.4-35 mg PN HCl/kg for 6 weeks and were then fed a diet deficient in vitamin B-6 for 0-8 weeks (69). Animals were sacrificed at weekly intervals and tissue samples were analyzed for total vitamin B-6 concentration. It was observed that vitamin B-6 was lost from spleen and thymus most rapidly. Kidney and heart vitamin B-6 fell steadily throughout the time of depletion. Brain was most slowly depleted of B-6. Only in liver did a previous high dietary intake of vitamin B-6 slow tissue depletion of the vitamin (69).

Tissue concentrations of vitamin B-6 are affected by the amount of protein in the diet. In rats fed a high protein diet, vitamin B-6

increased in the liver and decreased in other tissues, probably due to induction of vitamin B-6 dependent enzymes in the liver (70). Vitamin B-6 deficiency was aggravated by a high protein diet, resulting in severe decreases of vitamin B-6 in all tissues (70).

Feeding increasing amounts of vitamin B-6 to rats saturated all tissues but plasma and muscle (71). In plasma, PLP is bound by albumin. The capacity of albumin to bind PLP exceeds 800 ug/ml serum, accounting for the continuing rise in plasma PLP values as vitamin B-6 intake is increased (62).

Animal studies indicated that from 60-94% of vitamin B-6 in the muscle is bound as PLP to glycogen phosphorylase, thus phosphorylase is the major binding protein of PLP in muscle (36). With vitamin B-6 deficiency, muscle phosphorylase activity drops to 35% of the normal value, as does total vitamin B-6 content (66,72,73). Liver phosphorylase activity also decreases with vitamin B-6 deficiency but not as markedly as does the muscle enzyme activity (74,75). Krebs and Fischer (26) were the first to estimate that the PLP in phosphorylase represents at least half the total body vitamin B-6 and to suggest that muscle tissue might serve as a reservoir for vitamin B-6.

Black et al. (10) added support to the PLP reservoir hypothesis when they demonstrated expansion of the phosphorylase content of muscle with supplemental vitamin B-6. Rats fed vitamin B-6 at a level ten times that recommended by the NRC displayed steady increases of muscle phosphorylase and total muscle vitamin B-6 throughout the six week study. In contrast, PLP dependent alanine and aspartate

transaminase concentration increased for only two weeks and then reached a plateau. Muscle phosphorylase content did not increase in rats fed 10% of the recommended vitamin B-6 for 10 weeks.

Continued animal research by Black et al. (11) shed more light on the reservoir hypothesis. Feeding a vitamin B-6 deficient diet resulted in a decreased muscle phosphorylase content, as had been reported by others. However, upon measurement of total phosphorylase in an isolated muscle, it was seen that there was no net change in total muscle phosphorylase. Thus phosphorylase content had not decreased, rather the fall in enzyme concentration per gram of tissue was caused by normal tissue growth which appeared to dilute the enzyme present.

Rats suffering from anorexia after 10-11 weeks on the vitamin B-6 deficient diet (anorexia described as consumption of 50% of needed kilocalories) contained significantly lower concentrations of muscle phosphorylase. The researchers then compared muscle phosphorylase content in rats fed a normal diet with that of rats fed 10% normal energy or starved for 1-4 days. Lack of food rapidly and significantly depleted muscle phosphorylase while muscle alanine and aspartate aminotransferase content remained the same or decreased very little. Starvation appears to be the mechanism for preferential release ot PLP from its storage site in muscle tissue rather than vitamin B-6 deficiency itself. Glycogenolysis and a resultant release ot PLP from muscle could support the gluconeogenic enzymes of the liver that play an essential role in maintenance of blood glucose during times of caloric deprivation. Figure 2 illustrates current

understanding of the tissue interrelationships of pyridoxal and pyridoxal 5'-phosphate.

Levels of B-6 Vitamers in Blood

The occurrence of the six forms of vitamin B-6 and the variety of methods used for their analysis has made attempts to determine the major B-6 vitamers in the blood difficult to interpret. Kelsay et al. (76), using hydrolyzed blood, observed a preponderance of PL. Sauberlich and others (60) have indicated the majority of vitamin B-6 in blood or plasma as PLP, which forms PL upon hydrolysis. Whole blood is known to contain nearly equal amounts of PLP and PMP but only trace amounts of PMP are seen in plasma (61,77). PMP appears to be located primarily intracellularly, thus the difference between plasma and whole blood PMP concentration is probably accounted for by PMP contained within the erythrocyte and other cellular fractions (61,66). Plasma, erythrocyte, and whole blood vitamin B-6 values fell rapidly in subjects on a controlled vitamin B-6 depletion diet, and rose following vitamin B-6 supplementation, reflecting the vitamin B-6 intake (60,76,78). In a study by Lumeng et al. (79), plasma PLP of control subjects was found to remain fairly constant over a period of six months when a regular, adequate diet was consumed. A study by Wozenski et al. (7) revealed that an oral dose of only 0.5 mg PN produced significant changes in the plasma concentration of PLP and total vitamin B-6 of subjects fed a controlled diet containing 1.6 mg of vitamin B-6. The sensitivity of

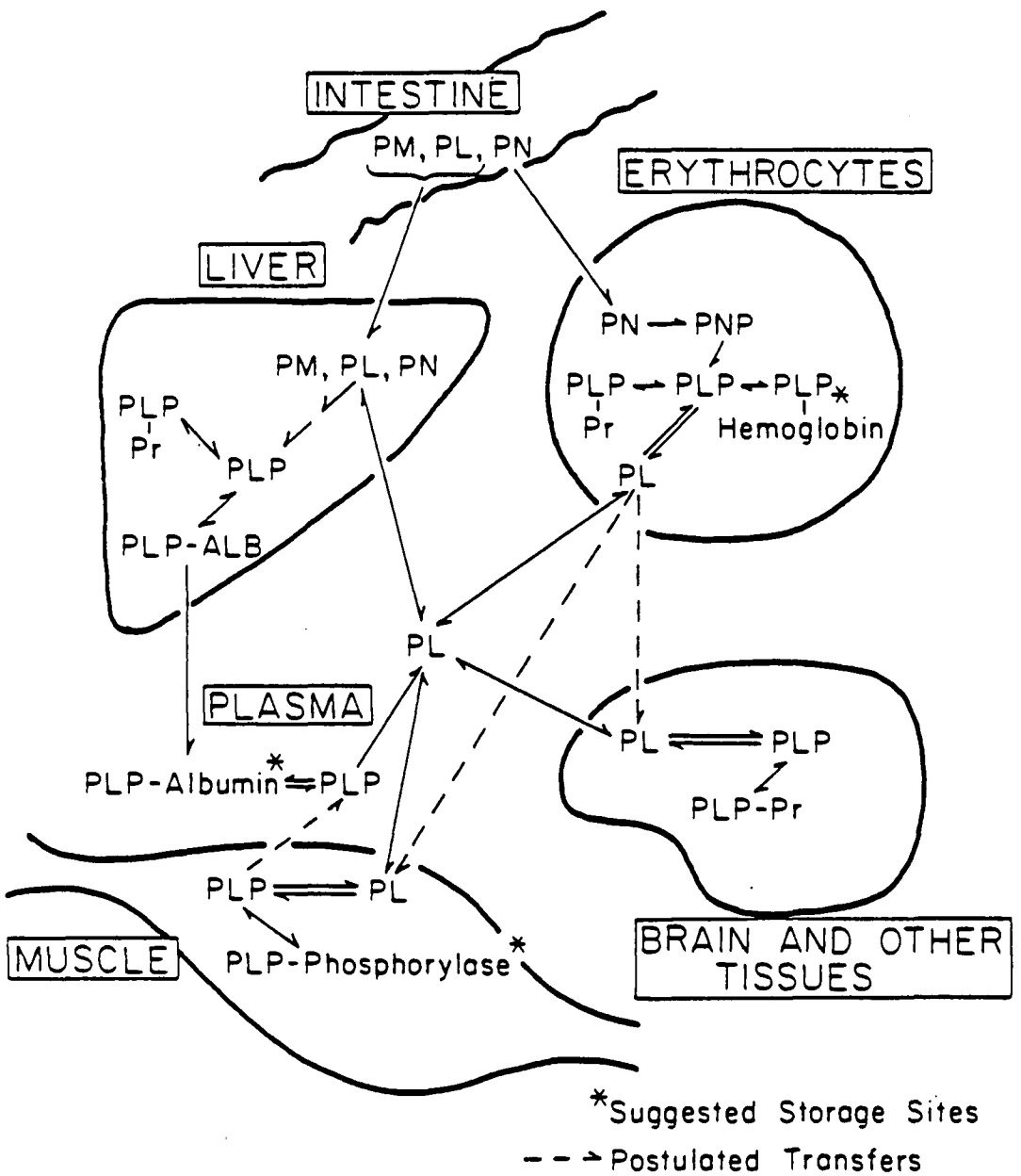

Figure 2 Tissue interrelationships of pyridoxal and pyridoxal 5'-phosphate. (6)

plasma PLP to changes in dietary intake and its correlation with other biochemical parameters of vitamin B-6 metabolism has led to the use of its measurement as an indication of vitamin B-6 status in humans (61,80). Lumeng and coworkers (71) report that in rats, plasma PLP correlated well with PLP content of skeletal muscle, the major storage pool of vitamin B-6.

A variety of investigators have reported normal plasma PLP values that range from 20-105 nM in adult males (79,81,82). Sex differences have been reported, with female plasma PLP values (14.2-79.6 nM) significantly lower than those of males (20.8-93.7 nM) (82,83). There also appears to be a decline of plasma PLP with increasing age (79,81,84). Due to the sensitivity of plasma PLP to the intake of vitamin B-6, an evaluation of plasma PLP must include information regarding dietary intake.

Levels of B-6 Vitamers in Urine

4-pyridoxic acid (4PA) is recognized as the major urinary metabolite of vitamin B-6 in man, accounting for about 50% of the ingested vitamin. Smaller amounts of the free vitamers, along with very little of the phosphorylated forms of the vitamin, are also found in urine (59,60). 4PA and total urinary vitamin B-6 respond quickly to changes in dietary vitamin B-6 (76,78). After 4 days of a vitamin B-6 depletion diet providing male subjects 0.16 mg of PN, urinary 4PA and vitamin B-6 levels decreased to 20% and 40% of their original concentrations (76). After 25 days of the depletion diet,

no 4PA was detected in urine. Supplementation with 0.6 or 0.9 mg of PN after vitamin B-6 depletion resulted in significant increases in 4PA and vitamin B-6 excretion. The increased excretion of 4PA and vitamin B-6 after supplementation was shown to correlate well with the level of vitamin B-6 intake. With consumption of physiological amounts of vitamin B-6, urinary vitamin B-6 was composed of approximately 2/3 PL and 1/3 PM, whereas a 0.16 mg PN/day depletion diet for 4 days caused nearly equal amounts of the two vitamers to be excreted. With normal vitamin B-6 intake, the form of vitamin B-6 taken has little effect on the vitamers found in urine. In contrast, with vitamin B-6 supplementation of above 50 mg, the form taken was found to affect the form excreted. Supplementation with PN was followed by increased excretion of PL and PN; PL supplementation caused increased excretion of only PL; PM supplementation caused increased excretion of both PL and PM. This seems to confirm that the preferred conversion pathway of the B-6 vitamers is to PLP, which is then oxidized and excreted as PL.

Wozenski et al. (7) fed 5 male subjects a diet containing 1.6 mg of vitamin B-6 and then supplemented them with 0.5, 1, 2, 4, or 10 mg of PN. Urine collected at 3, 8, 12, 16, and 24 hours after the dose was analyzed for urinary vitamin B-6 and 4PA. The results indicated that as the PN dose increased, greater amounts of vitamin B-6 and 4PA were excreted in the urine but that the % of the dose excreted as vitamin B-6 and 4PA decreased. In the range of 0.5 to 10 mg of PN, urinary vitamin B-6 fell from 9% to 7% and 4PA fell from 63% to 35% of the vitamin B-6 ingested.

Recommended Intakes of Vitamin B-6

Recommended Dietary Allowances (RDA's) as determined by the National Research Council (NRC) Food and Nutrition Board were first recorded in bulletin form in 1943 (85). At that time vitamin B6 was recognized as essential, yet human requirements for the vitamin were not yet known and an intake recommendation could not be made. The assumption was made that if one obtained enough of the other B vitamins through a varied diet, sufficient vitamin B-6 would be provided. The 1953 revision of the RDA's cited evidence from limited balance studies, in combination with amounts of vitamin B-6 found in a mixed diet, to suggest a daily vitamin B-6 intake of 1-2 mg as adequate (86). Vitamin B-6 remained untabulated in the 1958 and 1964 editions, but the council felt 1.5-2 mg a day a tentatively reasonable adult allowance (87,88). At this point it was felt that vitamin B-6 requirements paralleled those of thiamin, thus calling for greater intakes with increasing caloric needs. The council awaited further information before a more definitive allowance could be rcommended. The 1968 edition of the NRC bulletin tabulated a recommendation of 2 mg vitamin B-6 for adults (89). More recently (1980) the RDA has been revised to 2.2 mg for adult males and 2 mg for adult females (90).

Dietary recommendations for vitamin B-6 have come as a result of investigations utilizing depletion-repletion experiments to include collection of blood and urine for measurement of various parameters

of status. Determination of the RDA has been confounded by evidence that requirements for the vitamin increase with increased protein consumption. In recognition of this relationship, the Dietary Standard of Canada suggests a ratio of 0.02 mg vitamin B-6 per gram of protein eaten (91). Assuming a daily protein consumption of 110 grams by men and 100 grams by women, the NRC recommendation for vitamin B-6 would be adequate. Vitamin B-6 adequacy might be questionable for those with greater protein intakes.

Exercise Metabolism

Skeletal muscle, as the basic means for mobility, is able to adjust its energy production to meet large changes in energy demand. Energy expenditure from rest to a maximum power output of short duration can be increased by as much as 1000 fold in fractions of a second (92). Under some circumstances an increased energy expenditure can be sustained for several hours without rest. The muscle accomplishes these different rates of energy production through the selective use of energy substrates.

Substrate Availability

The substrates immediately available for energy are those stored within the muscle cell, namely the high energy phosphates, adenosine triphosphate (ATP) and creatine phosphate (CP), and glycogen. All can be used anaerobically, thus enabling energy metabolism to begin upon demand. The intracellular content of these substrates is limited such that energy for regeneration of ATP is made available through blood-borne glucose and free fatty acids (FFA). The time course of substrate use is the following:

1. $ATP \rightarrow ADP + Pi + energy$
2. $ADP + CP \rightarrow ATP + creatine$
3. $ADP + glucose\ (glycogen) \rightarrow ATP + lactate$
4. $ADP + O2 + substrate \rightarrow ATP + CO2$

Variation in exercise intensity will influence the choice of substrate throughout exercise and the length of time the effort can be sustained (93). For example, activities demanding maximum intensity such as a 100 m dash can be maintained for only short periods, with available ATP/CP being utilized. The lower the exercise intensity the greater the contribution of energy from aerobic metabolism and the longer the effort can be sustained. Activities can be placed in three general categories to determine the substrate and energy system used for performance (94):

MES: Muscular exertion of high intensity and short duration. Strictly anaerobic activities such as the 100 m dash or weight lifting utilize the ATP/CP system.

MEM: Muscular exertion of moderate intensity and medium duration. The availability of oxygen is limited, thus energy is derived via the ATP/CP system and anaerobic glycolysis. This occurs during events such as football or the 220 m sprint.

MEL: Muscular exertion of low intensity and long duration. Sufficient oxygen is available for aerobic metabolism of FFA. Examples of this

would be long distance activities such as running, swimming, and biking.

The conclusion that protein does not contribute to energy expenditure for muscular work has been based on observations that urinary nitrogen does not significantly increase above resting levels in individuals that have been exercised (92,95,96). More recently a number of authors have provided evidence in support of an active protein utilization during exercise, particularly during long-duration exercise (97-101). Interest in protein metabolism during exercise arose from observations of increased alanine release from working muscle and its uptake by liver during exercise (102). Felig et al. (103) proposed the existence of a glucose-alanine cycle, in which pyruvate-derived alanine carries amino groups rising from transaminations in muscle to the liver for conversion to glucose and urea. Gluconeogenesis from this alanine does not represent a new source of glucose (104). However, Lemon and Nagle (105) suggest that with prolonged glycogen-depleting exercise, as with short term starvation, there is a direct oxidation of the keto acids arising from the transamination of amino acids. Thus in some instances protein may contribute to calories used during exercise, though not to the extent of carbohydrate and fat.

Substrate Utilization

Muscle Glycogen

Skeletal muscle of the average 70 kg man contains about 350 g of glycogen. This locally stored glycogen can be used only by the exercising muscle since muscle lacks the enzyme glucose-6-phosphatase, which would allow glucose to be released into the bloodstream (106). Utilization of muscle glycogen is directly related to exercise intensity, with exercise at 90-100% VO2 max resulting in rapid glycogen degradation (107,108). During anaerobic exercise at this high intensity stored glycogen is never fully utilized. The high rate of glycogen degradation results in a rapid accumulation of lactate in the muscle to values which eventually inhibit further energy production (109). Exercise demanding 75-80% VO2 max will utilize essentially all of the muscle glycogen stores. The amount of glycogen available at the onset of aerobic exercise has been shown to be a determinant of exercise capacity (110,111). Bergstrom et al. (110) demonstrated that greater initial glycogen stores within the exercising muscles allowed subjects to work for a longer period of time until exhaustion.

Blood Glucose and Hepatic Glycogen

Closely related to muscle glycogen utilization are the uptake of glucose by the exercising muscle and hepatic glycogenolysis. Blood glucose itself represents a very small store of energy substrate to resting muscle (less than 2% total oxygen consumption) (112). However,during exercise blood glucose assumes an increasingly important role as an energy source. The uptake of glucose by muscle rises 7-20 times the resting levels after 10-40

minutes of bicycle exercise, the increment depending upon the intensity of exercise (113). This increase in glucose utilization accounts for 30-40% of the total oxidative metabolism of muscle. After 40 minutes of low intensity exercise blood glucose provides 75-90% of the carbohydrate being consumed by muscle, thereby substituting for the gradually diminishing muscle stores of glycogen (113,114).

During up to one hour of low intensity (30% VO2 max) exercise, the major part of the increase in muscle glucose uptake is derived from hepatic glycogen metabolism (115). Since liver glycogen stores are limited to approximately 70-90 g in post-absorptive man, hepatic glycogenolysis can be maintained for a limited period of time. Maintenance of blood glucose for prolonged exercise then necessitates increased gluconeogenesis and decreased glucose uptake by muscle. Indeed, the splanchnic uptake of gluconeogenic precursors (lactate, pyruvate, alanine, and glycerol) increased 2-10 fold after 4 hours of bicycle exercise at 30% VO2 max (115). This uptake of gluconeogenic precursors was sufficient to account for 45% of the hepatic glucose release, as opposed to only 25% at 40 minutes of exercise. Glucose uptake by muscle increases progressively until it peaks at 90-180 minutes and then it declines slightly. Thus as liver glycogen stores are depleted by prolonged exercise of mild intensity, gluconeogenic processes are accelerated and glucose uptake by muscle decreases slightly (113).

The arterial concentration of blood glucose falls gradually during low intensity prolonged exercise (95,115). At rest and up to

40 minutes of low intensity exercise splanchnic glucose production output exceeds muscle uptake such that blood glucose is maintained at near resting levels. As exercise continues beyond 40 minutes, glucose output appears to fail to keep pace with muscle utilization and a fall in arterial glucose concentration results.

The control process for the increased output of glucose by liver during exercise has not yet been fully determined, though it seems in part to be hormonally mediated. A fall in insulin (113), a rise in glucagon (102,115), and rises in plasma catecholamines (116,117) have been observed to occur during exercise. Felig and Wahren (118) examined the effect of maintaining insulin and glucagon at basal levels as well as the effect of hyperinsulinemia on splanchnic glucose output during exercise. Their results indicated that the fall in insulin and rise in glucagon are not essential for exercise to stimulate liver glucose production, but the magnitude of the increased glucose production is dependent on changes in the hormone levels.

Plasma Free Fatty Acids

The storage of triglyceride in adipose tissue accounts for 80-85% of one's body fuel stores, or well over 100,000 kilocalories of energy. In response to hormonal and nervous stimuli, triglyceride is hydrolyzed to FFA and glycerol. FFA are transported in the blood bound to albumin and are delivered to most tissues, where they then require oxygen for their subsequent catabolism. The plasma pool of FFA is relatively small and its turnover may occur

rapidly, especially during times of augmented uptake (119). In the resting state muscle depends almost entirely on FFA for energy, as evidenced by a respiratory quotient of near 0.7 (114).

The FFA mobilized from adipose tissue at rest are more than sufficient to meet the muscle's resting oxidative needs. With the onset of exercise this resting rate of fat mobilization becomes inadequate. An abrupt fall in arterial FFA occurs as the result of an increased rate of removal by working muscle. The rate of FFA release from adipose is then increased and increased arterial levels are observed five minutes after exercise begins (120). As exercise of low (30% VO2 max) or moderate (60%) intensity is continued over a long period of time, there is a progressive increase in plasma FFA concentration and a decrease in the respiratory quotient, indicating an increased reliance upon fat oxidation for energy (95,115,121,122). This was demonstrated in a study by Ahlborg et al. (115) where arterial levels of FFA increased threefold over 4 hours of bicycle exercise at 30% VO2 max, with FFA oxidation representing 65% of the total energy used at 240 minutes.

The uptake and oxidation of FFA by the muscle has been shown to be directly related to the concentration of FFA in the blood (123,124). Thus the utilization of FFA is primarily regulated by the rate at which they are mobilized from adipose tissue. A likely mechanism for the increased release of FFA during exercise is the release of norepinephrine from sympathetic nerve endings in response to muscular contraction (120,125,126). Plasma FFA have been increased above control values in rats and men by injecting heparin

after consumption of fat. The elevated FFA were shown to increase exercise endurance by slowing muscle and liver glycogen utilization and delaying hypoglycemia (127-129). Increased FFA oxidation leads to an accumulation of citrate (127). Citrate inhibits phosphofructokinase and ultimately slows the rate of glycolysis and glucose uptake (127,128,130,131).

Plasma lactate, which accumulates as a result of high intensity anaerobic work, serves to inhibit the release of FFA from adipose tissue. This was first demonstrated in a dog model by Issekutz et al. (132). This action of lactate on FFA release has been suggested as a means to prevent an efflux of substrate that is not of use during anaerobic work (133).

Training

Regular, repeated bouts of exercise expose the organism to a training load of greater intensity, duration, and frequency than that normally encountered, with the effect of improving the functions being trained (92). In general, 3-4 sessions per week for 20-30 minutes per session at an intensity level of 60% VO2 max will regularly improve cardiorespiratory fitness (92,134). A host of physiological and biochemical changes occur with aerobic training that cause the active person to differ from sedentary individuals (16,92,116,135,136,137).

The most objective indication of a training effect is an increase in the maximal aerobic capacity (92). Saltin et al. (138)

showed increases in VO2max of 4% in previously active young men, to 35% in previously sedentary subjects after a 50 day period of physical training. The greater oxygen consumption can be explained in part by increased maximal cardiac output and a wider arteriovenous oxygen difference (137). In addition to these changes in the cardiovascular system, major biochemical adaptations occur in skeletal muscle to increase its capacity for aerobic metabolism (135). Increased myoglobin concentration, which is seen in the muscle of exercised animals, may enhance oxygen transport into muscle mitochondria (135,139). Succinate dehydrogenase activity, considered representative of total mitochondrial protein and oxidative capacity of the muscle (140), is 2.8-3.4 fold greater in trained male runners as compared to sedentary men (141). There are also significant increases in the activity of enzymes considered rate limiting for the oxidation of long chain free fatty acids (142). In contrast, the activity of phosphorylase, phosphofructokinase, pyruvate kinase, and lactate deydrogenase (key glycogenolytic and glycolytic enzymes) are not significantly altered by endurance training (140,141,143).

Other examples of the adaptation to endurance exercise are lower blood (108,111,144,145) and muscle (108,145) lactate concentration, a slower rate of glycogen depletion (111,108), and a lower respiratory quotient (111,108) while performing at the same absolute workload in the trained as compared to the untrained state. Endurance training has been shown to enhance glycogen storage (15). The net effect of adaptation to endurance exercise allows the

trained individual to derive a greater percentage of energy from fat oxidation during submaximal exercise. This results in a decreased utilization of the limited carbohydrate stores and allows a longer performance time to exhaustion (16,135).

Diet and Exercise

As mentioned previously, there is a positive relationship between initial muscle glycogen concentration and exercise time to exhaustion. In turn, muscle glycogen content has been shown to be dependent upon the type of diet eaten. Bergstrom et al. (110) exercised subjects at 77% VO2 max after different diets. A normal mixed diet giving initial glycogen stores of 1.75 g/100 g wet muscle allowed a work time of 114 minutes. After 3 days of a protein and fat diet glycogen content was reduced to 0.63 g/100 g wet muscle and exercise was tolerated for only 57 minutes. When subjects ate a high carbohydrate diet for 3 days muscle glycogen reached 3.3 g/100 g wet muscle and exercise was prolonged for 167 minutes. Thus the inclusion of carbohydrate in the diet has a direct influence on the muscle glycogen stores and, consequently, the capacity for endurance exercise.

In an earlier study by Bergstrom and Hultman (146), subjects exercised one leg to exhaustion on a cycle ergometer as the other leg rested. Muscle biopsy revealed nearly depleted glycogen stores in the exercised legs while glycogen content of the rested legs was normal. A high carbohydrate diet returned glycogen levels to pre

exercise values within 24 hours and after an additional 2 days on the diet they rose to values twofold above the rested legs. The factor causing glycogen supercompensation evidently works locally within the exercising muscle and depends upon exertional depletion of existing glycogen stores.

The rate of muscle glycogen repletion after exhaustive exercise appears to be related to the muscle's glycogen synthetase activity, which is increased when the glycogen content of the muscle is low (147). Despite an activation of glycogen synthetase, skeletal muscle depleted of glycogen shows a slow and incomplete resynthesis of glycogen unless the intake of dietary carbohydrate is high (121). When glycogen depleting exercise was performed on 3 consecutive days during which a mixed diet was fed the effect of depletion was cumulative such that subjects began each day with less stored glycogen (121). When the same subjects ate a high carbohydrate diet (70% of kcals as carbohydrate), muscle glycogen was nearly complete within the 24 hours between each exercise period (148). Increased insulin levels, as a result of dietary carbohydrate, may play an additional role in glycogen synthetase activity (148-150). In general, glycogen synthesis after depletion increases in proportion to the amount of carbohydrate consumed. A diet containing 70% of the calories as carbohydrate is recommended for distance athletes involved in strenuous training (13,151).

The observations of Bergstrom and coworkers led Astrand to propose a carbohydrate loading regimen for endurance athletes (92,152). First, an exhaustive training bout is performed on a day

that is one week before the event. This is followed by 3 days of an almost exclusively fat and protein diet, along with exercise, to keep the glycogen content of the muscles low and presumably to increase glycogen synthetase to very high levels. The athlete should then eat a high carbohydrate diet, while reducing exercise, during the remaining days before competition to permit maximal glycogen storage. Carbohydrate loading in this way has been shown to increase the storage of muscle glycogen, but not without some problems. Subjects often find training difficult, and show signs of hypoglycemia during days on the low carbohydrate diet. Such drawbacks led Costill and Miller (151) to propose that the 3 days of low carbohydrate intake be substituted by a mixed diet. Indeed, muscle glycogen levels similar to those found after Astrand's "classical" regimen were measured in runners who followed the modified regimen (13).

Dietary attempts to increase plasma FFA levels, thus delaying muscle glycogen depletion, have been relatively unsuccessful (151). Coffee and tea, however, have been found to promote fat oxidation and improve endurance performance when taken 1 hour before exercise. Since subjects reported a less subjective effort with caffeine ingestion, researchers concluded that part of the performance improvements were related to the stimulating effect of caffeine on the central nervous system (153).

Sex

Men have generally been used as subjects for exercise research, though the increased participation of women in sports has stimulated a greater use of female subjects in recent years. It is of interest to review possible differences between the sexes in regards to exercise metabolism. There has been some investigation into the role of the female menstrual cycle on parameters of exercise. Bonen et al. (154) determined that when nutritional status is normal, substrate and hormonal responses to exercise are similar for the luteal and follicular phase of a woman's cycle. In another study, female subjects exercised on 5 different days during their menstrual cycle (155). There was no change in the balance of fuels used at a given workload as a function of the menstrual cycle.

The typical female described in the literature has a lower aerobic capacity, less strength, and more fat that her male counterpart (92). Nevertheless, women achieve a training response to regular aerobic exercise that is similar to that seen with men (111,156). Costill et al. (157) reported similar muscle enzyme activities and fiber composition between male and female track athletes, though males had larger muscle fiber area. Among these trained men and women who were similar in VO2 max, training, and fiber composition, there was no difference in the amount of energy derived from fat during a 60 minute treadmill run at 70% VO2 max (157). There also was no significant sex difference in the glycogen content of muscle (158). Nygaard (159) reports no difference between male and female subjects in their capacity for muscle glycogen storage.

Though it has been substantially narrowed in the past few years, a sex difference in distance running performance is commonly observed. Times by men for distances run are 10-30% faster than those of women (160-162). Researchers have attempted to determine the factors responsible for the performance difference. When trained male subjects exercised with extra weight to equalize the body fat difference between similarly trained women subjects, the sex difference in VO2 max (ml/min/kg total weight) was reduced by 65% (163). An additional study concluded that for men and women similarly trained, the sex difference in a 12 minute run performance is primarily due to differences in % body fat and VO2 max (ml/min/kg fat free weight) (164). Pate et al. (165) matched men and women according to similar performance in a 15 mile road race and asked them to complete a treadmill run to exhaustion. Percent body fat was determined and blood samples were analyzed for hematocrit, hemoglobin, and lactate. Comparison of the data indicated that when male and female distance runners display equal performance capacity, they are similar in body composition and in their cardiorespiratory response to exercise, though men have a significantly greater hemoglobin concentration (165). Thus it appears that the performance differences between men and women revolve around biological differences in % body fat and oxygen carrying capacity, which cause females to utilize more oxygen per unit weight at any given load. However,these differences do not appear to affect a woman's ability to metabolize fuels for exercise.

Metabolic Roles of Vitamin B-6 in Exercise Metabolism

PLP, as the active form ot vitamin B-6, plays an important role in energy metabolism during exercise as the cofactor for key gluconeogenic and glycogenolytic enzymes. PLP is the coenzyme for muscle and liver aminotransferases that maintain blood glucose during prolonged exercise via the branch chain amino acid-alanine-glucose cycle. The arterial concentration of alanine has been shown to rise 40% above resting levels after 40 minutes of low intensity exercise (115). During this same exercise period the contribution to glucose production from hepatic gluconeogenesis rose progressively over 4 hours of exercise (115). It appears that as muscle glycogen stores are depleted, gluconeogenic processes are accelerated.

As mentioned previously, PLP is the cofactor for glycogen phosphorylase. Phosphorylase, the rate limiting enzyme of glycogen degradation, catalyzes the phosphorolytic cleavage of glucose residues ot glycogen. The role of PLP in phosphorylase activity is not of the conventional Schiff base mechanism, though it has been recently established that the 5' phosphate group of PLP does play a catalytic role (166). Sansom et al. (37) present proposals on the contribution of the 5' phosphate group to phosphorylase catalysis, which include PLP as a nucleophile, an acid/base, and an electrophile.

The activity of phosphorylase is thought to be regulated by a shifting between phosphorylase b, the less active dimeric form, and

phosphorylase a, the more active tetrameric form (106). Toews et al. (167) obtained muscle biopsies from male subjects who had exercised to exhaustion for the purpose of determining the regulatory factors controlling muscle glycolysis during exercise. Results indicated that increased glycolysis during heavy exercise is derived primarily from glycogenolysis controlled by phosphorylase. Interestingly, the increase in glycogenolysis was not accompanied by a change in the phosphorylase a:total phosphorylase ratio. The authors concluded that the control of glycogenolysis during exercise must be by other than the b→a conversion of phosphorylse (167).

Black et al. (10,11) demonstrated in rats that phosphorylase acts as a reservoir for vitamin B-6. When animals were fed excess vitamin B-6 in their diet for 6 weeks, muscle phosphorylase activity increased steadily (10). When animals were fed a partial starvation diet, phosphorylase activity decreased (11), presumably with a concomitant release of PLP. During starvation it would be advantageous for the liver to have an endogenous source of PLP for gluconeogenic processes. Unfortunately Black and his coworkers did not look at blood levels of PLP in their animals.

It is not known if phosphorylase activity increases in man when fed excess vitamin B-6. Lawrence et al. (1) supplied trained and untrained swimmers with a 17 mg PN HCl supplement each day during a 6 month period. There was no difference in swim performance between the supplemented and placebo group at any time during the study. Significantly higher post exercise serum lactic acid levels were found in the swimmers taking the supplement. Though the researchers

could not explain the reason for such an increase in serum lactate, it is possible that the vitamin B-6 supplement imcreased levels of glycogen phosphorylase. An increase in phosphorylase activity may have caused glycogen to be utilized anaerobically to a greater extent in the supplemented group.

Endurance training increases the muscle mitochondrial levels of alanine transaminase and aspartate transaminase (135,168). Phosphorylase activity does not increase after long distance endurance training (135,169), but does increase after high intensity anaerobic exercise (170).

Influence of Exercise on Vitamin B-6 Metabolism

Russian investigators have shown an increased excretion of the vitamin B-6 metabolite, urinary 4PA after swimmming in rats (8) and after physical activity in humans (9). Wozenski (171) was the first from this laboratory to observe an alteration in vitamin B-6 metabolism after exercise in a human subject. An unexpected increase in PLP was detected in a subject's plasma 2 hours after a 0.5 mg oral dose of PN had been given. The subject reportedly had been running between the time of the PN dose and the 2 hour blood draw. Further investigations in this laboratory have yielded additional information about the effect of exercise on vitamin B-6 metabolism (3-6).

Leklem and Shultz studied vitamin B-6 metabolism in male adolescent cross country runners (3). Significant increases in plasma PLP and total plasma vitamin B-6 were observed in all subjects after three 1500 m runs. The researchers hyupothesize that exercise, viewed as an acute form of starvation, causes PLP to be released from muscle glycogen phosphorylase into the plasma.

Munoz (4) compared the vitamin B-6 responses to exercise in adolescent versus adult males, trained versus untrained males, and the response to running versus cycle ergometer exercise. Significant increases in plasma PLP and vitamin B-6 (PB6) were seen in all subjects after all exercise bouts. The post exercise increase of PLP in adult males after the bicycle exercise averaged 1.05 nmol/100 ml plasma. This increase is similar to increases reported 0.5-1 hour after the ingestion of 1-2 mg of pyridoxine (7). No significant differences were observed between trained and untrained subjects or between running and cycle exercise. Trained adult males did have significantly greater changes in PLP and PB6 when compared to adolescent males. Munoz suggested that this result was due to the larger muscle mass, thus the larger PLP store, of the trained adult male as compared to the adolescent male. Also significant was the drop in plasma PLP and PB6 post 30 minutes of exercise as compared to the increase immediately after exercise. The return to pre exercise levels may be an indication that the mobilized PLP was taken up by other tissues or metabolized and excreted.

Leklem et al. (5) compared the plasma PLP response to cycle ergometer and running exercise in trained men and women.

Significant increases in PLP were seen in all subjects after all exercise sessions. Plasma volume changes, calculated from hematocrit values, may have accounted for some, but not all, of the PLP changes seen after exercise. Cycle exercise resulted in PLP increases of 1.05 + 0.06 nmol/100 ml in men and 0.64 + 0.33 nmol/100 ml in women. Following the run, plasma PLP increased 1.73 + 0.65 nmol/100 ml in men and 0.65 + 0.36 nmol/100 ml in women. The smaller change in PLP in women as compared to men was most likely due to their smaller muscle mass, similar to the difference in adult males versus adolescent males noted previously.

Hatcher (6) studied the effects of a carbohydrate loading diet regimen and vitamin B-6 supplementation on vitamin B-6 metabolism during 50 minutes of cycle exercise. Four trained male cyclists were used as subjects. Plasma PLP and PB6 levels increased significantly after all exercise sessions. At 60 minutes post exercise plasma PLP and PB6 were significantly less than immediately post exercise. Exercise following the low carbohydrate diet resulted in a smaller increase in plasma PLP and PB6, presumably because of the lower level of PLP stored in the muscle due to the low carbohydrate intake. As compared to the day before exercise, a decreased excretion of urinary 4PA was observed after exercise in combination with the low carbohydrate diet. This could have been due to a combination of the low vitamin B-6 (1.55 mg) and the high protein (194 g) content of the high carbohydrate diet, and the increased demand for gluconeogenesis to maintain blood glucose during an exercise bout performed with low muscle glycogen stores.

Exercise after supplementation with 8 mg of vitamin B-6 resulted in greater increases in plasma PLP and vitamin B-6 than after exercise following unsupplemented diets, suggesting an increased storage of PLP in muscle.

deVos (12) investigated fuel metabolism during exercise in conjunction with Hatcher's diet and exercise study. The addition of supplemental vitamin B-6 to the high carbohydrate diet resulted in elevated plasma lactate levels post exercise when compared to the normal or low carbohydrate diets, though the increase was not significant. deVos concluded that vitamin B-6 supplementation (assuming increased phosphorylase activity as a result) may cause a more rapid emptying of muscle glycogen stores, which could then reduce endurance capacity.

III. Methods and Materials

Overview

A cooperative study was designed and conducted by several researchers, to determine the effect of vitamin B-6 and carbohydrate level of the diet on vitamin B-6 and fuel metabolism during exercise in aerobically trained and untrained young women and untrained post-menopausal women. The entire study involved 10 young women and 8 post-menopausal women. This paper focuses on 2 parameters of vitamin B-6 metabolism, plasma vitamin B-6 and urinary 4-pyridoxic acid, in the young trained and untrained women.

Subject Recruitment and Selection

Females aged 20-30 were recruited for this study through announcements made in Oregon State University health and nutrition classes and those placed in campus newsletters. From the 30 applications received, ten women meeting the following initial criteria were chosen as subjects:

1) Maintenance of normal body weight over the past year.
2) No use of oral contraceptives or vitamin/mineral supplements in the past six months.
3) Non-smoking.

4) Willingness to adhere to dietary restrictions throughout the seven experimental weeks.

Five of the ten subjects were identified as aerobically trained (134) and five as untrained, based upon reported physical activity histories. Subjects were given detailed information regarding the Oregon State University Human Subjects Committee approved experimental protocol and asked to sign an informed consent after receiving physician approval for study participation. Two weeks before the study began, subjects reported to Milam Hall metabolic laboratory in the fasting state. At this time, ten ml of blood were drawn and delivered to Good Samaritan Hospital for analysis to include values for glucose, creatinine, blood urea nitrogen, uric acid, total protein, albumin, cholesterol, sodium, potassium, chloride, calcium, alkaline phosphatase, total bilirubin, lactic acid dehydrogenase, phosphorus, glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, and triglyceride. A 5 gram xylose absorption test was also administered to subjects to screen for possible malabsorption (172).

Baseline Subject Data Collection

Subjects reported to the OSU Human Performance Laboratory one week before the study began for the collection of baseline data to determine group characteristics and to establish VO2 max (See Table 1.) The data collected included height, weight, and skinfold

TABLE 1 Physical statistics for trained and untrained groups.

Group	Mean ± S.D.	Range
Trained (n=5)		
Age (yr)	25.6±4.0	20-30
Height (cm)	158.3±3.5	153.8-162.5
Weight (kg)	55.8±6.0	47.7-64.5
Body Fat (%)[1]	20.1±4.2 (1)	13-24
VO2 max (ml/kg/min)[2]	40.5±3.7 (2)	37.1-46.5
Untrained (n=5)		
Age (yr)	24.4±3.2	21-29
Height (cm)	161.8±6.3	153.8-170.0
Weight (kg)	55.9±6.9	48.2-65.0
Body Fat (%)	20.4±1.5	18-22
VO2 max (ml/kg/min)	32.7±4.5	25.9-37.0

1. Percent body fat determined by skinfolds measured at seven sites(173).

2. VO2 max determined by graded exercise testing using the procedure of Wilmore and Costill (174).

measurements. Seven skinfold sites were used to determine percent body fat (173). Maximum oxygen uptake (VO2 max) was determined using a Monark bicycle ergometer (Quinton Instruments, Seattle, WA.). The cycle was adjusted so that the leg had a slight bend at the knee when the pedal was in the "down" position. This seat height was recorded for future use. The pedal rate was kept constant at 50 rpm with the aid of a metronome. Heart rate was monitored with an electrocardiograph (Quinton Instruments, Seattle, WA.) using a torso mounted limb lead system, with the negative reference electrode place at C5. Subjects initially pedaled three minutes with no resistance. Thereafter the exercise rate was increased by 150 kpm/min each three minutes until the subject could no longer maintain the 50 rpm pedal rate. Heart rate and oxygen consumption were measured during the last minute of each workload. Oxygen uptake was considered maximum when measured oxygen uptake reached a value that did not increase despite an increase in workload (174).

Subjects completed dietary intake records for 3 days prior to their VO2 max test. The diet records were used to estimate their current caloric and nutrient intake. Subjects also completed a calendar of their previous two months' menstrual cycles.

Experimental Design

A summary of the experimental design is presented in Figure 3. The diet and exercise study consisted of seven experimental weeks. An exericse session was administered at the end of each of four

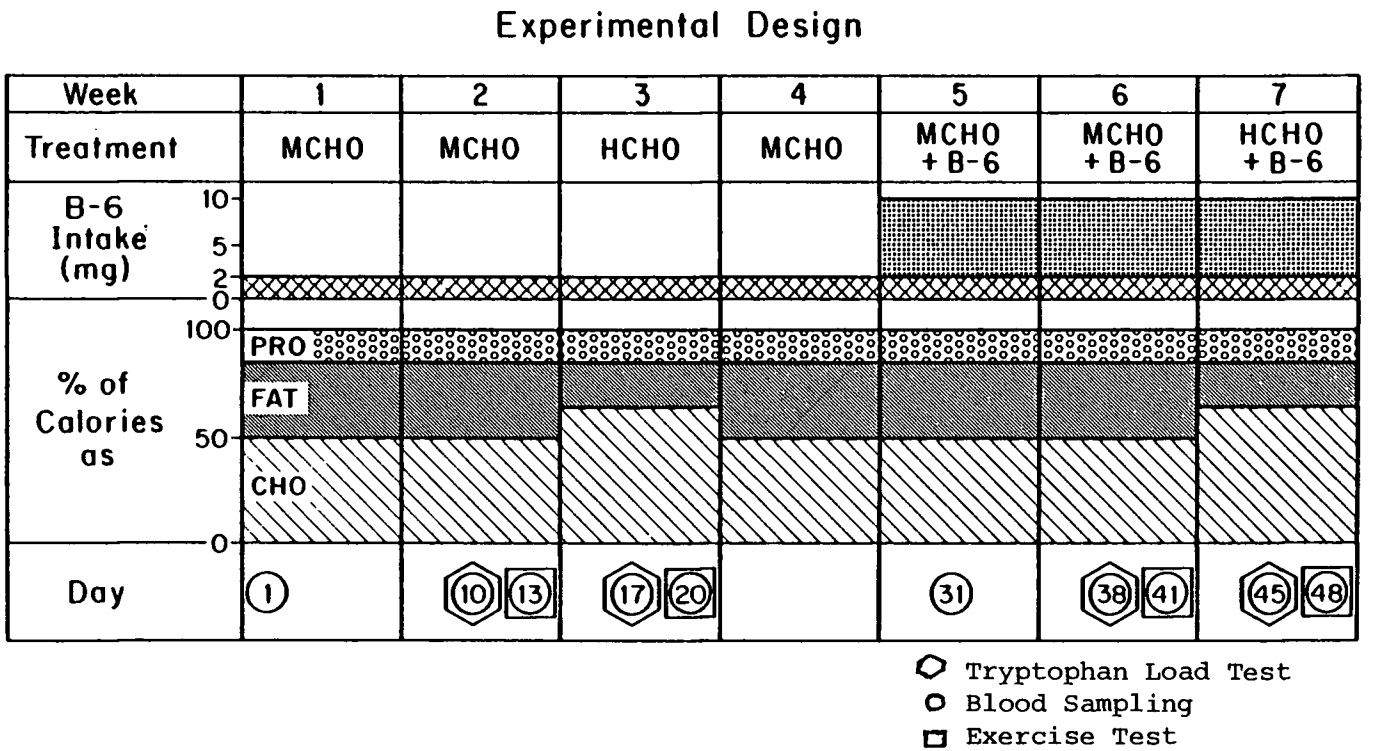

Figure 3 Experimental Design

dietary treatments. Subjects were fed a moderate carbohydrate diet (MCHO) during weeks 1 and 2. A high carbohydrate diet (HCHO) was fed week 3. At week 4 subjects were returned to the MCHO diet. No exercise session followed week 4. During weeks 5 and 6 the MCHO diet was fed with the addition of a daily vitamin B-6 supplement (MCHO+B-6). Throughout week 7 subjects were fed the HCHO diet plus supplemental vitamin B-6 (HCHO+B-6). Diet and exercise are explained in further detail below.

Diet

The design of the metabolic study includes four isocaloric dietary treatments referred to as moderate carbohydrate (MCHO), high carbohydrate (HCHO), MCHO plus supplemental vitamin B-6 (MCHO+B-6), and HCHO plus vitamin B-6 (HCHO+B-6). The diet is presented in Table 2.

The moderate carbohydrate diets (MCHO and MCHO+B-6) provided 49% of the total kilocalories (kcal) as CHO, 35% of the kcal as fat, and 16% of the kcal as protein. High carbohydrate diets (HCHO and HCHO+B-6) provided 63% of the kcal as carbohydrate, 22% of the kcal as fat, and 15% of the kcal as protein. The MCHO diet was manipulated with the intention of increasing the % of kcal from carbohydrate without changing protein. To increase kcal from carbohydrate, kcal from fat were decreased. This was accomplished without significantly altering the food items served. For example, skim milk replaced whole milk and ice milk replaced ice cream.

Non-supplemented diets (MCHO and HCHO) were developed to

TABLE 2 Composition of moderate and high carbohydrate diets.

Moderate Carbohydrate		High Carbohydrate	
Item	Grams	Item	Grams
BREAKFAST		BREAKFAST	
Wheat flakes, fortified	30	Wheat flakes, fortified	30
Milk, whole	200	Milk, skim	200
Raisins	20	Raisins	30
Muffin	40	Muffin	40
Orange juice, frozen reconstituted	170	Orange juice, frozen reconstituted	170
LUNCH		LUNCH	
Bread, whole wheat	50	Bread, whole wheat	50
Tuna, water pack	60	Tuna, water pack	60
Lettuce	10	Lettuce	10
Dill pickle	15	Dill pickle	15
Mayonnaise	24*	Salad dressing	14*
Egg white, cooked	45	Apple juice	200
Carrots, raw	50	Carrots, raw	50
Peaches, light syrup	100	Peaches, light syrup	100
Vanilla wafers	16	Vanilla wafers	32
DINNER		DINNER	
Rice, brown	45	Rice, brown	40
Milk, whole	200	Rice, white	20
Bread, white	25	Milk, skim	200
Turkey breast	60	Bread, white	50
Pears, light syrup	100	Turkey breast	60
Lettuce	50	Pears, light syrup	125
Cabbage, red	15	Lettuce	50
French dressing	20*	Cabbage, red	15
Carrots, canned	15	French dressing	20*
Green beans, canned	100	Carrots, canned	15
Ice cream	70	Peas, frozen	30
Margarine	15*	Ice milk	70
		Margarine	15*
		Honey	25*

* used to adjust calories

provide the subject with 2 mg of vitamin B-6. Supplementation with 8 mg of vitamin B-6 (MCHO+B-6 and HCHO+B-6) would provide each subject with 10 mg of vitamin B-6 during those diet periods. Analysis ot food composites throughout the study revealed that the MCHO and HCHO diets contained 2.3 and 2.4 mg vitamin B-6, respectively, with supplementation increasing that value by 8 mg.

Supplementation of the 8 mg of vitamin B-6 during MCHO+B-6 and HCHO+B-6 occurred via daily ingestion of PN-HCl in a 0.25% acetic acid solution. The solution was prepared in one large batch to contain 8 mg of vitamin B-6 per 10 ml. 10 ml portions were then frozen in vials at -20 C. Daily doses were thawed in the dark of a refrigerator for consumption by subjects with their breakfast. Subjects were instructed to rinse their emptied vial several times and to drink the rinse water.

Subjects were able to regulate their daily coloric intake within a small range through the discretionary use of margarine and french dressing. Margarine and dressing were weighed into containers marked with subject initials and number. If a subject did not consume all margarine or dressing, the remaining was returned, weighed, and that weight was recorded.

Food items for the metabolic study were purchased in one lot when possible. Exceptions to this were the fresh milk, eggs, lettuce, and cabbage. Milk was purchased weekly from a local dairy. Eggs were purchased biweekly from the OSU Poultry Science Department. One half ot the necessary cabbage and lettuce was purchased prior to the study and the remaining half at week 4.

Individual food portions were weighed to 0.01 gram. Food composites were prepared each Monday for analysis of total vitamin B-6 content.

Exercise

Subjects reported to Milam Hall on day 5 or 6 of weeks 2, 3, 6, and 7 at a designated time between 5:30 and 9:30 AM for each of the four exercise sessions. Subjects exercised at the same time on the same day of the week for each exercise session. The exercise session protocol is depicted in Figure 4. After resting for 10-15 minutes upon arrival, subjects were weighed and a pre-exercise blood sample was drawn. Blood pressure was then determined and recorded. Subjects were prepared for electrocardiograph monitoring using a Blackburn CM-5 lead pattern. Supine and seated ECG data was recorded. Subjects were assigned to exercise on one of two Monark cycle ergometers.

Subjects cycled at a predetermined warm-up workload which caused a heartrate of just over 100 beats per minute for 5 minutes. After the warm-up, subjects exercised for 20 minutes at an intensity that represented 80% of their determined VO2 max. Blood pressure was measured at the halfway point. At the end of the 20 minute ride, the workload was removed and subjects were instructed to continue pedaling for an active recovery of 5-10 minutes. A blood sample was drawn 2-3 minutes into the active recovery. Subjects were weighed after exercise and asked to rest quietly for 60 minutes without drinking or eating. Blood samples were drawn at post 30 and

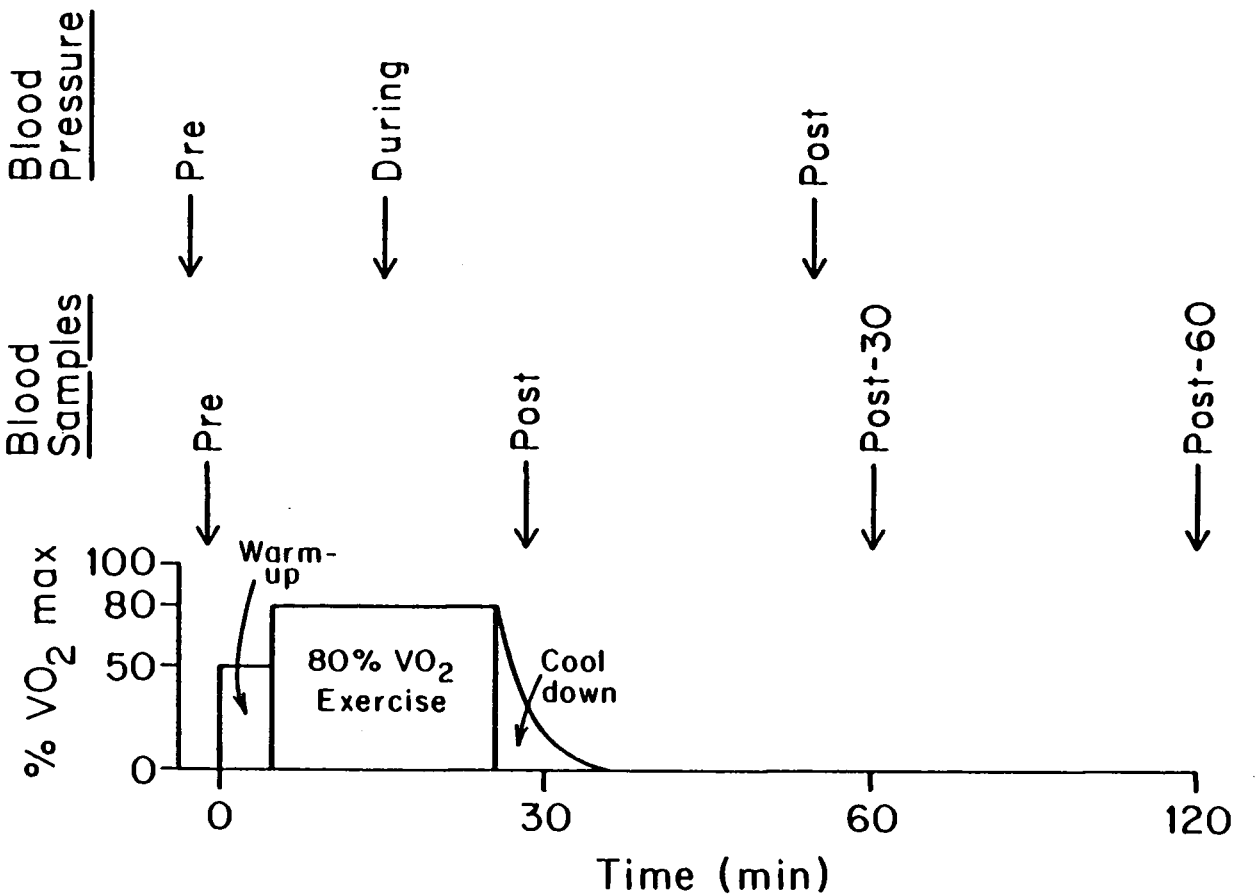

Figure 4 Exercise test protocol and blood sampling times.

post 60 minutes. Blood pressure was also recorded at these times. Subjects were allowed to eat breakfast after the post 60 minute samples were collected.

Daily Procedure

The OSU Foods and Nutrition metabolic laboratory, Room 105 Milam Hall, was the site of food preparation and meal consumption throughout the experimental period. Subjects were instructed to eat only the food provided. Alcohol and nutritional supplements were strictly prohibited. The consumption of caffeinated beverages was not allowed on the day before or the day of exercise.

Subjects reported to the laboratory each morning where they were weighed before receiving breakfast. Subjects recorded their previous day's activity on daily activity sheets. The activity sheets were also used to report approved drug use, beverage consumption, completeness of urine samples, and general feelings of well-being.

Weekday meals were eaten in the laboratory. Sack lunches were made available upon request. Foods for weekend, noon, and evening meals were packaged so that subjects could take them after breakfast for home preparation. Subjects were instructed to eat all food provided and to rinse utensils with water and drink the rinse water.

Sample Collection

Two registered medical technologists handled all blood drawing

responsibilities during the diet-exercise study. 20 ml samples were drawn from the anticubital vein of the forearm into heparinized tubes. Figure 4 illustrates the timing of blood draws with respect to exercise and recovery during each exercise session. In addition to exercise days, 20 ml blood was drawn on day 1 and on Wednesday of weeks 2, 3, 6, and 7. Plasma was separated and stored at -40° C. until analyzed.

Urine was collected in daily 24-hour samples under toluene. Each new day began after the first morning void of the bladder. Subjects were urged to begin urine collections at the same time each day. Urine was measured daily and samples were frozen at -20° C until analyzed.

Sample Analyses

Diet Composites

Composites of the experimental diet were made each week during the study. Foods were prepared and weighed as if for subject consumption and then divided into animal, plant, and dairy products. Composites were prepared by blending together the weighed foods with enough weighed distilled water to aid blending. The total weight was recorded and blended composites were stored at -40° C until analyzed. Aliquots of 1-2 mg were analyzed for total vitamin B-6 by an AOAC method (175).

Blood Analyses

Hemoglobin (Hgb) and hematocrit (Hct) were determined immediately following blood samplings. Hct analysis was performed in triplicate using the microhematocrit method. Hgb was determined in triplicate by the cyanomethemoglobin method. Following exercise sessions 1 and 2, Hct and Hgb were performed using a Coulter counter.

Plasma vitamin B-6 was determined using the microbiological method of Miller and Edwards (176). Saccharomyces uvarum 4228 #9080 (Am. Type Culture Collection), an organism that is responsive to the nonphosphorylated forms of vitamin B-6, was used for the assay. Due to limited sample, it was not possible to run duplicates. The 16 exercise plasma samples from each subject were determined in the same assay. An inter-assay control sample averaged 7.48 $\pm$ 0.46 nmol/100 ml (n=14), with a variablility of $\pm$ 6.0%.

Working in subdued light, 2 ml plasma was precipitated with 10 ml 10% TCA in centrifuge tubes and stirred 3-4 times during a 30 minute wait. The tubes were then centrifuged for 10 minutes and the supernatant was collected. A second and third washing of the samples was done using 5 ml TCA and the tubes were again centrifuged for 10 minutes. All supernatants were collected in 25 ml beakers and the precipitant discarded. The beakers were covered with watchglasses and autoclaved for 30 minutes at 102kPa to hydrolyze all the phosphorylated forms of vitamin B-6. When cool, the pH of the hydrolysates were adjusted to 4.5 with dilute KOH. Samples were then transferred to 100 ml glass stoppered cylinders.

Unsupplemented samples were diluted 2:1 with redistilled water, while supplemented samples were diluted 20:1.

Urine Analyses

Urinary creatinine was determined on a Technicon Autoanalyzer (Technicon Corporation, Tarryton, NY) using a modification of the method ot Pino et al. (177).

Urinary 4-pyridoxic acid was analyzed using the method of Reddy et al. (178). In this method, ion exchange resins serve to separate 4PA from interfering compounds in the urine. 4PA was determined by fluorescence using an Aminco Bowman Spectrophoro fluorometer (American Instrument Co, Inc. Silver Springs, MD). Urine samples from day 1, day 10, and the day before and the day of all exercise sessions were analyzed for each subject. All samples for each subject were analyzed in the same run, including at least 2 recoveries. An inter-assay control sample averaged 8.70 $\pm$ 0.74 umol/24 hr (n+35), with a variability of $\pm$ 8.5%.

Statistical Analyses

A three factor plot partially blocked ANOVA design with groups and diets as the whole plot factors and time (of blood or urine sampling) as the split plot factor, was used for the analysis of data. When significant interactions occurred, the main effects were

no longer considered of interest. The significant interactive effects were examined by performing hypothesis testing on differences between treatment means and on several contrasts of treatment means, where contrasts is defined as a comparison of two or more treatment means. The contrasts to be tested were determined before data was collected. Least significant difference was the test criterion used in testing the multiple comparisons (179).

For hypothesis testing "a" represents group, "b" represents diet, and "c" represents time. The letters are subscripted to indicate the specific group (1=trained, 2=untrained), diet (1=MCHO, 2=HCHO, 3=MCHO+B-6, 4=HCHO+B-6), and time (1=pre, 2=post, 3=p30, 4=p60 for plasma samples, and 1=day before exercise, 2=day of exercise, for urine samples). As an example, $a_1b_1c_1$ designates the mean or group 1 (trained) on diet 1 (MCHO) at time 1 (pre-exercise for plasma or day before exercise for urine). When the two groups were combined (n=10) "a" was ommitted from the hypothesis. The hypotheses tested were $(b_1c_1 - b_1c_2)$; $(b_1c_2 - b_1c_4)$; $[(b_1c_2 - b_1c_1) - (b_2c_2 - b_2c_1)]$; and $[(b_1c_2 - b_1c_4) - (b_2c_2 - b_2c_4)]$. These tests were repeated for all diets. The results were determined to be significant if the p value was less than the critical value of $\alpha = 0.05$.

IV. Results

Blood Analyses

Plasma Vitamin B-6

The concentration of plasma vitamin B-6 (PB6) was measured in aerobically trained and untrained young women for day 1, day 10, and before and after exercise to observe the effects of diet, exercise, and state of training on vitamin B-6 metabolism. The group mean PB6 values are presented in Tables 3 and 4. Values for each individual at each time and diet can be found in the appendix.

PB6 values ranged from 37.0 to 61.9 nM (x for untrained = 49.4 $\pm$ 8.68 nM, x for trained = 68.3 $\pm$ 16.4 nM) on day 1 of the diet-exercise study. These values increased to within a range of 32.5 to 112 nM (x for untrained = 73.8 $\pm$ 10.0 nM, x for trained 60.7 $\pm$ 16.7 nM) by day 10 and did not increase significantly for the remainder of time that the subjects were fed the non-supplemented diets. Subject #5 took antibiotics under the direction of her physician for the first 2 weeks of the study. The effect of the medication was to lower her PB6 values throughout that time period. On the day of the first exercise session (day 12 or 13) the PB6 values ranged from 37.9 to 112 nM (x for untrained = 81.2 $\pm$ 18.7 nM, x for trained = 58.1 $\pm$ 11.4 nm). The carbohydrate level of the diet had no significant effect on the pre-exercise PB6 values, though there was an expected main effect of diet indicating a significant difference between the non-supplemented and supplemented diets

TABLE 3 Group mean plasma vitamin B-6 values for day 1 and day 10 of the study.

	Groups		
	UT	T	COM
		(nM)	
Day 1	49.4 ± 8.69[2]	68.3 ± 16.4	58.9 ± 16.2
Day 10	73.8 ± 10.0	60.7 ± 16.7	71.1 ± 20.1

1. UT=untrained (n=5), T=trained (n=5), COM=groups combined (n=10)
2. Mean ± S.D.

TABLE 4 Group mean plasma vitamin B-6 values and differences from pre to post, post to p60, and pre to p60 minutes of exercise for all the exercise tests.

Test [1]		Groups [3]			Difference	Groups		
		UT	T	COM		UT	T	COM
		(nM)				(nM)		
MCHO	1 [2]	81.2 [4] ±18.7	58.1 ±11.4	69.7 ±19.3				
	2	94.6 ±21.8	66.2 ±12.3	80.4 ±22.7	pre-post	+13.4	+ 8.1	+10.7
	3	87.7 ±10.6	60.4 ±10.7	72.5 ±17.2				
	4	75.6 ±18.0	55.6 ±9.70	65.6 ±17.5	post-p60	-19.0	-10.6	-14.8
					pre-p60	- 5.6	- 2.5	- 4.1
HCHO	1	85.1 ±23.1	65.3 ±8.00	75.2 ±19.9				
	2	94.8 ±29.8	82.8 ±19.2	88.8 ±25.8	pre-post	+ 9.7	+17.5	+13.6
	3	80.4 ±23.5	62.6 ±7.00	68.5 ±16.9				
	4	68.9 ±9.30	61.6 ±8.00	64.9 ±9.30	post-p60	-25.9	-21.2	-23.9
					pre-p60	-16.2	- 3.7	-10.3
MCHO+B6	1	296 ±70.8	257 ±15.5	277 ±54.9				
	2	325 ±76.0	275 ±29.8	300 ±63.0	pre-post	+29.0	+18.0	+23.0
	3	301 ±70.0	267 ±15.9	284 ±53.6				
	4	285 ±65.1	248 ±14.4	267 ±50.6	post-p60	-40.0	-27.0	-33.0
					pre-p60	-16.2	-3.70	-10.3

TABLE 4 Group mean plasma vitamin B-6 values and differences from pre to post, post to p60, and pre to p60 minutes of exercise for all the exercise tests (continued).

Test		Groups			Difference	Groups		
		UT	T	COM		UT	T	COM
		(nM)				(nM)		
HCHO+B6	1	287 ±67.3	245 ±24.4	266 ±54.7				
	2	319 ±70.5	272 ±34.8	295 ±60.5	pre-post	+32.0	+27.0	+29.0
	3	300 ±61.2	252 ±37.4	276 ±56.0				
	4	286 ±68.4	231 ±20.0	258 ±57.3	post-p60	-33.0	-41.0	-37.0
					pre-p60	-1.80	-14.0	-8.00

1. MCHO=49% of total kcal as carbohydrate (CHO), 16% kcal protein (PRO), 35% kcal fat (FAT); HCHO= 63% CHO, 15% PRO, 22% FAT; MCHO+B-6= MCHO + 8 mg vitamin B-6 supplement; HCHO+B-6= HCHO + 8 mg vitamin B-6 supplement
2. 1=pre, 2=post, 3=p30, 4=p60 minutes of exercise
3. UT=untrained (n=5), T=trained (n=5), COM=groups combined (n=10)
4. Mean ± S.D.

($p<0.0001$). Pre-exercise PB6 values increased nearly fourfold as a result of the vitamin B-6 supplement (69.7 $\pm$ 19.3 nM with MCHO vs 277 $\pm$ 54.9 nM with MCHO+B6).

The results of the ANOVA for PB6 showed no significant main effects or interactive effects involving the two groups. Therefore the data for the groups were combined for further analysis of PB6 ($n=10$). Despite the lack of a statistically significant difference between the two groups, the trained women did display lower mean PB6 values throughout the controlled diet study when compared to the untrained group. The PB6 response to exercise was not notably different between the groups.

In response to exercise, there was a significant time (time related to blood sampling on the exercise day) x diet interaction ($p<0.0001$). Contrasts of means and differences in means showed a significant increase in PB6 from pre to post exercise and a significant decrease from post to p60 minutes of exercise (see Figures 5 and 6). The magnitude of the increase from pre to post and the decrease from post to p60 was significantly greater with the supplemented diets as compared to the non-supplemented diets (a pre to post increase of 10.7 nM with MCHO vs a pre to post increase of 23.3 nM with MCHO+B6). The magnitude of PB6 change from pre to post and from post to p60 also appeared to be affected by the level of dietary carbohydrate, though not significantly so. For example, the PB6 increase from pre to post with MCHO was 10.7 nM and with HCHO it was 13.6 nM. The decrease from post to p60 with MCHO was 14.8 nM while with HCHO it was 23.9 nM. A similar pattern occurred with the

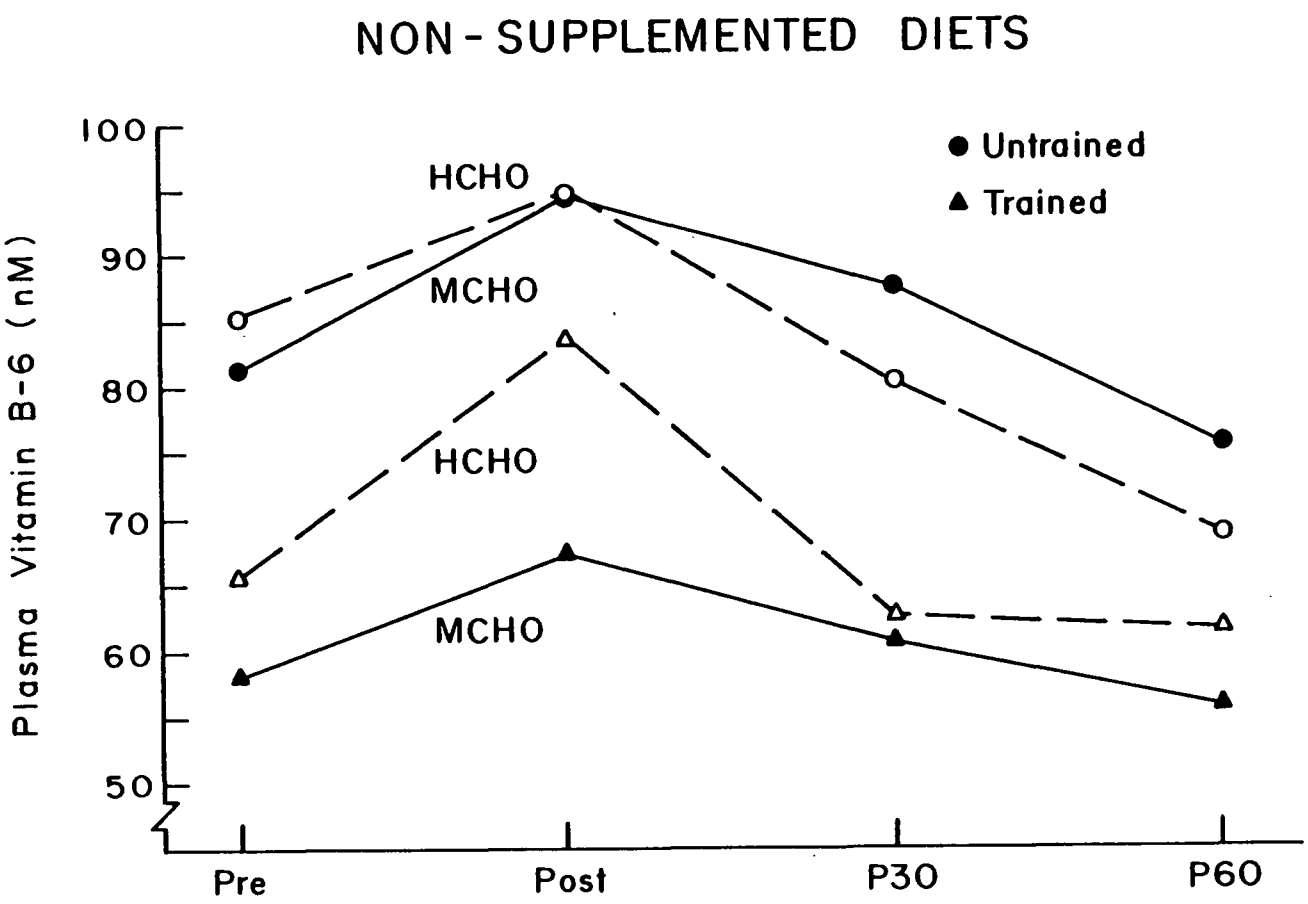

Figure 5 Plasma vitamin B-6 response to exercise with the non-supplemented diets.

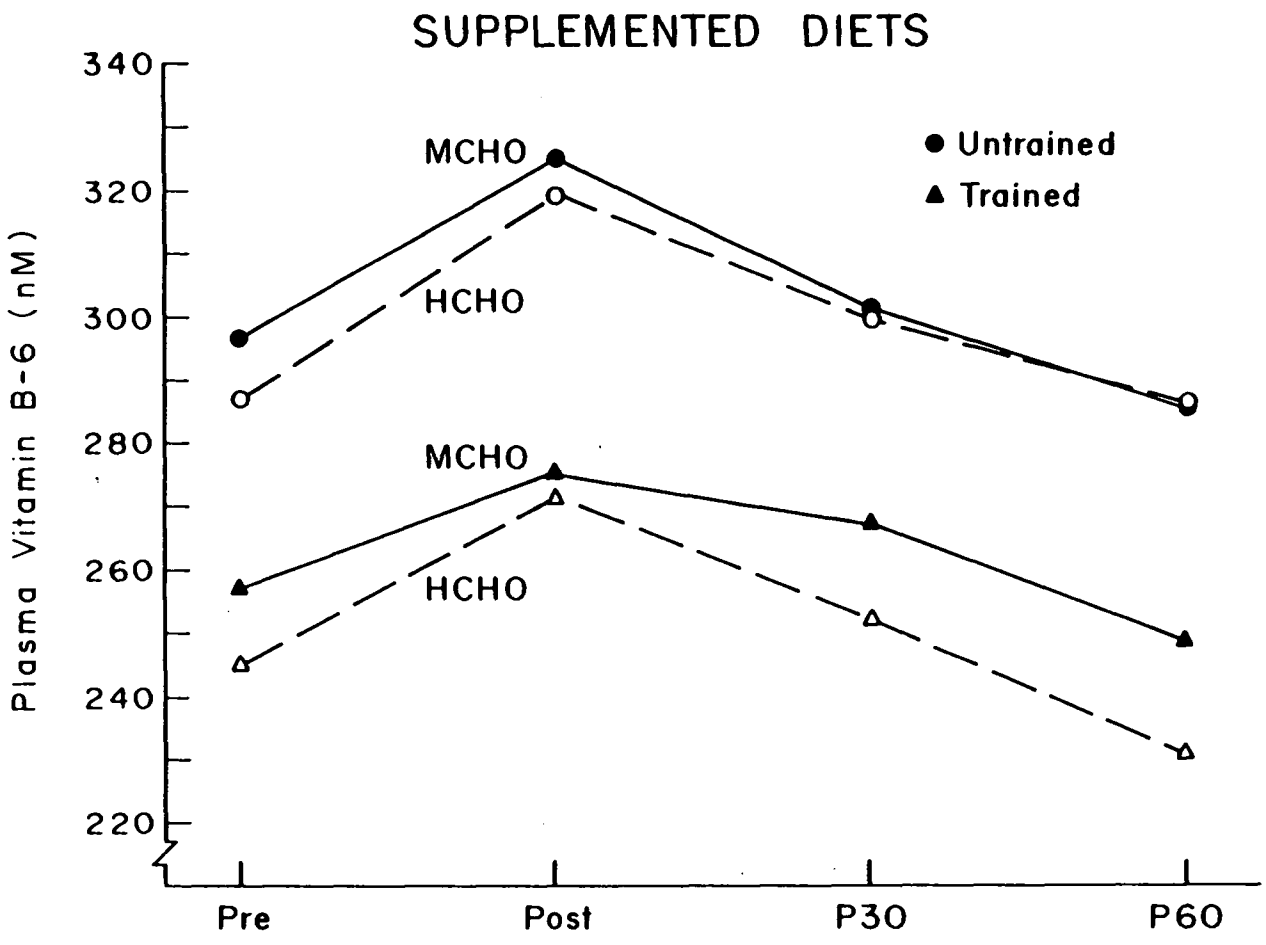

Figure 6 Plasma vitamin B-6 response to exercise with the supplemented diets.

supplemented diets (pre to post MCHO+B6 = 23.3 nM, pre to post HCHO+B6 = 29.6 nM; post to p60 MCHO+B6 = 33.2 nM, post to p60 HCHO+B6 = 37.1 nM). Thus high dietary carbohydrate combined with a vitamin B-6 supplement (HCHO+B6) elicited the greatest magnitude of PB6 change with exercise when compared to all other diets.

For the HCHO exercise session, the PB6 concentration had fallen below the pre-exercise mean value at p30 minutes and had decreased even further by the p60 minute blood sampling. For all other exercise sessions the p30 minute mean PB6 value approached the pre-exercise mean value and had fallen below it by p60 minutes.

Plasma Volume

Since plasma volume (PV) has been reported to decrease with exercise (180,181), a change seen in the concentration of plasma constituents in response to exercise could be due to a change in plasma volume. In the present study, PV changes were calculated from hematocrit values using the method of van Beaumont (182) to determine if the changes observed in PB6 were due to a concentration of the plasma (see Table 5). The mean PV change from pre to post exercise ranged from -7.16% to -13.3% for the four exercise sessions, with the mean PV change for all subjects over all exercise sessions being $-9.8 \pm 4.72\%$. With a -9.8% decrease in PV, a 7% increase in PB6 would be expected if the increase were due to hemoconcentration alone. However, the actual increase in PB6 from pre to post was 2.7-10.9% above the predicted changes. As PV returned to pre-exercise values at P60, PB6 values continued to fall

TABLE 5 Mean % plasma volume changes and plasma vitamin B-6 values predicted from % hematocrit changes from pre to post and from pre to post 60 minutes of exercise for all the exercise tests.

Test [1]	% chg Hct	% chg [2] PV	(nM) PB6 pre	PB6 post [3]	PB6 post [4]	% diff. [5]
MCHO	+10.1 +2.52	-13.3 +4.52	69.7 +19.3	80.4 +27.7	76.8 +21.9	+4.50
HCHO	+5.02 +2.22	-7.16 +3.00	75.2 +19.9	88.8 +25.8	79.1 +21.2	+10.9
MCHO+B-6	+5.30 +1.40	-8.20 +2.20	277 +54.9	300 +63.0	289 +55.0	+3.70
HCO+B-6	+8.39 +4.25	-10.3 +5.58	266 +54.7	295 +60.5	287 +59.7	+2.70

	% chg Hct	% chg PV	(nM) PB6 pre	PB6 p60 [6]	PB6 p60 [7]	% diff.
MCHO	+0.09 +4.20	+1.22 +5.89	69.7 +19.3	65.6 +17.5	68.9 +18.5	-5.00
HCHO	-2.47 +2.34	+4.23 +4.96	75.2 +19.9	64.9 +9.30	67.7 +10.6	-4.30
MCHO+B-6	-0.23 +3.13	+0.55 +5.30	277 +54.9	267 +50.6	280 +57.3	-4.90
HCHO+B-6	+0.39 +3.70	+1.55 +6.13	266 +54.7	258 +57.3	266 +52.2	-3.10

1. see 1. Table 4
2. % change PV calculated from method of van Beaumont (182)
3. PB6 values determined for post exercise
4. PB6 values predicted for post exercise from % Hct changes
5. % difference between actual and predicted PB6 values
6. PB6 values determined for post 60 minutes of exercise
7. PB6 values predicted for post 60 minutes of exercise from % Hct changes

below those seen at pre-exercise and were 3-5% lower than what would be expected if only plasma concentration were responsible for the changes. Thus, the changes reported in PB6 in response to exercise could be due partially to hemoconcentration, but most likely involve additional factors.

Urine Analyses

Urinary creatinine

Urinary creatinine was measured to aid in determining the completeness of the 24-hour urine collections. In addition, subjects were asked to comment on the completeness of their urine collection in their daily journal. When a daily creatinine value differed by more than 10% than the weekly mean value, or if all urine was not collected throughout a day, the urine sample for that day was not analyzed further. The mean daily creatinine values for the four exercise weeks were 1.22 ± 0.11, 1.29 ± 0.12, 1.24 ± 0.09, and 1.25 ± 0.11 g/24 hr for the trained group and 1.13 ± 0.14, 1.17 ± 0.14, 1.17 ± 0.13, and 1.23 ± 0.11 g/24 hr for the untrained group, respectively. Since the two groups were similar in weight and % lean body mass, and were consuming the same controlled diet, regular exercise is likely the explanation for the higher levels of urinary creatinine that were excreted by the trained group.

Urinary 4-pyridoxic Acid

Urinary 4-pyridoxic acid (4PA), the major metabolite of

vitamin B-6, was measured in aerobically trained and untrained women for day 1, day 10, and the day before and the day of exercise during this controlled metabolic study. Group mean urinary 4PA results are presented in Tables 6 and 7. Values for each individual at each time and diet can be found in the appendix.

4PA excretion ranged from 3.09 to 8.70 umol/24 hr on day 1 of the study (x for untrained = 4.69 ± 1.76 umol/24 hr, x for trained = 6.47 ± 1.54 umol/24 hr). Similar to the increase from day 1 to day 10 seen with PB6 values, the 4PA values increased to within a range of 5.19 to 7.67 umol/24 hr by day 10 (x for untrained = 6.07 ± 0.55 umol/24 hr, x for trained = 7.15 ± 0.50 umol/24 hr), and did not change significantly for the remainder of the time the subjects were fed the non-supplemented diets. On the day before the first exercise session (day 11 or 12) the 4PA excretion ranged from 6.19 to 8.00 umol/24 hr (x for untrained = 6.88 ± 0.73 umol/24 hr, x for trained = 7.55 ± 0.64 umol/24 hr). There was no effect of the carbohydrate level of the diet on 4PA excretion, though there was an expected main effect of diet indicating a significant difference between the non-supplemented and supplemented diets. The excretion of 4PA increased fivefold as a result of the vitamin B-6 supplement (7.21 ± 0.64 umol/24 hr with MCHO vs 37.6 ± 1.73 umol/24 hr with MCHO+B6).

The results ot the ANOVA for 4PA showed no significant main effects or interactive effects involving the two groups. Therefore the data for the groups were combined for further analysis of 4PA (n=10). Despite the lack ot a statistically significant difference

TABLE 6 Group mean urinary 4-pyridoxic acid excretion values for day 1 and day 10 of the study.

	Groups[1]		
	UT	T	COM
		(umol/24 hr)	
Day 1	4.69 ± 1.76[2]	6.47 ± 1.54	5.59 ± 1.73
Day 10	6.07 ± 0.55	7.15 ± 0.50	6.51 ± 0.72

1. UT=untrained (n=5), T=trained (n=5), COM=groups combined (n=10)
2. Mean ± S.D.

TABLE 7 Group mean urinary 4-pyridoxic acid excretion values and differences from the day before to the day of exercise for all the exercise tests.

Test[1]	Groups[3]	UT	T	COM	UT	T	COM
				(umol/24 hr)			
MCHO	1[2]	6.88[5] ± 0.73	7.55 ± 0.47	7.21 ± 0.64	difference[4]		
					+0.53	+0.04	+0.04
	2	7.41 ± 0.43	7.59 ± 0.93	7.25 ± 0.62			
HCHO	1	6.91 ± 1.01	7.37 ± 0.26	7.14 ± 0.69			
					+0.61	+0.19	+0.40
	2	7.52 ± 0.69	7.56 ± 0.55	7.54 ± 0.55			
MCHO+B6	1	36.7 ± 1.82	38.5 ± 1.53	37.6 ± 1.73			
					+2.26	+1.34	+1.40
	2	39.0 ± 3.13	39.8 ± 2.21	39.0 ± 2.80			
HCHO+B6	1	35.4 ± 2.20	38.1 ± 3.67	36.7 ± 3.00			
					+1.70	+2.59	+2.20
	2	37.1 ± 2.37	40.7 ± 1.50	38.9 ± 2.54			

1. see 1. Table 4
2. 1=day before exercise, 2=day of exercise
3. UT=untrained (n=5), T=trained (n=5), COM=groups combined (n=10)
4. difference in 4PA excretion between the day of exercise
5. Mean ± S.D.

between the 2 groups, the trained group consistently excreted greater amounts of 4PA than the untrained group while consuming the same amount of dietary vitamin B-6.

In response to exercise, there was a significant main effect involving time (time related to day of urine sampling) ($p<0.0005$). The 4PA means for all diets on the day before exercise were averaged and compared to the averaged means for the day of exercise. This treatment of the data revealed a greater excretion of 4PA on the day of exercise as compared to the day before exercise. (See Figures 7 and 8). The magnitude of the difference between the day before and the day of exercise was greater during the supplemented diets when compared to the non-supplemented diets, though not significantly so. This difference is especially evident when comparing the HCHO and the HCHO+B-6 diets. The difference in 4PA excretion between the day before and the day of exercise with the HCHO diet is 0.40 umol/24 hr. This same difference with the HCHO+B6 diet was 2.20 umol/24 hr.

The excretion of 4PA may be expressed as a percentage of dietary vitamin B-6 intake. When the % of vitamin B-6 excreted as 4PA was calculated from the combined group mean 4PA values on the day before exercise, 52.3% and 51.8% vitamin B-6 was excreted as 4PA with the MCHO and HCHO diets, respectively. The % 4PA excretion for the MCHO+B6 and HCHO+B6 diets was 61.0% and 59.6%, respectively. Thus the carbohydrate level of the diet had no significant effect on the % of vitamin B-6 excreted as 4PA, but the supplement resulted in a greater % 4PA excretion.

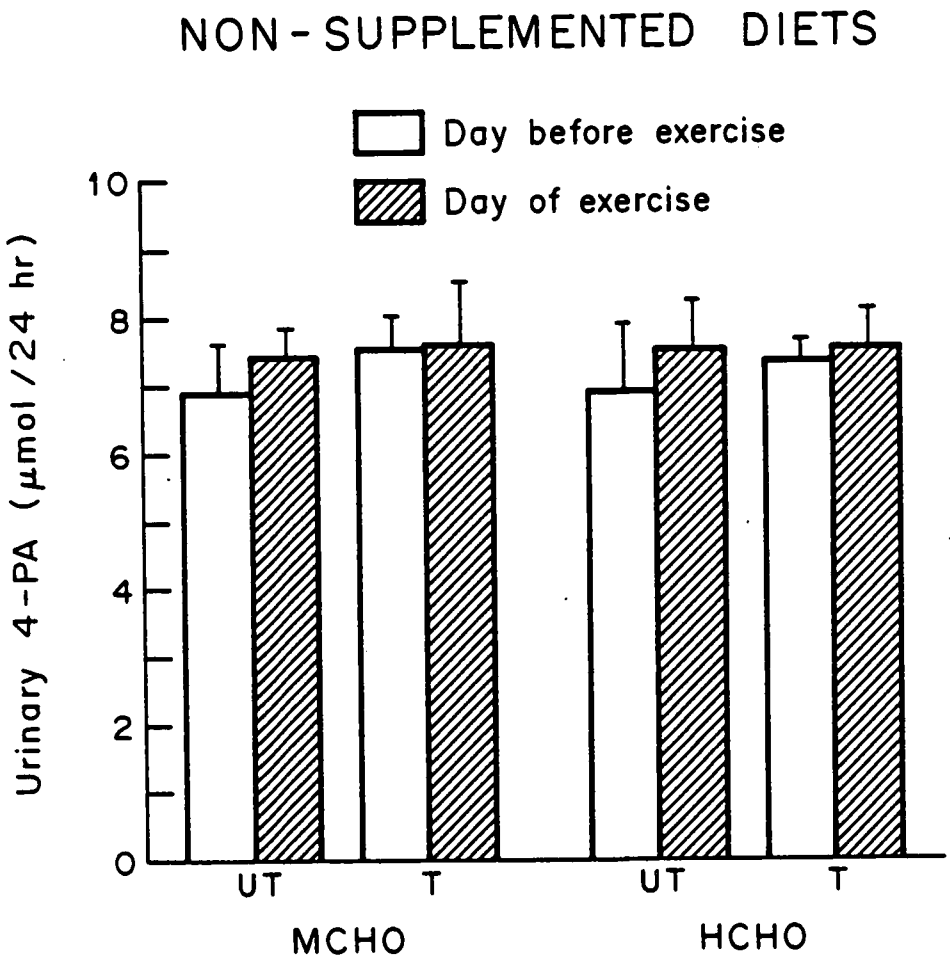

Figure 7 Urinary 4-pyridoxic acid excretion response to exercise with the non-supplemented diets.

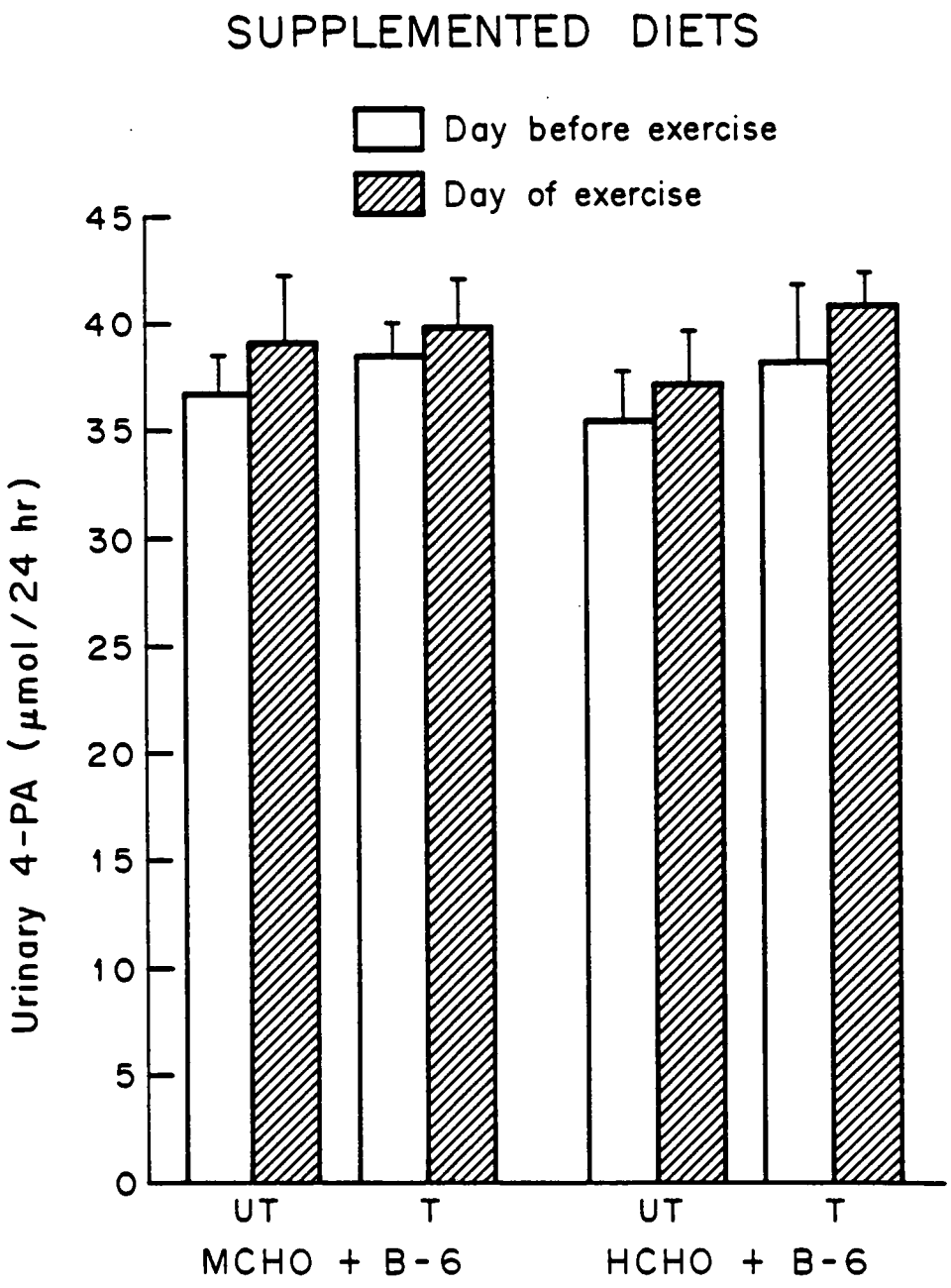

Figure 8 Urinary 4-pyridoxic acid excretion response to exercise with the supplemented diets.

V. Discussion

This laboratory has been the site of several studies observing the metabolism of vitamin B-6 during exercise in men (3-6). The investigation reported here is unique in its use of female subjects. Five aerobically trained and five untrained young women were recruited in order to detect possible differences between the two groups in their metabolic response to exercise. The importance of this investigation lies in the degree of control held over the diet and exercise variables. Subjects were fed accurately weighed food items over a seven week period, and sufficient time was allowed for adjustment to diet changes before each exercise session. In addition, 24-hour urine samples were collected to determine the excretion of 4PA. These procedures allowed a day to day evaluation of the intake of vitamin B-6 compared to the excretion of its primary metabolite, 4PA. VO2 max was determined for all subjects so that each could exercise at the same relative intensity. Thus the effect or a standardized exercise test on parameters of vitamin B-6 metabolism could be most clearly observed.

The non-supplemented diets, MCHO and HCHO, provided 2.3 and 2.4 mg of vitamin B-6, respectively. These amounts are slightly greater than the 2.0 mg recommended as the RDA for women. When PB6 values from day 1 of the study were compared to those from day 10 it was seen that as subjects consumed the MCHO diet, their PB6 values stabilized at higher levels, a possible indication that their intake of vitamin B-6 prior to the study was below the RDA. The slightly

higher intake of vitamin B-6 with the HCHO as compared to the MCHO diet had no apparent effect on PB6 levels.

The effect of the standardized exercise test was to significantly increase PB6 values above pre-exercise levels. By the end of a 60 minute recovery period, PB6 values had fallen below the pre-exercise levels. These changes seen in PB6 are similar in magnitude to those observed by others in men (3-6). Wozenski (7) observed PB6 levels to remain relatively stable in men during a 5 hour fast. Only 0.5-1 hour after an oral 1-2 mg PN dose have PB6 values been observed to increase to the extent that they are seen to do in response to exercise (7).

The first objective of this study was to examine the effect of an increase in the carbohydrate level of the diet on vitamin B-6 metabolism during exercise. In general, glycogen synthesis increases in proportion to the amount of consumed dietary carbohydrate (13), and a high carbohydrate diet has been shown to result in an increased utilization of total carbohydrate during exercise (14). The PB6 response to exercise, calculated as concentration changes, was determined for each of the four exercise sessions. The carbohydrate level of the diet had no significant effect on pre-exercise PB6 values. Comparisons of the pre to post and the post to p60 PB6 exercise response showed no significant difference between the MCHO and HCHO or between the MCHO+B6 and HCHO+B6 diets. Though not to a significant extent, the increased carbohydrate did appear to increase the magnitude of the changes in PB6 from pre to post and from post to p60 minutes of exercise.

The second objective of this investigation was to examine the effect ot a vitamin B-6 supplement on the metabolism of vitamin B-6 with exercise. A vitamin B-6 supplement was fed in addition to the diet, thus providing each subject with 10.3-10.4 mg of the vitamin, or 5 times the RDA. In addition to increasing pre-exercise PB6 values nearly fourfold, supplementation significantly increased the magnitude of the PB6 increase from pre to post and the decrease from post to p60. Though supplementation led to an elevation of pre-exercise PB6 values and a greater increase from pre to post exercise, it did not prevent PB6 values from dropping below pre-exercise levels at p60.

The measurement of PB6 at any time is a representation of the dynamic state ot tissue metabolism, release, and uptake of all three forms of vitamin B-6, both phosphorylated and nonphosphorylated. Any change in PB6 concentration that occurs without an intake of vitamin B-6, such as the change seen with exercise, must be explained by a modification of tissue metabolism, release, and uptake of the B6 vitamers.

Exercise is known to cause changes in plasma volume (180,181), so it is possible that the changes seen in PB6 with exercise are simply a result of hemoconcentration. A mean decrease in plasma volume ot 9.8% was seen at post exercise for all subjects over all exercise sessions. This decrease would be reflected in a 7% increase in PB6 if the change was due strictly to plasma concentration. However, the actual mean increases in PB6 from pre to post exercise were 2.7-10.9% above the values expected and the

actual mean decreases from post to p60 were 3-5% below the PB6 values that would be expected from hemoconcentration alone. Thus the changes in PB6 with exercise likely involve factors in addition to hemoconcentration.

If plasma volume changes are not the sole reason for the PB6 changes seen with exercise, several other possibilities can be considered. Total blood flow, due to an increased cardiac output, is increased fivefold during strenuous exercise (92). An increased blood flow through the liver could possibly account for the increased PB6 seen in the plasma during exercise. However, the increased blood flow is diverted primarily to the working muscle, with the liver receiving only 3-5% of the total flow. When this 3-5% is compared to the 20-25% of cardiac output that the liver receives during rest, it can be seen that the liver receives an essentially equivalent blood flow during rest and exercise. The rate of blood flow (ml/min) through the liver was shown to decrease during exercise by Ahlborg et al. (183).

Leklem and Shultz (3) have advanced four other possible sources of the increased PB6, and they are (1) release from the red blood cells, (2) release ot albumin from interstitial fluid, (3) release from tissues ot enzymes containing PLP, or (4) release from muscle stores. These researchers contend that the most likely of the four sources is the muscle. Black and coworkers (10,11) have demonstrated in the rat that muscle glycogen phosphorylase acts as a storage depot for vitamin B-6. This depot was shown to decrease under times of caloric deprivation rather than vitamin B-6

deficiency. Exercise can be viewed as an acute form of starvation since the utilization of energy substrate is similar in both situations (105,114). The hypothesis advanced by the results of this and previous studies is that the increased B-6 vitamers in the plasma with exercise are the result of PLP release from muscle phosphorylase.

Lumeng and coworkers (62) have presented data suggesting the liver as the sole source of plasma PLP (the major B-6 vitamer seen to increase with the PB6 exercise increase). It must be recalled though, that their organ ablation studies utilized fasted resting animals. In an exercise situation it would not be advantageous for the liver to release the cofactor (PLP) needed to aid in energy substrate regulation. It has been shown that the liver does not increase its storage of PLP with excess vitamin B-6 intake (184), as the muscle does in rats and is hypothesized to do in humans. The muscle offers itself as an ideal homeostatic mechanism for the release or PLP during times of need by other tissues. Black et al. (10,11) were able to relate the depletion of muscle glycogen phosphorylase to glucose homeostasis. Unfortunately they did not measure plasma levels of the B-6 vitamers.

If muscle phosphorylase behaves in the same manner in the human as it does in the rat, higher muscle levels of vitamin B-6 would be expected during the supplemented diet periods of this study. Supplementation did significantly increase the magnitude of the change seen in PB6 during exercise. Thus when vitamin B-6 was fed in excess, more was released from storage with exercise. This

observation lends more credence to the hypothesis that supplementation does increase muscle storage of vitamin B-6 in humans.

Though not to a significant extent, the higher level of carbohydrate increased the magnitude of the PB6 increase from pre to post and the decrease from post to p60 minutes of exercise. Since a high carbohydrate diet has been reported to result in an increased utilization ot carbohydrate during exercise (14), a greater reliance on muscle and liver glycogenolysis and gluconeogensis could be accompanied by a greater release ot vitamin B-6 from muscle phosphorylase. Alternately, it is possible that the additional week of supplementation with vitamin B-6 during the HCHO+B6 diet treatment further increased the muscle stores ot vitamin B-6 and that the HCHO+B6 exercise session resulted in an increased release of the vitamin from the muscle as compared to MCHO+B-6 exercise.

A third objective of this investigation was to examine the effect or exercise on urinary 4PA excretion while subjects were fed a well-controlled diet. As mentioned previously, the non-supplemented diets provided 2.3-2.4 mg of vitamin B-6 and the supplemented diets provided 10.3-10.4 mg of vitamin B-6. Shultz and Leklem (82) have shown a relationship between vitamin B-6 intake and 4PA excretion, thus suggesting that the excretion of 4PA could be used as an index for the evaluation of vitamin B-6 status. They propose 4.60-5.20 umol/24 hr as a marginal 4PA excretion range. Day 1 4PA values indicate that four subjects excreted less 4PA than what is considered marginal. After 10 days of the MCHO diet, which

supplied adequate vitamin B-6, 4PA excretion had stabilized at values within a range above the suggested marginal excretion values. Along with the increase in PB6 values that occurred from day 1 to day 10, the increase in 4PA excretion from day 1 to day 10 is an indication that the intake of vitamin B-6 by some subjects prior to the study was below recommended levels. The slightly higher intake of vitamin B-6 with the HCHO diets as compared to the MCHO diets had no apparent affect on the excretion of 4PA.

There was no significant effect of the carbohydrate level of the diet on 4PA excretion. Vitamin B-6 supplementation resulted in a fivefold increase in 4PA excretion. With all diet treatments, urinary 4PA was elevated the day of exercise as compared to the day before exercise. It must be emphasized again that this increase occurred while all subjects were consuming the same diet. The findings of Hatcher (6) suggest that 4PA excretion increases with exercise in men, however, it is difficult to make a definite conclusion from her data since the nutrient composition of the diet changed mid-week, the vitamin B-6 content of the diet was different during each of the three experimental weeks, and sufficient time for adjustment to the different diets was not allowed. 4PA excretion is reported to be increased in rats (8) and in humans (9) after exercise, but these studies did not appear to control diet intake. Thus the results ot this study show a significant increase in 4PA excretion with exercise under conditions of a very well-controlled diet. Total urinary vitamin B-6 (UB6) was measured during this study by Manore (185), who found no significant difference in UB6

with exercise. Thus the increase in 4PA excretion observed with exercise was related to the increased metabolism of PLP without a significant increase in the excretion of B6 vitamers.

An explanation for the increased excretion of 4PA with exercise is related to the decrease in PB6 from post to p60 to values below those at pre-exercise. This decrease likely represents an increased uptake of the B-6 vitamers by the liver for use as gluconeogenic coenzyme. Some ot the active vitamer, PLP, could then be oxidized and excreted in the urine as 4PA.

The final objective of this investigation was to examine the possibility of a difference in the metabolism of vitamin B-6 with exercise between a group of aerobically trained and a group of untrained women. Regular aerobic exercise has been shown to enhance the ability to store muscle glycogen (15) and to slow its rate of depletion during long duration exercise (108,111). Training increases the oxidative capacities ot the muscle but does not effect the activity of glycogenolytic and glycolytic enzymes (141). Five aerobically trained and five untrained young women were chosen for this study on the basis of their reported exercise habits. Their state of training was further confirmed by the determination of VO2 max. Though the trained individuals displayed VO2 max values that placed them in a "good" fitness category (186), none of them could be considered highly trained athletes undergoing a rigorous training program. Comparisons of PB6 and 4PA responses to exercise revealed no significant differences betweeen the two groups.

Though not to a significant extent, the trained group did

exhibit lower PB6 values throughout the study when compared to the untrained group. The trained group also had a greater excretion of 4PA. Importantly, these differences occurred while all received the same amount of vitamin B-6 in the diet. Since the trained group exercised throughout the experimental period to maintain their trained status and the untrained group did not, the differences between the two groups were likely due to regular exercise. The higher mean PB6 values of the untrained group were due primarily to one subject (#18) who displayed much higher PB6 values than the rest of the untrained subjects. Nevertheless, examination of individual values does reveal higher PB6 values among the untrained group as compared to the trained group. It is possible that if the level of training of the trained group had been higher, a statistically significant difference between the two groups would have been seen.

The results of this investigation have implications primarily for the woman who exercises regularly. Though there was no statistically significant difference between the trained and untrained groups in the measured parameters of vitamin B-6 metabolism, there was a trend for lower levels of PB6 and greater excretion of 4PA with exercise among the trained women. This occurred even while both groups were fed the same diet. If a person is consuming adequate amounts of vitamin B-6, as did the subjects in this study, vitamin B-6 status will not be compromised by exercise. However, if vitamin B-6 intake is less than adequate, status could possibly be compromised by regular exercise over the long term. Regular exercisers generally consume more kilocalories than

non-exercisers, which increases their chances of obtaining a diet adequate in vitamin B-6. This may not be the case for women who are restricting their food intake in addition to regularly exercising for weight loss. It cannot be suggested that a vitamin B-6 supplement is necessary for the trained woman, though it would be to her advantage to consume a diet high in all nutrients, including vitamin B-6. The moderate carbohydrate diet designed for this study contained 2.3 mg of vitamin B-6 per approximately 2000 kilocalories. Foods recommended for their vitamin B-6 content are whole grains, beans, and legumes.

The results ot this study lend more information concerning the effect or exercise on vitamin B-6 metabolism. The hypothesis advanced by others, that increased PB6 values with exercise are due to the release or PLP from muscle phosphorylase, cannot be determined with certainty until muscle biopsies are obtained in conjunction with blood and urine samples.

VI. Summary and Conclusions

Aerobically trained and untrained young women were subjects in this study in which the effect of dietary carbohydrate and vitamin B-6 supplementation on vitamin B-6 metabolism during exercise was examined. It has been hypothesized that muscle glycogen phosphorylase is the source of the increased plasma levels of vitamin B-6 that are seen with exercise (3,4,6). Phosphorylase has been shown in the rat to act as a storage depot for the active form of vitamin B-6, PLP. The muscle may offer itself as a homeostatic mechanism for the release or PLP needed for glucose production during starvation and exercise.

The four objectives of this investigation were: (1) to determine the effect of dietary carbohydrate on vitamin B-6 metabolism during exercise, (2) to determine the effect of a vitamin B-6 supplement on vitamin B-6 metabolism during exercise, (3) to study the effect of exercise on excretion of urinary 4PA under conditions of a controlled diet, and (4) to examine the possible differences in the metabolism of vitamin B-6 during exercise between aerobically trained and untrained young women.

The diet-exercise study consisted of seven experimental weeks. An exercise session was administered at the end of each of four isocaloric dietary treatments. Subjects were fed a moderate carbohydrate diet (49% of total kcal as carbohydrate) (MCHO) weeks 1 and 2. A high carbohydrate diet (63% of kcal as carbohydrate) (HCHO) was fed week 3. At week 4 subjects were returned to the MCHO

diet. During weeks 5 and 6 the MCHO diet was fed with the addition of a daily supplement of 8 mg PN (MCHO+B6). Throughout week 7 subjects were fed the HCHO diet plus supplemental vitamin B-6 (HCHO+B6). The vitamin B-6 content of the diets, as determined by weekly composite analysis, was 2.3 mg for MCHO and 2.4 mg for HCHO.

VO2 max was determined for each subject one week prior to the study. Exercise sessions were held on day 5 or 6 of weeks 2, 3, 6, and 7. Subjects reported for exercise testing after an overnight fast. The test consisted of 20 minutes of cycle ergometer exercise at 80% VO2 max which was preceded by 10 minutes of warm-up exercise and followed by 5-10 minutes of an active recovery. Blood samples were drawn pre exercise (pre), 2-3 minutes after exercise (post), 30 minutes after (p30), and 60 minutes after (p60) exercise. The samples were analyzed for plasma vitamin B-6 (PB6), hematocrit, and hemoglobin. Urine was collected in 24-hour aliquots and samples were analyzed for urinary 4-pyridoxic acid (4PA) and creatinine.

A three factor plot partially blocked ANOVA design, with groups and diets as the whole plot factors and time (of blood or urine sampling) as the split plot factor, was used for the analysis of data. Significant interactive effects were examined by performing hypothesis testing on differences between treatment means and contrasts of treatment means.

As reported previously, exercise was shown to alter the metabolism of vitamin B-6. PB6 was seen to increase significantly from pre to post and decrease from post to p60. For all diets, PB6 values at p60 had fallen below those seen pre-exercise. The

carbohydrate level of the diet had no effect on pre-exercise PB6 values. However, increased dietary carbohydrate did appear to increase the magnitude of the PB6 response to exercise, though not significantly so. This effect may have been related to an increased utilization of carbohydrate during exercise that reportedly occurs as dietary carbohydrate is increased (14). Supplementation of the diets with vitamin B-6 increased the magnitude of the PB6 response to exercise. This increased PB6 response seen with supplementation is likely a reflection of increased storage of PLP in the muscle, as has been shown to occur in rats, and is postulated to occur in humans. It was concluded that the change in plasma volume that accompanied exercise, as calculated from hematocrit, was not fully responsible for the changes seen in PB6.

Urinary excretion of 4PA was significantly increased the day of exercise as compared to the day before exercise. The carbohydrate level of the diet had no significant effect on 4PA excretion or on the percent of vitamin B-6 excreted as 4PA. Vitamin B-6 supplementation increased 4PA excretion fivefold and increased the magnitude of the difference between the day before and the day of exercise.

There was no significant difference between the trained and untrained groups with regard to their PB6 and 4PA responses to the standardized exercise test. However, there was a noticable trend for the trained subjects to have lower PB6 values and greater 4PA excretion than the untrained subjects throughout the controlled study.

The results of this study demonstrate that exercise alters the metabolism of vitamin B-6. They also suggest that vitamin B-6 status may be altered as a result of a regular exercise program, though it is not known to what extent status may be altered in the highly trained athlete undergoing a rigorous training program. This implied change in status will not be of concern if the exerciser obtains the RDA for vitamin B-6 in the daily diet, thus supplementation with vitamin B-6 is not considered necessary.

References

1. Lawrence JD, Smith JL, Bower RC, Riehl WP. The effect of tocopherol (vitamin E) and pyridoxine HCl (vitamin B-6) on the swimming endurance of trained swimmers. J Am College Health Assoc 1975;23:219-22.

2. Nutrition and Physical Fitness: A Statement by the American Dietetic Association. J A D A 1980;76:437-43.

3. Leklem JE, Shultz TD. Increased plasma pyridoxal 5'-phosphate and vitamin B-6 in male adolescents after a 4500-meter run. Am J Clin Nutr 1983;38:541-48.

4. Munoz KD. The influence of exercise on vitamin B-6 metabolism. Masters thesis. Oregon State University, 1982.

5. Leklem JE, Munoz K, Walter C. Vitamin B-6 metabolism in men and women following a run or cycle exercise. Fed Proc 1983;42:1066 (abstract).

6. Hatcher LH. Influence of controlled strenuous exercise on vitamin B-6 metabolism in man: effects of carbohydrate depletion-repletion diets and vitamin B-6 supplements. Masters thesis. Oregon State University, 1982.

7. Wozenski JR, Leklem JE, Miller LT. The metabolism of small doses of vitamin B-6 in men. J Nutr 1980;110:275-85.

8. Efremov VV, Zaburkin EM. Metabolism of pyridoxine and nicotinic acid during the physical stress of prolonged swimming in experimental animals. Voprosy Pitaniya 1972;31:39-43.

9. Borisov IM. Pyridoxic status of students in a physical culture institute. Voprosy Pitaniya 1977;36:48-52.

10. Black AL, Guirard BM, Snell EE. Increased muscle phosphorylase in rats fed high levels of vitamin B-6. J Nutr 1977;107:1962-68.

11. Black AL, Guirard BM, Snell EE. The behavior of muscle phosphorylase as a reservoir for vitamin B-6 in the rat. J Nutr 1978;108:670-77.

12. deVos A. Carbohydrate loading, vitamin B-6 supplementation, and fuel metabolism during exercise of differing intensity in post-absorptive man. Masters thesis. Oregon State University, 1982.

13. Sherman WM, Costill DL. The marathon: dietary manipulation to optimize performance. Am J Sports Med 1984;12:44-51.

14. Martin B, Robinson S, Robertshaw D. Influence of diet on leg uptake of glucose during heavy exercise. Am J Clin Nutr 1978;31:62-7.

15. Lamb DR, Peter JB, Jeffrees RN, Wallace HA. Glycogen, hexokinase and glycogen synthetase adaptations to exercise. Am J Physiol 1969;217:1628-32.

16. Saltin B, Rowell LB. Functional adaptations to physical activity and inactivity. Fed Proc 1980;39:1506-13.

17. Gyorgy P. Vitamin B-2 and the pellagra-like dermatitis in rats. Nature 1934;133:498-99.

18. Lepkovsky S. Crystalline factor I. Science 1938;87:169-70.

19. Keresztesy JC, Stevens JR. Vitamin B-6. J Am Chem Soc 1938;60:1267-68.

20. Gyorgy P. Crystalline vitamin B-6. J Am Chem Soc 1938;60:983-84.

21. Sauberlich HE. Human requirements for vitamin B-6. Vitam Horm 1964;22:807-23.

22. Harris SA, Folkers K. Synthetic vitamin B-6. Science 1939;89:347.

23. Stiller ET, Keresztesy JC, Stevens JR. The structure of vitamin B-6 I. J Am Chem Soc 1939;61:1237-42.

24. Snell ES, Guirard BM, Williams RJ. Occurrence in natural products of a physiologically active metabolite of pyridoxine. J Biol Chem 1942;143:519-30.

25. Spies ID, Bean WD, Ashe WF. A note on the use of vitamin B-6 in human nutrition. J A M A 1939;112:2414-15.

26. Spies ID, Ladisch RK, Bean WD. Vitamin B-6 (pyridoxin) deficiency in human beings. J A M A 1940;115:839-40.

27. Coursin DB. Convulsive siezures in infants with pyridoxine-deficient diet. J A M A 1954;154:406-8.

28. Molony CJ, Parmalee AH. Convulsions in young infants as a result of pyridoxine (vitamin B-6) deficiency. J A M A 1954;154:405-6.

29. Axelrod AE, Trakellis AC. Relationship of pyridoxine to immunological phenomena. Vitam Horm 1964;22:591-607.

30. Robson LC, Schwartz MR, Perkins WD. Vitamin B-6 and immunity. In: Human Vitamin B-6 Requirements. Washington DC:National Research Council, National Academy of Sciences, 1978:162-79.

31. DiSorbo DM, Litwack G. Use of PLP as a tool in the study of steroid receptors. Biochemical Actions of Hormones 1982;9:205-19.

32. Disorbo DM, Litwack G. Changes in the intracellular levels of pyridoxal-5-phosphate affect the induction of tyrosine aminotransferase by glucocorticoids. Biochem Biophys Res Comm 1981;99:1203-8.

33. Birch TW. The relation between vitamin B-6 and the unsaturated fatty acid factor. J Biol Chem 1938;124:775-93

34. Wakil SJ. Mechanism of fatty acid synthesis. J Lipid Res 1961;2:1-24.

35. Mueller JF. Vitamin B-6 in fat metabolism. Vitam Horm 1964;22:787-96.

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

37. Sansom MSP, Baby YS, Hajdu J, Stuart DI, Stura EA, Johnson LN. The role of pyridoxal phosphate in glycogen phosphorylase b; structure, environment and relationship to catalytic mechanism. In: Evangelopoulos AE, ed. Chemical and biological aspects of vitamin B-6 catalysis:part A. New York:AR Liss Inc, 1984:127-146.

38. Brin M. Chemistry, absorption, metabolism, catabolism, and toxicity. In: Human Vitamin B-6 Requirements. Washington DC: National Research Council, National Academy of Sciences, 1978:1-20.

39. Snell EE. Chemical structure in relation to biological activities of vitamin B-6. Vitam Horm 1958;16:78-127.

40. Siegel L, Melnick D, Oser BL. Bound pyridoxine (vitamin B-6) in biological materials. J Biol Chem 1943;149:361-67.

41. Gregory JF III, Kirk JR. The bioavailability of vitamin B-6 in foods. Nutr Rev 1981;39:1-8.

42. Kabir H, Leklem J, Miller LT. Measurement of glycosylated vitamin B-6 in foods. J Food Sci 1983;48:1422-25.

43. Leklem JE, Miller LT, Perera AD, Peffers DE. Bioavailability of vitamin B-6 from wheat bread in humans. J Nutr 1980;110:1819-28.

44. Gregory JF III, Kirk JR. Assessment of storage effects on vitamin B-6 stability and bioavailability in dehydrated food systems. J Food Sci 1978;43:1801-1815.

45. Scudi JV, Unna K, Antopol W. A study of the urinary excretion of vitamin B-6 by a colorimetric method. J Biol Chem 1940;135:371-76.

46. Yamada RH, Tsuji T. Vitamin B-6 absorption. In: Tryfiates GP,ed. Vitamin B-6 Metabolism and Role in Growth. Westport, Connecticut: Foods and Nutrition Press, 1978:335-55.

47. Booth CC, Brain MC. The absorption of tritium labeled pyridoxine hydrochloride in the rat. J Physiol 1962;164:282-94.

48. Brain MC, Booth CC. The absorption of tritium labeled pyridoxine hydrochloride in control subjects and in patients with intestinal malabsorption. Gut 1964;5:241-47.

49. Middleton HM III. Jejunal phosphorylation and dephosphorylation of absorbed pyridoxine HCl in vitro. Am J Physiol 1978; 253:E272-78.

50. Mehansho H, Hamm MW, Henderson LM. Transport and metabolism of pyridoxal and pyridoxal 5'-phosphate in the small intestine of the rat. J Nutr 1979;109:1542-51.

51. Hamm MW, Mehansho H, Henderson LM. Transport and metabolism of pyridoxamine and pyridoxamine 5'-phosphate in the small intestine of the rat. J Nutr 1979;109:1552-59.

52. Middleton HM III. Characterization of pyridoxal 5'-phosphate disappearance from in vivo perfused segments of rat jejunum. J Nutr 1982;112:269-75.

53. Middleton HM III. Effect of vitamin B-6 deficiency on in vitro uptake and metabolism of pyridoxine HCl by rat jejunum. Am J Clin Nutr 1980;33:2168-73.

55. Serebro HA, Solomon HM, Johnson JH, Hendrix TR. The intestinal absorption of vitamin B-6 compounds by the rat and hamster. Bull John Hopkins Hosp 1960;119:116-17.

56. Mehansho H, Buss DD, Hamm MW, Henderson LM. Transport and metabolism of pyridoxine in rat liver. Biochim Biophys Acta 1980;631:112-23.

57. McCormick DB, Gregory ME, Snell EE. Pyridoxal phosphokinases I. assay, distribution, purification, and properties. J Biol Chem 1961;236:2076-84.

58. Pogell BM. Enzymatic oxidation of pyridoxamine 5'-phosphate to pyridoxal 5'-phosphate in rabbit liver. J Biol Chem 1958;232:761-75.

59. Snell EE, Haskell BD. Chapter 1: Metabolism of water soluble vitamins Section C: The metabolism of vitamin B-6. Compr Biochem 1971;21:47-71.

60. Sauberlich HE, Canham JE, Baker EM, Raica N Jr, Herman YF. Biochemical assessment of the nutritional status of vitamin B-6 in the human. Am J Clin Nutr 1972;25:629-42.

61. Lumeng L, Lui A, Li T-K. Plasma content of B-6 vitamers and its relationship to hepatic vitamin B-6 metabolism. J Clin Invest 1980;66:688-95.

62. Lumeng L, Brashear RE, Li T-K. Pyridoxal 5'-phosphate in plasma: source, protein-binding, and cellular transport. J Lab Clin Med 1974;84:334-43.

63. Dempsey WB, Christensen HN. The specific binding of pyridoxal 5'-phosphate to bovine plasma albumin. J Biol Chem 1962;237:1113-20.

64. Anderson BB, Fulford-Jones CE, Child JA, Beard ME, Bateman CJT. Conversion of vitamin B-6 compound to active forms in the red blood cell. J Clin Invest 1971;50:1901-9.

65. Shane B. Vitamin B-6 and blood. In: Human Vitamin B-6 Requirements. Washington DC, National Research Council, National Academy of Sciences, 1976:111-28.

66. Lyon JB Jr, Bain JA, Williams HL. The distribution of vitamin B-6 in the tissues of two inbred strains of mice fed complete and vitamin B-6-deficient rations. J Biol Chem 1962;237:1989-91.

67. Thiele VF, Brin M. Chromatographic separation and microbiologic assay of vitamin B-6 in tissues from normal and vitamin B-6 depleted rats. J Nutr 1966;90:347-53.

68. Vanderslice JT, Maire CE, Beecher CR. Extraction and quantitation of B-6 vitamers from animal tissue and human plasma: A preliminary study. In: Leklem JE, Reynolds RD,eds. Methods in Vitamin B-6 Nutrition: Analysis and Status Assessment. New York: Plenum Press, 1981:123-47.

69. Debes SA, Ercanli FG. Depletion pattern of vitamin B-6 in tissues of rats fed graded levels of the vitamin. Fed Proc 1978;37:449 (abstract).

70. Itoh R, Okada M. Effect of dietary protein level on pyridoxal

content in tissue and excretion of pyridoxic acid into urine in normal or pyridoxine-deficient rats. J Nutr Sci Vitaminol 1973;19:523-8.

71. Lumeng L, Ryan MP, Li T-K. Validation of the diagnostic value of plasma pyridoxal 5'-phosphate measurements in vitamin B-6 nutrition of the rat. J Nutr 1978;108:545-53.

72. Lyon JB Jr, Porter J. The effect of pyridoxine deficiency on muscle and liver phosphorylase in two inbred strains of mice. Biochim Biophys Acta 1962;58:248-54.

73. Schweigert BS, Sauberlich HE, Elvehjem CA, Bauman CA. Dietary protein and the vitamin B-6 content of mouse tissue. J Biol Chem 1946;165:187-96.

74. Illingworth B, Kornfeld R, Brown DH. Phosphorylase and uridine diphosphoglucose-glycogen transferase in pyridoxine deficiency. Biochim Biophys Acta 1960;42:486-89.

75. Eisenstein AB. The effect of pyridoxine deficiency on liver and muscle phosphorylase. Biochim Biophys Acta 1962;58:244-47.

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

77. Contractor SF, Shane B. Estimation of vitamin B-6 compounds in human blood and urine. Clin Chim Acta 1968;21:71-77.

78. Baysal A, Johnson BA, Linkswiler H. Vitamin B-6 depletion in man:blood vitamin B-6, plasma pyridoxal 5'-phosphate, serum cholesterol, serum transaminases, and urinary vitamin B-6 and 4-pyridoxic acid. J Nutr 1966;89:19-23.

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

80. Li T-K, Lumeng L. Plasma PLP as an indicator of nutrition status: relationship to tissue vitamin B-6 content and hepatic metabolism. In: Leklem JE, Reynolds RD, eds. Methods in Vitamin B-6 Nutrition: Analysis and Status Assessment. New York: Plenum Press, 1981:289-96.

81. Rose CS, Gyorgy P, Buttler M, Andres R, Norris AH, Shock NW, Tobin J, Brin M, Spiegel H. Age differences in vitamin B-6 status of 617 men. Am J Clin Nutr 1976;29:847-53.

82. Shultz TD, Leklem JE. Urinary 4-pyridoxic acid, urinary vitamin B-6 and plasma pyridoxal 5'-phosphate as measures of vitamin

B-6 status and dietary intakes of adults. In: Leklem JE, Reynolds RD,eds. Methods in Vitamin B-6 Nutrition: Analysis and Status Assessment. New York: Plenum Press, 1981:297-320.

83. Chabner BA, DeVita VT, Livingston DM, Oliverio VT. Abnormalities of tryptophan metabolism and plasma pyridoxal phosphate in Hodgkins disease. New Eng J Med 1970;282:838-43.

84. Hamfelt A. Age variation of vitamin B-6 metabolism in man. Clin Chim Acta 1964;10:48-54.

85. Food and Nutrition Board. Recommended Dietary Allowance. Washington, DC: National Research Council, National Academy of Sciences, 1943.

86. Food and Nutrition Board. Recommended Dietary Allowance. Washington, DC: National Research Council, National Academy of Sciences, 1953.

87. Food and Nutrition Board. Recommended Dietary Allowance. Washington, DC: National Research Council, National Academy of Sciences, 1958.

88. Food and Nutrition Board. Recommended Dietary Allowance. Washington, DC: National Research Council, National Academy of Sciences, 1964.

89. Food and Nutrition Board. Recommended Dietary Allowance. Washington, DC: National Research Council, National Academy of Sciences, 1968.

90. Food and Nutrition Board. Recommended Dietary Allowance. Washington, DC: National Research Council, National Academy of Sciences, 1980.

91. Bureau of Nutritional Sciences. Dietary Standard for Canada, revised ed. Ottawa, Canada: Food Directorate, Health Protection Branch, Department of National Health and Welfare, 1975.

92. Astrand P-O, Rodahl K. Textbook of Work Physiology: Physiological Bases of Exercise. 2nd ed. San Francisco: McGraw-Hill, 1977.

93. Havel RJ. Influence of intensity and duration of exercise on supply and use of fuel. In: Muscle Metabolism During Exercise. Pernow P and Saltin B, eds. New York: Plenum Press, 1971:315-25.

94. Interval Training Conditioning for Sports and General Fitness. Fox, Edward, Matthews, ed. Philadelphia: WB Saunders, 1974.

95. Young DR, Pelligra R, Adachi RR. Serum glucose and free fatty

acids in man during prolonged exercise. J Appl Physiol 1966;21:1047-52.

96. Hedman R. The available glycogen in man and the connection between rate of oxygen intake and carbohydrate useage. Acta Physiol Scand 1957;40:305-10.

97. Gontzea I, Sutzescu R, Dimitrache S. The influence of adaptation to physical effort on nitrogen balance in men. Nutr Rep Int 1975;11:231-35.

98. Refsum HE, Stromme SB. Urea and creatine production and excretion in urine during and after prolonged heavy exercise. J Clin Lab Invest 1974;33:247-254.

99. White TP, Brooks GA. Amino acid and glucose oxidation during exercise. Med Sci Sports 1977;9:67 (abstract).

100. Dohm GL, Tapscott EB, Beecher GR. Effect of exercise on synthesis and degradation of muscle protein. Biochem J 1980;188:255-62.

101. Lemon PWR, Mullin JP. Effect of initial muscle glycogen levels on protein catabolism during exercise. J Appl Physiol: Respirat Environ Exercise Physiol 1980;48:624-29.

102. Felig P, Wahren J. Amino acid metabolism in exercising man. J Clin Invest 1971;50:2703-14.

103. Felig P, Pozefsky T, Marliss E, Cahill GF Jr. Alanine: key role in gluconeogenesis. Science 1970;167:1003-4.

104. Goldberg AL, Chang TW. Regulation and significance of amino acid metabolism in skeletal muscle. Fed Proc 1978;37:2301-07.

105. Lemon PWR, Nagle FJ. Effects of exercise on protein and amino acid metabolism. Med Sci Sport 1981;13:141-49.

106. Lehninger AL. Biochemistry. 2nd ed. New York: Worth Publ Inc, 1975.

107. Saltin B, Karlsson J. Muscle glycogen utilization during work of different intensities. Adv Exp Med Biol 1971;11:289-300.

108. Saltin B, Karlsson J. Muscle glycogen utilization during work of different intensities. In: Muscle Metabolism During Exercise. Pernow P and Saltin B, eds. New York: Plenum Press, 1971:289-99.

109. Karlsson J, Saltin B. Lactate, ATP and CP in working muscles during exhaustive exercise in man. J Appl Physiol 1970;29:57-64.

110. Bergstrom J, Hermansen L, Hultman E, Saltin B. Diet, muscle glycogen and physical performance. Acta Physiol Scand 1967;71:140-50.

111. Hermansen L, Hultman E, Saltin B. Muscle glycogen during prolonged severe exercise. Acta Physiol Scand 1967;71:129-39.

112. Andres R, Cader G, Zierler KL. The quantitatively minor role of carbohydrate in oxidative metabolism by skeletal muscle in intact man in the basal state. Measurements of oxygen and glucose uptake and carbon dioxide and lactate production in the forearm. J Clin Invest 1956;35:671-82.

113. Wahren J, Felig P, Ahlborg G, Jorfeldt L. Glucose metabolism during leg exercise in man. J Clin Invest 1971;50:2715-25.

114. Felig P, Wahren J. Fuel homeostasis in exercise. N Eng J Med 1975;293:1078-84.

115. Ahlborg G, Felig P, Hagenfeldt L, Hendler R, Wahren J. Substrate turnover during prolonged exercise in man. Splanchnic and leg metabolism of glucose, free fatty acids, and amino acids. J Clin Invest 1974;53:1080-90.

116. Hartley, HL. Growth hormone and catecholamine response to exercise in relation to physical training. Med Sci Sports 1975;7:34-36.

117. Winder WW, Hickson RC, Hagberg JM, Eshani AA, McLane JA. Training-induced changes in hormonal and metabolic responses to submaximal exercise. J Appl Physiol: Respirat Environ Exercise Physiol 1979;46:766-71.

118. Felig P, Wahren J. Role of insulin and glucagon in the regulation of hepatic glucose production during exercise. Diabetes 1979;28 (Suppl):71-75.

119. Dole VP. A relation between non-esterified fatty acids in plasma and the metabolism of glucose. J Clin Invest 1956;35:150-54.

120. Havel RJ. The fuels for muscular exercise. In: Johnson WR, Buskirk ER, eds. Science and Medicine of Exercise and Sport. 2nd ed. New York: Harper and Row, 1974.

121. Costill DL, Bowers R, Branham G, Sparks K. Muscle glycogen utilization during prolonged exercise on successive days. J Appl Physiol 1971;31:834-38.

122. Costill DL, Sparks K, Gregor R, Turner C. Muscle glycogen utilization during exhaustive running. J Appl Physiol 1971;31:353-56.

123. Armstrong DT, Steele R, Altszuler N, Dunn A, Bishop JS, DeBodo RC. Regulation of plasma free fatty acid turnover. Am J Physiol 1961;201:9-15.

124. Issekutz B Jr, Miller HI, Paul P, Rodahl K. Source of fat oxidation in exercising dogs. Am J Physiol 1964;207:583-89.

125. Rosell S, Ballard K. Adrenergic neuro-hormonal control of lipolysis in adipose tissue. In: Muscle Metabolism During Exercise. Pernow P and Saltin B, eds. New York: Plenum Press, 1971:111-17.

126. Haggendal J. Role of circulating noradrenalin and adrenalin. In: Muscle Metabolism During Exercise. Pernow P and Saltin B, eds. New York: Plenum Press, 1971:119-25.

127. Rennie MJ, Winder WW, Holloszy JO. A sparing effect of increased plasma free fatty acids on muscle and liver glycogen content in the exercising rat. Biochem J 1976;156:647-55.

128. Hickson RC, Rennie MJ, Conlee RK, Winder WW, Holloszy JO. Effect of increased plasma free fatty acids on glycogen utilization and endurance. J Appl Physiol: Respirat Environ Exercise Physiol 1977;43:829-33.

129. Costill DL, Coyle E, Dalsy G, Evans W, Fink W, Hoopes D. Effects of elevated plasma FFA and insulin on muscle glycogen usage during exercise. J Appl Physiol: Respirat Environ Exercise Physiol 1977;43:695-99.

130. Garland PB, Randle PJ, Newsholme EA. Citrate as an intermediary in the inhibition of phosphofructokinase in rat heart muscle by fatty acids, ketone bodies, pyruvate, diabetes, and starvation. Nature 1963;200:169-70.

131. Randle PJ, Newsholme EA, Garland PB. Regulation of glucose uptake by muscle 8. Effects of fatty acids, ketone bodies and pyruvate, and of alloxan diabetes and starvation, on the uptake and metabolic fate of glucose in rat heart and diaphragm muscles. Biochem J 1964;93:652-65.

132. Issekutz B Jr, Miller HI, Paul P, Rodahl K. Effect of lactic acid on free fatty acids and glucose oxidation in dogs. Am J Physiol 1965;209:1137-44.

133. Fredholm BB. Fat mobilization and blood lactate concentration. In: Muscle Metabolism During Exercise. Pernow P and Saltin B, eds. New York: Plenum Press, 1971:249-55.

134. American College of Sports Medicine. Position statement on the recommended quantity and quality of exercise for developing and

maintaining fitness in health adults. Med Sci Sport 1978;10:75-78.

135. Holloszy, JO. Adaptation of skeletal muscle to endurance exercise. Med Sci Sports 1975;7:155-64.

136. Veikko, Koivisto, Hendler R, Nadel E, Felig P. Influence of physical training on the fuel-hormone response to prolonged low intensity exercise. Metabolism 1982;31:192-97.

137. Schuer J, Tipton CM. Cardiovascular adaptations to physical training. Ann Rev Physiol 1977;39:221-51.

138. Saltin B, Blomqvist G, Mitchell JH, Hohnson RL Jr, Widenthal K, Chapman CB. Response to exercise after bedrest and after training. Circulation 1968;39 (Suppl):1-78.

139. Pattengale PK, Holloszy JO. Augmentation of skeletal muscle myoglobin by a program of treadmill running. Am J Physiol 1966;211:1313-20.

140. Holloszy JO, Oscai LB, Mole PA, Don IJ. Biochemical adaptations to endurance exercise in skeletal muscle. In: Muscle Metabolism During Exercise. Pernow P and Saltin B, eds. New York: Plenum Press, 1971:51-61.

141. Costill DL, Fink WJ, Pollck ML. Muscle fiber composition and enzyme activities of elite distance runners. Med Sci Sports 1976;8:96-100.

142. Mole PA, Oscai LB, Holloszy JO. Adaptation of muscle to exercise. Increase in the levels of palmityl CoA synthetase, carnitine palmityl transferase, and palmityl CoA dehydrogenase and in the capacity to oxidize fatty acids. J Clin Invest 1971;50:2323-30.

143. Baldwin KM, Winder WW, Terjung RL, Holloszy JO. Glycolytic enzymes in different types of skeletal muscle: adaptation to exercise. Am J Physiol 1973;225:962-66.

144. Ekblom B, Astrand P-O, Saltin B, Stenberg J, Wallstrom B. Effect of training on circulatory response to exercise. J Appl Physiol 1968;24:518-28.

145. Saltin B, Karlsson J. Muscle ATP, CP, and lactate during exercise after physical conditions. In: Muscle Metabolism During Exercise. Pernow P and Saltin B, eds. New York: Plenum Press, 1971:395-99.

146. Bergstrom J, Hultman E. Muscle glycogen synthesis after exercise: an enhancing factor localized to the muscle cells in man. Nature 1966;210:309-10.

147. Piehl K. Time course for refilling of glycogen stores in human muscle fibers following exercise-induced glycogen depletion. Acta Physiol Scand 1974;90:297-302.

148. Costill DL, Sherman WM, Fink WJ. The role of dietary carbohydrate in muscle glycogen resynthesis after strenuous running. Am J Clin Nutr 1982;34:1831-36.

149. Ivy JL. Role of insulin during exercise-induced glycogenesis: effect of cyclic AMP. Am J Physiol 1976;233:E509-13.

150. Adolfsson S, Ahren K. Control mechanisms for the synthesis of glycogen in striated muscle. In: Muscle Metabolism During Exercise. Pernow P and Saltin B, eds. New York: Plenum Press, 1971:257-72.

151. Costill DL, Miller JM. Nutrition for endurance sport: carbohydrate and fluid balance. Int J Sports Med 1980;1:2-14.

152. Astrand P-O. Something old and something new...very new. Nutrition Today 1968;3:9-11.

153. Costill DL, Dalsky GP, Fink WJ. Effects of caffeine ingestion on metabolism and exercise performance. Med Sci Sports 1978;10:155-58.

154. Bonen A, Haynes FJ, Watson-Wright W, Sopper MM, Pierce GN, Low MP, Graham TE. Effects of menstrual cycle on metabolic response to exercise. J Appl Physiol: Respirat Environ Exercise Physiol 1983;55:1506-13.

155. Stephenson LA, Kolka MA, Wilkerson JE. Metabolic and thermoregulatory responses to exercise during the human menstrual cycle. Med Sci Sports Exercise 1982;14:270-75.

156. Eisenman PA, Golding LA. Comparison of effects of training on VO2max in girls and young women. Med Sci Sports 1975;7:136-38.

157. Costill DL, Daniels J, Evans W, Fink W, Krahenbuhl, Saltin B. Skeletal muscle enzymes and fiber composition in male and female track athletes. J Appl Physiol 1976;40:149-54.

158. Costill DL, Fink WJ, Getchell LH, Ivy JL, Witzmann FA. Lipid metabolism in skeletal muscle of endurance-trained males and females. J Appl Physiol: Respirat Environ Exercise Physiol 1979;47:787-91.

159. Nygaard E. Women and exercise- with special reference to muscle morphology and metabolism. In: Biochemistry of Exercise. Vol IIB. Poortmans J and Niset G, eds. New York: Univ Park Press, 1981:161-75.

160. Burke EJ. Physiological effects of similar training programs in males and females. Res Quart 1977;48:510-517.

161. Fairbanks LC. Return to sports participation. Phys Sportsmed 1979;7:71-74.

162. Cureton KJ, Hershey LD, Tiburzi A. Body fatness and performance differences between men and women. Res Quart 1979;50:333-40.

163. Cureton KJ, Sparling PB. Distance running performance and metabolic responses to running with excess weight experimentally equated. Med Sci Sports Exercise 1980;12:288-94.

164. Sparling PB, Cureton KJ. Biological determinants of the sex difference in 12-minute run performance. Med Sci Sports Exercise 1983;15:218-23.

165. Pate RR, Barnes C, Miller W. A physiological comparison of performance-matched male and female distance runners. Med Sci Sports Exercise 1982;14:139 (abstract).

166. Helmreich EJM, Klein HW. The role of pyridoxal 5'-phosphate in the catalysis of glycogen phosphorylases. Angeu Chem Int Ed Engl 1980;19:441-55.

167. Toews CJ, Ward GR, Leveille R, Sutton JR, Jones NL. Regulation of glycogenolysis and pyruvate oxidation in human skeletal muscle in vivo. Diabetes Suppl 1979;28:100-2.

168. Benzi G. Endurance training and enzymatic activities in skeletal muscle. Med Sport (Karger, Basel) 1981, 13:165-74.

169. Huston RL, Weiser PC, Dohm GL, Askew EW, Boyd JB. Effects of training, exercise and diet on muscle glycolysis and liver gluconeogenesis. Life Sciences 1975;17:369-76.

170. Gillespie AC, Fox EL, Merola AJ. Enzyme adaptations in rat skeletal muscle after two intensisties of treadmill training. Med Sci Sports Exercise 1982;14:461-66.

171. Wozenski JR. The metabolism of vitamin B-6 in humans and guinea pigs. PhD thesis. Oregon State University, 1977.

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

173. Jackson AS, Pollock ML, Ward A. Generalized equations for predicting body density of women. Med Sci Sports Exercise 1980;12:175-82.

174. Wilmore JH, Costill DL. Semiautomated systems approach to the

assessment of oxygen uptake during exercise. J Appl Physiol 1974;365:618-20.

175. Official methods of analysis. Horwitz W, ed. Washington, DC: Association of Official Analytical Chemists, 1980:786-9.

176. Miller LT, Edwards M. Microbiological assay of vitamin B-6 in blood and urine. In: Leklem JE, Reynolds RD, eds. Methods in vitamin B-6 nutrition: analysis and status assessment. New York:Plenum Press, 1981:45-56.

177. Pino S, Benatti J, Gardyna H. An automated method for urine creatinine which does not require a dialyzer module. Clin Chem 1965;2:664-6.

178. Reddy SK, Reynolds MS, Price JM. The determination of 4-pyridoxic acid in urine. J Biol Chem 1958;223:691-701.

179. Steel RGD, Torrie TH. Principles and procedures of statistics. 2nd ed. New York,NY: McGraw-Hill, 1980.

180. Willarson JE, Guten B, Horvath SM. Exercise-induced changes in blood, red cell, plasma volumes in man. Med Sci Sports 1977;9:155-58.

APPENDICES

Subject #		Gluc mg/dl	Creat mg/dl	BUN mg/dl	UA mg/dl	TP g/dl	Alb g/dl	Chol mg/dl	Na^{+} meq/l	K^{+} meq/l	Cl^{-} meq/l	Ca^{++} mg/dl	Phos mg/dl	TBil mg/dl	SGOT U/l	LD U/l	SGPT U/l	AlP U/l
1	Before	89	0.8	10.8	4.1	7.4	4.7	133	139	3.8	106	10.2	2.9	0.5	29	137	18	49
	After	78	0.6	10.2	4.0	6.8	4.3	120	139	4.6	102	10.0	3.8	0.6	33	184	26	38
3	Before	85	0.9	10.9	4.2	6.4	4.4	144	140	4.4	102	9.5	3.8	0.6	23	140	20	31
	After	96	0.7	10.5	4.5	6.7	4.6	143	136	3.9	103	10.1	3.5	0.5	26	159	26	24
5	Before	84	0.7	12.2	4.9	6.6	4.3	157	138	4.4	103	9.2	3.8	0.7	24	114	21	40
	After	82	0.8	9.4	4.2	5.6	3.9	128	137	4.1	102	9.7	3.9	0.7	19	110	15	46
8	Before	86	0.8	13.8	3.8	6.8	4.5	161	137	4.0	105	9.6	3.9	0.6	35	150	29	30
	After	79	0.7	11.7	4.0	5.6	4.5	150	140	5.2	102	10.0	3.9	0.6	41	184	19	41
10	Before	88	0.9	12.8	4.1	6.6	4.8	191	141	4.7	107	9.8	3.4	0.3	26	149	21	41
	After	88	0.6	13.3	3.9	6.4	4.4	188	139	4.3	101	9.4	4.1	0.2	29	181	26	45
11	Before	89	1.0	13.9	4.5	6.9	4.5	184	140	4.2	105	9.8	3.4	0.5	23	174	13	27
	After	90	0.9	11.8	3.9	6.3	4.4	149	138	4.1	105	9.2	3.6	0.7	27	167	17	30
13	Before	88	0.7	6.8	4.8	6.8	4.5	146	138	3.5	105	9.5	4.4	0.5	27	140	12	24
	After	78	0.6	9.0	4.3	6.8	4.5	160	139	3.9	103	9.8	3.0	0.7	29	164	17	31
14	Before	83	0.8	12.8	4.3	6.9	4.7	195	138	3.7	102	10.6	4.0	0.7	26	171	16	24
	After	93	0.8	11.1	4.2	6.6	4.4	163	140	4.0	101	9.7	5.1	0.3	36	106	14	46
17	Before	90	0.9	15.5	4.0	7.1	4.4	121	139	4.5	104	9.8	3.4	0.7	22	129	13	24
	After	97	0.7	13.5	3.3	6.5	4.2	155	139	3.9	106	9.0	3.7	0.6	26	108	14	23
18	Before	84	1.0	12.6	3.9	7.0	4.8	186	138	4.6	108	9.9	3.6	0.6	22	147	23	40
	After	86	0.7	11.7	3.5	6.8	4.5	139	139	4.7	105	9.6	4.5	0.4	38	121	19	40

Appendix Table 1 Individual blood chemistry screen values before and after the study.

APPENDIX TABLE 2 Individual hematocrit values and plasma volume changes from pre to post exercise for all the exercise tests.

Subject #	Hct pre	Hct post	% chg Hct	PB6 (nM) pre	PB6 post[2]	PB6 post[3]	% chg PV[4]
			Moderate Carbohydrate Diet (MCHO)[1]				
1	36.6	39.0	+6.70	84.9	95.7	90.6	-9.80
3	34.7	39.0	+12.5	57.0	61.8	64.1	-17.0
5	36.5	39.9	+9.50	37.9	44.8	41.5	-13.7
8	35.5	40.1	+12.8	59.3	70.7	66.9	-19.7
10	32.8	35.8	+9.30	64.4	73.2	70.4	-12.6
11	35.2	37.3	+5.80	78.1	91.8	82.6	-8.50
13	37.0	40.4	+10.2	72.3	80.6	79.7	-4.70
14	36.7	40.4	+10.2	77.6	93.0	85.5	-14.0
17	33.5	40.2	+14.5	112	131	128	-19.5
			High Carbohydrate Diet (HCHO)				
1	33.2	34.8	+4.80	68.9	74.9	72.2	-6.80
3	33.8	36.6	+8.30	64.8	71.4	70.2	-11.5
5	36.9	39.8	+2.20	55.1	66.2	56.3	-3.40
8	35.8	37.7	+5.30	59.2	65.1	62.3	-7.80
10	34.1	35.4	+3.70	78.4	99.1	81.3	-5.40
11	33.6	35.7	+6.20	82.2	86.9	87.3	-8.80
13	33.6	35.8	+6.50	69.1	112	73.6	-9.20
14	32.0	34.5	+7.70	87.1	108	93.8	-10.6
17	35.8	36.1	+0.80	60.1	59.4	60.6	-1.30
18	34.2	35.8	+4.70	127	145	133	-9.20
			Moderate Carbohydrate Diet + Vitamin B-6 (MCHO+B-6)				
1	37.9	39.3	+3.70	207	217	214	-5.70
3	39.9	41.6	+4.10	231	220	240	-6.10
5	39.7	42.2	+7.50	274	309	291	-10.0
8	38.9	41.5	+6.50	250	278	266	-10.0
10	41.4	42.8	+3.40	259	286	268	-5.50
11	38.4	40.9	+6.70	275	304	293	-10.1
13	40.9	42.8	+4.50	271	283	283	-7.20
14	41.1	44.2	+7.50	344	376	370	-11.9
17	40.4	42.3	+4.70	250	288	262	-7.50
18	40.3	40.3	0	407	439	407	0

APPENDIX TABLE 2 Individual hematocrit values and plasma volume changes from pre to post exercise for all exercise tests. (Continued)

Subject #	Hct pre	Hct post	% chg Hct	PB6 pre (nM)	PB6 post[2] (nM)	PB6 post[3] (nM)	% chg PV[4]
			High Carbohydrate Diet + Vitamin B-6 (HCHO+B-6)[1]				
1	36.9	39.4	+6.90	208	217	222	+5.00
3	36.6	42.0	+14.8	207	215	238	-2.50
5	38.1	43.6	+14.4	275	325	315	-19.3
8	39.6	41.7	+5.20	231	272	243	-8.10
10	39.2	42.1	+7.60	247	274	266	-11.5
11	36.8	41.4	+12.4	247	328	278	-17.5
13	41.4	40.8	-0.96	266	273	255	+2.50
14	39.6	42.3	+6.80	334	374	357	-10.6
17	38.0	40.8	+7.30	252	267	270	-10.9
18	38.0	41.2	+8.60	393	412	427	-12.7

1. MCHO=49% of total kcal as carbohydrate (CHO), 16% kcal protein (PRO), 35% kcal fat (FAT); HCHO=63% CHO, 15% PRO, 22% FAT; MCHO+B-6=MCHO + 8 mg vitamin B-6 supplement; HCHO+B-6=HCHO + 8 mg vitamin B-6 supplement
2. PB6 value measured at post exercise.
3. PB6 value for post exercise predicted using % change in hematocrit
4. % change PV calculated from method of van Beaumont (182)

APPENDIX TABLE 3 Individual hematocrit values and plasma volume changes from pre to post 60 minutes of exercise for all the exercise tests.

Subject #	Hct pre	Hct p60	% chg Hct	PB6 (nM) pre	PB6 p60 [1]	PB6 p60 [2]	% chg PV [3]
			Moderate Carbohydrate Diet [4]				
1	36.6	34.1	-6.90	84.9	74.4	79.0	+11.7
3	34.7	34.4	-0.81	57.0	55.1	56.5	+1.25
5	36.5	35.2	-3.50	37.9	37.6	36.6	+5.68
8	35.5	37.0	+4.10	59.3	57.8	61.7	-6.08
10	32.8	33.4	+2.10	64.4	61.3	65.7	-3.02
11	35.2	35.1	-0.31	78.1	70.9	75.7	+0.48
13	37.0	35.0	-5.40	72.3	66.2	68.4	+9.07
14	36.7	36.7	0	77.6	74.2	77.6	0
17	33.5	35.3	+5.50	53.5	51.3	56.4	-7.83
18	35.1	34.9	-0.60	112	107	111	+0.93
			High Carbohydrate Diet				
1	33.2	32.6	-2.98	68.9	64.1	66.8	+2.90
3	33.8	33.1	-2.19	64.8	57.8	63.4	+3.40
5	36.9	34.0	-8.00	55.1	51.4	50.7	+13.8
8	35.8	35.8	0	59.2	57.6	59.2	0
10	34.1	34.2	+0.15	78.4	74.1	79.6	-0.22
11	33.6	32.7	-2.65	82.2	75.7	80.0	+4.10
13	33.6	33.1	-1.58	69.1	67.3	68.0	+2.41
14	32.0	30.6	-4.31	87.1	79.9	83.3	+6.63
17	35.8	34.7	-3.13	60.1	56.2	58.2	+5.03
18	34.2	----	----	127	----	127	-----
			Moderate Carbohydrate Diet + Vitamin B-6				
1	37.9	37.0	-2.29	207	202	202	+3.78
3	40.0	37.4	-6.38	231	223	216	+11.3
5	39.6	38.7	-2.45	274	258	267	+4.16
8	38.9	40.4	+3.73	250	258	259	-5.88
10	41.4	40.7	-1.71	259	241	255	+2.98
11	38.4	39.2	+2.08	275	263	281	-3.60
13	41.0	41.7	+1.71	271	262	317	-2.85
14	41.1	40.7	-1.70	344	314	338	+2.94
17	40.4	42.2	+4.28	250	253	261	-6.89
18	40.3	40.5	+0.30	407	394	408	-0.50

APPENDIX TABLE 3 Individual hematocrit values and plasma volume changed from pre to post 60 minutes of exercise for all the exercise tests. (Continued)

Subject #	Hct pre	Hct p60	% chg Hct	PB6 (nM) pre	PB6 p60[1]	PB6 p60[2]	% chg PV[3]
			High Carbohydrate Diet + Vitamin B-6[4]				
1	36.9	37.8	+2.36	208	209	213	-3.36
3	36.6	39.0	+6.56	207	206	221	-10.3
5	38.1	38.3	+0.52	275	267	276	-0.97
8	39.6	39.1	-1.41	231	223	228	+2.37
10	39.2	38.7	-1.12	247	228	244	+1.87
11	36.8	38.0	+3.18	247	253	255	-3.08
13	41.4	38.2	-7.87	266	231	245	+14.6
14	39.6	39.0	-1.44	334	336	329	+2.42
17	38.0	39.4	+3.55	252	237	261	-5.73
18	38.0	37.9	-0.18	393	394	392	+0.30

1. PB6 value measured post 60 minutes of exercise.
2. PB6 value predicted for post 60 minutes of exercise using % change in hematocrit.
3. % change PV calculated from method of van Beaumont (182)
4. see 1. Appendix Table 2

APPENDIX TABLE 4 Individual and mean plasma vitamin B-6 values for day 1, day 10, and all the exercise tests for the untrained group.

subject #		1	11	14	17	18	mean ± S.D.
				(nM)			
day 1		43.3	61.9	50.1	37.0	54.9	49.4 ± 8.69
day 10		67.1	83.9	83.5	61.0	112	73.8 ± 10.0
MCHO[1]	1[2]	84.9	78.1	77.6	53.5	112	81.2 ± 18.7
	2	95.7	91.8	93.0	61.9	131	94.6 ± 21.8
	3	81.1	80.7	83.1	----	106	87.7 ± 10.6
	4	74.4	70.9	74.2	51.3	107	75.6 ± 18.0
HCHO	1	68.9	82.2	87.1	60.1	127	85.1 ± 23.1
	2	74.9	86.9	108	59.4	145	94.8 ± 29.8
	3	63.5	79.1	84.2	53.4	122	80.4 ± 23.5
	4	64.1	75.7	79.9	56.2	---	68.9 ± 9.30
MCHO+B6	1	207	275	344	250	407	296 ± 70.8
	2	217	304	376	288	439	325 ± 76.4
	3	213	275	364	254	401	301 ± 70.0
	4	202	263	314	253	394	285 ± 65.1
HCHO+B6	1	208	247	334	252	393	287 ± 67.3
	2	217	328	374	267	412	319 ± 70.5
	3	217	308	343	247	385	300 ± 61.2
	4	209	253	336	237	394	286 ± 68.4

1. see 1. Appendix Table 2
2. 1= pre, 2= post, 3= p30, 4= p60 minutes of exercise

APPENDIX TABLE 5 Individual and mean plasma vitamin B-6 values for day 1, day 10, and all the exercise tests for the trained group.

subject #		3	5	8	10	13	mean ± S.D.
				(nM)			
day 1		61.2	49.6	85.5	55.1	90.1	68.3 ± 16.4
day 10		56.6	32.5	59.0	77.9	77.6	60.7 ± 16.7
MCHC[1]	1[2]	57.0	37.9	59.3	64.4	72.3	58.1 ± 11.4
	2	61.8	44.8	70.7	73.2	80.6	66.2 ± 12.3
	3	57.6	41.1	63.7	70.0	69.7	60.4 ± 10.7
	4	55.1	37.6	57.8	61.3	66.2	55.6 + 9.70
HCHO	1	64.8	55.1	59.2	78.4	69.1	65.3 ± 8.00
	2	71.4	66.2	65.1	99.1	112	82.8 ± 19.2
	3	60.7	55.7	59.8	75.7	61.3	62.6 ± 7.00
	4	57.8	51.4	57.6	74.1	67.3	61.6 ± 8.00
MCHO+B6	1	231	274	250	259	271	257 ± 15.5
	2	220	309	278	286	283	275 ± 29.8
	3	237	282	280	270	267	267 ± 15.9
	4	223	258	258	341	262	248 ± 14.4
HCHO+B6	1	207	275	231	247	266	245 ± 24.4
	2	215	325	272	274	273	272 ± 34.8
	3	194	309	266	242	251	252 ± 37.4
	4	206	267	223	228	231	231 ± 20.0

1. see Appendix Table 2
2. 1= pre, 2= post, 3= p30, 4= p60 minutes of exercise

APPENDIX TABLE 6 Individual and mean urinary 4-pyridoxic acid excretion values for day 1, day 10, and the day before and day after exercise for all the exercise tests for the untrained group.

subject #		1	11	14	17	18	mean ± S.D.
				(umol/24 hr)			
day 1		6.97	6.15	3.28	3.98	3.09	4.69 ± 1.76
day 10		6.15	6.47	5.19	6.17	5.98	6.07 ± 0.55
MCHO[1]	1[2]	7.78	6.48	6.38	7.56	6.19	6.88 ± 0.73
	2	8.09	7.55	7.18	6.96	7.27	7.41 ± 0.43
HCHO	1	8.52	5.83	6.38	6.77	7.04	6.91 ± 1.01
	2	8.52	7.12	7.98	6.96	7.04	7.52 ± 0.69
MCHO+B6	1	36.7	38.9	34.0	36.2	37.8	36.7 ± 1.82
	2	41.7	39.2	42.4	35.8	35.8	39.0 ± 3.13
HCHO+B6	1	39.1	33.6	35.2	35.0	34.0	35.4 ± 2.20
	2	39.1	37.8	37.0	33.1	38.4	37.1 ± 2.37

1. see 1. Appendix Table 2
2. 1=day before exercise, 2=day of exercise

APPENDIX TABLE 7 Individual and mean urinary 4-pyridoxic acid excretion values for day 1, day 10, and the day before and day after exercise for all exercise tests for the trained group.

subject #		3	5	8	10	13	mean ± S.D.
				(umol/24 hr)			
day 1		6.40	5.67	8.70	4.54	6.99	6.47 ± 1.54
day 10		-----	6.75	7.49	6.69	7.67	7.15 ± 0.50
MCHO[2]	1[1]	7.80	8.00	7.18	7.85	6.91	7.55 ± 0.47
	2	7.92	7.36	8.76	6.19	7.70	7.59 ± 0.93
HCHO	1	7.44	7.30	7.27	7.78	7.08	7.37 ± 0.26
	2	7.89	7.57	8.10	7.59	6.66	7.56 ± 0.55
MCHO+B6	1	38.6	38.5	40.2	36.0	38.9	38.4 ± 1.53
	2	37.5	39.4	42.3	41.9	40.6	39.8 ± 2.21
HCHO+B6	1	40.4	39.9	39.7	31.6	38.6	38.1 ± 3.67
	2	38.4	41.4	42.4	40.7	40.4	40.6 ± 1.50

1. 1=day before exercise, 2=day of exercise
2. see 1. Appendix Table 2

Diet and Exercise Study Calendar

Fall '82 Women's Diet & Exercise Study

SEPTEMBER '82

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	
			1	2	3	4	
5	6	7	8	9	10	11	
12	13	14	15	16	17	18	
19	Chemistry screen & 20	21	xylose test done 22	23	24	25	Pre-study data collection
26	VO_2 max determined 27	28	29	30			

OCTOBER '82

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	
					1	2	
3	Bld. (1) 4	(2) 5	(3) 6	(4) 7	(5) 8	(6) 9	week 1
(7) 10	(8) 11	(9) 12	Bld./T.L. (10) 13	(11) 14	Bld./exercise* (12) 15	Bld./exercise* (13) 16	week 2
(14) 17	(15) 18	(16) 19	Bld./T.L. (17) 20	(18) 21	Bld./exercise* (19) 22	Bld./exercise* (20) 23	week 3
(21) 24 / (28) 31	(22) 25	(23) 26	(24) 27	(25) 28	(26) 29	(27) 30	week 4

NOVEMBER '82

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	
	(29) 1	(30) 2	Bld. (31) 3	(32) 4	(33) 5	(34) 6	week 5
(35) 7	(36) 8	(37) 9	Bld./T.L. (38) 10	(39) 11	Bld./exercise* (40) 12	Bld./exercise* (41) 13	week 6
(42) 14	(43) 15	(44) 16	Bld./T.L. (45) 17	(46) 18	Bld./exercise* (47) 19	Bld./exercise* (48) 20	week 7
END STUDY Bld. 21	22	23	24	THANKSGIVING VACATION! 25	26	! 27	

Bld = blood
T.L. = Tryptophan Load Test
*Each subject will be exercised either Friday or Saturday in the week exercise is scheduled and bld will be drawn at that time. All exercise testing will be done in the morning before breakfast.
Any questions please call:

Phone #: OSU F & N Dept.--754-3561 (during working hours)
(home phones) Dr. Leklem--753-1072 Carol Walter--753-0640
Melinda Manore--758-9409 Caroll Lee--754-1860

Phone Questionnaire

NAME_____ AGE_____ POST-MENOAPUSAL: YES NO

ADDRESS_____ PHONE (HOME) _____

_____ PHONE (WORK) _____ HOURS:_____

HEIGHT _____ PRESENT WEIGHT_____ LENGHT OF TIME YOU HAVE MAINTAINED PRESENT WEIGHT _____

ORAL CONTRACEPTIVE USER: YES NO

USE VITAMINS: yes no

ACTIVITY LEVEL:

Do you have a current fitness program? YES NO If yes, please answer the following questions:

1. Type of fitness program: list normal weekly activities and intensity.

2. How long have you maintained this level of fitness?_____

Do you have any food preferences or foods you will not eat? List.

Are you currently on any medications? If yes, list.

Any other helpful information:

Would this person be a good subject?

Health History Questionnaire

HEALTH HISTORY QUESTIONNAIRE

DATE _____

NAME _____
last first middle

ADDRESS _____
street

_____
city state zip

PHONE _____ _____
area code

AGE (YEARS)_____

BIRTH DATE _____/ _____/ _____/
month day year

STATE (OR COUNTRY) OF BIRTH _____

PREDOMINATE STATE OF RESDIENCE: _____

NUMBER OF YEARS_____ CITY OR TOWN_____

HEIGHT _____/ _____/
feet inches

WEIGHT (lbs) _____ MOST WEIGHTED _____ WHAT YEAR____

LENGTH OF TIME YOU HAVE MAINTAINED YOUR CURRENT WEIGHT _____

RACE (Circle one):

a. American Indian b. Black c. Caucasian D. Chinese

e. Latin American f. Japanese g. other Oriental (specify)_____

h. Other (specify) _____

MARITAL STATUS (circle one): a. single b. married c. divorce/ separated
d. widowed

MENSTRUAL HISOTRY:

Are you sill having periods? (Circle one)

a. Yes, regularly b. Yes, irregularly c. No

Health History Questionnaire (continued)

If you are still having periods, please answer the following:
Do you take the "pill"?
a. Yes
b. Not now, but did in the past. Time expired since you last took the "pill". _____
c. Never took the pill.

If you no longer have periods, please answer the following:
At what age did your periods stop? _____
Have you had any bleeding since then? (circle any that apply)
a. none b. seldom c. often d. light e. heavy

PREGNANCY HISTORY

What is your pregnancy status now? (circle one)
a. never pregnant
b. pregnant now
c. not pregnant now, but pregnant within the last 12 months
d. not pregnant within the last 12 months
e. age at first pregnancy _____

MEDICAL HISTORY (circle any that apply, and give age at diagnosis)

	AGE
a. diabetes	_____
b. hypothyroidism	_____
c. hyperthyroidism	_____
d. goiter	_____
e. hypoadrenalism (Addison's disease)	_____
f. osteoporosis	_____
g. hepatitis	_____
h. cirrhosis	_____
i. gall stones	_____
j. kidney stones	_____
k. nephritis	_____
l. cystitis	_____
m. cancer (specify type) _____	_____
n. high blood pressure	_____
o. angina	_____
p. coronary thrombosis or any other type of heart problem (specify) _____	_____
q. mental depression requiring medication	_____
r. insomia requiring frequent medication	_____
s. allergies	_____

DRUG USE HISTORY (circle any which you take on a regular basis)

a. sleeping tablets
b. barbiturates
c. tranquilizers
d. blood pressure medication
e. diuretics
f. antibiotics
g. oral contraceptives
h. estrogen (female hormone)
i. thyroid (thyroxin)
j. insulin
k. cortsone
l. other steroids (specify)

Health History Questionnaire (continued)

SURGICAL HISTORY

Please specify any type of surgery you have had done and the date and age when it occurred.

Surgery	Date	Age

DIETARY HISTORY

Are you a vegetarian? (circle one) Yes No
If yes, circle one of the following:

a, lacto-ovo b. ovo c. lacto d. vegan

How long have you consumed a vegetarian diet? (circle one)

a. 1 year b. 2-5 years c. 6-10 years d. more than 10 years

Do you take vitamins? (circle one)

a. yes, daily
b. yes, frequently
c. never

If yes, what type and how long?

Type _____

How long (years) _____

FOOD PREFERENCES:

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

_____

_____

Physical Activity History

NAME _____ DATE _____

PHYSICAL ACTIVITY HISTORY

Please carefully review the following questions and answer them as accurately as possible. Place a check next to the appropriate answer for each question.

Occupational Activity

_____ I. Predomiately sedentary--setting position (desk worker, typist, light goods assembly line work, etc.)

_____ II. Light activity--some standing and walking (cashier, student, general office work, light factory work, police officer, etc.)

_____ III. Moderate activity--walking and material handling (waiter/ waitress, mail carrier, construction worker, heavy factory work, etc.)

_____ IV. Heavy activity--heavy manual labor (heavy construction laborer, lumberjack, farm laborer, long shoreman, etc.)

Recreational/Leisure Activity

Rate your current level of leisure activity on the following scale:

____Totally sedentary

____Very Light

____Light

____Very moderate

____ Moderate

____ Somewhat hard

____ Hard

____ Very hard

Which, if any, of the following exercises are you currently doing?

____ No exercise

____ Calisthenics

____ Lift weights

____ Walk for exercise

____ Jog/run

____ Ride a bicycle

____ Swim

____ Other (please describe)

Physical Activity History (continued)

How many days per week do you exercise?

____ None

____ One

____ Two

____ Three

____ Four

____ Five

____ Six

____ Seven

How much time do you spend on exercise each exercise day?

____ None

____ Less than 15 minutes

____ 15-30 minutes

____ 30-45 minutes

____ 45-60 minutes

____ 60-75 minutes

____ 75-90 minutes

____ More than 90 minutes

If you exercise, please rate the intensity of your exercise on the following scale:

____ 6

____ 7 very, very light

____ 8

____ 9 very light

____ 10

____ 11 fairly light

____ 12

____ 13 somewhat hard

____ 14

____ 15 hard

____ 16

____ 17 very hard

____ 18

____ 19 very, very hard

____ 20

How would you rate your current general state of physical fitness?

____ very, very good

____ very good

____ good

____ neither good nor poor

____ poor

____ very poor

____ very, very poor

Physical Activity History (continued)

How long have maintained your current level of physical fitness?

____ less than 6 months

____ 6 months - 1 year

____ 1-2 years

____ 2-3 years

____ more than 3 years

Briefly outline a normal week's activities:

Day	Activity	Minutes Engaged in activity	Intensity

Subject Consent Form

I, _____, give my consent to participate in this study. The study has been explained to me and all my questions have been answered. I agreed to consume all food and vitamin supplements provided and to provide a record of food consumed for three days prior to beginning of the experiment. I agree to participate in the five exercise periods scheduled in this experiment on the bicycle ergometer. I understand that there is a risk of a heart attack during a test such as this and that the type of exercise procedure to be conducted has been explained. I further agree to participate only after clearance of a physician is given. I agree to allow 23-30 mls of blood to be drawn periodically throughout the experimental period to monitor B-6 status and four times during each exercise test. I also agree to collect 24 hour urine samples throughout the experimental period.

I understand that I will be paid $100.00 should I complete the study. I understand that I am free to withdraw from the study at any time and that I can terminate the exercise part of the study at any time. I also understand that the investigators reserve the right to withdraw me from this study at any time.

All information concering me will be kept confidential.

Name _____ Date _____

Witness _____ Date _____

Physician Consent

The following individual _____ is being considered as a subject for a nutrition, diet and exercise study being conducted by the Foods and Nutrition Department at Oregon State University. The study will involve feeding the subject a normal or a high carbohydrate diet for seven weeks and exercising the subject four times on a stationary bicycle for 25 minutes at 80% VO_2max. Cardiac function will be monitored throughout the exercise periods on an ECG and blood pressures will be monitored pre, during and after exercise. All exercise will be conducted by experienced investigators.

Could you please conduct a routine physical on this individual to determine if there is any medical reason this person should not participate in the study. Results of the chemistry screen are available for your reference.

Please send the bill along with a list of itemized charges to:

Dr. James Leklem
Oregon State University
Foods and Nutrition Department
Corvallis, OR 97331

If you have any questions before releasing this person for participating in our study please call Dr. James Leklem at 754-3561.

Please sign the following statement if this individual is medically capable of participating in our study.

I have examined _____ on _____ and found her medically capable of participating in the 1982 Nutrition Department diet and exercise study.

Signed _____

Subject Responsibilities

As a subject in this study I will be expected to complete each of the following activities:

Prior to beginning study:

1. Complete health history and physical activity history questionnaires.

2. Record dietary intake for three days.

3. Physical examination by a physician and doctor's approval of physical health.

4. Assessment of VO_2 max and percent body fat.

5. Xylose absorption test with urine collection needed over a five hour period. An oral solution of 5 grams of xylose will be given under fasting conditions.

6. Fasting blood draw of 20-30 mls for blood chemistry screen and measurement of B-6 status.

During the metabolic study:

1. Daily weighing before breakfast.

2. Daily record of aerobic activities.

3. Collection of all urine (24 hours) throughout the study.

4. Consumption of all meals in the metabolic unit:
   a. All foods provided must be eaten.
   b. No additional food, snacks or drinks are allowed other than indicated.

5. Four times during the metabolic study a 2 gram dose of tryptophan will be given with the breakfast meal. This is a naturally occurring amino acid found in foods.

6. Throughout the study subjects must refrain from drug use. No nicotine, alcohol or other recreational drugs can be used for the duration of the study. Decaffeinated beverages will be provided when needed. Only decaffeinated beverages can be used the day before and the day of the exercise test. Use of any of these drugs will adversely alter the results of the experiment.

Subject Responsibilities (continued)

7. Weight will be maintained throughtout the study and diet will be adjusted to assure weight remains constant.

Exercise Requirements:

1. Each subject will participate in four exercise periods during the seven week experimental period. The exercise will consist of 30 minutes of aerobic activity at 80% VO_2 max on the bicycle ergometer.

2. ECG tracings will be monitored continuously throughout the exercise period.

3. Blood pressures will be taken pre, during and post-exercise.

4. All exercise periods will be done in post-absorptive (fasting) state on either Friday's or Saturday's, when convenient for the subject.

5. Venous blood (20-30 mls) will be drawn four times during each exercise period; pre, post, post-30 and post-60 minutes of exercise.

6. Subjects must maintain their classified level of fitness throughout the study.

7. Exercise periods will take approximately 1 1/2 hours, subject will arrive at least 15 minutes prior to their scheduled exercise time.

General Instructions

General Instructions for Fall '82 Diet & Exercise Study

COLLECTION OF URINE:

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

2. Label all containers carefully and clearly with your initials and date.

3. Each day:

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

4. Urine will be collected starting with breakfast on the day you start on the diet study. Return urine samples daily at any time convenient for you to Rm 106, Mlm Hall.

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

6. Please be careful not to spill or lose any urine. If this does happen, however, let us know immediately. The urine collections are a very critical part of this study.

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

EXERCISE TEST (4 times during the study)

1. Come at your scheduled time to Mlm Hall, Rm 107 in comfortable clothes for exercising.

2. Exercising tests will be done fasting (before breakfast).

3. Use only decaffinated beverages the day before and the day of your exercise test (ie. after the test is completed).

4. Breakfast will be provided after the exercise testing period has been completed.

OTHER

1. Eat all food given to you each day. Let us know if you are receiving too much or too little food and we will adjust your diet accordingly.

2. Record all activities every day. A journal will be provided for you at breakfast to fill in for the previous day's activities (# hrs. slept, working, exercising, etc.).

3. No alcoholic beverages, including beer and wine, are to be consumed during the study.

4. No vitamin or mineral supolements are to be consumed during the study.

5. No smoking or use of nicotine during the study.

OVER

General Instructions (continued)

FREE FOODS

May be eaten in any amount desired, but amounts eaten must be recorded except for condiments:

Coffee, tea	Condiments:
Diet beverages	Salt
	Pepper
	Spices, etc.

Note: Coffee and tea should be used black except for adding honey and milk already included in your diet allotment.

The names and phone numbers of people whom you may contact in case you have any questions or in case anything goes wrong:

	Residence	Office
Dr. James Leklem	753-1072	754-3561
Melinda Manore	758-9409	754-3561
Carol Walter	753-0640	754-3561
Caroll Lee	754-1860	754-3561

Daily Activity Sheet

Name _____

Date _____

DAILY ACTIVITY SHEET

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

Activity	Length of Time (fraction of hours)	Time of Day	Intensity
Work			
Sleep			
Sitting			
Walking			
Running			
Bicycling			
Swimming			
Other sports or activities (indicate type)			

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

   Coffee (cups) _____

   Tea (cups) _____

   Diet Pop _____

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

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

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

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

7. Other comments.

Foods & Nutrition Diet & Exercise Study
Instructions for Exercise Testing

TO: _____

You are scheduled to do your exercise test on _____ at _____ in Rm 107, Mlm Hall. The following procedures should be followed:

1) Wear comfortable clothing for exercise.
   a) halter top, swim suit top or bra with blouse that buttons in front
   b) shorts or loose slacks
   c) comfortable shoes for biking
2) Exercise will be done fasting, before breakfast.
3) No caffeinated beverages should be used the day before or the day of the exercise test.

The procedures of the exercise test are as follows:

1) Exercising for approximately 25 minutes at a % of your VO_2 max.
2) Blood will be drawn once before and 3 times after the exercise test (4 blood draws in all).
3) Breakfast will be served following the exercise test.
4) The exercise testing and breakfast will require approximately 2 hours.