AN ABSTRACT OF THE THESIS OF

Melinda M. Manore for the degree of Doctor of Philosophy in Foods and Nutrition presented on July 30, 1984

Title: The Effect of Two Carbohydrate Diets and Vitamin B-6 on Vitamin B-6 and Fuel Metabolism and Cardiac Function During Exercise in Trained and Untrained Women.

Abstract approved: James E. Leklem, Ph.D.

This study examined the effect of exercise on vitamin B-6 (B6) metabolism and the effect vitamin B-6 supplementation and dietary carbohydrate (CHO) have on fuel metabolism and cardiac function during exercise in women, 5 young/trained (YT), 5 young/untrained (YU), and 5 postmenopausal/untrained (PU) women were alternately fed four dietary treatments. The diets were fed in the following sequence: a moderate carbohydrate (MCHO) diet for 2 weeks; a high carbohydrate (HCHO) diet for 1 week; return to the MCHO diet for 1 week; a MCHO+B6 diet for 2 weeks and a HCHO+B6 diet for 1 week. The non-supplemented (NS) diets contained 2.3-2.4 mg B6, while the supplemented (S) diets included an additional 8 mg B6. Training was determined by $\dot{V}O_2$ max. Subjects were exercised at 80% $\dot{V}O_2$ max for 20 minutes on a cycle ergometer at the end of each dietary treatment. Blood was drawn pre, post, post 30-min and post60-min (p60) of exercise and analyzed for plasma pyridoxal 5'-phosphate (PLP), plasma B6 (PB6), glucose (G), free fatty acids (FFA), and lactate (LA). 24-hour urines were analyzed for 4-pyridoxic acid (4PA) and urinary B-6 (UB6). Electrocardiograph

tracings were taken at rest and at 80% maximal heart rate (MHR) and QT corrected (QT_c) and ST segment depressions measured. QT_c was calculated using Bazett's formula. ANOVA showed no difference among groups with respect to diet or time for PB6, 4PA or UB6. PLP and PB6 increased significantly from post to p60 for all diets. The magnitude of change of PB6 and PLP from pre to post and post to p60 was much greater with the S diets than the NS diets. 4PA excretion increased significantly from the day before exercise to the day of exercise for all diets. UB6 showed no significant change with exercise. ANOVA showed no difference among groups or diets for LA, however there was a significant increase from pre to post and significantly decreased from post to p60 for all diets. ANOVA showed significant time x group x diet interactions for FFA and significant diet x time and time x group interactions for G. G response was significantly different in PU than the YT or YU during exercise. S usually resulted in lower FFA response during exercise in all groups with the HCHO+B6 always producing the lowest FFA. Increased CHO also resulted in lower FFA during exercise. ANOVA of QT_c showed significant main effects for diet, group and time, but time x group was the only significant interaction. The MCHO diet showed significantly longer QT_c intervals than the other three diets. For all diets, QT_c increased from rest to 80% MHR in YT and PU but decreased or remained unchanged in YU. No ST segment depressions were seen.

THE EFFECT OF TWO CARBOHYDRATE DIETS AND VITAMIN B-6 ON VITAMIN B-6 AND FUEL METABOLISM AND CARDIAC FUNCTION DURING EXERCISE IN TRAINED AND UNTRAINED WOMEN

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

THE EFFECT OF TWO CARBOHYDRATE DIETS AND VITAMIN B-6 ON VITAMIN B-6 AND FUEL METABOLISM AND CARDIAC FUNCTION DURING EXERCISE IN TRAINED AND UNTRAINED WOMEN

CHAPTER I

INTRODUCTION

The rising interest in the prevention of disease, improving the quality of life and increasing one's overall well-being has stimulated an interest in the interrelationship between nutrition and fitness and its role in the improvement and maintenance of health and physical ability. One nutrient which is currently being investigated for its role in fuel metabolism and cardiac function during exercise is vitamin B-6. The effect exercise has on vitamin B-6 metabolism is also being investigated. It has already been established that in men exercise can alter vitamin B-6 metabolism (Hatcher et al., 1982; Leklem and Shultz, 1983). Both Hatcher et al. (1982) and Leklem and Schultz (1983) reported a significant rise in plasma pyridoxal 5'-phosphate during exercise. This rise was significant even after accounting for hemoconcentration. Black and co-workers (1977, 1978) established that the muscle glycogen phosphorylase enzyme can be a reservoir for vitamin B-6. However, the release of the stored vitamin only occurs during starvation and not when vitamin B-6 deficiency is introducted. Leklem and Shultz (1983) hypothesized that exercise may mimic starvation and thus stimulate the release of pyridoxal 5'-phosphate from the glycogen phosphorylase enzyme. The reason for this release has not been established, but it has been hypothesized that

the increased circulation of pyridoxal 5'-phosphate in the plasma during exercise may be to assist in vitamin B-6 dependent gluconeogenic processes. Whether these same alterations in vitamin B-6 metabolism will occur in women during exercise will be explored in this thesis.

The influence of diet on fuel metabolism and cardiac function during exercise was first examined by deVos et al. (1982), Goulard (1982), and Seimann (1982). They fed high and low carbohydate diets, with and without vitamin B-6 supplementation. Their results suggested that the amount of vitamin B-6 and carbohydrate in the diet may alter the use of glycogen, glucose, and free fatty acids during exercise. They hypothesized that this change in circulating fuels during exercise was brought about by alterations in the amount and rate at which the glycogen was stored and broken down. They also concluded that vitamin B-6 could alter cardiac function during high intensity exercise. Their results were based on measurement of ST segment and QT interval changes before, during, and after exercise. These studies were done in trained, male cyclists.

The literature indicates that women may be at greater risk of decreased vitamin B-6 status due to a variety of factors. Some of these factors include: increased need during pregnancy and lactation, decreased intake due to altered eating habits and/or dieting, altered absorption and/or the use of oral contraceptives (Chrisley and Driskell, 1979; Guthrie and Crocetti, 1983; Rose, 1978; Shane and Contractor, 1980). Women are also more likely to embark on severe weight reduction diets which may decrease vitamin B-6 status as well

as increase the risk of abnormal cardiac function (Isner et al., 1979; Van Itallii and Yang, 1984). Fuel metabolism and cardiac function in women during exercise has not been studied extensively. With these factors in mind, the purpose of this research study was:

1. To determine the effect of exercise on vitamin B-6 metabolism in trained and untrained women, both young and postmenopausal, while being fed a moderate or high carbohydrate diet with or without vitamin B-6 supplementation.
2. To determine the effect of diet, both moderate and high carbohydrate, and vitamin B-6, supplemented and non-supplemented, on cardiac function and fuel metabolism during exercise in women.

This thesis will include a review of the literature on vitamin B-6, fuel use during exercise, and cardiac function. The interrelationship between vitamin B-6, fuel metabolism and cardiac function during exercise will also be examined. Three articles to be submitted for publication follow the review of literature. The first article explores vitamin B-6 metabolism during exercise in women of differing ages and levels of training. It also examines the effect alterations in dietary carbohydrate have on vitamin B-6 metabolism during exercise. The second article reports changes in fuel metabolism during exercise in women consuming varying amounts of carbohydrate and vitamin B-6 in their diet. The last article addresses the effect diet and exercise may have on cardiac function in women. Changes in vitamin B-6 and carbohydrate were examined to see if cardiac function during exercise could be altered by dietary manipulation.

CHAPTER II

REVIEW OF LITERATURE

In reviewing the existing literature on the interrelationship between diet and exercise and, more specifically, the effect exercise can play in vitamin B-6 metabolism and its effect on fuel metabolism, an awareness is developed for the many variables involved. Because of this complexity one must always be aware that it is the varying degree to which these factors interrelate in a particular individual which decides the ultimate fuel utilized for energy during exercise.

The literature review on vitamin B-6 and fuel metabolism and cardiac function will be presented in four categories with each category having several parts. The first category is vitamin B-6 and covers the chemistry, functions, metabolism, and storage of this vitamin as well as vitamin B-6 status in women. The second category reviews fuel metabolism during exercise and discusses how diet may alter the fuels used during exercise. The contribution of carbohydrate, fat, and protein will be specifically addressed. For convenience and ease of understanding, these factors will be presented separately, however, one should not forget the interrelationship which exists between them. The third category is vitamin B-6 and exercise and will address the role vitamin B-6 may play in altering fuel metabolism during exercise as well as how exercise may alter vitamin B-6 metabolism. The final category will cover cardiac function during exercise and how it may be altered by diet.

VITAMIN B-6

Vitamin B-6 is a water-soluble vitamin which was first recognized by Gyorgy (1934) as a factor which could prevent skin lesions in rats. The crystalline compound was then isolated and reported by various investigators (Gyorgy, 1938; Lepkovsky, 1938; Keresztesy and Stevens, 1938), simultaneously. The vitamin was then characterized by Stiller et al. (1939) and Harris et al. (1939) and eventually synthesized by Harris and Folkers (1939), as being essential to man. This essentiality was first recognized in man in 1939 by Spies et al. (1939). They reported that patients eating a poor diet demonstrated non-descript symptoms such as weakness, irritability, insomnia, and nervousness. Surprisingly, relief was obtained within 24 hours after the administration of vitamin B-6, as pyridoxine. Further evidence of its essentiality was obtained in 1954 when infants fed formula diets in which the vitamin B-6 had been destroyed developed convulsions (Coursin, 1954).

Due to the structure of the vitamin B-6 molecule (3-hydroxy-4, 6-bis(hydroxy methly)-2-methyl pyridine) the term pyridoxine is used for the synthesized vitamin (Brin, 1978). However, further microbiological studies have shown two other natural forms of the vitamin exist, pyridoxal and pyridoxamine (Snell et al., 1942).

Chemistry, Sources, and Forms

Vitamin B-6 is the generic term used to describe all the biologically active forms of the vitamin. The forms include: pyridoxine (PN), the alcohol form; pyridoxal (PL), the aldehyde form;

pyridoxamine (PM), the amine form and the three 5'-phosphate forms, pyridoxine-5' phosphate (PNP), pyridoxal-5' phosphate (PLP) and pyridoxamine-5' phsophate (PMP). Fig. 2.1 shows the chemical structures of all six forms of vitamin B-6 and their biological interconversions. The major urinary metabolite of vitamin B-6, 4-pyridoxic acid (4PA) is also shown in Figure 1. Pyridoxine hydrochloride is the most common synthetic form of vitamin B-6 available. This is due to the fact that pyridoxine hydrochloride is a more stable compound and is easier to incorporate into products than other forms of the vitamin (Bauernfeind and Miller, 1978). Pyridoxine is rapidly destroyed by white light in neutral or alkaline solutions but stable in acid solutions (Gregory and Kirk, 1978).

In nature pyridoxal, pyridoxine and pyridoxamine are widespread in foods (Orr, 1969). PN is the major form found in plant foods. Examples of plant foods high in vitamin B-6 are nuts, beans, lentils, cereals, and some fruits such as bananas and avocados. The amount of vitamin B-6 found in a 100 gram portion of edible fruits, nuts, or vegetables may range from 0.40 mg in peanuts to 0.51 mg in bananas and 0.42 mg in avocados. However, fruits such as apples and peaches are low in vitamin B-6 with 0.03 and 0.027 mg per 100 grams, respectively (Orr, 1969). PL and PM are the predominate forms of vitamin B-6 found in animal products. Examples of animal products high in vitamin B-6 are beef liver and red meat, halibut, and tuna. The vitamin B-6 content of these foods is 0.84, 0.33, 0.43, and 0.43 mg/100 grams, respectively. In the American diet, meats supply

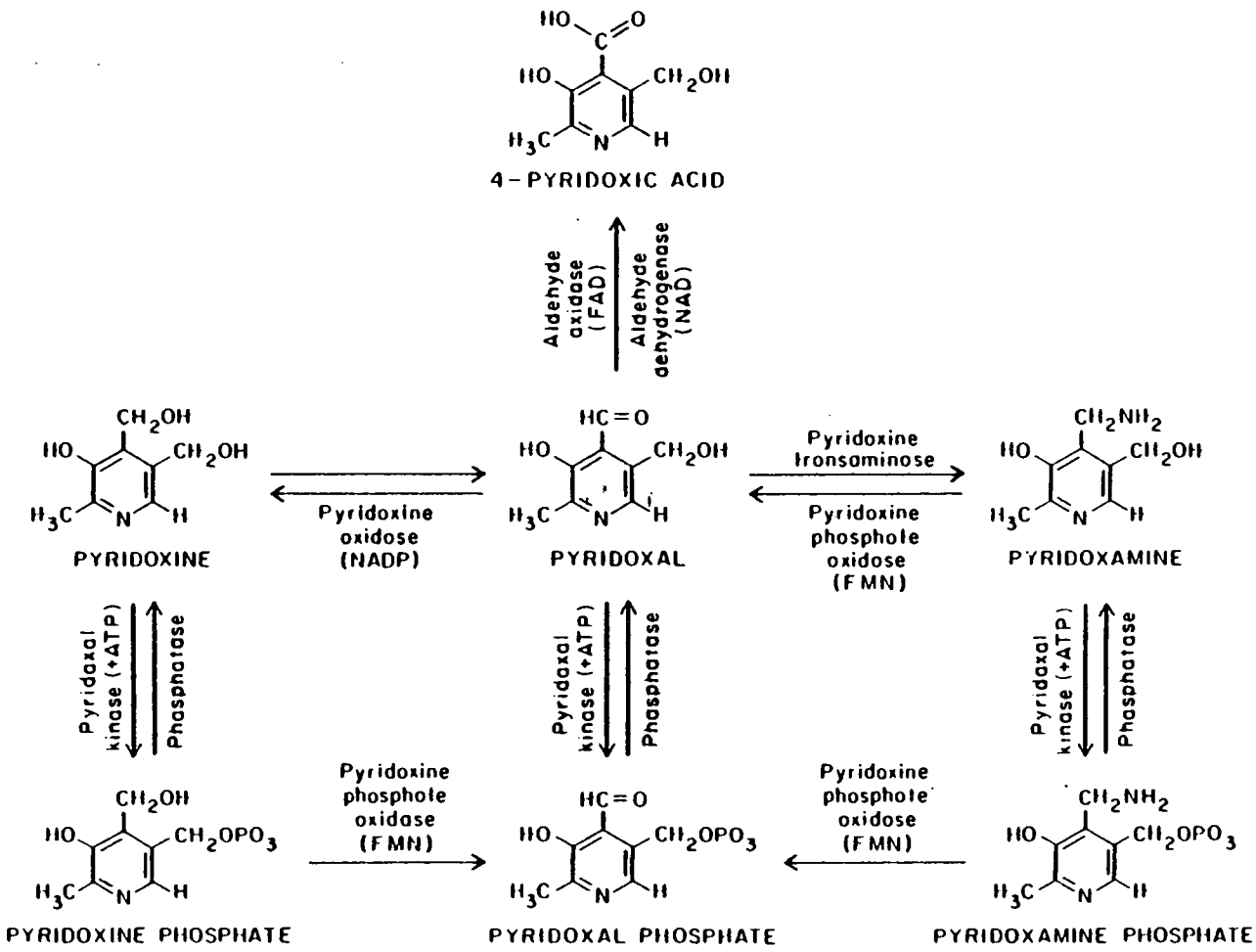


Figure 2.1. Interconversions and structures of B-6 vitamers.

approximately 40% of the vitamin B-6, while cereals, legumes, vegetables, and fruits combined supply approximately 46% of the vitamin (Sauberlich, 1981).

In food, vitamin B-6 is found in both the free and the bound form. In order for absorption to occur, the vitamin must be in the free form. Therefore, the bound nature of vitamin B-6 can interfere with the biological absorption of the vitamin. This can then decrease the amount of the vitamin which actually becomes available in the body for metabolic processes. The amount of a vitamin consumed in food vs. the amount actually absorbed for biological use is called bioavailability. Bioavailability is defined as the ratio of the amount of a particular nutrient which can be absorbed from a food to the total amount of the nutrient in that food. Thus, the availability of vitamin B-6 for absorption will directly influence the amount of the vitamin available in the body.

Many factors may contribute to the bioavailability of vitamin B-6 in the diet. Some of these factors are: the amount of the vitamin in the bound form in a food; other foods eaten in the meal; processing, storage, and cooking procedures used, as well as the ability of the body to digest the food eaten (Gregory and Kirk, 1978). In food, vitamin B-6 is known to bind to protein (Brin, 1978) and glucose (Yasumoto et al., 1977). In animal foods vitamin B-6 binds to protein through the formation of a Schiff base (Matso, 1957), while the binding of vitamin B-6 to glucose, forming pyridoxine-β-glucosides, is common in many plant foods (Kabir et al., 1983).

Metabolic Function

The biological functions of vitamin B-6 within the human body are numerous. One of its major functions is in the metabolism of amino acids. In amino acid metabolism, PLP is the cofactor for the amino-transferase enzymes which remove the α-amino group from the amino acid forming an α-keto acid. Glutamate transaminase, one of the most important of these enzymes, catalyzes the transfer of an amino group to α-keto glutarate.

α-amino acid + α-ketoglutarate <---> α-keto acid + glutamate

Another important transminase enzyme is alanine transaminase. It catalyzes the transfer of an amino group to pyruvate.

α-amino acid + pyruvate <----> α-keto acid + alanine

The alanine formed here can then transfer it's amino group to α-keto glutarate to form glutamate. These two transaminases funnel α-amino groups from a variety of amino acids to glutamate for conversion to NH4+ (Stryer, 1981, p. 408).

The particular way in which PLP functions in the transaminase enzymes is through the formation of a Schiff base. The PLP dependent transaminase enzymes form covalent Schiff base intermediates with their substrates. Fig. 2.2 shows the way PLP functions to remove the α-amino group from an amino acid. Snell and DiMari (1970) proposed the following mechanism for transaminase reactions:

Amino acid 1 + Enzyme-PLP <----> α-keto acid + Enzyme-PMP
α-keto acid 2 + Enzyme-PMP <----> Amino acid 2 + Enzyme-PLP

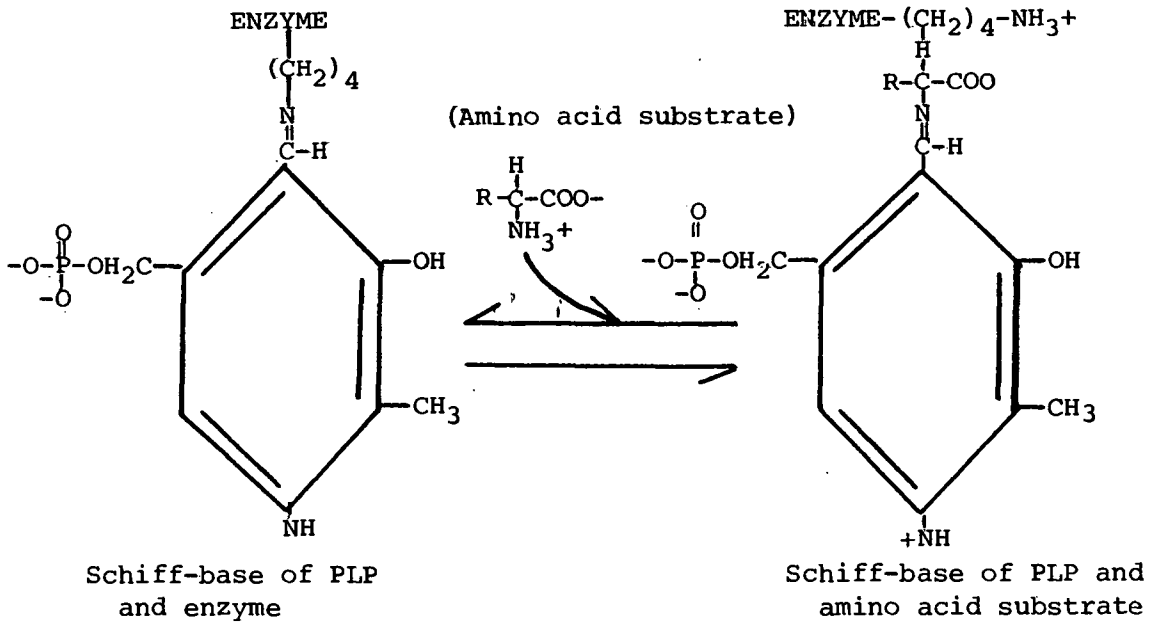


Figure 2.2. In absence of substrate, aldehyde group of PLP is Schiff-base linked to the α-amino group of a specific lysine residue at the active site. Add an amino acid substrate and a new Schiff-base linkage is formed. Amino acid-PLP Schiff-base formed remains tightly bound to the enzyme through covalent forces.

The second half of the reaction occurs by reversing the first step. The final outcome is regeneration of the enzyme pyridoxal 5'-phosphate complex (Enzyme-PLP) (Stryer, 1981).

Transaminases are not the only amino acid transformation reactions PLP is involved in. Other reactions which involve the α-amino group are decarboxylation, deaminations, reacemizations, and aldol cleavage (Stryer, 1981).

Another important function of PLP is in the metabolism of tryptophan. In tryptophan metabolism PLP is involved in the formation of serotonin, a neurotransmitter, via the action of a PLP dependent decarboxylase enzyme on 5-hydroxy tryptophan. Niacin, a vitamin, is produced as the result of tryptophan metabolism involving PLP dependent transaminase enzymes (Henderson and Hulse, 1978). The amino acids serine and threonine can also be directly deaminated into NH_4+ and pyruvate, and α-ketobutyrate and NH_4+, respectively. These direct deaminations are catalyzed by serine dehydrogenase and threonine dehydrogenase, both PLP dependent enzymes (Stryer, 1981). PLP is essential for cystathinone synthase, cystathinonase, and cysteine sulfinic acid decarboxylase, three enzymes involved in the metabolism of methionine (Sturman, 1978). PLP also plays a role in nucleic acid formation, because it is required for folate metabolism.

Recent interest in nutrition and the brain has reemphasized the important role vitamin B-6 plays in brain function. Both PLP and PMP can influence brain function by participating in the metabolism of protein, lipid, and carbohydrate as well as in the synthesis of hormones, neurotransmitters and other coenzymes (Ebadi, 1977). The importance of vitamin B-6 is emphasized when one realizes that major

neurotransmitters are synthesized by the aid of vitamin B-6 dependent enzymatic reactions. These include the formation of dopamine, norepinephrine, serotonin (Lovenberg et al., 1962) and indirectly acetycholine (Yamada et al., 1956).

Another major function of vitamin B-6 is in carbohydrate metabolism, where PLP is a cofactor for glycogen phosphorylase. This enzyme catalyzes the phosphorolytic cleavage of α-1,4 glycosidic bonds of glycogen starting at the non-reducing end of the polysaccharide (Helmreich and Klein, 1980). This cleavage of the glucose-1-phosphate from glycogen makes glucose available for energy production.

$$(\text{Glycogen})n + Pi \longleftrightarrow (\text{Glycogen})n\text{-}1 + \text{Glucose-1-phosphate}$$

PLP forms a Schiff base with a lysine residue in this enzyme, while the phosphate group of PLP attachs to the glucose residue. Therefore, vitamin B-6 plays an active role in glycogenolysis in the liver to maintain blood glucose and in the muscle when energy is needed.

Other metabolic functions in which vitamin B-6 participates are: the synthesis of aminolevulinic acid, an intermediate compound in the formation of porphyrin, a substance necessary for the formation of the porphyrin ring of hemoglobin (Richert and Schulman, 1959); as a modulator of hormone activity by interacting at the receptor sites and decreasing the effect of the hormone (Litwack, 1979); and in maintaining the integrity of the immune system where vitamin B-6 deficiency can impair both humoral and cell-mediated immune responses (Robson et al., 1977). Finally, vitamin B-6 plays a secondary role in

lipid metabolism where it is necessary for the synthesis of carnitine from lysine. Carnitine is needed to carry the fatty acid chains across inner mitochondrial membranes to the matrix where they are made accessible to oxidative enzymes. In this process PLP is necessary for the formation of glycine from β-hydroxy-3-methyl lysine and the subsequent formation of γ-3-methyl amino butyraldehyde which is then converted to γ-3-methyl amino butyrate and then to carnitine with the help of ascorbic acid and iron (Swell et al., 1961; Goswani and Coniglio, 1966).

Absorption

A linear relationship exists between the amount of vitamin B-6 absorbed in an oral load dose and 4-pyridoxic acid excretion (Booth and Brain, 1964; Johansson et al., 1966). This linear relationship has been interpreted to mean that vitamin B-6 is absorbed by passive diffusion. Absorption occurs in the jejunum and ileum with the rate of absorption for the three vitamers as follows: PL>PN>PM. Everted rings and sacs of rat intestines have been used as a means of measuring the transport of the B-6 vitamers across the intestinal mucosal. Using this method, Middleton (1977, 1979) has shown that the rate of pyridoxine hydrochloride absorbed in the rat jejunum is proportional to the concentration. In examining the absorption of small doses of PN, PL, and PM in five men, Wozenski et al. (1980) found that PL and PN produced a quicker response in plasma and urinary vitamin B-6 parameters than PM. Their work suggests that PM absorption is slower

and/or metabolized differently than PL or PN. However, Henderson (1984) reported that the absorption rate of the three non-phosphorylated vitamers was PL>PM>PN. The difference in the absorption rates reported by Henderson (1984) and Wozenski et al. (1980) is probably due to the fact that one used human subjects (an in vivo study) while the other used animals (an in vitro study).

Although it has been shown that some of the phosphorylated forms of the vitamin are transported across the intestinal membrane, most of these are hydrolyzed to the free form before absorption occurs (Middleton, 1979). Hydrolysis occurs via the membrane bound phophatases found on the surface of the tissue cells (Anderson et al., 1971; Lumeng and Li, 1974).

Transport and Interconversions

Once the various B-6 vitamers are transported across the intestinal mucosa, they are delivered via the blood to the various organs of the body. The methods by which the individual body tissues acquire vitamin B-6, convert it to PLP or PMP and then either utilize it, export it, or metabolize it to 4PA has received much attention. Snell and Haskell (1971) have reviewed the interconversions of the free and phosphorylated forms of vitamin B-6. Figure 2.3 shows the enzymes involved in each of the interconversions. PLP is the major cofactor form of vitamin B-6 in the body. However, since most of the vitamin B-6 ingested in food is either in the form of PN, PL, or PM, these vitamers must be converted to the active cofactors. This process takes place within various tissues of the body, the main sites being

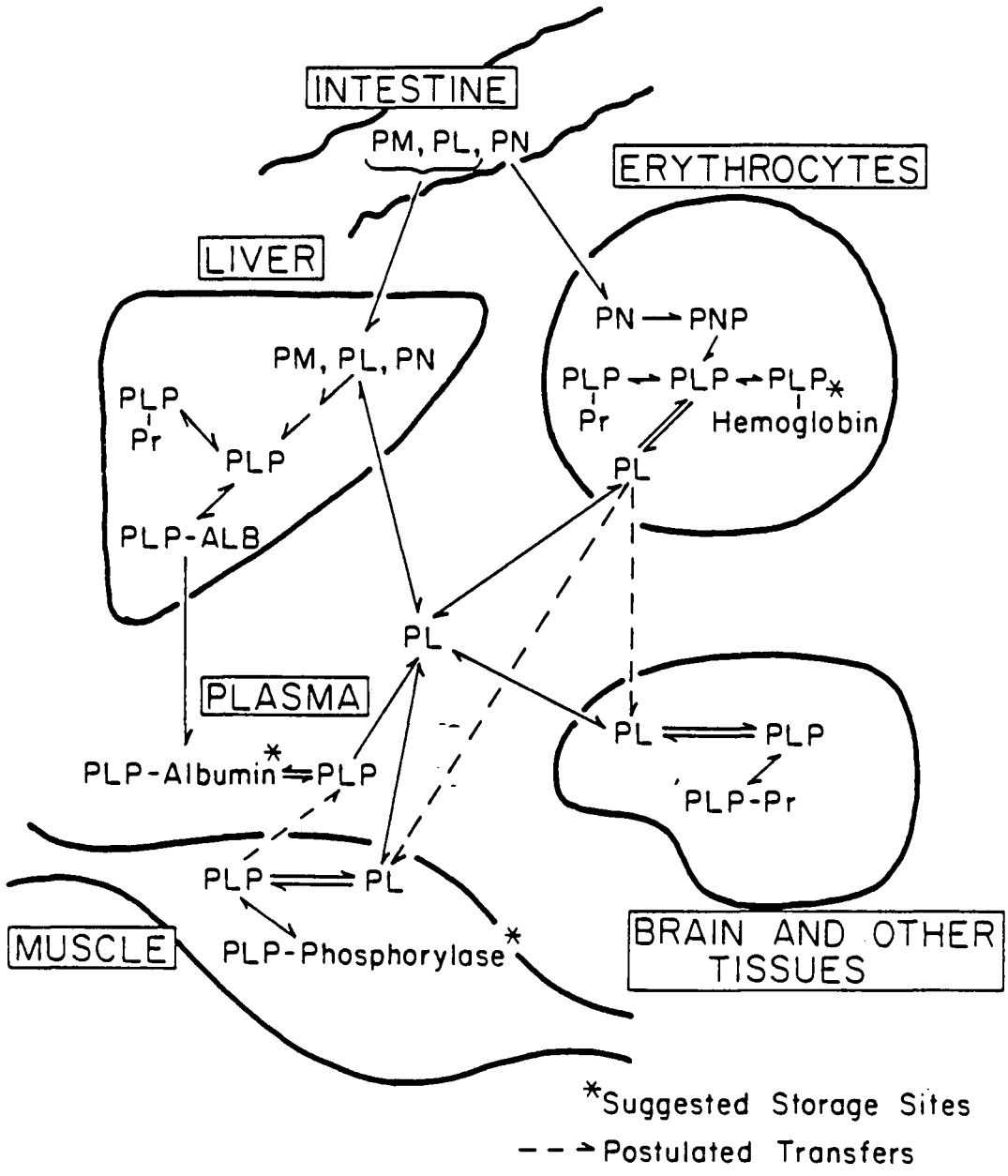


Figure 2.3 . Tissue interrelationships of pyridoxal and pyridoxal 5'-phosphate (Hatcher et al., 1982).

the liver and the red cell. The way cells receive and convert the B-6 vitamers has been explored by examining tritiated forms of PN (Mehansho et al., 1980). Through this method it has been determined that the preferred pathway of conversion of PN to PLP is through PNP via PL kinase (EC 2.7.1.35). PNP is then further converted to PLP via the cytosol PNP oxidase enzyme (EC 1.3.4.5). Once the vitamer has been converted to PLP, it can be used in the tissue or converted back to PL via alkaline phosphatase and re-released into the circulation. Conversion of PMP and PNP to PLP occurs through the PNP oxidase enzyme. PL kinase, which will convert all the free forms to their phosphorylated counterparts, is found in the brain, liver, spleen, kidney, muscle tissue, and the small and large intestines. However, PNP oxidase is confined to a few tissues. The highest activity is found in the liver, with only small amounts in the red cell, brain, and kidney. PNP oxidase is absent in the lung, heart, muscle, and pancreas (Pogell, 1958). This means that most tissues are capable of phosphorylating the B-6 vitamers but are unable to convert these vitamers to PLP. Therefore, many tissues depend on the liver and red cell to convert the dietary B-6 vitamers to PLP for subsequent release into the plasma (Mehansho et al., 1980).

As previously mentioned, the liver and red cell are the major sites of B-6 vitamer interconversion since they contain all the necessary enzymes. Therefore, PN, PM, and PL can be taken up and converted to PLP and/or PL for subsequent uptake by other tissues. Studies by Lumeng et al. (1974) have indicated that the liver is the main source of plasma PLP. These studies have been supported by

research in human subjects by Mitchell et al. (1976) who examined plasma PLP concentrations in normal subjects and patients with alcoholic cirrhosis, acute hepatitis or extrahepatic obstruction when given a 50 mg intravenous load of PN. Plasma PLP concentrations were significantly lower in the cirrhotic patients than controls.

The liver is unique in its ability to produce and release PLP which can then bind to albumin. Most tissues do not allow phosphorylated forms to cross their membranes. However, the tissues do have membrane-bound phosphatases which can hydrolyze PLP to PL for uptake into the tissue (Lumeng and Li, 1975, 1980). Once PL is in the cell, the cytosol kinases can convert it back to PLP. Suzue and Tachibana (1970) have suggested that PLP can cross into the red cell membrane without being hydrolyzed, but others disagree feeling that PLP is too tightly bound to albumin to cross the red cell membrane (Henderson, 1984).

The red cell can take up the B-6 vitamers and convert them to PL or PLP but cannot release the phosphorylated form from the cell (Anderson et al., 1971). This is due to the fact that the PLP found here is tightly bound to hemoglobin. Therefore, the red cell can only contribute PL to the plasma for transport to other tissues. However, it has been suggested by Anderson et al. (1971) that the red cell may deliver vitamin B-6 directly to the tissues without first releasing it in to the plasma. Fig. 2.3 shows the various tissue interrelationships of vitamin B-6.

Storage

In human tissues, PLP and PMP are the primary forms of vitamin B-6 with PLP being more prominent. The non-phosphorylated B-6 vitamers make up less than 10% of the total B-6 content of the body (Li and Lumeng, 1981). Johansson et al. (1966) suggested that the vitamin B-6 content of the body could be visualized as two compartments, a rapid-turnover compartment and a slow-turnover compartment. The rapid-turnover compartment consists of vitamin B-6 which is either excreted into the urine or passed to the slow-turnover compartment where it is bound to protein in the tissues. Shane (1978), using PN equivalents to express vitamin B-6 content, estimated that the total body stores in a healthy female to be 60 mg PN equivalents. Approximately 90% of this was in the slow-turnover compartment that has a half-life of 33 days. The remainder was in the fast-turnover compartment with a half-life of 15 hours. He estimated that the daily excretion rate was 2.6% or 1.5 mg PN, or the total body store. This model did not control diet and assumed an intake of 1.5 mg of vitamin B-6, which is below the 2.0 mg RDA for women.

Of the tissues which store vitamin B-6 in the body, the liver has the highest concentration of vitamin B-6. However the muscle, due to its mass, represents the single, largest storage site of vitamin B-6 in the body (Li and Lumeng, 1981). Lumeng et al., (1978) examined the relationship of plasma PLP concentration to the PLP content of the liver, brain, and skeletal muscle cf rats on varying levels of vitamin B-6 intake. They found the brain to be the slowest to alter its PLP content with increasing dietary B-6 and the skeletal

muscle and plasma the most responsive to changes in nutritional status. Their data supports the hypothesis that both plasma and skeletal muscle are storage reservoirs for vitamin B-6 and are capable of storing PLP during excessive intake and releasing it to other tissues during times of dietary deficiency.

In the skeletal muscle approximately 90% of the PLP is bound to the glycogen phosphorylase enzyme (Black, 1977). Black and co-workers (1977, 1978) have also found that muscle phosphorylase activity is lowered in B-6 deficient animals, but when an excess of vitamin B-6 is given, both muscle PLP content and glycogen phosphorylase activity increase proportionately. However, it was found that the muscle glycogen phosphorylase activity does not decrease with vitamin B-6 deficiency unless caloric deficit is also introduced. A decrease in glycogen phosphorylase is presumed to be concomitant with the release of PLP from the enzyme. Aspartate transaminase and alanine transaminase were also measured. On the vitamin B-6 deficient diet aspartate transaminase was approximately 70% of the initial level while alanine transferase decreased initially and then returned to near the original level. When the rats were partially starved they lost muscle phosphorylase while retaining alanine and aspartate aminotransferase. Therefore, they concluded that the PLP stored in the muscle cannot be used in times of vitamin B-6 deprivation unless there is also concurrent caloric restriction.

VITAMIN B-6 STATUS IN WOMEN

Recent evident has arisen which questions the nutritional adequacy of vitamin B-6 in the diets of women. The particular subgroups in question are the young women who may be pregnant, lactating or using oral contraceptives and the elderly women who may have altered metabolism and/or absorption of vitamin B-6 as well as marginal vitamin B-6 intakes.

Guthrie and Crocetti (1983) examined the vitamin B-6 intake of 2865 female respondents to a three-day diet record in the Nationwide Food Consumption Survey. They found that nearly 50% of the women ages 11 and older received less than 60% of the 1980 RDA for vitamin B-6. They also examined the intake of these women by age groups and found that vitamin B-6 intake decreased with increasing years. The age group, 23-51 years, had the highest percent of women (56.9%) under 60% of the 1980 RDA for vitamin B-6. Chrisley and Driskell (1979) also examined food records from 83 females ages 19 and older and found that all consumed less than the RDA for vitamin B-6. Women between the ages of 19-25 consumed 71.2% of the RDA, women ages 35-59 consumed 80% of the RDA and women over age 60 consumed 53% of the RDA for vitamin B-6. It was of interest to note that of the 43 males also included in this study none consumed below the RDA for vitamin B-6. Vir and Love (1980) assessed the vitamin B-6 status of oral contraceptive and non-oral contraceptive users by measuring the percentage stimulation of erythrocyte glutamic pyruvic transaminase (EGPT) activity. They found that there was no difference between the groups in percentage stimulation of EGPT, but dietary vitamin B-6 was low

(<2mg per day) in 92.3% of the women measured. These dietary assessments of young women seem to indicate that vitamin B-6 intake of this age group may be low.

The evidence and research pointing to low vitamin B-6 status of the elderly is more prevalent than that for younger women. As already mentioned, Guthrie and Crocetti (1983) found that 50% of the women in their study over the age of 51 years were consuming <60% of the 1980 RDA for vitamin B-6. These same results were echoed by Chrisley and Driskell (1979) where 53% of their female subjects older than 60 years of age were consuming <60% of the RDA for vitamin B-6. Ranke et al. (1960) also examined vitamin B-6 adequacy in the elderly and found that using serum glutamic oxalactic acid transaminase (SGOT) as a vitamin B-6 indicator, the older individuals consistently demonstrated lower SGOT values than the younger subjects. This study did not distinguish between males and females and SGOT is currently considered a questionable indicator of vitamin B-6 status. Hampton et al. (1977) examined the diet records of 20 women above the age of 60 and found that 50% of them consumed less than 50% of the RDA for vitamin B-6 and one fourth of these were classified as having subclinical vitamin B-6 deficiency. Chen and Fan-Chaing (1981) looked at erythrocyte glutamic-pyruvic transaminase activation as an indicator of vitamin B-6 deficiency and found that of the 77 women they studied, those between the ages of 65-74 had the highest glutamic-pyruvic transaminase activity when compared to younger age groups. When they combined male and female subject data

(n=139) they found that vitamin B-6 deficiency was evident in 56.6% of the institutionalized elderly (n=41) and 43.5% of the non-institutionalized elderly (n=79).

As these research papers have indicated, the vitamin B-6 status of women may be lower than that recommended by the RDA for optimal nutrition. The majority of the vitamin B-6 status research has evaluated vitamin B-6 status based on dietary records (24-hour recall or 2-3 day diet records) or the stimulation of vitamin B-6 dependent amino transaminase. However, to quantitatively evaluate the biological vitamin B-6 status of women, plasma parameters need to be measured and compared to normal ranges.

Blood Levels

Plasma PLP has been indicated by Shane (1978) as the most sensitive blood indicator of vitamin B-6 status. This recommendation is based on the fact that other plasma vitamin B-6 compounds fluctuate more over time than PLP when diet is held constant, thus making them poorer status indicators. Another advantage of using PLP as a status indicator is that it is the functional form of the vitamin in the body. An example of the sensitivity of plasma PLP to dietary changes was demonstrated by Brown et al. (1975). They measured PLP during an adequate vitamin B-6 diet of 2.0 mg and then again on a vitamin B-6 deficient diet of 0.19 mg. Their results showed that the plasma levels of PLP rapidly decreased during the depletion state and then were quickly restored on an intake of 2.0 mg of vitamin B-6 for 28 days.

Assessing what the "normal" plasma values for PLP are for "healthy adults" has not been easy. Li and Lumeng (1980) reported plasma PLP concentrations of healthy subjects on normal, unsupplemented diets as 49.8 pmol/mo. Shultz and Leklem (1981) reported that in vegetatarian and non-vegetarian adults the plasma PLP concentrations were significantly lower in the adult women (38±14 nM) than the adult men (52±19 nM). They have outlined guidelines for what may be considered the marginal limits of plasma PLP for women. They suggest the range of the marginal limit for PLP be 31.6-35.6 nM (7.8-8.8 ng/ml).

Excretion

Urinary 4-pyridoxic acid (4PA) is the major excretory product of vitamin B-6 metabolism and appears to increase when tissue concentrations increase. 4PA is formed in the liver by the action of either a NAD-dependent aldehyde dehydrogenase or a FAD-dependent aldehyde oxidase on PL (Figure 1). 4PA has not been considered a good indicator of status because it seems to parallel intake and may only reflect the recent dietary vitamin B-6 consumption and not the severity of any deficiency which may be present. Total urinary vitamin B-6 is also considered an indicator of immediate dietary intake and therefore not a good status indicator. When large doses of vitamin B-6 are fed, the quantity of 4PA and free vitamers increase in the urine. Rabinowitz and Snell (1949) demonstrated this by feeding 100 mg doses of PL, PN, and PM and found that approximately 70-90% of the dose appears in the urine as 4PA within 36 hours, depending on the vitamer. Wozenski and co-workers (1980) also found that the excretion of 4PA

after a 10 mg PN dose was 7.42±1.77 μmoles/24 hours on the day after the dose, compared to 4.43±0.97 μmoles/day the day before the test dose. However, they also noted that as the size of the dose increased, from 1 to 10 mg PN, less of the dose was recovered as urinary 4PA and vitamin B-6. This may suggest that as the size of the dose increases more of the dose is either being retained by the body or converted to other products which were not examined. They also compared the rate of metabolism and excretion of equimolar doses of PN, PL, and PM and found that PL was more rapidly converted to plasma PLP and urinary 4PA than the other two forms.

Shultz and Leklem (1981) examined the 4PA excretions of vegetarians and non-vegetarians on self-selected diets. They found that there was a significant sex difference in 4PA excretion with the males excreting significantly more 4PA than the females. The mean 4PA excretion for the males was 7.46±4.34 μmol/24 hour compared to 5.57±3.09 μmol/24 hours for the women. They also found that 4PA excretion seemed to decrease slightly with age. Recommendations for marginal range limits for 4PA excretion for women were given as 4.6-5.2 μmol/24 hours.

Factors Affecting Status

Three major factors which have been studied in relationship with vitamin B-6 status in women are protein intake, orgal contraceptive use, and age. As previously mentioned, numerous investigators have reported low dietary vitamin B-6 intake in women, especially elderly women (Baker et al., 1979; Driskell, 1978). Factors which may

contribute to the decreased nutritional and vitamin B-6 status of the elderly woman are numerous and may include such things as: altered food selection (due to the loss of teeth, alterations in taste and smell); increased use of medications; fixed incomes; depression resulting from the loss of a spouse and/or friends; inability to prepare their own food; immobility; accumulation of chronic diseases as well as the biological changes of aging which may alter absorption of nutrients (Russell, 1983).

Protein intake may also affect vitamin B-6 status by altering the biological need for the vitamin. PLP is essential for the transaminase enzymes which function in amino acid metabolism. Therefore, with increasing protein intake more vitamin B-6 is needed for amino acid transamination. It has therefore been suggested that vitamin B-6 intake be based on protein intake, with 0.02 mg vitamin B-6 being recommended for every gram of protein consumed (Donald, 1978; Guthrie and Crocetti, 1983). Shultz and Leklem (1980) also suggested that measurement of inadequate vitamin B-6 nutrition be based on a vitamin B-6/protein ratio. This recommendation comes from the recognition of the interrelationship between vitamin B-6 and protein metabolism. However, regardless of the recommendations that vitamin B-6 intake be based on protein consumption, the current RDA for adult women is 2.0 mg vitamin B-6.

The relationship between oral contraceptive use and the need for vitamin B-6 has been the topic of numerous research projects as well as attracting much interest in the popular press. It was

first noticed by Rose (1965) that women using oral contraceptives exhibited an abnormality of tryptophan metabolism similar to that seen in vitamin B-6 deficiency. In examining the tryptophan results of oral contraceptive users more closely, it was observed that not only did they excrete abnormal amounts of kynurenic acid, xanthurenic acid, kynurenine, and 3-OH kynurenine (as seen in vitamin B-6 deficiency) but they also excreted more quinolinic acid, 3-OH-anthranilic acid and N'methyl nicotinainide (Rose, 1978). The later metabolites are not excreted in increased amounts in vitamin B-6 deficient individuals. This observation led to the postulate that oral contraceptives increase the turnover of the tryptophan-nicotinic acid metabolic pathway, while shunting less of the vitamin through the tryptophan hyroxylase enzyme for eventual serotonin production. Leklem et al. (1975) found that oral contraceptive users deplete vitamin B-6 more quickly than non-oral contraceptive users and do not replete as quickly. However, it is doubtful that oral contraceptives produce a true vitamin B-6 deficiency and Rose (1978) concludes that although oral contraceptive users demonstrate abnormal tryptophan metabolism only about 15-20% have direct biochemical evidence of vitamin B-6 deficiency.

FUEL METABOLISM DURING EXERCISE

The study of work physiology encompasses two broad sets of processes which supply the energy needed to do work (Burke, 1980). The "energy transport system" involves the transport of food and

oxygen from the environment to the cells for survival. This process is accomplished through the use of the bodies' digestive, respiratory, and circulatory systems which are regulated by the endocrine and nervous systems. The "energy transfer system" involves the enzymes and coenzymes at the cellular level which participate in the transfer of energy from food to the "energy currency" of the body, adenosine triphosphate (ATP). ATP has the unique property of being able to store and release energy. Thus, the energy which was consumed in the form of food is ultimately transferred to ATP to be used in the metabolic reactions needed for the cells to perform work, such as muscle contraction, nerve transmission, circulation, and digestion. Therefore, fuel metabolism (energy transfer) during exercise involves the catabolism of food from the diet or the catabolism of energy stored in the body to the functional form of energy, ATP.

Aerobic and Anaerobic Metabolism

Although ATP is the ultimate source of energy for the cells, large quantities are not stored in the body. In fact, only about 4-6 mmoles of ATP are stored per kg of muscle at any one time (Kraemer and Fleck, 1982). This quantity of ATP could be depleted in less than a second of intense muscular work (Beis and Newsholem, 1975). Therefore, ATP must be constantly resynthesized to provide a continuous flow of energy. During anaerobic conditions (without oxygen) ATP can be synthesized from two sources. The first source is creatine phosphate (CP), an energy rich compound, which can

anaerobically split off a phosphate group, which then can bind to ADP creating ATP.

ATP--------->ADP + P + energy-------> for biological work.

CP + ADP -------> ATP + C

2ADP --------> ATP + AMP

Cells store creatine phosphate in larger quantities than ATP. The creatine phosphate reserve in the body can support all-out work for approximately 5-8 second (Katch and McArdle, 1977).

The second source of anaerobic energy used to resynthesize ATP is the anaerobic glycolysis of glucose. In this process, a molecule of glucose is broken down into two, three-carbon molecules of pyruvic acid with two molecules of ATP being produced. The production of ATP through anaerobic glycolysis of glucose represents only a small fraction (5%) of the ATP's that could be produced if the molecule were completely degraded to carbon dioxide and water by aerobic (with oxygen) reactions (Newsholem, 1981). Within the muscle, the by-product of anaerobic glycolysis is lactic acid.

If exercise or work is to continue longer than a few seconds, the body must turn to alternative sources of energy. These alternative sources require the use of oxygen for the complete metabolism of glucose and free fatty acids (FFA) through the tri-carboxcylic acid cycle (TCA) to carbon dioxide and water. The TCA cycle operates only under aerobic conditions (Stryer, 1981). The rate at which the TCA cycle will produce ATP depends on the need for ATP in the body.

During exercise the body does not rely solely on either the aerobic or anaerobic systems to meet energy demands. The proportion

of each system used during exercise depends on many factors, such as: the type of activity being engaged in; the intensity, duration and frequency of the activity; and the anaerobic and aerobic power of the individual performing the activity. For example, a trained marathon runner would rely more on the aerobic system to supply energy to the working muscles through the metabolism of FFA, while a sprinter or weight lifter would rely more on the anaerobic system to meet energy demands.

Work and Oxygen Consumption

In order to fully appreciate the human potential for physical activity one must understand the relationship between work and oxygen consumption. The terms oxygen consumption, oxygen intake, oxygen uptake, or $\dot{V}O_2$ all mean the same thing and refer to an individual's use of oxygen (in liters) during any given minute. $\dot{V}O_2$ max refers to an individual's maximal ability to take up oxygen. $\dot{V}O_2$ max is measured under maximal effort exercise and usually is corrected for body weight (ml/kg/min) so that individuals of different statures can be compared. One's maximal aerobic power ($\dot{V}O_2$ max) depends mainly on heredity and training state (Burke, 1980).

As an individual proceeds from rest to a given submaximal workload, the oxygen consumption does not rise immediately to the needed level. There usually is a 2-4 minute lag called oxygen deficit, during which the body adjusts to the new demands for oxygen brought on by the working muscles. Since, the oxygen deficit indicates that not quite enough oxygen is being supplied to meet

energy demands, these energy demands have to be met by anaerobic sources of energy. As the workload increases, oxygen consumption continues to rise until a "steady state" is reached. At this point the oxygen consumption levels off, indicating that metabolically the oxygen demands (which reflect energy demands) are being met by the oxygen supply. An individual is usually capable of working under a steady state condition until fatigue factors set in. If the work load continues to rise, oxygen consumption rises until the point of $\dot{V}O_2$ max has been reached. At this point the body reaches its maximal ability to deliver oxygen to the muscles regardless of any increased demand. Once a person has reached $\dot{V}O_2$ max, an increase in the workload does not produce an increase in the extraction of oxygen by the body and anaerobic metabolism must again supply the additional energy needed.

Figure 2.4 illustrates the change in oxygen consumption as the duration of the exercise continues until a steady state is reached (athlete A) (Burke, 1980). In the example, athlete A has a higher $\dot{V}O_2$ max than athlete B. Although both athletes are working at the same workload, athlete A is more trained and is working within his/her ability to supply oxygen to the muscles, while athlete B is less trained and cannot supply the needed energy aerobically. When the individuals stop exercising, $\dot{V}O_2$ does not immediately return to resting levels. This gradual recovery period, termed the oxygen debt, is needed to eliminate excess lactic acid and to replace ATP and CP stores. Since athlete B incurred a larger oxygen debt, his/her recovery period will be longer. The superior aerobic training

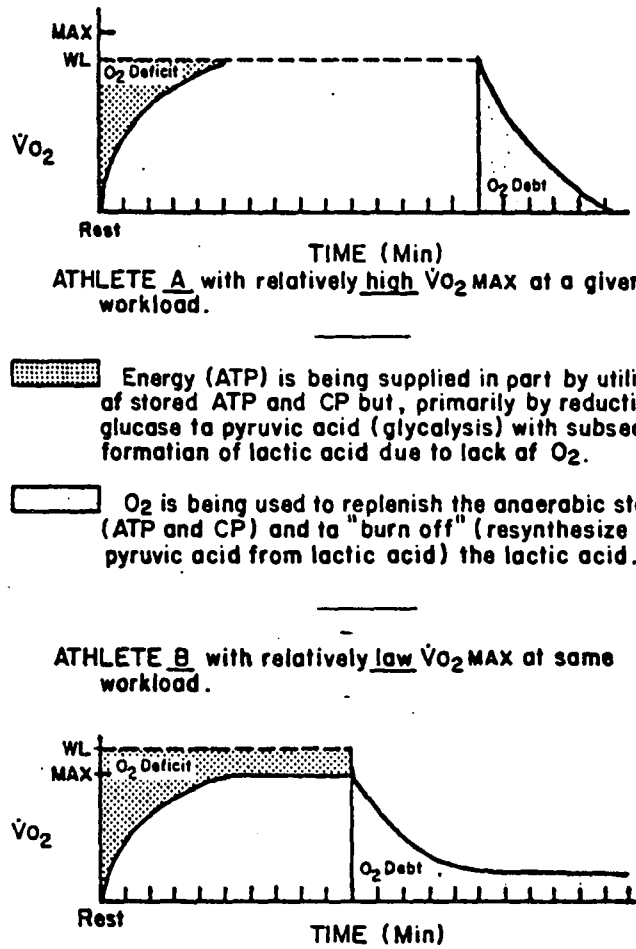


Figure 2.4 - Two athletes with different aerobic power ($\dot{V}O_2$ max) (Burke, 1980).

of athlete A leads to two major advantages over athlete B. First, athlete A is capable of working longer than athlete B since athlete B can only supply energy anaerobically for a limited time. Secondly, athlete B is competing under a condition of general fatigue, due to lactic acid build-up, which will eventually decrease endurance and skill (Newsholem, 1981).

Other metabolic differences which exist between a trained individual and his or her less trained counterpart are numerous. Some of these differences include: more relative muscle and less relative fat; thicker, larger and stronger connective tissue (Holloszy and Booth, 1976); increased vital capacity (more air in one maximal effort); decrease in total volume of air as well as number of breaths needed during submaximal work (Davies, 1981); increased pulmonary perfusion and smaller residual volume; increased stroke volume and larger, stronger heart; increased oxidative enzymes which result in greater fat utilization during exercise (Gollnick and Saltin, 1972; Holloszy and Booth, 1976); increased number of capillaries in skeletal muscle which could alter deliver and uptake of substrates and oxygen (Anderson and Henriksson, 1977) and increased maximal cardiac output.

Endogenous and Exogenous Fuels

When work is demanded of the body, it can turn to numerous stores of energy, both outside and inside the muscle, to provide the energy needed. As already mentioned, during anaerobic work the body

draws mainly on the endogenous energy stores within the muscle, such as ATP, creatine phosphate and glucose (stored as glycogen). During aerobic work, the body draws on both its' endogenous stores of energy as well as its exogenous stores, such as fat, to meet the energy demands. Protein, both endogenous and exogenous, can also play a part in providing glucose to the working muscle through the Cori cycle or the metabolism of the carbon skeletons of the amino acids through the TCA cycle (Felig and Wahren, 1971). The following section reviews fuel utilization during exercise and will separately examine the contribution made by carbohydrate, fat, and protein under various exercise modalities. Although these fuel sources are discussed separately it should be kept in mind that during exercise all make an important contribution to providing fuel for energy. However, the proportion of fuel provided by each source may vary, depending on the prevailing conditions.

Carbohydrate metabolism

During exercise glycogen stored in the muscles and glucose obtained from the liver via circulation are the primary carbohydrate stores available for energy production. However, there are many factors which can influence the intensity and degree of carbohydrate utilization during exercise. Some of these factors include: pre-exercise diet; initial glycogen level; physical conditioning; circulating substrate and hormones; specific exercise engaged in and the intensity; duration, and frequency of that activity (Hickson, 1982; Locksley, 1980).

For many years measurement of respiratory quotient (RQ) (carbon dioxide production/oxygen consumption) during exercise had been the main means of measuring the participation of fats and carbohydrates as fuels during exercise (Lewis and Gutin, 1973). An RQ of 1.00 indicates that carbohydrates are the sole fuel being utilized, while a value near .70 indicates that fats are the major fuels being metabolized. Today measurements of arterial/venous blood during exercise, muscle biopsy and labeled substrate studies have greatly extended our knowledge of fuel metabolism during exercise (Hickson, 1982).

Blood and hepatic glucose

At rest plasma glucose levels supply energy mainly to the brain and nervous system with only about 10% of the blood glucose going to the muscle to meet energy demands (Andres et al., 1956). Liver glycogen is the primary source of blood glucose and is activated in rapid response to hypoglycemia. Muscle glycogen cannot contribute directly to blood glucose since muscle does not contain the enzyme glucose-6-phosphatase (Felig and Wahren, 1974).

Ahlborg and Felig (1982) and Ahlborg et al. (1974) reported that leg glucose uptake rose 10 to 16-fold after 40 minutes of exercise. It has also been reported by Felig and Wahren (1979) that glucose uptake by the muscles can increase anywhere from 7-40% depending on the intensity of the exercise. This exercise-induced glucose uptake requires little insulin, and insulin levels generally decrease during exercise (Ahlborg et al., 1974; Ahlborg and Felig, 1982; Felig and Wahren, 1979; Vranic et al., 1976). The increased

muscle utilization of glucose during exercise may be reflected in a slight increase or no change in glucose during the initial stages of exercise (Calles et al., 1983; Ahlborg and Felig, 1982). Wahren et al. (1971) have reported a fall in plasma glucose during exercise. These conflicting results probably reflect the individuality of the subjects as well as the intensity and duration of the exercise. However, strenuous, exhaustive exercise has resulted in hypoglycemia (blood glucose, <45 mg./dl.) because hepatic output cannot keep up with muscle glucose uptake (Ahlborg and Felig, 1982).

To specifically monitor the net exchange of glucose and lactate across the legs and forearm during prolonged (3-3.5 hours) exercise, Ahlborg and Felig (1982) catheterized 10 male subjects and exercised them at 58% $\dot{V}O_2$ max on a cycle ergometer. Their results showed that arterial concentrations of glucose remained unchanged up to 40 minutes of exercise and then fell, with the most marked drop occurring during the third hour of exercise with values 40% below basal level. Arterial lactate level rose 0.50-0.60 mmol/ liter during the first 2 hours or exercise and increased by a further 0.5 mmol/liter between the second and third hour. At 90-120 minutes of exercise splanchnic glucose production increased to 3.5 times the basal resting value. During the third hour of exercise, splanchnic glucose output fell by >50%. Splanchnic uptake of glucose precursors increased during exercise. Lactate uptake increased 2.5 fold during the first 120 minutes of exercise and increased a further 75% to four times the basal value during the

third hour of exercise. During recovery there was an additional increase in lactate uptake to a level five to six times the basal rate. The uptake of pyruvate and glycerol also increased during the last hour of exercise, reaching a level six times basal, and increased even further during the recovery period. As previously mentioned, leg glucose uptake increased 16-fold by 90 minutes of exercise but fell by 27% during the last hour of exercise. There was a significant release of lactate by the leg during exercise as it increased 6-fold over the basal level by 90 minutes. However, after 120 minutes of exercise no significant net output of lactate was observed in the leg. This decrease in lactate removal from the leg probably means the glycogen stores have decreased substantially and that the leg muscles are relying more on blood glucose for energy.

Ahlborg and Felig (1982) also monitored the exchange of fuel in the forearm which was not being exercised and found a significant uptake of glucose in the basal state, but when leg exercise was introduced, this uptake tappered off until only 15% of the basal rate was being taken up after three hours of exercise. A significant net release of lactate was also observed in the forearm. Their data suggest that during strenuous leg exercise the forearm muscle is a site of increased lactate release due to muscle glycogen breakdown and contributes to the delivery of lactate to the liver for gluconeogenesis. They also suggested that there may be a redistribution of muscle glycogen stores after prolonged exercise as the leg muscles decrease lactate output while forearm lactic acid increases, even during recovery.

Thus during exercise, hepatic glucose output increases progressively as exercise continues, first through glycogenolysis, and later through enhanced gluconeogenesis, as three-carbon substrates (primarily alanine, lactate, glycerol, and pyruvate) become available. If liver glycogen is depleted, either through starvation or prolonged exercise, and the intensity of the effort is such that the carbohydrate requirement for the working muscle exceeds the glycogen content, blood glucose will be utilized. Gluconeogenesis alone cannot maintain the blood glucose at high work intensities, and hypoglycemia occurs, contributing to the termination of exercise (Ahlborg and Felig, 1982).

It should be mentioned that major hormonal changes occur during exercise to further preserve the blood glucose and to mobilize the stored fat. Of major importance are the rise in catecholamines and glucagon and the fall in insulin (Felig and Wahren, 1975; Wahren, 1979). Epinephrine stimulates glycogenolysis and inhibits insulin release (Deibert, 1980) while norepinephrine mobilizes FFA from the adipose tissue. The falling insulin levels minimize peripheral glucose uptake while allowing lipolysis and glycogenolysis to occur (Felig and Wahren, 1975). Therefore, stored muscle glycogen is a more readily available substrate for the energy metabolism in the working muscle cell than exogenous glucose. Because the fall in insulin decreases peripheral glucose uptake, it prevents the central nervous system from competing equally with the contracting muscles for blood glucose and helps prevent the onset of hypoglycemia. Glucagon levels also rise to stimulate liver gluconeogenesis and

further helps to maintain blood glucose (Felig and Wahren, 1975, 1979; Wahren, 1979).

In post-exercise recovery, glucose metabolism is used to initiate repletion of glycogen stores in the muscle as well as the liver. Glucose uptake by the muscle remains three to four times the basal level for at least 40 minutes after exercise when strenuous exercise has been engaged in (Ahlborg and Felig, 1982). Repletion of liver glycogen is facilitated by a rapid decline in splanchnic glucose output, which reaches basal levels by 40 minutes (Felig and Wahren, 1975) while splanchnic uptake of gluconeogenic substrates continues. Ahlborg and Felig, (1982) found that splanchnic uptake of lactate increased five to six times the basal rate, as did pyruvate and glycerol. In exercise which is less strenuous, the level of plasma glucose usually returns to basal level within 20-40 minutes post-exercise.

Glycogen

Glycogen is a polysaccharide stored in the muscles and liver of humans. As previously mentioned, liver glycogen is used mainly to maintain blood glucose while muscle glycogen is a source of endogenous energy within the muscle. By using needle biopsy techniques, the amount of glycogen in the muscles of an individual can be estimated. On a normal mixed diet, it is estimated that the glycogen content in the thigh muscle ranges from about 1.0-2.5 grams per 100 grams of wet muscle tissue (Hermansen et al., 1979). In an untrained individual the glycogen content may be approximately 1.5 g/100 gm of muscle tissue. This may represent 300-400 grams of glycogen in the body or

approximately 1,200-1,500 kcals (Hermansen et al., 1979). However, in a trained individual on the same mixed diet, 2.0-2.5 g/100 gm of muscle glycogen is common.

Hermansen and co-workers (1967) exercised twenty subjects (10 trained and 10 untrained) on a cycle ergometer at 80% $\dot{V}O_2$ for 90 minutes and took quadriceps muscle biopsies every 20 minutes. The glycogen content of the muscle fell from a mean of 1.6 g/100 gm to 0.10 g/100 gm during this 90 minute period. The RQ was higher in the untrained group (.95) than the trained (.90), indicating that FFA were playing a greater role in fuel contribution in the trained group.

During exercise the amount of glycogen stored in the muscle is important for two reasons. First, if the intensity of the exercise is high, carbohydrate becomes a more important energy source than fat. This is due to the fact that carbohydrate can be metabolized anaerobically, whereas fat requires oxygen for metabolism. At a moderate work load (50% $\dot{V}O_2$ max) about 50% of the energy comes from fat and 50% from carbohydrate, but with increasing workloads (70-80% $\dot{V}O_2$ max) the muscle seems to prefer carbohydrate to fat. Oxidation of carbohydrate yields about 5 kcal/liter of oxygen while fat oxidation yields approximately 4.7 kcal/liter. Since oxygen uptake is a major limiting factor during strenuous exercise, more energy is gained from oxidation of carbohydrates than from oxidation of fat (Hermansen et al., 1979).

Second, the amount of energy stored as carbohydrates and fat in the body is different. In a 70 kg man, fat accounts for approximately

80,000 to 100,000 kcal while the amount of energy stored as carbohydrate is less than 2,000 kcal. Therefore, during a high intensity exercise level (70-80% of $\dot{V}O_2$ max), the pre-exercise glycogen content of the muscle will determine the length of time the optimal pace can be sustained. Also, the shape of the glycogen depletion curve is disproportionately high in the initial states of exercise. As much as 20% of the muscle glycogen stores can be consumed within the first five minutes of exercise (Loskley, 1980). This "glycogen burst", perceived by the runner as the discomfort before the "second wind" begins, is due to the recruitment of the glycolytic muscle fibers for anaerobic glycolysis at the beginning of the run, before the blood flow is redistributed to the working muscles, bringing oxygen and substrates for oxidative metabolism (Holloszy, 1982). The glycogen burst is reflected by the rising levels of lactate in blood and muscle at the onet of exercise which gradually clears as exercise continues (Ahlborg and Felig, 1982).

Two critical factors are involved in glycogen depletion during exercise. The first is that the rate of utilization is directly proportional to exercise intensity (% $\dot{V}O_2$ max) and the second is that glycogen depletion determines the duration of the exercise. Recognizing these two important factors has led researchers to look for ways of altering muscle glycogen metabolism during exercise as well as the amount of glycogen stored prior to the exercise event (Hickson, 1983).

The influence of diet on exercise performance was first described by Christensen and Hansen in 1939. They showed that subjects ingesting a carbohydrate-rich diet could work substantially longer than those ingesting a protein-fat diet (210 minutes to exhaustion vs. 80 minutes). Since then numerous researchers have explored the effect of dietary carbohydrate and endurance (Bergstron et al., 1967; Costill and Miller, 1980; Karlsson and Saltin, 1971; Sherman and Costill, 1984). Bergstron and co-workers (1967) were one of the first groups to quantify the amount of glycogen in the muscle with the number of minutes an individual could exercise before exhaustion set in. They found that when pre-exercise muscle glycogen content was 1.7 g/100 g wet muscle weight and exercise intensity was 75% $\dot{V}O_2$ max, 114 minutes of exercise could be performed. However, when muscle glyocgen was lowered to 0.63 gm/100 gm the same work could only be performed for 57 minutes. When a carbohydrate rich diet was fed for three days, muscle glycogen increased to 3.51 g/100 g and exercise time to exhaustion rose to 167 minutes. The rate of muscle glycogen resynthesis following exhaustive exercise appears to be related to the muscle glycogen synthetase activity and the carbohydrate content of the diet (Costill and Miller, 1980). Depletion of the muscle glycogen through exercise results in a marked elevation of glycogen synthetase activity which subsequently facilitiates muscle glyocen storage. It appears that without first activating the enzymatic step, increased glycogen storage will not occur. Thus, a high carbohydrate diet will not necessarily increase muscle glycogen

stores above "normal" levels unless the diet is preceded by strenuous exercise which brings about muscle glycogen depletion (Costill and Miller, 1980).

Costill and Miller (1980) have shown (Fig. 2.5) that even though activation of glycogen synthetase occurs with strenuous exercise, incomplete resynthesis of muscle glycogen will occur unless the diet is rich in carbohydrate. They have demonstrated that when subjects are limited to a mixed diet of 40% carbohydrate, small restoration of glycogen occurs between two-hour training sessions scheduled 24 hours apart. However, when the same subjects were fed a high carbohydrate diet of 70% carbohydrate, muscle glycogen was almost completely replenished after 24 hours. They also reported that the subjects perceived that exercise sessions on the high carbohydrate diet were less difficult. Jacobs et al. (1982) confirmed these results in elite soccer players who, after a regular season game followed by a free day and then a day of light training, were unable to restore their glycogen levels to those seen in sedentary individuals. These results confirm that a high carbohydrate diet is necessary for those involved in frequent strenuous activity to maintain normal glycogen levels. Depletion of muscle glycogen has not only been shown to decrease endurance, but Heigenhauser (1983) demonstrated that with glycogen depletion a 14% decrease in maximum power occurs as well as increased heart rate and ventilation.

Research done by Hermansen et al. (1967) and others (Bergstron, 1967; Gollnick et al., 1972; Karlsson and Saltin, 1971) has resulted

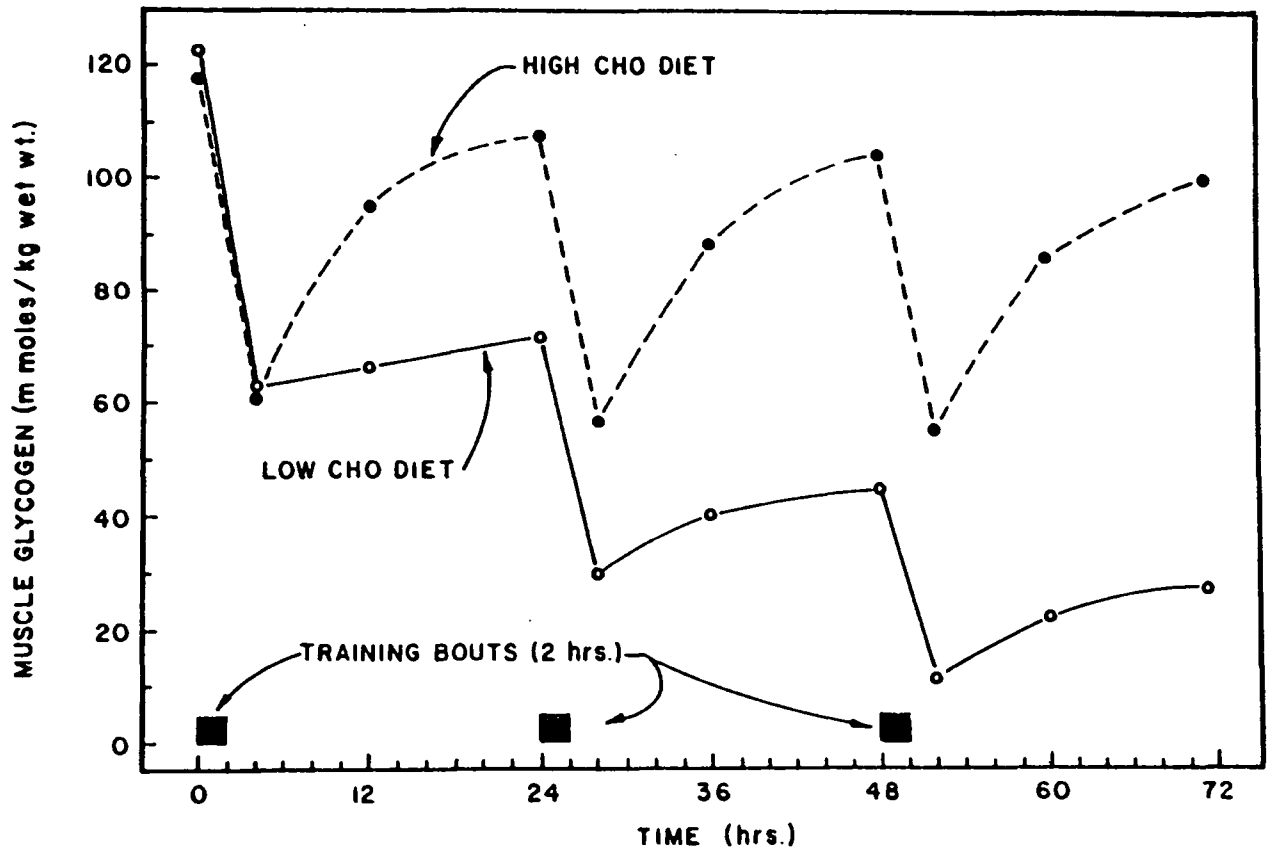


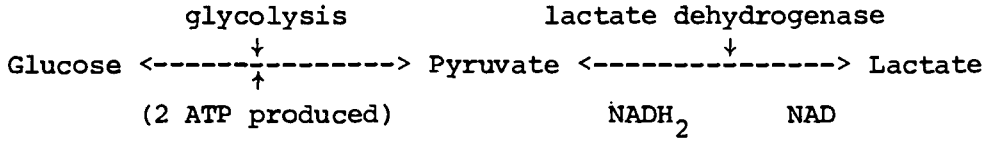
Figure 2.5. Muscle glycogen and resynthesis patterns during repeated days of strenuous running exercise while consuming a low carbohydrate diet or a high carbohydrat diet (40 and 70% of the calories derived from carbohydrate, respectively). Low-carbohydrate resulted in progressive reduction in muscle glycogen, whereas the high-carbohydrate diet "normalized" muscle glycogen stores on a day-to-day basis. Adapted from Costill and Miller, 1983.

in a "carbohydrate loading" regimen used to superload muscle glycogen. This regimen involves exercising to exhaustion and eating a low carbohydrate diet (less than 10%) for 2-3 days in order to starve the muscle of carbohydrate. This is then followed by three days of a high carbohydrate diet (greater than 90%) and rest. The event is then performed on the seventh day. This regimen is known to increase muscle glycogen to twice its normal level (Bergstron et al., 1967). Problems have arisen with this procedure and some athletes have complained of dizziness, irritability, and difficult workouts during the low carbohydrate portion of the diet and muscle heaviness after the carbohydrate loading occurs (Sherman and Costill, 1984). Recently, Sherman (1983) examined a less-stringent regimen of diet and exercise to see if similar results of carbohydrate loading could be obtained without the adverse side effects of the low carbohydrate days. Three dietary treatments were fed to eight trained male runners ($\dot{V}O_2$ max = 63 ml/kg/min) who exercised between 90-120 minutes per day over a five day period. Each person ran on a treadmill at 73% $\dot{V}O_2$ max and then rested on the sixth day. The three diets consisted of a control diet of 50% carbohydrate for six days, a modified diet for 3 days of 50% carbohydrate followed by 3 days of 70% carbohydrate and a modified-classical diet which consisted of 3 days of 10% carbohydrate and 3 days of 70% carbohydrate. Muscle glycogen rose from 130, 130, 80 mmol/kg to 160, 205, 207 mmol/kg for the control, modified and modified-classical treatments, respectively. These results seem to indicate that the modified regimen can produce results similar to that of the classical

carbohydrate loading diets without the dietary restriction of 10% carbohydrate for three days and the undesirable side effects this restriction brings about. It should be emphasized that carbohydrate loading or supercompensation allows runners to maintain their high running intensity for a longer period of time, but does not increase their speed as compared to controls (Karlsson and Saltin, 1971).

Lactate

Lactate is the by-product of anaerobic metabolism (glycolysis) of glucose to produce energy during exercise. Glycolysis is the sequence of reactions that convert glucose into pyruvate with the concomitant production of ATP (Stryer, 1981).



The production of lactate occurs in anaerobic glycolysis to rid the system of the $NADH_2$ build-up which occurs in the cytoplasm due to the glycolytic breakdown of glucose to pyruvate. The regeneration of NAD+ in the reduction of pyruvate to lactate sustains the continued operation of glycolysis under anaerobic conditions. However, continued anaerobic glycolysis causes increased lactic acid accumulation which will eventually result in a decrease in metabolic pH (Kraemer and Fleck, 1982). This increased acidity in the muscle will sooner or later slow down and eventually stop the glycolysis, and with it exercise.

Lactate dehydrogenase (LDH) is the enzyme responsible for the conversion of pyruvate to lactate. LDH is found in two predominant forms. The H form which predominates in the heart and the M form which

is in the skeletal muscle. These two forms differ in the rate at which they reduce pyruvate to lactate. The H form is inhibited at low concentrations of pyruvate while the M form is inhibited at a higher substrate concentration of pyruvate. Therefore, the M form of LDH can reduce pyruvate to lactate at a much higher rate. The H-LDH is favored in systems which use aerobic oxidation of glucose such as the heart and the slow twitch fibers of the muscles, while the M-LDH would be favored where anaerobic systems are preferred, such as in fast twitch fibers (Sjodin, 1976).

Lactate production during exercise depends mainly on the intensity of the exercise and the training state of the individual involved in the exercise (Cunningham and Faulkner, 1969; Margaria et al., 1964; Ready and Quinney, 1982). During high intensity cycle exercise in men, Karlsson and Saltin (1971) found the greatest levels of lactate accumulation in muscle and blood were reached at the highest workloads which resulted in exhaustion in 2 to 3 minutes and 5 to 7 minutes. When lower workloads were used, resulting in exhaustion in 15 to 20 minutes, significantly lower levels of lactic acid were produced. For the short, high intensity times of 2-3 minutes and 5-7 minutes, mean peak plasma lactates were 13.4 and 13.3 mmol/L, respectively. However, at the lower workload, where exhaustion did not occur until 15-20 minutes, mean values of lactate were 9.1 mmol/L of blood.

At lower intensity workloads of less than 30% $\dot{V}O_2$ max, Ahlborg et al. (1974) found that lactate rose only slightly from rest (1.06 mmol/L) to 40 minutes of exercise (1.31 mmol/L) and remained the same

until 240 minutes or exercise had occurred at which time the lactate had risen to 1.80 mmol/L. In a more recent report, Alhborg and Felig (1982) used a $\dot{V}O_2$ max of 58% for three hours and found lactate increased .50-.60 mmol/L during the first 2 hours of exercise and increased another 0.5 mmol/L during the third hour of exhaustive work. In examining the post exercise lactate levels of cross country skiers, Astrand et al. (1963) found that exhaustive exercise caused plasma lactate values to drop as the time of exercise increased. They examined the plasma lactate levels of cross-country skiers in races varying from 10-85 km in length. They found a mean lactate level of 12.5 mEq/L at the end of the 10 km race (35-36 minutes), 6.4 mEq/L at the end of the 30 km race (130-136 minutes) and 3.5 mEq/L at the end of a 50-km race lasting 186-198 minutes. Their results suggest that fatigue factors other than those caused by lactic acid build-up were occurring.

Maughn and Leiper (1983) examined the post-exercise lactate levels of elite and non-elite male and female marathon runners and found the mean lactate levels for males to be 1.88 mmol/L and females 2.32 mmol/L. They also reported that post-lactate concentration was not related to running speed or fractional utilization of aerobic capacity. Schnabel and Kindermann (1983) compared maximal lactate levels (during maximal exhaustion) in 55 males divided into five groups. The groups included: 440 meter high performance runnings, 400 meter lower performance runners, middle-distance runners, long-distance runners, and marathon runners. They

found maximal lactates progressively decreased from a high of 17.47 mmol/ml in 400 meter high performance runners to 10.13 mmol/ml in marathon runners. Controls had a mean maximal lactate value of 15.54 mmol/ml. Research reporting lactate levels of women at various training intensities is limited. As already mentioned, Maugh and Leiper (1983) found steady state lactate values of female vs. male marathon runner to be higher by approximately 0.50 mm/L. Hughson and Green (1982) found male lactate levels to be higher than females at fast and slow ramp work. Unfortunately, males and females worked at different relative workloads.

Lactic acid build-up during endurance activities is not as critical as when high intensities are involved. This is due to the aerobic state of endurance activities which allow some of the lactate produced during exercise to defuse out of the muscle into the blood stream where it can be transported to the liver to participate in gluconeogenesis (Bonen and Bekastro, 1976). This occurs through the reoxidizing of lactate to pyruvate. Pyruvate can then participate in the Cori cycle to produce glucose or can be shunted into the TCA cycle for the production of energy through oxidative pathways.

When exercise is terminated, much of the lactic acid which has accumulated in the muscle during exercise can diffuse out into the blood. Peak post-exercise lactate acid levels occur approximately 2-9 minutes after exercise has ceased (Fjuitsuka et al., 1982; Mader et al., 1978). Plasma lactate levels will drop after this initial peak due to the fact that lactate is quickly being taken up by the

liver for gluconeogenesis for the regeneration of blood glucose and the resynthesis of glycogen (Ahlborg and Felig, 1982).

Fat metabolism

In the body, triglycerides stored in the adipose tissue make up the largest deposit of stored energy in man. For example, a man weighing 70 kg with 13% body fat has approximately 9.1 kg of body fat, a large portion of which can be used for energy production. In order for this stored energy to be made available during exercise the triglycerides must be hydrolyzed to FFA and glycerol. This breakdown of the triglyceride molecule during exercise is stimulated by both hormonal and nervous systems which occur in response to exercise (Bjorntorp, 1981; Pequignot et al., 1980). FFA are bound to albumin then transported via the plasma to the muscle. It appears that the uptake and oxidation of FFA in the muscles is in direct relationship to the arterial concentration. Hagenfeldt (1979) measured the uptake of FFA in the leg and the arm both during rest and exercise and found the uptake to be linearly related to the inflow and concluded that the rate of lipolysis in the adipose tissue seemed to be the most important factor influencing the rate of disappearance of plasma FFA and its muscle uptake. Therefore, the rate-limiting step in muscle FFA use during exercise is the mobilization from the adipose tissue storage site.

At the onset of work involving large muscle groups, such as running, the plasma level of FFA falls due to the increased uptake by the working muscle (Hagenfeldt, 1979). However, the subsequent

mobilization is very rapid as norepinephrine stimulation of adipose lipase occurs (Ahlborg and Felig, 1982; Pequignot et al., 1980; Steinberg, 1964). After this initial lag, mobilization surpasses uptake and the plasma levels of FFA and glycerol rise. As exercise continues at a submaximal workload, the plasma levels continue to rise and may be as high as four to six times the basal level (Ahlborg and Felig, 1982; Hagenfeldt, 1979; Locksley, 1980). As FFA mobilization increases, turnover of FFA also increases, reflecting increased muscle utilization of FFA substrate (Hagenfeldt, 1979). Therefore, except for the initial lag period, mobilization continues to supply FFA faster than the demand. Costill et al. (1979) examined the relative contribution of FFA to the energy demands of exercise and capacity of the skeletal muscle to metabolize lipids in endurance trained males and females. They found that male and female runners of similar $\dot{V}O_2$ max, training mileage and fiber composition, tested during a 60-minute treadmill test at 70% $\dot{V}O_2$ max, had similar muscle lipid metabolism, enzyme activity and calculated RQ.

The relationship of FFA utilization during exercise is related to the intensity of the exercise ($\dot{V}O_2$ max). This means that at rest and during prolonged light exercise fats, in the form of FFA, can supply over 90% of the oxidative needs of the muscle (Locksley, 1980). But as intensity increases, the percent of energy supplied by fats decreases since not enough oxygen is being supplied to the muscle to meet all the energy needs through oxidative pathways. As intensity increases to 60% $\dot{V}O_2$ max, the amount of fat used declines

in direct proportion to the amount of carbohydrate substrate required. This reciprocal relationship creates a continuum of fuel utilization from fats (principally at exogenous FFA) for low-intensity prolonged energy use, to carbohydrate (principally as endogenous glycogen) for high-intensity short-duration use.

In exercise of long duration and fairly high intensity, such as a marathon pace of 70-85% $\dot{V}O_2$ max, the utilization of carbohydrate relative to fat may be approximately 70:30 (Costill and Miller, 1980). At this intensity, muscle glycogen has been identified as the limiting factor (Sherman and Costill, 1984). Since FFA serve as an alternative to carbohydrate for muscular energy, factors that enhance FFA mobilization and oxidation result in the sparing of muscle glycogen and generally improve endurance performance (Costill et al., 1977; Costill and Dalsky, 1978). Costill et al. (1977) found that by artifically increasing FFA in the plasma of subjects during a 30 minute treadmill run, at 70% $\dot{V}O_2$ max, glycogen depletion was decreased by 40% compared to controls.

In addition to the FFA that can be generated by the breakdown of triglyceride in the adipose tissue, a portion of lipids oxidized during porlonged exercise are derived from plasma and intramuscular triglyceride hydrolysis (Costill et al., 1973; Essen, 1977). Depletion rates are related to the amount stored, and not to the intensity of duration of the exercise. While the direct contribution of plasma triglyceride is relatively small during exercise, intramuscular lipid stores have been shown to decrease as much as 30% to 50% during long distance runs up to 100 km (Essen, 1977). The decreased importance

of the endogenous lipid supply to total fat metabolism during exercise is probably related to the fact that FFA uptake is not rate-limiting in the muscle (Golnick, 1977).

Training contributes to the body's ability to utilize fat as energy in many ways. First, with training the lipid mobilization process in adipose tissue is more efficient (Holloszy, 1982). It is hypothesized that this change is due to an increased sentivity to catecholamines produced by training and/or a decrease of plasma insulin levels with exercise. This may mean that the working muscle is more adapted to using lipid substrate (Bjorntrop, 1981). Training has also been shown to increase the concentration of aerobic enzymes responsible for lipid oxidation, while also increasing the body's ability to transport oxygen which is necessary for the aerobic combustion of fat (Holloszy, 1982). Increased lipid oxidation decreases the need for anaerobic glycolysis and the accumulation of lactic acid which inhibits the mobilization of FFA (Issekutz et al., 1965). Therefore, lipid mobilization and combustion are facilitated by physical training and explains why plasma FFA levels during exercise in trained individuals are generally higher than those seen in the untrained state (Sutton, 1978).

Protein metabolism

The role protein plays in exercise metabolism is related to the question of whether or not protein serves as a significant fuel source during exercise. In the past, protein has seldom been considered as

an important source of energy during exercise based on unchanged 24-hour urinary nitrogen excretion values (Astrand and Rodahl, 1977). However, in recent years evidence has been accumulating to suggest that exercise does influence protein metabolism and use during exercise. In a recent study done by Lemon and Mullin (1980), sweat urea excretion was calculated. They found that protein could account for approximately 10.4% of the energy expenditure in carbohydrate depleted subjects and 4% of the energy in carbohydrate-loaded subjects. Decombaz et al. (1979) also found a 44% increase in urea production after a 100-km road race. However, they did not measure sweat urea which has been shown by Lemon and Mullin (1980) to contribute significantly to the total urea production during exercise. Gontzea et al. (1975) went a step further in answering the question of urea production during exercise and did a nitrogen balance study in subjects who were initially untrained and sedentary and then placed on a 20-day training period. Subjects were in nitrogen balance during the control period prior to training but went into negative nitrigen balance during the first four days of training. However, as training continued, nitrogen balance returned to nearly zero by the 12th day. Adaptation to training decreased excretion of nitrogen after exercise. This adaptation to training may account for some of the discrepancy seen in the literature in reference to increased urea nitrogen excretion and exercise. However, although training may minimize urea excretion during exercise, the intensity and duration of the exercise may also play a major role in the amount of urea excreted. As in the study done by Decombaz et al. (1979), a 100-km race definately qualifies

as extreme exercise which may bring about a higher urea nitrogen excretion than might be seen if these same trained subjects were running a 10-km race.

The effect intensity of exercise can have on protein catabolism for energy is supported by the research done by Felig and Wahren (1971). They examined the flux of amino acids across the leg and splanchnic bed during a 40-minute exercise period as work intensity was increased. They found that the peripheral release and splanchnic uptake of alanine exceeded that of the other amino acids and accounted for 35-40% of the total net amino acid exchange. Arterial alanine rose from 20-25% with mild exercise to 60-96% with more strenuous exercise. Splanchnic alanine uptake during exercise exceeded that of other amino acids and increased 15-20% during mild to moderate exercise. However, at heavier workloads splanchnic uptake of other amino acids were also increased. Ahlborg et al. (1974) also measured the metabolism of splanchnic and leg amino acids during strenuous exercise of 4 hours duration at 30% $\dot{V}O_2$ max. They found the arterial concentration of alanine rose 40% above the resting level after 40 minutes of exercise, while all other amino acids showed no significant change. A direct linear relationship was also found between arterial pyruvate and alanine levels at rest and 40 minutes of exercise. It was of interest to note that at the end of four hours of exercise the liver was producing a significant splanchnic output of valine, isoleucine, and leucine and the leg uptake of these branched chain amino acids was almost equal to the liver output. These branched chain amino acids can be taken up by the muscle and converted to alanine. This exchange of alanine from the

muscle to the liver is termed the glucose-alanine cycle. It is currently believed that the glucose-alanine cycle is the main pathway through which catabolized protein is shunted to the liver to produce energy during exercise (Lemon and Nagle, 1981).

In this pathway, protein can be catabolized in the muscle and the resulting amino acids converted to alanine which is transported to the liver and degraded to pyruvate and eventually converted to glucose. The liver releases the glucose back into the blood stream where it can be transported back to the muscle for energy. Branch chain amino acids can be released by the liver and taken up by the muscle, converted to alanine and then re-released by the muscle into the plasma to return to the liver for gluconeogenesis.

Although the contribution of protein to fuel metabolism during exercise is still controversial most researchers now agree that exercise does affect protein metabolism. The extent of the effect has yet to be determined but is influenced by training, diet, and intensity and duration of the exercise. Evans et al. (1983) have estimated that 5.5% of the total caloric cost of exercise is provided by protein.

DIETARY MANIPULATION OF FUEL METABOLISM DURING EXERCISE

As previously mentioned, FFA spare glycogen use and depletion during exercise. This occurs because not only is the uptake and transport of FFA not rate-limiting but several steps in the glycolytic pathway are inhibited when FFA substrates are available (Locksley, 1980).

Furthermore, FFA seem to have a direct depressant action on membrance glucose transport through unknown mechanisms (Locksley, 1980). However, as previously described, the intensity of an exercise largely determines the amount of glycogen which will be broken down.

It is now known that a high carbohydrate diet can increase muscle glycogen stores and prolong exercise. However, other changes in fuel metabolism occur when diet is altered. Maughan et al. (1978) noted that a low carbohydrate diet not only caused glycogen depletion but also caused lower RQ's, blood lactate, blood pyruvate, blood glucose, and plasma triglycerides than when a high carbohydrate diet was fed. Plasma FFA and plasma glycerol were also higher than controls on the low carbohydrate diet and lower than controls on the high carbohydrate diet. Carbohydrate contributed less to fuel metabolism on the low carbohydrate diet than the high carbohydrate diet. In examining the effect a high fat diet would have on fuel use during exercise and endurance, Miller et al. (1984) fed a high fat diet and a normal diet to two groups of male Sprague-Dawley rats. The animals were exercised to exhaustion on a rodent treadmill. The high fat, low carbohydrate diet allowed the rodents to run significantly longer than controls. Adaptations to high fat diet included lower liver and muscle glycogen, decreased glycogen breakdown during exercise, decreased lactate production, and elevated ketone levels. It also caused increased enzyme activities of muscular 3-hydroxy-Coenzyme A dehydrogenase (35-110%) and citrate synthase (15-20%). These results seem to indicate that adaptations by the muscle due to diet apparently increased the

the animal's ability to oxidize fat during glycogen depletion. It should be noted that the amount of urea production was not measured so that the reliance on protein for energy is not known. These results have not been reproduced in man. However, high fat diets, as those seen in glycogen depletion do cause higher FFA during exercise and lower plasma glucose levels. Because glycogen is the limiting factor in endurance events, dietary manipulation has been emphasized to replenish glycogen, even though a high carbohydrate diet may contribute to somewhat high plasma levels of glucose and lactate and lower FFA, indicating that there may be more carbohydrate burned for energy.

The ultimate purpose of a high carbohydrate diet for individuals engaged in physical activity is to replenish glycogen stores, increase endurance, and maintain health by reducing the reliance on body protein for energy substrates. Low carbohydrate/high fat diets produce higher plasma FFA and higher urea excretion values which may indicate increased protein utilization for fuel substrate (Lemon and Nagle, 1980). Low carbohydrate/high fat diets are also contraindicated for overall nutritional health as they have been implicated in increased cancer and cardiovascular disease (Carroll, 1979).

INTERRELATIONSHIP BETWEEN VITAMIN B-6 AND EXERCISE

The relationship between vitamin B-6 and exercise involves two main theories. The first is based on recent research which suggests that exercise alters vitamin B-6 metabolism. The second states that

increased dietary vitamin B-6 increases storage of PLP in the muscle glycogen phosphorylase which in turn may increase the breakdown of muscle glycogen during exercise, ultimately altering the fuels used during exercise. In reviewing the literature on this topic, each of these theories will be dealt with separately.

Until recently it was assumed that vitamin B-6 was not stored to any great extent in the human body. However, in 1964 Krebs and Fisher, in researching glycogen phosphorylase, suggested that this enzyme might be a repository for vitamin B-6 in animals. This hypothesis was pursued by Black and co-workers (1977). They found a linear relationship between increased vitamin B-6 intake and increased glycogen phosphorylase in rats. They concluded that glycogen phosphorylase acts as a reservoir for vitamin B-6 in animals. In 1978, Black et al. further tested this theory by feeding rats normal to excessive amounts of vitamin B-6 to increase glycogen phosphorylase and then placed the animals on a vitamin B-6 deficient diet for eight weeks. They found that the glycogen phosphorylase did not give up its stored vitamin B-6 unless starvation was also induced. They then concluded that the muscle glycogen phosphorylase acts as a reservoir for vitamin B-6 and that starvation or caloric restriction and not vitamin B-6 deficiency causes depletion of the enzyme. Black and co-workers (1978) also examined aspartate aminotransferase and alanine aminotransferase. They PLP dependent enzymes decreased irregularly during the vitamin B-6 deficient diet. On the vitamin B-6 deficient diet the final concentration of glycogen phophorylase and aspartate amino transferase was approximately 70% of the initial level while alanine amino transferase

decreased initially and then returned to near the original level. When the rats were partially starved, they lost muscle phosphorylase while retaining alanine and aspartate aminotransferase. When totally starved, the rats lost more muscle phosphorylase than during the partial starvation period. However, during total starvation, they completely retained alanine aminotransferase and lost some aspartate aminotransferase. Chen and Marlatt (1975) examined the PLP dependent glutamic pyruvic transaminase in trained rats which were fed various levels of vitamin B-6 and then exercised on a treadmill. However, transaminase activity was examined when the animals were in a resting state, not immediately after exercise. They found transaminase activity to increase with higher levels of dietary vitamin B-6 but found no change in erythrocyte glutamic-pyruvic transaminase activity due to training. Critz and Merrick (1964) examined glutamic-oxalacetic transaminase levels in the muscle and liver of female rats immediately after exercise. They found that both treadmill work and swimming elevated skeletal muscle and liver activity of this enzyme. They also noted that the duration of the work appeared to be a more important factor in regulating glutamic-oxalacetic transminase than the rate of the work.

Leklem and Shultz (1983) observed that plasma PLP was increased in male runners after a 4500 meter run. After accounting for hemoconcentration, the plasma PLP values were still well above resting values. They hypothesized that this increased PLP in the plasma was a result of the muscle glycogen phosphorylase giving up its

bound PLP and that exercise mimics starvation. Thus, their conclusion supports that of Black et al. (1977, 1978). This theory was further examined by Hatcher et al. (1982). They observed a significant rise in PLP with exercise and a significant decrease during the recovery period in male cyclists. They also accounted for hemoconcentration and still found a significant rise in PLP. In investigating if supplementation of vitamin B-6 would increase the magnitude of the rise of PLP seen with exercise over a non-supplemented diet, a high carbohydrate diet supplemented with vitamin B-6 was fed. It was hypothesized that the high carbohydrate diet would increase the glycogen and that vitamin B-6 supplementation would increase the glycogen phosphorylase. When the subjects were exercised, an even greater rise in plasma PLP was seen over the non-supplemented diet. Hatcher et al. (1982) concluded that exercise does imitate starvation in its effect on the glycogen phosphorylase causing the reservoir to release PLP. They also concluded that supplementation causes an even greater storage and release of PLP. After exercise had ceased, uptake of PLP back into the muscle or continued uptake into the liver could account for the dramatic decrease in plasma PLP during the recovery period.

Leklem et al. (1982) examined whether men and women differ in the changes in plasma PLP levels during exercise and found that both running and cycling produced higher plasma PLP values in the men than in women. The men had exercise-induced PLP increases of 105-173 nM whereas the increase in the women ranged from 64-65 nM.

The mechanism by which the glycogen phosphorylase enzyme is degraded and the release of PLP occurs is not known. Exercise can

induce protein catabolism within the muscle for energy production (Felig and Wahren, 1971). This may be the way in which the PLP is released from glycogen phosphorylase for subsequent release into the plasma. Gani et al. (1978) have developed a micellular model which imitates the glycogen phosphorylase enzyme in many ways. In this model system, they have proposed a method for removal of PLP from the enzyme by a reaction with cysteine. Whether this system works in the environment of the muscle cell has yet to be determined.

The significance of the release of PLP from the glycogen phosphorylase enzyme during exercise or starvation is probably related to the role this vitamin plays in gluconeogenesis. During both exercise and starvation the body depends on liver production of glucose to maintain blood glucose levels. The liver can produce glucose through glycogenolysis of its stored glycogen (a B-6 dependent process) or through gluconeogenesis in which the alanine-glucose cycle contributes to glucose production (a B-6 dependent transamination process). Angel (1980), in examining the effect of vitamin B-6 deficiency on gluconeogenesis in rats, found that deficiency of vitamin B-6 impaired gluconeogenesis from amino acids. This impairment was due to decreased transamination.

Increased circulation of PLP in the plasma caused by exercise may not only mean that the vitamin is more available to assist in gluconeogenesis but may also mean it is more susceptible to metabolic degradation to 4PA and excretion in the urine. It should be remembered that individuals who are low or deficient in vitamin B-6 have decreased

4PA excretions (Donald et al., 1971; Shin and Linkswiler, 1974). Therefore, exercise induced excretion would be relative to the amount of 4PA excreted when activity was not being engaged in.

Although relatively little research has been done in the area of 4PA excretion during exercise, Borisov (1977) did find increased urinary 4PA excretion in 297 students during the summer months when compared to the winter, spring, and autumn seasons. Activity levels were not reported, but one might expect students to be more active in the summer than other seasons. The author suggested that active students need 1½ to 2 times the standard recommendations of vitamin B-6. Hatcher et al. (1982) also examined 4PA excretion of trained cyclists on non-exercise and exercise days and found that 4PA excretion (expressed as % of vitamin B-6 intake) ranged from 47.1-64.9% on non-exercise days to 60.4-71.7% on exercise days.

The way in which vitamin B-6 may alter fuel metabolism is related to glycogen phosphorylase being a storage site for vitamin B-6. PLP is essential for the activity of glycogen phosphorylase as well as for the maintenance of the stable tetramer form of the enzyme (Helmreich and Klein, 1980). PLP does not act via a Shiff base mechanism in glycogen phosphorylase as it does in so many other vitamin B-6 dependent enzymes. Its participation in glycogen phsophorylase seems to be through the phosphate group of PLP directly participating in the catalytic reaction (Helmreich and Klein, 1980; Stryer, 1981). Thus, PLP is essential in the enzymatic function of glycogen phosphorylase.

As Black et al. (1977) have demonstrated, glycogen phosphorylase can be increased in the muscle. Increased concentration of the enzyme

may initiate increased glycogen breakdown. This hypothesis was examined by deVos et al. (1982), who found that cyclists on a high carbohydrate and vitamin B-6 supplemented diet produced higher lactate values during exercise than when fed a high, moderate, or low carbohydrate diet without vitamin B-6 supplementation. If glycogen is being broken down more quickly due to increased glycogenolysis, one would expect less reliance on FFA to be occurring and thus lower plasma FFA. Also, if lactate is high, due to increased anaerobic glycolysis in the muscle, this could inhibit FFA mobilization (Issekutz et al., 1965).

Lawrence et al. (1975) attempted to assess whether a 51 mg dose of vitamin B-6 would increase the endurance of swimmers. They found that supplementation had no effect on increased swimming endurance and that training appeared to be the major factor in determining endurance. It should be noted that the vitamin B-6 group had the highest lactate levels after a test swim as compared to the other treatment group or the control group. No attempt was made to control diet or other variables which may also contribute to performance ability.

Therefore, the interrelationship between vitamin B-6 and exercise could involve two factors: 1) the alteration of vitamin B-6 metabolism due to the effect exercise has on the release of PLP from the muscle glycogen phosphorylase enzyme and 2) changes in the fuels used during exercise due to increased glycogen breakdown brought about by increased glycogen phosphorylase concentration (due to vitami B-6 supplementation.

CARDIAC FUNCTION

This review of cardiac function will focus specifically on the following items: 1) the assessment of cardiac muscle functions and the general use of the electrocardiography (ECG), 2) the specific clinical uses of the QT interval and ST segment as diagnostic tools and 3) how diet and exercise may independently or together modify cardiac function, as measured through changes in the QT interval and the ST segment.

ECG: Measurement of Cardiac Function

The heart is a unique muscle in that it is capable of self-excitation, a process which causes a rhythmical contraction to occur. The movement of this excitation through the heart can be monitored through the electrical potentials produced by the individual cardiac muscle cells. The ECG is used to monitor the summation of this movement of electrical impulses by making a tracing or graph of the variations in voltage produced by the heart muscle during different phases of the cardiac cycle. These voltage variations are produced during depolarization and repolarization of the cardiac muscle cells. In order to trace these changes in voltage, electrodes are placed at various axes of the heart. Figure 2.6 graphically depicts how the various waves of the ECG are made (Scheidt, 1983). Passage of current toward the positive end of a bipolar lead causes a positive or upward movement of the stylus. Conversely, passage of current away from the positive pole of a bipolar electrode causes a negative deflection, or downward movement of the stylus. By placing leads at various

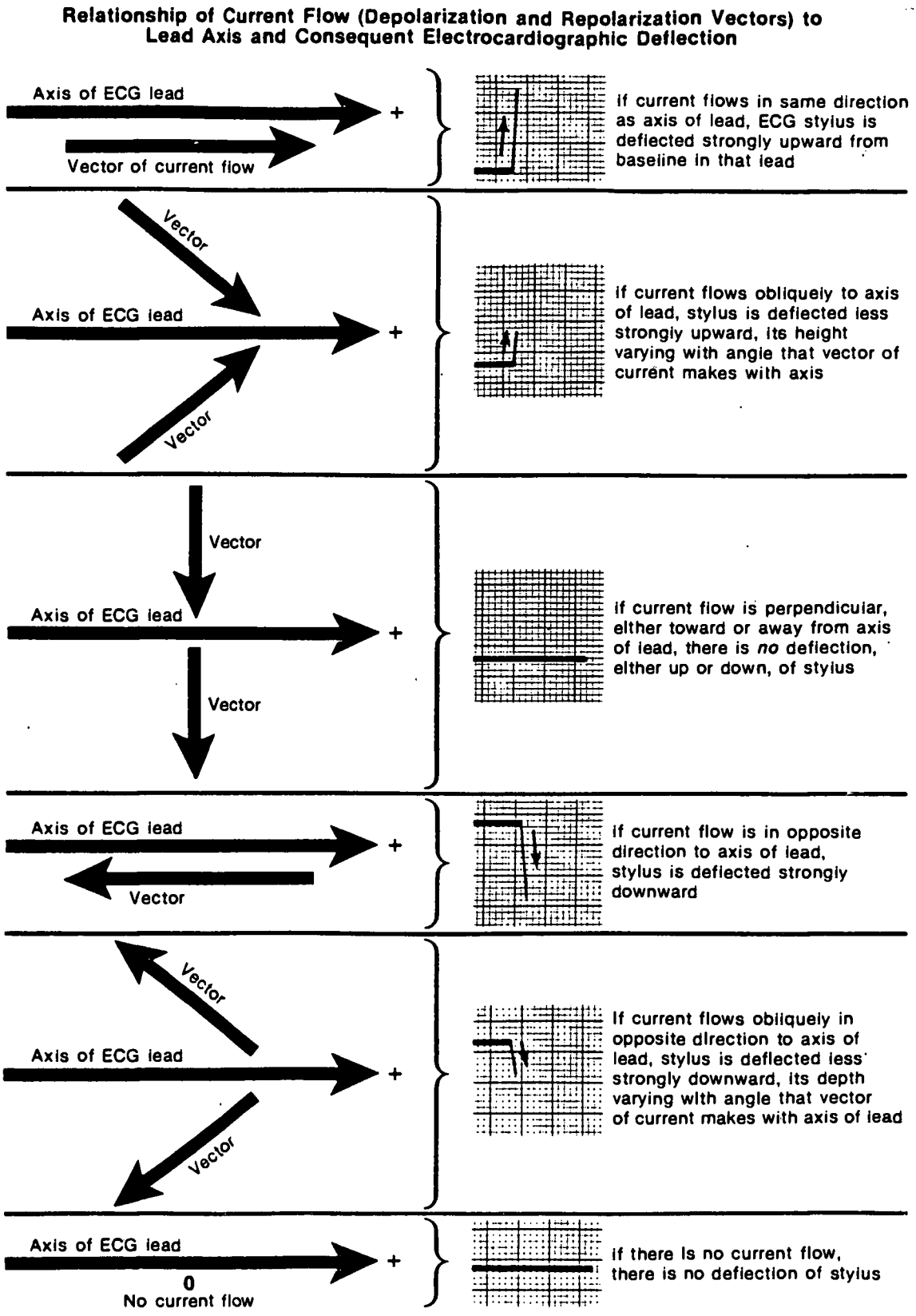


Figure 2.6. Relationship of current flow (depolarization and repolarization vectors) to lead axis and consequent electrocardiographic deflection (Scheidt, 1983).

axes of the heart it is possible to examine the electrical activity of the heart from several angles.

A normal ECG configuration (depicting a normal cardiac cycle) is shown in Fig. 2.7. This tracing of the cardiac cycle has been divided into several intervals, waves, and segments which correspond to specific cardiac sequences. If there is a deviation in the sequence of the cardiac events or the time it takes for these events to occur, changes or deviations from the normal ECG configuration will also occur. It is through this process that the ECG can be used to monitor cardiac function and serve as a diagnostic tool to detect deviations from normal cardiac patterns.

The particular sections of the ECG configuration which will be addressed in this review are the QT interval and the ST segment (Figure 2.7) (Scheidt, 1983).

QT Interval

The abundant research now available on how QT intervals change with certain pathologic conditions has made them an important diagnostic tool in clinical electrocardiography. The cardiac events which correspond with the QT interval are the depolarization and subsequent repolarization of the ventricals, see Fig. 2.8. However, as the heart beat quickens the duration of the QT interval is also affected. The QRS complex (which represents the depolarization of the ventricals) portion of the QT interval is independent of heart rate. The shortening of the QT interval results from a rate-proportional shortening of the ventricular muscle action potentials in the repolarization state.

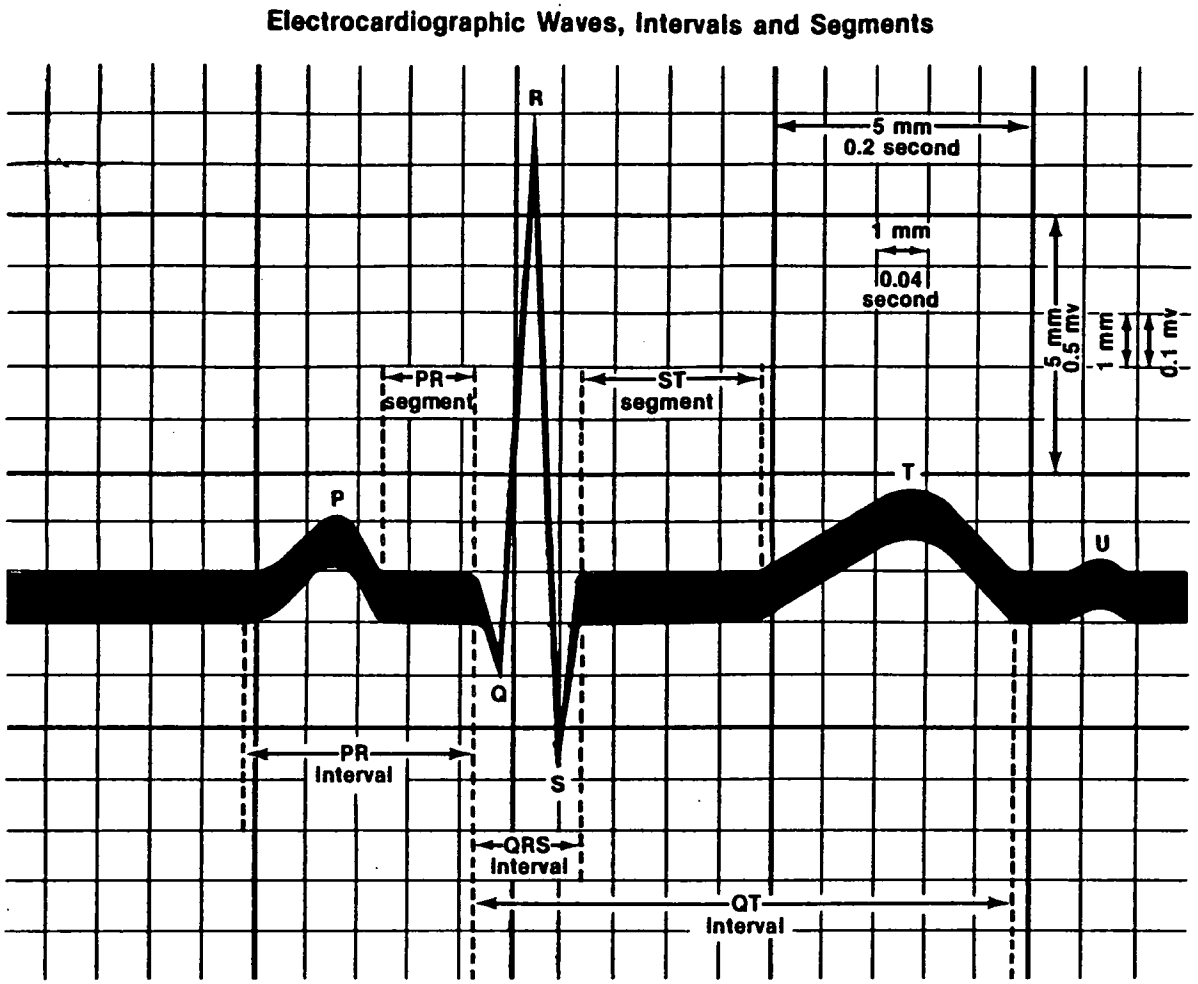


Figure 2.7. Electrocardiographic waves, intervals, and segments (Scheidt, 1983).

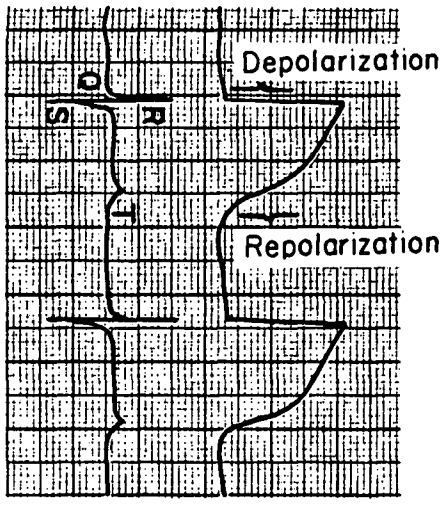


Figure 2.8. Depolarization and repolarization of cardiac muscle which corresponds to the QT interval in the ECG configuration (Guyton, 1981).

This in turn results in a shortening of the ST-T component of the QT interval. The QT interval is measured from the beginning of the QRS complex to the end of the T wave (Guyton, 1981).

It is the relationship between the duration of the QT interval and heart rate which has received much attention over the past 50 years. Prolongation of the QT interval may occur in association with use of quinidine-like drugs (Roden et al., 1983), congenital syndromes such as Romano-Ward and Jarvell and Lange-Nielsen syndromes (Schwartz et al., 1975; Inoh et al., 1981; Leistner et al., 1983), hypothermia (Emslie-Smith et al., 1959), electrolyte disturbances such as hypocalcemia (Surawicz, 1964) as well as in myocardial diseases such as mitral valve prolapse (Pudde et al., 1983), myocardial infarction, ventricular arrhythmias and ischemia (Ahnve et al., 1978; Taylor et al., 1981). It has also been implicated as contributing to Sudden Infant Death Syndrome (Schwartz et al., 1981; Guntheroth, 1982). QT prolongation occurs when there is a delayed or expanded repolarization phase, uniform or regional, in the ventricular myocardium. The elongated QT interval associated with this prolonged repolarization phase increases the probability of ventricular tachycardia and fibrillation (Taylor et al., 1981) by increasing the chance that a premature beat may occur. It is for this reason that the long QT interval is significant in the diagnosis of disturbed metabolic functions within the cardiac muscle and is used in the assessment of coronary abnormalities.

Because the QT interval is dependent on heart rate, a prolonged QT interval is relative to the heart rate at which it is being measured.

In recognizing this relationship between heart rate and length of the QT interval, many investigators have attempted to develop formulas for adjusting or correcting the QT interval based on heart rate. Simonson et al. (1962) reviewed the current formulas in the literature and compared them. Of the equations they reviewed those of, Bazett (1920), Fredericia (1920), and Ashman (1936) are the most frequently used clinically today. The square root formulas of Bazett (1920) and the logarithmic formula of Ashman (1936) are used most frequently in North America, while the cube root formula of Fredericia is used in Europe.

The Bazett formula results in a corrected or derived QT interval, QT_c, from the formula:

$$QT_c = \text{QT interval} \; / \; \sqrt{\text{R-R interval}}$$

Both the QT interval and the R-R interval (cardiac cycle length) are measured in seconds. The QT_c may also be regarded as a constant K. The value of the QT_c or K corresponds to the QT duration at a heart rate of 60 beats per minute. For at this rate, the R-R interval is 1.0 second, and since the square root of 1.0 second is still 1.0 second, the QT will be constant. Bazett (1920) reported mean K values of 0.368 for men (range = 0.341-0.392) and women 0.399. The normal range of K is often given as 0.350-0.430 seconds. Table 2.1 gives a summary of the various formulas, sample sizes, and K's reported in the literature (adapted from Simonson et al., 1962). For those investigators who used the square root formula, K values range between 0.368-0.397 for men and 0.399-0.415 for women. However, regardless of the formula used, women and the elderly generally have longer QT

Table 2.1. Suggested formulas for relationship between R-R and Q-T intervals.

Authors	Size and composition of group	Lead	Formula* QT_c
Bazett (1920)	M: 20. F: 19. Normal subjects	II	$k\sqrt{R\text{-}R}$ M:k=0.368 (range 0.342-0.392) F:k=0.399
Shipley & Halloran (1936)	M & F: 200. Normal subjects. Age 22-35 yr.	II	$k\sqrt{R\text{-}R}$ M: K= 0.397 K= 0.415
Hegglin & Holzmann (1937)	M & F: 700. Normal subjects and patients without HD	II	$k\sqrt{R\text{-}R \pm 0.04}$ k=0.39 (for M & F)
Fridericia (1920)	M & F: 50. Normal subjects. Age 30-81 yr.	II	$k\sqrt[3]{100\ R\text{-}R}$ k= 0.0822
Schlomka & Raab (1936)	M & F: 336. Subjects without HD	II	$k\sqrt[3]{100\ R\text{-}R}$ (AGE) 20-30: k=0.0795 40-50: k=0.0802 60-70: k=0.0815 over70: k=0.0826
Ashman (1939)	M: 432. F:425. Children: 200. Subjects without HD	II	$K \log(10[R\text{-}R + K_2])$ Young M: K =0.373 Young F: K =0.385 Elderly M: K =0.380 Elderly F: K =0.390 Children: K =0.376 For all groups: K_2=0.07
Ashman & Hull (1945)	2,000 cases		$K \log (10[R\text{-}R + K_2])$ Revised constants: Young F: K =0.385 Elderly M: K =0.380 Elderly F: K =0.385 Children: K =0.375 For all groups: K_2=0.07
Schlamowitz (1946)	M: 650. Healthy soldiers. Age 18-44 yr.	II	0.205 R-R + 0.167
Mayeda (1934)	M: 153. F: 65. Normal Subjects and patients without HD, age 18-64 yr. HR: 54.5-115.8	II	$(M\ [100\ R\text{-}R])^n$ M=0.2574 n=0.604

*R-R interval measured in seconds; F: Female; M: Male; HD: heart disease; k or K: constant developed from measurement of Q-T interval in seconds.

intervals than young men. The advantage of using QT_c in monitoring cardiac function is that once the QT interval is corrected for heart rate, a single criterion can be used to compare all QT_c values. In the literature a $QT_c > 0.440$ seconds is usually considered to indicate cardiac abnormalities. Maximum QT interval measurements are given in 2.2 for various heart rates (Scheidt, 1983).

Puddu et al. (1981) reported that in monitoring patients just prior to the onset of ventricular fibrillation, QT intervals were lengthened and QT_c was > 0.440 seconds in all cases. Ahnve et al. (1978) also found that in ventricular arrhythmic and ventribular tachycardia prolonged QT intervals could be measured before the event occurred. They observed that the QT interval was significantly lengthened relative to the control values ($QT_c = 0.404$ seconds) in days 1-4 (QT_c range = 0.427-0.448) after the infarction had occurred and returned to control level by the 14th day ($QT_c = -.407$). Prolongation of the QT interval has also been implicated in sudden death. Schwartz and Wolf (1978), examined QT_c values in myocardial infarct patients and healthy controls over a seven year period, with resting ECG's taken every two months. The QT_c among the controls was 0.418±.020 and among the deceased the QT_c was 0.443±.027, with the latter being signiciantly longer than the value for surviving patients. They concluded that the fact that it was necessary to add 1½ standard deviations to the QT_c of the controls in order to reach 0.440 seconds supports the concept that 0.440 seconds is a proper limit for QT_c. They reported that the only control whose QT_c was prolonged (0.457) died suddenly and a fresh myocardial infarction was found at the

Table 2.2. Maximum QT intervals at various heart rates (adapted from Scheidt, 1983).

Heart rate	Maximum QT (seconds)		Heart rate	Maximum QT (seconds)	
	male	female		male	female
300	.19	.20	68	.38	.41
250	.20	.22	65	.38	.42
214	.21	.23	62	.39	.43
187	.23	.25	60	.40	.44
166	.24	.26	57	.41	.45
150	.25	.28	55	.42	.46
136	.26	.29	52	.42	.47
125	.28	.30	50	.44	.48
115	.29	.32	46	.45	.50
107	.30	.33	43	.47	.51
100	.31	.34	41	.48	.53
93	.32	.35	39	.49	.54
88	.33	.36	37	.51	.56
83	.34	.37	35	.52	.57
78	.35	.38	34	.53	.58
75	.36	.39	32	.54	.60
71	.37	.40	30	.57	.62

autopsy. Of the 21 patients who had mean QT_c values of > 0.440 seconds, 16 (77%) died suddenly. They also reported that a QT_c of > 0.440 seconds was present in 57% of the deceased in contrast to 18% of the survivors and values > 0.450 seconds were found in 36% of the deceased and in only 8% of the survivors. The calculated risk of sudden death for patients with a previous myocardial infarction or prolonged QT intervals was respectively, 2.16 and 2.36 times greater than for those with a normal QT_c.

Taylor et al. (1981) also assessed changes in ventricular repolarization during acute myocardial infarction in 32 patients who were admitted within 2.0±1.8 hours after the onset of a myocardial infarction. The initial corrected QT interval upon hospitalization was longer (0.52±0.07 seconds) in the 14 patients developing ventricular tachycardia within the first 48 hours as compared to the QT_c (0.47±0.03) in the 8 patients with frequent ventricular premature beats and to QT_c (0.46±0.03) in the 10 patients with infrequent ventricular premature beats. Five days after the infarction the QT_c had shortened in all but the ventricular tachycardia patients, suggesting a greater initial abnormality of repolarization in these patients.

As previously mentioned, long QT intervals have been reported in congenital syndromes such as Jervell and Long-Nielsen and Romano-Ward Syndrome. In both of these conditions prolonged QT intervals are associated with sudden death brought on by ventricular fibrillation (Schwartz, 1978). In reviewing case studies of individuals with these syndromes, Schwartz et al. (1975) reported prolonged QT_c values of 0.50-0.61 seconds. Leistner et al. (1983) also measured the QT interval

in an infant with the Roman-Ward Syndrome and found mean QT_c intervals of 0.53 seconds compared to normal infant values of 0.345-0.434. Inoh et al. (1981) compared the QT_c intervals of Romano-Ward Syndrome patients with those of patients with myocardial diseases and found that the myocardial patients had QT_c of 0.45-0.475 while Romano-Ward Syndrome patients had QT_c of 0.475-0.70. These studies give strong evidence that elongation of the QT interval can predispose one to the increased probability of ventricular fibrillation.

QT Interval: Exercise and Dietary Influence

The literature contains relatively few research studies evaluating QT interval changes with exercise and diet. Especially scarce are studies examining the effect that dietary manipulation has on QT intervals during exercise. The studies which have been done will be reviewed here.

Yu et al. (1949) made one of the first attempts to monitor QT interval changes before, during, and after exercise. The exercise procedure they used included a 10 minute rest period in a chair followed by a walk on a motor driven treadmill at 2.6 or 1.7 mph or less, depending on each individual's tolerance and a 10 minute recovery period. ECG measurements were taken the last three minutes of rest, during each minute of exercise and the last three minutes of recovery. Ninety-seven tests were done on 77 subjects. These subjects were divided into four groups: healthy, normal controls, or patients with heart disease, congenital heart disease, or pulmonary disease. In the control group they observed a significant increase in the QT_c

(measured by Bazett's formula) from rest to exercise and then a shortening of the QT_c back to resting values at the end of the recovery period. However, in the heart disease patients the QT_c increased only slightly in the first three minutes of exercise and then from the fourth minute on there was a tendency for the QT_c to decline until the end of the exercise. It lengthened slightly during the recovery period. In the congenital heart disease patients, the responses were even more abnormal with the QT_c remaining unchanged in the first minutes of exercise and then progressively shortened until the end of the exercise time. However, the pulmonary group had responses similar to the control group. They concluded that an abnormal response of QT_c during exercise is one that shortens in comparison with that observed during rest. Normal subjects showed a significant QT_c increase from rest to exercise as compared to coronary heart disease patients.

Estimations of normal QT intervals during exercise are extrapolated from resting data using formulas such as Bazett's. In an attempt to establish normal QT intervals during exercise, Fisher (1979) examined 44 subjects on the cycle ergometer and treadmill to establish actual (not predicted) QT intervals during exercise. He observed that the normal QT interval is usually longer than predicted during exercise. He also noticed that no difference occurred between QT intervals during exercise due to age, sex, cycle or treadmill, or steady state exercise vs. rapidly progressive exercise. These results seem to support the earlier work of Yu et al. (1949) who also reported increased QT_c during exercise in normal healthy adults.

Other researchers who have monitored QT intervals in response to exercise are Greensberg et al. (1979), Roman and Bellet (1965) and Masters and Rosenfeld (1961). They measured QT_c immediately after exercise and during the recovery period and found that prolonged QT_c were predictive of coronary insufficiencies. These studies measured QT_c using the formula, QT_c = QT interval (in sec.)/0.4 $\sqrt{\text{R-R interval}}$ (in sec.). They used an upper limit of 1.08 seconds.

The measurement of QT intervals in athletes or those aerobically trained are minimal and the results are mixed. Beckner and Winsor (1954) and Smith et al. (1969) have reported that QT intervals during exercise do not significantly differ between controls and athletes. But others, Beswick and Jordon (1961) and Van Ganse (1970) have found that QT intervals in athletes during exercise are longer as compared to controls. However, after exercise the response of the QT interval seems to be similar in both athletes and controls (though some athletes may exhibit a longer QT interval here also). In consideration of the discrepancy in the literature, Lichtman et al. (1973) cautions the evaluation of the ECG's of athletes. They felt that abnormalities which may occur are probably due to the anatomic and electrophysiologic effects of training on the cardiovascular system and do not suggest cardiovascular disease.

The effect of diet on QT intervals has been confined to research on cardiac cycle changes due to severe dieting and/or starvation have more recently been in the news. This was due to the fact that during 1977 and early 1978, 58 deaths occurred in obese adults who were or had been reducing on low-calorie diets from collagen or gelatin

hydrolases (liquid protein diets). In examining the cause of these deaths, Isner et al. (1979) and later Van Itallie and Yang (1984) closely examined data on 17 of these obese subjects who had no other complicating disorders such as diabetes or coronary heart disease, except for their obesity. Of the 17 patients, 16 were women with an average age of 37 and had lost approximately 35% of their pre-diet weight ($\bar{x}$= 41 kg) over a five month period. In the initial examination of the data, Isner et al. (1979) observed that 10 patients on whom they had ECG data all had episodes of ventricular tachycardia and nine of the 10 had prolonged QT intervals which was unassociated with recognized causes of QT interval prolongation. Attempts to determine a cause of death failed and they had to conclude that a definite cause-effect relationship could not be established between the liquid-protein diets and death. All had died of sudden and unexpected ventricular tachydysrhythmias with prolonged QT intervals. However, it was observed that the less obese died sooner than the more obese, which suggested to the investigators that a maximum safety period existed. Van Itallie and Yang (1984) re-examined the data on the 17 obese subjects in reference to the observation made by Isner et al. that the less obese died sooner. In charting months of survival against body mass index they found that a positive correlation of 0.824 existed. They also re-examined the QT interval data and heart weights at death. They found that there was a decrease in heart weight proportional to the decrease in body weight. This indicated that the myocardial mass was not spared on the liquid-protein diets. Histological examination showed myocardial atrophy, varying degrees of

fiber attenuation, disappearance of myofibrils, but no significant coronary atherosclerosis. They concluded that the link between the low calorie liquid-protein diets and death was due to myocardial protein depletion followed by cardiac arrhythmia. The cardiac depletion manifested itself in long QT intervals, which indicated that repolarization was not occurring properly. Both Isner et al. (1979) and Van Itallie and Yang (1984) concluded that starvation or low calorie restriction can result in prolonged QT intervals and that repeated ECG's should be done on any individual who embarks on a semistarvation diet for weight loss. Their conclusions were recently reaffirmed in the examination of three anorexia nervosa patients (all women) who died of sudden cardiac death. Isner et al. (1983) examined ECG's taken seven days prior to death and noticed elongated QT intervals. The QT_c for the three women were, 0.58, 0.46, 0.45 seconds, all above the 0.440 second cut off.

Pringle et al. (1983) retrospectively reexamined the ECG's of 13 obese patients (11 women, 2 men) who underwent therapeutic starvation in a three year period. All 13 patients fasted for seven weeks, eight for 12 weeks, and four for 17 weeks. By the end of the eighth week all subjects had significant prolongation of the QT_c interval. The significance of the prolongation of the QT_c was illustrated by one patient who suffered two cardiac arrests during the study. Linet et al. (1983), in treating 14 obese women on a 1200 kcal high protein diet for four weeks, a very-low-calorie-diet (1.2 g/kg of protein) for 7 weeks and a weight maintenance diet for 4 weeks, reported no abnormal

cardiac function during the experimental period. However, subjects in this study received numerous vitamin and mineral supplements, including 4 mg of vitamin B-6 daily, whereas subjects in the study of Pringle et al. (1983) study were not given supplements other than vitamins A, B, C, and D, potassium, and folic acid. However, the types of B vitamins administered were not reported. Linet et al. (1983) attributed the success of their diet regimen to the numerous vitamin and mineral supplements given.

Research examining the effect of carbohydrate on ECG's has been limited. A carbohydrate load (100 g glucose or a high CHO diet) produces altered ECG readings ½ to 1 hour after the meal as compared to fasting values (Rochlin and Edwards, 1954; Simonson and Keys, 1950). Changes seen in the ECG are T wave elongation, flattening, and inversion. Riley et al. (1972) has more recently examined the influence of a glucose ingestion on resting and exercise ECG measurements. They examined 35 subjects (18 men and 17 women) who were tested on separate days after a glucose. The glucose was given in the form of a cola drink. One hour after the ingestion of the test drink, a resting ECG was taken and then each subject underwent a graded treadmill exercise test. Exercise intensity was gradually increased to 90% maximal heart rate (MHR). ECG changes which occurred were decreased T wave amplitude, ST segment depression, increased R wave amplitude and increased heart rate.

The examination of the effect of an altered carbohydrate diet (over several days) on QT intervals during exercise was examined

by Goulard (1982). He found that in trained male cyclists exercised at 60, 80, and 90% of MHR, produced a "D" value (the difference between QT_c at rest and QT_c during exercise) on the carbohydrate depletion diet that was significantly different than the "D" value when the control diet was fed. When a carbohydrate loading diet was fed and exercise done at 90% maximal heart rate he also found a significant difference in the "D" value on the carbohydrate loading diet vs. control diet. This indicates an inadequate response of the heart to meet the demands of exercise on the depletion and the loading diets. When vitamin B-6 was added (8 mg) to the loading and depletion diets, cardiac response improved at the 60% maximal heart rate, but not at the higher intensities. He concluded that the carbohydrate depletion diet and the carbohydrate loading diet placed unfavorable demands on the heart and that supplementation with vitamin B-6 improved this response at low intensity workloads.

ST Segment

The ST segment is that portion of the ECG complex from the end of the QRS complex to the beginning of the T wave. It represents the beginning of ventricular repolarization. This portion of the ECG complex is normally isoelectric at rest. The measurement of the ST segment is done from the J point (the junction between QRS and ST segment) to the beginning of the T wave, see Figure 8. Any deviations from the isolectric line greater than 1 mm for .08 seconds or longer in leads I, II, or III is normally considered abnormal (Winsor, 1977).

Elevation of the ST segment has been associated with aneurysm of left ventricle, myocardial infarction and coronary arterial spasm (Feyter et al., 1981). ST segment depression (downsloping or horizontal from the J point) is associated primarily with myocardial ischemia, coronary insufficiency and left ventricular hypertrophy (Boden et al., 1981; Scheidt, 1983).

ST Segment: Exercise and Dietary Influence

Monitoring the ECG complex during exercise stress testing has become a popular and useful tool in helping to assess coronary "capabilities" or limitations. Bruce and McDonough (1969) have outlined three reasons the exercise stress tests is useful in this way. First, it can be used to identify pathophysiological risk factors for chronic coronary disease which are not apparent by examining the cardiovascular system at rest. Second, by quantitatively assessing the functional aerobic capacity one can define the magnitude of impairment. Finally, the prediction of prognosis depends on both the impairment and its component risk factors. Numerous investigators have utilized the maximal and submaximal exercise stress test to assist in the diagnosis of potential coronary problems as well as confirming diagnosis based on other criteria. However, it is a well established practice not to daignose coronary artery disease based on ECG data alone.

The ability of ST segment depression to predict or assist in predicting coronary artery disease has been studied extensively in

in conjunction with exercise stress testing. Gobel et al. (1981) correlated the findings of coronary arteriography with the results from an exercise stress test on 116 men with ischemic heart disease and 105 healthy controls. Eighty-eight percent of the coronary patients were identified successfully by the exercise stress test. They found the ST segment depression, reduced exercise tolerance and persistence of ST segment changes for more than two minutes following exercise were the three most important factors for predicting two or three vessel coronary artery disease. Bruce et al. (1980) identified four variables from the maximal stress test which were significantly associated with identifying subsequent primary coronary heart disease in healthy men. ST segment depression was one of these four variables. However, they were quick to emphasize that it is a misconception to think that exertional myocardial ischemia can be solely assessed by ST segment depression alone. Other factors which may also contribute to the ischemia also need to be considered. Nahormek et al. (1979) also used exercise stress testing to assist in the recognition of coronary artery disease. They found that the predictive value of ST segment depression improved with the increasing magnitude of the depression as well as the frequency. Stuart and Ellestad (1976) reported the predictive value of ST segment changes in a 6 year follow-up study of 438 patients who underwent maximal treadmill stress testing. They found the incidence of coronary disease (death, myocardial infarction, or angina pectoris) as follows: 13% in 84 subjects who stress test produced 2 mm downsloping ST segment depression, 9% in 230 subjects with 2 mm

horizontal ST depression and 9% in 124 subjects with upsloping ST segments with 2 mm ST depression measured for 0.08 seconds from the J point.

Although identification of ST segment depression has been used extensively to assist in identifying coronary artery insufficiencies many researchers warn of the possibility of false positive stress tests. Ellstad et al. (1977) identified eleven other criteria in addition to ST segment depression which could help correctly classify exercise stress tests. Some of these criteria were maximal heart rate, maximal systolic blood pressure, contour of ST segment depression, chest pain during testing, and time of the onset of ST segment depression. Froelicher et al. (1981), Spirito et al. (1983) and Quyyumi et al. (1983) all warn that changes in ST segments can occur in healthy subjects. Quyyumi et al. (1983) followed 120 healthy volunteers on 24 hour ambulatory ECG monitoring. They found that ST changes often interpreted as myocardial ischemia are frequently seen in 24 hour ECG monitoring of healthy volunteers. Spirito et al. (1983) also monitored ST segment changes in healthy young athletes. They concluded that abnormal exercise tests results occur commonly in both athletes and non-athletes. They found abnormal ST segment responses in 9% of the athletes tested. Abnormal responses were attributed to the increased left ventricular mass often seen in athletes. Bjuro et al. (1980) concluded the reason for the prevalence of false positive stress testing results using the ST segment depression is that the criterion is not stringent enough and proposed a more stringent criterion be used.

The literature indicates that ST segment changes are often used to assist in the prediction, diagnosis, and/or prognosis of coronary disease. However, researchers are cautioned to the many factors which may cause false positive results in an exercise stress test, including abnormal ST segment responses. Other criteria, besides ST segment abnormalities, should be examined before coronary ischemia is diagnosed.

Research examining the effect of diet and ST segment changes is rare. As already mentioned in the discussion on QT intervals, a glucose load can alter the ST segment response during exercise (Riley et al., 1972). McHenry et al. (1981) have also reported that apparently healthy men given a glucose tolerance test prior to performing an exercise stress test produced abnormal ST segment depression. When these same subjects returned two weeks later and an exercise stress test was performed under fasting conditions, the ST segment responses were normal. They concluded that a glucose tolerance test or a glucose load could contribute to a false positive response in the exercise stress test. The effect of an altered carbohydrate diet (not a glucose load) on ST segment length was examined by Siemann (1982). A carbohydrate depletion and repletion diet was fed with and without an 8 mg supplement of vitamin B-6 to male cyclists. Subjects were exercised at 60, 80, and 90% of maximal heart rate. Vitamin B-6 did not significantly alter the work loads achieved. However, the ST segment was significantly abbreviated during the carbohydrate-depletion phase at 60, 80, and 90% of maximal heart rate and significantly abbreviated during the carbohydrate-loading phase at the 90% maximal

heart rate. He concluded that while the external work output of the body was increased through carbohydrate-loading, the cost of the increased work capacity manifested itself in an abbreviated ST segment. ST depression is more commonly cited in the literature as an indication of cardiac ischemia, not ST segment abbreviation. ST segment depression was not measured in this study.

CHAPTER III

CHANGES IN PLASMA PYRIDOXAL 5'-PHOSPHATE AND URINARY 4-PYRIDOXIC ACID DURING EXERCISE IN TRAINED AND UNTRAINED WOMEN CONSUMING TWO CARBOHYDRATE DIETS WITH AND WITHOUT VITAMIN B-6 SUPPLEMENTATION. [1,2]

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ABSTRACT

To assess the effect of exercise on vitamin B-6 metabolism in women, 5 young/trained, 5 young/untrained, and 5 postmenopausal/ untrained women were alternatively fed four dietary treatments and then exercised. A moderate carbohydrate (CHO) (49%) (MCHO) diet for 2 weeks; a high-CHO (64%) (HCHO) diet for 1 week; a MCHO+B-6 diet for 2 weeks and HCHO+B-6 diet for 1 week. A 1 week MCHO diet separated the non-supplemented (NS) (2.3-2.4 mg B-6) and supplemented (S) (10.3-10.4 mg B-6) diets. Training state was determined by $\dot{V}O_2$ max. At the end of each dietary treatment the subjects were exercised at 80% $\dot{V}O_2$ max for 20 minutes. Blood was drawn, pre, post, post-30 min and post-60 min (p60) of exercise and analyzed for plasma pyridoxal 5'-phosphate (PLP) and vitamin B-6 (PB6). 24-hour urine collections were analyzed for 4-pyridoxic acid (4PA) and urinary B-6 (UB6). ANOVA showed no difference among groups with respect to diet or time for PB6, PLP, 4PA, or UB6. PLP and PB6 increased significantly from pre to post and decreased significantly from post to p60 for all diets. The magnitude of the change of PB6 and PLP from pre to post and post to p60 was much greater with the S diets than the NS diets. 4PA excretion increased significantly ($p<.0001$) from the day before exercise to day of exercise for all diets (change in 4PA = 0.50 [NS]; 1.99 [S] μmoles/24 hr). Urinary vitamin B-6 excretion showed no change. While there was a significant change in vitamin B-6 metabolism with exercise, neither training, increased CHO or age had an effect on this change.

INTRODUCTION

The increased interest in the prevention of disease, improving the quality of life, and increasing ones' overall well-being has stimulated an increased interest in the interrelationship between fitness and nutrition. The role nutrition plays in the improvement of physical ability and the nutritional needs of individuals involved in physical activity is still a relatively unexplored area of nutrition research. This is especially true in the area of micronutrient metabolism and needs for those involved in physical activity.

When the body is involved in physical exertion, certain metabolic processes occur to assure adequate energy is provided to the exercising muscles of the body. Two of these processes include the breakdown of muscle and liver glycogen to glucose and the deamination of amino acids, making the carbon skeletons available for gluconeogenesis. Both of these gluconeogenic processes involve vitamin B-6 dependent reactions. Pyridoxal 5'-phosphate, the active form of vitamin B-6, is essential to glycogenolysis, since it is an integral part of the glycogen phosphorylase enzyme (Krebs and Fisher, 1964). This enzyme cleaves glucose-1-phosphate from glycogen, thus providing glucose for energy production. Pyridoxal 5'-phosphate is also a cofactor for the amino-transferase enzymes which degrade amino acids.

For many years it has been assumed that vitamin B-6, a water soluble vitamin, was not stored to any great extent in the body. Recently, Black and co-workers (1977) have reported an increase in glycogen phosphyorylase in the muscles of rats supplemented with vitamin B-6. They also noted that this stored form of the vitamin was not made

available to the body during times of dietary vitamin B-6 deficiency unless a caloric deficit also existed (Black et al., 1978). In examining this theory in man, Leklem and Shultz (1983) and others (Hatcher et al., 1982; Munoz, 1983) observed an increase in plasma pyridoxal 5'-phosphate in men, after various types of strenuous exercise. Leklem and Shultz have hypothesized that exercise simulates the release of pyridoxal 5'-phosphate from the muscle glycogen phosphorylase enzyme to facilitate gluconeogenesis in other parts of the body.

Numerous investigators have reported that dietary vitamin B-6 intake in adult women is low to marginal (Chrisley and Driskell, 1979; Guthrie and Crocetti, 1983; Vir and Love, 1980). The effect that exercise and various dietary regimes may have on the metabolism of this nutrient has not been examined in women to determine if altered B-6 metabolism may affect vitamin B-6 status. Therefore, our research project was designed to examine the effect of exercise on vitamin B-6 metabolism in women and to determine if age, level of physical fitness, carbohydrate level of the diet, and vitamin B-6 supplementation would alter this metabolism. The vitamin B-6 status of these subjects is being examined in another research paper (Lee, 1985).

METHODS

Experimental Design

This seven week metabolic study was designed to include four dietary treatments, each of which was followed by an exercise testing period (Figure 3.1). Weeks 1 and 2 consisted of a moderate carbohydrate (MCHO) diet followed by a 1 week high carbohydrate (HCHO) diet. Subjects were then returned to the MCHO diet for 1 week before administration of a MCHO + vitamin B-6 diet (MCHO+B6) for 2 weeks and a HCHO + vitamin B-6 (HCHO+B6) for 1 week. The first week of the MCHO diet was designed to normalize all subjects to the same dietary level of vitamin B-6 and allow subjects time to adapt to the diet and routine of the study. The administration of the MCHO diet for one week after the HCHO diet was designed to assure no confounding overlap of the dietary treatments and to have the premenopausal subjects at the same relative point in their menstrual cycle during the last two exercise testing sessions as experienced in the first two exercise sessions.

Subjects

Fifteen healthy female volunteers were recruited for this study. They were divided into three groups, each with an N=5; young/trained, young/untrained, and postmenopausal/untrained according to age and aerobic training. Physical characteristics of each of the groups are given in Table 3.1. Untrained was defined as performing a maximum of 40 minutes of aerobic activity during a one week period with exercise occurring less than two days a week. Trained was defined as performing a minimum of 120 minutes of aerobic activity during a one

week period with exercise occurring a minimum of three days a week. Actual level of training was determined prior to the beginning of the study by measuring $\dot{V}O_2$ max using the Wilmore and Costill (1974) protocol. Subjects had to have maintained their classified level of fitness (trained or untrained) for at least six months prior to beginning the study. They were also required to maintain this level of fitness throughout the experimental period. To monitor this, subjects were required to report all aerobic exercise and the duration and intensity of that exercise in a daily journal. Subjects were also required to provide researchers with the following information prior to beginning the study: completed health history questionnaire in which no abnormal health problems were recorded, complete a three day dietary record and an exercise history questionnaire. The following criteria were also required before participation began: normal blood chemistry screen, normal cardiac function as determined by electrocardiograph tracings at rest and maximal heart rate, normal xylose absorption test (Harris, 1969) to assess carbohydrate absorption (means ± S.D. = 31.6±8.19%), doctor's approval for participation in the study, blood sample for plasma vitamin B-6 and pyridoxal 5'-phosphate assessment, blood pressure within normal limits at rest and during exercise, no vitamin supplements used for at least four weeks prior to beginning study, no nicotine, oral contraceptives or estrogen replacement therapy used. Subjects were familiarized with the experimental procedure and signed informed consent forms. This study was approved by the Oregon State University Committee for Protection of Human Subjects.

Dietary Treatments

The dietary procedure for this study consisted of four dietary treatments, two of which were supplemented with vitamin B-6 and two which were not supplemented (Figure 3.1). The specific foods and amounts served for each meal and each diet are given in Table 3.2. The initial 2000 kcal moderate carbohydrate (MCHO) diet consisted of 49% carbohydrate, 35% fat, and 16% protein. The initial 2000 kcal high carbohydrate (HCHO) diet consisted of 64% carbohydrate, 22% fat, and 16% protein. The vitamin B-6 level of the unsupplemented diets were maintained at 2.3 mg for the MCHO and 2.4 mg for the HCHO diet. The supplemented diets repeated the MCHO and HCHO dietary treatments but included an additional 8.0 mg of pyridoxine (vitamin B-6) given as an oral solution of pyridoxine hydrochloride at the breakfast meal. The level of supplementation was designed to provide excess vitamin B-6 without introducing a pharmacological effect. This was also a level used in a previous study (Hatcher et al., 1982).

All food served in the metabolic study was purchased in bulk from the same lot when possible. Foods which could not be purchased this way were thoroughly mixed prior to beginning the study and frozen in aliquots for daily use. This was done to assure uniformity of the nutrient content of the diets. Each food item fed was carefully weighed before being served to each subject. Weekly food composites were made and analyzed for vitamin B-6. This was done to assess the actual amount of the vitamin being consumed by each subject and to determine if storage and food preparation were altering the vitamin content of the food.

11 subjects were placed on a 1800-2000 kcal level at the beginning of the study. Each person was weighed daily and calorie adjusted as necessary to assure that weight was maintained throughout the study. Additional calories were added in the form of carbohydrate of fat containing foods so as to keep the carbohydrate/fat ratio the same on the MCHO and HCHO diets. These foods contained essentially no vitamin B-6. All meals were prepared in the metabolic kitchen. No alcoholic beverages were allowed throughout the study and no caffeinated beverages were allowed the day before and the day of the exercise testing.

Exercise Procedure

Prior to beginning the metabolic diet study each subject was put through a graded exercise test on a Monark cycle ergometer to assess maximum heart rate and $\dot{V}O_2$ max. The subject's heart rate and cardiac cycles were monitored continually during the test using an electrocardiograph. Blood pressures were monitored before and after exercise. Rules for termination of exercise tests as outlined by the American College of Sports Medicine (1980) were followed.

Using the data obtained from the $\dot{V}O_2$ max stress test, subjects were exercised on a Monark cycle ergometer at 80% of the $\dot{V}O_2$ max for 20 minutes during each of the exercise testing sessions (Figure 3.1). This exercise protocol is one recommended by the American College of Sports Medicine (1980) as the average conditioning intensity and duration for asymptomatic adults.

The specific exercise procedure for each exercise session included five minutes of warm-up followed by 20 minutes of exercise at 80% $\dot{V}O_2$ max and a 5-10 minute cool-down period (Figure 3.2). The experimental design was such that each subject was exercised on the 5th or 6th day of the week of each dietary treatment. This was done to allow 5 or 6 days to adapt to the diet before each exercise testing session. It also allowed time for a 24-hour post-exercise urine collection to reflect any changes in urinary excretion due to the diet and exercise combination. Subjects were monitored on an electrocardiograph before, during, and after the exercise period. Unusual irregularities in heart function or in the general well-being of the subject would have caused exercise to be terminated. Blood pressures were also taken pre, during, and post exercise, see Figure 3.2. All exercise testing was done fasting, prior to eating breakfast. Subjects were not allowed to take in fluids until after the last blood sample was drawn. Body weight was measured before and immediately after exercise. All exercise was performed at a moderate temperature of 20-24°C and at a mean relative humidity of 48% (range = 42-64%).

Daily Procedures

All subjects were asked to follow a number of daily procedures to assure compliance with the metabolic study, assist in monitoring subjects well-being and assure that accurate records were kept. These daily procedures included keeping a journal of activities (minutes exercised, non-caloric beverages consumed, medications taken,

menstrual periods); recording weight; returning unused portions of margarine, salad dressing, or honey used to increase calories and accurately collecting each day's urine.

Blood and Urine Collection

Blood was drawn during each of the four exercise testing sessions. The first sample was drawn prior to beginning exercise, after the subject had rested for 10-15 minutes. The second sample was drawn two minutes after the termination of the 20 minute exercise period. The third and fourth samples were drawn 30 and 60 minutes, respectively, after the post exercise draw. The subjects were asked to sit or rest quietly during the 60 minute period following exercise. All blood samples were collected in heparinized tubes, kept on ice until centrifuged (no more than 30 minutes) and plasma was removed and then stored at -40°C.

Throughout the metabolic study, subjects were required to collect 24-hour urine samples. Portions of each day's 24-hour urine collection were frozen for subsequent analysis. Those urines collected the day before and the day of exercise were analyzed to assess the effect of the exercise treatment.

Analyses

Plasma and urinary B-6 were analyzed using a microbiological method of Miller and Edwards (1981). Interassay variation of a plasma B-6 control sample (n=14) was 6.1%, and that of urinary B-6 (n=18) was 3.0%.

Plasma pyridoxal 5'-phosphate was determined by a modified method of Chabner and Livingston (1970). Interassay variation of a pyridoxal 5'-phosphate control sample (n=22) was 6.5%. Urinary 4-pyridoxic acid was analyzed by a fluorometric procedure described by Reddy et al. (1958). Interassay variation of a control sample (n=35) was 8.5%. Hemoglobin determination and hematocrits were done on all samples and hematoicrit values used to calculate changes in plasma volume as described by van Beaumont (1972). Vitamin B-6 content of the diet was assayed weekly using a standard microbiologic procedure (ADAC, 1980). Xylose absorption was assessed by the method of Harris (1969). Creatinine was determined (Pino et al., 1965) and used to assess completeness of 24-hour urine collections. Plasma albumin was also determined (Slater et al., 1975).

Statistical Analysis

A three-way split plot partially blocked ANOVA design with groups and diets as whole plot factors and time (of the blood sampling or the urine collection) the split plot factor was used in the analysis of the data. When significant interactions occurred main effects were no longer of interest. The significant interactive effects were examined by performing hypothesis testing on several contrasts of treatment means where contrasts is defined as a comparison involving two or more treatment means (Neter and Wasserman, 1974). Contrasts to be tested were specified in advance of data collection. Least significant difference was the test criterion used in testing multiple comparisons (Steel and Torrie, 1980).

For the purpose of hypothesis testing "a" designates group, "b" designates diet and "c" represents time. These letters are subscripted to indicate the specific group (1=young/trained; 2=young/untrained; 3=postmenopausal/untrained), diet (1=MCHO; 2=HCHO; 3=MCHO+B-6; 4= HCHO+B-6) and time (1=pre; 2=post; 3=post30; 4=post60 for plasma samples). For example, $a_1b_1c_1$ designates the mean of the five subjects in group 1, on diet 1, at time 1. When groups were combined (n=15) "a" was omitted. The specific hypotheses tested in this study are as follows: $[b_1c_1-b_1c_2]$; $[b_1c_2-b_1c_4]$; $[b_1c_2-b_1c_1]-[b_2c_2-b_2c_1]$; $[b_1c_2-b_1c_4]-[b_2c_2-b_2c_4]$. These hypotheses were tested for all diets Results were determined to be significant if the p-value was less than the critical value of $\alpha = .05$.

RESULTS

At the beginning of the metabolic diet the mean plasma pyridoxal 5'-phosphate levels for each group were 42.3±14.1 nM for the young/trained, 35.4±14.8 nM for the young/untrained and 29.6±12.4 nM for the postmenopausal/untrained women. After being fed the metabolic diet (MCHO, 2.3 mg B6) for 10 days the mean plasma values were 47.1±9.0 nM for the young/trained, 48.0±18.2 nM for the young/untrained and 47.2± 15.5 nM for the postmenopausal/untrained. At the end of the HCHO diet (2.4 mg B6), after being in the metabolic study for 19 days, the mean plasma pyridoxal 5'-phosphate values were similar to those seen after 10 days in the study. The mean plasma pyridoxal 5'-phosphate values for the HCHO diet were 48.0±8.8 nM for the young/trained, 61.2±24.2 nM for the young/untrained, and 44.0±17.8 nM for the post-menopausal/untrained.

The pyridoxal 5'-phosphate and plasma vitamin B-6 means ± S.D. for each group, diet, and time are given in Table 3.4. In the analysis of pyridoxal 5'-phosphate and plasma vitamin B-6, time represents the four blood draws taken pre, post, post-30 and post-60 of minutes exercise. The results of the ANOVA (Table 3.3) for plasma vitamin B-6 and pyridoxal 5'-phosphate showed no significant main effects or any interactive effects involving the groups. Therefore, in analyzing these response variables, we could average across groups and use the combined n=15. The results showed a significant diet x time interaction ($p<0.0001$) for both plasma vitamin B-6 and pyridoxal 5'-phosphate. Contrasts of means and differences in means resulted in a significant increase from pre to post and a significant decrease from post to

post-60 for all diets. However, the magnitude of the increase from pre to post was significantly greater with the vitamin B-6 supplemented diets than with the non-supplemented diets. The same was true of the decrease from post to post-60 (Figure 3.3 and 3.4). When subjects were fed the non-supplemented diets, the magnitude of change from pre to post for pyridoxal 5'-phosphate was 8 nM for the MCHO diet and 9 nM for the HCHO diet. When these diets were supplemented, the magnitude of the increase from pre to post was 14 nM for the MCHO+B6 and 21 nM for the HCHO+B6. The magnitude of the decrease was also greater from post to post-60 minutes for the vitamin B-6 supplemented diets. With the non-supplemented diets there was a decrease of 11 nM for the MCHO and 10 nM for the HCHO diet while with the supplemented diets there was a decrease of 27 nM for the MCHO+B6 diet and 35 nM for the HCHO+B6 diet. Changes of similar magnitude were seen in the plasma vitamin B-6 values.

In examining the fasting values (pre) for plasma vitamin B-6 with the non-supplemented diets there was no significant difference between the two diets (68.7 nM, MCHO; 70.6 nM, HCHO). The mean fasting value seen for the MCHO+B6 diet (268 nM) was somewhat higher than the HCHO+B6 diet (259 nM), although the difference was not significant. For pyridoxal 5'-phsophate the mean fasting value (pre) for the two non-supplemented diets were similar (50.8 nM, MCHO; 51.1 nM, HCHO). When the diets were supplemented with 8 mg of pyridoxine the fasting values rose three fold. For the supplemented diets, the MCHO+B6 diet had a higher fasting value (200 nM) thn the HCHO+B6 diet (186 nM), but again the difference was not significant. Pyridoxal 5'-phsophate made up

approximately 73% of the total plasma vitamin B-6, ranging from 72-77% on the non-supplemented diets and 70-75% on the supplemented diets. This percentage remained fairly constant regardless of the time period examined.

The fasting (pre) values for pyridoxal 5'-phosphate (nM) in each of the three groups (young/trained, young/untrained, postmenopausal/ untrained) on the four different diets (MCHO, HCHO, MCHO+B-6, HCHO+B-6) were as follows: young/trained (47.1±9.0, 48.0±8.8, 204±19, 175±24), young/trained (58.0±18.2, 61.2±24.2, 210±54, 203±56) and postmenopausal/untrained (47.2±15.2, 44.0±17.8, 187±25, 181±41), respectively. Although the pre values were not significantly different for the three groups the young trained and postmenopausal/ untrained women had values which more closely resembled each other on the two non-supplemented diets than the young, untrained group. It should be noted here that the fasting values represent only one day and are not a mean of several days.

The results of the ANOVA indicated no significant effect of group and no interactive effects between the factors for 4-pyridoxic acid. Significant main effects for 4-pyridoxic acid were time ($p<0.0005$) and diet ($p<0.0001$), (Table 3.4). There were only two time periods in the analysis for 4-pyridoxic acid, the day before exercise and the day of exercise. The day of exercise was the 24-hour urine collection on the day in which the exercise test occurred. The significant time effect indicates that there was a significant increase in 4-pyridoxic acid excretion from pre exercise to the day of exercise on all the

diets. The increase from the day before exercise to the day of exercise in μmoles/day was 0.42 on the MCHO diet, 0.59 on the HCHO diet, 1.77 on the MCHO+B6 diet and 2.21 on the HCHO+B6 diet (Figure 3.5). Therefore, a 0.50 μmole/24 hour change was seen with the non-supplemented diets whereas a 1.99 μmole/24 hour change was seen with the supplemented diets. For urinary vitamin B-6 no significant interactions were found and diet was the only significant main effect ($p<0.0001$). While the excretion of vitamin B-6 on the day of exercise was slightly greater than the day prior to exercise, this difference was not significantly different for any of the diets. Means ± S.D. are given in Table 3.5 for urinary 4-pyridoxic acid and vitamin B-6 excretion.

To assess whether the increased 4-pyridoxic acid excretion seen the day of the experimental sessions was also reflected in the young trained consistently excreting more 4-pyridoxic acid throughout the metabolic study as compared to the other non-exercising groups, urinary 4-pyridoxic acid excretions across the four diets were examined. The mean 4-pyridoxic acid excretion for the two non-supplemented and the two supplemented diets were determined for each individual. These means represented the average of 5 (for the untrained) and 6 (for the trained) days of 4-pyridoxic acid excretion for each diet. In neither the young/untrained nor the postmenopausal/untrained groups were the 4-pyridoxic acid values for the days of the experimental exercise session used, therefore only 5 days of each week were averaged. Another ANOVA was then done on the mean 4-pyridoxic acid

values of the non-supplemented and supplemented dietary treatments. The mean values for each group and diet are given in Table 3.6. The results of the ANOVA indicated no significant differences between the three groups in 4-pyridoxic acid excretion throughout the entire metabolic study ($F=2.8596$; $DF=2.12$; $p=.0965$). However, the young/trained individuals consistently had higher 4-pyridoxic acid excretions than the two untrained groups for both the non-supplemented and supplemented diets. As expected, supplementation produced higher values for all the vitamin B-6 parameters measured in this study.

Plasma volume changes were calculated using the van Beaumont (1972) method to assess if changes seen in plasma pyridoxal 5'-phosphate with exercise were due to hemoconcentration alone. The mean plasma volume changes seen in the four exercise sessions ranged from -7 to -11%. Using -11%, the maximal change seen in plasma volume, one would expect an approximate 7% increase in plasma pyridoxal 5'-phosphate due to plasma concentration alone. However, the mean increase in plasma pyridoxal 5'-phosphate in all the exercise sessions ranged from 2-17% above what would be expected due to hemoconcentration. Also, when plasma volumes returned to their pre exercise level by the post-60 blood draw, the plasma pyridoxal 5'-phosphate values continue to fall in the plasma and were as much as 23% below the pre values at this point. Albumin changes were also calculated and the percent increase in albumin seen with exercise was similar to the expected percent increase one would calculate using the van Beaumont method based on hematocrit. Therefore, the changes we observed in plasma pyridoxal

5'-phsophate and vitamin B-6 during exercise are due to other factors besides that related to hemoconcentration. Body weight measurements done before and immediately after exercise indicated that weight loss ranged between 0-2.2 kg during the exercise sessions, with a mean loss of .62 kg.

The mean body weight for the young/trained, young/untrained, and postmenopausal/untrained at the beginning of the diet study was 56.8±5.7, 56.7±6.7, and 59.86 kg, respectively. The mean body weight for the young/trained, young/untrained, and postmenopausal/untrained at the end of the 49 day study was 55.3±5.4, 55.6±5.7, and 59.4±4.9 kg, respectively.

DISCUSSION

The results of this study indicate that exercise produced a significant rise in plasma levels of vitamin B-6 and pyridoxal 5'-phosphate in women. This increase was followed by a significant decrease from post-exercise to post-60 minutes of exercise. Furthermore, the supplemented diets contributed to a greater magnitude in the rise and fall of these vitamin B-6 parameters during exercise than the non-supplemented diets. This increase in plasma pyridoxal 5'-phosphate with exercise is similar to what has been reported in men during and after strenuous exercise (Hatcher et al., 1982; Leklem and Shultz, 1983; Munoz, 1980). It has been reported that pyridoxal 5'-phosphate is stored in the glycogen phosphorylase of muscle by Black et al. (1977, 1979). However, they have also reported that the glycogen phosphorylase did not give up its stored vitamin B-6 unless starvation or a caloric deficit was induced. Leklem and Shultz (1983) hypothesized that exercise mimics starvation, thus stimulating the release of pyridoxal 5'-phosphate from the glycogen phosphorylase enzyme. Therefore, the increase seen in plasma pyridoxal 5'-phosphate with exercise may be due to the release of pyridoxal 5'-phosphate from the muscle reservoir. The significance of the release of pyridoxal 5'-phosphate from the glycogen phosphorylase enzyme during exercise is probably related to the role this vitamin plays in gluconeogenesis. During exercise the body depends on liver production of glucose to maintain blood glucose levels (Ahlborg and Felig, 1982; Lemon and Nagle, 1981). The liver can produce glucose through glycogenolysis

of its stored glycogen (a vitamin B-6 dependent process) or through gluconeogenesis in which the alanine-glucose cycle contributes to glucose production (a vitamin B-6 dependent transamination process). Vitamin B-6 deficiency in animals has been shown to decrease gluconeogenesis due to decreased transamination (Angle, 1980). Others have shown transaminase enzymes to increase with vitamin B-6 supplementation (Black et al., 1978), but show no change with training (Chen and Marlatt, 1978).

In the present study, the changes observed in pyridoxal 5'-phosphate and vitamin B-6 were 2-17% greater than what would be expected due to a decrease in plasma volume resulting from hemoconcentration of the plasma. Also, when plasma volumes returned to their pre-exercise level by the post-60 blood draw, the plasma pyridoxal 5'-phosphate values continued to fall, indicating that something other than plasma concentration was contributing to the change.

We also compared trained vs. untrained subjects relative to their vitamin B-6 metabolism during exercise and found that all three groups responded similarly, regardless of training level. Training increases the body's ability to store muscle glycogen as well as the ability to utilize FFA more effectively during aerobic activity, sparing the muscle glycogen (Costill and Miller, 1980; Sherman and Costill, 1984). We were interested in investigating whether increased muscle glycogen storage (in the trained individuals) would alter the way in which muscle glycogen phosphorylase, the proposed reservoir of vitamin B-6 storage, would respond during exercise. However, no difference between the groups was demonstrated and the changes seen in vitamin

B-6 metabolism depended on the amount of vitamin B-6 in the diet, not the level of training. It should be noted that the trained individuals used in this study were not highly trained athletes, but individuals who participated in a regular aerobic fitness program. Therefore, changes seen in highly trained athletes may vary from what was observed in this study. Leklem and Shultz (1983) observed mean increases in pyridoxal 5'-phosphate of 12.8 nM from pre to post exercise in male adolescents involved in a cross-country training program. However, at the beginning of the training period the nM change seen with exercise (a 4500 meter run) was 7.6, while at the end of the training period the nM change was 18.3. Because dietary intakes were not monitored, it is difficult to determine if the increased change observed at the end of the training program was due to diet or increased fitness.

Because plasma pyridoxal 5'-phosphate is reported to decrease with age (Rose et al., 1974), we examined the magnitude of the increase from pre-exercise to post-exercise in each group and diet to determine if the postmenopausal/untrained group produced the same response during exercise as the two younger groups. It was observed that regardless of the pre (resting) pyridoxal 5'-phosphate value, plasma pyridoxal 5'-phosphate levels increased during exercise in all groups approximately the same nM amount, with greater increases being observed with the supplemented diets. Therefore, supplementation seemed to contribute to more vitamin B-6 storage in the muscle and its subsequent release during exercise. This supports the theory of Black et al. (1977, 1978) which suggests that the muscle glycogen

phosphorylase is a storage reservoir for vitamin B-6. The greater magnitude of change seen in pyridoxal 5'-phosphate from pre to post with the HCHO+B-6 diet as compared to the MCHO+B-6 diet may be due to the fact that the subjects had been on the supplemented diet a week longer. An additional week of supplementation may have contributed to a greater amount of vitamin B-6 being stored in the muscle. Therefore, if more vitamin B-6 had been stored, a greater amount may be available for release during exercise.

The increase in pyridoxal 5'-phosphate in the plasma during exercise has been reported to be related to altered fuel metabolism in men (deVos et al., 1982). It was hypothesized that a high carbohydrate diet would increase the muscle glycogen and that vitamin B-6 supplementation would increase the glycogen phosphorylase enzyme. Increased concentration of this enzyme due to vitamin B-6 supplementation may initiate increased glycogen breakdown. This may in turn increase the muscle's utilization of glucose for energy and decrease the reliance on FFA for energy during exercise. deVos et al. (1982) observed that male cyclists on a high carbohydrate and vitamin B-6 supplemented diet produced higher plasma lactate levels during exercise than when fed a high, moderate or low carbohydrate diet without vitamin B-6 supplementation. This increase in lactate may indicate that the muscles are breaking down muscle glycogen more quickly with supplementation of vitamin B-6.

In this study, the level of carbohydrate in the diet did not alter vitamin B-6 metabolism during exercise in the three groups. It has been observed by Leklem et al. (1982, 1984) that a glucose

load will produce a decrease in plasma pyridoxal 5'-phosphate levels. They hypothesized that a glucose load would produce a response opposite to that seen in starvation or exercise (when energy needs are in demand) and would stimulate the removal of pyridoxal 5'-phosphate from the plasma for storage in the muscle phosphorylase. With the MCHO and HCHO diets the pre values for pyridoxal 5'-phosphate were very similar. However, when supplementation was introduced the HCHO+B-6 diet showed a slightly lower plasma pyridoxal 5'-phosphate response. The decrease in pyridoxal 5'-phosphate from post-exercise to post-60 minutes of exercise was similar to the decrease observed by Hatcher et al. (1982) in male cyclists after exercise. After exercise has ceased, the uptake of pyridoxal 5'-phosphate back into the muscle or continued uptake by the liver could account for the dramatic decrease in plasma pyridoxal 5'-phosphate during the recovery period.

Urinary 4-pyridoxic acid and urinary vitamin B-6 were examined to determine whether the sudden increase of plasma vitamin B-6 and pyridoxal 5'-phosphate during exercise and their subsequent decrease after exercise was followed by an increased urinary loss of vitamin B-6 metabolites. Urinary vitamin B-6 excretion showed no change with exercise. However, urinary 4-pyridoxic acid showed an increased excretion with exercise regardless of the diet consumed. The excretion increased 5-8% from the day before exercise to the day of exercise. This would indicate that exercise is contributing to an increased metabolism of the vitamin to its primary urinary metabolite. Urinary 4-pyridoxic acid was measured on some subjects the day after

exercise. In examining this data, it was observed that the increased excretion seen with exercise was often followed by a day of lower excretion. At times the 4-pyridoxic acid excretion the day after exercise fell below the pre-exercise level, indicating that the body may be conserving the vitamin after the increased excretion related to the exercise. The trained group exercised regularly through the metabolic study to maintain their fitness level. This frequency of exercise may have meant that they were consistently excreting higher amounts of 4-pyridoxic acid than their non-exercising counterparts. In examining the mean 4-pyridoxic excretion over the entire metabolic study, the young trained group was not significantly different from the untrained groups (Table 3.6). However, they did consistently excrete higher levels of the metabolite than either of the untrained groups, regardless of the level of vitamin B-6 in the diet. Therefore, it appears that the young trained individuals may be losing more vitamin B-6 in urinary excretion. Whether this would compromise their vitamin B-6 status over a long period of time is doubtful, unless dietary intake was consistently low or other confounding problems occurred. Borisov (1977) has reported increased 4-pyridoxic acid excretion in students studying and training at a sports institute. Hatcher et al. (1982) have reported increased 4-pyridoxic acid excretion in trained cyclists on exercise days as compared to non-exercise days.

It should be emphasized that the changes seen in vitamin B-6 metabolism with exercise were observed while all subjects were on a

controlled metabolic diet. Twenty-four hour urines were also collected daily. All subjects received 2.3 to 2.4 mg of vitamin B-6 on the non-supplemented diets, an amount slightly higher than the RDA.

Current literature on the effect of exercise on nutrient metabolism in women is limited, especially in the elderly female population. This research focused on women, who may be at greater risk of being vitamin B-6 deficient, to see if exercise alters their metabolism and ultimate need for vitamin B-6. From our results it cannot be concluded that female athletes or other individuals currently involved in a cardiovascular/respiratory fitness program need more vitamin B-6 than that recommended by the Recommended Dietary Allowances (1980). However, research has not been done on high endurance athletes who spend long hours training each day for such grueling events as triathalons, marathons, or ultramarathons and may be on marginal vitamin B-6 intake. Also, examination of the effect supplementation has on fuel metabolism should be carefully considered before athletes or those involved in endurance fitness programs supplement with high amounts of vitamin B-6. Vitamin B-6 may alter availability of FFA and glucose to and in the functioning muscle, subsequently altering performance.

In conclusion, this study indicated that neither level of physical training, age, nor level of carbohydrate seemed to significantly affect vitamin B-6 metabolism during exercise in women. All groups demonstrated the same increase in plasma vitamin B-6 parameters with exercise regardless of the diet.

Table 3.1. Physical statistics for young/trained, young/untrained, and postmenopausal/untrained groups.

	Means ± S.D.	Range
Young/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 (%)	20.1±4.2 (1)	13-24
$\dot{V}O_2$ max (ml/kg/min)	40.5±3.7 (2)	37.1-46.5
Young/untrained (n=5)		
Age (yr)	24.4±3.2	21-29
Height (cm)	161.8±6.3	153.8-170.00
Weight (kg)	55.9±6.9	48.2-65.0
Body Fat (%)	20.4±1.5	18-22
$\dot{V}O_2$ max (ml/kg/min)	32.7±4.5	25.9-37.0
Postmenopausal/untrained (n=5)		
Age (yr)	55.8±4.8	49-61
Height (cm)	155.8±4.5	150.8-160.8
Weight (kg)	58.8±6.0	51.8-68.2
Body Fat (%)	27.0±3.4	22.31
$\dot{V}O_2$ max (ml/kg/min)	24.4±1.6	23.9-27.6

(1) Percent body fat determined by skinfolds measured at seven sites (Jackson et al., 1980).

(2) $\dot{V}O_2$ max determined by graded exercise testing using the Wilmore and Costill (1974) procedure on a cycle ergometer.

Table 3.2. 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*

*Variable amounts used to adjust calories. Amounts listed are average amounts used.

Table 3.3. Significant* F-test values from 3-way ANOVA.

Variable (1)	Factor (2)	DF	F-value	P-value*
PB6	D	3, 36	374.0331	.0001
	T	3, 108	85.3587	.0001
	D x T	9, 108	7.9452	.0001
PLP	D	3, 36	398.5720	.0001
	T	3, 108	93.0836	.0001
	D x T	9, 108	7.9822	.0001
4PA	D	3, 36	2235.6088	.0001
	T	1, 36	14.5205	.0005
UB6	D	3, 36	277.0676	.0001

* only those interactions or main effects with p values of <.05 are reported

(1) PB6 = plasma vitamin B-6; PLP = pyridoxal 5'-phosphate; 4PA = 4-pyridoxic acid; UB6 = urinary vitamin B-6.

(2) T = time (pre, post, post30, post60 for PB6 and PLP); time = (day before exercise, day of exercise for UB6 and 4PA).

G = group (young/trained; young/untrained; postmenopausal/ untrained).

D = diet (MCHO, HCHO, MCHO+B-6, HCHO+B-6).

Table 3.4. Pyridoxal 5'-phosphate levels over time with groups combined (n=15) for the four different dietary treatments.

	TIME[1]			
DIET[2]	pre	post	post30	post60
	nM			
MCHO	50.8±14.7[3]	58.8±18.2	50.0±14.9	47.9±13.9
HCHO	51.1±18.4	60.2±22.5	51.6±17.6	50.0±18.5
MCHO+B-6	200±35.1	214±36.7	194±36.8	187±31.0
HCHO+B-6	186±41.5	207±43.9	181±35.3	172±37.4

Plasma vitamin B-6 levels over time with groups combined (n=15) for the four different dietary treatments.

	TIME			
	pre	post	post30	post60
DIET		nM		
MCHO	68.7±18.1	77.2±21.6	68.2±16.9	63.7±16.7
HCHO	70.6±19.8	78.8±24.6	68.8±18.9	68.0±20.2
MCHO+B-6	268±48.6	291±55.3	276±47.6	256±46.8
HCHO+B-6	259±48.6	287±52.0	269±49.6	250±51.1

(1) Pre = resting, post = at termination of exercise, post30 = 30 minutes after exercise was terminated, post60 = 60 minutes after exercise was terminated.

(2) MCHO = 49% carbohydrate, 35% fat, 16% protein; HCHO = 64% carbohydrate, 22% fat, 16% protein; MCHO+B-6 = MCHO diet plus 8 mg vitamin B-6 added; HCHO+B-6 = HCHO diet plus 8 mg vitamin B-6 added.

(3) Mean ± S.D.

Table 3.5. 4-pyridoxic acid levels the day before and the day of exercise with groups combined, n=15.

	TIME	
	Day Before Exercise[1]	Day of Exercise
	µmoles/24 hours	
DIET[2]		
MCHO	7.10 ± .77[3]	7.52 ± .73
HCHO	7.04 ± .66	7.64 ± .73
MCHO+B-6	37.7 ± 2.24	39.5 ± 2.95
HCHO+B-6	37.1 ± 4.14	39.3 ± 3.00

Urinary vitamin B-6 levels the day before exercise and the day of exercise with groups combined, n=15.

	TIME	
	Day Before Exercise	Day of Exercise
	µmoles/24 hours	
DIET		
MCHO	0.93 ± .31	1.00 ± .24
HCHO	1.010 ± .23	1.02 ± .32
MCHO+B-6	5.98 ± 1.4	6.20 ± 1.54
HCHO+B-6	6.05 ± 1.3	6.37 ± 1.49

(1) Indicates the 24-hour period in which exercise occurred.

(2) Abbreviations as in Table 3.4.

(3) Mean ± S.D.

Table 3.6. Mean 4-pyridoxic acid excretion of groups fed the supplemented and non-supplemented diets.

	μmoles/24 hours
Young/Trained (1)	
non-supplemented	7.36 ± .52
supplemented	36.50 ± .90
Young/Untrained (2)	
non-supplemented	6.56 ± .71
supplemented	32.64 ± .51
Postmenopausal/Untrained (2)	
non-supplemented	7.11 ± .65
supplemented	34.58 ± 4.17

(1) Values represent the mean of 12 days (including the 2 days of exercise testing).

(2) Values represent the mean of 10 days (does not include the 2 days of exercise testing).

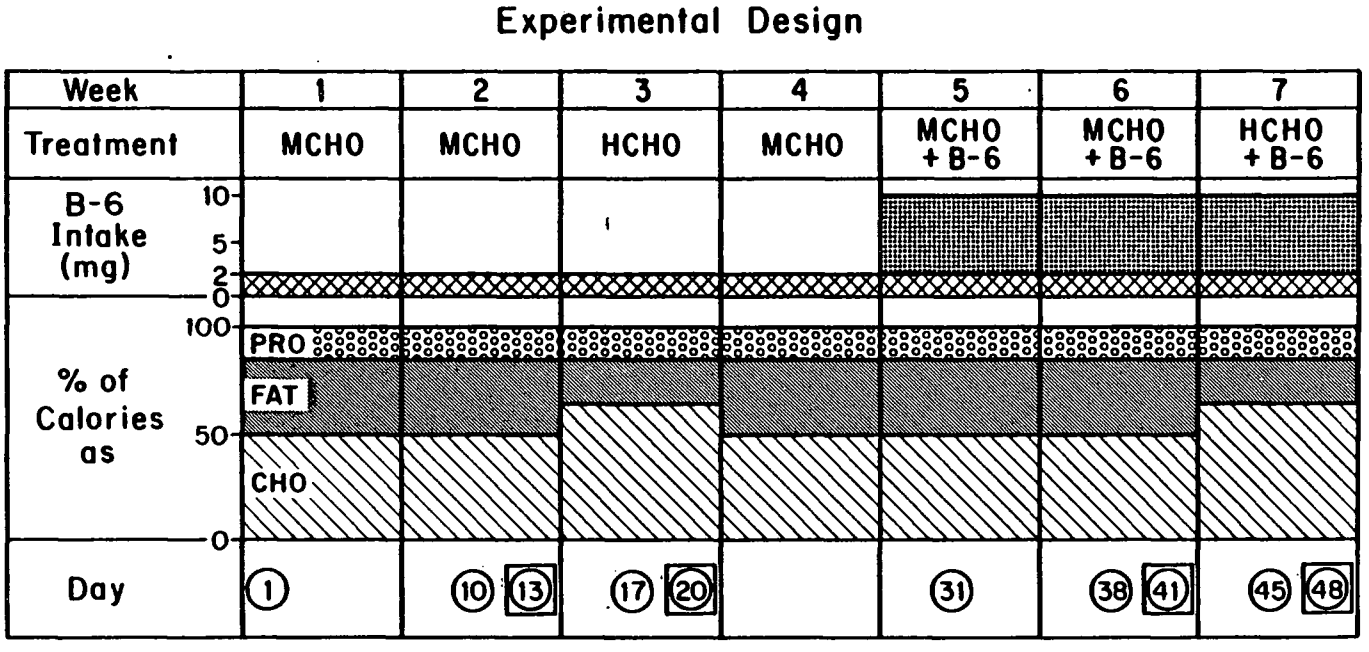

Figure 3.1. Experimental Design. MCHO = moderate carbohydrate diet (49%) (2.3 mg B-6); HCHO = high carbohydrate diet (63%) (2.4 mg B-6); +B-6 = supplementation of 8 mg of pyridoxine hydrochloride added; day = day during metabolic study on which exercise testing occurred as well as blood and urine analysis; ☐ = represents exercise testing; = represents days blood and urine analysis done.

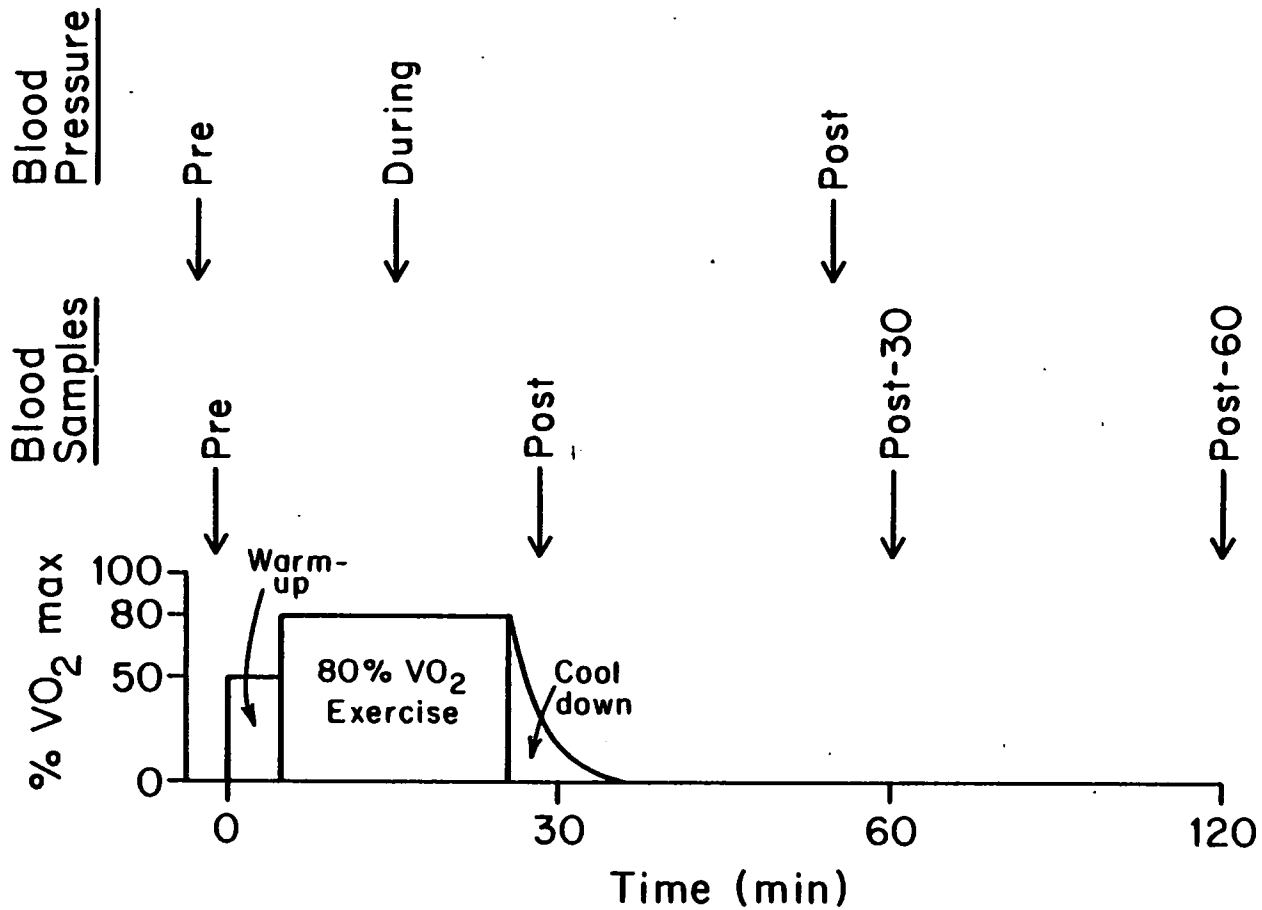

Figure 3.2. Exercise procedure. Exercise consisted of 5 minutes of warm-up followed by 20 minutes at 80% $\dot{V}O_2$ max followed by a 5-10 minute cool down period. Times for blood draws and blood pressures are marked.

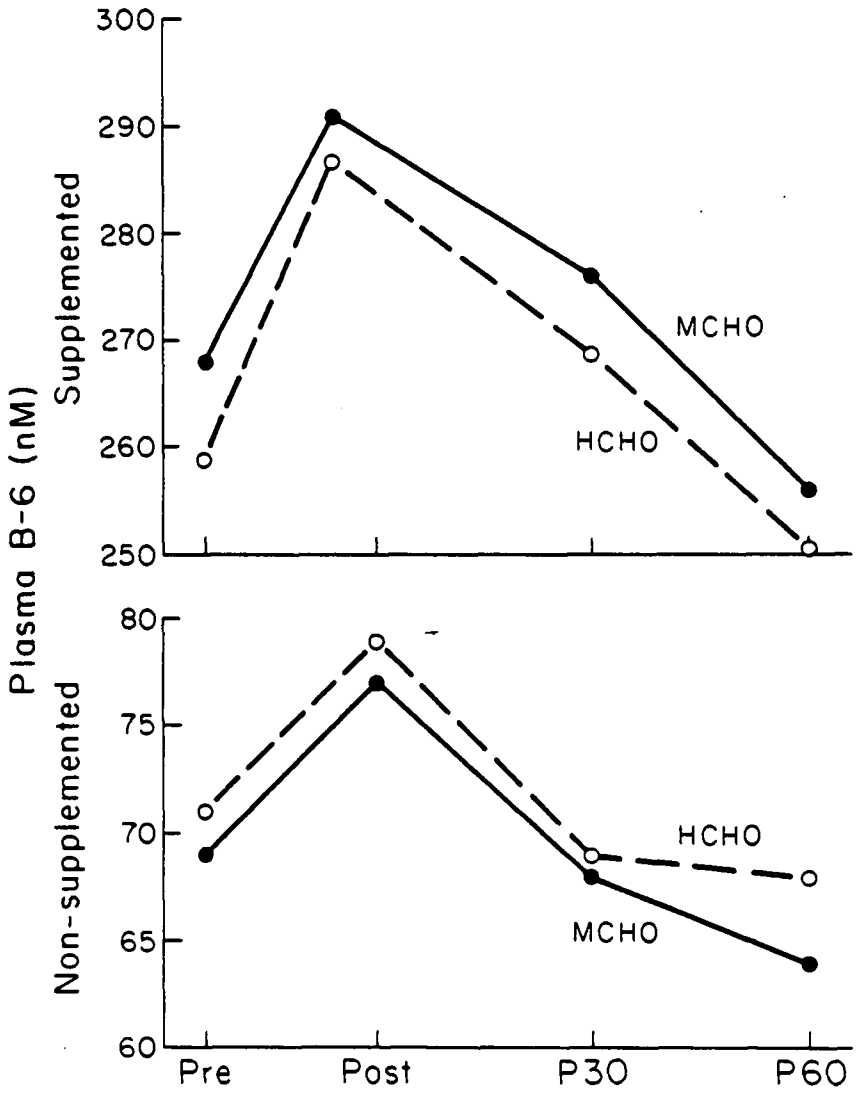

Figure 3.3. Plasma Vitamin B-6: Changes over time with exercise. All groups combined, n=15. Abbreviations for diet are given in Figure 3.1. Changes pre to post and post to p60 are significantly different.

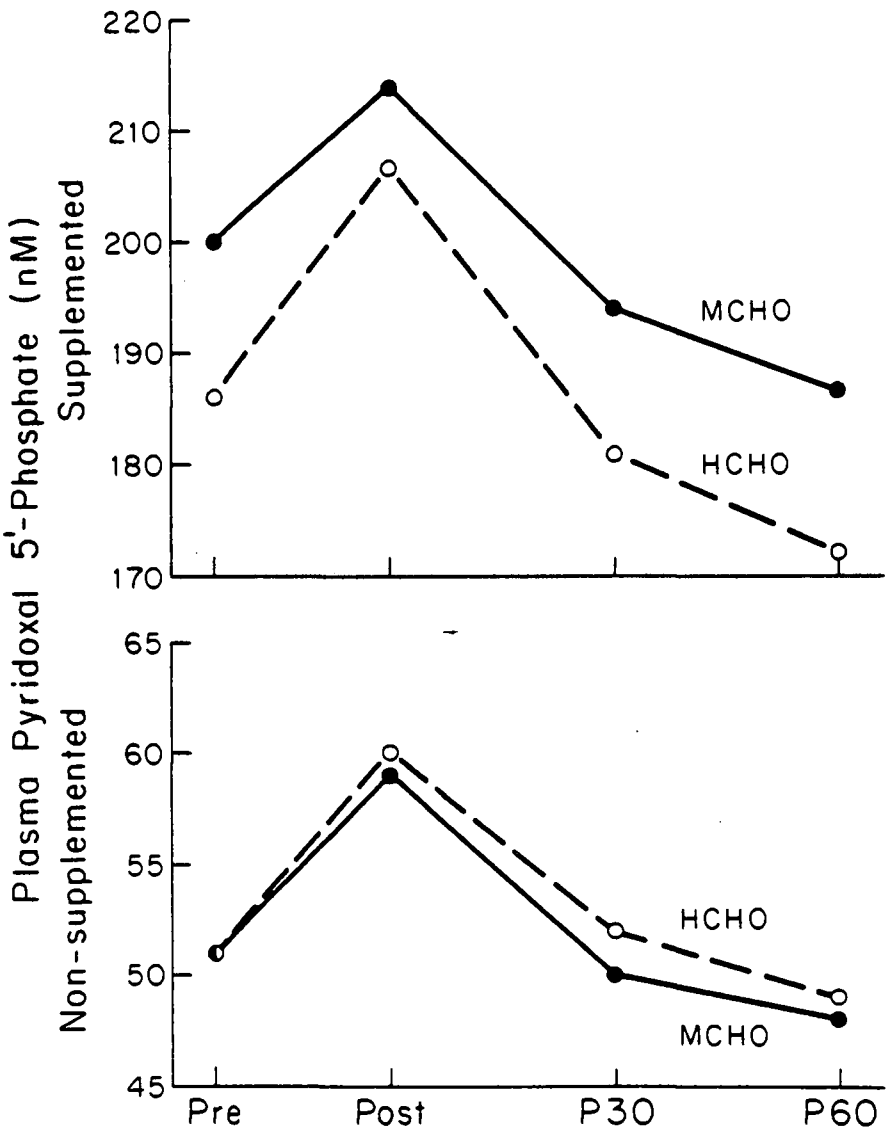

Figure 3.4. Plasma pyridoxal 5'-phosphate: Changes over time with exercise. All groups combined, n=15. Abbreviations for diet are given in Figure 3.1. Changes pre to post and post to p60 are significantly different.

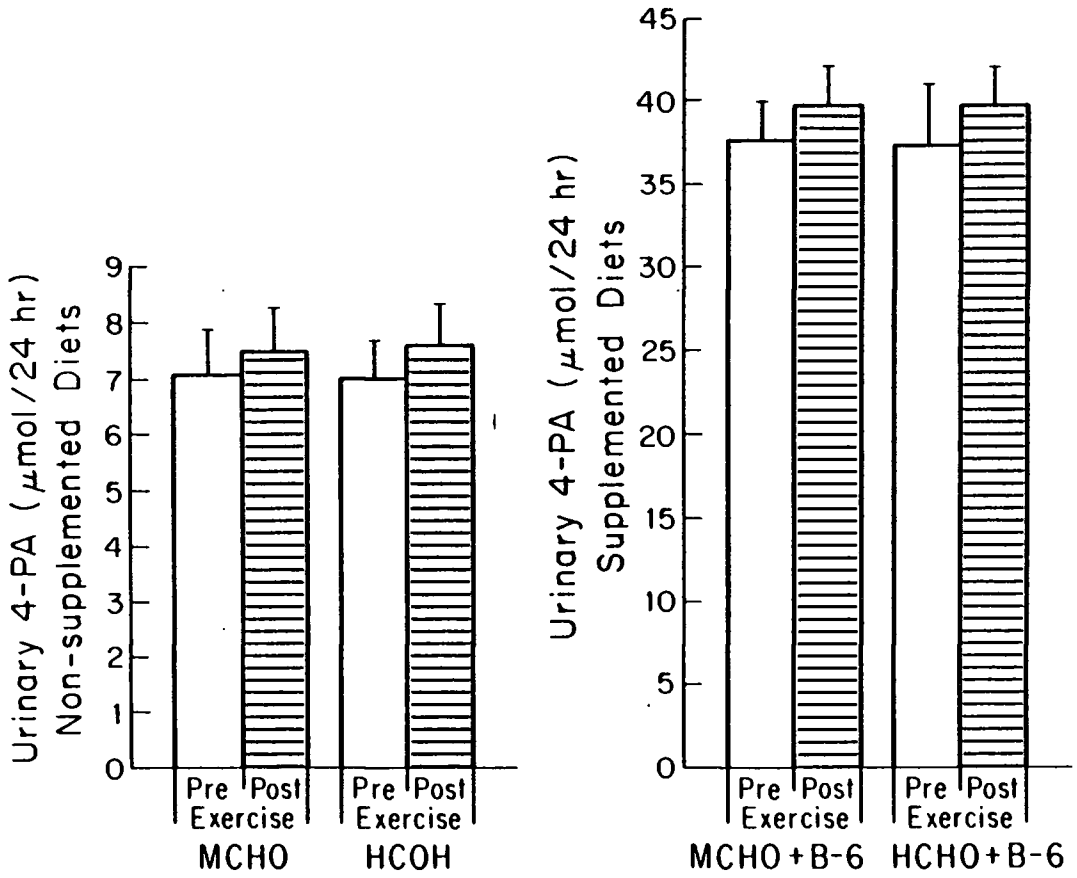

Figure 3.5. Urinary 4-Pyridoxic Acid (4PA): Excretion the day before (pre) and the day of exercise (post). Error bar represents one standard deviation. Changes pre to post significantly different ($p<.0001$) for all diets.

CHAPTER IV

CHANGES IN FUEL METABOLISM DURING EXERCISE IN TRAINED AND UNTRAINED WOMEN FED TWO CARBOHYDRATE DIETS WITH AND WITHOUT VITAMIN B-6 SUPPLEMENTATION. [1,2]

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ABSTRACT

Pyridoxal 5'-phosphate (PLP) is essential to both gluconeogenesis and glycogenolysis as a cofactor for glycogen phosphorylase and the alanine-glucose cycle. Glycogen phosphyorylase is also proposed as a storage reservoir for vitamin B-6 (Black et al., 1978). To examine the effect of vitamin B-6 (B-6) and carbohydrate (CHO) on fuel metabolism during exercise, 5 young/trained (YT), 5 young/untrained (YU), and 5 postmenopausal/untrained (PU) women were alternately fed 4 diets. A moderate-CHO (49%) (MCHO) for 2 weeks; a high-CHO (63%) (HCHO) for 1 wk; MCHO+B-6 for 2 weeks and HCHO+B-6 for 1 week. A 1 week baseline MCHO diet separated the non-supplemented (NS) (2.3-2.4 mg B6) and supplemented (S) (8 mg B6 added) treatments. Training was determined by $\dot{V}O_2$ max. Subjects were exercised at the end of each dietary period at 80% $\dot{V}O_2$ for 20 minutes on a cycle ergometer. Blood was drawn pre, post, post30-min. and post60-min. (p60) of exercise and analyzed for plasma PLP, glucose (G), free fatty acids (FFA) and lactate (LA). ANOVA showed no difference among groups or diet for LA, though LA showed a significant increase from pre to post and significant decrease from post to p60 on all diets. Additionally, ANOVA showed significant time x group x diet interactions for FFA and significant diet x time and time x group interactions for G. S usually resulted in a lower FFA response during exercise in all groups with the HCHO+B-6 always producing the lowest FFA. Increased CHO also resulted in lower FFA during exercise. ANOVA showed no difference in PLP for groups with respect to diet or time. PLP

increased significantly from pre to post and decreased significantly from post to p60. These results indicate that both CHO and vitamin B-6 can alter fuel metabolism during exercise in women.

INTRODUCTION

During endurance activities certain metabolic processes occur to assure adequate energy is provided to the working muscles of the body. These processes include the breakdown of muscle and liver glycogen for glucose production, the deamination of amino acids with their carbon skeletons being converted to glucose and the lypolysis of fat in the adipose tissue contributing free fatty acids and glycerol for energy production (Alhborg and Felig, 1982; Alhborg et al., 1974). In the trained individual the body can more readily turn to lipid metabolism as its major source of energy during exercise while sparing the muscle's glycogen. The adaptation of fuel metabolism due to training assures muscle glycogen will be spared and that greater endurance can be achieved (Constill et al., 1977; Bjorntrop, 1981). The same is not true of the untrained individual who must rely more on the fuel provided by muscle glycogen since lipid metabolism is limited by the cardiovascular/respiratory systems ability to deliver oxygen to the working muscles during exercise (Sutten, 1978).

It is now known that muscle glycogen levels and the fuels used during exercise can be altered by diet (Jacobs et al., 1982). A high fat diet is known to decrease glycogen storage while producing higher plasma free fatty acids (FFA) during exercise (Maughan et al., 1978). In contrast, if a diet is high in carbohydrate, plasma FFA are decreased during exercise (compared to a high fat diet) but adequate glycogen replacement is assured. Since FFA utilization and uptake in the working muscle is dependent on plasma concentration (Hagenfeldt,

1979), an athlete or any individual engaged in an endurance activity would benefit from a diet that assures adequate glycogen replacement while producing the highest plasma FFA concentrations during exercise. Currently the trade off has been to recommend diets high in carbohydrate for endurance athletes so adequate glycogen replacement is assured, since glycogen is the limiting factor in endurance performance (Costill et al., 1977).

Pyridoxal 5'-phosphate, the active form of vitamin B-6, is essential to glycogenolysis since it is an integral part of the glycogen phosphorylase enzyme (Krebs and Fisher, 1964). This enzyme cleaves glucose-1-phosphate from glycogen, thus providing glucose for energy production. Pyridoxal 5'-phosphate is also essential to gluconeogenesis as a cofactor for amino transferase enzymes which participate in the glucose-alanine cycle. For many years it has been assumed that vitamin B-6, a water soluble vitamin, was not stored to any great extent in the body. Recently, Black and co-workers (1977) have reported an increase in glycogen phosphorylase in the muscles of rats supplemented with vitamin B-6. They also noted that this stored form of the vitamin was not made available to the body during times of dietary vitamin B-6 deficiency unless a caloric deficit also existed (Black et al., 1978). In examining this theory in man, Leklem and Shultz (1983) and Munoz (1982) observed an increase in plasma pyridoxal 5'-phosphate in males after various types of strenuous activities. They have hypothesized that exercise simulates a condition of acute starvation in the body (since an energy demand is being imposed) and that pyridoxal 5'-phosphate

is being released from muscle glycogen phosphorylase to facilitate gluconeogenesis in other parts of the body. Hatcher et al. (1982) also observed that male cyclists, who were glycogen depleted and repleted on low and high carbohydrate diets, with and without vitamin B-6 supplementation, had significantly higher plasma pyridoxal 5'-phosphate values after exercise when fed a high carbohydrate diet supplemented with vitamin B-6. These results seemed to indicate that by increasing glycogen phosphorylase through vitamin B-6 supplementation and increasing muscle glycogen through carbohydrate loading the body actually broke down the muscle glycogen more quickly. This outcome would not be favorable for an endurance athlete who wants to use muscle glycogen sparingly, while relying on FFA for the majority of the energy needed during exercise. These conclusions were supported by deVos et al. (1982) who reported significantly increased lactate and glucose during the same feeding and exercise period when compared to low and normal carbohydrate diets with or without vitamin B-6 supplementation.

The examination of fuel metabolism in women is limited in the literature. Costill et al. (1979) reported that lipid metabolism in endurance trained males and females did not differ, but little research has been done on the effect of altered dietary carbohydrate and fuel metabolism in women. This is especially true of older women or women who are less trained. Alteration of carbohydrate in the diet is recommended for those involved in endurance activities to increase glycogen stores (Costill and Miller, 1980). Another ergoenic aid

often used by those involved in physical fitness and sport is vitamin supplementation. Therefore, since vitamin B-6 supplementation may be used by athletes and non-athletes alike and because the majority of the work in this area has been done in men, our resaerch was designed to examine if the addition of vitamin B-6 to a moderate or high carbohydrate diet would alter fuels used during exercise in women of various ages and levels of training.

This study was part of a larger research project in which the effect of age, exercise, and carbohydrate on vitamin B-6 metabolism was examined in women.

METHODS

Experimental Design

Four diets were fed during this seven week metabolic study, with each diet being followed by an exercise testing session (Figure 4.1). The first 2 weeks consisted of a moderate carbohydrate (MCHO) diet followed by a 1 week high carbohydrate (HCHO) diet. Subjects were then returned to the MCHO diet for 1 week before administration of a MCHO + vitamin B-6 diet (MCHO+B-6) for 2 weeks and a HCHO + vitamin B-6 (HCHO+B-6) for 1 week. The first week on the MCHO diet was designed to normalize all subjects to the same dietary level of vitamin B-6 and carbohydrate and allow subjects time to adapt to the diet and routine of the study. The administration of the MCHO diet for one week after the HCHO diet was designed to assure no confounding overlap of the dietary treatments and to have the premenopausal subjects at the same relative point in their menstrual cycle during the last two exercise testing sessions as experienced in the first two exercise sessions.

Subjects

Fifteen healthy female volunteers were recruited for this study. They were recruited for one of three groups, each with an n=5; young/trained, young/untrained, and postmenopausal/untrained according to age and aerobic training. Physical characteristics of each of the groups are given in Table 4.1. Untrained was defined as performing less than 40 minutes of aerobic activity during a one week period with

exercise occurring no more than two days a week. Trained was defined as performing a minimum of 120 minutes of aerobic activity during a one week period with exercise occurring at least three days a week. Actual level of training was determined prior to the beginning of the study by measuring $V\dot{O}_2$ max using the Wilmore and Costill procedure (1974). Subjects had to have maintained their classified level of fitness (trained or untrained) for at least six months prior to beginning the study. They were also required to maintain this level of fitness throughout the experimental period. To monitor this, subjects were required to report all aerobic exercise and the duration and intensity of that exercise in a daily journal. Subjects were also required to provide researchers with the following information prior to beginning the study: completed health history questionnaire which established health status, complete a three day dietary record, and an exercise history questionnaire. The following criteria was also required before participation began: normal blood chemistry screen (which included glucose, calcium, albumin, creatinine, cholesterol, urea nitrogen, and triglycerides), normal cardiac function as determined by electrocardiograph tracings at rest and maximal heart rate, a normal xylose absorption (Harris, 1969) test to assess carbohydrate absorption ($\bar{x}$ + S.D. = 31.6±8.19%), doctor's approval for participation in the study, blood sample for plasma pyridoxal 5'-phosphate assessment, blood pressures within normal limits at rest and during exercise, no vitamin supplements used for at least four weeks prior to beginning study. No nicotine, oral contraceptives, or

estrogen replacement therapy were used during the study. Subjects were informed of the experimental procedure and signed informed consent forms. This study was approved by the Oregon State University Committee for Protection of Human Subjects.

Dietary Treatments

This study included four dietary treatments in the dietary procedure, two of which were supplemented with vitamin B-6 and two which were not supplemented (Figure 4.1). The specific dietary composition of the diets and the amounts served for each meal are given in Table 4.2. The two non-supplemented treatments consisted of a moderate carbohydrate (MCHO) diet (49% carbohydrate; 35% fat; 16% protein) and a high carbohydrate (HCHO) diet (64% carbohydrate; 22% fat; 16% protein). The unsupplemented diets had a vitamin B-6 level of 2.3 mg for the MCHO and 2.4 mg for the HCHO diet. The supplemented diets repeated the MCHO and HCHO dietary treatments but included an additional 8.0 mg pyridoxine (vitamin B-6) given as an oral solution of pyridoxine hydrochloride at the breakfast meal. The level of supplementation was designed to provide excess vitamin B-6 without introducing a pharmacological effect.

To minimize variation in nutrient composition, all foods served in this dietary study were purchased in bulk from the same lot when possible. Foods which could not be purchased this way were thoroughly mixed prior to beginning the study and frozen in aliquots for daily use. Mixing was done to assure uniformity of the nutrient content of the

diets. All food items fed were carefully weighed to a tenth of a gram before being served to each subject. Food composites were made weekly and analyzed for vitamin B-6. This was done to assess the actual amount of the vitamin being consumed by each subject and to determine if storage and food preparation were altering the vitamin content of the food.

At the beginning of the study all subjects were placed on a 1800-2000 kcal diet. Subjects were weighed daily and calories adjusted as needed to assure that weight was maintained throughout the study. If weight loss occurred, additional calories were added in the form of carbohydate or fat containing foods (such as margarine, honey, and salad dressing or mayonnaise). The carbohydrate/fat ratio was maintained on each diet even though extra calories were added. Foods used to increase caloric level of the diets contained essentially no vitamin B-6. All meals were prepared in the Foods and Nutrition Department metabolic kitchen. No alcoholic beverages were allowed throughout the study and no caffeinated beverages were allowed the day before and the day of the exercise testing.

Exercise Procedure

Each subject was put through a graded exercise test on a Monark cycle ergometer to establish maximum heart rate and $\dot{V}O_2$ max using the Wilmore and Costill procedure (1974). Exercise testing was done prior to beginning the metabolic study. The subject's heart rate and cardiac cycles were monitored continually during the test using an electrocardiograph. Blood pressures were monitored before, during, and after

exercise. Rules for termination of exercise tests as outlined by the American College of Sports Medicine (1980) were followed. Using the information obtained from the $\dot{V}O_2$ max exercise test, subjects were exercised on a Monark cycle ergometer at 80% of the $\dot{V}O_2$ max for 20 minutes during each of the exercise testing sessions (Figure 4.2).

The procedure for each exercise session included five minutes of warm-up followed by 20 minutes of exercise at 80% $\dot{V}O_2$ max and a 5-10 minute cool-down period (Figure 4.1). The experimental design was such that each subject was exercised on the 5th or 6th day of the week of each dietary treatment. This was done to allow 5 or 6 days to adapt to the diet before each exercise testing session. It also allowed for a urine collection the day after exercise to assess any post-exercise metabolic changes under the same experimental dietary treatment. Subjects were monitored on an electrocardiograph before, during, and after the exercise period. Any irregularities in heart function or in the general well-being of the subject would have caused exercise to be terminated. Blood pressures were also taken pre, during, and post exercise (Figure 4.2). All exercise testing was done in the morning in a fasted state. Subjects were not allowed to take in fluids until after the last blood sample was drawn. Body weight was measured before and immediately after exercise. All exercise was performed at a moderate temperature of 20-24°C and at a relative humidity of 48% (range = 42-64%).

Daily Procedures

A number of daily procedures were required of each subject to assure compliance with the metabolic study, assist in monitoring subjects well-being, and assure that accurate records were kept. These daily procedures included keeping a journal of activities (minutes exercised, non-caloric beverages consumed, medications taken, menstrual periods); recording weight and returning unused portions of margarine, salad dressing, or honey used to increase calories.

Blood Collection

During each of the four exercise testing sessions blood was drawn four times (Figure 4.2). The first sample was drawn prior to beginning exercise, after the subject had rested for 10-15 minutes. The second sample was drawn two minutes after the termination of the 20 minute exercise period. The third and fourth samples were drawn 30 and 60 minutes, respectively, after the post exercise draw. The subjects were asked to sit or rest quietly during the 60 minute period following exercise. All blood samples were collected in heparinized tubes, kept on ice until centrifuged (no more than 30 minutes) and plasma was removed and then stored at -40°C.

Analyses

Plasma pyridoxal 5'-phosphate was determined by a modified method of Chabner and Livingston (1970). Interassay variation of a pyridoxal 5'-phosphate control sample (n=22) was 6.5%. Glucose was

analyzed in duplicate using a modified procedure of Hoffman (1937) on a Technicon autoanalyzer. Interassay variation of a glucose control sample (n=31) was 3.1%. Plasma lactate was determined using a Sigma Chemical Co. kit and the method described by Hohorst (1963). Interassay variation of a lactate control sample (n=6) was 8.0%. Plasma FFA concentration was determined by using a modified method of Trout et al. (1960). Interassay variation of a FFA control sample (n=9) was 3.8%. Hemoglobin determination and hematocrits were done on all samples and hematocrit values used to calculate changes in plasma volume as described by van Beaumount (1972). Vitamin B-6 content of the diet was assayed weekly using a procedure by ADAC (1980). Xylose absorption was assessed using a method by Harris (1969).

Statistical Analysis

A three-way split plot partially blocked ANOVA design with groups and diets as whole plot factors and time (of the blood sampling) the split plot factor was used in the analysis of the data. When significant interactions occurred main effects were no longer of interest. The significant interactive effects were examined by performing hypothesis testing on several contrasts of treatment means where contrasts is defined as a comparison involving two or more treatment means (Neter and Wasserman, 1974). Contrasts to be tested were specified in advance of data collection. Least significant difference was the test criterion used in testing multiple comparisons (Steel and Torrie, 1980).

For the purpose of hypothesis testing "a" designates group, "b" designates diet, and "c" represents time. These letters are subscripted to indicate the specific group (1=young/trained; 2=young/untrained; 3=postmenopausal/untrained), diet (1=MCHO; 2=HCHO; 3=MCHO+B-6; 4=HCHO+B-6) and time (1=pre; 2=post; 3=post30; 4=post60 for plasma samples). For example, $a_1b_1c_1$ designates the mean of the five subjects in group 1, on diet 1, at time 1. Examples of hypotheses tested are as follows: $(a_1b_1c_1 - a_2b_1c_1)$; $[(a_1b_1c_2 - a_1b_1c_1) - (a_1b_2c_2 - a_1b_2c_1)]$; and $[(b_1c_2 - b_1c_2) - (b_2c_2 - b_2c_1)]$. When groups were combined (n=15) "a" was omitted from the hypothesis. Results were determined to be significant if the p-value was less than the critical value of $\alpha = .05$.

RESULTS

The results of the ANOVA showed significant diet x time ($p<.0373$) interactions for glucose (Table 4.3). In examining the diet x time interaction (Figure 4.3), with groups combined, feeding the MCHO diet resulted in significantly higher glucose values from pre to post-exercise than when the two supplemented diets were fed. The MCHO diet was also significantly different from the other three diets with respect to the decrease seen from post-exercise to post-60 minutes of exercise. The time x group interaction (Figure 4.4), with diets combined, showed that the postmenopausal/untrained group had a significantly lower glucose response over time than either the young/trained or the young/untrained groups. Plasma glucose values for each group, diet, and time are given in Table 4.4. Pre (resting) plasma glucose levels were similar within each group regardless of the diet administered.

The results of the ANOVA for FFA showed a significant 3-way interaction between group x diet x time ($p<.0471$) (Table 4.3). A 3-way interaction indicates that the groups responded differently across time with the different diets. In examining the changes over time with the four different diets, the young/trained group showed a significant increase from pre to post-exercise and a significant decrease from post-exercise to post-60 minutes of exercise for all diets (Figure 4.5). The untrained groups (young/untrained and postmenopausal/untrained) showed a significant increase from pre to post-exercise with all diets except the HCHO+B6 diet. For the young/untrained group there was a significant decrease from post to post-60 only with the MCHO

diet. FFA levels for the postmenopausal/untrained group did not decrease significantly from post to post-60 with any of the diets.

The concentration of the FFA in the plasma during exercise is directly proportional to the uptake of FFA by the working muscle (Hagenfeldt, 1979). If supplementation causes increased glycogen breakdown, then one might expect lower FFA in the blood due to the fact that there is less of a need for FFA as a fuel source. Therefore, we examined the effect supplementation had on the peak FFA values reached during exercise with each diet. Supplementation generally caused a decrease in the peak exercise FFA value produced in each group when comparing MCHO to MCHO+B-6 and HCHO to HCHO+B-6. The two exceptions were in the young/trained and the postmenopausal/ untrained when comparing the MCHO and MCHO+B-6 diets. Although FFA production decreased with supplementation in all but these two cases, these decreases were not significant. The peak FFA values in the young/trained group ranged from 1.51 mEq on the MCHO+B-6 diet to a low of 1.26 mEq on the HCHO+B-6 diet, while the untrained groups ranged from a high of 1.17 mEq (young/untrained; MCHO diet) and 1.05 mEq (postmenopausal/untrained; MCHO+B-6 diet) to a low of 0.63 mEq (young/trained; HCHO+B-6 diet) and 0.74 mEq (postmenopausal/untrained; HCHO+B-6 diet). Although one particular diet did not consistently produce the highest FFA (it was either the MCHO or the MCHO+B-6 diet) the diet which gave the lowest FFA value during exercise was always the HCHO+B-6 diet. This was true regardless of the group examined. The young/trained group had significantly higher peak FFA

during exercise than the two untrained groups, regardless of the diet (Figures 4.5a, b, c, d).

The effect of carbohydrate on FFA production during exercise was also examined. As reported by other researchers (Maughan et al., 1978) the FFA values decreased in all groups when switching from a MCHO diet to a HCHO diet, regardless of supplementation. However, this decrease was not statistically significant.

In comparing the resting FFA values of the groups, when the MCHO diet was supplemented with vitamin B-6 the resting FFA values decreased in the young/trained and young/untrained, but the postmenopausal/ untrained group remained unchanged. When the HCHO diet was fed, a depression of the resting FFA values occurred below those seen on the MCHO diet. When supplementation was fed with the HCHO diet the resting FFA values dropped to their lowest point.

Lactate values for each diet (with groups combined) are given in Table 4.6. The results of the ANOVA for lactate showed no significant interactive effects, and time was the only significant main effect ($p<.0001$)(see Table 4.3). This indicates that there were no group or diet differences in the response of lactate over time with exercise. Therefore, groups were combined and a n=15 used. There was a significant increase from pre to post-exercise and a significant decrease from post to post-60 minutes of exercise with lactate, regardless of the diet administered (Figure 4.6). However, in contrast to the FFA levels, the highest lactate values were seen with the HCHO+B-6 diet.

In assessing whether exercise altered plasma pyridoxal 5'-phosphate levels in women during exercise, pyridoxal 5'-phosphate was examined at each of the four blood draws: pre, post, post-30, and post-60 minutes of exercise. The results of the ANOVA for pyridoxal 5'-phosphate showed no significant main effects or any interactive effects involving groups (Table 4.3). Therefore, groups were combined in the analysis of pyridoxal 5'-phosphate, and a n=15 was used. However, pyridoxal 5'-phosphate did show a significant diet x time interaction ($p<.0001$). Contrasts of differences in means showed a significant increase in pyridoxal 5'-phosphate from pre to post-exercise and a significant decrease from post to post-60 minutes of exercise (Table 4.7). The supplemented diets showed a significantly greater magnitude of change from pre to post-exercise and from post-exercise to post-60 than the non-supplemented diets. The actual pyridoxal 5'-phosphate values for each diet and time periods (with groups combined) are given in Table 4.7.

The van Beaumont (1972) method was used to calculate plasma volume changes. This was done to help evaluate if the changes seen in plasma pyridoxal 5'-phosphate with exercise were due to hemoconcentration alone. The mean plasma volume changes seen in the four exercise sessions ranged from -7 to -11%. Using the largest mean changes in plasma volume, -11%, one would expect an approximate 7% increase in plasma pyridoxal 5'-phosphate due to plasma concentration alone. However, the mean increase seen in plasma pyridoxal 5'-phosphate in all the exercise sessions ranged from 2-17% above what would be

expected due to just hemoconcentration. Also, at post-60, when plasma volumes had returned to their pre-exercise level the plasma pyridoxal 5'-phosphate values continued to fall and were as much as 23% below the pre-exercise values in some subjects. This change cannot be accounted for by the change in plasma volume. Therefore, the changes we observed in plasma pyridoxal 5'-phosphate during exercise are due to other factors, in addition to those related to hemoconcentration.

The mean body weight for the young/trained, young/untrained, and postmenopausal/untrained at the beginning of the diet study was 56.8±5.7, 56.7±6.7, and 59.86 kg, respectively. The mean body weight for the young/trained, young/untrained, and postmenopausal/untrained at the end of the 49 day study was 55.3±5.4, 55.6±5.7, and 59.4±4.9 kg, respectively.

DISCUSSION

In examining the relationship between vitamin B-6 and fuel metabolism during exercise it is helpful to consider previous studies as well as the results we observed, both which indicate that there may be a relationship between these two variables. With exercise a significant rise in plasma pyridoxal 5'-phosphate was observed from resting to post-exercise (taken after 20 minutes of exercise at 80% $\dot{V}O_2$ max). This significant increase was followed by a significant decrease from post-exercise to post 60-minutes of exercise. Supplementation of the diet produced a greater magnitude in the rise and fall of pyridoxal 5'-phosphate during exercise than the non-supplemented diets. These changes were the same in all three groups. An increase in pyridoxal 5'-phosphate during exercise has been reported in men after participating in a 4500 meter run (Leklem and Shultz, 1983). The increase in pyridoxal 5'-phosphate during exercise and its subsequent decrease after exercise has also been reported in male cyclists exercising for 60-minutes (Hatcher et al., 1982). The change in pyricoxal 5'-phosphate during exercise may be related to the cofactor role pyridoxal 5'-phosphate plays in glycogen phosphorylase. Glycogen phosphorylase serves as a storage reservoir for pyridoxal 5'-phosphate in the muscle. During exercise pyridoxal 5'-phosphate may be released from the muscle glycogen phosphorylase into the plasma where it can participate in gluconeogenesis in other parts of the body. The particular way pyridoxal 5'-phosphate is released from the glycogen phosphorylase enzyme and released into the plasma has not been

elucidated. Gani et al. (1978) have developed a micellar model of glycogen phosphorylase and have proposed a mechanism for the removal of pyridoxal 5'-phosphate from the micell using a reaction with cystine. An increase in vitamin B-6 in the diet has been shown to increase glycogen phosphorylase in the muscles of rats (Black et al., 1977, 1978). This increase in enzyme concentration in the muscle may increase the rate of breakdown of glycogen during exercise for energy production. If increased vitamin B-6 in the diet does increase utilization of glycogen during exercise, then one would expect a change in circulating FFA and lactate values during exercise reflecting this alteration in fuel metabolism in the muscle.

In examining the fuel responses during exercise to the manipulation of carbohydrate and vitamin B-6, it was observed that both supplementation and carbohydrate tended to result in a decrease in circulating FFA. The decrease in FFA due to supplementation was seen in all but two situations (young/trained, and postmenopausal/untrained MCHO vs. MCHO+B-6), and the decrease in FFA due to carbohydrate was observed in all three groups. This decrease in FFA with increased carbohydrate support others (Maughan et al., 1978) who have found that increasing carbohydrate in the diet depresses circulating FFA during exercise. However, recommendation of a HCHO diet to athletes, particularly those involved in endurance activities, is still necessary to assure that their glycogen stores are replenished as quickly as possible. But, the addition of supplemented vitamin B-6 beyond the requirement of this diet regimen may be contraindicated, since it could lower

plasma FFA during exercise and also causes a more rapid utilization of glycogen for energy in the muscles. It should be emphasized the subjects in this study were only exercised for 20 minutes.

Although the lactate level was not significantly different between the groups or the diets, it is of interest to note that the HCHO+B-6 diet produced the highest lactate and lowest FFA values, indicating more glucose was being utilized for fuel by the working muscle. The plasma glucose level showed little change between the diets except for the MCHO, which produced a significantly higher glucose response than the other diets. We feel this response may be falsely high and could have been due to anxiety experienced by some of the subjects at the first exercise session. However, regardless of the diet, the postmenopausal/untrained women did have significantly lower glucose responses than the young women. This may be due to an increased uptake of plasma glucose by the exercising muscle for energy since the postmenopausal women had less relative muscle mass and hence less glycogen stored for anaerobic energy needs.

Therefore, it can be concluded that both carbohydrate and vitamin B-6 may alter fuel metabolism during exercise, with vitamin B-6 supplementation causing the greatest decrease in exercise FFA values and the highest lactate values. These changes were seen after 20 minutes of exercise at 80% $\dot{V}O_2$ max, the changes which may occur with supplementation of a high carbohydrate diet in longer endurance activities, such as a marathon, have not been examined.

Based on this research it cannot be recommended to endurance athletes or those involved in endurance activities to supplement their

high carbohydrate diet with vitamin B-6 above that which is recommended by the RDA. This research and that of Hatcher et al. (1983) has shown that exercise does increase the pyridoxal 5'-phosphate in the plasma during exercise followed by a plasma decrease back to pre or below pre values. This change in vitamin B-6 metabolism may mean that the muscle glycogen phosphorylase enzyme is releasing pyridoxal 5'-phosphate from the muscle, subsequently causing an increase in plasma pyridoxal 5'-phosphate. Supplementation of vitamin B-6 caused an even greater increase in the plasma level of pyridoxal 5'-phosphate. Numerous researchers (Bergstron et al., 1967; Hermansen et al., 1967; Costill and Miller, 1980) have shown that increased glycogen stores improve endurance. If higher plasma pyridoxal 5'-phosphate is an indication of increased glycogen breakdown during exercise, then supplementation with vitamin B-6 would hinder endurance. High levels of vitamin B-6 in the diet may also alter the degree to which FFA are utilized for energy during exercise. This change in utilization of FFA may be due to the fact that the muscle is relying more on the immediate energy of glycogen (if supplementation with vitamin B-6 speeds up glycogen breakdown) and less on the exogenous energy of FFA. This alteration in fuel metabolism (increased glycogen breakdown) caused by supplementation of vitamin B-6 increases the reliance on the limited sources of fuel in the body (carbohydrate) and thus could alter the utlimate performance of the athlete in an endurance event. These results indicate that both dietary vitamin B-6 and carbohydrate may alter the fuels made available for energy during exercise in women.

Table 4.1. Physical statistics for young/trained, young/untrained, and postmenopausal/untrained groups.

	Means ± S.D.	Range
Young/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 (%)	20.1±4.2 (1)	13-24
$\dot{V}O_2$ max (ml/kg/min)	40.5±3.7 (2)	37.1-46.5
Young/untrained (n=5)		
Age (yr)	24.4±3.2	21-29
Height (cm)	161.8±6.3	153.8-170.00
Weight (kg)	55.9±6.9	48.2-65.0
Body Fat (%)	20.4±1.5	18-22
$\dot{V}O_2$ max (ml/kg/min)	32.7±4.5	25.9-37.0
Postmenopausal/untrained (n=5)		
Age (yr)	55.8±4.8	49-61
Height (cm)	155.8±4.5	150.8-160.8
Weight (kg)	58.8±6.0	51.8-68.2
Body Fat (%)	27.0±3.4	22.31
$\dot{V}O_2$ max (ml/kg/min)	24.4±1.6	23.9-27.6

(1) Percent body fat determined by skinfolds measured at seven sites (Jackson et al., 1980).

(2) $\dot{V}O_2$ max determined by graded exercise testing using the Wilmore and Costill (1974) procedure on a cycle ergometer.

Table 4.2. 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*

*Variable amounts used to adjust calories. Amounts listed are average amounts used.

Table 4.3. Significant* F-test values from the 3-way ANOVA.

Variable	Factor (1)	DF	F-Value	p-Value*
Glucose	T	3, 108	42.21	.0002
	T x G	6, 36	2.82	.0235
	D x T	9, 108	2.08	.0373
FFA	D	3, 36	10.04	.0001
	T	3, 108	79.05	.0001
	T x G	6, 36	3.02	.0170
	G x D x T	18, 108	1.72	.0471
Lactate	T	3, 108	358.53	.0001
PLP	D	3, 36	398.57	.0001
	T	3, 108	93.08	.0001
	D x T	9, 108	7.98	.0001

* Only those interactions or main effects with p-values <.05 are reported.

(1) T = time (pre, post, p30, p60).

G = group (young/trained; young/untrained; postmenopausal/untrained)

D = diet (MCHO, MCHO+B6, HCHO, HCHO+B6)

Table 4.4. Plasma glucose levels over time with exercise and four different diets for each group.

	TIME[1]			
	pre	post	post30	post60
	mg/100 ml			
young/trained (n=5)				
MCHO[2]	88.7±4.0[3]	104.7±15.7	90.5±8.7	84.3±11.6
HCHO	88.7±3.9	106.8±22.7	94.1±14.1	92.2±4.1
MCHO+B-6	87.6±5.3	96.9±9.4	86.5±2.8	90.4±3.7
HCHO+B-6	91.3±4.0	102.6±6.8	88.2±8.4	95.2±5.8
young/untrained (n=5)				
MCHO	91.3±4.3	118.0±30.4	95.9±6.0	86.8±5.0
HCHO	91.9±7.4	93.9±7.4	88.9±3.0	90.2±3.3
MCHO+B-6	91.9±6.6	96.7±16.1	91.2±15.1	88.5±4.4
HCHO+B-6	90.3±3.7	95.9±8.2	87.2±7.7	86.5±7.3
postmenopausal/untrained (n=5)				
MCHO	99.9±5.2	95.7±8.5	92.4±4.3	95.3±4.0
HCHO	97.7±4.3	96.4±7.5	94.7±5.6	98.9±3.7
MCHO+B-6	100.1±3.7	100.9±5.9	94.7±3.0	96.9±4.8
HCHO+B-6	98.7±1.4	98.0±6.1	96.4±5.4	97.6±1.4

(1) Pre = resting, post = at termination of exercise, post30 = 30 minutes after exercise was terminated, post60 = 60 minutes after exercise was terminated.

(2) MCHO = 49% carbohydrate, 35% fat, 16% protein; HCHO = 64% carbohydrate, 22% fat, 16% protein; MCHO+B-6 = MCHO diet plus 8 mg vitamin B-6 added; HCHO+B-6 = HCHO diet plus 8 mg vitamin B-6 added.

(3) Mean ± S.D.

Table 4.5. Plasma FFA levels over time with exercise and four different diets for each group.

	TIME[1]			
	pre	post	post30	post60
	mEq/L			
young/trained (n=5)				
MCHO[1]	.893±.063[2]	1.422±.361	.880±.222	.977±.258
HCHO	.645±.152	1.352±.611	.838±.417	.739±.224
MCHO+B-6	.817±.231	1.507±.794	.853±.372	.839±.196
HCHO+B-6	.535±.286	1.262±.559	.722±.254	.779±.252
young/untrained (n=5)				
MCHO	.907±.361	1.166±.288	.694±.971	.688±.208
HCHO	.625±.188	.859±.332	.842±.444	.870±.333
MCHO+B-6	.678±.201	.945±.351	.815±.300	.884±.399
HCHO+B-6	.487±.206	.627±.157	.464±.179	.591±.206
postmenopausal/untrained (n=5)				
MCHO	.700±.122	1.009±.156	.596±.259	1.043±.366
HCHO	.603±.155	.964±.162	.718±.192	.808±.308
MCHO+B-6	.740±.314	1.054±.260	.685±.207	.988±.109
HCHO+B-6	.532±.185	.738±.149	.545±.102	.637±.127

(1) Abbreviations are in Table 4.4.

(2) Mean ± S.D.

Table 4.6. Plasma lactate levels over time with exercise and four different diets, with groups combined, n=15.

	TIME[1]			
	pre	post	post30	post60
DIET[1]	µmol/L			
MCHO	.617±.294[2]	2.88±.320	1.14±.126	.681±.191
HCHO	.689±.266	2.89±.001	1.30±.584	.799±.336
MCHO+B-6	.626±.223	2.49±.796	1.18±.569	.773±.323
HCHO+B-6	.695±.300	2.93±.769	1.39±.833	.725±.282

(1) Abbreviations listed in Table 4.4.

(2) Mean ± S.D.

Table 4.7. Plasma pyridoxal 5'-phosphate levels over time with groups combined (n=15) for the four different dietary treatments.

	TIME[1]			
	pre	post	post30	post60
	nM			
DIET[1]				
MCHO	50.8±14.7[2]	58.8±18.2	50.0±14.9	47.9±13.9
HCHO	51.1±18.4	60.2±22.5	51.6±17.6	50.0±18.5
MCHO+B-6	200±35.1	214±36.7	194±36.8	187±31.0
HCHO+B-6	186±41.5	207±43.9	181±35.3	172±37.4

(1) Abbreviations are in Table 4.4.

(2) Mean ± S.D.

Experimental Design

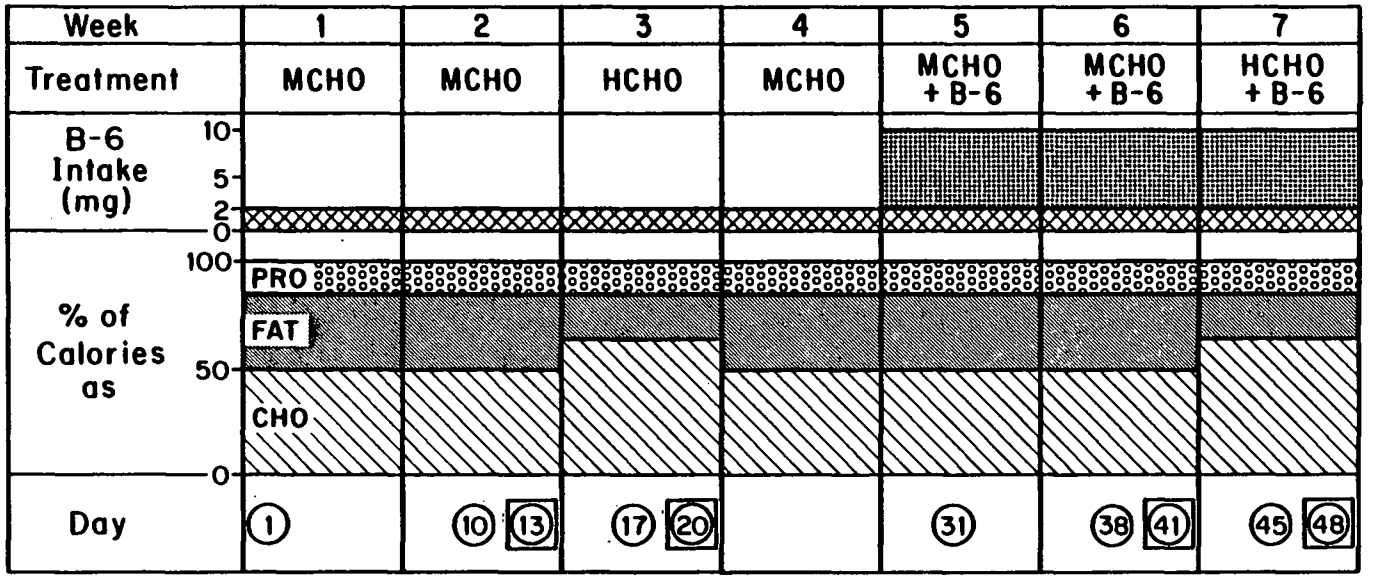

Figure 4.1. Experimental Design. MCHO = moderate carbohydrate diet (49%) (2.3 mg B-6); HCHO = high carbohydrate diet (63%) (2.4 mg B-6); +B-6 = supplementation of 8 mg of pyridoxine added; day = day during metabolic study on which exercise testing occurred as well as days blood was drawn; ☐ represents exercise testing; represents days blood was drawn.

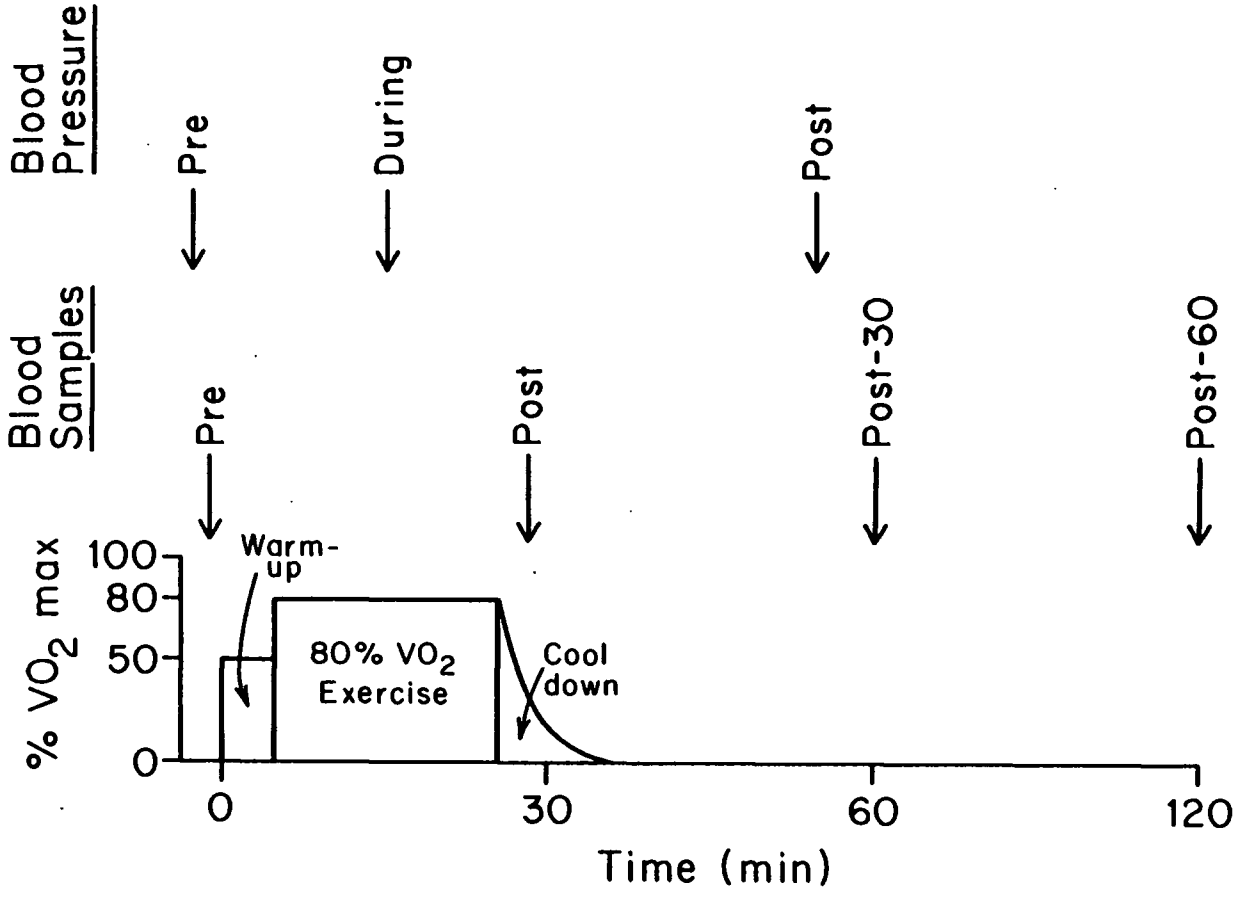

Figure 4.2. Exercise procedure. Exercise consisted of 5 minutes of warm-up followed by 20 minutes at 80% $\dot{V}O_2$max followed by a 5-10 minute cool-down period. Times for blood draws and blood pressures are marked.

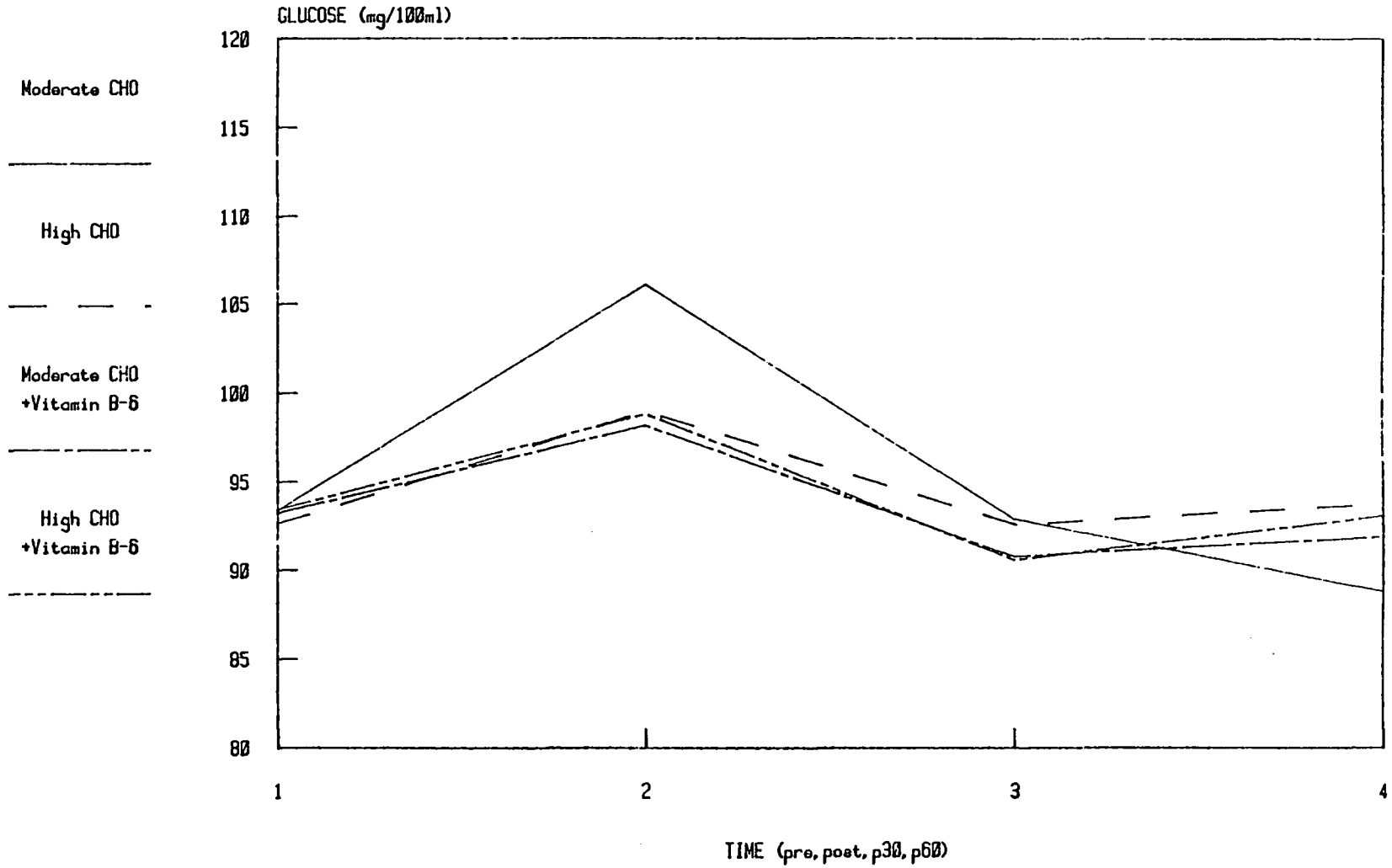

Figure 4.3. Glucose: changes over time with exercise. Diet x time interaction, all groups combined. Abbreviations for diet are given in Figure 4.1. The change in level of glucose with the MCHO diet was significantly different than that for the other diets.

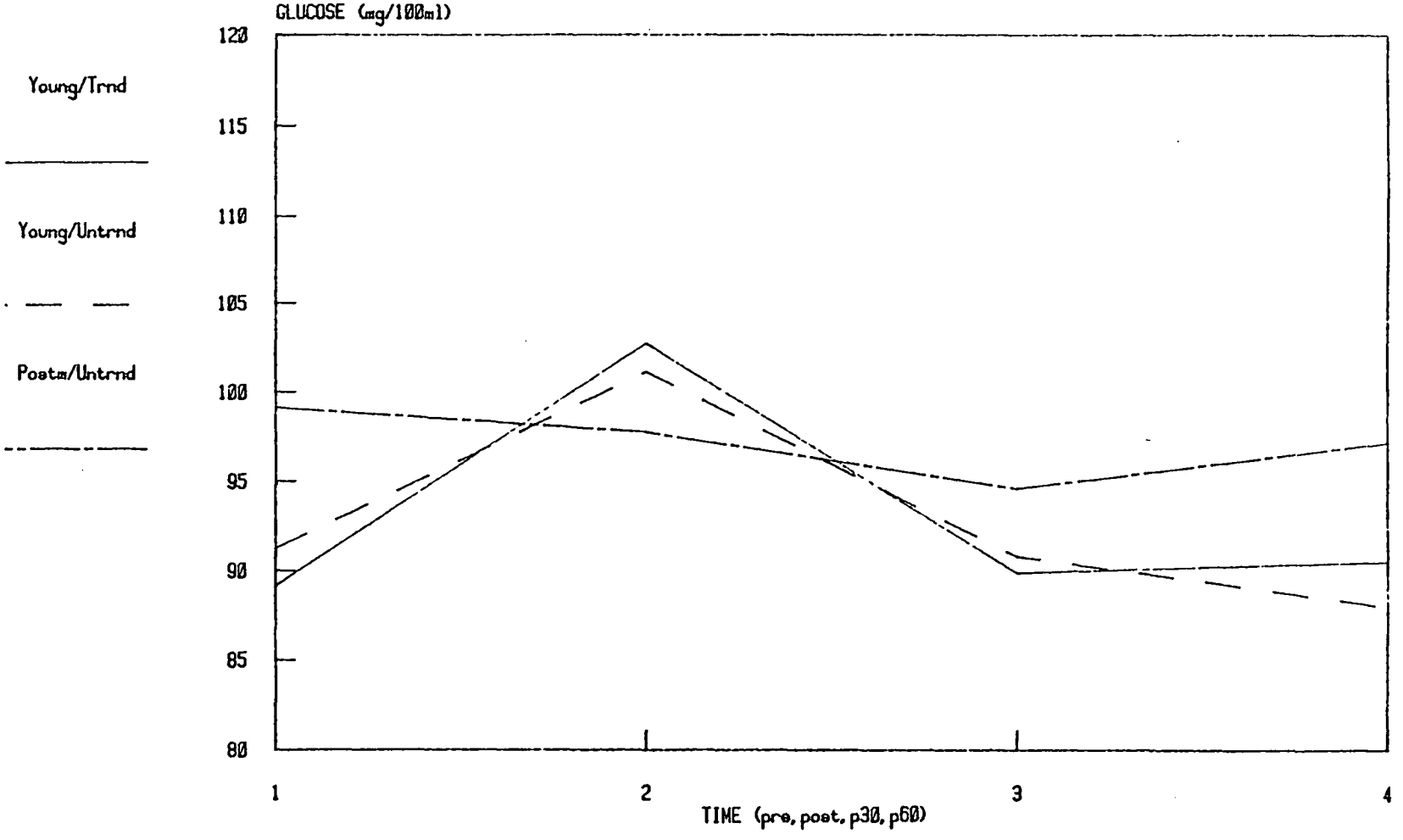

Figure 4.4. Glucose: changes over time with exercise. Time x group interaction, all diets combined. Abbreviations for diet are given in Figure 4.1. The changes in glucose levels for the postmenopausal/untrained group were significantly different than that for the two young groups.

Figures 4.5(a-d). FFA: changes over time with exercise. Time x group x diet interactions. Abbreviations for diets are given in Figure 4.1. Significant differences are given in the text.

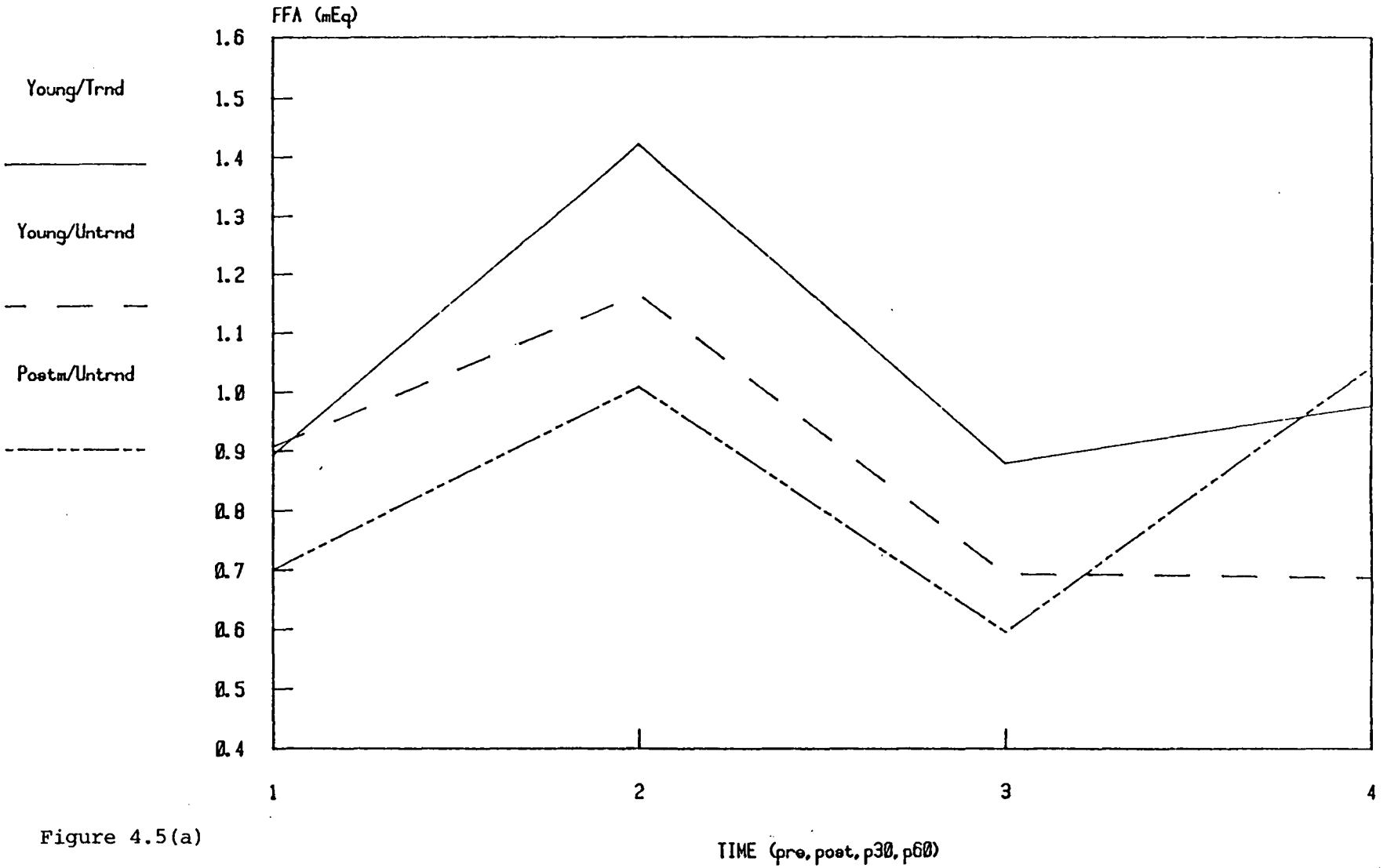
FFA (mEq)
1.6
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
Young/Trnd
Young/Untrnd
Postm/Untrnd
1
2
3
4
TIME (pre, post, p30, p60)

Figure 4.5(a)

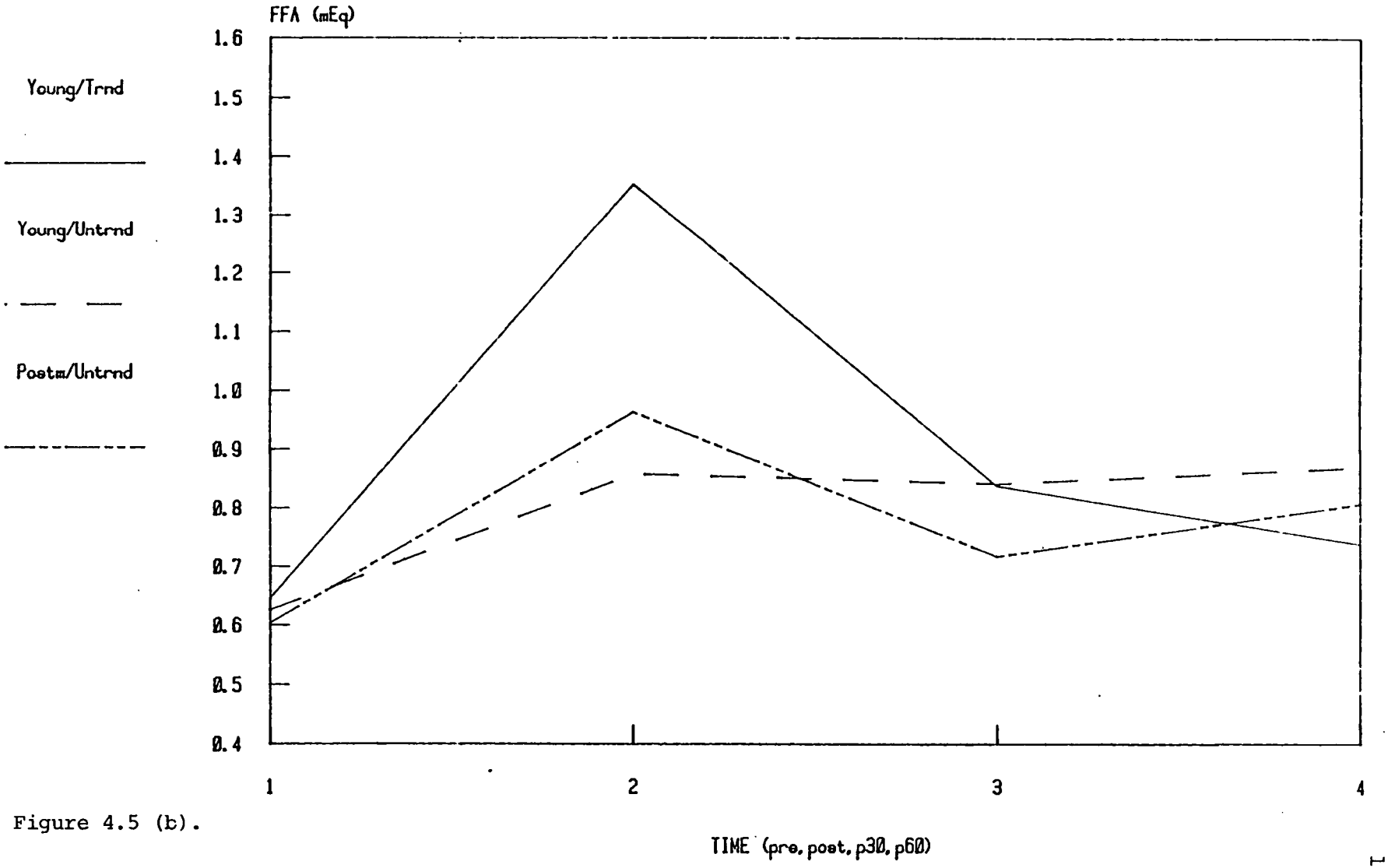
FFA (mEq)
1.6
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
Young/Trnd
Young/Untrnd
Postm/Untrnd
1
2
3
4
TIME (pre, post, p30, p60)

Figure 4.5 (b).

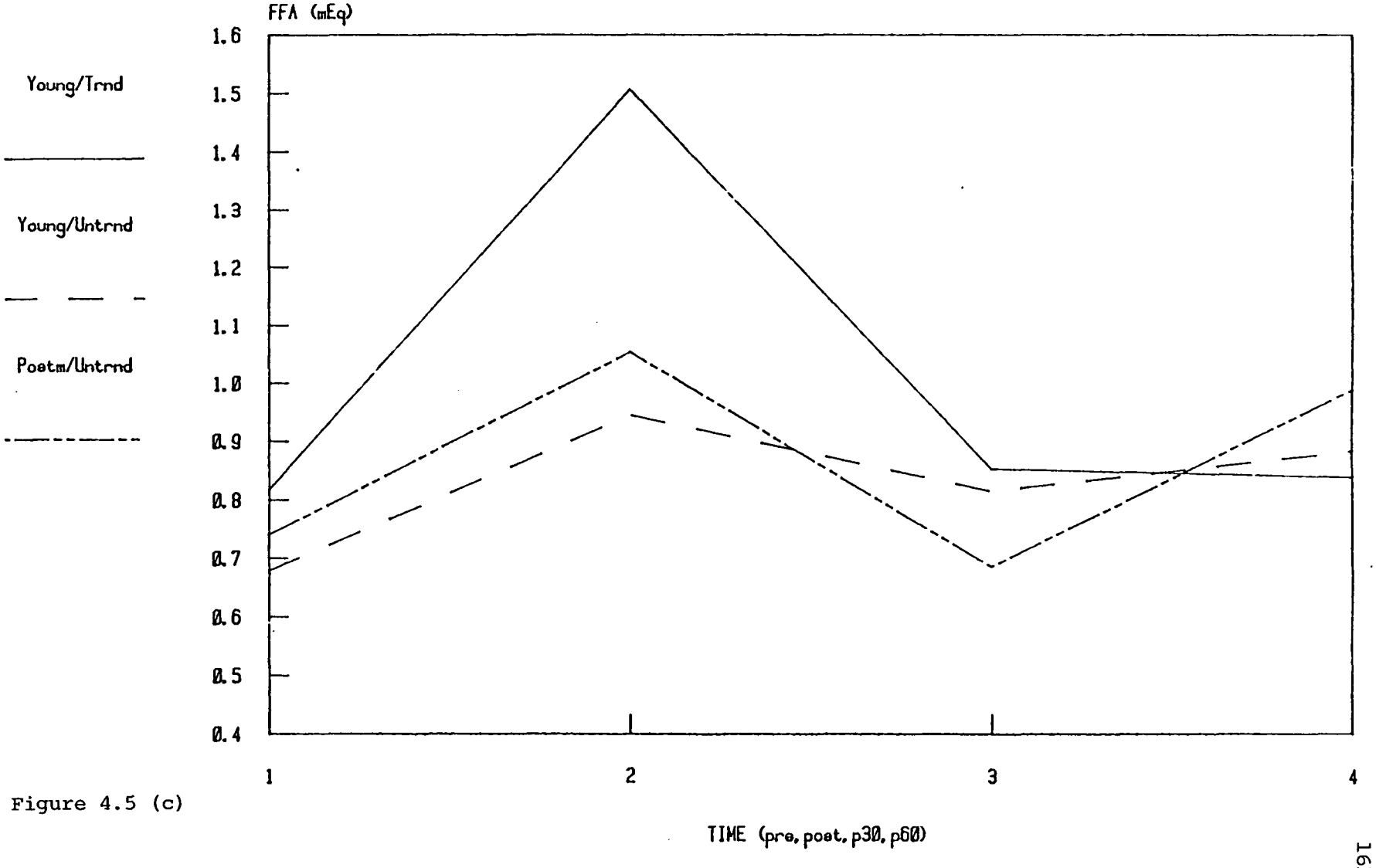
FFA (mEq)
1.6
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
Young/Trnd
Young/Untrnd
Postm/Untrnd
1
2
3
4
TIME (pre, post, p30, p60)

Figure 4.5 (c)

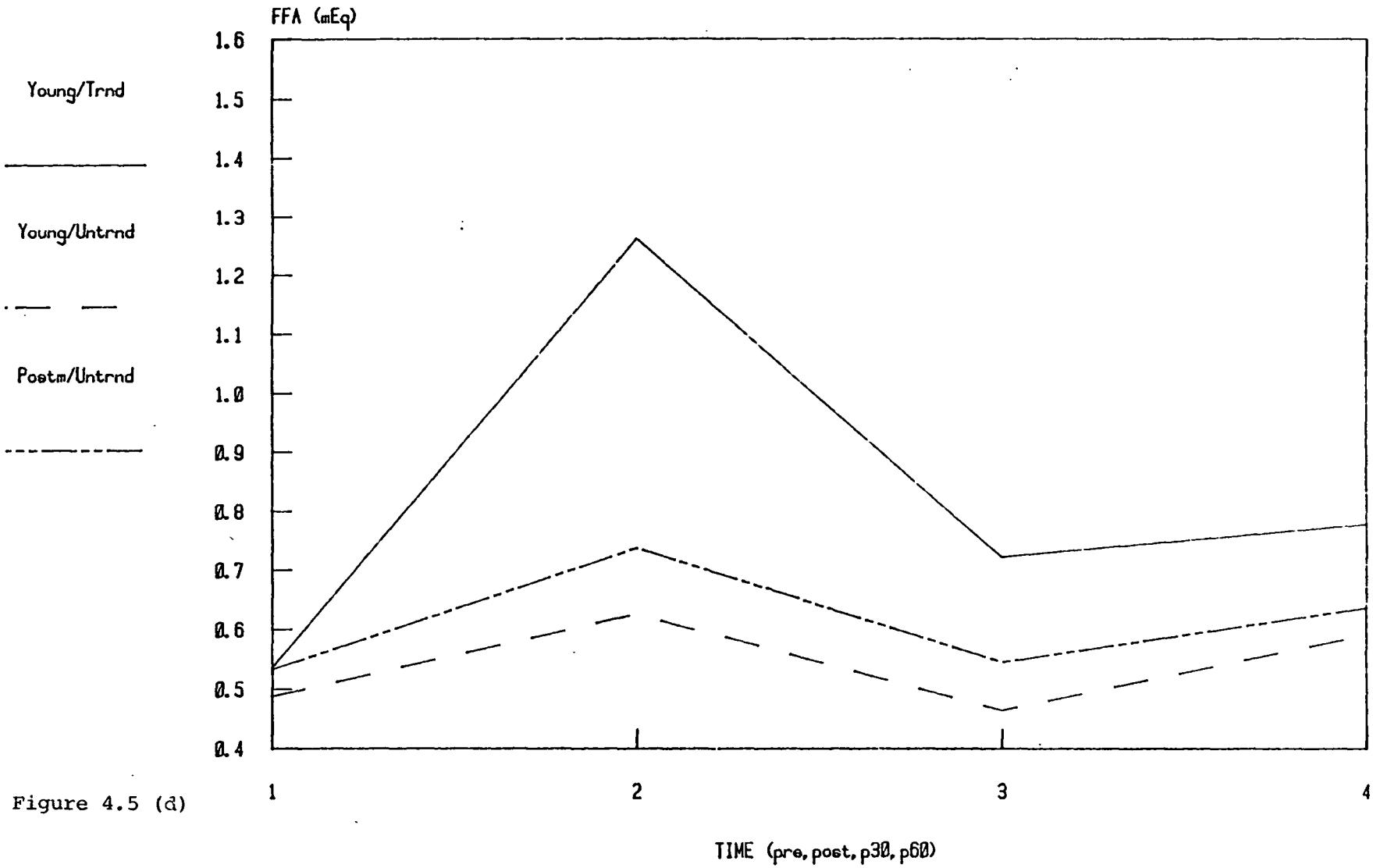
FFA (mEq)
1.6
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
Young/Trnd
Young/Untrnd
Postm/Untrnd
1
2
3
4
TIME (pre, post, p30, p60)

Figure 4.5 (d)

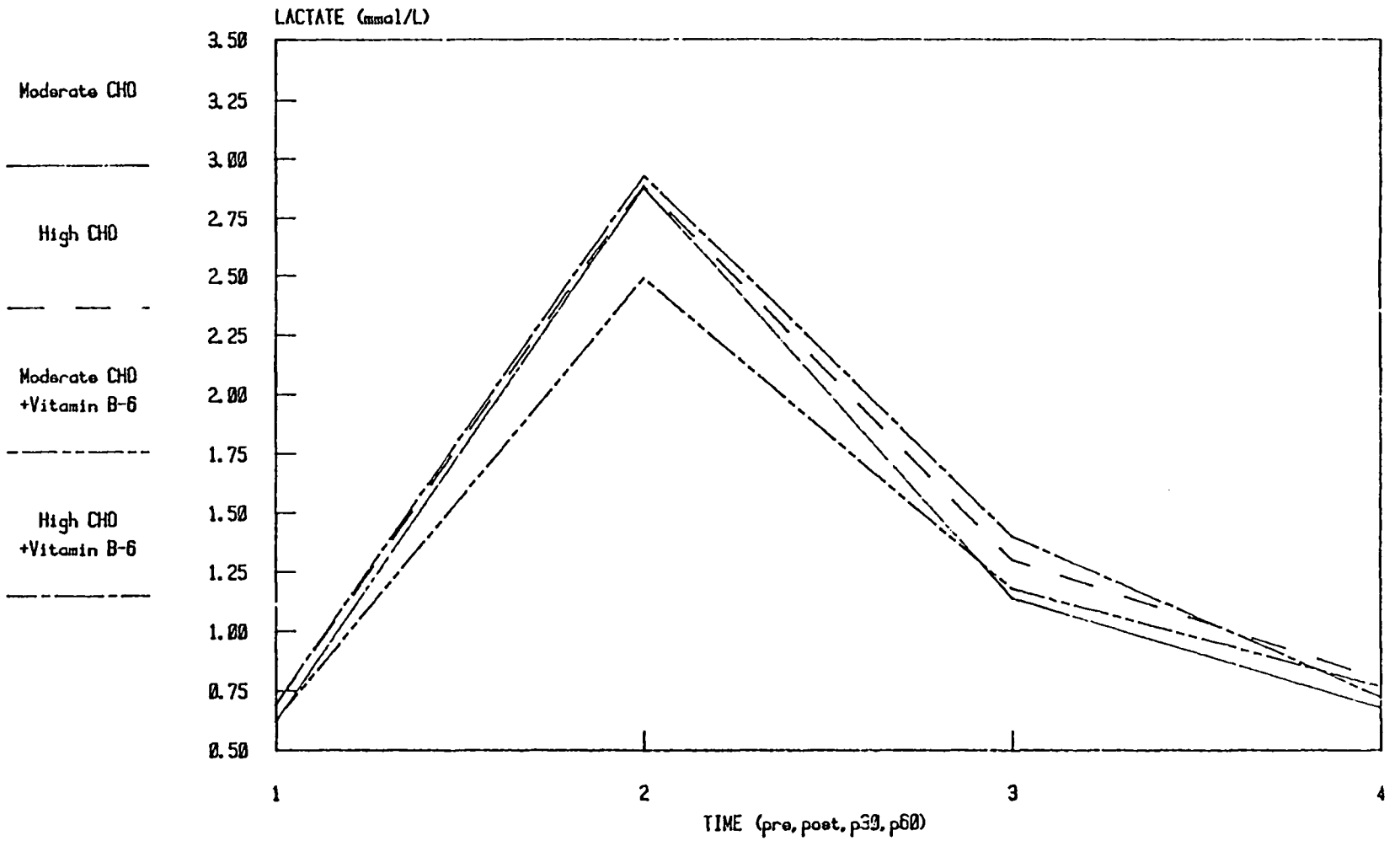

Figure 4.6. Lactate: changes over time with exercise. All groups combined. Time was a significant main effect. No significant interaction was observed. Abbreviations for the diets are given in Figure 4.1.

CHAPTER V

CHANGES IN QT_c IN WOMEN DURING EXERCISE FED TWO CARBOHYDRATE DIETS WITH AND WITHOUT VITAMIN B-6 SUPPLEMENTATION [1,2]

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ABSTRACT

Pyridoxal 5'-phosphate, the active form of vitamin B-6 (B6) is an essential cofactor in glycogenolysis. Thus, the changes in B6 status may alter fuel substrates available for the heart and working muscles during exercise (EX). We investigated whether dietary carbohydrate (CHO) and B6 supplementation could alter cardiac function as determined by QT intervals and ST segment depressions in 5 young/trained (YT); 5 young/untrained (YU); and 5 postmenopausal/untrained (PU) women who were alternately fed 4 dietary treatments. The diets were administered in the following sequence: a moderate CHO (49%) (MCHO) for 2 weeks; a high CHO (64%) (HCHO) for 1 week; return to baseline MCHO for 1 week; MCHO+B6 for 2 weeks, and HCHO+B6 for 1 week. The non-supplemented diets included 2.3-2.4 mg B6 while the supplemented diets included an additional 8 mg of vitamin B6. Training status was established by measuring $\dot{V}O_2$ max. EX responses were determined for each dietary treatment. The EX involved 20 minutes of exercise on a cycle ergometer at 80% $\dot{V}O_2$ max. ECG tracings were monitored before, during, and after EX and QT corrected (QT_c) was measured at rest and 80% maximal heart rate (MHR). QT_c was measured using Bazett's formula. ST segment depressions were also measured at 80% MHR. No significant ST segments were observed on any dietary treatment during EX. ANOVA of QT_c showed significant main effects for diet, group, and time but showed time x group the only significant interaction. The MCHO diet showed significantly longer QT_c intervals than the other three diets. For all diets QT_c increased from rest to 80% MHR in YT and PU but decreased or remained unchanged in YU. The change from rest to 80%

MHR was significantly different between the groups, with the YT and PU being different than the YU group. For the experimental design used, cardiac function as measured by QT_c intervals, showed that diet (B6 and/or CHO) can alter this function in women during exercise.

INTRODUCTION

Recent popular literature (Gruberg and Raymone, 1981) has rekindled the interest in vitamin B-6 and cardiac function. In the late 50's and early 60's research done by Olsen (1954) and Seronde (1960) indicated that vitamin B-6 deficiency could cause cardiac lesions in rats. In addition, Levy et al. (1959) and Boxer et al. (1957) examined coronary patients and found that they were low in whole blood vitamin B-6. The role vitamin B-6 may play in cardiac function, other than that for protein metabolism, is as a cofactor in reactions related to fuel metabolism. Vitamin B-6 is essential for both gluconeogenesis and glycogenolysis. Pyridoxal 5'-phosphate, the active form of vitamin B-6, is essential for the glycogen phosphorylas enzyme (Helmreich and Klein, 1980) which catalyzes both the breakdown of glycogen in the liver for blood glucose as well as in heart muscle for energy. Pyridoxal 5'-phosphate also plays an important role in the transamination of amino acids for the alanine-glucose cycle which also contributes to glucose availability during exercise (Lemon and Nagel, 1981). Therefore, the availability of vitamin B-6 may alter the fuel available to the heart during exercise.

Recent research (Leklem and Shultz, 1983) has shown that during exercise plasma pyridoxal 5'-phosphate levels increase. This may be due to the release of pyridoxal 5'-phosphate from the muscle storage reservoir, glycogen phosphorylase. Supplementation of vitamin B-6 has also been shown to increase the glycogen phosphorylase enzyme in the muscle (Black et al., 1977, 1978). This increase in the glycogen

phosphorylase enzyme may speed the breakdown of glycogen in the muscles for energy and alter the fuels circulating in the blood. This would in turn alter the fuels available to the heart muscle for energy during exercise.

Several studies have been done which indicate that women may have low vitamin B-6 status (Chrisley and Driskell, 1979; Gutherie and Crocetti, 1983; Vir and Love, 1980). Especially at risk are the young women who may be using oral contraceptives, are pregnant or lactating, and elderly women who may have marginal dietary intake of vitamin B-6 as well as impaired absorption of the vitamin. Chronic dieting, which is often done by women, may also contribute to low dietary intakes of vitamin B-6 as well as impair cardiac function. Isner et al. (1979) and Van Itallii and Yang (1984) have identified catabolism of cardiac mass in individuals who have participated in rapid weight loss using liquid protein diets. In these patients, death occurred due to ventricular tachydysrhythmia, elongated QT_c intervals were observed prior to death.

Research in our laboratory has indicated that alterations in diet (level of carbohydrate) can influence cardiac function during exercise in men as measured by QT_c intervals and ST segments (Goulard, 1982; Seimann, 1982). Other researchers (Riley et al., 1972; Rochlin and Edwards, 1954; Simonson and Keys, 1950) have also found that carbohydrate in the form of a glucose load or a high carbohydrate meal can alter cardiac function during exercise ½ to 1 hour after the meal.

In this research project we have explored the cardiac function of women, both trained and untrained and young and postmenopausal, to see if level of dietary vitamin B-6 and/or level of dietary carbohydrate would alter cardiac function during exercise. Cardiac function was monitored by measured QT_C intervals and ST segment depressions at rest and during exercise on each of the dietary treatments. Controlled dietary metabolic conditions were imposed to control as many factors as possible which could influence the outcome of the results. This study was part of a larger project examining vitamin B-6 metabolism and fuel metabolism in women during exercise.

METHODS

Experimental Design

This seven week metabolic study was designed to include four dietary treatments, each of which were followed by an exercise testing period (Figure 5.1). The diets were administered in the following sequence: a moderate carbohydrate (MCHO) diet for 2 weeks; a high carbohydrate (HCHO) diet for 1 week; return to the MCHO diet for 1 week; a MCHO + vitamin B-6 (MCHO+B-6) diet for 2 weeks and a HCHO + vitamin B-6 (HCHO+B-6) diet for 1 week. The first week of the MCHO diet was designed to normalize all subjects to the same dietary level of vitamin B-6 and allow subjects time to adapt to the diet and routine of the study. The administration of the MCHO diet for one week after the HCHO diet was designed to assure no confounding overlap of the dietary treatments and to have the premenopausal subjects at the same relative point in their menstrual cycle during the last two exercise testing sessions as experienced in the first two exercise sessions.

Subjects

Fifteen healthy female volunteers were recruited for one of three groups, each with an n=5; young/trained, young/untrained, and postmenopausal/untrained using the criteria of age and aerobic training status. Physical characteristics of each of the groups are given in Table 5.1. Subjects in the young/trained and young/untrained

groups were between the ages of 20-30 and the postmenopausal/untrained women were between the ages of 49-61 years. Untrained was defined as performing less than 40 minutes of aerobic activity during a one week period with exercise occurring no more than two days a week. Trained was defined as performing a minimum of 120 minutes of aerobic activity during a one week period with exercise occurring a minimum of three days a week. Training level was confirmed prior to the beginning of the study by measuring $\dot{V}O_2$ max on a Monark cycle ergometer using the procedure described by Wilmore and Costill (1974). The classified level of fitness (trained or untrained) had to have been maintained for at least six months prior to beginning the study. Maintenance of the classified level of fitness was required throughout the experimental period. To monitor this, subjects were required to report the duration, intensity, and frequency of all aerobic exercise in a daily journal. Subjects were also required to provide researchers with the following information prior to beginning the study: a completed health history questionnaire establishing normal health status, a three day dietary record and an exercise history questionnaire. The following criteria also had to be met before participation began: normal blood chemistry screen, normal cardiac function as determined by electrocardiograph tracings at rest and maximal heart rate, normal xylose absorption test to assess carbohydrate absorption (means + S.D. = 31.6±8.19%), doctor's approval for participation in the study, blood pressure within normal limits at rest and during exercise, no vitamin supplements used for at least four weeks prior to beginning study. No

nicotine, oral contraceptives, or estrogen replacement therapy could be used during the study. Subjects were familiarized with the experimental procedure and signed informed consent forms. This study was approved by the Oregon State University Committee for Protection of Human Subjects.

Dietary Treatments

Four dietary treatments were administered in this study, two which were supplemented with vitaminB-6 and two which were not supplemented (Figure 5.1). The specific foods and amounts served for each meal and each diet are given in Table 5.2. The same foods were fed each day of the respective periods. The two non-supplemented treatments consisted of a moderate carbohydrate (MCHO) diet (49% carbohydrate; 35% fat; 16% protein) and a high carbohydrate (HCHO) diet (64% carbohydrate; 22% fat; 16% protein). The vitamin B-6 level of the unsupplemented diets were maintained at 2.3 mg for the MCHO and 2.4 mg for the HCHO diet. The supplemented diets repeated the MCHO and HCHO dietary treatments but included an additional 8.0 mg of pyridoxine (vitamin B-6) given as oral solution of pyridoxine hydrochloride with the breakfast meal. The level of supplementation was low enough to provide excess vitamin B-6 without introducing a pharmacological effect.

All food served in the metabolic study was purchased in bulk from the same lot when possible. Foods which could not be purchased this way were thoroughly mixed prior to beginning the study and

frozen in aliquots for daily use. This was done to assure uniformity of the nutrient content of the diets. Each food item fed was carefully weighed before being served to each subject. Weekly food composites were made and analyzed for vitamin B-6. This was done to assess the actual amount of the vitamin being consumed by each subject and to determine if storage and food preparation were altering the vitamin content of the food. Little alteration of the vitamin B-6 content of the diets occurred during the experimental period.

All subjects were placed on a 1800-2000 kcal level at the beginning of the study. Each person was weighed daily and calories adjusted as necessary to assure that weight was maintained throughout the study. Additional calories were added in the form of carbohydrate or fat containing foods to keep the carbohydrate/fat ratio the same for the MCHO and HCHO diets. These foods contained essentially no vitamin B-6. All meals were prepared in the Foods and Nutrition Department metabolic kitchen. No alcoholic beverages were allowed throughout the study and no caffeinated beverages were allowed the day before and the day of the exercise testing.

Exercise Procedure

A maximal exercise test was performed on each subject prior to beginning the study to establish maximum heart rate and $\dot{V}O_2$ max. Exercise testing was done on a Monark cycle ergometer. The subject's heart rate and cardiac cycles were monitored continually during the test using an electrocardiograph (ECG), significant irregularities

disqualified the subjects the subjects from the study. Blood pressures were monitored before and after exercise. Rules for termination of exercise tests as outlined by the American College of Sports Medicine (1980) were followed.

During each of the experimental exercise testing sessions, subjects were exercised on a Monark cycle ergometer at 80% of their $\dot{V}O_2$ max for 20 minutes (Figure 5.1). The specific procedure for each exercise session included five minutes of warm-up followed by 20 minutes of exercise at 80% $\dot{V}O_2$ max and a 5-10 minute cool-down period. The experimental design was such that each subject was exercised on the 5th or 6th day of the week of each dietary treatment. This was done to allow 5 or 6 days to adapt to the diet before each exercise testing session. Subjects were monitored on an electrocardiograph before, during, and after the exercise period. Blood pressures were taken pre, during, and post exercise using a sphygmonanometer. All exercise testing was done in a fasted state in the morning. All exercise was performed at a moderate temperature of 20-24°C and at a relative humidity of 48% (range = 42-64%).

ECG Analysis

ECG tracings were taken during rest, exercise, and recovery at normal paper speed (25 mm/sec.). A CM5 ECG exercise lead system was used. Using Basett's (192) formula, (QT_c = QT interval/$\sqrt{\text{R-R interval}}$) QT_c was calculated at rest and at 80% of maximal heart rate (MHR). Five consecutive QT intervals were read at both rest and 80% MHR.

ST segment depressions were also measured at 80% MHR. The ST segment depression criteria used was a horizontal or downsloping ST segment of 1 mm or more for at least 0.08 seconds. More than one ST segment depression needed to occur in the set of ECG configurations read before a ST segment depression would be recorded.

Analyses

Plasma pyridoxal 5'-phosphate was determined by a modified method of Chabner and Livingston (1970). Interassay variation of a plasma control sample (n=22) was 6.5%.

Daily Procedures

Subjects followed a number of daily procedures to assure compliance with the metabolic study, assist in monitoring subjects well-being and assure that accurate records were kept. These daily procedures included keeping a journal of activities (minutes exercised, non-caloric beverages consumed, medications taken, menstrual periods); recording weight; returning unused portions of margarine, salad dressing, or honey used to increase calories.

Statistical Analysis

A three-way split plot partially blocked ANOVA design with groups and diets as whole plot factors and time as the split plot factor was used in the analysis of the QT_c data. When significant

interactions occurred main effects were no longer of interest. The significant interactive effects were examined by performing hypothesis testing on several contrasts of treatment means where contrasts is defined as a comparison involving two or more treatment means (Neter and Wasserman, 1974). Contrasts to be tested were specified in advance of data collection. Least significant difference was the test criterion used in testing multiple comparisons (Steel and Torrie, 1980).

For the purpose of hypothesis testing "a" designed group, "b" designated diet, and "c" represented time. These letters are subscripted to indicate the specific group (1=young/trained; 2=young/untrained; 3=postmenopausal/untrained), diet (1=MCHO; 2=HCHO; 3=MCHO+B-6; 4=HCHO+B-6) and time (1=resting heart rate; 2=80% maximal heart rate). For example, $a_1b_1c_1$ designates the mean of the five subjects in group 1, on diet 1, at time 1. When groups were combined (n=15) "b" was omitted. Interaction hypotheses tested were: $[a_1c_2 - a_1c_1]$, $[(a_1c_2 - a_1c_1) - (a_2c_2 - a_2c_1)]$. These hypotheses were tested for all groups. Main effect hypothesis tested was $b_1 - b_2$. This hypothesis was repeated for each diet combination. Results were determined to be significant if the p-value was less than the critical value of $\alpha = .05$.

RESULTS

We examined the effect of diet (MCHO, HCHO, MCHO+B-6, HCHO+B-6) on QT_c intervals in young/trained, young/untrained, and postmenopausal/untrained women at rest and during exercise at 80% MHR. Results of the ANOVA (Table 5.3) showed significant main effects for group ($p=.0500$), diet ($p=.0060$) and time ($p<.0001$). However, because there was a significant interactive effect of time x group ($p=.0014$) the main effects of time and group were not examined. No significant three way interaction of group x time x diet occurred so we examined the group x time interaction by averaging over diet. Examination of the group x time interaction involved examining contrasts of means and differences in means. In testing the difference between rest and 80% MHR during exercise within each group, contrasts of means showed that the QT_c interval for the young/untrained group was significantly different than that of the young/trained and postmenopausal/untrained groups. The young/untrained showed a non-significant decrease in QT_c (seconds) from rest to 80% MHR (rest=0.411±.020; 80%=0.406±.016) while the young/trained and postmenopausal/untrained showed a significant increase in QT_c (seconds) from rest to 80% MHR. The young/trained increased from a resting value of 0.389±.017 to a 80% MHR value of 0.422±.019 while the postmenopausal/untrained mean increased from a resting QT_c of 0.410±.021 to 0.434±.021 at 80% MHR.

Contrasts of differences between means showed that the change from rest to 80% MHR was significantly different between the groups. The change seen in QT_c from rest to 80% MHR was 0.033 for the young/trained; -0.005 for the young/untrained and 0.024 for the

postmenopausal/untrained. The young/trained and postmenopausal/untrained groups were again significantly different than the young/untrained in their QT_c response to exercise. The group x time data is given in Table 5.2.

In examining the significant main effects of diet, the MCHO diet showed significantly longer QT_c intervals than the other three diets, regardless of group or time. The QT_c for the MCHO+B-6, HCHO, and HCHO+B-6 were all very similar, 0.407±.021, 0.408±.024, 0.410±.023, respectively. The QT_c interval for the MCHO diet was 0.422±.022. These means represent the three groups (3x5) combined across the two time periods on each of the diets, therefore, n=30 (15x2).

ECG complexes on which QT intervals were measured were also examined for ST segment depression. Criteria for ST segment depression was 1 mm depression for 0.08 seconds measured during exercise at 80% maximal heart rate. No ST segment depressions, which met the above criteria, were observed in any group for any diet or time.

Resting plasma pyridoxal 5'-phosphate levels were examined for each group during each dietary treatment. On the non-supplemented diets the mean (±S.D.) pyridoxal 5'-phosphate values for the young/trained, young/untrained, and postmenopausal/untrained were 47.5±.7 nM, 59.5±2.1 nM and 45.5±2.1 nM, respectively. The supplemented diets produced mean (+S.D.) plasma pyridoxal 5'-phosphate values of 189.5±21.5 nM for the young/trained; 206.5±5.0 nM for the young/untrained and 184±4.2 nM for the postmenopausal/untrained women. The two supplemented and the two non-supplemented diets produced similar

plasma pyridoxal 5'-phosphate levels, regardless of the carbohydrate level. Before the study began, blood was drawn and the plasma was analyzed for pyridoxal 5'-phosphate. This was done to establish baseline data for each subject and to assess the amount of vitamin B-6 being provided by their free living diet. The pre-study plasma pyridoxal 5'-phosphate values for the young/trained, young/untrained, and postmenopausal/untrained women were 42.3±14.2, 35.4±14.8, 29.6± 12.4 nM, respectively.

DISCUSSION

Examination of cardiac function, as measured by QT_c intervals, showed that for the experimental design used, diet can alter cardiac function in women during exercise. It is important to consider that the changes observed were seen under controlled metabolic conditions. Each diet was fed long enough to stabilize plasma vitamin B-6 levels before exercise was engaged in. All subjects received a diet which was slightly more than the RDA for vitamin B-6 during the first two exercise sessions and supplemented with 8 mg vitamin B-6 during the last two exercise sessions. Subjects were fed the same foods daily and all foods were weighed to the tenth of a gram. All exercise was done after an overnight fast. The results show that cardiac function as measured by QT_c, was significantly different on the supplemented diets and/or the high carbohydrate diet with no supplementation than on the MCHO diet. This may be due to the fact that with increased vitamin B-6 more glucose is available to the cardiac muscle during exercise. In addition, vitamin B-6 may be further increasing glucose availability via its involvement in glycogenolysis. The carbohydrate effect may have been due to the increased glycogen storage which may occur when a high carbohydrate diet is fed (Sherman and Costill, 1984). Because the MCHO diet was the first dietary treatment administered, heart rates at rest were examined to see if anxiety could have contributed to the alteration in QT_c intervals observed on this diet. However, resting heart rates for each subject were similar for each of the dietary treatments.

Yu et al. (1949) examined the effect of exercise on QT_c intervals in healthy adults during exercise and found that QT_c intervals significantly increased from rest to exercise. Two of our groups, the young/trained, and the postmenopausal/untrained, demonstrated a significant increase in QT_c from rest to exercise, while the young/ untrained group showed a slight decrease. The explanation of this is uncertain.

Others (McHenry et al., 1981; Riley et al., 1972) have shown that a glucose load prior to exercise can produce altered cardiac function as demonstrated by the reversal of the polarity of the T wave, decrease in T wave area, and amplitude and horizontal ST segment depressions. Since we exercised subjects after an overnight fast and after they had been on the diet for 5 to 6 days it is unlikely that the diet caused an acute alteration of cardiac function comparable to those seen with a glucose load.

The literature reports that resting QT_c intervals increase with age (Simonson et al., 1962). Our results support these findings, the postmenopausal/untrained group had longer QT_c intervals at rest and during exercise than the two young groups. None of the groups demonstrated mean QT_c intervals longer than 0.440, the critical point often used by researcher to designate excessively long QT_c intervals.

ST segments showed no significant alterations during exercise as a result of the dietary treatments in the three groups. The fact that no ST segment depressions were observed with the diets fed may have been due to the fact that 1) healthy subjects were recruited, and

2) alterations in cardiac function due to diet may be too subtle to manifest themselves in significant ST segment depressions.

Since women may be at greater risk of being vitamin B-6 deficient by consuming diets marginal in vitamin B-6 as well as low in calories, they may be prone to abnormal cardiac function during exercise. At the present time no research is available on cardiac function during exercise in women on low vitamin B-6 intakes. However, many popular weight loss diets, such as the liquid protein diets, may increase the need for vitamin B-6 for protein metabolism at a time when there is a decreased intake of the vitamin (Canham et al., 1969). Pringle et al. (1983) monitored QT_c intervals in 11 obese women on a therapeutic starvation diet and found significantly prolonged QT_c intervals after eight weeks of dieting. The QT_c intervals were elongated enough to contribute to two cardiac arrests in one of the patients. Linet et al. (1983) also fed a very-low-calorie/high protein diet for 15 weeks to 14 overweight women and observed no alteration in cardiac function. They attributed lack of cardiac abnormalities to the numerous vitamin and mineral supplements they fed, including 4 mg of vitamin B-6. Our results showed normal QT_c intervals in all groups during exercise receiving an adequate vitamin B-6 diet and calories. All subjects maintained their weight during the study. However, the groups did respond differently to the dietary treatments with increased carbohydrate and/or increased vitamin B-6 producing a shorter QT_c. Therefore, alteration of cardiac function as determined by mean QT_c intervals, can result from dietary carbohydrate manipulation and vitamin B-6 supplementation but effects seem to depend on age and level of training.

In summary, this study demonstrate that diet is capable of altering cardiac function as measured by QT_c intervals in women. The reason for this alteration is still unknown but may be related to the changes in fuel metabolism which can occur when either carbohydrate or vitamin B-6 are manipulated in the diet. Evidence that low calorie diets and/or starvation diets can contribute to cardiac muscle loss and cardiac abnormalities, combined with the fact that women may have lower vitamin B-6 status, should stimulate exploration of these two factors in high risk groups.

Table 5.1. Physical statistics for young/trained, young/untrained, and postmenopausal/untrained groups.

	Means ± S.D.	Range
Young/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 (%)	20.1±4.2 (1)	13-24
$\dot{V}O_2$ max (ml/kg/min)	40.5±3.7 (2)	37.1-46.5
Young/untrained (n=5)		
Age (yr)	24.4±3.2	21-29
Height (cm)	161.8±6.3	153.8-170.00
Weight (kg)	55.9±6.9	48.2-65.0
Body Fat (%)	20.4±1.5	18-22
$\dot{V}O_2$ max (ml/kg/min)	32.7±4.5	25.9-37.0
Postmenopausal/untrained (n=5)		
Age (yr)	55.8±4.8	49-61
Height (cm)	155.8±4.5	150.8-160.8
Weight (kg)	58.8±6.0	51.8-68.2
Body Fat (%)	27.0±3.4	22.31
$\dot{V}O_2$ max (ml/kg/min)	24.4±1.6	23.9-27.6

(1) Percent body fat determined by skinfolds measured at seven sites (Jackson et al., 1980).

(2) $\dot{V}O_2$ max determined by graded exercise testing using the Wilmore and Costill (1974) procedure on a cycle ergometer.

Table 5.2. 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*

*Variable amounts used to adjust calories. Amounts listed are average amounts used.

Table 5.3. Significant* F-test values from 3-way ANOVA

Variable	Factor (1)	DF	F-values	p-value*
QT_c	group	2,12	3.8688	.0500
	diet	3,36	4.8790	.0060
	time	1,36	37.2655	<.0001
	group x time	2,12	11.9853	.0014

* Only those interactions or main effects with p-values of <.05 are reported.

(1) Time = rest and 80% MHR
Group = young/trained, young/untrained, and postmenopausal/untrained (n=5 for each group)
Diets = MCHO, HCHO, MCHO+B-6, and HCHO+B-6

Table 5.4. QT_c intervals for group x time interaction (1).

	Seconds
Young/trained	
rest	0.389±0.017
80% MHR	0.422±0.019
Young/untrained	
rest	0.411±0.020
80% MHR	0.406±0.016
Postmenopausal/untrained	
rest	0.410±0.021
80% MHR	0.434±0.021

(1) Means combined across diets, n=20 (5 subjects in each group x 4 diets).

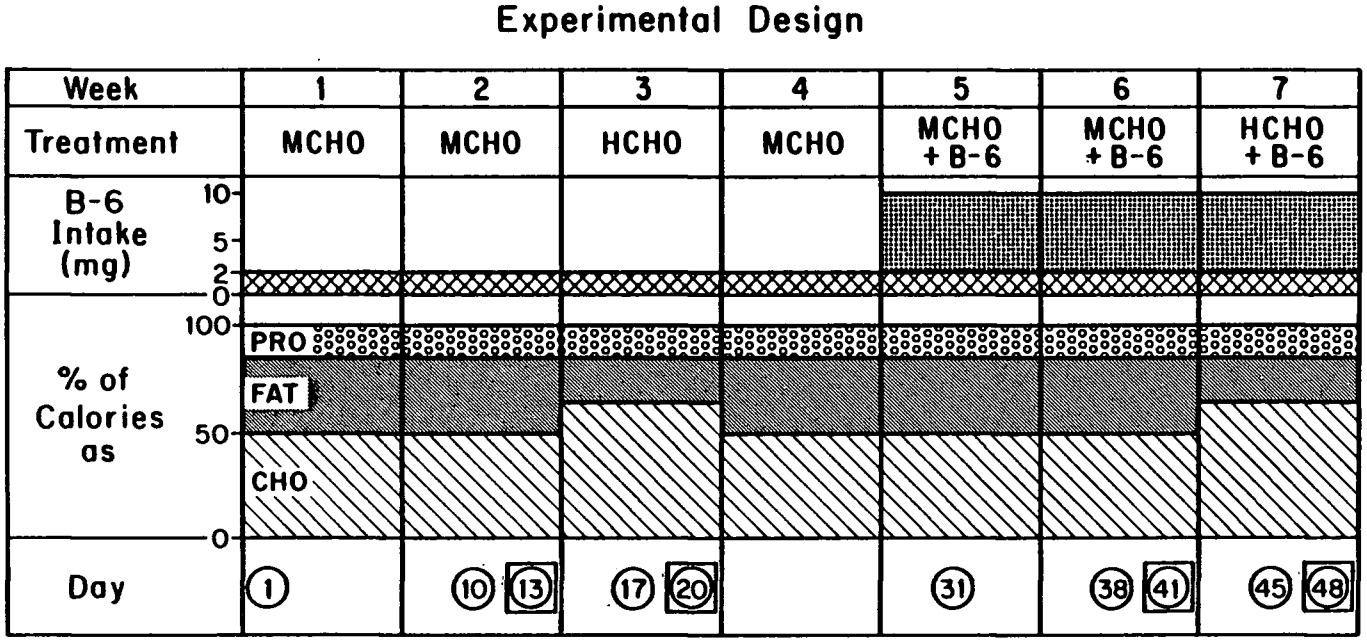

Figure 5.1. Experimental design. MCHO = moderate carbohydrate diet (49%) (2.3 mg B-6); HCHO = high carbohydrate diet (63%) (2.4 mg B-6); +B-6 = supplementation of 8 mg of pyridoxine added; day = day during metabolic study on which exercise testing occurred. ☐ represents exercise testing.

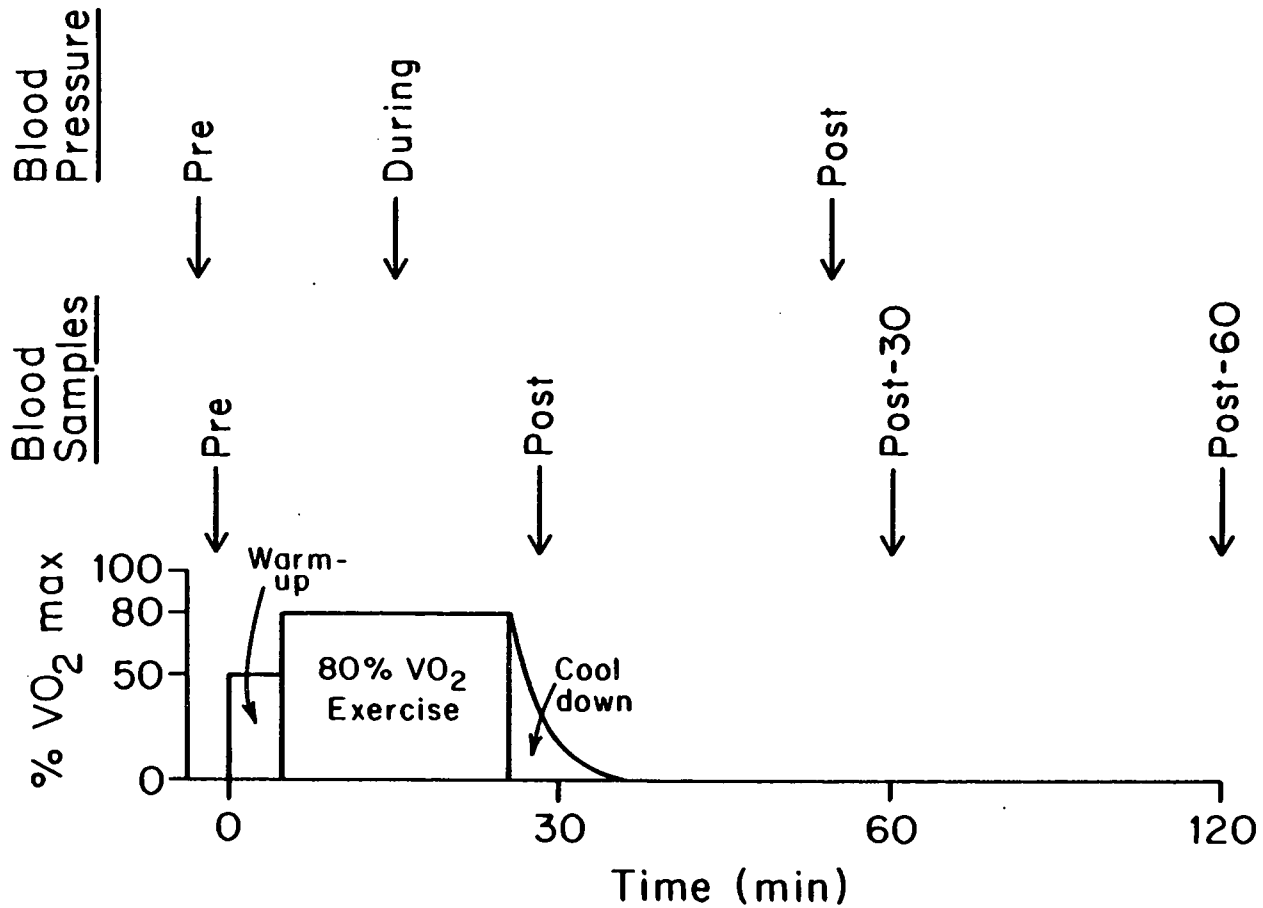

Figure 5.2. Exercise procedure. Exercise consisted of 5 minutes of warm-up followed by 20 minutes at 80% $\dot{V}O_2$ max followed by a 5-10 minute cool-down period. Times for blood draws and blood pressures are marked.

CHAPTER VI

CONCLUSIONS

The objectives of this study were to 1) determine the effect of exercise on vitamin B-6 metabolism in trained and untrained women, both young and postmenopausal, while being fed a moderate and high carbohydrate diet with and without vitamin B-6 supplementation, and 2) to determine the effect of two levels of dietary carbohydrate, with and without vitamin B-6 supplementation, on cardiac function and fuel metabolism during exercise in women.

In this study fifteen women were recruited for three groups: young/trained, young/untrained, and postmenopausal/untrained, each with an n=5. The young women were between the ages of 20-30 and the postmenopausal women were between the ages of 49-61. Subjects were classified according to training level based on measured $\dot{V}O_2$ max and exercise history. These subjects took part in a seven week metabolic study which was designed to include four dietary treatments. The dietary treatments were administered in the following sequence: a MCHO diet for 2 weeks, a HCHO diet for 1 week, a return to the MCHO for 1 week, a MCHO+B-6 diet for 2 weeks, and a MCHO diet for 1 week. At the end of each dietary treatment the subjects were exercised on a cycle ergometer for 20 minutes at 80% of $\dot{V}O_2$ max. Blood was drawn pre, post, post 30 and post 60 minutes of exercise and analyzed for plasma pyridoxal 5'-phosphate, plasma vitamin B-6, glucose, FFA, lactate, and albumin. During the exercise testing

sessions electrocardiograph measurements were taken and QT intervals and measured at rest and 80% MHR. Electrocardiograph tracings were also monitored for ST segment depressions at 80% MHR. The criteria for ST segments depressions was a horizontal or downsloping ST segment of 1 mm or more for .08 seconds. QT_c intervals were calculated using Bazett's (1920) formula. Daily 24-hour urine collections were taken and the day of exercise and the day after exercise were analyzed for 4-pyridoxic acid and urinary vitamin B-6.

A three-way split plot partially blocked ANOVA design with groups and diets as whole plot factors and time (of blood sampling or the urine collection) the split plot factor was used in the analysis of the data. When significant interactions occurred, main effects were no longer of interest. The significant interactive effects were then examined by performing hypothesis tests on the contrasts of treatment means or differences between treatment means.

The results of this study indicated that exercise does alter vitamin B-6 metabolism in women. Both plasma pyridoxal 5'-phosphate and vitamin B-6 significantly increased from pre to post and significantly decreased from post to post-60 for each of the diets. However, the magnitude of this change was larger on the supplemented diets as compared to the non-supplemented diets. This increase in plasma pyridoxal 5'-phosphate with exercise is similar to what has been reported in men during and after strenuous exercise (Hatcher et al., 1982; Leklem and Shultz, 1983; Munoz, 1980). The muscle glycogen phophorylase enzyme is a storage site for vitamin B-6,

which gives up it's stored vitamin only during times of starvation (Black et al., 1977, 1978). Leklem and Shultz (1983) hypothesized that exercise mimics starvation, thus simulating the release of pyridoxal 5'-phosphate from the glycogen phosphorylase enzyme. The significance of the release of pyridoxal 5'-phosphate from the glycogen phosphorylase enzyme during exercise is probably related to the role this vitamin plays in gluconeogenesis. During exercise the body depends on liver production of glucose to maintain blood glucose levels. The liver can produce glucose through glycogenolysis of it's stored glycogen (a vitamin B-6 dependent process) or through gluconeogenesis in which the alanine-glucose cycle contributes to glucose production (a vitamin B-6 dependent transamination process).

The effect of training and carbohydrate were also examined to determine if either contributed to alterations of vitamin B-6 metabolism during exercise. Our results showed that neither training nor carbohydrate significantly affect the metabolism of vitamin B-6 during exercise. The level of vitamin B-6 in the diet seemed to be the most significant factor contributing to the alteration of vitamin B-6 metabolism during exercise.

Urinary vitamin B-6 and 4-pyridoxic acid were also examined to assess whether exercise, carbohydrate, or age would alter the amount of these compounds excreted in the urine. The only factor which contributed significantly to alterations in 4-pyridoxic acid excretion was exercise. All groups showed a significantly greater excretion of 4-pyridoxic acid the day after exercise as compared to the day

of exercise. Urinary vitamin B-6 showed no significant change throughout the metabolic study, regardless of the factors introduced.

Examining the effect of the four dietary treatments on each group showed that the postmenopausal/untrained group had significantly lower glucose responses over time during exercise than either the young/trained or the young/untrained groups. This response in the postmenopausal women may indicate that the exercising muscles were taking up plasma glucose at a faster rate than could be met by the gluconeogenic processes of the body.

Changes in FFA with exercise indicated that age, training, and diet contributed to each group responding differently with the different diets. The factor which contributed the most to altered FFA metabolism between the groups during exercise was training. The young/trained group consistently had higher FFA during exercise, regardless of the diet served. Since the concentration of the FFA in the plasma during exercise is directly proportional to the uptake of FFA by the working muscles (Hagenfeldt, 1979), we examined the effect supplementation had on the peak FFA values reached during exercise with each diet. If supplementation causes increased glycogen breakdown, then one might expect lower FFA in the blood due to the fact that there is less need for FFA as a fuel source. It was observed that supplementation generally caused a decrease in peak exercise FFA levels reached by each group when comparing the MCHO diet to the MCHO+B-6 diet and the HCHO diet to the HCHO+B-6 diet. The effect carbohydrate had on peak FFA production during exercise

was also examined. FFA levels decreased in all groups when changing the diet from a MCHO to HCHO, regardless of the supplementation.

In examination of the lactate levels during exercise, neither diet, age, nor training contributed to variations in the response to exercise. The only significant response in lactic acid levels occurred over time with exercise. All groups demonstrated a significant increase in lactate levels from rest to post-exercise and a significant decrease from post-exercise to post-60 minutes of exercise, regardless of the diet administered. However, the highest lactate values were always seen on the HCHO+B-6 diet, while concurrently producing the lowest FFA values. This may indicate that the HCHO+B-6 diet contributed to more glucose being utilized for fuel by the working muscles as compared to the other three diets.

The relationship between diet and cardiac function has not been studied extensively. However, the death of 58 individuals during the years of 1977 and 1978, who were reducing on very-low-calorie liquid-protein-diets, has stimulated interest in this relationship (Van Itallie and Yang, 1984). Other investigators (Isner et al., 1979, 1983; Linet et al., 1983; Pringle et al., 1983) have been examining the cardiac function of individuals on semistarvation diets, restricted diets or very-low-carloie high-protein-diets and have reported mixed results. Several studies have also indicated that women may have low vitamin B-6 status (Chrisley and Driskell, 1979; Guthrie and Crocetti, 1983; Vir and Love, 1980). The role vitamin B-6 may play in cardiac function, other than that for protein metabolism, is as a cofactor in reactions related to fuel metabolism.

Vitamin B-6 is essential for both gluconeogenesis and glycogenolysis. The chronic dieting, mentioned above, may also contribute to low dietary intakes of vitamin B-6. Therefore, we examined the effect of diets which altered carbohydrate and vitamin B-6 levels in women during exercise. The results showed that the MCHO diet produced significantly longer QT_c intervals than the other three diets. This may be due to the fact that vitamin B-6 is improving the availability of glucose to the cardiac muscle during exercise as well as assisting in the glycogen breakdown in the cardiac muscle itself. The carbohydrate effect may have been due to the increased glycogen storage which may occur when a high carbohydrate diet is fed. The QT_c intervals were also significantly different for the groups during exercise, with the young/untrained demonstrating no change or a slight decrease from rest to 80% of MHR while the young/traine and postmenopausal/untrained demonstrated a significant increase in QT_c with exercise. The explanation for this is uncertain. However, it should be remembered that all mean QT_c intervals in the groups were within the normal limits. Electrocardiograph configurations were also examined for ST segment depressions, none were observed.

In summary, this study indicates that exercise can alter vitamin B-6 metabolism in women, but neither training, increased carbohydrate or age have an effect on this change. Our results also indicate that both carbohydrate and vitamin B-6 can alter fuel metabolism and cardiac function, as measured through QT_c intervals, during exercise in women. The fact that exercise may influence both the release of

vitamin B-6 from the muscle glycogen phosphorylase enzyme into the plasma and increase the excretion of 4-pyridoxic acid may be factors to consider in assessing the vitamin B-6 status of exercising women. The increased release of vitamin B-6 from the muscle on a diet supplemented with vitamin B-6 may indicate that the muscle enzyme concentration has increased, and may catalyze the increased breakdown of muscle glycogen. This increase in muscle glycogen would be unfavorable for an individual involved in endurance activities, since muscle glycogen needs to be spared. Finally, the fact that alteration in vitamin B-6 and carbohydrate can influence cardiac function may be of interest to those involved in monitoring women who are chronic dieters or using semistarvation diets.

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APPENDIX

APPENDIX I

COMPREHENSIVE DATA TABLES

STATISTICAL MODEL USED FOR 3-WAY ANOVA

$$Y_{ijk\ell} = \mu + \alpha_i + \delta_{i\ell} + \beta_j + \alpha\beta_{ij} + \theta_{ij\ell} + \gamma_k + \alpha\gamma_{ik} + \lambda_{ik\ell} + \beta\gamma_{jk} + \alpha\beta\gamma_{ijk} + \varepsilon_{ijk\ell}$$

μ = overall mean yield

α_i = effect of the ith group (3 groups) i = 1,2,3 a=3

β_j = effect of the jth diet (4 diets) j = 1,2,3,4 b=4

γ_k = effect of the kth time (4 times) k = 1,2,3,4 c=4

$\alpha\beta_{ij}$ = interaction of (group x diet)

$\alpha\gamma_{ik}$ = interaction of (group x time)

$\beta\gamma_{jk}$ = interaction of (diet x time)

$\alpha\beta\gamma_{ijk}$ = interaction of (group x diet x time)

$\delta_{i\ell}$ = random error of group ~ $N(0,\sigma_a^2)$

$\theta_{ij\ell}$ = random error of (group x diet) units ~ $N(0,\sigma_{ab}^2)$

$\lambda_{ik\ell}$ = random error of (group x time) units ~ $N(0,\sigma_{ac}^2)$

$\varepsilon_{ijk\ell}$ = random error of time, (time x diet) (time x diet x group) ~ $N(0,\sigma_e^2)$ (subject = 5 in each group; r=5; ℓ=1,2,3,4,5)

PLASMA VITAMIN B6 (nmoles/ml)

Individual Values for Each Diet and Time

	Subject #	pre	post	post30	post60
Young/Trained					
	3	.0570	.0618	.0576	.0551
	5	.0379	.0448	.0411	.0376
Moderate CHO	8	.0593	.0707	.0637	.0578
	10	.0644	.0732	.0700	.0613
	13	.0723	.0806	.0697	.0662
	3	.0648	.0714	.0607	.0578
	5	.0551	.0662	.0557	.0514
High CHO	8	.0592	.0651	.0598	.0576
	10	.0784	.0991	.0757	.0741
	13	.0691	.0764	.0613	.0673
	3	.2309	.2197	.2373	.2235
	5	.2736	.3095	.2816	.2577
Moderate CHO & B6	8	.2501	.2783	.2800	.2577
	10	.2592	.2864	.2704	.2409
	13	.2712	.2831	.2672	.2672
	3	.2067	.2147	.1936	.2062
	5	.2752	.3247	.3095	.2672
High CHO & B6	8	.2315	.2716	.2656	.2228
	10	.2465	.2744	.2417	.2283
	13	.2656	.2728	.2505	.2315
Young/Untrained					
	1	.0849	.0957	.0811	.0744
	11	.0781	.0918	.0807	.0709
Moderate CHO	14	.0776	.0930	.0831	.0742
	17	.0535	.0619	.0490	.0513
	18	.1122	.1308	.1061	.1073
	1	.0689	.0749	.0635	.0641
	11	.0822	.0869	.0791	.0757
High CHO	14	.0871	.1076	.0842	.0799
	17	.0509	.0573	.0534	.0562
	18	.1272	.1455	.1222	.1272

PLASMA VITAMIN B6 (nmoles/ml) (CONTINUED)

Individual Values for Each Diet and Time

	Subject #	pre	post	post30	post60
Young/Untrained					
	1	.2070	.2168	.2134	.2017
	11	.2752	.3039	.2752	.2632
Moderate CHO & B6	14	.3438	.3757	.3637	.3142
	17	.2496	.2879	.2536	.2529
	18	.4068	.4395	.4012	.3941
	1	.2076	.2367	.2170	.2091
	11	.2465	.3278	.3079	.2529
High CHO & B6	14	.3342	.3741	.3430	.3358
	17	.2520	.2668	.2469	.2371
	18	.3932	.4116	.3845	.3936
Postmenopausal/Untrained					
	2	.0594	.0549	.0543	.0502
	6	.0696	.0739	.0642	.0573
Moderate CHO	7	.0544	.0636	.0592	.0523
	15	.0600	.0636	.0565	.0570
	16	.0899	.0972	.0869	.0827
	2	.0462	.0503	.0499	.0428
	6	.0714	.0732	.0675	.0695
High CHO	7	.0551	.0538	.0545	.0509
	15	.0616	.0638	.0559	.0596
	16	.0813	.0911	.0890	.0867
	2	.2664	.2704	.2664	.2328
	6	.2443	.2728	.2513	.2288
Moderate CHO & B6	7	.2372	.2536	.2389	.2372
	15	.2425	.2879	.2656	.2640
	16	.2667	.2807	.2672	.2091
	2	.2592	.2688	.2792	.2505
	6	.2601	.2738	.2536	.2431
High CHO & B6	7	.2183	.2465	.2230	.2010
	15	.2292	.2728	.2648	.2303
	16	.2601	.2632	.2601	.2403

PYRIDOXAL 5'-PHOSPHATE (nmoles/ml)

Individual Values for Each Diet and Time

	Subject #	pre	post	post30	post60
Young/Trained					
Moderate CHO	3	.0495	.0538	.0446	.0374
	5	.0315	.0358	.0268	.0285
	8	.0473	.0544	.0467	.0472
	10	.0527	.0568	.0477	.0476
	13	.0543	.0629	.0518	.0536
High CHO	3	.0510	.0554	.0445	.0455
	5	.0349	.0495	.0421	.0378
	8	.0438	.0491	.0442	.0414
	10	.0572	.0607	.0540	.0438
	13	.0531	.0647	.0578	.0537
Moderate CHO & B6	3	.1856	.1759	.1534	.1517
	5	.2335	.2501	.2288	.2236
	8	.2059	.2082	.1884	.1728
	10	.1881	.2210	.1922	.1665
	13	.2071	.2283	.2061	.1988
Hogh CHO & B6	3	.1535	.1705	.1526	.1543
	5	.2087	.2477	.2083	.2182
	8	.1573	.1857	.1655	.1453
	10	.1620	.1758	.1539	.1498
	13	.1920	.2098	.1712	.1640
Young/Untrained					
Moderate CHO	1	.0448	.0763	.0537	.0569
	11	.0593	.0707	.0571	.0560
	14	.0611	.0697	.0611	.0559
	17	.0389	.0407	.0366	.0360
	18	.0859	.1017	.0839	.0772
High CHO	1	.0459	.0518	.0448	.0403
	11	.0526	.0629	.0572	.0533
	14	.0654	.0748	.0655	.0599
	17	.0408	.0424	.0376	.0404
	18	.1012	.1227	.0971	.1012

PYRIDOXAL 5'-PHOSPHATE (nmoles/ml) (CONTINUED)

Individual Values for Each Diet and Time

	Subject #	pre	post	post30	post60
Young/Untrained					
	1	.1490	.1713	.1572	.1535
	11	.1990	.2146	.1995	.1934
Moderate CHO & B6	14	.2401	.2582	.2115	.2115
	17	.1744	.1781	.1726	.1713
	18	.2863	.2963	.2859	.2651
	1	.1527	.1652	.1437	.1285
	11	.1707	.2056	.1721	.1787
High CHO & B6	14	.2335	.2439	.2111	.2118
	17	.1706	.1807	.1586	.1478
	18	.2873	.3152	.2684	.2535
Postmenopausal/Untrained					
	2	.0364	.0324	.0265	.0283
	6	.0610	.0687	.0617	.0557
Moderate CHO	7	.0353	.0435	.0389	.0347
	15	.0363	.0459	.0457	.0387
	16	.0671	.0688	.0644	.0635
	2	.0253	.0265	.0225	.0232
	6	.0608	.0748	.0613	.0648
High CHO	7	.0327	.0423	.0387	.0381
	15	.0360	.0445	.0391	.0377
	16	.0651	.0793	.0681	.0688
	2	.1850	.2066	.1510	.1509
	6	.2295	.2469	.2405	.2034
Moderate CHO & B6	7	.1759	.1845	.1748	.1748
	15	.1645	.1708	.1650	.1913
	16	.1783	.2030	.1848	.1709
	2	.1754	.2155	.1814	.1753
	6	.2327	.2517	.2220	.2124
High CHO & B6	7	.1374	.1449	.1422	.1237
	15	.1450	.1752	.1614	.1400
	16	.2121	.2178	.2075	.1832

4-PYRIDOXIC ACID (umoles/24-hr.)

Individual Values for Each Diet and Time

	Subject #	Day Before Exercies	Day of Exercise
Young/Trained			
Moderate CHO	3	7.800	7.920
	5	8.000	7.360
	8	7.180	8.760
	10	7.850	6.190
	13	6.910	7.700
High CHO	3	7.440	7.890
	5	7.300	7.570
	8	7.270	8.100
	10	7.780	7.590
	13	7.080	6.660
Moderate CHO & B6	3	38.60	37.50
	5	38.54	39.40
	8	40.20	42.30
	10	36.00	41.90
	13	39.00	40.60
High CHO & B6	3	40.40	38.40
	5	39.90	41.40
	8	39.70	42.50
	10	31.60	40.70
	13	38.70	40.50
Young/Untrained			
Moderate CHO	1	7.780	8.090
	11	6.480	7.550
	14	6.380	7.102
	17	7.560	6.960
	18	6.190	7.260
High CHO	1	8.520	8.520
	11	5.830	7.120
	14	6.380	7.980
	17	6.770	6.960
	18	7.040	7.040

4-PYRIDOXIC ACID (umoles/24-hr.) (CONTINUED)

Individual Values for Each Diet and Time

	Subject #	Day Before Exercise	Day of Exercise
Young/Untrained			
	1	36.70	41.70
	11	38.90	39.20
Moderate CHO & B6	14	34.08	42.44
	17	36.21	35.81
	18	37.81	35.86
	1	39.20	39.20
	11	33.70	37.86
High CHO & B6	14	35.26	37.04
	17	35.02	33.11
	18	34.01	38.42
Postmenopausal Untrained			
	2	6.450	7.030
	6	7.490	8.120
Moderate CHO	7	7.820	7.510
	15	5.380	8.680
	16	7.180	6.500
	2	6.400	6.270
	6	7.280	8.960
High CHO	7	7.260	8.340
	15	6.290	7.680
	16	7.000	7.850
	2	34.12	35.16
	6	39.36	37.29
Moderate CHO & B6	7	40.52	42.88
	15	35.19	36.79
	16	40.87	43.95
	2	33.22	34.64
	6	35.98	42.12
High CHO & B6	7	41.94	44.62
	15	31.53	38.17
	16	45.92	40.50

URINARY VITAMIN B6 (umoles/24-hr.)

Individual Values for Each Diet and Time

	Subject #	Day Before Exercise	Day of Exercise
Young/Trained			
Moderate CHO	3	1.35	1.02
	5	.340	.950
	8	.950	1.15
	10	.950	1.07
	13	.680	.920
High CHO	3	1.10	1.78
	5	.630	.390
	8	.990	.980
	10	1.24	1.20
	13	.880	.800
Moderate CHO & B6	3	5.17	8.60
	5	6.33	5.67
	8	4.34	6.60
	10	5.25	6.01
	13	5.30	4.85
High CHO & B6	3	7.67	6.25
	5	4.53	5.67
	8	6.02	6.28
	10	5.23	5.44
	13	5.78	4.89
Young/Untrained			
Moderate CHO	1	1.52	1.46
	11	.880	1.14
	14	.780	.670
	17	1.10	1.12
	18	1.26	1.46
High CHO	1	1.47	1.36
	11	1.03	.990
	14	.930	.720
	17	1.06	1.01
	18	1.39	1.33

URINARY VITAMIN B6 (umoles/24-hr.) (CONTINUED)

Individual Values for Each Diet and Time

	Subject #	Day Before Exercise	Day of Exercise
Young/Untrained			
Moderate CHO & B6	1	7.12	7.47
	11	6.13	4.25
	14	5.57	6.08
	17	7.58	6.14
	18	8.29	8.05
High CHO & B6	1	6.87	7.69
	11	6.63	7.13
	14	5.75	6.53
	17	7.18	7.57
	18	6.89	9.07
Postmenopausal Untrained			
Moderate CHO	2	.880	.870
	6	.930	.960
	7	.690	.840
	15	.820	.880
	16	1.11	1.14
High CHO	2	.880	.810
	6	.910	.880
	7	.510	.700
	15	.770	.750
	16	1.04	.910
Moderate CHO & B6	2	8.68	8.99
	6	4.66	4.90
	7	5.70	3.67
	15	5.03	5.73
	16	4.59	5.92
High CHO & B6	2	8.83	9.02
	6	4.93	4.59
	7	4.85	4.36
	15	4.98	4.95
	16	4.55	6.07

GLUCOSE (mg/100ml)

Individual Values for Each Diet and Time

	Subject #	pre	post	post30	post60
Young/Trained					
Moderate CHO	3	86.7	117.3	88.0	94.9
	5	94.2	125.3	105.7	64.5
	8	83.7	98.1	86.3	86.7
	10	89.1	89.1	88.7	88.9
	13	90.6	93.7	83.9	86.7
High CHO	3	88.2	110.0	94.9	97.3
	5	90.8	143.2	117.9	95.6
	8	87.4	104.5	86.5	90.5
	10	93.7	90.9	89.7	90.0
	13	83.4	85.3	81.7	87.4
Moderate CHO & B6	3	86.5	92.7	88.7	93.3
	5	90.4	92.8	83.6	88.5
	8	92.3	106.9	83.2	86.9
	10	90.2	106.4	88.6	95.4
	13	78.9	85.7	88.2	87.8
High CHO & B6	3	91.6	97.0	84.9	93.7
	5	94.5	109.7	102.7	102.5
	8	87.2	104.8	81.4	89.5
	10	95.8	107.5	86.9	99.9
	13	87.4	93.9	84.9	90.4
Young/Untrained					
Moderate CHO	1	88.6	160.1	101.3	80.9
	11	96.8	114.0	92.9	90.5
	14	94.8	131.7	96.1	93.2
	17	86.5	78.3	87.4	85.7
	18	89.9	106.1	101.8	83.6
High CHO	1	85.5	90.8	89.0	86.7
	11	92.9	97.4	84.3	89.1
	14	92.7	104.5	90.4	88.1
	17	91.4	85.1	92.3	93.0
	18	94.3	91.7	88.7	94.3

GLUCOSE (mg/100ml) (CONTINUED)

Individual Values for Each Diet and Time

	Subject #	pre	post	post30	post60
Young/Untrained					
	1	89.9	90.5	86.3	89.5
	11	93.5	95.8	79.0	86.3
Moderate CHO & B6	14	102.	124.3	117.2	95.2
	17	88.8	90.1	90.1	88.1
	18	85.0	82.8	83.4	83.5
	1	88.1	95.6	88.0	87.2
	11	89.1	103.2	87.1	86.2
High CHO & B6	14	96.3	103.9	98.5	96.1
	17	91.0	92.9	85.4	87.4
	18	87.0	84.1	76.9	75.7
Postmenopausal/Untrained					
	2	102.7	96.5	97.2	99.1
	6	101.1	98.1	94.5	90.7
Moderate CHO	7	100.1	87.2	85.7	97.7
	15	102.7	108.2	93.5	91.2
	16	97.7	88.4	91.1	97.9
	2	102.	96.3	97.0	97.5
	6	95.1	96.6	91.9	103.3
High CHO	7	102.5	94.7	102.1	100.3
	15	96.1	107.7	95.4	100.2
	16	93.0	86.7	87.1	93.4
	2	104.6	100.8	95.1	103.8
	6	100.2	110.1	95.4	97.8
Moderate CHO & B6	7	98.8	96.2	94.4	96.6
	15	94.8	102.1	90.1	95.8
	16	101.9	95.5	98.5	90.4
	2	100.1	96.5	96.50	95.6
	6	98.7	106.0	93.70	97.6
High CHO & B6	7	99.8	92.5	89.00	99.6
	15	96.8	102.6	102.1	97.6
	16	98.0	92.5	100.9	97.5

FFA (mEq)

Individual Values for Each Diet and Time

	Subject #	pre	post	post30	post60
Young/Trained					
	3	.8551	1.9808	1.2171	.8248
	5	.9085	1.0915	.9346	.7672
Moderate CHO	8	.8060	1.5274	.8408	1.2886
	10	.9268	1.1265	.7970	.7770
	13	.9674	1.3819	.6122	1.2279
	3	.6992	2.4253	1.4734	.8039
	5	.8405	1.0654	.9555	.7620
High CHO	8	.5174	.9851	.4129	.5672
	10	.7022	1.0017	.5275	1.0665
	13	.4672	1.2938	.8199	.4941
	3	1.1177	2.7235	1.2223	1.0654
	5	.7254	.7777	.6731	.6522
Moderate CHO & B6	8	.4925	.9055	.3433	.6716
	10	.8419	1.2962	.8220	.7770
	13	.9088	1.8329	1.2050	1.0273
	3	.4900	2.1638	.9451	.8038
	5	.5475	.9817	.7777	.5946
High CHO & B6	8	.2189	.6666	.3184	.5871
	10	.9967	1.2762	.6522	1.2013
	13	.4230	1.2227	.9141	.7069
Young/Untrained					
	1	1.5096	1.6620	.7268	.7058
	11	.9767	1.1265	.9068	.9767
Moderate CHO	14	.7121	1.0770	.4242	.4646
	17	.7063	1.0477	.8418	.7816
	18	.6309	.9180	.5723	.5137
	1	.8949	1.357	1.0631	1.3257
	11	.6672	1.0266	.8120	.9767
High CHO	14	.3788	.6161	.4697	.4646
	17	.5456	.7414	.6059	.9473
	18	.6367	.5547	.7188	(.6367)

FFA (mEq) (CONTINUED)

Individual Values for Each Diet and Time

	Subject #	pre	post	post30	post60
Young/Untrained					
	1	.7005	1.1891	.8319	1.4623
	11	.7271	.9168	.9518	1.0765
Moderate CHO & B6	14	.5859	.6061	.5555	.3939
	17	.9623	1.3992	1.2335	.7548
	18	.4141	.6133	.5019	.7422
	1	.4641	.7478	.5955	.7636
	11	.6273	.6772	.5025	.7471
High CHO & B6	14	.3687	.5151	.4040	.3586
	17	.7464	.7816	.6310	.7113
	18	.2266	.4141	.1856	.3731
Postmenopausal/Untrained					
	2	.8634	1.168	1.0053	1.3993
	6	.6270	1.0703	.6054	1.2324
Moderate CHO	7	.6486	1.0571	.6162	1.2541
	15	.5960	.7525	.3734	.5202
	16	.7663	.9975	.3799	.8067
	2	.7583	1.1734	.9632	.9895
	6	.6865	1.0000	.8486	1.1676
High CHO	7	.6865	1.0421	.6595	.6054
	15	.4949	.7727	.4697	.3939
	16	.3889	.8318	.6510	.8820
	2	1.2522	1.4939	.9370	1.1051
	6	.4973	.8276	.5243	.9351
Moderate CHO & B6	7	.7563	1.0378	.8216	1.0162
	15	.4697	.9040	.4343	.8283
	16	.7264	1.0045	.7062	1.0577
	2	.8319	.9475	.6480	.7478
	6	.3351	.5784	.4486	.4973
High CHO & B6	7	.5514	.6324	.6054	.7946
	15	.4848	.8232	.4242	.5656
	16	.4552	.7063	.6008	.5808

LACTATE (mg/100ml)

Individual Values for Each Diet and Time

	Subject #	pre	post	post30	post60
Young/Trained					
	3	3.36	19.41	7.60	6.18
	5	4.29	20.31	7.59	5.08
Moderate CHO	8	4.23	21.25	9.99	5.96
	10	5.36	15.96	5.23	5.17
	13	4.44	38.66	11.67	7.17
	3	3.86	21.53	8.46	4.87
	5	7.43	24.50	8.74	7.01
High CHO	8	8.94	29.06	22.25	12.87
	10	3.76	10.41	5.44	4.28
	13	9.32	32.82	11.12	7.69
	3	6.18	18.30	10.27	4.12
	5	2.57	18.58	14.76	5.29
Moderate CHO & B6	8	5.70	19.35	8.79	4.54
	10	2.45	18.95	10.70	8.11
	13	5.02	19.25	6.41	5.72
	3	3.46	21.93	9.16	4.37
	5	5.44	34.75	27.27	12.88
High CHO & B6	8	6.90	17.38	5.85	4.96
	10	2.92	17.74	5.12	3.04
	13	5.13	21.72	6.64	5.66
Young/Untrained					
	1	4.97	43.24	17.64	5.28
	11	4.20	24.00	8.62	5.42
Moderate CHO	14	5.89	39.58	14.67	6.98
	17	3.94	19.36	10.10	4.67
	18	5.16	27.34	10.93	7.71
	1	4.92	20.98	10.78	5.43
	11	4.26	27.56	7.52	5.48
High CHO	14	5.94	35.71	18.25	11.99
	17	2.72	22.85	20.86	5.38
	18	7.49	30.34	15.09	(8.0)

LACTATE (mg/100ml) (CONTINUED)

Individual Values for Each Diet and Time

	Subject #	pre	post	post30	post60
Young/Untrained					
	1	5.28	15.32	8.36	6.29
	11	4.15	24.71	7.17	7.23
Moderate CHO & B6	14	9.76	42.11	21.07	12.39
	17	8.16	13.59	6.16	4.80
	18	3.50	16.03	7.77	4.66
	1	5.28	20.52	14.16	8.41
	11	4.73	40.46	8.74	5.72
High CHO & B6	14	4.60	27.97	14.72	6.45
	17	4.99	21.47	7.49	5.55
	18	4.72	23.58	7.49	7.50
Postmenopausal/Untrained					
	2	9.72	20.42	11.79	6.54
	6	3.56	26.22	5.71	3.77
Moderate CHO	7	5.65	27.01	10.63	5.97
	15	13.40	28.61	15.36	11.10
	16	5.05	17.99	5.77	5.00
	2	7.85	32.89	10.68	5.73
	6	5.18	24.34	5.81	3.56
High CHO	7	5.60	30.41	6.91	4.56
	15	11.15	28.76	12.54	12.04
	16	4.64	17.94	11.29	8.97
	2	5.68	29.56	15.17	11.33
	6	5.71	25.28	6.60	5.86
Moderate CHO & B6	7	7.28	24.23	5.76	5.44
	15	5.64	22.51	21.22	12.84
	16	7.37	27.89	9.48	5.72
	2	11.99	37.43	11.74	9.67
	6	10.21	27.22	10.00	4.08
High CHO & B6	7	5.03	28.42	26.43	4.30
	15	10.46	29.36	24.05	8.17
	16	7.94	25.41	8.30	7.17

ALBUMIN (gm/100ml)

Individual Values for Each Diet and Time

	Subject #	pre	post	post30	post60
Young/Trained					
	3	4.48	5.21	4.68	4.64
	5	4.14	4.70	4.13	3.96
Moderate CHO	8	4.53	5.09	4.52	4.20
	10	4.52	4.89	4.63	4.45
	13	4.87	5.18	4.64	4.77
	3	4.73	5.15	4.80	4.50
	5	4.30	4.64	4.22	4.21
High CHO	8	4.53	4.68	4.42	4.45
	10	4.86	4.99	4.66	4.79
	13	4.47	5.02	4.61	4.60
	3	4.53	5.12	4.89	4.68
	5	4.40	4.82	4.31	4.25
Moderate CHO & B6	8	4.58	4.82	4.70	4.38
	10	4.51	5.11	4.71	4.86
	13	5.06	5.20	4.88	5.16
	3	4.51	5.13	4.72	4.77
	5	4.17	4.75	4.30	4.49
High CHO & B6	8	4.76	4.95	4.56	4.71
	10	4.69	5.30	4.65	4.66
	13	5.06	5.12	4.65	4.61
Young/Untrained					
	1	4.60	5.15	4.60	4.48
	11	4.82	5.29	4.81	4.74
Moderate CHO	14	4.72	5.35	4.81	4.86
	17	4.42	4.80	4.60	4.54
	18	4.88	5.48	4.73	4.93
	1	4.59	5.10	4.81	4.64
	11	4.90	5.10	4.75	4.71
High CHO	14	4.73	5.29	4.84	4.78
	17	4.54	4.81	4.63	4.63
	18	4.97	5.30	4.70	4.97

ALBUMIN (gm/100ml) (CONTINUED)

Individual Values for Each Diet and Time

	Subject #	pre	post	post30	post60
Young/Untrained					
	1	4.60	4.90	4.69	4.65
	11	4.75	5.13	4.72	4.92
Moderate CHO & B6	14	5.00	5.52	5.08	5.03
	17	4.51	4.82	4.70	4.97
	18	4.58	4.99	4.72	4.67
	1	4.41	4.92	4.46	4.56
	11	4.34	4.96	4.60	4.49
High CHO & B6	14	4.79	5.48	4.94	4.73
	17	4.39	4.76	4.73	4.74
	18	4.72	5.14	4.63	4.63
Postmenopausal/Untrained					
	2	4.72	4.73	4.61	4.52
	6	4.13	4.53	4.17	4.16
Moderate CHO	7	4.36	5.02	4.66	4.47
	15	4.75	5.10	4.44	4.41
	16	4.73	5.05	4.83	4.75
	2	4.46	4.70	4.54	4.43
	6	4.16	4.38	4.15	4.29
High CHO	7	4.81	4.97	4.57	4.62
	15	4.45	4.98	4.58	4.62
	16	4.56	4.74	4.52	4.62
	2	4.76	4.90	4.64	4.61
	6	4.13	4.37	4.25	4.07
Moderate CHO & B6	7	4.65	4.96	4.59	4.59
	15	4.42	4.86	4.43	4.64
	16	4.87	5.07	4.68	4.77
	2	4.58	4.92	4.51	4.56
	6	4.05	4.34	4.04	4.04
High CHO & B6	7	4.52	4.81	4.68	4.53
	15	4.25	4.90	4.25	4.25
	16	4.91	4.54	4.80	4.60

QT_C INTERVALS AT REST AND 80% MHR

Individual Values for Each Diet and Time

	Subject #	Seconds Rest	80% MHR
Young/Trained			
Moderate CHO	3	.404	.407
	5	.428	.451
	8	.393	.425
	10	.419	.408
	13	.373	.396
High CHO	3	.380	.451
	5	.435	.427
	8	.375	.432
	10	.376	.420
	13	.376	.421
Moderate CHO + B-6	3	.392	.422
	5	.402	.460
	8	.411	.427
	10	.381	.414
	13	.381	.420
High CHO + B-6	3	.370	.442
	5	.394	.401
	8	.385	.483
	10	.408	.438
	13	.394	.390
Young/Untrained			
Moderate CHO	1	.421	.420
	11	.436	.428
	14	.441	.421
	17	.424	.424
	18	.436	.431
High CHO	1	.411	.405
	11	.400	.401
	14	.441	.421
	17	.424	.424
	18	.436	.431

QT_c INTERVALS AT REST AND 80% MHR (CONT.)

Individual Values for Each Diet and TIme

	Subject #	Seconds Rest	80% MHR
Young/Untrained	1	.411	.399
	11	.427	.392
Moderate CHO + B-6	14	.394	.407
	17	.387	.401
	18	.396	.407
	1	.409	.375
	11	.415	.416
High CHO + B-6	14	.431	.421
	17	.381	.383
	18	.438	.429
Postmenopausal/Untrained			
	2	.423	.424
	6	.428	.492
Moderate CHO	7	.443	.421
	15	.405	.466
	16	.446	.429
	2	.383	.408
	6	.429	.447
High CHO	7	.403	.404
	15	.454	.464
	16	.431	.450
	2	.415	.416
	6	.387	.438
Moderate CHO + B-6	7	.393	.421
	15	.391	.445
	16	.427	.446
	2	.401	.433
	6	.422	.425
High CHO + B-6	7	.403	.454
	15	.395	.456
	16	.421	.430

PLASMA VOLUME CHANGES

Individual Values for the Moderate CHO

Subject #	Hct pre	Hct post	% chg	PLP nM pre	PLP post	% chg	% chg PV
1	36.6	39.0	6.67	44.8	76.3	70.0	- 9.8
2	37.2	36.1	-3.14	36.4	32.4	-12.35	5.1
3	34.7	39.0	12.47	49.5	53.8	8.68	-17.0
5	36.5	39.9	9.50	31.5	35.8	13.65	-13.7
6	34.8	35.4	1.70	61.0	68.7	12.62	- 2.5
7	36.5	36.8	.01	35.3	43.5	23.2	- 1.4
8	35.5	40.1	12.80	47.3	54.4	15.01	-19.7
10	32.8	35.8	9.28	52.7	56.8	7.78	-12.6
11	35.2	37.3	5.79	59.3	70.7	19.20	- 8.5
13	37.0	38.2	3.07	54.3	62.9	15.84	- 4.7
14	36.7	40.4	10.22	61.1	69.7	14.08	-14.7
15	41.6	43.2	3.92	36.3	45.9	26.44	- 6.4
16	37.2	39.6	6.50	67.1	68.8	2.53	- 9.7
17	33.5	36.7	9.63	38.9	40.7	4.62	-13.2
18	35.1	40.2	14.54	85.9	101.7	15.52	-19.5

Individual Values for the High CHO

1	33.2	34.8	4.78	45.9	51.8	12.85	- 6.8
2	34.8	35.8	2.73	25.3	26.5	4.51	- 4.1
3	33.8	36.6	8.25	51.0	55.4	8.63	-11.5
5	36.9	37.8	2.19	34.9	49.5	41.83	- 3.4
6	34.9	36.2	3.34	60.8	74.8	23.03	- 5.0
7	35.1	34.9	-.01	32.7	72.3	29.36	+ .6
8	35.8	37.7	5.27	43.8	49.1	12.10	- 7.8
10	34.1	35.4	3.66	57.2	60.7	6.12	- 5.4
11	33.6	35.6	6.23	52.6	64.9	23.38	- 8.8
13	33.6	35.8	6.51	53.1	64.7	21.84	- 9.2
14	32.0	34.5	7.75	65.4	74.8	14.37	-10.6
15	38.8	40.8	5.07	36.0	44.6	23.89	- 7.8
16	34.7	36.8	5.97	65.1	79.3	21.81	- 8.6
17	35.8	36.1	.80	40.8	42.4	3.92	- 1.3
18	34.2	35.8	4.70	101.2	122.7	21.25	- 6.8

PLASMA VOLUME CHANGES

Individual Values for the Moderate CHO + B6

Subject #	Hct_{pre}	Hct_{post}	% chg	PLP_{pre} nM	PLP_{post}	% chg	% chg PV
1	37.9	39.3	3.69	149.0	171.3	14.97	-5.7
2	40.2	45.5	13.31	185.0	206.6	11.68	-19.4
3	39.9	41.6	4.05	185.6	175.9	-5.22	-6.1
5	39.7	42.2	6.43	233.5	250.1	7.11	-10.0
6	38.8	40.8	6.43	229.5	246.9	7.58	-7.9
7	42.1	43.3	3.04	175.9	184.5	4.89	-5.1
8	38.9	41.5	6.53	205.9	208.2	1.12	-10.0
10	41.4	42.8	3.36	188.1	221.0	17.49	-5.5
11	38.4	40.9	6.65	199.0	214.6	7.84	-10.1
13	40.9	42.8	4.47	207.1	228.3	10.24	-7.2
14	41.1	44.2	7.55	240.1	258.2	7.54	-11.9
15	42.3	47.4	12.15	164.5	170.8	3.83	-18.8
16	43.6	44.2	1.35	178.3	203.0	13.85	-2.4
17	40.4	42.3	4.70	174.4	178.1	2.12	-7.5
18.	40.3	40.3	- .99	286.3	296.3	3.49	+.25
	Individual Values for the High CHO + B6						
1	36.9	36.4	6.86	152.7	165.2	7.95	-10.2
2	40.2	42.8	6.46	175.4	215.5	22.86	-10.1
3	35.6	41.9	14.76	153.5	170.5	11.07	- 2.5
5	38.1	43.6	14.37	208.7	247.7	18.69	-19.3
6	39.6	41.2	4.03	232.7	251.7	8.17	- 6.4
7	42.1	43.3	5.36	137.4	144.9	5.46	- 8.3
8	39.6	41.7	5.15	157.3	185.7	18.05	- 8.1
10	39.2	42.1	7.56	162.0	175.8	8.52	-11.5
11	36.8	41.4	12.41	170.7	205.6	20.46	-17.5
13	41.4	40.8	- .96	192.0	209.8	9.28	+ 2.5
14	39.6	42.3	6.86	233.5	243.9	4.45	-10.6
15	40.2	46.0	14.43	145.0	175.2	20.83	-21.1
16	39.0	41.7	7.00	212.1	217.8	2.69	-10.7
17	38.0	40.8	7.29	170.6	180.7	5.92	-10.9
18	37.9	41.2	8.58	287.3	315.2	9.71	-12.7

PLASMA VOLUME CHANGES

Individual Values for the Moderate CHO

Subject #	Hct_{pre}	Hct_{p60}	% chg	PLP_{pre} nM	PLP_{p60}	% chg	% chg PV
1	36.6	35.1	-6.89	44.8	56.9	21.27	+11.64
2	37.2	34.9	-7.55	36.4	28.3	-22.25	+10.78
3	34.7	34.4	- .81	49.5	37.4	-32.35	+ 1.25
5	36.5	35.2	-3.48	31.5	28.5	-10.53	+ 5.68
6	34.8	33.9	-2.33	61.0	57.0	- 6.55	+ 3.80
7	36.5	35.0	-4.11	35.3	34.7	- 1.70	+ 6.75
8	35.5	37.0	4.08	47.3	47.2	- .21	- 6.08
10	32.8	33.4	2.00	52.7	47.6	- 9.68	- 3.02
11	35.2	35.1	- .31	59.3	56.0	- 5.56	+ .48
13	37.0	35.0	1.99	54.3	53.6	- 1.29	+ 9.07
14	36.7	36.7	.00	61.1	55.9	- 8.51	.00
15	41.6	39.7	-4.57	36.3	38.7	- 6.61	+ 8.20
16	37.2	38.6	3.82	67.1	63.5	- 5.37	- 5.85
17	33.5	35.3	5.50	38.9	36.0	- 7.46	- 7.84
18	35.1	34.9	.60	85.9	77.2	-10.13	+ .93

Individual Values for the High CHO

1	33.2	32.6	-1.99	45.9	40.1	-12.10	+2.99
2	34.8	34.7	- .43	25.3	23.2	- 8.30	+ .66
3	33.8	33.1	-2.19	51.0	45.5	-10.78	+3.38
5	37.0	34.0	-8.01	34.9	37.8	+ 8.31	+13.81
6	35.0	35.0	.00	60.8	64.8	+ 6.50	.00
7	35.1	34.8	- .71	32.7	38.1	+ 6.58	+1.11
8	35.9	35.8	- .11	43.8	41.4	- 5.48	+ .17
10	34.1	34.2	+ .15	57.2	43.8	-23.43	- .22
11	33.6	32.7	-2.65	52.6	53.3	+ 1.22	+4.10
13	33.6	33.1	-1.58	53.1	53.7	+ 1.13	+2.41
14	32.0	30.6	-4.31	65.4	59.9	- 8.41	+6.63
15	38.8	37.5	-3.35	36.0	37.7	+ 4.72	+5.66
16	34.7	34.1	-1.36	65.1	68.8	+ 5.68	-2.10
17	35.8	34.7	-3.13	40.8	40.4	- .98	-5.03
18	34.2	34.2	.00	101.2	101.2	.00	.00

PLASMA VOLUME CHANGES

Individual Values for the Moderate CHO + B-6

Subject #	Hct pre	Hct p60	% chg	PLP nM pre	PLP p60	% chg	% chg PV
1	37.9	37.0	-2.30	149.0	153.5	+ 3.02	+ 3.78
2	40.2	40.4	.57	185.0	150.9	-18.43	- .95
3	40.0	37.4	-6.38	185.6	151.7	-18.27	+11.35
5	39.7	39.7	-2.45	233.5	223.6	- 4.24	+ 4.16
6	38.8	37.6	-3.22	229.5	203.4	-11.37	+ 5.44
7	42.1	40.0	-4.95	175.9	174.8	- .63	+ 8.98
8	38.9	40.4	3.73	205.9	172.8	-16.08	- 5.88
10	41.4	40.7	-1.71	188.1	166.5	-11.48	+ 2.98
11	38.4	39.2	+2.68	199.0	193.4	- 2.81	+ 3.60
13	41.0	41.7	1.71	207.1	198.8	- 4.01	- 2.85
14	41.1	40.4	-1.70	240.1	211.5	-11.91	+ 2.94
15	42.3	44.2	4.54	164.5	191.3	+16.29	- 7.53
16	43.4	40.7	-6.58	178.2	170.9	- 4.15	+12.49
17	40.4	42.2	4.28	174.4	171.3	- 1.78	- 6.89
18	40.3	40.5	+ .30	286.1	265.1	- 7.41	- .50

Individual Values for the High CHO + B-6

1	36.9	37.8	2.36	152.7	128.5	- 15.85	- 3.36
2	40.2	41.4	2.91	175.4	175.3	.06	- 4.73
3	36.6	39.0	6.56	153.5	154.3	.52	-10.30
5	38.1	38.3	.60	208.7	218.2	4.60	- .97
6	39.6	38.0	-4.11	232.7	212.4	- 8.72	7.11
7	38.6	37.9	-1.81	137.4	123.7	- 9.97	- 3.01
8	39.6	39.1	-1.41	157.3	145.3	- 7.63	- 2.37
10	39.2	38.7	-1.12	162.0	149.8	-11.70	- 1.87
11	36.8	38.0	3.18	170.7	178.7	5.04	- 3.08
13	41.4	38.2	-7.87	192.0	164.0	-14.58	-14.58
14	39.6	39.0	-1.44	233.5	211.8	- 9.29	- 2.42
15	40.2	40.8	1.54	145.0	140.0	- 3.45	- 2.54
16	39.0	39.5	1.28	212.1	183.2	-13.63	- 2.08
17	38.0	39.4	3.55	170.6	147.8	-13.36	- 5.73
18	38.0	37.9	-. 18	287.3	253.5	-11.76	+ .30

Individual Chemistry Screen Values Pre and Post Diet Study

Young/ Trained

		Gluc	Creat	BUN	UA	TP	Alb	Chol	Na^+	K^+	Cl^-	Ca^{++}	Phos	TBil	SGOT	LO	SGPT	AIP
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
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

Units:

Gluc	mg/dl	Alb	gms/dl	CL^-	mEq/L	SGPT	U/L
Creat	mg/dl	Chol	mg/dl	Ca	mg/dl	AIP	U/L
Bun	mg/dl	Nat	mEq/L	TBil	mg/dl		
UA (uric acid)	mg/dl	K+	mEq/L	SGOT	U/L		
				LD	U/L		
TP	gms/dl						

Individual Chemistry Screen Values Pre and Post Diet Study

Young/ Untrained

		Gluc	Creat	BUN	UA	TP	Alb	Chol	Na^+	K^+	Cl^-	Ca^{++}	Phos	TBil	SGOT	LD	SGPT	AIP
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
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
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	26	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

Units:

Gluc	mg/dl	Alb	gms/dl	CL^-	mEq/L	SGPT	U/L
Creat	mg/dl	Chol	mg/dl	Ca	mg/dl	AIP	U/L
Bun	mg/dl	Nat	mEq/L	TBil	mg/dl		
UA (uric acid)	mg/dl	K+	mEq/L	SGOT	U/L		
				LD	U/L		
TP	gms/dl						

Individual Chemistry Screen Values Pre and Post Diet Study

Postmenopausal/Untrained

		Gluc	Creat	BUN	UA	TP	Alb	Chol	Na^+	K^+	Cl^-	Ca^{++}	Phos	TBil	SGOT	LD	SGPT	AIP
2	Before	100	0.7	13.6	4.8	7.6	4.3	154	136	5.3	104	9.6	3.2	0.3	35	159	20	48
	After	91	0.8	11.8	5.8	8.1	4.3	180	140	4.7	106	9.8	2.9	0.5	29	155	19	40
6	Before	91	0.8	10.0	4.3	6.2	3.9	232	137	3.9	101	9.1	3.6	0.4	27	228	19	40
	After	88	0.8	13.5	4.8	5.9	3.8	250	141	4.2	104	9.1	4.0	0.6	32	196	22	40
7	Before	85	0.7	10.2	4.8	6.5	4.1	183	137	4.7	99	9.7	3.4	0.6	27	200	19	65
	After	95	0.7	10.4	4.9	6.6	4.3	171	140	5.0	105	9.9	3.9	0.5	25	195	24	59
15	Before	78	0.9	11.2	5.9	7.3	4.1	211	135	4.1	99	9.8	3.5	0.5	33	191	28	50
	After	99	0.8	13.8	5.6	6.9	4.0	166	143	5.0	105	9.7	3.9	0.5	32	147	33	45
16	Before	91	0.7	13.8	5.4	6.6	4.4	257	137	4.9	98	9.8	4.2	0.3	33	186	50	71
	After	88	0.8	11.5	5.4	6.2	4.2	243	138	4.5	102	9.9	4.4	0.5	47	179	36	69

Units:

Gluc	mg/dl	Alb	gms/dl	CL^-	mEq/L	SGPT	U/L
Creat	mg/dl	Chol	mg/dl	Ca	mg/dl	AIP	U/L
Bun	mg/dl	Nat	mEq/L	TBil	mg/dl		
UA (uric acid)	mg/dl	K+	mEq/L	SGOT	U/L		
				LD	U/L		
TP	gms/dl						

APPENDIX II

FORMS USED IN THE PRESENT STUDY

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 ____ Moderate

____Very Light ____ Somewhat hard

____Light ____ Hard

____Very moderate ____ Very hard

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

____ No exercise ____ Jog/run

____ Calisthenics ____ Ride a bicycle

____ Lift weights ____ Swim

____ Walk for exercise ____ Other (please describe)

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

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

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

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)

_____

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.

_____

_____

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.

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 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 supplements are to be consumed during the study.

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

OVER

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

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	xylose test done 21	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?

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.

The following individual _____ ie being considered as a subject for a nutrition, diet and exercise study being conducted by the Foode 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 exerciee periods on an ECG and blood preesures will be monitored pre, during and after exercise. All exercise will be conducted by experienced inveetigators.

Could you please conduct a routine physical on thie 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 thie individual ie 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 _____

CONSENT FORM

I, _____, give my consent to partici-

in this study. The sutdy has been explained to me and all my questions have been answered. I agree to consume all food and vitamin supplements provided and to provide a record of food consumed for three days prior to beginng 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 20-30 mls of blood to be drawn periodically during the experimental period to monitor B-6 status and four times during each exercise test. I also agree to collect 24 hour urine samples throughtout 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 concerning me will be kept confidential.

NAME _____ DATE _____

WITNESS _____ DATE _____

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 $\dot{V}O_2$ 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.