# AN ABSTRACT OF THE THESIS OF

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Title:   Supplemental Vitamin B-6 and Endurance Exercise Effects on Plasma Catecholamines of Trained Male Cyclists

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This study examined the effect of vitamin B-6 supplementation and exhaustive submaximal exercise on plasma catecholamine concentrations, and the relationship between plasma catecholamines and fuel use, heart rate and oxygen consumption. Five trained men (age=18-35 years; $VO_2max$=53 ml $O_2$/kg/min.) participated in two controlled dietary periods that were identical except for the addition of 20 mg/d pyridoxine (PN) supplementation during the second period. On the seventh morning of each period, fasted subjects exercised to exhaustion on a cycle ergometer at 74.5% ± 7.8 $VO_2max$. Blood was drawn pre-exercise (twice), 60 minutes into exercise, immediately post-exercise and 60 minutes post-exercise. Plasma was analyzed for norepinephrine, epinephrine, glucose, pyridoxal 5'-phosphate (PLP), lactic acid, glycerol and free fatty acids (FFA). Heart rate and oxygen consumption were measured pre-exercise and at 10-minute intervals during exercise. Mean plasma PLP concentration was significantly higher during the supplemented versus the nonsupplemented trial at all time points. There were no statistically significant differences in mean plasma catecholamine concentrations or mean plasma fuel concentrations between the nonsupplemented and supplemented trials at any of the time points examined. There were significant changes in the mean plasma concentrations of norepinephrine, lactic acid, glycerol and FFA over time in both trials. Respiratory exchange ratios (R) were higher during the supplemented trial compared to the nonsupplemented trial, but the differences did not attain statistical significance. There were no significant differences in mean exercise times to exhaustion or mean heart rates between the trials. The overall mean oxygen consumption during exercise was consistently higher during the supplemented versus the nonsupplemented trial and the

difference attained significance (p=0.016) at one time point (10 min.). The mean oxygen consumption during rest was lower during supplementation versus nonsupplementation, but the difference was not statistically significant. The percent plasma volume change (PVC) was significantly greater at post-exercise, relative to pre-exercise, during the supplemented versus the nonsupplemented trial. The percent PVC also increased significantly over time during the supplemented but not the nonsupplemented trial. These results suggest that 20 mg/d of vitamin B-6 supplementation does not effect plasma catecholamine concentrations, fuel utilization or heart rate at rest or during submaximal exercise to exhaustion. The results may suggest a higher oxygen consumption during exhaustive exercise after PN supplementation.

Supplemental Vitamin B-6 and Endurance Exercise Effects on Plasma
Catecholamines of Trained Male Cyclists

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Jennifer Charity Young

# TABLE OF CONTENTS

# LIST OF FIGURES

# LIST OF TABLES

LIST OF TABLES (Continued)

# LIST OF APPENDIX TABLES AND FORMS

# Supplemental Vitamin B-6 and Endurance Exercise Effects on Plasma Catecholamines of Trained Male Cyclists

## INTRODUCTION

Catecholamines (norepinephrine, epinephrine, dopamine) play a key role as neurotransmitters in the central and sympathetic nervous systems, and as hormones in the sympathoadrenal system. They allow the body to respond rapidly to anxiety or anticipation, low blood glucose, hypoxia, exercise and a host of "fight or flight" conditions such as trauma, pain and hypotension (Berne and Levy, 1988). During exercise, the catecholamines influence heart rate (Christensen et al., 1979; Orizio et al., 1988), metabolic rate (Sjostrom, 1985; Mathews et al., 1990) and substrate mobilization (Landsberg and Young, 1980 & 1992; Mazzeo, 1991). The catecholamines also have a stimulatory effect on resting metabolic rate (Tremblay et al., 1992; Poehlman et al., 1992).

Pyridoxal 5'-phosphate (PLP), the major coenzymatic form of vitamin B-6, is essential to more than 100 enzymatic reactions in the body (Sauberlich, 1985). PLP is involved in amino acid and carbohydrate metabolism, immune function, niacin formation, red cell metabolism and formation, and nervous system function (Leklem, 1988b). During exercise, the body requires PLP to mobilize and produce glucose through the processes of glycogenolysis and gluconeogenesis (Cori and Illingsworth, 1957; Merrill and Burnham, 1990).

The catecholamines cannot be synthesized without the presence of PLP. PLP acts as the coenzyme for L-DOPA decarboxylase to catalyze the synthesis of dopamine, from which norepinephrine and, subsequently, epinephrine are formed (Mathews and van Holde, 1990). The basis of this thesis relies on the role of PLP in catecholamine synthesis. Research in animal brains has correlated the distribution of L-DOPA decarboxylase as well as pyridoxal kinase (required for PLP formation) with the distributions of norepinephrine and dopamine (Siow and Dakshinamurti, 1990). In addition, vitamin B-6 supplementation has resulted in an increase in L-DOPA decarboxylase activity in animal brains (Roberge, 1977). An acute dose of vitamin B-6 (pyridoxal or pyridoxine) stimulated the secretion of adrenal norepinephrine and epinephrine in rats (Lau-Cam, 1991). These studies suggest that an increase in PLP availability enhances catecholamine synthesis and/or release in animals.

In humans, two conditions may make more PLP available for catecholamine synthesis: vitamin B-6 supplementation and exercise. Plasma and tissue concentrations of PLP have been shown to rise in response to vitamin B-6 supplementation (Lui et al., 1985; Rose et al., 1976); plasma PLP concentration also rises during exercise, and this effect is enhanced by vitamin B-6 supplementation (Dunton et al., 1992 & 1994). Hence, vitamin B-6 supplementation coupled with endurance exercise presents the optimal conditions for examining any effect on catecholamine synthesis or release. For obvious reasons, plasma catecholamine concentrations, rather than tissue concentrations, must be used as an indicator of any change in catecholamine synthesis.

This study will be the first to explore any effect of vitamin B-6 supplementation on plasma catecholamine concentrations in humans. In addition to the proposed effect on catecholamine synthesis, some evidence has suggested that vitamin B-6 supplementation results in greater carbohydrate metabolism relative to fat metabolism (Leklem, 1985; Virk, 1992 & 1994), and lower heart rates and oxygen consumption (Virk, 1992) during exercise. Given the role of the catecholamines in these processes, part of this thesis will also analyze any changes in plasma substrates, heart rate or oxygen consumption with vitamin B-6 supplementation.

## Hypothesis

Oral vitamin B-6 supplementation causes an additional increase in plasma catecholamines stimulated by exhaustive endurance exercise in trained male cyclists.

## Objectives

1)      To examine the change in plasma catecholamine concentrations produced by exhaustive endurance exercise in trained male cyclists during vitamin B-6 supplemented and unsupplemented states

2)      To analyze the relationship between the rise in plasma catecholamine concentrations and fuel use during exhaustive endurance exercise in the vitamin B-6 supplemented and unsupplemented trials

3)      To evaluate heart rate changes during exhaustive endurance exercise and resting metabolic rate (RMR) in vitamin B-6 supplemented and unsupplemented conditions

LITERATURE REVIEW

**Vitamin B-6**

Introduction

Vitamin B-6 is a water-soluble B vitamin that exists in three primary forms, pyridoxal (PL), pyridoxine (PN) and pyridoxamine (PM). The vitamers are derivatives of 3-hydroxy-2-methylpyridine. Each of the forms may also be phosphorylated. A glycosylated form of pyridoxine has also been identified as 5'-O-(B-D-glycopyranosyl) conjugate.

Pyridoxal 5'-phosphate (PLP) is the major coenzymatic form of vitamin B-6. It is involved in over 100 enzymatic reactions in the body (Sauberlich, 1985), playing an important role in amino acid and carbohydrate metabolism, immune function, niacin formation, red cell metabolism and formation, nervous system function and hormone modulation (Leklem, 1988b).

Structure, Sources, Requirements

The B-6 vitamers are found in a wide variety of foods, generally in the phosphorylated form. Table 1 lists the vitamin B-6 content and percentages of the three primary forms for selected foods. PN and PM are the predominant forms in plants, while animal foods contain primarily PM and PL. In addition, some plants contain relatively high levels of the glycosylated PN. Research suggests that the bioavailability of the glucoside is somewhat lower than the phosphorylated or non-phosphorylated forms (Trumbo and Gregory, 1989). Meat, fish and poultry are rich in vitamin B-6 and contain no detectable pyridoxine B-glucoside (Leklem, 1988a). Legumes, such as navy beans and soybeans; whole grains; fruits, especially watermelon and bananas; and some vegetables, particularly potatoes, contain significant amounts of vitamin B-6. Milk and eggs are relatively low in B-6 content. Heating of food, such as toasting of bread, causes loss of vitamin B-6 through binding of PL and PLP to proteins or peptide lysyl residues (Gregory and Kirk, 1977).

**Table 1. Vitamin B-6 Content of Selected Foods and Percentages of the Three Forms**

| Food | Vitamin B-6 (mg/100 g) | PN (%) | PL (%) | PM (%) |
|------|------------------------|--------|--------|--------|
| **Vegetables** | | | | |
| Broccoli, raw | 0.195 | 29 | 65 | 6 |
| Cabbage, raw | 0.160 | 61 | 31 | 8 |
| Carrots, raw | 0.150 | 75 | 19 | 6 |
| Cauliflower, raw | 0.210 | 16 | 27 | 5 |
| Corn, sweet | 0.161 | 6 | 68 | 26 |
| Potatoes, raw | 0.250 | 68 | 18 | 14 |
| Spinach, raw | 0.280 | 36 | 49 | 15 |
| Tomatoes, raw | 0.100 | 38 | 29 | 33 |
| **Fruits** | | | | |
| Apples, red delicious | 0.030 | 61 | 31 | 8 |
| Apricots, dried | 0.169 | 81 | 11 | 8 |
| Apricots, raw | 0.070 | 58 | 20 | 22 |
| Avocados, raw | 0.420 | 56 | 29 | 15 |
| Bananas, raw | 0.510 | 61 | 10 | 29 |
| Grapefruit, raw | 0.034 | - | - | - |
| Oranges, raw | 0.060 | 59 | 26 | 15 |
| Peaches, canned | 0.019 | 61 | 30 | 9 |
| Raisins, seedless | 0.240 | 83 | 11 | 6 |
| **Beans & Legumes** | | | | |
| Beans, white, raw | 0.560 | 62 | 20 | 18 |
| Lentils | 0.600 | 69 | 13 | 18 |
| Lima beans, canned | 0.090 | 75 | 15 | 10 |
| Lima beans, frozen | 0.150 | 45 | 30 | 25 |
| Peanut butter | 0.330 | 74 | 9 | 17 |
| Soybeans, dry, raw | 0.810 | 44 | 44 | 12 |
| **Nuts** | | | | |
| Almonds, skinless | 0.100 | 52 | 28 | 20 |
| Filberts | 0.545 | 29 | 68 | 3 |
| Pecans | 0.183 | 71 | 12 | 17 |
| Walnuts | 0.730 | 31 | 65 | 4 |
| **Cereals/Grains** | | | | |
| Barley, pearled | 0.224 | 53 | 42 | 6 |
| Bread, white | 0.040 | - | - | - |
| Bread, whole wheat | 0.180 | - | - | - |
| Cornmeal | 0.250 | 11 | 51 | 38 |
| Oatmeal, dry | 0.140 | 12 | 49 | 39 |
| Rice, brown | 0.550 | 78 | 12 | 10 |
| Rice, white | 0.170 | 64 | 19 | 17 |
| Rye flour, light | 0.090 | 64 | 22 | 14 |
| Wheat cereal, flakes | 0.292 | 79 | 11 | 10 |
| Wheat flour, white | 0.060 | 55 | 24 | 21 |

**Table 1,**
**Continued**

| Food | Vitamin B-6 (mg/100 g) | PN (%) | PL (%) | PM (%) |
|------|------------------------|--------|--------|--------|
| Wheat flour, whole | 0.340 | 71 | 16 | 13 |
| Meat/poultry/fish | | | | |
| Beef, raw | 0.330 | 16 | 53 | 31 |
| Chicken breast | 0.683 | 7 | 74 | 19 |
| Flounder fillet | 0.170 | 7 | 71 | 22 |
| Halibut | 0.430 | - | - | - |
| Pork, ham, canned | 0.320 | 8 | 8 | 84 |
| Salmon, canned | 0.300 | 2 | 9 | 89 |
| Sardine, canned oil | 0.280 | 13 | 58 | 29 |
| Tuna, canned | 0.425 | 19 | 69 | 12 |
| Milk/eggs/cheese | | | | |
| Cheddar | 0.080 | 4 | 8 | 88 |
| Egg, whole | 0.110 | 0 | 85 | 15 |
| Milk, cow, homog. | 0.040 | 3 | 76 | 21 |
| Milk, human | 0.010 | 0 | 50 | 50 |

Values taken from Orr, 1969

The current Recommended Dietary Allowance (RDA) for vitamin B-6 is 2.0 mg/d for males age 15+ and 1.6 mg/d for nonpregnant, nonlactating females age 19+ (National Research Council, 1989). The RDA should include a margin of safety that provides for variations in individual requirements and in the bioavailability of B-6 from different food sources. Tarr et al (1981) estimated that foods representing the "average" American diet exhibit 71-79% bioavailability of vitamin B-6 (Tarr et al, 1981). The need for vitamin B-6 increases as protein intake rises (Miller et al, 1985) probably due to the role of PLP in amino acid metabolism. The RDA for vitamin B-6 for adults was established by using twice the RDA for protein (125 g/d for males and 100 g/d for females) and a vitamin B-6/protein ratio of 0.016 mg/g (National Research Council, 1989). Therefore, the RDA for vitamin B-6 is geared to handle substantially higher amounts of protein than the RDA for that macronutrient, which is approximately 50 g/d for adult women and 60 g/d for adult men. However, it has been proposed that a higher vitamin B-6/protein ratio is required (Dietary Std. Canada, 1975). In addition, some evidence suggests that physical activity (Leklem and Shultz, 1983; Manore et al, 1987a) and other factors listed in Table 2 may affect vitamin B-6 requirements.

**Table 2. Factors Effecting Vitamin B-6 Requirements**

1. Dietary
   - a. Physical structure of food
   - b. Forms of vitamin B-6; effects of processing
   - c. Binding of vitamin B-6 forms
   - d. Protein intake
   - e. Nutrient interaction-riboflavin, carbohydrate
2. Physiological/Biochemical
   - a. Physical activity-increased loss
   - b. Protein-enzyme induction
   - c. Increased catabolism/turnover-phosphatase activity, illness
   - d. Impaired phosphorylation and/or interconversion, competing pathways, nutrient deficiencies, drugs
   - e. Pregnancy-demand of fetus
   - f. Growth-increased cell mass, repair
   - g. Excretion rate-urinary, sweat, menstrual loss
   - h. Lactation-adequate levels in milk
   - i. Gender-differences in metabolism
   - j. Age differences in metabolism
3. Genetic
   - a. Defects in apoenzymes requiring PLP-altered binding to apoenzyme
   - b. Altered levels of apoenzymes requiring PLP-biochemical individuality
4. Defect in delivery to tissues
   - a. Impaired gastrointestinal absorption
   - b. Impaired transport-albumin, synthesis, and binding, phosphatase activity
5. Disease prevention/treatment (?)
   - a. Heart disease
   - b. Cancer
   - c. Diabetes mellitus
   - d. Premenstrual syndrome
   - e. Renal disease
   - f. Alcoholism

Adapted from Leklem, 1988a & 1993

## Basic Functions

Amino acid metabolism requires PLP for aminotransferase, decarboxylation, side-chain cleavage and dehydratase reactions (Merrill and Burnham, 1990). Through aminotransferase (transamination and deamination) reactions, amino acids can be made available as substrates for conversion to glucose in the process of gluconeogenesis in the liver. In addition, alanine aminotransferase requires PLP as a coenzyme for converting alanine to pyruvate, another step in the gluconeogenic process. PLP is involved in carbohydrate metabolism as a component of glycogen phosphorylase (Cori and Illingsworth, 1957), the enzyme that catalyzes the breakdown of glycogen to glucose for energy (glycogenolysis). The role of PLP in the metabolism of fuels in the body will be more thoroughly discussed in the Vitamin B-6 and Exercise chapter.

PLP is also a coenzyme in the synthesis of niacin (from tryptophan), heme and nucleic acids; the function of steroid hormone receptors; development and maintenance of the immune system; and metabolism of neurotransmitters (Leklem, 1988b). In the nervous system, decarboxylation enzymes require PLP to catalyze the synthesis of the following neurotransmitters: dopamine, epinephrine, norephinephrine, tyramine, tryptamine, serotonin, histamine, gamma-aminobutyric acid (GABA) and taurine. PLP is also needed to synthesize sphingolipids and the phospholipids, phosphatidylethanolamine and phospatidylcholine (lecithin). The synthesis of sphingolipids and polyamines is crucial to the development of the nervous system (Dakshinamurti, 1982).

In the majority of enzymatic reactions, PLP covalently binds to enzymes via a Schiff base with an amino group of lysine in the enzyme and forms a Schiff base with the amino group of the substrate in a transamination reaction (Leussing,1986). As coenzyme to glycogen phosphorylase, on the other hand, PLP appears to use its phosphate group in the coenzymatic role instead of binding with the aldehyde moiety in the classic Schiff base reaction (Helmreich and Klein, 1980).

## Absorption, Metabolism, Storage

Absorption of B-6 has been extensively studied in the rat (Henderson, 1985; Middleton, 1977, 1978, 1982, 1985). The three major nonphosphorylated forms of B-6, PL, PM and PN, are largely absorbed by a nonsaturable, passive process in the proximal jejunum.

Since phosphorylated compounds do not readily cross cell membranes, phosphorylated forms must be hydrolyzed by alkaline phosphatase before they can be absorbed. After uptake, the forms are metabolically trapped through phosphorylation (Henderson, 1985). Some research indicates that there may be a saturable component of uptake, particularly in the duodenum (Middleton, 1985). If there is a saturable mechanism in humans, there would be implications for the absorption of large supplemental doses of B-6. After absorption, the B-6 vitamers are transported to the liver, where metabolism of vitamin B-6 primarily takes place.

Basically, the three primary forms are converted to PLP, and excess B-6 is converted to 4-pyridoxic acid (via PLP to PL) then excreted in the urine. The nonphosphorylated forms enter the hepatocytes by a facilitated process and diffusion followed by metabolic trapping through phosphorylation (Mehansho et al, 1980; Kozik and McCormick, 1984). Phosphorylated forms must be hydrolyzed by the plasma membrane alkaline phosphatase prior to diffusion into hepatocytes (Mehansho et al, 1980; Kozik and McCormick, 1984). PL, PN and PM are phosphorylated at the 5' position by pyridoxal kinase in the cytosol (McCormick et al, 1961). Adenosine triphosphate (ATP) serves as the phosphate source and zinc acts as a cofactor in the reaction. PMP and PNP are then converted to PLP via pyridoxine phosphate oxidase, which requires flavin mononucleotide (FMN) (Kazarinoff and McCormick, 1975). Excess PL from liver interconversion or the circulation is irreversibly converted to 4-pyridoxic acid (4-PA) by aldehyde dehydrogenase or aldehyde oxidase. The dehydrogenase requires nicotinamide-adenine dinucleotide (NAD) and the oxidase uses flavin adenine dinucleotide (FAD) as a coenzyme. In the human liver, aldehyde oxidase activity has been detected, while aldehyde dehydrogenase activity has not (Merrill and Henderson, 1990). The kidney may also play a role in the oxidation of PLP (Merrill and Henderson, 1990). The liver releases PLP, PL and 4-PA into the plasma. PLP remaining in the liver is bound to apo-enzymes, which is thought to result in metabolic trapping (Lumeng and Li, 1980). 4-PA is the end-product of B-6 metabolism and is excreted in the urine by the kidney. The amount of 4-PA excreted is primarily related to B-6 intake, with higher intakes resulting in higher excretion. Given an adequate intake of B-6, urinary 4-PA excretion accounts for 40-60% of the daily intake (Leklem, 1988b).

PLP and PL are the main forms of B-6 in the plasma that are available to other tissues. Under fasting conditions with normal B-6 intake, PLP accounts for 60-70% of total plasma B-6 (Lumeng et al, 1985; Leklem and Shultz, 1983; Leklem, 1990). PL is the next most abundant form, with lower levels of PN and PM, and very low or absent levels of PMP and PNP (Coburn and Mahuren, 1983). PN would be available following a meal

or supplementation (primarily as PN-HCl) if the intake was high enough and the PN escaped metabolism in the liver. In the circulation, PLP and PL are bound to albumin (Dempsey and Christensen, 1962), with PLP bound more tightly (Anderson et al, 1974). Binding of PLP protects it from hydrolysis and permits its delivery to tissues. Since the phosphate molecule of phosphorylated forms must be removed at cell membranes by alkaline phosphatase prior to uptake, PL seems to be the primary form that crosses cell membranes (Merrill and Henderson, 1990).

Erythrocytes may play an important role in B-6 transport and metabolism. Erythrocytes rapidly absorb PN and PL by a simple diffusion process (Mehansho and Henderson, 1980) and contain adequate kinase and PNP oxidase to convert PN, PL and PM to PLP. However, both PLP and PL are tightly bound to hemoglobin, with PL bound more tightly at a different site on the molecule (Ink et al, 1982). PL concentration in erythrocytes is 4 to 5 times greater than in plasma since PL has a greater affinity for hemoglobin than for albumin (Ink and Henderson, 1984). Thus, the issue of whether or not PL or PLP is available from erythrocytes to other tissues remains controversial (Leklem, 1988b).

The highest levels of B-6 in humans are found in the muscle, plasma, liver, brain, kidney and spleen (Leklem, 1988a; Shin et al, 1983). In most tissues aside from blood, the major forms of B-6 are PLP and PMP (Dakshinamurti, 1982). As in plasma and erythrocytes, PLP is bound to proteins in other tissues. Binding to protein probably protects PLP from hydrolysis and serves a storage function (Fonda and Harker, 1982). PN, PL and PM can be converted to their respective phosphorylated forms in most tissues since kinase activity is present; however, PNP and PMP cannot be converted to PLP in many tissues since the oxidase enzyme is absent (McCormick et al, 1961; Pogell, 1958). The kinase and oxidase enzymes are limited in human tissues other than the liver (Merrill and Henderson, 1990), brain (McCormick and Snell, 1959) and non-nucleated erythrocytes. Therefore, PL is the only B-6 vitamer that serves as a source for PLP in tissues lacking oxidase. For example, muscle tissue cannot convert PN or PM to PLP.

Water-soluble vitamins, such as B-6, are generally not stored to any large degree and excess intake is excreted in the urine. Excretion of 4-PA does rise with large intakes of B-6, but the excretion does not account for all the extra B-6 ingested (Leklem, 1988a). Hence, B-6 must be retained somewhere in the body or excreted in other forms. The main tissues that appear to be effected by vitamin B-6 supplementation are blood and muscle. Plasma and tissue (muscle) PLP levels in humans have been shown to rise in response to increased B-6 intake (Lui et al., 1985; Ubbink et al., 1987; Coburn et al., 1991).

Rose et al (1976) found dramatic increases in plasma PLP in men supplemented with 5 mg PN/d or more. When 25 mg/d or 50 mg/d of PN were administered, plasma PLP increased 4- to 5-fold and plateaued 2-4 days after supplementation began (Lumeng et al., 1974). Metabolic balance studies in men have shown that plasma PLP levels plateau within 7-10 days after a change in B-6 intake between 0.5 and 10 mg/d (Lui et al., 1985). Coburn et al (1988) performed human muscle biopsies and found that the amount of PLP in muscle is far greater than in plasma, suggesting that PLP can be concentrated in muscle. Total-body B-6 is estimated at approximately 1000 $\mu$mol in a 70 kg man, with 850 $\mu$mol and 900 $\mu$mol contained in the muscle of males and females, respectively (Coburn et al, 1988). Studies with rats and mice have indicated that muscle contains 50% of the total body pool of vitamin B-6, largely as PLP bound to glycogen phosphorylase (Krebs and Fischer, 1964; Black et al, 1978). More recent research in humans estimates the value closer to 70% (Coburn et al, 1988). In rats, the glycogen phosphorylase content and PLP content of muscle increased with increasing B-6 intake (Black et al, 1977). Coburn et al (1991) demonstrated that six weeks of 166 mg/d PN-HCl supplementation (0.948 mmol/d) in men increased the vitamin B-6 content of human muscle. However, the increase in vitamin B-6 content of human muscle with PN-HCl supplementation was small (25%) compared to observed increases in rats (Coburn et al., 1991; Black et al., 1977 & 1978). Hence, muscle appears to be the prime storage site for B-6, but few studies have analyzed the PLP content of other human tissues (Leklem, 1988a).

## Regulation of PLP Synthesis and Release

Nutrients required for PLP synthesis include B-6, zinc, magnesium and riboflavin. With adequate dietary intake, bioavailability and metabolism of these nutrients, they should not limit PLP synthesis. In particular, absorption of B-6 is largely a passive, nonsaturable process as previously described. As such, intestinal absorption of B-6 does not normally play a limiting role in providing substrate (B-6) for PLP synthesis. `

Varying levels of enzyme activity provide a mechanism for regulating PLP synthesis and release from liver (Merrill and Henderson, 1990). In humans, the rates of phosphorylation of PN and PL are much greater than the rates of dephosphorylation (Merrill and Henderson, 1990). Hence, kinase activity appears to be favored over the phosphatase activity at physiological pH. The phosphorylation of PN and PM to the 5'-phosphates is somewhat faster than the conversion of PNP and PMP to PLP. Thus, the

PNP oxidase appears to be the limiting enzyme in the pathway (Leklem, 1988b). In addition, the rate of oxidation of PL to 4-PA is similar to the rate of phosphorylation; thus, once PLP is converted to PL, a substantial portion is rapidly oxidized to 4-PA (Merrill et al., 1984). These mechanisms may help prevent accumulation of the highly reactive PLP in the liver. Also recall that most tissues lack PNP oxidase, further controlling PLP synthesis.

Feedback inhibition of enzymes catalyzing PLP formation also provide a mechanism for controlling cellular PLP concentrations. PNP oxidase is highly regulated by product inhibition, thus high PLP concentrations limit flux through the pathway (Merrill et al, 1979; Dakshinamurti, 1982). In rat brain (Dakshinamurti, 1982) and rabbit brain (Ebadi et al., 1970), pyridoxal kinase activity is also inhibited by PLP. Ebadi compared the activity of pyridoxal kinase with PLP concentration in different regions of the rabbit brain, and revealed an inverse relationship between the tissue concentration of PLP and the activity of pyridoxal kinase.

In addition to enzyme regulation, protein binding may help to control cellular PLP concentrations (Lumeng and Li, 1980; Dakshinamurti, 1982). Also, clearance of circulating PLP depends on the activity of alkaline phosphatase (Merrill and Henderson, 1990; Whyte et al., 1985) since PLP must be dephosphorylated before uptake. The uptake of PLP into certain tissues may involve a saturable mechanism (Spector and Greenwald, 1978).

The exact relationship between hepatocyte uptake of PL versus release has not been elucidated. It has been shown that the rate of PN uptake by rat hepatocytes at physiological concentrations is close to the rate of metabolism (Kozik and McCormick, 1984). PLP appears to be released from storage (muscle) during a caloric deficit, as occurs during food restriction or exercise. When rats were made vitamin B-6 deficient, the content of muscle glycogen phosphorylase (containing PLP) did not decrease. In contrast, when rats were made calorie-deficient, the glycogen phosphorylase content of muscle did decrease (Black et al, 1978). Thus, a caloric deficit appears to release PLP associated with glycogen phosphorylase while a B-6 deficiency does not. Coburn et al (1991) examined the vitamin B-6 content of human muscle after six weeks of very low vitamin B-6 intakes. Their conclusions indicate that, like rats, human muscle content of vitamin B-6 does not decrease with decreased vitamin B-6 intake.

# Catecholamines

## Introduction

Catecholamines (dopamine, norepinephrine, epinephrine) are amines derived from the amino acid tyrosine, wherein the amino group is retained and a second hydroxyl group is introduced in the ortho-position on the benzene ring.

The catecholamines are localized predominantly in neurons of the central nervous system (CNS), nerve endings in the sympathetic division of the autonomic nervous system and chromaffin cells of the adrenal medulla. They function as neurotransmitters in the CNS and sympathetic nervous system, exerting their effects upon a target tissue or membrane close to the secretion site. The catecholamines also function as hormones in the sympathoadrenal system, stimulating activities in tissues remote from the secretory site. In general, catecholamines in the CNS relay signals that regulate mental, emotional, motor, neuroendocrine and homeostatic processes. Catecholamines released from the sympathoadrenal system effect cardiovascular, visceral and metabolic processes to maintain homeostasis in the face of changing environmental or physiological conditions.

## Synthesis, Storage and Release

The catecholamines are synthesized in neurons of the central nervous system, neurons in the sympathetic division of the autonomic nervous system and chromaffin cells of the adrenal medulla (Berne and Levy, 1988; Zubay, 1988).

The catecholamines share a common biosynthetic pathway that starts with the aromatic amino acid tyrosine (Mathews and van Holde, 1990). The hydroxylation of tyrosine to L-DOPA by tyrosine hydroxylase requires molecular oxygen, a tetrahydropteridine cofactor and NADPH. In the second reaction, L-DOPA decarboxylase requires pyridoxal 5'-phosphate (PLP). The decarboxylase catalyzes synthesis of dopamine from L-DOPA (3,4-dihydroxyphenylalanine). Dopamine beta-hydroxylase catalyzes the beta-hydroxylation of the dopamine side chain to form norepinephrine in the presence of molecular oxygen and ascorbic acid, which functions as a hydrogen donor. Methylation of norepinephrine is then catalyzed by phenylethanolamine N-

methyltransferase to give epinephrine. In this reaction, S-adenosylmethione is the methyl donor.

The first reaction, catalyzed by tyrosine hydroxylase, is the rate-limiting step in the sequence (Perlman and Chalfie, 1977; Kopin, 1977; Udenfriend, 1966). Tyrosine hydroxylase and L-DOPA decarboxylase are cytosolic enzymes; hence, the first two reactions take place in the cytoplasm of neurons and chromaffin cells. However, dopamine beta-hydroxylase is localized in chromaffin granules in the adrenal medulla and the corresponding granulated vesicles of neurons. Therefore, dopamine formed in the second reaction must be taken up into the granules before it can be hydroxylated to form norepinephrine. Phenylethanolamine N-methyltransferase, which catalyzes the formation of epinephrine from norepinephrine, is found in the cytosol of chromaffin cells and in the cytosol of small numbers of neurons in the CNS that use epinephrine as a neurotransmitter (Coupland, 1972; Saavedra et al., 1975). Accordingly, norepinephrine must diffuse out of the granule into the cytosol before it can be converted to epinephrine. Finally, dopamine, norepinephrine and epinephrine are stored in specific granules within central neurons, sympathetic nerve terminals or in the adrenomedullary chromaffin cells.

Within the body, epinephrine is located almost exclusively in the chromaffin cells, where it is stored in high concentrations. In about 85% of the chromaffin granules, the norepinephrine diffuses back into the cytoplasm where it is N-methylated to form epinephrine. The epinephrine is taken back up into the chromaffin granule for storage as the predominant adrenal medullary catecholamine. In approximately 15% of the adrenomedullary chromaffin granules, norepinephrine is stored after synthesis (Berne and Levy, 1988). The level of catecholamines in mammalian adrenal is on the order of several millimoles per kilogram (milligrams per gram) of tissue (Landsberg and Young, 1992).

Norepinephrine is found in the peripheral sympathetic nerves, the CNS and the adrenal medulla. Since virtually all the norepinephrine outside the CNS and adrenal medulla is located in the sympathetic nerve endings, the norepinephrine content of a particular tissue reflects the degree of its sympathetic innervation. Heavily innervated organs have a norepinephrine concentration of about 1.0 $\mu$g norepinephrine/g tissue; for example, in the heart the norepinephrine concentration ranges from 5 to 10 $\mu$mol/kg (1 to 2 $\mu$g/g) of tissue. The actual concentration in the nerve ending itself is much greater and has been estimated to be in the range of 5 to 50 mmol/kg (1 to 10 mg/g) of nerve cytoplasm (Dahlstrom et al., 1966). As already described, dopamine is present primarily in the brain where it occurs in high concentrations.

To release the catecholamines, the granules are extruded from the nerve ending or adrenal medulla in response to neuronal depolarization. The final common effector

pathway activating the adrenal medulla consists of cholinergic preganglionic fibers in the greater splanchnic nerve. Acetylcholine is the neurotransmitter that stimulates depolarization of the chromaffin cell or neural membrane by increasing its permeability to sodium. This, in turn, induces an influx of calcium ions, which probably produce microfilament contraction and draw the respective catecholamine-containing granules to the cell membrane. The granules fuse with the membrane and discharge their contents by exocytosis. Epinephrine, norepinephrine, ATP, dopamine beta-hydroxylase and other materials are then released into the circulation. The extruded catecholamines can then bind to specific receptors located on the postsynaptic neuron or enter the circulation (Landsberg and Young, 1992).

The catecholamines interact with specific receptors at various cells throughout the body to stimulate or inhibit transmission of nervous signals, physiological events (e.g., increasing depth and frequency of heartbeats) or metabolic processes (e.g., glycogenolysis). Dependent upon the type of receptor, the catecholamine-receptor complex may cause a direct response, such as increased permeability of a membrane to ions, or may initiate a regulatory cascade, in which the intensity of an initial signal is amplified through a series of enzyme activations until the desired result is achieved (Kebabian et al., 1972; Miller et al., 1974; Iversen, 1975). Basically, receptors on target tissues give specificity to circulating hormones, while receptors and anatomical connections (hard wiring) bestow specificity to neurons.

Central Nervous System

Dopamine and norepinephrine are widely used as neurotransmitters in the CNS (Landsberg and Young, 1992; Krstulovic, 1986; Trendelenburg and Weiner, 1989), while epinephrine appears only in certain selected regions of the CNS (Saavedra et al., 1975; Trendelenburg and Weiner, 1989). Neurotransmitters are synthesized in neurons, the basic functional unit of the nervous system that provides an organized network of point-to-point connections. In response to stimuli, neurons secrete neurotransmitters into a synaptic cleft, where they travel a short distance across the synapse to chemically stimulate depolarization of another effector cell or membrane. Basically, this action eventuates an action potential and transmission of a nerve signal.

The majority of studies that report catecholamine concentrations in the CNS have examined the rat brain. Dopamine is present in high concentrations, in the range of 0.73 to

0.82 $\mu$g/g of whole brain tissue (Krstulovic, 1986). Research examining the limbic regions of the human brain reports dopamine concentrations ranging from <.03 to 3.4 $\mu$g/g of tissue (Farley et al., 1977). Dopaminergic neurons play important roles in controlling mental, motor and neuroendocrine functions. Neurons that contain high levels of dopamine are prominent in the limbic lobe, basal ganglia, septum, hypothalamus, and midbrain regions known as the substantia nigra and the ventral tegmentum (Trendelenburg and Weiner, 1989). Some of the axons of these neurons travel to the forebrain, where they may play a role in emotional responses. Other dopaminergic axons terminate in the corpus striatum, where they are believed to play an important role in control of complex movements. The degeneration of these dopaminergic synapses occurs in Parkinson's disease and is believed to be a major cause of the muscular tremors and rigidity that characterize this disease. Dopaminergic fibers are also directed to various parts of the cerebral cortex and limbic system; dysfunction of these fibers has been proposed as a cause of schizophrenia (Landsberg and Young, 1992).

Although dopamine is commonly regarded as a neurotransmitter in the CNS, it is also found in the carotid body (Favier et al., 1983) and kidney (Lee, 1986). However, the amount of dopamine in mammalian tissues is low (Holzbauer and Sharman, 1972; Dinerstein et al., 1983), generally less than 10% of the norepinephrine concentration. The specific origin of plasma dopamine is uncertain; some may be derived from the adrenal medulla, some from sympathetic nerves and some from specialized organs, e.g., carotid body. Distinct peripheral autonomic dopaminergic nerves may exist but have not been conclusively demonstrated. Dopamine exists in adrenergic nerves as a precursor of norepinephrine, but is not known to be released in sufficient quantities from these nerves to elicit distinct dopaminergic responses. Plasma levels of dopamine in humans are low (about 0.25 pmol/mL) and metabolism is rapid (Trendelenburg & Weiner, 1989).

Norepinephrine concentrations in the rat have been reported in the range of 0.28 to 0.35 $\mu$g/g of whole brain tissue (Krstulovic, 1986). Norepinephrine concentrations in the subcortical areas of the human brain reportedly range from 0.02 to 2.26 $\mu$g/g of tissue (Farley and Hornykiewicz, 1977). Neurons containing a high concentration of norepinephrine are located in the limbic lobe, hypothalamus and parts of the medulla (Trendelenburg and Weiner, 1989; Holzbauer and Sharman, 1972). The highest levels are found in the locus ceruleus in the pons, the neurons of which project to the cerebral cortex, hypothalamus, cerebellum and spinal cord. These neurons are important in controlling neuroendocrine systems; maintaining visceral homeostasis; altering emotional states, including mood and dreaming; arousal; and responding to stressful conditions such as exercise (Berne and Levy, 1988).

The amount of epinephrine in brain (Saavedra et al., 1975) is small, reportedly less than one-tenth the norepinephrine level (Trendelenburg and Weiner, 1989). Like the noradrenergic system, neurons containing epinephrine are found in the brain stem, specifically in the medulla oblongata, and these neurons have extensions to the hypothalamus (Landsberg and Young, 1992; Trendelenburg and Weiner, 1989).

Sympathoadrenal System

The term *sympathoadrenal system* refers to the anatomical and physiological unit made up of the sympathetic nervous system and the adrenal medulla. The sympathetic nerves originate in the spinal cord and extend to various organs, including the heart, bronchi, blood vessels and adrenal medulla. Norepinephrine is the peripheral adrenergic neurotransmitter, synthesized and stored in sympathetic nerve endings and released in the innervated tissues. Most of the preganglionic fibers of the sympathetic system are cholinergic, that is, they release acetylcholine; most of the postganglionic fibers of the sympathetic system are noradrenergic, that is, they release norepinephrine.

The adrenal medulla represents essentially an enlarged and specialized sympathetic ganglion. Cholinergic preganglionic axons innervate the medulla without relay through a postganglionic neuron, and the neuronal cell bodies of the medulla do not have axons. Instead, they discharge their catecholamines directly into the bloodstream, thus functioning as endocrine rather than nerve cells (Landsberg and Young, 1992; Guyton, 1987). A pair of normal human adrenals contain approximately 33 $\mu$mol (6.0 mg) of catecholamines in the chromaffin granules (Perlman and Chalfie, 1977). The catecholamines released from the adrenal medulla act as hormones, stimulating physiological or metabolic activities in remote tissues. Of the total catecholamine concentration released, the human medulla releases approximately 80% as epinephrine and 20% as norepinephrine (i.e., 4:1 ratio of epinephrine to norepinephrine) (Berne and Levy, 1988).

Plasma levels of catecholamines are influenced by the amount of catecholamine synthesis, secretion from the sympathoadrenal system and rate of clearance. Essentially all the circulating epinephrine is derived from adrenal medullary secretion. Circulating norepinephrine is released from the medulla and also derived from the sympathetic nerve terminals, having escaped local uptake from synaptic clefts. Some tissues also contribute to plasma levels of norepinephrine. Esler et al (1984) estimated that at rest, the kidneys and skeletal muscle each contribute about 20% of the total overflow of norepinephrine into

plasma. Most attempts to differentiate sympathetic from adrenomedullary responses in humans have relied on measurements of norepinephrine and epinephrine in urine or plasma. However, it is difficult to separate the response of the sympathetic nerve endings from the response of the adrenal medulla, since norepinephrine is secreted from them both and the adrenal medulla may release substantial quantities of norepinephrine when stimulated (Landsberg & Young, 1992). Hence, the relative importance of the two limbs of the sympathoadrenal system in different physiological situations in humans is difficult to assess and, therefore, not well defined.

When the adrenal medulla is activated in association with the rest of the sympathetic nervous system, the body's organs are stimulated in two different ways simultaneously, directly by the sympathetic nerves and indirectly by the medullary hormones. Norepinephrine and epinephrine released from the adrenal medulla are also capable of stimulating structures of the body that are not innervated by direct sympathetic fibers (Landsberg & Young, 1992). For instance, the metabolic rate of every cell of the body is increased by these hormones, especially by epinephrine, even though only a relatively small proportion of all the cells in the body are innervated by sympathetic fibers (Guyton, 1987).

As a whole, the sympathoadrenal system causes excitatory effects in some organs but inhibitory effects in others. In general, sympathetic stimulation increases the rate and force of contraction of heart muscle; decreases gastrointestinal peristalsis; constricts blood vessels, except in the heart and skeletal muscles; and has metabolic effects causing release of glucose from the liver, glycogenolysis in muscle, and increased metabolic rate. Mass responses of the sympathetic system prepare the body for "flight or flight," (Cannon, 1929) increasing the capability of the body to perform more vigorous muscle activity than would otherwise be possible. Massive sympathetic discharge increases arterial pressure, blood flow to active muscles concurrent with decreased blood flow to organs that are not needed for rapid activity, rates of cellular metabolism throughout the body, blood glucose concentration and muscle strength (Guyton, 1987).

In contrast to emergency situations, a graded response may occur in accordance with the intensity of the stimuli. During exposure to cold or physical exercise, for instance, the initial response is predominantly one of sympathetic stimulation, but as the severity of the cold or the intensity and duration of exertion increase, the secretion of adrenomedullary catecholamines increases (Leduc, 1961; Young and Landsberg, 1983). Once in the circulation, norepinephrine and epinephrine have almost the same effects on the different organs as those caused by direct sympathetic stimulation; for instance, they constrict certain blood vessels, cause increased cardiac activity, inhibit the gastronintestinal

18

tract and influence energy metabolism. However, the effects last about ten times as long because the hormones are more slowly removed from the blood than from uptake into noradrenergic nerve endings (Guyton, 1987). Overall, catecholamines released during exercise promote changes in blood-flow distribution, enhanced cardiac contractility, liver and muscle glycogenolysis, gluconeogenesis and adipose tissue lipolysis (McArdle et al., 1986; Landsberg & Young, 1992). All of these actions are beneficial to the exercise response.

The components of the sympathoadrenal system also can be differentially effected by various physiological stimuli. For example, renal and adrenal nerve activity responds similarly to changes in blood pressure but not to hypoglycemia; during hypoglycemia the adrenal medulla is markedly stimulated while the sympathetic nervous system is suppressed (Niijima, 1976). As previously discussed, it is difficult to separate the response of the adrenal medulla from the response of the sympathetic nervous system.


Regulation


Catecholamine release at the sympathetic nerve endings and the adrenal medulla is the direct consequence of a downward flow of impulses from sympathetic centers within the CNS (Landsberg and Young, 1992). The state of these centers is controlled by many factors: The intrinsic activity of the hypothalamic and brain stem nuclei that constitute the sympathetic centers, other regions in the brain stem, hypothalamus, limbic lobe and cerebral cortex; and visceral and somatic afferents that directly or indirectly relay information about the environment from the periphery. Afferent pathways involved in regulation of sympathetic activity include baroreceptors in the large systemic arteries and other organs (e.g., adrenal gland) (Niijima and Winter, 1968); volume sensors in the heart and great veins (Thoren et al., 1976); chemoreceptors in the aortic arch, carotid body, liver, muscle and kidney (Niijima, 1981; Lang and Hood, 1976; Recordati et al., 1980); and cutaneous and visceral pain and temperature sensors. Sympathoadrenal activity is also controlled by the composition of the extracellular fluid, including concentrations of electrolytes, substrates and hormones, as well as temperature and tonicity. For instance, low levels of the metabolic fuels glucose and oxygen, and alterations in the ionic state of the plasma stimulate the sympathoadrenal system.

The sympathetic nerve ending, due to its relationship to the adrenergic synapse and effector tissue, is subject to local regulatory influences that affect the adrenal medulla to a

lesser degree. For example, a fall in pH or a decrease in temperature diminishes the amount of norepinephrine that is released from stimulated sympathetic nerves (Vanhouette et al., 1981). Further, norepinephrine in the synaptic cleft has the potential to stimulate as well as suppress its own release, depending upon the varied stimulation of different receptors.

Since the sympathoadrenal system is an efferent limb of the nervous system, the onset of the effects of released catecholamines is rapid and quickly terminated. Catecholamines are active at exceedingly low concentrations, in the micromolar to picomolar ranges. Catecholamine-mediated events take place in seconds compared with the minutes, hours or days that characterize the time course of action of other hormones. In fact, anticipation of a particular acitivity such as exercise may activate the sympathoadrenal system before the exercise begins, thereby stimulating a variety of catecholamine-responsive processes in advance (Mason et al, 1973).

The rate of release of a hormone is determined ultimately by the rate of its synthesis. Nerve stimulation of the adrenal medulla or sympathetically innervated organ results in release of catecholamines without much change in the catecholamine level within the tissue (Kopin, 1977). Likewise, catecholamine levels in the adrenal or sympathetically innervated tissues change little despite marked increases in sympathetic activity or norepinephrine turnover (Landsberg and Axelrod, 1968). The stability of catecholamine levels in tissues in the face of increased sympathetic activity is the result of simultaneous increase in catecholamine synthesis. Recapture of released norepinephrine at nerve endings also contributes to the stability of norepinephrine stores. Changes in sympathoadrenal activity are coupled to catecholamine synthesis in two ways. In the short run, changes in the activity of tyrosine hydroxylase adjust to the level of synthesis according to the rate of catecholamine release. (Recall that tyrosine hydroxylase catalyzes the rate-limiting step in catecholamine synthesis.) In the long run, sustained increases in impulse traffic in the sympathoadrenal system result in induction of tyrosine hydroxylase synthesis. Thus, regulation of catecholamine synthesis involves changes in either the activity or the rate of synthesis of tyrosine hydroxlase.

The rate of tyrosine hydroxylation in vivo depends on the concentration and availability of specific substrates and endogenous inhibitors. In particular, the substrate tyrosine and the cofactor tetrahydropteridine could potentially be limiting. Since the human liver can synthesize tyrosine from phenylalanine, tyrosine is not normally considered to be an *indispensable* amino acid. An indispensable amino acid is one that cannot be synthesized by the body and must be ingested. Tyrosine may become indispensable if the diet is deficient in phenylalanine or there is a metabolic problem in

forming tyrosine, such as a deficiency of phenylalanine hydroxylase (phenylketonuria). With an adequate intake of quality protein, however, availability of tyrosine would not be a limiting factor in the synthesis of the catecholamines under normal circumstances. On the other hand, catecholamine synthesis has been shown to fluctuate with macronutrient intake and tyrosine administration. This dietary effect may differentially influence the CNS and the peripheral sympathoadrenal system. In rats fed a protein-free diet, urinary dopamine decreased while urinary norepinephrine and epinephrine increased (Agharanya and Wurtman, 1985). Tyrosine or meals that increase the relative plasma level of tyrosine administered to rats has been shown to increase brain dopamine and norepinephrine concentrations only when neurons increase their firing rate (Fernstrom, 1983; Milner and Wurtman, 1986). Administration of tyrosine to rats or humans has been shown to elevate urinary excretion of dopamine, norepinephrine and epinephrine (Agharanya and Wurtman, 1985). However, tyrosine administration (7.5 g/d) with meals decreased plasma norepinephrine levels in non-stressed men (Benedict et al, 1983). In rats, isocaloric protein supplementation (casein) raised urinary dopamine and epinephrine; and addition of tyrosine equal to the tyrosine content of casein had similar results on urinary dopamine compared to casein ingestion, but did not effect epinephrine excretion (Kaufman et al, 1989). In regard to carbohydrate and fat intake, sucrose and lard suppressed urinary dopamine, and sucrose reduced epinephrine excretion in rats (Kaufman et al, 1989). In humans, resting plasma norepinephrine was slightly higher and plasma epinephrine was slightly lower following a diet and exercise protocol that produced low liver glycogen and high muscle glycogen compared to a protocol that produced normal liver glycogen and high muscle glycogen (Lavoie et al, 1983). Resting plasma norepinephrine and epinephrine in humans was slightly higher, epinephrine moreso than norepinephrine, following a normal carbohydrate diet (45.5% CHO) compared to a high carbohydrate diet (77.2% CHO) (Hall et al, 1983). These changes in resting human catecholamine levels following dietary carbohydrate and glycogen store manipulation did not appear to be statistically significant (Lavoie et al, 1983; Hall et al, 1983).

Since tyrosine hydroxylase is found only in tissues that synthesize catecholamines (Landsberg and Young, 1992), tyrosine must be taken up into neurons or the chromaffin cells of the adrenal medulla before synthesis of catecholamines can occur. There is no evidence, however, that the tyrosine uptake is rate-limiting. Under physiological conditions, the striatal concentration of tyrosine appears to fully saturate the enzyme (Murrin et al., 1976). In fact, striatal tyrosine can be depleted by 70% to 80% before a decrease in dopamine synthesis is observed (Biggio et al., 1976).

In contrast to tyrosine, tyrosine hydroxylase is probably not saturated with the tetrahydrobiopterin cofactor at physiological tyrosine levels (Vaccro et al., 1980). Tyrosine hydroxylase is also inhibited through negative feedback by catechols (DOPA, norepinephrine and dopamine) in the cytosol, which are thought to act by antagonizing activation by the reduced pterine cofactor (Udenfriend, 1966). Acute stimulation of the sympathetic innervation to the medulla via acetylcholine activates tyrosine hydroxylase, possibly by decreasing cytoplasmic catecholamine levels and relieving the feedback inhibition, freeing the pteridine cofactor (Geffen and Livett, 1971). Because physiologically relevant alterations in tetrahydrobiopterin may also occur (Abou-Donia, 1981), changes in cofactor concentration may contribute to alterations in tyrosine hydroxylase activity. Thus, both direct activation of tyrosine hydroxylase and release of negative feedback inhibition are probably involved in increasing tyrosine hydroxylase activity in response to nerve stimulation.

In addition to stimulation by acetylcholine, tyrosine hydroxylase activity and the resultant catecholamine synthesis is stimulated by other endogenous substances. Cyclic AMP (cAMP) increases tyrosine hydroxylase activity (Haycock et al., 1982; Chalfie et al., 1979; Zubay, 1988). Unlike depolarization, however, cAMP does not stimulate catecholamine secretion and does not require calcium (Chalfie et al., 1979). Adrenocorticotropic hormone (ACTH) helps to sustain the levels of tyrosine hydroxlase and dopamine beta-hydroxylase, which catalyzes formation of norepinephrine, under stressful conditions (Berne and Levy, 1988). ACTH is released from the anterior pituitary and also stimulates production and release of cortisol and aldosterone. In effect, it helps regulate the output of adrenomedullary hormones. Control of its secretion is performed by hypothalamic ACTH-releasing factor, the secretion of which is controlled by neural input to the hypothalamus by factors such as anxiety, stress and exercise (McArdle et al., 1986).

Prolonged stimulation of the sympathoadrenal system increases concentrations of both tyrosine hydroxylase and dopamine beta-hydroxylase in the adrenal medulla and sympathetic nerves, thus helping to ensure maintenance of catecholamine output during continuous demand. Induction of the hydroxylase enzymes may involve a cAMP-dependent protein kinase (Kumakura et al., 1979). Therefore, both activation and induction of tyrosine hydroxylase may be regulated by cAMP. Induction of tyrosine hydroxylase appears to increase the capacity of sympathetic neurons or chromaffin cells to synthesize catecholamines in response to increased physiological demand.

Furthermore, cortisol released into the circulation from the adrenal cortex in response to ACTH specifically induces phenylethanolamine N-methyltransferase and therefore selectively stimulates epinephrine synthesis (Berne and Levy, 1988; Wurtman

and Axelrod, 1966). The anatomical relationship between the adrenal medulla and the cortex subserves this action, since blood from the cortex with a high concentration of cortisol directly perfuses the chromaffin cells. Hence, high levels of glucocorticoids may be important in regulating the capacity of the chromaffin cell to form epinephrine. However, the N-methyltransferase is not known to be rate limiting in epinephrine synthesis since epinephrine secretion is controlled by the nervous system. In addition, some forms of brain phenylethanolamine N-methyltransferase are not considered inducible by glucocorticoids (Landsberg and Young, 1992).

Although hydroxylation of tyrosine is the rate-limiting step in catecholamine synthesis, substrate or coenzyme availability to the other enzymes in the pathway may influence the ultimate rate of catecholamine synthesis. Specifically, L-DOPA decarboxylase requires PLP as coenzyme and L-DOPA as substrate in order to catalyze the reaction synthesizing dopamine, from which norepinephrine and, subsequently, epinephrine may be formed. Enzyme activity is, therefore, indirectly controlled through availability of PLP and L-DOPA. Inhibition in the activity of tyrosine hydroxylase, as described above (Abou-Donia, 1981; Udenfriend, 1966), reduces the substrate available for L-DOPA decarboxylase. The role of PLP availability in regulation of catecholamine synthesis will be discussed in depth later in this review.

Uptake of the catecholamines into storage granules in neurons, sympathetic nerve endings and chromaffin cells of the adrenal medulla also appears to play a role in regulating catecholamine levels. Dopamine formed in the cytosol must be taken up by the granules before dopamine beta-hydroxylase can catalyze the formation of norepinephrine. The uptake is stereospecific, energy-requiring, saturable and competitive with regard to substrate (Perlman and Chalfie, 1977; Kopin, 1977). In addition, dopamine beta-hydroxylase is found only in tissues that synthesize and store catecholamines (Kaufman and Friedman, 1965). Norepinephrine must leave the granule to be converted to epinephrine by phenylethanolamine N-methyltransferase. The final uptake of dopamine, norepinephrine, and epinephrine for storage by the secretory granules requires ATP and magnesium (Berne and Levy, 1988; Landsberg and Young, 1992). Catecholamines remaining in the cytosol are subject to potential oxidation by enzyme action, as will be described later. As already discussed, the catechols remaining in the cytosol also inhibit the activity of tyrosine hydroxylase.

Transport of catecholamines in plasma does not seem to influence regulation. At physiological levels, 50 to 60% of the catecholamines in human plasma are loosely bound to albumin (Danon and Sapira, 1972), globulins and lipoproteins (Sager et al., 1987). However, the significance of protein binding is unclear since water-soluble catecholamines

do not require protein binding for transport. It is possible that protein binding protects the catecholamines from oxidation.

Deactivation and Excretion

The biological effects of catecholamines are terminated rapidly by the following mechanisms: reuptake into presynaptic nerve terminals, oxidative deamination, methylation, conjugation with sulfate and glucuronide, and renal excretion. After release of dopamine and norepinephrine from neurons, free neurotransmitter in the synaptic cleft that has not reacted with receptors is taken up into the presynaptic nerve ending in only a few seconds. Uptake into nerves plays a less important role in the deactivation of epinephrine.

Norepinephrine secreted by sympathetic nerve endings may be removed by uptake into nerve endings, or it may diffuse away from nerve endings into the blood. Research examining venous versus arterial concentrations of radiolabelled norepinephrine has reported that only about 15% of arterio-venous differences in plasma $^3$H-norepinephrine could be attributed to neuronal uptake (Goldstein et al, 1985). Norepinephrine that diffuses into the blood, and norepinephrine and epinephrine secreted into the blood by the adrenal medulla can remain active for several minutes until they diffuse into some tissue where they are rapidly destroyed by enzymes; this occurs mainly in the liver (Guyton, 1987). Clearance of catecholamines from the circulation is believed to occur via a beta-adrenergic mechanism (Cryer et al, 1980) with a half-life on the order of 1-3 min. (Peronnet et al, 1988; Vendasalu, 1960; Berne and Levy, 1988). The metabolic clearance rate of epinephrine is reported to be 3.5 to 6.0 L/minute, and that of norepinephrine is 2.0 to 4.0 L/minute (Berne and Levy, 1988).

Monoamine oxidase catalyzes the oxidative deamination of intraneuronal dopamine and norepinephrine and of circulating catecholamines. Dopamine or norepinephrine bound to postsynaptic membranes or free in the presynaptic nerve ending is destroyed by monoamine oxidase. Monoamine oxidase is present in most tissues; its concentration is low in skeletal muscle and blood and high in liver, kidney, intestine and stomach (Tipton, 1973). The monoamine oxidase is located in the mitochondria of sympathetic nerve endings and chromaffin cells. The catecholamines stored in granules are protected from oxidation; thus, monoamine oxidase plays a role in limiting catecholamine storage.

Catechol O-methyltransferase catalyzes the meta-O-methylation of circulating norepinephrine and epinephrine after uptake into tissues, and locally released norepinephrine in effector tissues, such as the heart (Kopin, 1977). The enzyme is primarily extraneuronal, with the highest concentrations found in the liver and kidney (Giachetti, 1978). Methylation is more important than deamination in the metabolism of circulating (Kopin et al., 1961) and locally released (Kopin et al., 1983) catecholamines. PLP and pyridoxine (25-50-75-100 $\mu$g/ml) have been shown to inhibit catechol O-methyltransferase in vitro, with PLP having the more potent effect (Bettini et al., 1985).

Phenol sulfotransferase catalyzes conjugation of the phenolic hydroxyl group of the catecholamines with sulfate (Kopin, 1985; Weinshilboum, 1988). Ingested catechols are conjugated to an important degree, and catechols in the diet appear in plasma and urine principally as conjugates (Davidson et al., 1981). The sulfotransferase is concentrated in platelets, brain, liver and gut (Weinshilboum, 1988).

Catecholamines are excreted in the urine, but the renal mechanisms that are involved are poorly understood. In humans, most of the catecholamines are excreted as deaminated metabolites; a small fraction is excreted unchanged or as O-methylated amines (Ruthven and Sandler, 1965). Under normal circumstances, epinephrine and its metabolites account for a minor proportion of excretory products compared to norepinephrine and its metabolites.

# Vitamin B-6 and Catecholamines

## Introduction

As previously described, pyridoxal 5'-phosphate (PLP) is the major coenzymatic form of vitamin B-6 and acts as coenzyme for L-DOPA decarboxylase in the conversion of L-DOPA to dopamine. Dopamine can then be hydroxylated to norepinephrine, which can then be methylated to give epinephrine. Thus, the amount of PLP in a given tissue may play a role in regulating L-DOPA decarboxylase and catecholamine synthesis in neurons and the chromaffin cells of the adrenal medulla. General regulation of PLP availability via absorption, metabolism in the liver, transport in the blood and uptake into tissues has already been described. Specifically, enzyme kinetics, negative inhibition of enzyme activity and protein binding provide mechanisms for controlling cellular PLP concentrations (Merrill and Henderson, 1990; Merrill et al., 1979; Dakshinamurti, 1982; Ebadi et al., 1970; Lumeng and Li, 1980). Additional mechanisms regulate the levels of PLP that are available to synthesize catecholamines within neurons, namely saturable transport systems (Spector, 1978a & 1978b), feedback of monoamines on pyridoxal kinase (Ebadi, 1979) and affinity for DOPA decarboxylase (Dakshinamurti, 1990).

## Regulation of Vitamin B-6 Transport to the Brain

Research has shown that radiolabelled pyridoxine (PN) readily passes into rat and mice brains from blood after intravenous injection (Tiselius, 1973). After injection of [$^3$H,$^{32}$P]pyridoxine-5'phosphate (PNP), most of the PNP recovered in brain and cerebrospinal fluid was not labeled with $^{32}$P (Spector and Greenwald, 1978). Total vitamin B-6 levels in mammal brains appear to be better maintained than in liver and kidney during a vitamin B-6 deficiency, and do not show a significant increase with high vitamin B-6 intake (Spector, 1978b). Hence, it would appear that a homeostatic mechanism exists to regulate vitamin B-6 levels in the brain. Spector has performed both *in vitro* (1978a) and *in vivo* (1978b) experiments which indicate that the mechanism regulating brain levels of vitamin B-6 involves control of vitamin B-6 entry into and exit from cerebrospinal fluid (CSF) and brain. Spector (1978a) found that *in vitro* rabbit brain

and choroid plexuses (site of blood-CSF transfer, or blood-brain barrier) accumulated radiolabelled PN by a saturable process. The transport of the [³H]pyridoxine into both tissues was inhibited by PN, PL and PM and, to a lesser degree, by PLP, PMP and PNP. Most of the [³H]pyridoxine taken up was phosphorylated and retained. Brain slices released only nonphosphorylated vitamers and choroid plexus released mainly phosphorylated vitamers into artificial CSF. This indicates that the choroid plexus, not the brain, is the source of phosphorylated B-6 vitamers released to the CSF.

During *in vivo* studies using adult New Zealand white rabbits, Spector (1978b) measured the endogenous concentrations of vitamin B-6 (all phosphorylated and nonphosphorylated forms except PN and PNP, which he considered negligible) in CSF, choroid plexus, brain and plasma. He then measured the entry of radiolabelled vitamin B-6 into the same tissues and plasma 2.5 hours after intravenous injection of various amounts of radiolabelled PN. Basal (control) concentrations of B-6 vitamers (PL, PLP, PM & PMP), expressed as $\mu$mol/L or $\mu$mol/kg, showed the following pattern: plasma<CSF<brain<choroid plexus; the percentage of phosphorylated vitamin B-6 correlated with the amount of total vitamin B-6 (i.e., the greatest percent of PLP and PMP was in the choroid plexus). After intravenous infusion of PN, the pattern of B-6 vitamer (PL, PLP, PM & PMP) concentrations showed the following: CSF<brain<plasma<choroid plexus, regardless of the quantity of PN injected. Note that plasma concentrations of B-6 vitamers after PN infusion exceeded the levels in CSF and brain, whereas prior to PN infusion the vitamer levels were lower than in CSF or brain. The ratio of total vitamin B-6 to plasma vitamin B-6 progressively decreased in CSF, brain and choroid plexus as the level of PN infusion increased. These findings suggest that movement of vitamin B-6 between blood and CSF, and subsequently into brain and choroid plexus, involves one or more saturable mechanisms. That is, after intravenous injection of PN, the relative entry of B-6 vitamers from plasma into CSF, choroid plexus and brain decreased compared to control conditions.

From both in vitro and in vivo studies, Spector and Greenwald (1978) reported that nonphosphorylated B-6 vitamers are the principal forms transported into brain and choroid plexus. However, phosphorylated vitamers are the forms that accumulate in these tissues. Hence, concentration of vitamin B-6 in the brain is dependent upon PL kinase.

The exact mechanism of transport regulation in and out of the brain is unclear, but Spector suggests that the saturation of either a carrier transport system and/or an enzymatic phosphorylation reaction may be responsible. Dakishnamurti (1982) surmised that the unknown mechanism regulating the transfer of vitamin B-6 to brain and CSF from plasma

can operate to maintain vitamin homeostasis at high plasma levels as well as maintain high brain levels even at very low plasma concentrations of vitamin B-6.

## Regulation of PLP Synthesis in Brain

The regulation of pyridoxal kinase appears to influence the synthesis of catecholamines in the brain (Ebadi and Govitrapong, 1979). Levels of PLP, the catecholamines themselves and L-DOPA play a part in this regulation. Recall that PL kinase catalyzes the phosphorylation of PN, PM and PL to their respective phosphorylated forms in the cytosol of the cell. PMP and PNP can then be converted to PLP by PNP oxidase.

Dakshinamurti (1990) reported that the activity of pyridoxal kinase is unevenly distributed throughout the brain (measured as $\mu$mol of PLP formed/g tissue/hr.). Dakshinamurti also reported a positive correlation between the varying distributions of pyridoxal kinase activity in brain and the concentrations of catecholamines discovered by other researchers; for instance, pyridoxal kinase activity showed a positive relationship with the concentration of norepinephrine in hypothalamus (McGeer et al., 1963) and the level of dopamine in striatum (Montagu, 1957).

In contrast, Ebadi and Govitrapong (1979) found an inverse relationship between the activity of pyridoxal kinase and acute concentrations of certain monoamines in the rabbit brain. For instance, a given level of serotonin, dopamine, norepinephrine, histamine, GABA or tyramine were all shown to be potent inhibitors of pyridoxal kinase. When Ebadi et al (1970) treated rabbits acutely with drugs that reduced the concentrations of these amines, the activity of PL kinase increased. Conversely, when the concentration of these amines was increased by compounds that inhibited their metabolism, the activity of pyridoxal kinase decreased.

Although the amines inhibited the enzyme, their precursor amino acids, such as tyrosine in the case of the catecholamines, did not inhibit the kinase. Amine metabolites that lacked biological activity did not inhibit pyridoxal kinase. However, L-DOPA, the substrate for DOPA decarboxylase, shared inhibitory activity with dopamine and norepinephrine, but became a more potent inhibitor after decarboxylation to dopamine. Ebadi and Govitrapong (1979) found that acute administration of L-DOPA reduced the concentration of PLP in rat basal ganglia, but the chronic oral administration of L-DOPA (100 mg/kg/d) did not alter the concentration of PLP. This suggests an adaptive increase in the activity of pyridoxal kinase.

In addition, increased levels of dopamine in rat basal ganglia have been observed after an acute PN injection (Dakshinamurti, 1990). It could be surmised that levels would normalize with chronic administration due to negative feedback of dopamine and PLP on pyridoxal kinase and/or PNP oxidase activity.

The studies reviewed herein examined the relation of catecholamines and PL kinase activity in the brain. It may also be possible that PL kinase in the adrenal medulla is similarly influenced, but additional research would be required to substantiate this theory.

## Regulation of DOPA Decarboxylase

Changes in catecholamine concentrations logically reflect DOPA decarboxylase activity (Landsberg and Young, 1992). From the above discussion, it follows that decarboxylase activity is indirectly controlled through availability of PLP. Hence, a deficiency of PLP would seemingly limit decarboxylase activity. However, PLP has a strong affinity for DOPA decarboxylase, particularly in the brain (Dakshinamurti et al., 1976; Siow and Dakshinamurti, 1990).

Regardless of the method used to deplete pyridoxine (dietary or employment of anti-metabolites like 4-deoxypyridoxine or penicillamine), a very significant decrease in the brain serotonin content was seen in pyridoxine-deficient young rats with no change in brain catecholamines (Dakshinamurti et al., 1976). Similar results were obtained in various brain regions of pyridoxine-deficient adult rats (Dakishnamurti et al., 1976). Recall that PLP is required for 5HTP decarboxylase to catalyze the synthesis of serotonin.

The possibility that the decrease in brain serotonin seen in pyridoxine-deficient rats was the result of inanition (a weak state from lack of food and water) or due to a generalized malnutrition was methodically ruled out. Further metabolism of serotonin by monoamine oxidase or the transport of its metabolite, 5-hydroxyindoleacetic acid, to CSF were also not altered in pyridoxine deficiency. Thus, the decreased levels of serotonin seen in pyridoxine deficiency appear to be attributable to decreased availability of PLP required for 5HTP decarboxylase. Indeed, Siow and Dakshinamurti (1990) discovered that pyridoxine deficiency differentially affects DOPA and 5HTP decarboxylase activities in the rat brain because PLP is more tightly bound (has a greater affinity) to DOPA decarboxylase that to 5HTP decarboxylase. Thus DOPA decarboxylase is less sensitive to a pyridoxine deficiency than is 5HTP decarboxylase.

It has been reported that the distribution of DOPA decarboxylase in the individual hypothalamic nuclei correlated directly with the distributions of norepinephrine and dopamine, but inversely with the distribution of serotonin (Siow and Dakshinamurti, 1990). This could be attributed to PLP's greater affinity for DOPA decarboxylase as opposed to 5HTP decarboxylase.

DOPA decarboxylase may have different properties within various regions of the brain. For instance, research has shown that brainstem DOPA decarboxylase in rats has a lower affinity for PLP than the enzyme in other regions of the brain; and DOPA decarboxylase from the brainstem exhibits different kinetic characteristics than the enzyme in the corpus striatum (Siow and Dakshinamurti, 1990).

Although research indicates that the synthesis of catecholamines in the young or mature brain is not effected by a pyridoxine deficiency, perinatal or neonatal mammals may be negatively impacted. Bhagavan et al. (1977) reported that pyridoxine deficiency resulted in a more marked reduction (75-80%) of PLP in all regions of the neonatal rat brain as compared with decreases of PLP (50-60%) in postweanling rats. Bhagavan and associates did not report the effects of the PLP reduction on catecholamine production, nor on DOPA decarboxylase activity. However, a decrease in glutamic acid decarboxylase activity was thought to lead to seizures due to reduced GABA production.

Guilarte et al. (1987) found a significant decrease in corpus striatal dopamine concentration and DOPA decarboxylase activity in rats whose mothers were pyridoxine deficient during the last week of gestation and throughout lactation. The author believes that the time of onset of the pyridoxine deficiency is an important factor in determining its effects on the development of the dopaminergic system in the corpus striatum. The development of the dopaminergic system appears to be most vulnerable perinatally.

DOPA decarboxylase also seems to have different properties in varying tissues. Siow and Dakshinamurti (1990) compared properties of DOPA decarboxylase from neuronal and non-neuronal tissues using data from their research and the research of other investigators. Common features exist, but so do obvious differences, such as kinetics and stability to heat. The authors do not indicate whether enzymes in neuronal or non-neuronal tissues have diverse affinities for PLP. Nevertheless, Bhagavan and Coursin (1973) proposed that differential depletion of PLP from various tissues occurs during pyridoxine deficiency. They found a depletion of PLP from the brain of 50%, liver losses of about 75% and blood concentrations nearly negligible (over 95% loss) after 6-8 weeks of feeding a vitamin B-6 deficient diet.

Research by Roberge (1977) provided an example of tissue differences with regard to decarboxylase activity. Cat brain and liver decarboxylase activity responded differently

to excess PN. (The researcher did not differentiate between DOPA decarboxylase and 5HTP decarboxylase.) Pyridoxine (50 mg/kg by mouth) given for 7 consecutive days significantly increased decarboxylase activity (22%) in the neostriatum of cat brain but no change occurred in the liver.

Roberge (1977) also demonstrated an interrelationship between PN and L-DOPA that affected decarboxylase activity differently in the cat brain and liver. The administration of L-DOPA and pyridoxine together (100 and 50 mg/kg, by mouth, respectively) for 7 days increased decarboxylase activity in the brain to the same extent as did L-DOPA and pyridoxine given individually. On the other hand, liver decarboxylase activity remained normal when both drugs were administered together, and decreased significantly after L-DOPA administration but not after pyridoxine treatment.

In cats treated with L-DOPA, actinomycin D prevented the increased decarboxylase activity in the neostriatum but had no effect on the enzymatic activity in the liver (Roberge, 1977). In addition, denatured supernatant from livers of cats treated with L-DOPA contained a dialysable compound that inhibited decarboxylase activity in the supernatant from livers of untreated cats. No inhibitor was formed in the brain with L-DOPA administration. The inhibitor did not appear to be formed in the liver when L-DOPA and pyridoxine were administered together. These results may explain the mechanism by which L-DOPA exerts its beneficial effects in Parkinson's patients and why pyridoxine administered with L-DOPA reduces its therapeutic effectiveness. The author suggested that pyridoxine protected the enzyme in the liver and favored a more rapid degradation of L-DOPA peripherally, resulting in less L-DOPA available for the CNS. However, the inhibitor manufactured in the liver could possibly negatively impact decarboxylase activity in the brain as well as in the liver.

The research reviewed here has all been performed with animals. As such, direct application to humans must be approached with caution. For instance, DOPA decarboxylase appears to display different characteristics between tissues and even in various regions of the brain. There may well be variations between species as well. Further, neither DOPA decarboxylase activity in the chromaffin cells nor the effect of vitamin B-6 status on catecholamine synthesis in the adrenal medulla have been examined.

In humans, DOPA decarboxylase activity and/or catecholamine levels have not been examined under conditions of PN supplementation. Nevertheless, the literature would suggest that vitamin B-6 supplementation could enhance catecholamine synthesis through greater availability of PLP. Plasma and tissue (muscle) PLP levels in humans have been shown to rise in response to increased vitamin B-6 intake (Lui et al., 1985; Ubbink et al., 1987). Metabolic balance studies in men have shown that plasma PLP

levels plateau within 7-10 days after a change in vitamin B-6 intake between 0.5 and 10 mg/d; this research also reported a direct relationship between fasting plasma PLP level and body store during the period of PN supplementation in humans (Lui et al., 1985). Hence, with greater intake of vitamin B-6 (e.g., supplementation), more PLP may be available to neurons and the adrenal medulla from plasma and/or tissue, allowing greater DOPA decarboxylase activity and catecholamine production.

Aside from its role in synthesis of catecholamines, PLP may play a role in stimulating their release. Research by Lau-Cam (1991) reveals a role for PLP as an instigator of catecholamine release. The investigator administered a single, 100-300 mg/kg ip dose of PN or PL to rats and measured an almost immediate and gradual mobilization of the liver glycogen and an accompanying elevation of the serum glucose. The dose also stimulated the secretion of adrenal catecholamines and the accumulation of liver cAMP. PL had a more potent effect than PN. A pretreatment with ß-adrenoreceptor blocking agents conferred significant protection against the glycogen depletion, as would be expected given the role of the catecholamines in activation of glycogenolysis. In addition, PL was shown to possess virtually no effect on the liver glycogen of adrenalectomized rats. However, there was still a slight rise in blood glucose that possibly could have been stimulated by norepinephrine release from the brain, which would promote glycogenolysis and gluconeogenesis in the liver.

From the data, Lau-Cam (1991) suggests that PLP acts as a ß-adrenoreceptor agonist, stimulating the release of epinephrine and norepinephrine. More work must be performed to determine if physiologically viable amounts of vitamin B-6 intake may affect catecholamine release under certain conditions (e.g., low blood glucose, strenuous exercise).

## Vitamin B-6 and Exercise

Introduction

Research indicates that exercise causes an increased metabolic flux of vitamin B-6 in the body as evidenced by increased plasma PLP and urinary 4-PA (Leklem and Shultz, 1983; Manore et al, 1987a & b; Dunton et al, 1992). B-6 supplementation has been shown to enhance this effect (Manore et al, 1987a; Dunton et al, 1992). Altering B-6 intake may modify fuel usage during exercise due to its role as a coenzyme in glycogenolysis and gluconeogenesis . In particular, it has been proposed that greater intakes of B-6 result in higher glycogen (Leklem, 1985) and glucose (Virk, 1992) usage relative to fat utilization. Vitamin B-6 may also play a role in lowering heart rate and oxygen consumption during exercise (Virk, 1992).

PLP and 4-PA Response to Exercise

Plasma PLP concentration in humans has been shown to rise during exercise and return to resting levels or below by 60 minutes post-exercise (Leklem, 1985). The rise in plasma PLP with exercise does not appear to be dependent upon exercise intensity nor duration (Crozier et al., 1994). PN supplementation has been shown to enhance the rise in plasma PLP during exercise (Manore et al, 1987a; Dunton et al, 1992). Leklem and Shultz (1983) conducted the first study that showed altered plasma PLP levels as a result of middle-distance running. They investigated the effect of a 4500-meter run (approximately three miles) on plasma PLP and vitamin B-6 metabolism in seven male, athletes ages 15-18 years. A mean increase in PLP of $7.7 \pm 3.1$ to $18.3 \pm 7.7$ nmol/L of plasma (range of 3.5 to 29.8 nmol/L of plasma) was observed after exercise. Pre-run plasma PLP levels ranged from 47.6 to 68.0 nmol/L; post-run values ranges from 57.5 to 86.3 nmol/L. Manore and associates (1987a) assessed the effect of exercise on plasma PLP and vitamin B-6 metabolism in five young, trained women; five young, untrained women; and five postmenopausal, untrained women fed diets differing in carbohydrate and vitamin B-6. The four diets consisted of moderate (49%) carbohydrate (2.3 mg/d B-6), high (64%) carbohydrate (2.4 mg/d B-6), and each carbohydrate intake plus B-6

supplementation (8 mg/d PN-HCl). At the end of each dietary phase, subjects exercised on a cycle ergometer at 80% $VO_2$ max for 20 minutes. Plasma PLP increased significantly from pre- to post-exercise and decreased significantly from post- to post-60 min. for all groups and diets. This pattern remained consistent regardless of the level of training, increased carbohydrate intake or age. With the PN supplemented diets, the magnitude of the PLP increase from pre- to post-exercise was significantly greater compared to the unsupplemented diets as was the PLP decrease from post- to post-60 min.

Dunton and colleagues (1992) analyzed the effect of exhaustive cycling on plasma PLP in six male, trained cyclists fed two diets differing only in vitamin B-6 content (2.3 mg/d and 22.3 mg/d total). During the unsupplemented period, mean plasma PLP values were 31.4 ± 12.5 nmol/L pre-exercise, 36.3 ± 12.8 nmol/L at 60 minutes of exercise, 30.0 ± 13.4 nmol/L immediately post-exercise and 25.3 ± 9.65 nmol/L 60 minutes post-exercise. This amounted to a plasma PLP increase of 19 ± 11% during exercise, a mean PLP decrease of 7 ± 15% from pre- to post-exercise and a PLP decrease of 18 ± 13% from pre- to post-60 minutes. During the supplemented period, mean plasma PLP values were 199 ± 47.6 nmol/L pre-exercise, 261 ± 65.2 nmol/L at 60 minutes of exercise, 201 ±39.5 nmol/L immediately after exercise and 168 ± 33.0 nmol/L 60 minutes after exercise. This amounted to a plasma PLP increase of 28 ± 20% during exercise, a slight PLP increase of 3 ± 18% from pre- to post-exercise and a PLP decrease of 14 ± 15% from pre- to -post-60 minutes. Of note was the observed peak in plasma PLP concentration at 60 minutes of exercise, followed by a decrease in plasma PLP concentration to the end of exercise and through recovery. Plasma PLP concentrations at the end of exercise were similar to resting values while post-60 minute PLP values were lower than resting values.

Leklem (1988a) has hypothesized that increased plasma PLP concentration during exercise results from release of PLP from glycogen phosphorylase and subsequently from muscle. This idea rests on the assumption that exercise produces an acute case of caloric deprivation (Leklem and Shultz, 1983), which has been shown to stimulate a rise in rat plasma PLP via release from glycogen phosphorylase (Black et al, 1978). Leklem (1988a) has proposed a mechanism for the exercise-induced release of PLP from muscle. As in rats, greater B-6 intake coincides with greater levels of muscle glycogen phosphorylase and PLP. During exercise there would be an increased rate of glycogen breakdown and subsequent lactic acid production. The increased lactate would decrease the pH in muscle as low as 6.5 to 6.6, facilitating release of PLP from glycogen phosphorylase. PLP could then "leak" from the muscle into the circulation, elevating

plasma PLP levels. The circulating PLP would then be available to other tissues. In particular, the liver uses PLP as a coenzyme for gluconeogenic reactions to maintain blood glucose.

If the increased plasma PLP during exercise results from glycogen breakdown in the muscle, it follows that decreased glycogen use would reduce the rise in plasma PLP. It has been thought that glucose ingestion would reduce the amount of PLP released (Leklem, 1985). However, a study by Hofmann and collegues (1991) suggests that PLP is released largely from the liver, rather than muscle, during exercise. The researchers analyzed the effect of water or glucose ingestion on plasma PLP levels after treadmill running in six trained males. After running at 65% $VO_2$ max, the subjects' PLP levels increased by a magnitude of 19-26% regardless of which fluid was administered. The authors proposed that the liver releases PLP during exercise to provide the coenzyme to working skeletal muscle. No metabolic fuels were measured in this study.

In addition to elevating plasma PLP concentration, exercise has been shown to increase urinary 4-PA excretion (Leklem and Shultz, 1983; Manore et al, 1987a; Dunton et al, 1992; Leklem, 1985; Rokitzki et al, 1994). Rokitzki et al (1994) reported a loss of 0.8-0.9 mg vitamin B-6 in trained runners two hours after a marathon race. This loss was based on an absolute urinary 4-PA excretion of 2.05 $\mu$mol and metabolization of vitamin B-6 to 4-PA of 40-50%. The reported loss is about 40% of the RDA for vitamin B-6. They concluded that the vitamin B-6 loss did not require vitamin supplementation, but could be replaced with a balanced diet.

The rise in 4-PA excretion after exercise appears to be independent of age or carbohydrate content of the diet. Training may effect the amount of 4-PA excretion after exercise. Manore et al (1987a) noted no difference in 4-PA excretion between trained and untrained women on an exercise day, but reported higher urinary 4-PA in the trained women five to six days before an exercise day. The difference was not statistically significant. Dreon and Butterfield (1986) compared the 4-PA excretion of four trained men to a group of inactive men. Both groups consumed the same diet (4.2 mg B-6/d). Baseline excretion of 4-PA was lower in the trained runners than in the control subjects, but urinary 4-PA increased slightly in the trained group after a methionine challenge. The researchers hypothesized that active individuals have a labile pool of pyridoxine that can be redistributed under conditions of increased need, such as exercise. This would indicate that exercise may promote storage of vitamin B-6.

PLP Role in Fuel Utilization

During exercise, PLP aids in metabolizing carbohydrate and amino acids in muscle and liver to maintain adequate levels of blood glucose (Leklem, 1985). Glycogenolysis involves the breakdown of glycogen to glucose in the liver and muscle. PLP serves as a coenzyme to glycogen phosphorylase to convert glycogen to glucose-1-phosphate (Chastiotis et al., 1982; Cori and Illingsworth, 1957), the first step in the glycogenolytic pathway. During gluconeogenesis non-carbohydrate precursors (i.e., amino acids, lactic acid and glycerol) are used to synthesize glucose in the liver. In the Cori-alanine cycle (Cori, 1931; Felig et al., 1970), PLP acts as a coenzyme to aminotransferase enzymes for conversion of amino acids to alanine in muscle, and to alanine aminotransferase for synthesis of pyruvate from alanine in the liver. Pyruvate may then be converted to glucose and exported to the circulation. Lactic acid formed in the muscle during strenuous exercise also travels to the liver where it can be transformed to pyruvate, then subsequently to glucose and released to the circulation.

Due to PLP's role in fuel metabolism, it has been hypothesized that PLP can act as a fuel modifier during exercise, and that supplemental vitamin B-6 may increase the rate of glycogenolysis (Leklem, 1985; Virk, 1992). If increased vitamin B-6 in the diet does increase glycogen use during exercise, one would expect a change in circulating plasma free fatty acids (FFA) and lactate. Research has produced variable results when attempting to correlate fuel use during exercise with vitamin B-6 supplementation.

An initial experiment (deVos, 1982) examined the influence of B-6 on fuel metabolism in conjunction with a glycogen depletion-repletion regimen (medium carbohydrate-low carbohydrate-high carbohydrate) and supplemental B-6 (8 mg/d) in four young, trained males. On completing the depletion and repletion phases with and without supplemental B-6, subjects exercised for 50 min. on a cycle ergometer at stepwise increases in intensity. Blood was drawn pre-exercise, during exercise, post-exercise, post-30 min. and post-60 min., and analyzed for plasma PLP, glucose, lactate, and FFA. As expected, the mean change in plasma PLP during exercise was greater for the B-6 supplemented periods compared to the unsupplemented periods. The lowest mean change in plasma PLP concentrations with exercise followed the low carbohydrate (LC) diet. Since subjects exercised heavily during the LC diet, glycogen reserves would have been low. Leklem (1985) notes that under these conditions, PLP would have been used for gluconeogenesis (via aminotransferase activity) to maintain blood glucose and muscle reserves would be depleted. This would explain the smaller increase in plasma

PLP after exercise seen with the LC diet. In contrast, the low carbohydrate plus B-6 supplementation (LC+B-6) diet produced the largest increase in plasma PLP during exercise. Assuming that muscle glycogen reserves were depleted due to the low carbohydrate intake and exercise, the question then arises as to where the excess PLP was stored. It may have been released from the liver, and there may have been less muscle uptake. Plasma FFA concentrations were from 30% to 75% higher after the LC and LC+B-6 diets than after the control, high carbohydrate (HC) and high carbohydrate plus B-6 supplementation (HC+B-6) diets. This is not surprising, as glycogen stores would be decreased after a low carbohydrate diet and the body would depend more on fat for energy. The B-6 supplement did not appear to effect plasma FFA levels. Plasma glucose values during exercise were from 3% to 4% lower and plasma lactate values were 57% higher for the HC and HC+B-6 diets compared to controls. The HC+B-6 diet produced higher post-exercise lactate levels than the HC diet, but the difference was not statistically significant. deVos proposed that the simultaneously low glucose and high lactate levels indicated that glucose was derived mainly from muscle glycogen in the HC and HC+B-6 conditions. The lowest lactate levels were found post-exercise after the LC+B-6 diet, and these values were significantly lower than the post-exercise values for the LC diet. Leklem (1985) suggests that with the B-6 supplement, there was less muscle glycogen reserve due to greater glycogen phosphorylase activity prior to the exercise day. This would account for the low lactate levels seen with the LC+B-6 diet.

Manore and Leklem (1988) assessed the effect of two levels of carbohydrate and vitamin B-6 supplementation (2.4 to 10.4 mg/d) on glucose, FFA and lactate during exercise in the same groups of women as described above (i.e., Manore et al., 1987a). As previously noted, the increase in plasma PLP during exercise was significantly greater with the B-6 supplemented diets compared to the unsupplemented diets. Circulating FFA generally decreased at rest and during exercise with an increase in dietary carbohydrate regardless of the group. The effect of supplemental vitamin B-6 on peak plasma FFA concentrations was more variable and seemed to depend on level of training and a high carbohydrate diet. Nevertheless, in all groups the HC+B-6 diet produced the lowest plasma FFA concentrations during exercise compared to the other diets. This finding is consistent with the hypothesis that higher vitamin B-6 intake could lead to a more rapid utilization of glycogen and less dependence on FFAs. However, lactate levels were similar for all groups and diets. It is possible the differences in carbohydrate intake were not great enough to increase carbohydrate metabolism sufficiently to increase plasma lactate levels. In addition, the subjects were only exercised for 20 min., which may not have been long enough to generate noticeable

changes in lactate production. In general, plasma glucose concentrations showed little change between the diets, but the post-menopausal/untrained women had a significantly lower glucose response to exercise than the young women regardless of diet.

Lawrence et al (1975) serendipitously provided data on fuel modification by vitamin B-6 when investigating the effects of vitamin E (alpha-tocopherol) on swimming endurance. Seventy-two trained, male and female swimmers were given either a placebo, 51 mg/d PN-HCl or 900 IU alpha-tocopherol for six months. None of the treatments significantly effected swimming performance, but subjects supplemented with B-6 showed a slight decrease in improvement compared with the other groups. More importantly, this group showed significantly higher plasma lactate levels with exercise than subjects not supplemented with B-6. Compared with the Manore et al (1987a) study, the B-6 dosage was larger, the duration of the diet was longer, the number of trained subjects was larger, and the exercise type and duration differed with this research. These factors may explain the contrasting lactate results.

Recently, Virk (1992) assessed fuel use during cycle ergometer exercise in six male, trained cyclists fed two diets differing only in vitamin B-6 content (2.3 mg/d and 22.3 mg/d). At the end of each dietary phase, subjects exercised to exhaustion at 64-75% $VO_2$ max. Blood was drawn pre-exercise, during exercise (60 min.), post-exercise, post-30 min. and post-60 min., and analyzed for plasma glucose, lactate, glycerol and FFA. The only significant difference in plasma substrates was lower plasma FFA concentrations at pre-exercise in the supplemented state compared to the unsupplemented condition. In addition to plasma substrates, expired air was collected for 2 min. at 10 min. intervals during the exercise periods to determine respiratory exchange ratios (R). The R value is calculated from oxygen consumption and carbon dioxide production to estimate the relative amounts of carbohydrate, fat and protein being used for fuel. The numerical value of R reflects the varying amounts of oxygen required for oxidation of a given substrate: a value of 1.0 reflects carbohydrate metabolism (1 mole of carbon dioxide produced per mole of oxygen consumed); a value of 0.70 reflects fat utilization (Virk; McArdle et al., 1986). Hence, an increase in the R value indicates increased carbohydrate combustion. Although Virk did not find statistically different R values between the supplemented and unsupplemented periods, subjects maintained higher R values during the supplemented exercise period. From the data, Virk concluded that B-6 supplementation did not significantly effect fuel utilization during submaximal exercise to exhaustion under the conditions of this study.

An earlier study (Campuzano, 1988) also analyzed respiratory gases to determine if PN supplementation effected fuel metabolism. Campuzano assessed the effect of one

hour cycle ergometry at 72% $VO_2$ max in six trained males fed a constant diet supplemented with 20 mg/d PN (given as PN-HCl) six days prior to exercise and on the exercise day itself. The results of gas collection during exercise indicated that carbohydrate metabolism increased from 43% without supplementation to 52% with supplementation, but the difference was not statistically significant. Fat utilization decreased from 45% without supplementation to 36% with supplementation during exercise (not significant). Furthermore, resting R values were higher with supplementation compared to the control diet (0.86 versus 0.80), indicating greater carbohydrate (glycogen) usage during the B-6 supplementation period.

Research to date indicates a role for PLP in modifying fuel use during exercise, in particular increasing glycogen use, as determined by plasma lactate levels, and decreasing FFA combustion. The results of these studies may be confounded by variables such as carbohydrate content of the diet, training level of the subjects and the exercise protocol, particularly with regard to intensity and duration.

In addition to its influence on fuel metabolism via its coenzymatic role in glycogenolysis and gluconeogenesis, PLP may also effect fuel use through stimulation of catecholamine synthesis and/or release. It has been suggested earlier in this review that elevated plasma PLP concentrations during exercise may stimulate an increase in plasma catecholamines similar to the rise in plasma catecholamines seen with an acute injection of PL in the Lau-Cam (1991) study. With exercise and/or B-6 supplementation, more circulating plasma PLP may be available to neurons and the adrenal medulla, allowing greater DOPA decarboxylase activity and catecholamine production. Catecholamines activate the glycogen cascade system, thus allowing another avenue for PLP to indirectly enhance glucose production.

## Vitamin B-6 Influence on Heart Rate and Basal Metabolic Rate

A prior study in this lab (Virk, 1992) found significantly lower heart rates in male cyclists during exhaustive exercise after B-6 supplementation (20 mg PN/d for 6 days) compared to the unsupplemented condition. The mean resting heart rate was also lower after the supplemented period, although the difference was not statistically significant. Other research has reported no effect of B-6 supplemention on resting heart rates (Manore et al., 1987b).

Virk (personal communication) has suggested that PLP functions like a cardiac beta-adrenoreceptor blocker during exercise, which would account for the decreased heart rate. This proposal is based on similar reported effects of propranolol (a beta-blocker) and B-6 supplementation with regard to plasma free fatty acids, heart rate and R values. Tremblay et al (1992) found decreased heart rate, lower plasma free fatty acids and a higher R value in trained men after being given propranolol. This trend has also been noted during exercise after B-6 supplementation (Virk, 1992).

Tremblay and associates also reported that basal metabolic rate (BMR) declined in trained men when the beta-blocker was administered. BMR is calculated from oxygen consumption and denotes the energy (kcal/min.) used to sustain basal metabolic functions. Although basal metabolic rate has not been examined in B-6/exercise research, oxygen consumption in trained men was lower after B-6 supplementation compared to the unsupplemented trial during exercise (Virk, 1992). Campuzano (1988) reported higher R values at rest and during exercise in trained males after B-6 supplementation. This may indicate lower oxygen consumption in the supplemented condition. Thus, PLP may function to decrease BMR and to lower heart rate during exhaustive exercise and at rest, possibly by acting like a beta- adrenoreceptor blocker (binding beta-adrenoreceptors).

## Catecholamines and Exercise

Introduction

When the catecholamines are released during exercise, they have metabolic effects that regulate oxygen uptake and metabolic rate, mobilize energy reserves from storage and maintain extracellular fluid balance. They also have cardiovascular effects that promote changes in blood-flow distribution and enhance cardiac output (heart rate x stroke volume) (McArdle et al., 1986; Abboud et al., 1976). This chapter will focus on the response of plasma norepinephrine and epinephrine to exercise, variables effecting this response, and the influence of the catecholamines on fuel mobilization, oxygen uptake and metabolic rate, and heart rate.

Catecholamine Response to Exercise

Circulating levels of plasma catecholamines increase during exercise, and the magnitude of the elevation depends upon the duration and intensity of the exercise (McArdle et al., 1986; Trendelenburg and Weiner, 1989; Berne and Levy, 1988; Rogers et al., 1991; Favier et al., 1983; Kjaer et al., 1985). Altogether, the concentration of catecholamines rises in plasma as the exercise effort becomes more strenuous, with the highest levels found at the end of maximal exercise or prolonged submaximal exercise to exhaustion. After exercise, plasma catecholamine concentrations generally return to resting values within one hour.

Reported normal resting levels of catecholamines in human plasma are about 1.0-2.0 pmol/mL for norepinephrine and 0.10-0.25 pmol/mL for epinephrine (Trendelenburg and Weiner, 1989; Landsberg and Young, 1992; Berne and Levy, 1988). Resting plasma norepinephrine levels are generally at least three to four times greater than epinephrine levels (Mazzeo, 1991). In the exercise studies to be reviewed in this chapter (Hartley et al., 1972a & b; Galbo et al., 1975; Rogers et al., 1991; Young et al., 1992; Hagberg et al., 1979), resting norepinephrine levels in men generally ranged from 1.4 pmol/mL (Hagberg et al., 1979) to 3.3 pmol/mL (Rogers et al., 1991), and resting epinephrine levels generally ranged from 0.11 pmol/mL (Hartley et al., 1972a) to 0.49 pmol/mL (Rogers et al., 1991). The resting plasma NE:E ratios of the reviewed studies generally

ranged from 6:1 (Galbo et al., 1975) to 8:1 (Young et al., 1992). Exercise stimulated a two-fold to 10-fold increase in both plasma catecholamines relative to resting values. Peak exercise values uncovered in this review were 34.5 pmol/mL for norepinephrine and 5.6 pmol/mL for epinephrine during maximal exercise (Rogers et al., 1991).

During exercise, the magnitude and pattern of change over time differs between plasma norepinephrine and epinephrine. This variation reflects differential activation of sympathetic nerves and the adrenal medulla (Young, J.B. et al., 1984; Peronnet, F. et al., 1981) in response to the duration and intensity of the exercise challenge (Hartley et al., 1972a & b; Rogers et al., 1991). Mild to moderate exercise affects the SNS, releasing primarily norepinephrine. Norepinephrine concentration continues to rise with successively increasing exercise levels, and may increase before the onset of exercise (anticipatory response). Intense or prolonged exercise activates the adrenal medulla, in addition to sympathetic nerves, releasing epinephrine. Epinephrine increases only gradually at moderate exercise levels and escalates more dramatically at strenuous efforts. Mazzeo (1991) reports that a decrease in the plasma NE:E ratio during exercise reflects a greater relative reliance on adrenal activity compared with SNS activity.

Most of the research that has reported absolute catecholamine values involved short-term exercise or graded exercise to maximal effort. In a recent study, Rogers et al. (1991) determined the plasma catecholamine response of 10 well-trained men ($VO_2$ max=55.2 ml $O_2$/kg/min.) to submaximal exercise (approx. 52% $VO_2$ max, 4 min.) and maximal exercise (graded to 100% $VO_2$ max). Subjects (age 33 ± 7 yrs.) were tested in the exercise mode in which they were trained: Eight runners exercised on a treadmill, and two cyclists rode a cycle ergometer. Resting catecholamine levels (standing on the treadmill or sitting on the cycle) were 3.3 ± 0.29 pmol/mL and 0.49 ± 0.11 pmol/mL for norepinephrine and epinephrine, respectively. Relative to resting, plasma norepinephrine rose two-fold to 5.3 ± 0.65 pmol/mL during submaximal exercise and 10-fold to 34.5 ± 4.2 pmol/mL at maximal exercise. Relative to resting, plasma epinephrine rose two-fold to 0.93 ± 0.27 during submaximal exercise and 11-fold to 5.6 ± 1.2 pmol/mL at maximal exercise. The maximal values are the highest peak norepinephrine and epinephrine levels found in this review.

Two different groups of researchers (Hartley et al., 1972a & b; and Galbo et al., 1975) examined the catecholamine responses to graded and prolonged exercise in a given group of young men. Hartley and associates (1972a & b) found increases in circulating norepinephrine and epinephrine concentration in response to graded bicycle exercise and to prolonged bicycle exercise to exhaustion in seven trained men ($VO_2$ max=47.9 ml $O_2$/kg/min) ages 20-24. Higher plasma concentrations of norepinephrine and lower

epinephrine levels were attained at the end of graded exercise compared to the exhaustive exercise. In both studies, resting norepinephrine levels were substantially higher than in the other exercise studies reviewed herein. The higher observed resting norepinephrine concentrations may be due to various factors, including anxiety (Mason et al., 1973), time of day (Turton and Deegan, 1974), method of catecholamine analysis (Trendelenburg and Weiner, 1989) or feeding (Landsberg and Young, 1978). These factors effecting plasma catecholamine concentrations will be discussed in some detail later. The Hartley studies reported mean resting norepinephrine concentration was $8.8 \pm 0.59$ pmol/mL 20 minutes prior to and immediately prior to graded exercise; increased to $10.6 \pm 0.59$ pmol/mL during mild exercise (42% $VO_2$ max, 8 min.); increased to $13.5 \pm 0.59$ pmol/mL during moderate exercise (75% $VO_2$ max, 8 min.); and rose two-fold relative to resting to $20.0 \pm 2.3$ pmol/mL during heavy exercise (98% $VO_2$ max, 5 min.). Sixty minutes after heavy exercise ended, norepinephrine was slightly higher than the resting value ($10.0 \pm 0.59$ pmol/mL vs. $8.8$ pmol/mL). Mean resting levels of norepinephrine prior to prolonged exercise were $12.3 \pm 0.59$ pmol/mL 20 minutes before exercise and $11.8 \pm 0.59$ pmol/mL immediately before exercise. These are the highest resting norepinephrine levels reported herein. Norepinephrine concentration increased about two-fold to $17.1 \pm 0.59$ pmol/mL after 40 min. of exercise (73% $VO_2$ max); and was slightly higher at exhaustion ($17.7 \pm 1.8$ pmol/mL). Sixty minutes after prolonged exercise, plasma norepinephrine was slightly lower than resting values ($11.2 \pm 1.2$ pmol/mL). Mean resting epinephrine rose 20 minutes before graded exercise from $0.110 \pm 0.11$ to $0.164 \pm 0.16$ pmol/mL immediately prior to exercise; increased to $0.383 \pm 0.27$ pmol/mL during mild exercise; rose to $0.60 \pm 0.16$ pmol/mL during moderate exercise; and peaked at $2.3 \pm 0.60$ pmol/mL during heavy exercise, a 14-fold increase relative to pre-exercise. The difference from resting values was significant only at the end of heavy exercise. Sixty minutes after heavy exercise ended, epinephrine remained elevated relative to baseline ($0.55 \pm 0.27$ pmol/mL vs. $0.164$ pmol/mL). Mean resting levels of epinephrine prior to prolonged exercise decreased from $1.1 \pm 0.55$ pmol/mL 20 minutes pre-exercise to $0.55 \pm 0.55$. Note that these resting values are three to 10 times higher than the resting epinephrine values reported in the graded study, although the same analysis method was used. It may be presumed that a typographical error as far as decimal placement occurred when recording resting epinephrine values in one or the other of the studies. During prolonged exercise, epinephrine increased at 40 min. to $1.6 \pm 0.55$ pmol/mL; and rose further to $4.4 \pm 1.1$ pmol/mL at exhaustion, an eight-fold increase above pre-exercise. The increase above resting levels was only statistically significant at exhaustion. Sixty minutes after prolonged exercise, epinephrine had returned to reported resting levels ($1.1 \pm 0.55$

pmol/mL). Hence, plasma norepinephrine and epinephrine concentrations changed somewhat differently depending on the intensity and duration of the exercise. During graded exercise, concentrations of both catecholamines increased in plasma throughout exercise and remained slightly elevated above resting values at post-60 minutes. However, prolonged exercise resulted in differing responses between the catecholamines: plasma norepinephrine did not change much from 40 minutes to exhaustion and was slightly lower at post-60 minutes relative to rest; plasma epinephrine concentration increased from 40 minutes to exhaustion and returned to baseline at post-60 minutes. Further, peak levels were realized after graded exercise for plasma norepinephrine, but after exhaustive exercise for plasma epinephrine.

Galbo et al. (1975) examined the plasma catecholamine responses of eight men, ages 20-28, to graded exercise (mild: 47% $VO_2$ max, 10 min.; moderate: 77% $VO_2$ max, 10 min.; and heavy: 100% $VO_2$ max, <4 min.) and to prolonged exercise (76% $VO_2$ max to exhaustion). This study involved treadmill running rather than cycling. The eight subjects were not described as "trained," but their mean $VO_2$ max was higher than the subjects in the Hartley et al. (1972a & b) studies (54.7 ml $O_2$/kg/min. vs. 47.9 ml $O_2$/kg/min.). The absolute catecholamine levels attained were lower than the values reported by Hartley et al., but the magnitude of change was greater for plasma norepinephrine and lesser for plasma epinephrine. Like the results from Hartley et al., plasma norepinephrine concentration was higher at the end of maximal exercise than exhaustive exercise, and did not change much from 40 minutes to the end of exercise in the prolonged trial. During graded exercise, norepinephrine increased from 2.3 ± 0.35 pmol/mL (resting) to 2.6 ± 0.53 pmol/mL (mild), 7.6 ± 1.7 pmol/mL (moderate) and 13.0 ± 2.3 pmol/mL (heavy), with the latter value reflecting a six-fold increase relative to pre-exercise. During prolonged exercise, norepinephrine increased from a resting value of 2.0 ± 0.35 to 8.47 ± 1.09 pmol/mL at 40 minutes and increased somewhat to 8.9 ± 0.47 pmol/mL at exhaustion, a four-fold increase above pre-exercise. Unlike the results from Hartley et al., plasma epinephrine concentration was higher at the end of graded exercise rather than at the end of exhaustive exercise, and did not increase dramatically from 40 minutes to end exercise in the prolonged trial. During graded exercise, epinephrine remained unchanged from resting to mild exercise (0.38 ± 0.055 pmol/mL), and rose to 0.66 ± 0.11 pmol/mL (moderate) and 2.3 ± 0.71 pmol/mL (heavy), a six-fold increase relative to pre-exercise. During prolonged exercise, epinephrine rose from a resting value of 0.27 ± 0.055 to 1.31 ± 0.32 pmol/mL at 40 minutes and to 1.8 ± 0.22 pmol/mL at exhaustion, a seven-fold increase above pre-exercise. Both catecholamines returned to resting values 30 minutes after graded exercise, but 30 minutes after prolonged exercise

mean norepinephrine (2.9 ± 0.53 pmol/mL) and epinephrine (0.60 ± 0.11) remained elevated above resting values.

Kindermann et al. (1982) examined the plasma catecholamine responses in 17 young men during two types of treadmill exercise: "prolonged" aerobic exercise of 50 min. at the anaerobic threshold of 4 mmol/l blood lactate; and a single bout of anaerobic short-term high-intensity exercise at 156% of maximal exercise capacity, determined in a progressive test, leading to exhaustion within 1.5 min. The subjects were fit, with a mean $VO_2$ max of 55.1 ml $O_2$/kg/min. During anaerobic exercise, plasma epinephrine and norepinephrine increased 15-fold; during aerobic exercise, epinephrine increased 3-4 fold and norepinephrine increased 6-9 fold. The researchers interpret these results as an indication that anaerobic exercise results in higher emotional stress and adrenal activity than aerobic exercise.

Five studies that examined the catecholamine response to prolonged submaximal exercise unfortunately provided only graphical results. They will be summarized here to note the pattern of plasma norepinephrine and epinephrine change over the course of a prolonged effort. Each of these studies looked at runners exercising on a treadmill. An early study (Hall et al., 1983) looked at the catecholamine response in seven runners (VO2 max=63.4 ml $O_2$/kg/min, age=36.9 ± 2.3 yr) maintaining a pace to attempt completion of a marathon within three hours. A 7-8 fold rise in both norepinephrine and epinephrine occurred, with peak values at the end of exercise (exhaustion). Twenty minutes post-exercise, catecholamine levels were slightly elevated above resting values.

O'Brien and associates (1993) compared the difference in catecholamine levels in 12 trained men ($VO_2$ max=56.7 ml $O_2$/kg/min, ages=30 ±5.5 yr) running either a "fast" marathon (2 hr., 45 min.) or a "slow" marathon (3 hr., 45 min.). The fast and slow paces translated to an intensity of 73.3% $VO_2$ max and 64.5% $VO_2$ max, respectively. Plasma norepinephrine increased significantly in both groups, but obtained significantly higher values in the fast group throughout the marathon. Plasma epinephrine was significantly elevated from pre-exercise values by 150 minutes in the fast group, and was significantly higher in the fast group versus the slow group by 120 minutes of exercise. In the slow group, epinephrine did not increase significantly until the end of exercise. Hence, even with exercise of long duration, an increased level of intensity incurs a greater relative catecholamine response.

Friedmann and Kindermann (1989) exercised eight trained men ($VO_2$ max=65.1 ml $O_2$/kg/min, ages=29.3 ± 4.8 yr) at 80.2% $VO_2$ max until exhaustion (90 ± 25.5 min.). This level of intensity was described as the anaerobic threshold for these athletes. Both catecholamines showed a peak value at the end of exercise. From resting values,

norepinephrine rose 5-7 times while epinephrine rose 4-6 times. Peak values were lower relative to the peak values depicted in the O'Brien et al. study (1993).

Two final studies examined submaximal exercise, but not to exhaustion. They provide additional background on the response of plasma catecholamines to different intensities of aerobic exercise, since the duration of exercise was controlled. Farrell et al. (1987) looked at catecholamine levels in seven trained men ($VO_2$ max=61.1 ml $O_2$/kg/min, ages=27.4 ± 6.8 yr) during treadmill exercise at 40% $VO_2$ max (80 min.), 60% $VO_2$ max (80 min.) and 80% $VO_2$ max (40 min.). Norepinephrine and epinephrine increased significantly above resting values in each exercise test. As expected, the increases were greatest during the 80% $VO_2$ max run, and greater during the 60% $VO_2$ max run than the 40% $VO_2$ max run. Nieman et al. (1993) compared the epinephrine response in 10 trained men ($VO_2$ max=66.0 ml $O_2$/kg/min, ages=22.1 ± 1.3 yr) to two levels of exercise: 50% $VO_2$ max and 80% $VO_2$ max for 45 minutes each. Epinephrine increased in both trials and, as expected, was significantly higher in the 80% $VO_2$ max run at the end of exercise compared to the 50% $VO_2$ max run. By one hour post-exercise in both tests, epinephrine levels were similar to those at rest.

Hence, the above studies illustrate the changes in plasma norepinephrine and epinephrine concentrations in response to exercise of varying intensity and duration. They suggest that intensity may enhance the increase in plasma catecholamines greater than duration. In addition, they suggest a different relative increase in plasma norepinephrine and plasma epinephrine with different exercise protocols. However, it is difficult to discern if the plasma concentration of one catecholamine versus the other is more dependent on either intensity or duration, as research has not reported consistent findings with regard to this issue. Plasma epinephrine may have a greater relative response to maximal or anaerobic exercise than plasma norepinephrine (Hartley et al., 1972a; Rogers et al., 1991; Young et al., 1992), although some research indicates that catecholamine concentrations increase the same relative amount (Galbo et al., 1975; Kindermann et al., 1982). Results regarding relative increases in plasma norepinephrine and plasma epinephrine with prolonged or aerobic exercise are even less consistent: some researchers indicate that plasma epinephrine rises relatively more than plasma norepinephrine (Hartley et al., 1972b; Galbo et al., 1975), others show that plasma norepinephrine rises relatively more than plasma epinephrine (Kindermann et al., 1982; Friedmann and Kindermann, 1989).

Two studies (Young et al., 1992; Hagberg et al., 1979) help elucidate the plasma catecholamine response in the minutes following an exercise challenge. Young et al. (1992) examined catecholamine concentrations immediately after an exercise period.

Thirteen healthy men (ages 24-34 yr) walked and ran on a treadmill (modified Bruce protocol) until maximum effort was reached. The $VO_2$ max of the subjects was not determined. Mean plasma norepinephrine increased from a resting value of $2.9 \pm 0.33$ pmol/mL to $16.5 \pm 3.0$ pmol/mL at maximal exercise, a 5.5-fold increase. One minute later, the concentration had risen further to $21.8 \pm 4.3$ pmol/mL, seven times greater than the resting level. At minutes two and three after exercise, the concentration did not decrease appreciably below the immediate post-exercise value. At 10 minutes after exercise, the value had fallen to $6.3 \pm 0.62$ pmol/mL, a two-fold elevation above the resting value. Plasma epinephrine rose from a resting value of $0.34 \pm 0.07$ pmol/mL to $2.7 \pm 0.22$ pmol/mL at maximal exercise, an eight-fold increase. One minute later, it had risen further to $3.0 \pm 0.82$ pmol/mL, after which it fell to $1.7 \pm 0.35$ pmol/mL at the two-minute point. At the end of the 10-minute post-exercise period, the value was $0.56 \pm 0.17$ pmol/mL, about a two-fold elevation above the resting value. Like Young et al. (1992), Hagberg et al. (1979) found an increase in plasma norepinephrine during the first minute after exercise. Five healthy men trained for two months ($VO_2$ max=52.4 ml $O_2$/kg/min, ages 28-34 yr) performed intense, short-term cycle ergometer exercise (approx. 100% $VO_2$ max). Plasma norepinephrine was approximately 23.5 pmol/mL at the end of exercise (from a resting mean of $1.41 \pm 0.23$ pmol/mL), increased slightly in all subjects after one minute post-exercise (no numerical values were reported), then decreased with a half-time of 2.8 min. between one and 11 min. post-exercise. These two studies indicate that timing of blood draw at the end of exercise may lead to disparity of catecholamine measurement results amongst exercise studies.

Mechanisms proposed to explain the fluctuations in plasma catecholamines during exercise involve changes in catecholamine clearance and secretion. Leuenberger and associates (1993) used steady-state infusions of $^3$H-labeled NE to determine the magnitude and time course of the arterial NE spillover into the plasma (an index of NE release at sympathetic nerve terminals) and clearance during sustained bicycling at low ($\sim$25% max) and moderate-to-high ($\sim$65% max) intensity in young, healthy men. The mean $VO_2$ max of the subjects was 40.3 ml $O_2$/kg/min. During 30 min. of low-level exercise, NE spillover increased ($1.45 \pm 0.13$ to $3.14 \pm 0.30$ nmol/min/m; $m$ refers to normalization for body surface area in m$^2$) and leveled off at 20-30 min.; NE clearance remained unchanged. During 20 min. of moderate-to-high intensity exercise, there was a substantial and progressive rise in arterial NE spillover ($2.15 \pm 0.27$ to $13.52 \pm 1.62$ nmol/min/m), and NE clearance decreased ($0.91 \pm 0.05$ to $0.80 \pm 0.05$ l/min/m). From these data, the authors suggested an interaction between the effects of exercise intensity and duration on

NE spillover; and at moderate-to-high intensity, a small decrease in NE clearance contributed to the observed rise in plasma NE.

Warren et al. (1984) intravenously infused subjects with epinephrine at rest and during moderate, constant bicycle exercise. The researchers found that the adrenal medulla did not respond to mild or moderate exercise, indicated by no increase in plasma epinephrine. They also reported that epinephrine secretion and clearance were actually reduced, with alterations in epinephrine levels influenced by changing clearance rates. In contrast to Warren et al. (1984), Kjaer and associates (1985) have indicated that changes in secretion, rather than clearance, account for the response of plasma epinephrine during exercise. They gave intravenous infusions of $^3$H-labeled epinephrine and drew arterial blood from subjects at rest and during exercise. They found small changes in epinephrine clearance that coincided with relative exercise intensity. At mild exercise epinephrine clearance increased, but at loads from 30% to 76% of $VO_2$ max, clearance decreased with intensity. Since the changes in clearance could not account for the rise in plasma epinephrine concentration during exercise, the authors attribute the rise in epinephrine to increased adrenomedullary secretion. The authors point to methodology, such as calculation of clearance and site of blood collection, to explain the disagreement with the Warren et al. (1984) results. Subjects in the two studies also varied slightly in age and gender, and may have differed in training status. The Warren et al. research included five men and a woman, ages 19-29; training status was not reported. The Kjaer et al. research included six trained men ($VO_2$ max=70 ml $O_2$/kg/min.) and six untrained men ($VO_2$ max=45 ml $O_2$/kg/min.), ages 20-26.

In summary of the above research, plasma norepinephrine concentration increases at lower exercise intensities than epinephrine, even rising before exercise in an anticipatory response. Norepinephrine rises steadily and may plateau during prolonged submaximal exercise. Epinephrine rises little at lower intensities then increases at a given level of intensity relative to an individual's aerobic capacity, most likely coinciding with the anaerobic threshold (indicated by increased plasma lactate). After exercise, the catecholamines may increase above exercise values during the first minute, remain elevated above resting values for up to 30 minutes or longer, and return to resting values by one hour post-exercise.

Variables Effecting Plasma Catecholamine Levels

Variation in the catecholamine levels among the studies reviewed may arise from many factors. The effects of exercise intensity and duration on plasma catecholamines have already been discussed. Other factors that may effect catecholamine levels at rest and during exercise include training status, anxiety level, prior diet, caffeine and tobacco use, age and gender of subjects; method of blood collection and body position of the subjects; time of day; and method of catecholamine analysis. Since most study designs vary by multiple factors, or do not report these factors, it is difficult to pinpoint which element may be causing differing results between studies. Research conditions should control for as many of these variables as possible to provide accurate results and allow comparison among research.

Physical training influences the sympathoadrenal response to exercise. The magnitude of the effect appears to be dependent upon the level of training and whether the catecholamine response to absolute or relative workloads is compared. Furthermore, plasma norepinephrine and epinephrine secretion at rest and during exercise respond differently to training. Endurance training results in lower resting plasma norepinephrine and a reduced response to a given absolute workload; whereas training may result in higher resting plasma epinephrine and no difference or a reduced response to an absolute effort (McArdle et al., 1986; Kjaer et al., 1985, 1986; Trendelenburg and Weiner, 1989; Mazzeo et al., 1986; Hartley et al. 1972b). Norepinephrine responses to relative workloads are identical or lower in trained versus untrained individuals; whereas epinephrine responses to relative workloads are identical or higher in trained compared to untrained subjects (Kjaer et al., 1985, 1986; Hartley et al., 1972a, 1972b). It has been suggested that a higher plasma epinephrine concentration during maximal exercise of the same relative intensity in trained subjects compared with untrained subjects indicates that physical training increases the capacity to secrete adrenomedullary hormones (Kjaer, 1992). This theory is supported by research with rats, revealing increased adrenal and cardiac epinephrine concentrations after endurance training (Mazzeo et al., 1986). Norepinephrine turnover and tyrosine hydroxylase activity are attenuated in the heart of trained rats (Mazzeo and Grantham, 1989). This reduced norepinephrine response could result in less norepinephrine spillover to the circulation, contributing to observations of reduced plasma norepinephrine with training (Mazzeo, 1991). Clearance of norepinephrine (Hagberg et al., 1979) and epinephrine (Kjaer et al., 1985) is not changed

with training, indicating that sympathetic and adrenomedullary secretion may be altered with endurance training.

Kjaer and associates (1985) found significantly higher plasma epinephrine concentrations in six trained versus six untrained young men (ages 20-26 yrs.) in response to exhaustive graded bicycle exercise of the same relative intensity. Norepinephrine levels at exhaustion did not differ significantly between the two groups; however, at identical work loads, norepinephrine was significantly lower in the trained compared to the untrained men. Kjaer and associates examined the $VO_2/VO_2$ max and found it was identical at exhaustion in the trained and sedentary groups; thus, the authors suggested that trained subjects may have an increased responsiveness of the adrenal medulla at strenuous exercise levels. Trendelenburg and Weiner (1989) have suggested that training results in metabolic changes that allow more strenuous or prolonged exertion before recruiting greater adrenal medullary secretion.

In contrast, Rogers et al. (1991) reported minimal differences in the release of norepinephrine and epinephrine during exercise between 10 well-trained men (age $33\pm7$ yrs.) and nine minimally trained men (age $28\pm5$ yrs.). The investigators did find higher plasma norepinephrine and epinephrine levels in the well-trained versus the minimally trained men in response to maximal graded treadmill or cycling exercise of the same relative intensity. At identical submaximal work (four min.), plasma norepinephrine and epinephrine concentrations were higher in the well-trained versus the minimally trained men. Since these differences in catecholamine concentrations between the two groups did not reach statistical significance, Rogers and associates suggested that minimal training may be equal to extensive training in effecting catecholamine metabolic systems.

The level of training between the "trained" and "sedentary" groups in the Kjaer study was much more distinct (70 ml $O_2$/kg/min. vs 45 ml $O_2$/kg/min.) than the level of training between the "trained" and "minimally trained" groups in the Rogers study (55 ml $O_2$/kg/min. vs 42 ml $O_2$/kg/min.). It is plausible that there was not enough difference in the level of training between the "trained" and "minimally trained" subjects in the Rogers research to result in a significant difference in catecholamine concentrations between the groups at a given work level. Differences in duration and intensity of exercise could also have contributed to the differing conclusions of the Kjaer et al. (1985) and Rogers et al. (1991) research. Rogers and associates did not report exercise durations; hence, they cannot be compared to the Kjaer et al. research. However, the duration of the Kjaer et al. exercise protocol was presumably longer than the duration of the Rogers et al. exercise protocol since the former involved submaximal exercise to exhaustion, while the latter elevated the exercise intensity to maximum effort. Discrepancies may also arise from the

differing types of exercise used as it has been shown that plasma catecholamine levels are influenced by the amount of muscle used (Kjaer et al., 1991).

Interestingly, the Rogers et al. (1991) study reported significantly higher resting plasma norepinephrine levels but similar epinephrine levels between the trained and minimally trained men. The greater level of resting norepinephrine in trained subjects could have been the result of sympathoadrenal response in anticipation of exercise, which could be adaptively more sensitive in trained subjects than in untrained subjects (Mason et al., 1973; McArdle et al., 1986). The Kjaer study found significantly higher resting plasma epinephrine but not higher resting plasma norepinephrine in trained versus untrained men. This would support the theory of heightened adrenomedullary secretion capacity after endurance training.

Body position during blood draw has been shown to effect resting plasma catecholamine levels. Assuming an upright position (sitting or standing up) has been found to significantly increase norepinephrine (Vendsalu, 1960) and epinephrine levels (Warren et al., 1984). The norepinephrine levels attained during quiet standing are reportedly about double those found in the supine position. Warren et al. (1984) measured a significant rise in plasma epinephrine from $0.12 \pm 0.03$ to $0.19 \pm 0.03$ pmol/mL when subjects moved from the supine position to standing. These observed changes may be influenced by plasma volume changes (Harrison, 1985). Some of the exercise studies described above collected resting blood samples from standing subjects (Young et at., 1992; Rogers et al., 1991) or sitting subjects (Hagberg et al., 1979; Rogers et al., 1991). In other studies, it is unclear whether resting samples were obtained from supine or sitting subjects (Hartley et al., 1972a & 1972b) or from supine or standing subjects (Galbo et al., 1975). Exercise samples were also taken in different positions (i.e., sitting or standing).

Anxiety associated with blood collection in some people may be sufficient to raise the levels of circulating catecholamines (Callingham, 1975), and plasma catecholamine levels in humans have been shown to differ with the site from which blood is obtained (Vendsalu, 1960). These factors probably did not contribute appreciably to varying catecholamine results in the studies reviewed herein: All of the studies obtained blood samples from an antecubital vein and most of the protocols called for insertion of a catheter and a period of waiting to allow the effects of the procedure to dissipate. Much harder to control is the anticipatory rise in plasma norepinephrine levels noted before physical activity (Mason et al., 1973). Anticipation of exercise may have contributed to the substantially higher resting norepinephrine concentrations in the Hartley et al. studies (1972a & b) compared to the other research.

Plasma catecholamines vary diurnally, with peak levels of both norepinephrine and epinephrine in the late morning or early afternoon and the lowest point in the early hours of the morning, during sleep (Turton and Deegan, 1974). Most of the research covered here appears to have taken resting blood samples in the morning around 8 a.m., except the Hartley et al. (1972a & b) research took resting samples at 9:40 a.m. and 10 a.m. Taking blood samples in the late morning may have contributed to the higher resting norepinephrine levels reported in the Hartley et al. studies. Reported resting epinephrine concentrations were higher in one Hartley et al. study (1972b) but lower in the other study (1972a) compared to the other research reviewed here. It has already been mentioned that resting epinephrine data from one or the other Hartley et al. research (1972a & b) most likely displayed a typographical error.

Methods used for analyzing the plasma catecholamines also can lead to varying results between studies. Methods used in the studies reviewed here included ethylenediamine-fluorimetric (Hartley et al., 1972a & b), catechol-O-methyltransferase (COMT) single-isotope derivative (Hagberg et al., 1979), COMT double-isotope derivative (Galbo et al., 1975) and HPLC-ECD (Rogers et al., 1991; Young et al., 1992). Trendelenburg and Weiner (1989) report that there is considerable variation among "normal" resting values of the catecholamines regardless of the method used. The authors emphasize the importance of standardizing blood draw conditions to reduce variations in results. They note that the ethylenediamine/fluorimetric method is not widely used and produces values several-fold higher than the COMT and HPLC-ECD methods. Good agreement between the COMT and HPLC-ECD methods has been reported (Hjemdahl et al., 1979; Goldstein et al., 1981). Hjemdahl (1984) examined results from 34 laboratories performing radioenzymatic, HPLC-ECD and fluorimetric assays. Fluorimetric assays resulted in high and variable catecholamine measurements, and the method was considered unacceptable. The study reported reasonable agreement between the levels of norepinephrine and epinephrine measured with radioenzymatic and HPLC assays: resting norepinephrine and epinephrine levels were about 1.8 and 0.2 pmol/mL, respectively, in a basal pool; and 11 and 0.7 pmol/mL, respectively, in an exercise sample. There was variability between methods as well as between laboratories using the same method. Hence, the catecholamine analysis method chosen by Hartley et al. (1972a & b) could well have resulted in falsely high and variable catecholamine measurements, whereas the analyses methods chosen by Galbo et al. (1975), Rogers et al. (1991), Young et al. (1992) and Hagberg et al. (1979) should not have contributed to a disparity in catecholamine results, given good standardization and quality control.

Whether subjects are fasted or fed prior to a blood draw has been shown to effect plasma catecholamines. During fasting, plasma norepinephrine and norepinephrine turnover decreases (Landsberg and Young, 1978; Young et al., 1984; Berne and Levy, 1988), while epinephrine secretion modestly increases (Young et al., 1984; Landsberg and Young, 1992; Berne and Levy, 1988). After feeding, plasma norepinephrine and norepinephrine turnover increases (Landsberg and Young, 1978; Berne and Levy, 1988). The acute response of epinephrine to feeding has not been uncovered during this literature search, but epinephrine secretion has been reported to increase four to five hours after a meal or glucose ingestion (Landsberg and Young, 1978 & 1992; Berne and Levy, 1988). In the studies reviewed herein, two noted that subjects were fasted (Galbo et al., 1975; Rogers et al., 1991) and two did not note whether or not subjects were fasted (Young et al., 1992; Hagberg et al., 1979). Hartley and associates (1972a & b) fed the subjects a "usual" breakfast that was two hours and 40 minutes prior to the first resting blood draw and four hours prior to the start of exercise. The seemingly high catecholamine results reported by these groups could have been partially attributed to this feeding schedule.

Dietary intake over time has been shown to influence the sympathoadrenal system. As previously discussed in Chapter 2 of this review, variations in protein and tyrosine intake (Agharanya and Wurtman, 1985; Fernstrom, 1983; Benedict et al., 1983; Kaufman et al., 1989), fat intake (Kaufman et al., 1989) and carbohydrate intake (Kaufman et al., 1989; Lavoie et al., 1983; Hall et al., 1983) may influence resting plasma and urinary catecholamines in rats and humans. Increased urinary catecholamine excretion is generally accepted to indicate an increased release of catecholamines (Bellet et al., 1969). During exercise, plasma norepinephrine and epinephrine levels in men rose significantly more after a diet and exercise protocol that produced low liver glycogen and high muscle glycogen compared to a protocol that produced normal liver glycogen and high muscle glycogen (Lavoie et al., 1983). Plasma norepinephrine and epinephrine levels in humans also rose higher during exercise after a normal carbohydrate diet (45.5% CHO) compared to a high carbohydrate diet (77.2% CHO) (Hall et al., 1983). As hypothesized in this thesis, vitamin B-6 supplementation may effect catecholamine synthesis. It is conceivable that niacin or ascorbic acid supplementation could also influence catecholamine synthesis. Recall that NADPH is required to convert tyrosine to L-DOPA, and ascorbic acid is needed to convert dopamine to norepinephrine. Sodium intake has also been shown to effect plasma norepinephrine but not plasma epinephrine in men (Nicholls et al., 1980). The highest norepinephrine values occurred at low salt intakes, lowest at intermediate salt intake (100-180mEq/d) and intermediate at high salt intake (>180 mEq/d).

Acute and chronic use of caffeine has been shown to alter plasma catecholamine levels in humans. Plasma and urinary norepinephrine and epinephrine rise in response to acute caffeine ingestion (Robertson et al., 1978; Bellet et al., 1969), but this response is blunted in habitual caffeine users (Robertson et al., 1981; Jung et al., 1981). Furthermore, habitual caffeine users show similar plasma norepinephrine and epinephrine responses during exercise after either caffeine or placebo administration (Tarnopolsky et al., 1989; Van Soeren et al., 1993), whereas nonusers show increased plasma epinephrine concentrations after caffeine ingestion above placebo values during exercise (Van Soeren et al., 1993). However, chronic caffeine users have displayed a two-fold greater epinephrine response to exercise compared to nonusers after placebo ingestion (Van Soeren et al., 1993); both users and nonusers displayed identical norepinephrine responses. In short, caffeine users have shown very large plasma epinephrine responses to exercise but no significant responses to acute caffeine ingestion, while nonusers have demonstrated increased plasma epinephrine levels during exercise after caffeine ingestion. Most of the exercise studies covered in this review note that subjects were in a fasted condition, so acute caffeine ingestion would not be a confounding variable. However, the Hartley et al. studies (1972a & b) fed subjects a "usual" breakfast, which may have included caffeinated beverages. Habitual caffeine use may have contributed to variations in reported plasma catecholamines. Chronic caffeine use has not been a controlled factor in most of the exercise studies described in this review. Only the Rogers et al. (1991) study indicated that diet was restricted in catecholamine and sympathetic stimulants for two days prior to testing.

Aside from the effects of caffeine ingestion upon a subject's catecholamine response, caffeinated and decaffeinated coffee can interfere with HPLC determination of epinephrine (Goldstein, 1986). Dihydrocaffeic acid is a catechol metabolite of caffeic acid, which is a non-caffeine constituent of both caffeinated and decaffeinated coffee. The chromatographic peak of the acid has been shown to coincide with that of epinephrine. Hence, if HPLC is the chosen method for catecholamine analysis, caffeinated as well as decaffeinated food and beverage consumption should be controlled.

Cigarette smoking has been shown to evoke increases in plasma norepinephrine and epinephrine (Cryer et al., 1976). None of the studies reviewed herein noted whether or not subjects were smokers. The studies indicated that subjects were "healthy," which may indicate that they did not smoke. Galbo et al. (1975) prohibited subjects from tobacco use at least 10 hours prior to testing, but this does not indicate whether or not subjects were chronic tobacco users. A history of smoking could contribute to variable catecholamine measurements.

In normotensive humans, plasma levels of catecholamines and urinary catecholamines have been found to increase with age (Trendelenburg and Weiner, 1988; Lehmann and Keul, 1986a; Fleg et al., 1985) and to rise higher during exercise in older individuals ages 60-80 years compared to younger people ages 20-50 (Lehmann and Keul, 1986a; Fleg et al., 1985; Devalon et al., 1989). Based on this research, age would not have contributed to any variability in catecholamine measurements among the studies reviewed here; all the studies used subjects that were at least 20 years old but no more than 40 years old. As far as gender is concerned, most research has not seen any differences in plasma catecholamine levels between men and women at rest or during exercise (Trendelenburg and Weiner, 1989; Favier et al., 1983). One study (Lehmann and Keul, 1986b) found lower urinary excretion of norepinephrine and epinephrine in healthy women than in men during the first half of life expectancy ($\leq$39 yrs.), but similar excretion of both catecholamines in the second half of life expectancy ($\geq$40 yrs.). The studies reviewed here all involved male subjects, so gender would not contribute to any variability in catecholamine measurements. Research has not revealed any significant race differences in plasma catecholamine levels (Trendelenburg and Weiner, 1989).

## Role of Catecholamines in Fuel Mobilization

Catecholamines play an important role in energy metabolism by stimulating glucose production in the liver (Naveri et al., 1985; Rizza et al., 1979; Vranic et al., 1984; Mazzeo, 1991; Landsberg and Young, 1980 & 1992) mobilization of free fatty acids and glycerol from adipose tissue (Berne and Levy, 1988; Wahrenberg et al., 1987; Crass et al., 1975) and breakdown of glycogen (Landsberg and Young, 1980 & 1992; Vranic et al., 1984; Mazzeo and Marshall, 1989; Stainsby et al., 1984; Podolin et al., 1991) and triglyceride (Stankiewicz-Choroszucha & Gorski, 1978) in working muscle. Catecholamines may also effect protein metabolism (Mathews et al., 1990). The catecholamines interact with insulin and glucagon in the regulation of these processes, with the effects of the catecholamines and glucagon generally opposing those of insulin (Landsberg and Young, 1980; Galbo and Gollnick, 1984; Kindermann et al., 1982).

Epinephrine and norepinephrine stimulate glycogenolysis by activating adenylate cyclase at liver and muscle receptor sites, which activates conversion of ATP to cyclic AMP (cAMP). cAMP is the "second messenger" that elicits the regulatory cascade of glycogen breakdown (Mathews and van Holde, 1990). Glycogenolysis and

gluconeogenesis in the liver lead to increased blood glucose; glycogen breakdown in the muscle provides the working tissue with energy and, during more strenuous exercise, increases plasma lactate levels, providing additional substrate to the liver for glucose production. Epinephrine, compared to norepinephrine, is a particularly potent player in making glucose available for working muscles and the central nervous system. When plasma glucose is reduced, the adrenal medulla is markedly stimulated, while the sympathetic nervous system is suppressed (Landsberg and Young, 1992; Vranic et al. 1984). Plasma epinephrine increases as plasma glucose level is reduced within the normal physiological range (5.3 to 3.3 mmol/L) (Santiago et al., 1980; Christensen et al., 1979; Galbo et al., 1975). During insulin-induced hypoglycemia, plasma epinephrine rises as much as 10- to 50-fold and plasma norepinephrine shows a small increase (Landsberg and Young, 1992) or no increase (Vranic et al. 1984). Galbo et al. (1977) report no influence of blood glucose concentration on plasma norepinephrine concentration during prolonged (>60 min. ) exhaustive exercise. Epinephrine stimulates glucose production while inhibiting insulin-mediated glucose uptake by muscle from the circulation (Berne and Levy, 1988; Rizza et al., 1979). Epinephrine infusion in dogs caused a transient increase in glucose production and a sustained inhibition of glucose clearance, resulting in hyperglycemia (Vranic et al., 1984). Exaggerated epinephrine release during hypoglycemic exercise in dogs prevented severe hypoglycemia by inhibiting glucose utilization and stimulating glucose production (Vranic et al., 1984). Hepatic glucose production in humans has been shown to increase exponentially with the exercise workload in parallel with the rise in plasma epinephrine (Kjaer et al., 1984 & 1991). During exhaustive exercise, blood glucose concentration tends to decrease with a responding increase in plasma epinephrine (Galbo et al., 1975; Christensen et al., 1979; Hartley et al., 1972b). In humans administered propranolol ( a ß-adrenergic receptor blocking agent) before treadmill exercise, biopsies of the vastus lateralis muscle revealed a lower glycogen depletion rate than exercise without propranolol (Galbo et al., 1976).

Plasma lactate is commonly used as an indicator of muscle glycogenolysis during exercise. Research has revealed a correlation between plasma epinephrine and lactate in exercising humans (Mazzeo and Marshall, 1989; Warren et al., 1984; Stainsby et al., 1984; O'Brien et al., 1993; Podolin et al., 1991). Stainsby et al. (1984) have reported that intravenous infusions of epinephrine and norepinephrine (increasing plasma concentrations three-fold) significantly enhanced maximal net lactate output at 40 minutes of exercise versus rest during isometric contractions in the gastrocnemius muscle of dogs. Arterial and venous lactate concentrations increased continuously during epinephrine infusion but remained constant during norepinephrine infusion. Epinephrine also has been reported to

help modulate lactate production by adipose tissue in animals (DiGirolamo et al., 1992). Mazzeo and Marshall (1989) investigated the relationship among plasma catecholamines, blood lactate threshold (determined from the nonlinear increase in blood lactate concentration versus oxygen consumption) and ventilatory threshold in trained cyclists and cross-country runners performing graded bicycle ergometer exercise and treadmill running. They found that plasma norepinephrine and epinephrine shifted in an identical manner and occurred simultaneously with the blood lactate threshold regardless of testing protocol or training status. Warren and associates (1984) found a significant increase (approx. four-fold over resting) in plasma epinephrine concentration only at a workload that coincided with the only point at which plasma lactate was also significantly higher than baseline; hence the authors report that plasma epinephrine rose when anaerobic metabolism commenced. Epinephrine infusions in men during cycle ergometer exercise above the lactate threshold produced an increase in the respiratory exchange ratio, indicating increased use of carbohydrate with higher plasma epinephrine concentrations (Gaesser et al., 1994). Podolin et al. (1991) also examined the relationship between the lactate threshold, plasma catecholamines and ventilatory threshold during graded exercise, but looked at subjects (male) under normal and glycogen-depleted conditions. With bicycle ergometry, there was a high correlation between plasma lactate and norepinephrine and between lactate and epinephrine under both conditions. The glycogen-depletion protocol resulted in a shift in the lactate threshold to a later work rate ($75.3 \pm 1.9\%$ $VO_2$ max versus $65.6 \pm 2.3\%$ $VO_2$ max) that was coordinated with a shift in the epinephrine and norepinephrine inflections. O'Brien et al. (1993) found a significant correlation between blood lactate and epinephrine in trained runners engaged in a treadmill marathon. Mean plasma epinephrine, norepinephrine and lactate were significantly higher in subjects placed in a "fast" group ($73.3\%$ $VO_2$ max) versus a "slow" group ($64.5\%$ $VO_2$ max): epinephrine was $4.9 \pm 1.09$ pmol/mL and $6.56 \pm 1.09$ pmol/mL; norepinephrine was $22.9 \pm 8.23$ pmol/mL and $14.7 \pm 5.29$ pmol/mL; and lactate was $2.1 \pm 0.3$ mM and $1.2 \pm 0.2$ mM in the fast and slow groups, respectively.

Catecholamines activate adipose tissue lipase, thereby mobilizing free fatty acids for energy (Berne and Levy, 1988), the preferred substrate for cardiac muscle. One bout of exercise has been shown to increase peripheral responsiveness of ß-receptors to catecholamines in adipose tissue of humans (Wahrenberg et al., 1987). Norepinephrine and epinephrine have been shown to stimulate *in vitro* rat myocardial triglyceride lipolysis and fatty acid oxidation in a concentration-dependent fashion (Crass et al., 1975). When exogenous free fatty acids were present, mobilization of heart triglyceride was minimized. The relative role of circulating norepinephrine and epinephrine in regulation of lipolysis

during exercise has not been determined (Kjaer, 1992). Galbo et al. (1975) found a positive correlation between plasma free fatty acids and epinephrine during prolonged exercise in men. A reduced increase in the exercise-induced rise of glycerol and FFA has been shown when the epinephrine response is blocked (Kjaer et al., 1990; Galbo et al. 1976) with propranolol, indicating an important role for epinephrine in FFA mobilization. Epinephrine may also mobilize glycerol from peripheral tissues as a substrate for gluconeogenesis (Landsberg and Young, 1992). A recent study (Wahrenberg et al., 1991) examined the adrenergic regulation of lipolysis in men and women after 30 min. of bicycle exercise at 75% of estimated $VO_2$ max. Both genders showed a marked increase in the plasma level of glycerol during exercise and a concomitant increase in the plasma levels of norepinephrine and epinephrine. There was a significant correlation between plasma norepinephrine and plasma glycerol before and during exercise, but no correlation between epinephrine and glycerol. Norepinephrine stimulated lipolysis in isolated adipocytes of both genders before exercise, but norepinephrine elicited a greater lipolytic response in adipocytes from men than from women after exercise.

Although the catecholamines aid in mobilization of glycerol for hepatic gluconeogenesis, it is not clear what impact catecholamine changes have on protein turnover during exercise in vivo (Kjaer, 1992). In humans, the acute effect of epinephrine infusion to elevate plasma levels to the range seen in stress and trauma (0.5-2 $\mu g$/min. for 8.5 hr.) during rest in men did not increase protein turnover (Mathews et al., 1990). Peak plasma epinephrine concentration was $4.1 \pm 0.11$ pmol/mL, a value plausibly reached with maximal or exhaustive exercise (Hartley et al., 1972b; Rogers et al., 1991). Amino acid levels were, however, depressed with epinephrine infusion, with the largest drops occurring for the essential amino acids (-27%) and the branch-chain amino acids (-22%). For example, plasma leucine (an essential BCAA) concentration dropped from $123.4 \pm 8.0$ $\mu M$ at time zero to $87.0 \pm 8.6$ $\mu M$ at peak plasma epinephrine concentration.

The fuel mixture used during exercise depends upon the intensity and duration of effort, as well as the fitness and nutritional status of the exerciser (Ahlborg et al., 1974; Jansson and Kaijser, 1982). Stored muscle glycogen and blood-borne glucose are the prime contributors of energy in the early minutes of exercise and during high-intensity exercise in which oxygen supply does not meet the demands for aerobic metabolism. Hepatic gluconeogenesis and glycogenolysis produce glucose released to the blood. Circulating FFA released from adipocytes and FFA from breakdown of triglycerides in muscle contribute approximately the same amount of energy as glucose during moderate exercise of less than an hour. During prolonged aerobic exercise, it has been widely accepted that glycogen stores become reduced and an increasingly greater percentage of

energy is supplied through fat metabolism. Recent research (O'Brien et al., 1993) has indicated that carbohydrate, not lipid, is the primary fuel during sustained exercise requiring high power outputs such as the marathon. As glycogen is depleted, lactate and amino acids also serve as energy sources through conversion to glucose in the liver.

Training has been shown to increase utilization of FFA from muscle triglyceride (Hurley et al., 1986), accompanied by a reduction in glycogen breakdown (Coggan et al., 1990) and gluconeogenesis (Coggan et al., 1995). It has also been suggested that the apparent glycogen sparing effect of endurance training results from an enhanced capability to utilize blood glucose as a fuel (O'Brien et al., 1993). Kjaer et al. (1986) found higher plasma epinephrine, glucose and FFA in trained versus untrained subjects during running at three different intensities at identical relative workloads. Blood lactate was similar in the two groups; glycerol concentrations increased and tended to be higher in the trained than in the untrained subjects at 100% $VO_2$ max; FFA concentration decreased with intensity but was higher in the trained than in the untrained group at 60% $VO_2$ max. The rate of glucose appearance increased with exercise intensity and was more marked in trained than in untrained subjects, whereas rate of glucose disappearance increased similarly in the two groups and matched the rate of glucose appearance of the untrained men. Accordingly, the plasma glucose concentration increased with work intensity in the trained subjects and was higher than in the untrained subjects at all intensities. Glucose clearance increased during exercise and at high work loads was significantly higher in untrained than in trained subjects. Glucose and lactate values peaked one minute after exercise and were higher in trained than in untrained subjects; glycerol values peaked 15 min. after exercise and were significantly higher in trained than in untrained subjects; FFA concentration increased rapidly after exercise and was initially significantly higher in trained than in untrained subjects. The authors suggest that the higher epinephrine secretion in the trained subjects versus the untrained subjects may contribute to higher hepatic glucose production, muscle glycogenolysis and lipolysis, and lower glucose clearance during maximal exercise.

Aside from training, variations in dietary intake can introduce a compounding variable to research examining the influence of the catecholamines on fuel use. Many studies have shown the influence of diet on fuel use and performance. For instance, individuals consuming a high-carbohydrate diet show greater muscle glycogen and endurance compared to individuals on a lower carbohydrate or high-fat diet (Hall et al., 1983). Individuals with low glycogen stores must rely more heavily upon fat mobilization and gluconeogenesis relative to individuals with high glycogen stores. A diet deficient in carbohydrate has been shown to deplete muscle and liver glycogen, and effect performance in intense, short-term exercise as well as prolonged, submaximal endurance activities

(Lavoie et al., 1983; Costill et al., 1982). Hence, research attempting to elucidate the impact of catecholamines on fuel use should control dietary intake.

## Catecholamine Influence on Oxygen Consumption and Resting Metabolic Rate

The resting metabolic rate (RMR) refers to resting heat production (kcal/min.) in the fasting state at normal temperatures, which includes heat generated in the maintenance of homeostasis (Landsberg and Young, 1992). It is similar to the basal metabolic rate (BMR), but somewhat higher since it is not measured directly upon waking. RMR, like BMR, is calculated from oxygen consumption.

Infusions of catecholamines have been shown to increase oxygen consumption by a mechanism that is predominantly beta-receptor mediated (Landsberg and Young, 1983b). Tremblay et al. (1992) reported that basal metabolic rate (BMR) declined in trained men when the adrenergic beta-blocker propanolol was administered. In 1968, Svedmyr showed that infusions of epinephrine (0.1 $\mu$g/kg/min) and norepinephrine (0.15 $\mu$g/kg/min) increased oxygen consumption with Vmax calculated to be 23% and 15% of basal metabolic rate, respectively, in young healthy males. More recently, Sjostrom (1985) and Mathews et al. (1990) found that epinephrine infusions elevated metabolic rate in a dose-dependent fashion. Sjostrom (1985) calculated an epinephrine threshold concentration of 0.65 pmol/mL (4.3 ng/kg fat-free mass/min.) for a detectable response in metabolic rate in six women. Mathews and colleagues (1990) found a significant increase in metabolic rate (1.204 kcal/min vs 1.125 kcal/min) at an epinephrine infusion rate of 0.5 $\mu$g/min. compared to no infusion in five men. This response corresponded to a plasma epinephrine concentration of 1.14 ± 0.109 pmol/mL. The plasma epinephrine concentrations observed to increase metabolic rate in these two studies are higher than typically seen at rest in healthy individuals, but are in the range seen during exercise.

Increases in resting endogenous norepinephrine have been associated with increased metabolic rate. Poehlman et al. (1992) found a 9% increase in RMR, despite no changes in body composition, and increased levels of resting arterialized norepinephrine (1.21 ± 0.23 pmol/mL to 1.56 ± 0.46 pmol/mL) after moderate endurance training in older individuals (age 69 ± 6). According to Landsberg and Young (1983b), the catecholamines have only a small effect on RMR, but are considered to be major mediators of heat production in excess of RMR (facultative thermogenesis). Exercise-induced heat production/oxygen consumption falls into this latter category. Hence, with increased

catecholamine levels during a given workload, oxygen consumption would be expected to increase. Indeed, plasma norepinephrine and epinephrine reportedly increase in parallel with changes in oxygen uptake during exercise (Galbo et al., 1975; Christensen et al., 1979).

## Catecholamine Effects on Heart Rate

The effects of catecholamines on the heart are mediated by beta-1 receptors and include increased heart rate, enhanced contractility and augmented conduction velocity, all of which contribute to an increase in cardiac output (Landsberg and Young, 1992). Norepinephrine appears to have a more potent effect on heart rate than epinephrine. When heart rate is mediated by the sympathetic nervous system, a rise in plasma norepinephrine is correlated with an increase in heart rate (Christensen et al., 1979; Orizio et al., 1988; Perini et al., 1993; Kindermann et al., 1982). An increase in urinary excretion of norepinephrine, but not epinephrine, has also been significantly correlated with heart rate during exercise (Gillberg et al., 1986). During exercise, the initial rise in heart rate is due to withdrawal of vagal tone, but further increases in heart rate are mediated by the sympathetic nervous system, and thus, this latter rise correlates with a rise in plasma norepinephrine. In addition, the increase in heart rate that occurs upon standing is mediated by sympathetic nerves and plasma norepinephrine increases (Christensen and Brandsborg, 1973). Christensen and associates (1979) report that there is no rise in plasma norepinephrine during exercise provided the increase in heart rate at steady state is less than approximately 25 beats/min. When exercise requires higher increases in heart rate, the increases are paralleled by increases in plasma norepinephrine. Propranolol administration in humans has been shown to decrease resting heart rate (Tremblay et al., 1992), diminish the increase in heart rate and cardiac output during exercise, and increase plasma norepinephrine concentration (Christensen et al., 1975; Galbo et al., 1976).

As previously discussed, plasma norepinephrine levels during a given absolute workload decrease with training. It is also well-documented that heart rate is reduced with training. The literature indicates a role for plasma catecholamines in the reduction of heart rate, but other factors apparently are also involved. Winder and associates (1978) examined plasma catecholamine and heart rate responses to a five-minute strenuous bicycle ergometer test in men before and during seven weeks of endurance training. The investigators found a decrease in norepinephrine (17.6 pmol/mL to 8.8 pmol/mL) and

epinephrine (2.7 pmol/mL to 0.93 pmol/mL) during exercise from pre-training to post-training. Heart rate declined in parallel with the plasma catecholamines to the end of the third week of training, but continued to decrease after the catecholamine response had plateaued. Hagberg et al. (1979) found the post-exercise heart rates of men to be 25 beats/min. higher after detraining, while plasma norepinephrine was not significantly different. These data would indicate that a more rapid recovery of heart rate in trained individuals is not necessarily due to a more rapid recovery from the sympathetic response to exercise. Winder and associates noted that factors such as increased parasympathetic tone or reduced adreno-receptor sensitivity could be involved in the training-induced decrease in heart rate. Down-regulation of norepinephrine receptors may be partly responsible for training-related bradycardia, dependent upon the type of training (Mazzeo, 1991). In animals, treadmill training has been shown to produce no change in cardiac receptor number or affinity (Scarpace et al., 1992); but swim training has been shown to result in a decrease in ß-receptor number and maximal binding capacity in the heart (Werle et al., 1990).

# METHODS

## Overview

This research was a follow-up of a series of exercise and nutrition studies at Oregon State University that have evaluated changes in vitamin B-6 metabolism and fuel use in men following differing amounts of exercise and vitamin B-6 supplementation. This study examined the influence of vitamin B-6 supplementation and exhaustive exercise on plasma catecholamine levels, fuel utilization, heart rate and resting metabolic rate (RMR). Trained, young male cyclists exercised twice to exhaustion at 75% of their $VO_2$ max on a cycle ergometer, once in the unsupplemented state and once in the vitamin B-6 supplemented state. There were approximately two weeks between exercise tests. A pre-study training ride familiarized the subjects with the test procedure and equipment, and allowed for accurate setting of the workload. For six days before and three days after each test (including test day), the subjects were fed the same diet. The diet was nutritionally balanced and controlled for all nutrients, including vitamin B-6. Respiratory gases and blood were collected before, during (when possible) and after each exercise session to assess changes in plasma catecholamine levels, fuel utilization and B-6 metabolism. Plasma was analyzed for norepinephrine, epinephrine, glucose, lactic acid, glycerol, free fatty acids and PLP. Twenty-four-hour urine collections were also analyzed for 4-PA.

## Subjects

Five healthy trained male cyclists, age 18-35 years, volunteered to participate in the study, which was approved by the OSU Human Subjects Committee (6/26/92). "Trained" was defined as approximately 120-180 minutes of aerobic activity, preferably cycling, three days per week and maintained for at least one year. Subjects were asked to maintain their fitness regimens throughout the investigation and abstain from racing during the controlled dietary periods. This was intended to minimize the chance of a training effect and maintain each individual's physical condition between exercise trials. Daily exercise was monitored by reviewing subject logs that described the intensity and

duration of daily training. $VO_2$ max was measured pre-study and body composition was measured mid-study to determine the subjects' fitness levels.

The subjects met the qualifications of the American College of Sports Medicine (1986) for placement in the "Apparently Healthy Individuals" classification of people undergoing exercise testing. Apparently Healthy Individuals are defined as "those who are apparently healthy and have no major coronary risk factors." Major coronary risk factors include: high blood pressure (>145/95), high total cholesterol/HDL cholesterol ratio(>5), cigarette smoking, abnormal resting ECG, family history of coronary or other atherosclerotic disease prior to age 50, or diabetes mellitus. Subjects were required to abstain from taking vitamin supplements or using drugs known to interfere with vitamin B-6 metabolism, sympathoadrenal function or analysis for at least 4 weeks prior to the start of the study. A 20 ml sample of blood was taken prior to the start of the study to check for normal plasma PLP levels. Informed consent was signed after subjects were familiarized with the study. All subjects met the following qualifications:

1.     Normal health history based on a questionnaire
2.     Normal blood chemistry screen
3.     Normal ECG at rest and at maximal heart rate
4.     Normal blood pressure at rest (i.e., ≤120/80) and with exercise
5.     No nicotine for at least one year
6.     Signed an informed consent
7.     Underwent a $VO_2$ max test to assess fitness level.

## Subject Risks/Benefits

The risk in maximal exercise testing is one death per 10,000 in large, varied populations. Trained personnel administered the tests and monitored the subjects for signs of exercise tolerance in accordance with guidelines set by the American College of Sports Medicine (1986).

As a benefit of participation, each subject received a stipend of $50 from Oregon State University upon completion of the study. They also received a copy of all blood chemistry, body composition and exercise information free of charge. Finally, each subject received 18 days of nutritionally balanced meals free of charge.

## Prestudy Testing

One week prior to the start of the 32-day study protocol, each subject completed a $VO_2$ max test on the cycle ergometer (protocol adapted from the American College of Sports Medicine guidelines, 1986). Workload was increased in 30-watt increments until a plateau in oxygen consumption was observed or until subjects requested to stop the test. The highest oxygen consumption value or peak $VO_2$ obtained during the max test was used to set subsequent workloads during the exhaustive sessions. The max test lasted 8-10 minutes. All exercise testing was conducted at the OSU Human Performance Lab (room 19 Women's Building) on the same cycle ergometer (Quinton Instruments). In addition to the max test, subjects completed a preliminary practice ride wherein the exercising workload was gradually set and the subjects were stabilized at 75% ± 1% of their $VO_2$ max. The preliminary test lasted approximately 20 minutes. At this session, subjects also were familiarized with the gas collection equipment and cycle ergometer.

## Study Protocol

Diet

Subjects progressed through two controlled dietary periods. Refer to Table 3 for a list of foods and amounts fed daily. The diet contained 60% of kcals as carbohydrate, 17% of kcals as protein and 23% of kcals as fat in three meals and one snack. The diet met at least 100% of the Recommended Dietary Allowances (RDAs) for all nutrients for men 18-35 years of age. Caloric content was 3,715 kcals and was adjusted to maintain body weight. Each controlled dietary period provided 2.3-2.4 mg of vitamin B-6 from food sources. Following dietary period I, 20 mg/day of vitamin B-6 (as pyridoxine hydrochloride in a gelatin capsule) was supplemented for the remainder of the study. The total vitamin B-6 intake during dietary period II was 22.3 mg/day (2.3 mg from dietary sources and 20 mg from the supplement). Each subject received a placebo capsule during dietary period I. Subjects did not know when they were receiving the B-6 supplement.

## Table 3. Foods Consumed Daily During Controlled Diet Periods I and II

Breakfast: 200 g orange juice
     50 g raisins
     60 g Life cereal
     220 g 1% milk
     60 g whole wheat bread
     200 g pears, lite syrup, drained
     20 g margarine (used throughout the day;
           more available upon request)
     coffee, tea as desired
     jelly as desired
     1 capsule

Lunch: Tuna salad sandwich
     60 g water-packed tuna, drained
     20 g egg white, cooked, chopped
     35 g salad dressing
     15 g pickle relish
     10 g lettuce, iceberg
     60 g whole wheat bread
    Salad
     80 g iceberg lettuce, chopped
     15 g red cabbage, chopped
     50 g kidney beans, drained
     10 g carrot, grated
     20 g French dressing (more available upon request)
     240 g apple juice
     10 each vanilla wafers
     12 g gelatin (flavored with 240 g "Koolade")
     coffee, tea as desired

Dinner: 110 g turkey
     70 g white rice
     50 g cheddar cheese
     200 g green beans, drained
     70 g carrot sticks
     30 g whole wheat bread
     220 g 1% milk
     120 g vanilla frozen yogurt
     200 g peaches, lite syrup
     12 g gelatin (flavored with 240 g "Koolade")
     coffee, tea as desired

Snack: 2 pkts (of 3 each) graham crackers
     200 g grape juice

Meals during the diet period were prepared and served at the Oregon State University Metabolic kitchen (room 105 Milam Hall). All food used during the dietary periods was purchased from the same lot when possible. During meal preparation, food items were weighed to the nearest 0.1 g to assure uniform nutrient intake. Two food

composites from each dietary period were made and analyzed by microbiological assay for vitamin B-6 content (Polansky, 1981). No alcoholic beverages were allowed throughout the study and no caffeinated beverages were allowed the day before, day of or day after the exercise tests.

## Exercise Tests to Exhaustion

Two identical exercise-to-exhaustion sessions were conducted on the seventh day of each controlled dietary period. Two subjects were exercised per day. Each subject arrived at the Human Performance Lab one hour before the start of the exercise test following a 12-14 hour fast. All testing was done at a temperature of 20-24 °C and relative humidity of <60%. A fan was provided for evaporative cooling. Resting blood pressure, blood draws, ECG and metabolic gases were taken in a seated position. Subjects were weighed and had a resting blood pressure taken. They rested for 1/2 hour and had 20 ml of blood drawn. All blood was drawn by a medical technologist. The subjects rested another 1/2 hour and then had another 20 ml of blood drawn. Five ECG electrodes were placed for three limb leads (Quinton Instruments, Model 630 A), and a resting ECG was taken from which heart rate (beats/min.) was calculated. Resting metabolic rate (RMR) was determined by oxygen consumption. The bike seat height was adjusted and subjects performed a 2-minute warm-up at the lowest workload. Subjects were put on the gas collection equipment (Sensor Medics Metabolic Cart Model 2900) and the workload was gradually increased until they attained 75% of $VO_2$ max (as determined in the preliminary test) at 80 revolutions per minute (RPM). Once 75% of $VO_2$ max was attained, subjects were taken off the gas collection equipment, but were put on again for 3 minutes at 10 minute intervals during the test. Respiratory gases were used to calculate R values and measure oxygen consumption. Timing of the exercise session began once subjects reached 75% of $VO_2$ max. Subject were instructed to maintain the workload until exhausted, or prior to exhaustion as indicated by the American College of Sports Medicine guidelines for stopping an exercise test. Based on a previous exhaustive study completed in Fall 1990 in the Department of Nutrition & Food Management at OSU (Virk, Dunton, 1992) each exhaustive exercise test was estimated to take about 2 hours. Exhaustion was defined as the inability to remain within $80 \pm 5$ RPM for 20 seconds.

ECG and heart rate were monitored continuously during each exercise test. Prior to each gas collection (i.e., at ten-minute intervals), an ECG was printed and the heart rate recorded for approximately one minute. Heart rate was calculated by counting the number of 1 mm boxes (0.04 sec) between two successive R waves in the ECG printout and dividing that number into the constant 1500 (the constant 1500 is used since 1500 x 0.04 = 60 and the heart rate is calculated in beats per 60 seconds) (Goldberger and Goldberger, 1990). When subjects neared exhaustion, more frequent recordings were made, time recorded and heart rate calculated. Irregularities in heart function or in general well-being of the subjects were considered cause to terminate the exercise. In accordance with guidelines set by the American College of Sports Medicine (1986), the test would be terminated with the appearance of any of the following:

1. Subject requests to stop

2. Failure of the monitoring system

3. Progressive angina

At 60 minutes of exercise, the workload was decreased for approximately two minutes to allow for the collection of 20 ml blood. Following the blood collection, subjects were given 120 ml of water to drink. The workload was placed at the previously defined level (75% $VO_2$ max) and the test continued until exhaustion. RPE (rate of perceived exertion) was obtained from subjects once every 10 minutes, which helped identify the degree of fatigue. Subjects were not coached or encouraged to continue during the exhaustive tests. They were not aware of cycle times since clocks and watches were concealed. When exhaustion was reached, subjects dismounted from the bicycle and were immediately seated in a chair. A 20 ml sample of blood was drawn within 1-2 minutes after exhaustion. Subjects were given 120 ml of room-temperature water. An ending ECG was taken, and heart rate continued to be monitored until it reached 100 beats/min after stopping the exercise. Subjects were then weighed and sat quietly for the next hour. 20 ml of blood was drawn at 60 minutes post-exercise. The testing was then complete and subjects were allowed food and water.

## Daily Procedures

Subjects were asked to follow several daily procedures to ensure compliance with the metabolic study and to monitor daily activity levels. Subjects kept a daily log during

each nine-day dietary period (period I & II). The log contained information on completeness of intake, food items consumed to adjust weight, beverage consumption, any medications taken, overall health, accuracy of daily urine collections, daily body weight, and the amount, type and perceived intensity of daily exercise. Subjects performed 24-hour urine collections each day of the dietary periods.

## Mid-Study Period

Subjects ate a self-selected diet during this period and maintained their usual exercise habits. Three 24-hour dietary recalls were completed by the subjects on days 16-19 to assess usual patterns of nutrient consumption. The recalls were analyzed by Dunton for nutrient content with Food Processor II software. A fasting blood draw (20 ml) occurred mid-study. Subjects completed their daily logs and 24-hour urine collections were made for four days.

Body composition was determined by underwater weighing. Residual lung volume was indirectly determined using the oxygen dilution technique, wherein dilution of the lungs' original nitrogen concentration occurs through rapid re-breathing of about five liters of oxygen (Wilmore, 1969). Subjects were weighed on dry land. Each subject was seated in the underwater weighing chair, which is attached to a mechanical crank. The subject was lowered to chin level and asked to submerge and expel all the air in his lungs while remaining seated in the chair. A submersion weight was recorded. Three to four trials were conducted until three consistent weight readings were attained. Body density and percent body fat were calculated using the Seri equation (Seri, 1956). Body composition data aids in assessing each subject's degree of fitness.

## Sample Collection

Blood (20 ml) was drawn nine times throughout study, including prestudy, the start of each period, exercise days and mid-study. All blood samples were collected into heparinized tubes and kept on ice until centrifuged (30 min. maximum). The plasma was removed and stored in aliquots at -40°C for the fuel analyses. Plasma for catecholamine determination was portioned into 0.7 ml aliquots, mixed with 5 mM

glutathione (35 $\mu$l of 0.1 M glutathione in 0.7 ml of plasma) as a protectant against oxidation and stored at -70°C.

All urine was collected during periods I and II into bottles provided. Approximately 10 ml of toluene were added to the collection bottles (prior to collection) to act as a preservative. Urine was also collected days 16-19 mid-study. Each morning, daily collections were thoroughly mixed, portioned into small bottles and frozen at -20 °C.

**Analyses**

Plasma Catecholamines

Plasma catecholamines were determined by High-Performance Liquid Chromatography with Electrochemical Detection (HPLC-ECD) by a method modified from Goldstein et al. (1981). The modification included using a mobil phase recipe recommended by Phenomenex since it produced a better separation. Due to limited amounts of sample, single determinations were made to maximize catecholamine concentration within a given sample and thereby maximize detection. The intra-assay (n=2, 2) coefficient of variation (CV) and interassay (n=5) CV for plasma norepinephrine in an exercised male control was 2.68% and 11.54%, respectively. The intra-assay (n=2, 2, 2, 2) CV and interassay (n=5) CV for plasma norepinephrine in a rested female control was 13.5% and 9.4%, respectively. Standard curves during the five days of sample analyses were linear (r=.9999), with a 2.5% CV for the slope. The intra-assay (n=2, 2) CV and interassay (n=5) CV for plasma plasma epinephrine in an exercised male control was 12.4% and 12.6%, respectively. Plasma epinephrine in a rested female control apparently fell below the detection limit of the system. The detection limit for the epinephrine standard was between 0.50 nM and 1.0 nM. The 1.0 nM standard was consistently detected, while the 0.50 nM standard was not. The 0.50 nM standard would correspond to an initial plasma sample containing 0.10 pmol epinephrine/mL of plasma. Other researchers have also reported a similar detection limit for plasma epinephrine (Hallman et al, 1978; Goldstein et al, 1981; Gerlo and Malfait, 1985). Standard curves during the five days of sample analyses were linear (r=.9999), with a 1.5% CV for the slope. In general, catecholamines were adsorbed on alumina, eluted with perchloric acid and separated from each other and from other readily oxidizable substances by HPLC. This process is

described in detail below. The effluent from the column flowed through an electrochemical detector that oxidized catechols to quinones. The current generated by the oxidation reaction was measured and the output was recorded, from which the norepinephrine and epinephrine concentrations were calculated from a standard curve.

The HPLC-ECD system consisted of a Shimadzu LC-10AD solvent delivery module, SIL-10A autoinjector, SCL-10 system controller, CR501 Chromatopac integrator and L-ECD 6A electrochemical detector with a glassy carbon working electrode; a Phenomenex UltraCarb 5 ODS (20) reverse-phase C18 250 x 4.6 mm column and UltraCarb 5 ODS (20) 30 x 4.6 mm guard column; and a Supelco HPLC column water jacket. The ECD applied potential was +0.72 V (versus a Ag/AgCl reference electrode). The column temperature was maintained at 34.5-35.0 °C. Integrator parameters were as follows: width 2.5, slope 200, minimum area 400, attenuation 3, speed 3, stop time 18 min. Total run time (system controller) was 25 minutes for each sample.

Reagents included acid-washed alumina (BAS); hydrochloric acid (HCl) (VWR Scientific), perchloric acid (PCA) (Baker), phosphoric acid (Baker) and nitric acid (Baker); sodium hydroxide (NaOH) (Fisher); dihydroxybenzylamine (DHBA•HBr), epinephrine (bitart), norepinephrine (NE•HCl), sodium octyl sulfate (SOS) and tris base (Sigma); disodium EDTA (dihydrate) (American Scientific); sodium metabisulfite (NaMBS) (Mallinckrodt); and methanol (MeOH) (Baker). Nanopure water was used in all dilutions. Glassware was rinsed in 5% nitric acid and rinsed in nanopure water.

The mobile phase contained 100 mM sodium phosphate, 1.0 mM EDTA, 0.85 mM SOS and 10% MeOH adjusted to pH 3.0 with NaOH. Mobile phase was made fresh each night, filtered (0.2 $\mu$) and degassed twice, and covered tightly with parafilm until use the following morning. Mobile phase was filtered and degassed once before addition of MeOH and once after addition of MeOH (twice total). Mobile phase was pumped at 1.0 ml/min.

Extraction of catecholamines was performed in conical plastic tubes with lids. To each tube was added 1.0 ml freshly thawed, mixed and centrifuged plasma; 50 $\mu$l 5 mM NaMBS (made fresh daily); 50 $\mu$l 100 nM DHBA (made fresh daily from 10 $\mu$M stock); 200 $\mu$l 3M-4g% Tris-EDTA (adjusted to pH 8.6 with HCl and stored in refrigerator); and approximately 15 mg alumina (stored in dessicator). Samples were mixed for 15 min. and microcentrifuged approx. 1 min. Supernatant was then removed and discarded. The alumina was washed three times with nanopure water, then centrifuged approx. 5 min. to remove excess water. Catecholamines were desorbed with 200 $\mu$l 0.1 M PCA. Tubes were vortexed, allowed to stand 15 min. then centrifuged

approx. 1 min. Supernatant was filtered (0.2$\mu$) into HPLC sample vials with 200 $\mu$l-capacity polypropylene inserts (National Scientific). 125 $\mu$l of sample were autoinjected.

Catecholamine standards and the internal standard (DHBA) were prepared with 0.1 M PCA as the solvent. Dilutions were made fresh daily from stock standards (10$\mu$M) stored at -20°C. Standard concentrations were 1.0 nM, 2.0 nM, 5 nM, 10 nM and 20 nM for both norepinephrine and epinephrine with an additional standard concentration of 30 nM for norepinephrine. These concentrations corresponded to 0.1, 0.2. 0.5, 1.0, 2.0 and 3.0 absolute pmol injected onto the column. DHBA concentration was kept constant at 25 nM in each standard (2.5 pmol onto column). 125 $\mu$l of standard was autoinjected. Peak heights (x) and nM concentration (y) were used to calculate standard curves for norepinephrine and epinephrine through linear regression. Peak heights of samples were used to calculate nM concentrations from the standard curves. nM sample values were then multiplied by 0.200 (dilution factor) to attain pmol/mL catecholamine in the original plasma.

## Plasma Fuels

Plasma glucose was measured by the glucose oxidase method (Trinder, 1969) using a Technicon Autoanalyzer System II (Alkpem). Samples were analyzed in duplicate. Ricky Virk performed the analyses. The intra-assay (n=7) coefficient of variation for plasma glucose (CV) was 2.3% for controls.

Lactic acid was analyzed with a kit from Sigma Chemical Co. (Procedure No. 726-UV/826-UV) and measured spectrophotometrically (Henry, 1968) with a Beckman DU 40 spectrophotometer. Samples were analyzed in duplicate. Lactic acid is converted to pyruvic acid in the presence of lactate dehydrogenase and excess NAD. The absorbance of the NADH formed in the reaction can be measured and directly related to the plasma lactic acid concentration. Ricky Virk performed the analyses. The intra-assay (n=2) and interassay (n=3) CV's for controls for plasma lactate were <1.0% and 1.8%, respectively.

Plasma free fatty acids (FFA) were analyzed by a colorimetric method (Faholt, 1973) using a Beckman spectrophotometer. Samples were analyzed in triplicate. After FFA extraction by chloroform-heptane-methanol and phosphate buffer, the copper soaps of FFA are determined colorimetrically with diphenylcarbazide and a concentration

determined directly from palmitic acid standards. Ricky Virk and the author performed the analyses. The intra-assay (n=6, 6, 3) and interassay (n=3) CV's for plasma FFA controls were 4.4% and 14.5%, respectively (Ricky Virk).

Plasma glycerol was analyzed with a kit from Sigma Chemical Co. (Procedure No. 320-UV) and measured spectrophotometrically (Beckman) using a modified enzymatic method (Pinter et al., 1967) for triglycerides that omits the saponification step. Samples were analyzed in duplicate. Glycerol phosphate is formed from glycerol with the ultimate conversion of NADH to NAD through coupled reactions. The amount of NADH oxidized, represented by a decrease in absorbance, is equivalent to the amount of glycerol in the sample. Ricky Virk and the author performed the analyses. The intra-assay (n=2) and interassay (n=4) CV's for plasma glycerol controls were 4.7% and 14.4%, respectively (Ricky Virk).

Substrate utilization during exercise was assessed through computerized analysis of R values (Sensor medics Metabolic Cart Model 2900). The ratio of $CO_2$ production to $O_2$ consumption determines the relative contributions of fat and carbohydrate for energy. The computerized printout also provided $VO_2$ data on an absolute and relative basis.

Other

Hematocrit and hemoglobin were measured by microhematocrit and cyanomethemoglobin methods, respectively. Hemoglobin was analyzed in triplicate and hematocrit was analyzed in duplicate. Karin Hardin and Dr. Jim Ridlington performed the analyses. Hemoglobin and hematocrit values were used to calculate plasma volume changes according to the following equations:

% plasma volume change=$\{[(Hb_1/Hb_2)(100\text{-}Hct_2/(100\text{-}Hct_1)] -1\}(100)$
(Dill and Costill, 1974)

% plasma volume change=$(100/100\text{-}Hct_1)[100(Hct_1\text{-}Hct_2)/Hct_2]$
(Van Beaumont, 1972)

$Hb_1$=initial hemoglobin concentration (e.g., pre-exercise); $Hb_2$=final hemoglobin concentration (e.g., post-exercise); $Hct_1$=initial hematocrit %; $Hct_2$=final hematocrit %

Other graduate students analyzed dietary B-6 content by a standard microbiological procedure (AOAC, 1980) and plasma PLP (Chabner and Livingston, 1970). The PLP interassay (n=21) CV for a control plasma was 3.1% (Nancy Dunton).

## Statistics

The data were analyzed by standard statistical techniques: single analysis of variance (ANOVA) and multiple range tests (Statgraphics Software 5.0), paired t-tests (two-tailed) (Excel Software 4.0), and product-moment correlation coefficients (r) based on linear regression best-fit equations. ANOVA was used to determine if changes occurred over time within each test for each variable studied. A 95% confidence interval (CI) using the Bonferroni method was used for the multiple range tests. This method provides an error rate of at most 5.0% (Mize and Shultz, 1985). Paired t-tests were used to determine if a difference existed between a given time point in each exercise test; null hypotheses were rejected at the 0.05 level of significance. Linear regression was used to plot standard curves, from which sample concentrations could be calculated.

# RESULTS

The characteristics of the five healthy male cyclists that participated in the study are listed in Table 4. Five blood samples were taken during each exhaustive exercise test: two samples pre-exercise (pre1 and pre2), 60 minutes of exercise (Dx), at exhaustion (post-exercise), and 60 minutes after the end of exercise (post60). Two subjects did not attain Dx, likely due to the level of conditioning, motivation and/or over-estimation of the workload intensity. Heart rate was recorded at rest, at intervals throughout exercise and post-exercise. Oxygen consumption was measured at rest and at intervals throughout exercise.

The data were analyzed in two ways: comparisons of each variable were made between the vitamin B-6 supplemented (S) and nonsupplemented (NS) conditions at each time point, and comparisons of each variable across time were made within each trial. Percent differences in concentrations of two variables between NS and S, and between time points within a trial were calculated to allow some comparison of the magnitude of change, particularly when no statistically significant differences were found. With regard to percent differences, "no difference" indicates that the difference between two values was <1%. A difference of <1% was considered negligible and likely a result of experimental variation. Comparisons of variables at specific time points between the trials used the following formula: (conc. suppl.-conc. nonsuppl./conc.nonsuppl.)100. Comparisons of variables across time within a trial used the following formula: (conc. time 2-conc. time 1/conc. time 1)100.

## Plasma Volume Changes

Table 5 lists mean plasma volume changes (PVC %), hematocrit (Hct) and hemoglobin (Hb) values, and the associated standard deviations for the nonsupplemented (NS) and vitamin B-6 supplemented (S) exercise tests. (Individual values are listed in Table A.1 in the Appendix.) Percent PVC values were calculated using two different methods (Dill and Costill, 1974; Van Beaumont, 1972) to compare pre-exercise (mean of pre1 and pre2) plasma volume to plasma volume at Dx, post-exercise and post60. The only significant difference was an increase (p<.05) in the percent PVC for S versus NS at exhaustion. The significance was reached using the data from the Dill and Costill equation, although

## Table 4.  Subject Characteristics

| Subject | Age (years) | Body Wt. (kg) | Height (cm) | Body Fat (%) | VO2 max (ml/kg/min) |
|---|---|---|---|---|---|
| 1 | 25 | 79.7 | 189 | 5.01 | 59.3 |
| 2 | 27 | 73.9 | 183 | 11.8 | 61.9 |
| 4 | 37 | 104 | 191 | 16.1 | 43.4 |
| 5 | 21 | 74.6 | 188 | 9.70 | 55.6 |
| 6 | 35 | 82.3 | 157 | 13.5 | 49.5 |
| Mean (n=5) | 29 | 83.0 | 182 | 11.2 | 53.9 |
| std. deviation | 6.8 | 12.5 | 14.1 | 4.19 | 7.51 |
| Body weights were taken immediately prior to hydrostatic weighing (mid-study). | | | | | |
| Body fat percentages were determined from hydrostatic weighing. | | | | | |
| VO2 max was determined on a cycle ergometer (pre-study). | | | | | |

## Table 5. Mean Hematocrit, Hemoglobin, Plasma Volume Changes

| | Test | Pre1 | Pre2 | During | Post | Post60 | |
|---|---|---|---|---|---|---|---|
| **Hct (%)** | NS | 44.50 | 44.85 | 47.58 | 46.75 | 44.55 | |
| | | sd=3.13 | sd=3.21 | sd=3.12 | sd=3.08 | sd=2.02 | |
| | | n=5 | n=5 | n=3 | n=5 | n=5 | |
| | S | 44.50 | 44.30 | 48.67 | 48.15 | 44.95 | |
| | | sd=3.57 | sd=3.77 | sd=3.78 | sd=2.61 | sd=2.91 | |
| | | n=5 | n=5 | n=3 | n=5 | n=5 | |
| **Hb (gm/dl)** | NS | 14.81 | 14.87 | 16.00 | 16.14 | 15.42 | |
| | | sd=0.82 | sd=0.64 | sd=1.05 | sd=0.70 | sd=0.55 | |
| | | n=5 | n=5 | n=3 | n=5 | n=5 | |
| | S | 15.08 | 15.01 | 16.92 | 16.63 | 15.64 | |
| | | sd=1.29 | sd=1.29 | sd=1.36 | sd=1.25 | sd=1.10 | |
| | | n=5 | n=5 | n=3 | n=5 | n=5 | |

| | | *Dill and Costill* | | | *Van Beaumont* | | |
|---|---|---|---|---|---|---|---|
| **PVC (%)** | Test | During | Post | Post60 | During | Post | Post60 |
| | NS | -9.04 | -11.31* | -3.37 | -6.92 | -7.71 | 0.72 |
| | | sd=3.41 | sd=6.85 | sd=5.82 | sd=4.34 | sd=8.77 | sd=7.85 |
| | | n=3 | n=5 | n=5 | n=3 | n=5 | n=5 |
| | S | -13.87 | -16.10 | -4.69a | -10.33 | -13.85 | -2.16 |
| | | sd=2.02 | sd=5.76 | sd=4.50 | sd=2.60 | sd=6.46 | sd=4.36 |
| | | n=3 | n=5 | n=5 | n=3 | n=5 | n=5 |

Hct=Hematocrit; Hb=Hemoglobin; PVC=plasma volume change. NS=nonsupplemented exercise test to exhaustion; S=B-6 supplemented exercise test to exhaustion. Pre1=0.5 hr before exercise; Pre2=before exercise; During=1.0 hour into exercise; Post=immediately after exercise; Post60=60 min after exercise. Dill and Costill (1974) & Van Beaumont (1972) refer to methods of calculating PVC.

*=significantly different than S value; a=significantly different than post

values used from the Van Beaumont equation approached significance (p=.078). Using either method, the mean percent PVC was somewhat greater with S versus NS at all time points. In other words, plasma volume decreased more with S: The difference in percent PVC between the tests was 3.4-4.8% at Dx, 4.1-6.1% post-exercise and 1.3-2.9% post60, depending on the method of calculation. Individual percent PVC values varied somewhat using either method of calculation: only two of three subjects had a greater PVC at Dx, three of five subjects had a greater PVC at post-exercise, and two of five subjects had a greater PVC at post60 with S.

A significant change in the mean percent PVC over time was found for S (p<.05) but not for NS, using either method of PVC calculation. Percent PVC changed significantly (95% CI Bonferroni) between post-exercise and post60 from -15.43% to -4.69% (Dill and Costill) or from -13.86% to -2.16% (Van Beaumont) with S. With NS, mean percent PVC post-exercise was also greater (more negative) than the percent PVC at post60, but this difference did not achieve statistical significance. Individual Hb and Hct values decreased or showed no change and percent PVC decreased (became more positive) from post to post60 in both trials. In both trials, mean percent PVC increased (became more negative) from Dx (n=3) to post-exercise (n=5). However, the three individuals who attained Dx displayed a decrease in percent PVC (i.e., increased plasma volume) from Dx to post-exercise in both trials. The high percent PVC values of the two individuals who did not attain Dx drove down the mean percent PVC at post-exercise.

**Plasma PLP**

Table 6 lists individual plasma PLP concentrations, mean concentrations and standard deviations for the nonsupplemented (NS) and vitamin B-6 supplemented (S) exercise tests. Figure 1 provides a graphical comparison of the mean plasma PLP concentrations. The PLP analyses wre performed by another graduate student, and the data are included here for comparative purposes. Mean plasma PLP concentrations were significantly higher with S compared to NS at all time points studied (p<.005).

There were no significant changes in mean plasma PLP levels over time in either exercise test. Both NS and S showed a similar mean pattern of plasma PLP response over time except during rest, but individual trends varied. Plasma PLP decreased slightly from pre1 to pre2 during the supplemented trial (5%), but remained unchanged during rest in the nonsupplemented trial. Mean plasma PLP concentrations in both trials increased from

## Table 6. Plasma PLP Concentrations (nmol/L)

| Subject | Test | Pre1 | Pre2 | During | Post | Post60 |
|---|---|---|---|---|---|---|
| 1 | NS | 36.7 | 37.3 | 38.7 | 30.0 | 29.4 |
|  | S | 179 | 176 | 193 | 174 | 156 |
| 2 | NS | 29.3 | 29.4 |  | 36.4 | 28.7 |
|  | S | 205 | 191 |  | 227 | 172 |
| 4 | NS | 17.8 | 17.4 |  | 23.5 | 15.9 |
|  | S | 133 | 134 |  | 157 | 126 |
| 5 | NS | 32.2 | 33.1 | 34.9 | 29.8 | 24.3 |
|  | S | 177 | 162 | 169 | 179 | 144 |
| 6 | NS | 44.8 | 45.4 | 53.8 | 43.3 | 36.4 |
|  | S | 192 | 173 | 204 | 159 | 132 |
| Mean | NS | 32.2* | 32.5* | 42.5* | 32.6* | 26.9* |
|  |  | sd=9.93 | sd=10.3 | sd=10.0 | sd=7.52 | sd=7.54 |
|  |  | n=5 | n=5 | n=3 | n=5 | n=5 |
|  | S | 177 | 169 | 189 | 179 | 146 |
|  |  | sd=27.3 | sd=21.4 | sd=18.0 | sd=28.3 | sd=18.6 |
|  |  | n=5 | n=5 | n=3 | n=5 | n=5 |

Individual values are means of samples analyzed in duplicate by Nancy Dunton.
NS=nonsupplemented exercise test to exhaustion; S=B-6 supplemented exercise test to exhaustion
Pre1=0.5 hr before exercise; Pre2=before exercise; During=1.0 hour into exercise; Post=immediately after exercise; Post60=60 min after exercise; *=significantly different than S value

**Figure 1. Plasma PLP Concentrations**
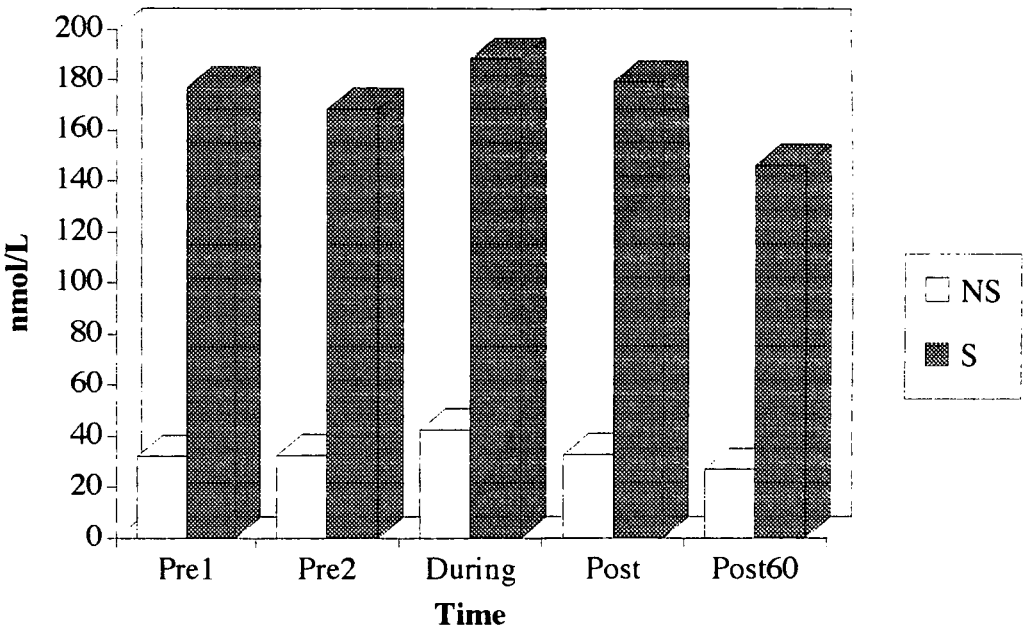
pre-exercise to Dx (n=3), decreased from Dx to post-exercise (n=3) and decreased from post-exercise to post60 (n=5). The mean PLP concentration at post-exercise was no different than the pre-exercise concentration with NS, but was slightly higher (4%) with S. At post60, mean plasma PLP was lower in NS and S (17% and 15%, respectively) relative to resting values. The magnitude of the increase from pre-exercise (n=5) to Dx (n=3) was greater with NS (31%) versus S (9%). Calculating pre-exercise mean plasma PLP values with data from the three individuals who attained Dx still results in a somewhat greater magnitude of increase in plasma PLP from rest to Dx with NS (11%) versus S (7%).

## Plasma Norepinephrine

Table 7 lists the individual plasma norepinephrine concentrations, mean concentrations and standard deviations for the nonsupplemented (NS) and vitamin B-6 supplemented (S) exercise tests. There were no significant differences in mean plasma norepinephrine concentrations between the two tests at any of the time points studied. The mean (n=3) pre-exercise plasma norepinephrine levels during S were somewhat lower than during NS: 10% lower at pre1 and 24% lower at pre2. Mean plasma norepinephrine was slightly higher at Dx (3%, n=3), post-exercise (2%, n=5) and post60 (8%, n=5) during S versus NS. However, differences in individual norepinephrine concentrations between the trials varied and no consistent increase or decrease in plasma norepinephrine was seen from NS to S. The mean directional changes in plasma norepinephrine at pre-exercise, Dx and post60 do not represent the response of the majority of subjects at those time points. Although the pre-exercise point showed lower mean plasma norepinephrine levels during S, only one individual of the three with paired data (i.e., NS and S samples) for pre-exercise actually showed a decreased plasma norepinephrine response with supplementation (54% pre1; 66% pre2); the other two individuals had higher pre-exercise plasma norepinephrine values (25-40% pre1; 5-59% pre2). Of the three subjects who reached Dx, only one showed an increased (24%) plasma norepinephrine level with supplementation in accordance with the mean response; one individual had a lower plasma norepinephrine concentration (14%), and the other individual had no change. Although the post60 point showed a mean increase in plasma norepinephrine during S compared to NS, only two of the subjects actually had higher (47-79%) plasma norepinephrine levels; the other three displayed lower plasma norepinephrine concentrations (15-44%). At post-

**Table 7. Plasma Norepinephrine Concentrations (pmol/mL)**

| Subject | Test | Pre1 | Pre2 | During | Post | Post60 |
|---------|------|------|------|--------|------|--------|
| 1 | NS | 1.14 | 1.71 | 4.61 | 4.27 | 1.71 |
|   | S  | 0.52 | 0.59 | 5.74 | 4.71 | 1.45 |
| 2 | NS | 0.65 | 0.84 |      | 5.86 | 1.10 |
|   | S  | 0.82 | 0.89 |      | 4.97 | 0.93 |
| 4 | NS | 0.52 | 0.53 |      | 4.11 | 0.87 |
|   | S  | 0.73 | 0.84 |      | 5.35 | 1.55 |
| 5 | NS |      | 0.56 | 3.65 | 4.65 | 0.87 |
|   | S  |      |      | 3.61 | 3.48 | 0.48 |
| 6 | NS |      | 0.74 | 5.03 | 6.32 | 1.25 |
|   | S  |      |      | 4.34 | 7.25 | 1.84 |
| Mean | NS | 0.77 | 0.87 | 4.43a | 5.04b | 1.16c |
|   |    | sd=0.32 | sd=0.48 | sd=0.71 | sd=0.99 | sd=0.35 |
|   |    | n=3 | n=5 | n=3 | n=5 | n=5 |
|   | S  | 0.69 | 0.77 | 4.56a | 5.15b | 1.25c |
|   |    | sd=0.15 | sd=0.16 | sd=1.08 | sd=1.37 | sd=0.54 |
|   |    | n=3 | n=3 | n=3 | n=5 | n=5 |

NS=nonsupplemented exercise test to exhaustion; S=B-6 supplemented exercise test to exhaustion
Pre1=0.5 hr before exercise; Pre2=before exercise; During=1.0 hour into exercise; Post=immediately after exercise; Post60=60 min after exercise. a & b=sig. diff. than rest; c=sig. diff. than During & Post

exercise, the majority of individual responses corresponded to the mean directional change in plasma norepinephrine: three of five subjects, including two who attained Dx, had higher plasma norepinephrine levels during S versus NS (10-30%).

There was a significant change in mean plasma norepinephrine concentration over time in each of the exercise tests (p<.0001). Both tests showed significantly higher plasma norepinephrine concentrations at Dx and post-exercise versus pre-exercise, and significantly lower plasma norepinephrine levels at post60 compared to Dx and to post-exercise (95% CI Bonferroni). The pattern in both tests was the same: mean plasma norepinephrine increased during rest, rose significantly above the mean resting value at Dx, increased (not significantly) from Dx to post-exercise, then declined significantly from post-exercise to post60. Resting samples were obtained for five subjects during NS but only three subjects during S, while post60 samples were obtained from all five subjects in both trials. Performing a comparison with matched sample sizes (n=3) from the same individuals for each trial results in a greater elevation in plasma norepinephrine at post60 relative to rest in S (79%) versus NS (37%). The magnitude of the increase in mean plasma norepinephrine to peak values at post-exercise was a dramatic 512% and 605% relative to average resting values in the NS and S tests, respectively. Comparing pre- and post-exercise values from the three individuals for whom complete resting values were detected still results in a greater magnitude of plasma norepinephrine increase during S (586% vs 428%). However, only one individual (subj1) actually had a greater elevation in plasma norepinephrine concentration at post-exercise versus rest during S; the other two subjects had a greater elevation between these points during NS. The magnitude of the decrease in mean plasma norepinephrine concentration from post-exercise to post60 in both tests was about 75%.

Significant mean plasma norepinephrine changes over time in both trials were followed by all individual subjects for whom data were obtained. The mean increase in plasma norepinephrine from Dx to post-exercise in both trials was dictated by the majority of subjects during NS but not during S: during NS, two subjects had an increase (26%) in plasma norepinephrine concentration and one had a decrease (7%) between these points; during S, two subjects had a decrease (4-18%) and one had an increase (67%) in plasma norepinephrine concentration between Dx and post-exercise. The three subjects with detected pre1 and pre2 norepinephrine values for both trials all showed an increase in plasma norepinephrine between these points in each trial. Based on data from these three subjects, the magnitude of norepinephrine increase from pre1 to pre2 was greater during NS (33%) versus S (13%). In accordance with the mean trend, all three subjects with

matched data for pre-exercise and post60 had elevated plasma norepinephrine concentrations at post60 relative to rest in both trials.

## Plasma Epinephrine

Individual plasma epinephrine concentrations, mean concentrations and standard deviations for the nonsupplemented (NS) and vitamin B-6 supplemented (S) exercise tests are listed in Table 8. The detection limit of the HPLC-ECD system for the epinephrine standard was between 0.50 nM and 1.0 nM, corresponding to 0.05 and 0.10 absolute pmol.

There were no significant differences in mean plasma epinephrine concentrations between the two tests at any of the time points studied. However, mean plasma epinephrine concentrations were higher at all time points during S versus NS except post-exercise, which showed no change. The differences amounted to 19% at pre1 (n=2) and 30% at pre2 (n=4); 7% at Dx (n=3) and 15% at post60 (n=5). For some people, plasma epinephrine could not be detected; only subjects with both NS and S values at a given time point (paired values) were used to compare epinephrine values at that time point. In support of the mean response, most of the individual pre2 and post60 minute values were higher during S: three of four subjects with paired epinephrine values had increased values at pre2 (15-96%) and post60 (5-65%). Although these were the same four subjects, the three who supported the mean change were not necessarily the same three individuals. The mean directional changes in plasma epinephrine at pre1 reflects only the response of one individual: of two subjects with paired values, one had higher plasma epinephrine and one had lower plasma epinephrine during S. The mean response in plasma epinephrine at Dx does not represent the response of the majority of subjects: of the three subjects with paired values, one had higher plasma epinephrine (41%), one had lower plasma epinephrine (36%) and one had no change in plasma epinephrine concentration from NS to S. The "no difference" in mean plasma epinephrine at post-exercise between the trials reflects the decreased plasma epinephrine values of three individuals (18-53%) and increased values of two individuals (10-147%).

There were no significant changes in mean plasma epinephrine concentration over time for either NS or S. The pattern in both tests was the same except from Dx to post-exercise: mean plasma epinephrine concentration increased during rest, rose above the mean resting value at Dx (n=3), then declined from post-exercise to post60 (n=5). From

## Table 8. Plasma Epinephrine Concentrations

| Subject | Test | Pre1 | Pre2 | During | Post | Post60 |
|---------|------|------|------|--------|------|--------|
| 1 | NS | | 0.23 | 0.76 | 1.17 | 0.42 |
| | S | 0.38 | 0.46 | 1.07 | 0.95 | 0.44 |
| 2 | NS | 0.33 | 0.37 | | 0.41 | 0.34 |
| | S | 0.45 | 0.42 | | 0.31 | 0.29 |
| 4 | NS | 0.26 | 0.28 | | 0.32 | 0.25 |
| | S | | 0.35 | | 0.35 | 0.41 |
| 5 | NS | | 0.23 | 0.55 | 0.72 | 0.32 |
| | S | | | 0.35 | 0.34 | |
| 6 | NS | 0.18 | 0.24 | 0.48 | 0.47 | 0.19 |
| | S | 0.16 | 0.24 | 0.48 | 1.17 | 0.24 |
| Mean | NS | 0.26 | 0.27 | 0.59 | 0.62 | 0.30 |
| | | sd=0.08 | sd=0.06 | sd=0.14 | sd=0.34 | sd=0.09 |
| | | n=3 | n=5 | n=3 | n=5 | n=5 |
| | S | 0.33 | 0.37 | 0.63 | 0.63 | 0.34 |
| | | sd=0.15 | sd=0.10 | sd=0.38 | sd=0.41 | sd=0.10 |
| | | n=3 | n=4 | n=3 | n=5 | n=4 |

NS=nonsupplemented exercise test to exhaustion; S=B-6 supplemented exercise test to exhaustion
Pre1=0.5 hr before exercise; Pre2=before exercise; During=1.0 hour into exercise; Post=immediately after exercise; Post60=60 min. after exercise

Dx to post-exercise (n=3), mean plasma epinephrine increased (4.5%) with NS, but remained unchanged with S. Peak mean epinephrine concentrations were attained at post-exercise (n=5) with NS, but at Dx (n=3) with S. The magnitude of the increase from pre-exercise to peak levels was greater with NS (133%) than S (81%). Omitting the data for subj5 still results in a greater magnitude of plasma epinephrine rise from pre2 to post-exercise (n=4) in NS versus S (111% vs. 89%). The magnitude of the decrease in mean plasma epinephrine concentration from post-exercise to post60 in both tests was about 48%.

In accordance with the mean response, all subjects had an increased plasma epinephrine level above resting at Dx and post-exercise, and a decrease in plasma epinephrine from post-exercise to post60 in both trials. Peak epinephrine concentration achieved at post-exercise with NS and at Dx with S reflect the results of two out of three subjects (subj1 and subj5). In contrast to the mean response from Dx to post-exercise with S, which showed no change, two of three subjects had a decrease in plasma epinephrine and one (subj6) had an increase in plasma epinephrine concentration between these points. From Dx to post-exercise with NS, two of three subjects had an increase in plasma epinephrine and one (subj6) had no change in plasma epinephrine concentration between these points. Hence the mean response reflected the results of two out of three subjects. Individual trends in plasma epinephrine concentrations from pre1 to pre2 showed higher pre2 plasma epinephrine relative to pre1 in two of three subjects with detected values with S; the third subject (subj2) had a decrease in plasma epinephrine concentration between these points with S. The two subjects with detected pre1 and pre2 data for NS both had an increase in plasma epinephrine concentration between these points. Based on the data of these two subjects (subj2 and subj6), the magnitude of plasma epinephrine increase from pre1 to pre2 was greater with NS (18%) versus S (7%). Trends in the mean epinephrine responses at post60 relative to pre-exercise were not dictated by the response of most subjects: only two individuals of five showed an elevation in post60 epinephrine relative to resting with NS, and only one individual (subject 2) of four showed a decrease in epinephrine relative to resting with S.

## Plasma Glucose

Table 9 lists individual plasma glucose concentrations, mean concentrations and standard deviations for the nonsupplemented (NS) and vitamin B-6 supplemented (S) exercise

## Table 9.  Plasma Glucose Concentrations (mmol/L)

| Subject | Test | Pre1 | Pre2 | During | Post | Post60 |
|---|---|---|---|---|---|---|
| 1 | NS | 4.57 | 4.60 | 4.69 | 5.10 | 4.84 |
|  | S | 4.38 | 4.40 | 4.87 | 4.68 | 4.68 |
| 2 | NS | 4.78 | 4.71 |  | 5.44 | 4.81 |
|  | S | 4.61 | 4.67 |  | 4.15 | 4.83 |
| 4 | NS | 4.94 | 5.04 |  | 5.53 | 4.79 |
|  | S | 4.49 | 4.98 |  | 6.19 | 4.75 |
| 5 | NS | 4.46 | 4.37 | 4.64 | 4.37 | 4.40 |
|  | S | 5.05 | 4.87 | 4.83 | 4.61 | 4.68 |
| 6 | NS | 5.31 | 5.42 | 5.08 | 4.72 | 5.29 |
|  | S | 5.50 | 5.46 | 5.13 | 5.14 | 5.37 |
| Mean | NS | 4.81 | 4.83 | 4.80 | 5.03 | 4.83 |
|  |  | sd=0.33 | sd=0.41 | sd=0.24 | sd=0.49 | sd=0.32 |
|  |  | n=5 | n=5 | n=3 | n=5 | n=5 |
|  | S | 4.81 | 4.88 | 4.94 | 4.95 | 4.86 |
|  |  | sd=0.46 | sd=0.39 | sd=0.16 | sd=0.77 | sd=0.29 |
|  |  | n=5 | n=5 | n=3 | n=5 | n=5 |

Individual values are means of samples analyzed in duplicate.
NS=nonsupplemented exercise test to exhaustion; S=B-6 supplemented exercise test to exhaustion
Pre1=0.5 hr before exercise; Pre2=before exercise; During=1.0 hour into exercise; Post=immediately after exercise; Post60=60 min after exercise

tests. There were no significant differences in mean plasma glucose concentrations between the two tests at any of the time points studied. There was $\leq 1\%$ difference in observed mean glucose values between the trials at pre-exercise, post-exercise and post60. Mean plasma glucose concentration was slightly higher (3%) at Dx with S versus NS. Individual pre-exercise values largely showed little difference between the trials, except one subject (subj5) who had a 12% higher plasma glucose value in S versus NS.

Plasma glucose concentrations appeared to achieve a small rise with exercise in both trials, but there were no significant changes in mean plasma glucose over time for either exercise test.

## Plasma Lactic Acid

Table 10 lists individual plasma lactate concentrations, mean concentrations and standard deviations for the nonsupplemented (NS) and vitamin B-6 supplemented (S) exercise tests. Figure 2 provides a graphical comparison of the mean plasma lactate concentrations. There were no statistically significant differences in mean plasma lactate levels between the two tests at any of the time points studied. However, mean plasma lactate was higher pre-exercise (9%) and at Dx (48%), and lower post-exercise (6%) with S versus NS. There was no difference in mean plasma lactate levels at post60 between the two tests: two individuals had higher (15-30%) plasma lactate and three individuals had lower plasma lactate (7-31%) at post60 with supplementation. The majority of subjects at pre-exercise and post-exercise accounted for the mean trends: three of the five subjects had higher (14-38%) plasma lactate levels pre-exercise after supplementation; three of the five subjects, including two who attained Dx, showed lower (10-26%) plasma lactate levels at post-exercise after supplementation. All three subjects who exercised longer than 60 minutes had higher (7-80%) plasma lactate levels at Dx with S compared to NS. With a greater sample size, it is possible the difference in plasma lactate concentrations between NS and S at Dx may have achieved statistical significance (with n=3, p=0.16).

There were significant changes in mean plasma lactate concentration over time in both exercise tests (p<.001), but the tests showed a somewhat different time course during exercise. Both tests had significantly higher plasma lactate concentrations at Dx and post-exercise versus pre-exercise, and significantly lower plasma lactate levels at post60 compared to Dx and to post-exercise (95% CI Bonferroni). In addition, plasma lactate showed a significant decrease with S from Dx to post-exercise, while plasma lactate

## Table 10. Plasma Lactic Acid Concentrations (mmol/L)

| Subject | Test | Pre1 | Pre2 | During | Post | Post60 |
|---|---|---|---|---|---|---|
| 1 | NS | 1.07 | 1.74 | 3.21 | 3.15 | 1.28 |
| | S | 1.28 | 1.28 | 3.43 | 2.34 | 1.47 |
| 2 | NS | 1.14 | 0.95 | | 3.34 | 1.15 |
| | S | 0.94 | 0.87 | | 2.51 | 1.07 |
| 4 | NS | 0.83 | 0.73 | | 2.08 | 0.98 |
| | S | 0.91 | 0.87 | | 2.90 | 0.88 |
| 5 | NS | 0.88 | 0.95 | 1.91 | 3.12 | 1.29 |
| | S | 0.98 | 1.25 | 3.30 | 2.79 | 0.89 |
| 6 | NS | 1.11 | 1.33 | 2.81 | 3.34 | 1.34 |
| | S | 1.49 | 1.88 | 5.04 | 3.62 | 1.75 |
| Mean | NS | 1.01 | 1.14 | 2.64a | 3.01b | 1.21c |
| | | sd=0.14 | sd=0.40 | sd=0.67 | sd=0.53 | sd=0.15 |
| | | n=5 | n=5 | n=3 | n=5 | n=5 |
| | S | 1.12 | 1.23 | 3.92a | 2.83b,d | 1.21c |
| | | sd=0.25 | sd=0.41 | sd=0.97 | sd=0.49 | sd=0.38 |
| | | n=5 | n=5 | n=3 | n=5 | n=5 |

Individual values are means of samples analyzed in duplicate. NS=nonsupplemented exercise test to exhaust.; S=B-6 supplemented exercise test to exhaustion. Pre1=0.5 hr before exercise; Pre2=before exercise; During=1.0 hour into exercise; Post=immediately after exercise; Post60=60 min after exercise
a & b=sig. diff. than rest; c=sig. diff. than During & Post; d=sig. diff. than During

**Figure 2. Plasma Lactic Acid Concentrations**
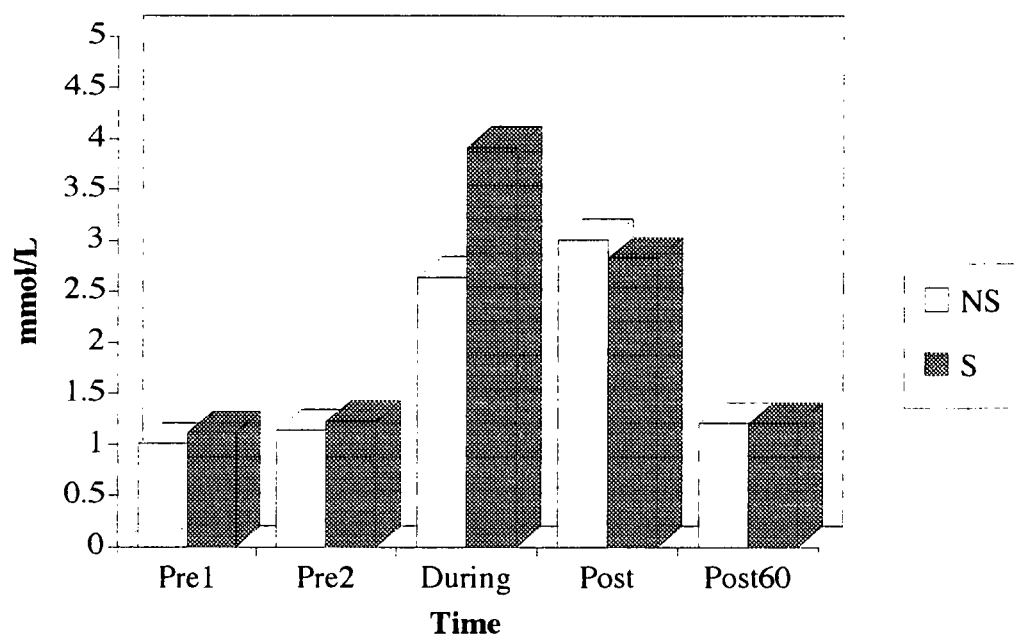
increased (nonsignificant) with NS between these two time points. In other words, plasma lactate levels peaked at Dx with S then began to decline; plasma lactate concentrations with NS achieved the highest concentration post-exercise before starting to decline. Mean plasma lactate concentrations increased similarly from pre1 to pre2 with NS (13%) and S (10%) and decreased similarly from post-exercise to post60 with NS (60%) and S (57%). The magnitude of plasma lactate increase from pre-exercise (n=5) to Dx (n=3) was greater with S (234%) than NS (145%). The magnitude of the increase in plasma lactate concentration from pre-exercise to Dx is still greater with S than NS (188% vs. 123%) when data is examined from the three subjects who attained Dx. From Dx to post-exercise (n=3), plasma lactate rose 14% with NS and declined 28% with S. At post60, the mean plasma lactate level was elevated above the mean pre-exercise value in NS (13%), and slightly elevated above the mean pre-exercise value in S (3%).

Significant mean plasma lactate changes in both trials resulted from a change similar to the mean in each individual's plasma lactate concentration. Nonsignificant changes in mean plasma lactate were not dictated by all subjects for any observed trend, but in some cases by the majority of subjects. With NS, the majority of subjects had an increase in plasma lactate from pre1 to pre2: three subjects had an increase in plasma lactate (7-63%); two subjects had a decrease in plasma lactate (12-16%). With S, the majority of the subjects did not have an increase in plasma lactate from pre1 to pre2: two of five subjects had an increase in plasma lactate (26-27%), two had a decrease (5-8%) and one had no change. From Dx to post-exercise with NS, two of three subjects had an increase in plasma lactate concentration in accordance with the mean response, while one subject (subj1) had a decrease in plasma lactate between these points. At post60 with S, three of five individuals had an elevated plasma lactate concentration relative to resting, one subject had no difference and one subject had a lower lactate level at post60 relative to resting. At post60 with NS, four subjects displayed elevated plasma lactate concentrations while one subject had a lower plasma lactate level relative to resting values.

## Plasma Free Fatty Acids

Individual free fatty acid (FFA) concentrations, mean concentrations and standard deviations for the nonsupplemented (NS) and vitamin B-6 supplemented (S) exercise tests are listed in Table 11, and means are shown in Figure 3. There were no significant differences in mean plasma FFA concentrations between the two tests at any of the time

## Table 11. Plasma Free Fatty Acid Concentrations (mmol/L)

| Subject | Test | Pre1 | Pre2 | During | Post | Post60 |
|---|---|---|---|---|---|---|
| 1 | NS | 0.25 | 0.39 | 0.63 | 1.82 | 1.08 |
|   | S | 0.25 | 0.37 | 0.56 | 1.21 | 1.05 |
| 2 | NS | 0.46 | 0.57 |  | 1.63 | 0.71 |
|   | S | 0.55 | 0.38 |  | 0.88 | 0.49 |
| 4 | NS | 0.72 | 0.57 |  | 0.93 | 0.55 |
|   | S | 0.41 | 0.43 |  | 0.99 | 0.57 |
| 5 | NS | 0.57 | 0.44 | 0.76 | 1.16 | 1.18 |
|   | S | 0.32 | 0.29 | 0.45 | 0.46 | 0.72 |
| 6 | NS | 0.31 | 0.29 | 0.45 | 1.18 | 1.18 |
|   | S | 0.37 | 0.36 | 0.38 | 1.81 | 1.27 |
| Mean | NS | 0.46 | 0.45 | 0.61 | 1.34a,b | 0.94 |
|   |   | sd=0.19 | sd=0.12 | sd=0.15 | sd=0.37 | sd=0.29 |
|   |   | n=5 | n=5 | n=3 | n=5 | n=5 |
|   | S | 0.38 | 0.37 | 0.46 | 1.07a | 0.82 |
|   |   | sd=0.11 | sd=0.05 | sd=0.09 | sd=0.49 | sd=0.99 |
|   |   | n=5 | n=5 | n=3 | n=5 | n=5 |

Individual values are means of samples analyzed in triplicate.
NS=nonsupplemented exercise test to exhaustion; S=B-6 supplemented exercise test to exhaustion
Pre1=0.5 hr before exercise; Pre2=before exercise; During=1.0 hour into exercise; Post=immediately after
exercise; Post60=60 min after exercise. a=sig. diff. than rest; b=sig. diff. than During

**Figure 3. Plasma Free Fatty Acid Concentrations**
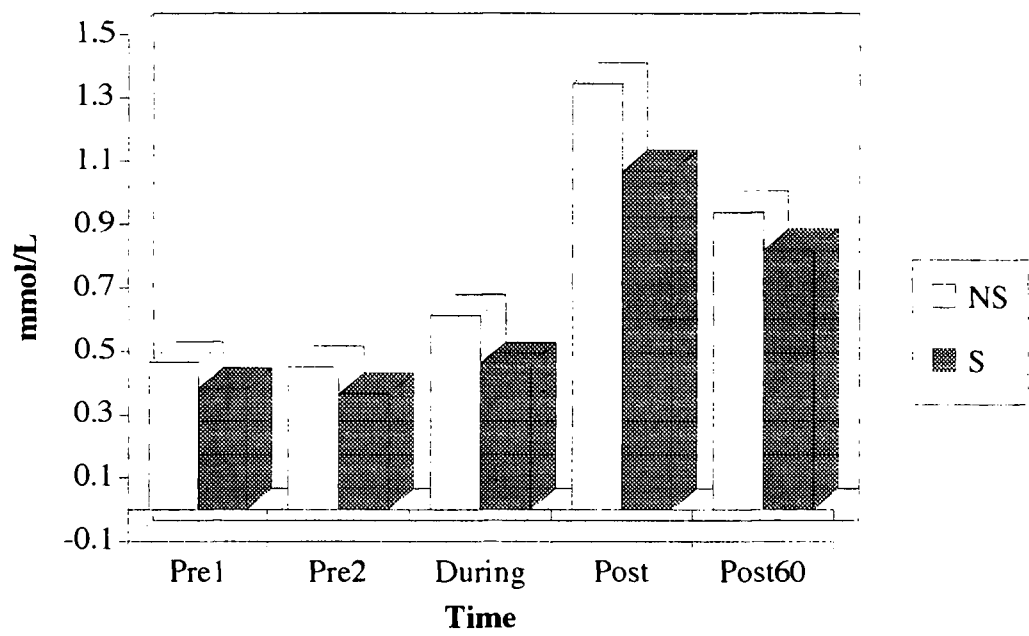
points studied. However, mean plasma FFA concentrations were lower at all time points with S compared to NS. The percent decrease amounted to 18% pre-exercise, 24% at Dx, 20% post-exercise and 13% at post60. All three of the subjects who exercised longer than 60 minutes had lower plasma FFA concentrations at Dx with S compared with NS (12-40%); significance may have been reached with a greater sample size (with n=3, p=0.18). Individual plasma FFA responses were lower in four of five subjects pre-exercise with S (3-40%); significance may have been reached with a greater sample size (with n=5, p=0.16). Individual directional changes in plasma FFA between the trials were more variable at post-exercise and post60: only three of the five subjects had lower plasma FFA levels post-exercise (33-60%) and post60 (2-39%) with S versus NS.

There were significant changes in mean plasma FFA levels over time in both NS (p<.001) and S (p<.05), but more contrasts achieved significance in NS than in S (95% CI Bonferroni). The pattern of mean plasma FFA response was the same in both tests, FFA values decreased during rest, increased above resting levels at Dx (n=3), continued to rise to post-exercise (n=3 or n=5), then declined to post60. For both NS and S, there was a significant rise in plasma FFA concentrations from pre-exercise to post-exercise. The magnitude of the mean increase from rest to post-exercise was similar for NS (193%) and S (186%). With NS, a significant increase in plasma FFA concentration was also found from Dx to post-exercise. Mean plasma FFA concentrations also increased from Dx to post-exercise with S, but the increase did not reach statistical significance. The magnitude of the increase in plasma FFA from rest to Dx in both trials (nonsignificant) and the decrease from post-exercise to post60 in both trials (nonsignificant) was about 10% greater with NS versus S. At post60 in both trials, plasma FFA levels were elevated relative to pre-exercise values, with a slightly greater difference occurring with S (119%) versus NS (105%).

All individuals had an increase in plasma FFA concentrations above resting levels at post-exercise in both trials (n=5), and all three individuals who attained Dx had increased plasma FFA concentrations at post-exercise relative to Dx with NS. Although comparison of FFA means between Dx and post-exercise with S did not attain significance, all three individuals who reached Dx had higher plasma FFA concentrations by post-exercise. In accordance with the small mean decrease (3-4%) in plasma FFA from pre1 to pre2 in both trials, three of five individuals had a decrease in plasma FFA between these points in both tests. All individuals with S and four of five subjects with NS had higher plasma FFA concentrations at post60 relative to resting values.

## Plasma Glycerol

Individual plasma glycerol concentrations, mean concentrations and standard deviations for the two tests are listed in Table 12, and means are shown in Figure 4. There were no significant differences in mean plasma glycerol concentrations between the two tests at any of the time points studied. Mean plasma glycerol concentrations were somewhat higher pre-exercise (9%) and lower at Dx (15%), post-exercise (19%) and post60 (14%) with S versus NS. The majority of subjects followed these mean trends at each time point; however, some variation of individual directional changes in plasma glycerol and the small sample size prevented these trends from achieving significance. In particular, significance may have been reached for post-exercise directional changes with a larger sample size (with n=5, p=0.14).

There were significant changes in mean plasma glycerol over time in both exercise tests (p<.001) as was seen for FFA, but more contrasts achieved significance with NS than with S (95% CI Bonferroni). Mean plasma glycerol levels showed the same pattern in both tests: plasma glycerol values were higher at Dx relative to rest (n=3) and continued to increase through post-exercise (n=3 or n=5), then declined to post60. Both NS and S showed a significant rise in plasma glycerol from pre-exercise to peak mean levels at post-exercise, and a significant decrease in plasma glycerol levels from post-exercise to post60. All individual plasma glycerol responses followed this pattern in each test (n=5). The magnitude of the mean plasma glycerol increase at post-exercise relative to pre-exercise was greater with NS (242% vs.155%), and the magnitude of the decrease in plasma glycerol from post-exercise to post60 was about the same in both trials (47%). In NS, a significant increase in plasma glycerol was also found from pre-exercise to Dx (136%) and from Dx to post-exercise (44%). All individual plasma glycerol levels with NS followed this pattern (n=3). Mean plasma glycerol levels in both trials remained elevated (not significantly) above pre-exercise at post60, with a larger difference occurring in NS (74% vs. 36%).

## Respiratory Exchange Ratios

Table 13 lists individual respiratory exchange ratios (R), mean R values and standard deviations for the nonsupplemented (NS) and vitamin B-6 supplemented (S) exercise

## Table 12. Plasma Glycerol Concentrations (mmol/L)

|  | Test | Pre1 | Pre2 | During | Post | Post60 |
|---|---|---|---|---|---|---|
| 1 | NS | 0.12 | 0.12 | 0.32 | 0.58 | 0.39 |
|  | S | 0.19 | 0.19 | 0.36 | 0.50 | 0.30 |
| 2 | NS | 0.19 | 0.19 |  | 0.70 | 0.30 |
|  | S | 0.25 | 0.19 |  | 0.49 | 0.24 |
| 4 | NS | 0.16 | 0.17 |  | 0.57 | 0.23 |
|  | S | 0.19 | 0.20 |  | 0.53 | 0.22 |
| 5 | NS | 0.28 | 0.27 | 0.62 | 0.71 | 0.38 |
|  | S | 0.22 | 0.20 | 0.41 | 0.38 | 0.24 |
| 6 | NS | 0.24 | 0.21 | 0.45 | 0.77 | 0.39 |
|  | S | 0.26 | 0.24 | 0.40 | 0.82 | 0.44 |
| Mean | NS | 0.20 | 0.19 | 0.46c | 0.67a,d | 0.34b |
|  |  | sd=0.06 | sd=0.05 | sd=0.15 | sd=0.09 | sd=0.07 |
|  |  | n=5 | n=5 | n=3 | n=5 | n=5 |
|  | S | 0.22 | 0.21 | 0.39 | 0.54a | 0.29b |
|  |  | sd=0.03 | sd=0.02 | sd=0.03 | sd=0.17 | sd=0.09 |
|  |  | n=5 | n=5 | n=3 | n=5 | n=5 |

Individual values are means of samples analyzed in duplicate. NS=nonsupplemented exercise test to exhaust.; S=B-6 supplemented exercise test to exhaustion. Pre1=0.5 hr before exercise; Pre2=before exercise; During=1.0 hour into exercise; Post=immediately after exercise; Post60=60 min after exercise; a & c=sig. diff. than rest; b=sig. diff. than Post; d=sig. diff. than During

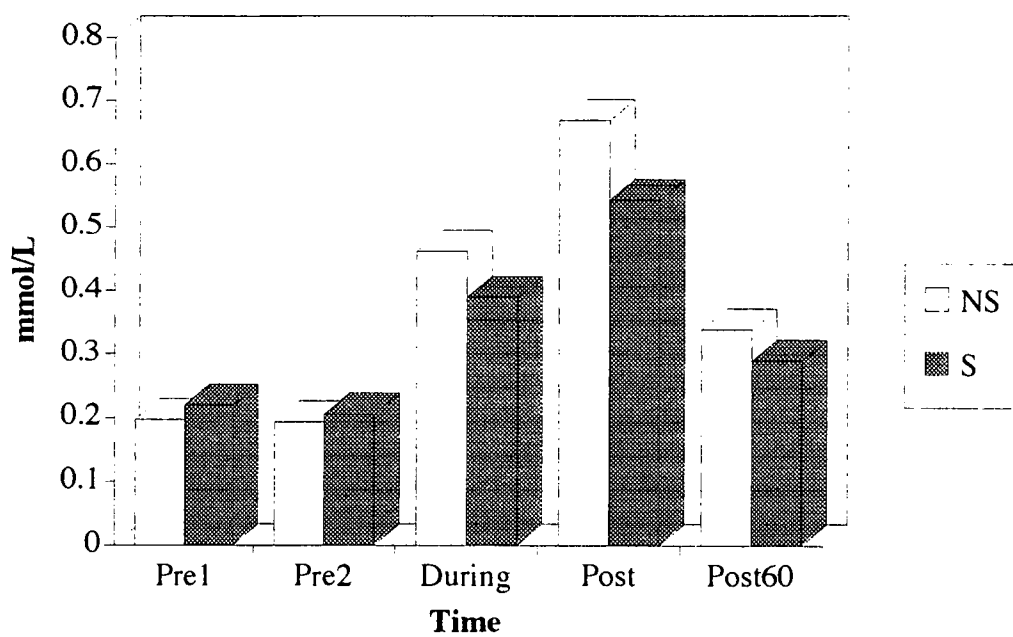**Figure 4. Plasma Glycerol Concentrations**

## Table 13. Respiratory Exchange Ratios (R)

| Subj/Test | Rest | 10 | 20 | 30 | 40 | 50 | 60 | 70 | 80 | 90 | 100 | 110 | 120 | 130 | 140 | 150 | 160 | 170 | 180 | 190 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **1** | | | | | | | | | | | | | | | | | | | | |
| NS | 0.85 | 0.97 | 0.94 | 0.93 | 0.91 | 0.95 | 0.95 | 0.96 | 0.93 | 0.94 | 0.91 | 0.90 | 0.93 | 0.90 | | | | | | |
| S | 0.89 | 0.95 | 0.96 | 0.96 | 0.94 | 0.95 | 0.94 | 0.95 | 0.94 | 0.92 | 0.91 | 0.90 | | | | | | | | |
| **2** | | | | | | | | | | | | | | | | | | | | |
| NS | 0.95 | 0.93 | 0.94 | 0.91 | 0.91 | 0.92 | | | | | | | | | | | | | | |
| S | 0.90 | 0.89 | 0.89 | | | | | | | | | | | | | | | | | |
| **4** | | | | | | | | | | | | | | | | | | | | |
| NS | 0.83 | 0.91 | 0.92 | 0.91 | 0.89 | 0.87 | | | | | | | | | | | | | | |
| S | 0.93 | 0.92 | 0.91 | 0.91 | 0.90 | 0.91 | | | | | | | | | | | | | | |
| **5** | | | | | | | | | | | | | | | | | | | | |
| NS | 1.03 | 0.86 | 0.86 | 0.86 | 0.89 | 0.87 | 0.85 | | 0.83 | 0.82 | | | | | | | | | | |
| S | 1.01 | 0.90 | 0.86 | 0.89 | 0.88 | 0.88 | 0.87 | 0.85 | 0.86 | | | | | | | | | | | |
| **6** | | | | | | | | | | | | | | | | | | | | |
| NS | 0.88 | 0.96 | 0.93 | 0.92 | 0.93 | 0.93 | 0.91 | 0.90 | 0.89 | 0.90 | 0.90 | 0.88 | 0.91 | 0.88 | 0.88 | | | | | |
| S | 0.96 | 0.97 | 0.98 | 0.98 | 0.98 | 0.97 | 0.99 | 0.95 | 0.93 | 0.93 | 0.90 | 0.92 | 0.92 | | 0.89 | 0.88 | 0.89 | 0.88 | 0.89 | 0.89 |
| **Mean** | | | | | | | | | | | | | | | | | | | | |
| NS | 0.91 | 0.93 | 0.92 | 0.91 | 0.91 | 0.91 | 0.90 | 0.93 | 0.88 | 0.89 | 0.9 | 0.89 | 0.92 | 0.89 | 0.88 | | | | | |
| sd | 0.08 | 0.04 | 0.03 | 0.03 | 0.02 | 0.04 | 0.0503 | 0.04 | 0.05 | 0.06 | 0.01 | 0.01 | 0.01 | 0.01 | | | | | | |
| | n=5 | n=5 | n=5 | n=5 | n=5 | n=5 | n=3 | n=2 | n=3 | n=3 | n=2 | n=2 | n=2 | n=2 | n=1 | | | | | |
| S | 0.94 | 0.93 | 0.92 | 0.93 | 0.92 | 0.93 | 0.93 | 0.92 | 0.91 | 0.92 | 0.9 | 0.91 | 0.92 | | 0.89 | 0.88 | 0.89 | 0.88 | 0.89 | 0.89 |
| sd | 0.05 | 0.03 | 0.05 | 0.04 | 0.04 | 0.0403 | 0.06 | 0.06 | 0.04 | 0.01 | 0.01 | 0.01 | | | | | | | | |
| | n=5 | n=5 | n=5 | n=4 | n=4 | n=4 | n=3 | n=3 | n=3 | n=2 | n=2 | n=1 | | | n=1 | n=1 | n=1 | n=1 | n=1 | n=1 |

NS=nonsupplemented exercise test to exhaustion; S=B-6 supplemented exercise test to exhaustion. All values were taken with subjects in the seated position.

Resting values are means of six values taken over approximately two min. immediately prior to exercise.

Exercise values are means of three-minute gas collection periods; the noted time is the third minute of the collection period.

tests. There were no significant differences in mean R values between the two tests at any of the time points recorded (i.e., up to and including time 110 min.). At two time points, the difference in mean R values between the trials approached significance (p=0.09): at 30 minutes of exercise (n=4) and at 80 minutes of exercise (n=3), mean R values were higher with S compared to NS. Most of the mean R values were somewhat higher with S versus NS: Of 12 time points compared, eight mean R values were higher (2-4%), including the resting value; one was slightly lower (1.4%); and three remained unchanged (difference <1%) between NS and S.

No significant change in the mean R value over time was found in either exercise test. In general, R values decreased over time in both tests, but the pattern of change varied somewhat. The slight difference in pattern variations over time with NS and S likely do not reflect an effect of PN supplementation, but rather the changing sample size as subjects reached exhaustion. For example, a different pattern from 10 to 30 minutes of exercise between the trials likely occurred due to exhaustion of subject 2 at 22 minutes with S. Subject 2 had a low exercising R value (0.89) relative to the other subjects at 10 and 20 minutes of exercise with S; when he dropped out after 20 minutes, a rise in mean R value was seen at 30 minutes.

## Heart Rate

Table 14 lists individual heart rates, mean heart rates and standard deviations for the nonsupplemented (NS) and vitamin B-6 supplemented (S) exercise tests. Figure 5 provides a graphical comparison of the mean heart rates. There were no significant differences in mean heart rates between the two tests at any of the time points recorded. There was no consistent pattern of increase or decrease in heart rate with S compared to NS.

As expected, there was a significant change in mean heart rate over time in both exercise tests (p<.001). The pattern was the same in both tests, with heart rate increasing about 95 beats/min. from resting to 15 minutes of exercise (i.e., from about 57 beats/min. to 150 beats/min.) then basically leveling off throughout the remainder of exercise with a slight increase near exhaustion (approx. 5 beats/min), and decreasing post-exercise to about twice the resting value. Statistical significance (95% CI Bonferroni) was reached in both tests for comparisons between resting heart rate and exercising heart rate as well as resting heart rate versus post-exercise heart rate; and between post-exercise heart rate and

## Table 14. Heart Rates (beats/min.)

| Subj Test | Rest | Warm | Minutes 5 | 15 | 25 | 35 | 45 | 55 | 65 | 75 | 85 | 95 | 105 | 115 | 125 | 135 | 145 | 155 | 165 | 175 | 185 | Final | Post |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **1** | | | | | | | | | | | | | | | | | | | | | | | |
| NS | 47 | 77 | 136 | 136 | 132 | 134 | 132 | 132 | 134 | 136 | 138 | 145 | 145 | 150 | 148 | | | | | | | 141 | 93 |
| S | 44 | 80 | 129 | 136 | 136 | 136 | 134 | 136 | 137 | 140 | 150 | 150 | 141 | | | | | | | | | 148 | 93 |
| **2** | | | | | | | | | | | | | | | | | | | | | | | |
| NS | 47 | 93 | 153 | 158 | 167 | 167 | 167 | | | | | | | | | | | | | | | 171 | 92 |
| S | 50 | 93 | 145 | 158 | | | | | | | | | | | | | | | | | | 155 | 86 |
| **4** | | | | | | | | | | | | | | | | | | | | | | | |
| NS | 75 | 103 | 150 | 158 | 167 | 167 | 170 | | | | | | | | | | | | | | | 173 | 115 |
| S | 81 | 110 | 155 | 161 | 164 | 167 | 173 | 173 | | | | | | | | | | | | | | 176 | 127 |
| **5** | | | | | | | | | | | | | | | | | | | | | | | |
| NS | 53 | 103 | 145 | 141 | 150 | 148 | 143 | 167 | 145 | | | 150 | | | | | | | | | | 150 | 115 |
| S | 50 | 90 | 138 | 138 | 145 | 143 | 153 | 145 | 145 | 150 | | | | | | | | | | | | 148 | 105 |
| **6** | | | | | | | | | | | | | | | | | | | | | | | |
| NS | 65 | 95 | 155 | 155 | 158 | 161 | 164 | 164 | 161 | | | 167 | 167 | 173 | 173 | | 180 | 180 | | | | 176 | 132 |
| S | 65 | 103 | 158 | 161 | 161 | | 161 | | 167 | 164 | 164 | 167 | 167 | 167 | | 176 | 173 | 184 | 184 | 180 | 180 | 187 | 134 |
| **Mean** | | | | | | | | | | | | | | | | | | | | | | | |
| NS | 57 | 94 | 148 | 150 | 155 | 155 | 155 | 154 | 147 | 136 | 152 | 156 | 159 | 161 | 148 | 180 | 180 | | | | | 155 | 106 |
| sd | 12.3 | 10.6 | 7.6 | 10.4 | 14.6 | 14.3 | 16.7 | 19.4 | 13.6 | | 14.6 | 15.6 | 19.8 | 16.3 | | | | | | | | 14.8 | 21.0 |
| | n=5 | n=5 | n=5 | n=5 | n=5 | n=5 | n=5 | n=3 | n=3 | n=1 | n=3 | n=2 | n=2 | n=2 | n=1 | n=1 | n=1 | | | | | n=5 | n=5 |
| S | 58 | 95 | 145 | 151 | 151 | 149 | 155 | 151 | 150 | 151 | 157 | 158 | 154 | 167 | | 176 | 173 | 184 | 184 | 180 | 180 | 163 | 111 |
| sd | 15.0 | 11.6 | 12.0 | 12.7 | 13.3 | 16.3 | 16.4 | 19.3 | 15.5 | 12.0 | 9.9 | 12.0 | 18.4 | | | | | | | | | 19.3 | 17.3 |
| | n=5 | n=5 | n=5 | n=5 | n=4 | n=3 | n=4 | n=3 | n=3 | n=3 | n=2 | n=2 | n=2 | n=1 | | n=1 | n=1 | n=1 | n=1 | n=1 | n=1 | n=5 | n=5 |

NS=nonsupplemented exercise test to exhaustion; S=B-6 supplemented exercise test to exhaustion. All values were taken with subjects in the seated position.
Individual resting values are means of five or six values; warmup and exercise values are means of three values.
Final exercising HR fell between the last recorded time point and the next designated time point. Post is the mean of three values immediately after exercise.

# Figure 5. Heart Rates
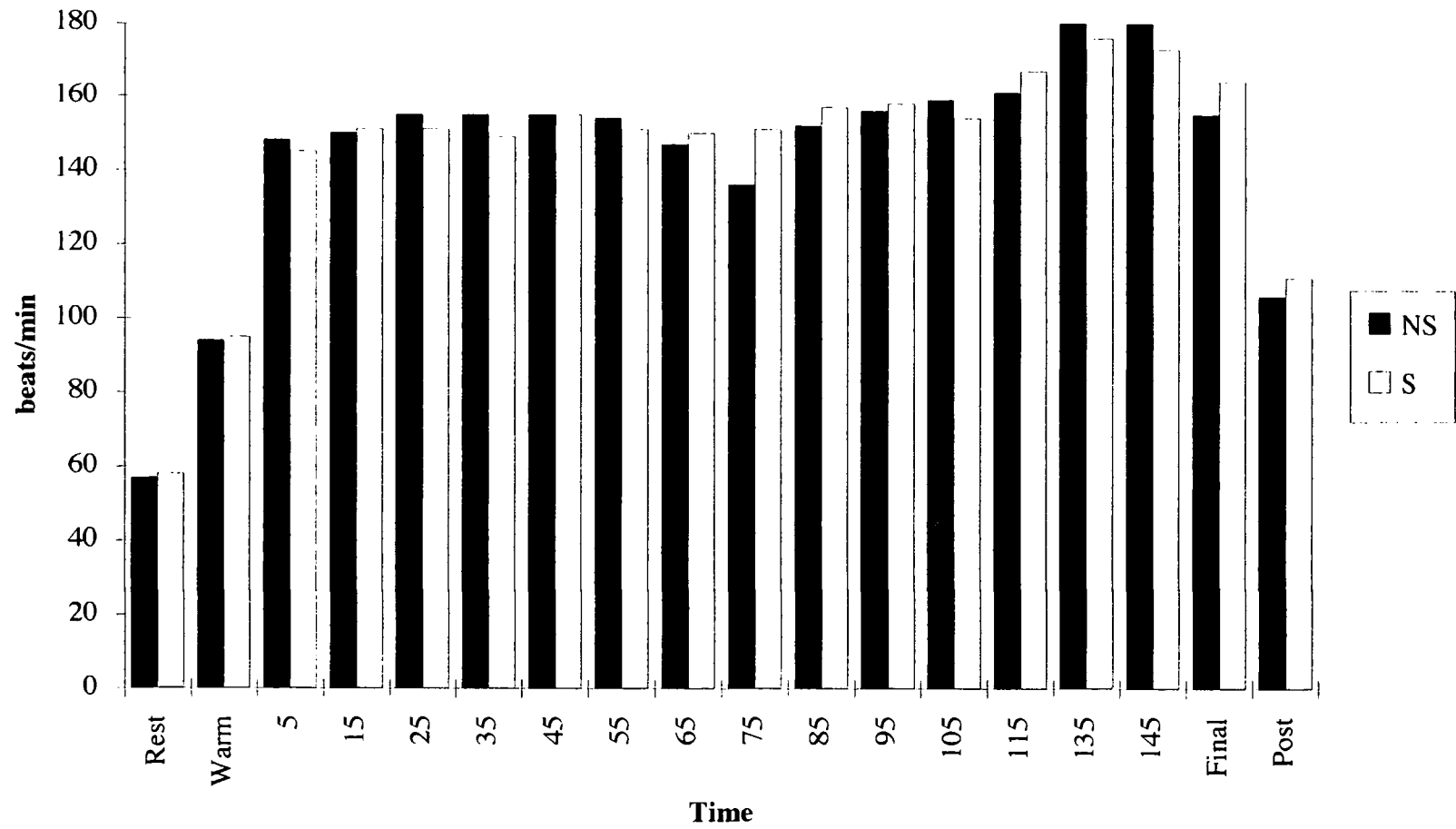
exercising values. Not surprisingly, all individual heart rates showed this pattern. More contrasts between post-exercise and exercising heart rate achieved significance with NS compared to S (12 of 16 time points versus 6 of 16 time points), suggesting a greater magnitude of change in heart rate from exercise to post-exercise with NS. The difference in number of significant points between the trials likely is due to the lower standard error calculated for NS.

## Oxygen Consumption

Table 15 lists individual oxygen consumption ($VO_2$) values, mean $VO_2$ values and standard deviations for the nonsupplemented (NS) and vitamin B-6 supplemented (S) exercise tests. There was one significant increase (p=0.016) in $VO_2$ at 10 minutes into exercise (n=5) from NS to S. Most of the mean exercising $VO_2$ values were higher with S: Of 11 time points compared during exercise, nine mean $VO_2$ values were higher (3-11%) and two remained unchanged (difference <1%) when comparing S to NS. The p values at five exercise time points appeared to be approaching significance: 30 minutes of exercise (n=4, p=0.18), 40 minutes of exercise (n=4, p=0.13), 50 minutes of exercise (n=4, p=0.10) and 60 minutes of exercise (n=3, p=0.072). In contrast to the general increase in $VO_2$ during exercise after PN supplementation, the mean resting $VO_2$ was lower (17%) in S than in NS. Statistical comparison of resting $VO_2$ between the trials appeared to approach significance (n=5, p=0.12).

As expected, a significant change in the mean $VO_2$ response over time was found for both exercise tests (p<.001). Resting values in both tests were significantly different than all exercising $VO_2$ values. In general, the mean as well as individual trend in both tests involved a dramatic increase in $VO_2$ from resting to 10 minutes into exercise, then a fairly constant $VO_2$ until a modest rise during the final two-three time points. The pattern and magnitude of these changes were somewhat different between the two trials, which likely reflect the changing sample size as subjects reached exhaustion. For example, as with the variation in R values, the different pattern observed to 30 minutes reflects a reduced sample size at 30 minutes with S compared to NS. Subject 2 had a high exercising $VO_2$ value (50 ml/kg/min) relative to the other subjects at 10 and 20 minutes of exercise with S; when he dropped out after 20 minutes, a decrease in mean R value was seen at 30 minutes.

## Table 15. Oxygen Consumption (ml/kg/min)

| Subj / Test | Rest | Minutes 10 | 20 | 30 | 40 | 50 | 60 | 70 | 80 | 90 | 100 | 110 | 120 | 130 | 140 | 150 | 160 | 170 | 180 | 190 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **1** | | | | | | | | | | | | | | | | | | | | | |
| NS | 5.49 | 40.34 | 39.69 | 39.50 | 39.62 | 39.80 | 39.48 | 41.21 | 41.56 | 41.94 | 43.25 | 41.12 | 43.78 | 43.57 | | | | | | |
| S | 3.83 | 43.86 | 43.44 | 43.62 | 42.84 | 45.63 | 46.25 | 47.21 | 49.43 | 47.98 | 47.74 | 47.09 | | | | | | | | |
| **2** | | | | | | | | | | | | | | | | | | | | | |
| NS | 4.49 | 46.21 | 47.88 | 47.29 | 46.89 | 46.79 | | | | | | | | | | | | | | |
| S | 4.88 | 50.05 | 49.93 | | | | | | | | | | | | | | | | | |
| **4** | | | | | | | | | | | | | | | | | | | | | |
| NS | 4.49 | 33.84 | 34.68 | 33.0 | 34.50 | 32.49 | | | | | | | | | | | | | | |
| S | 3.76 | 35.6 | 36.03 | 35.45 | 36.63 | 37.27 | | | | | | | | | | | | | | |
| **5** | | | | | | | | | | | | | | | | | | | | | |
| NS | 6.15 | 36.68 | 36.49 | 37.78 | 37.07 | 37.63 | 36.69 | | 35.90 | 37.03 | | | | | | | | | | |
| S | 5.16 | 37.87 | 38.44 | 37.02 | 37.85 | 37.99 | 40.57 | 38.58 | 38.43 | | | | | | | | | | | |
| **6** | | | | | | | | | | | | | | | | | | | | | |
| NS | 6.16 | 39.12 | 40.10 | 39.26 | 40.25 | 39.71 | 39.32 | 40.14 | 41.06 | 40.77 | 40.64 | 40.98 | 42.62 | 42.53 | 42.31 | | | | | |
| S | 5.81 | 40.30 | 39.13 | 40.59 | 40.15 | 41.00 | 41.86 | 40.42 | 40.14 | 40.53 | 41.28 | 41.38 | 41.70 | | 42.27 | 41.98 | 43.77 | 42.58 | 43.14 | 43.73 |
| **Mean** | | | | | | | | | | | | | | | | | | | | | |
| NS | 5.36 | 39.34* | 39.77 | 39.37 | 39.67 | 39.28 | 38.50 | 40.67 | 39.51 | 39.91 | 41.94 | 41.05 | 43.20 | 43.05 | 42.31 | | | | | |
| sd | 0.84 | 4.62 | 5.06 | 5.14 | 4.64 | 5.14 | 1.57 | 0.76 | 3.13 | 2.56 | 1.84 | 0.10 | 0.82 | 0.73 | | | | | | |
| | n=5 | n=5 | n=5 | n=5 | n=5 | n=5 | n=3 | n=2 | n=3 | n=3 | n=2 | n=2 | n=2 | n=2 | n=1 | | | | | |
| S | 4.69 | 41.54 | 41.39 | 39.17 | 39.37 | 40.50 | 42.89 | 42.07 | 42.67 | 44.25 | 44.51 | 44.23 | 41.70 | | 42.27 | 41.98 | 43.77 | 42.58 | 43.14 | 43.73 |
| sd | 0.88 | 5.66 | 5.47 | 3.66 | 2.74 | 3.80 | 2.98 | 4.55 | 5.91 | 5.27 | 4.57 | 4.04 | | | | | | | | |
| | n=5 | n=5 | n=5 | n=4 | n=4 | n=4 | n=3 | n=3 | n=3 | n=2 | n=2 | n=2 | n=1 | | n=1 | n=1 | n=1 | n=1 | n=1 | n=1 |

NS=nonsupplemented exercise test; S=B-6 supplemented exercise test. All values were taken with subjects seated on the cycle ergometer.
Resting values are means of six values taken over approximately two minutes immediately prior to exercise.
Exercise values are means of three-minute gas collection periods; the noted time is the third minute of the collection period. *Significant difference from S value

## Kilocalorie Intake, Exercise Times, Pre- and Post-Exercise Body Weights

Table 16 lists individual kilocalorie (kcal) intake, mean kilocalorie intake and standard deviations for the nonsupplemented (NS) and vitamin B-6 supplemented (S) diet periods. There was no significant difference in kcal intake between the two periods, nor between any of the kcal intakes among all the kcal intakes (supplemented and nonsupplemented). The mean kcal intake did not change between the two periods (percent difference <0.5%).

Table 17 lists individual times to exhaustion, mean exercise durations and standard deviations for the nonsupplemented (NS) and vitamin B-6 supplemented (S) exercise tests. There was no significant difference in mean exercise time between the two periods, nor between any of the exercise times among all the times (supplemented and nonsupplemented). The mean exercise time decreased somewhat (3%) with S compared to NS, but individual directional changes in exercise time varied: three individuals had decreased exercise times (12-58%), and two individuals exercised longer (7-30%) with S versus NS. Omitting the data from the subject (subj2) who had a 58% difference in exercise time between the trials does not result in any significant difference in mean exercise times between NS and S; it does, however, result in a 4% increase in mean exercise time from NS to S.

Table 17 lists individual pre- and post-exercise body weights, mean body weights and standard deviations for the nonsupplemented (NS) and vitamin B-6 supplemented (S) exercise tests. There was no significant difference in mean pre-exercise body weights, mean post-exercise body weights nor the difference of pre- and post-exercise body weights between the two exercise tests. Note that only three pairs of values were available to perform the post-exercise comparisons. Of these three pairs, one subject showed no change in pre- or post-exercise weights (<.5%); one subject showed a slight decrease in pre-and post-exercise weights (1%); and one subject showed a slight increase in pre-and post-exercise weight during the supplemented versus the nonsupplemented tests. For the two subjects with only paired (nonsupplemented and supplemented) values for pre-exercise, there was no change in body weight between S and NS.

There was no significant change in mean body weight from pre-exercise to post-exercise in NS. There was no significant change in mean body weight from pre- to post-exercise with S regardless of whether three values or five values were used for the post-exercise mean calculation. There was a slight decrease (2%) in mean body weight from pre-to post-exercise in S (n=3) and NS (n=5). All individual body weights also decreased from pre- to post-exercise in NS and S.

## Table 16. Kilocalorie Intake During B-6 Supplemented and Unsupplemented Diets

| Subject | Diet Period | Mean Kcals | Std. Deviation |
|---|---|---|---|
| 1 | NS | 3944 | 235 |
|  | S | 4171 | 490 |
|  |  |  |  |
| 2 | NS | 3766 | 131 |
|  | S | 3544 | 93.5 |
|  |  |  |  |
| 4 | NS | 3816 | 198 |
|  | S | 3810 | 196 |
|  |  |  |  |
| 5 | NS | 3973 | 184 |
|  | S | 3993 | 139 |
|  |  |  |  |
| 6 | NS | 3724 | 211 |
|  | S | 3637 | 310 |
|  |  |  |  |
| Group (n=5) | NS | 3845 | 109 |
|  | S | 3831 | 256 |
|  |  |  |  |
| NS=nonsupplemented diet period I; S=B-6 supplemented diet period II. | | | |
| Mean Kcals for each period were averaged from nine daily values. | | | |

**Table 17. Body Weights (kg) and Exercise Times to Exhaustion (min)**

| Subject | Test | | Pre Weight | | Post Weight | Time |
|---------|------|---|-----------|------|-------------|------|
| 1 | NS | | 80.9 | | 78.4 | 130.0 |
| | S | | 80.9 | | | 110.3 |
| | | | | | | |
| 2 | NS | | 74.1 | | 72.7 | 53.6 |
| | S | | 74.3 | | | 22.3 |
| | | | | | | |
| 4 | NS | | 105.4 | | 103.9 | 52.3 |
| | S | | 104.1 | | 102.5 | 55.8 |
| | | | | | | |
| 5 | NS | | 76.4 | | 75.2 | 92.0 |
| | S | | 76.4 | | 74.5 | 80.9 |
| | | | | | | |
| 6 | NS | | 85.4 | | 82.0 | 146.1 |
| | S | | 86.6 | | 82.9 | 190.3 |
| | | | | | | |
| Mean | NS | | 84.4 | | 82.4 | 94.8 |
| | | sd | 12.5 | | 12.5 | 42.9 |
| | | n | 5 | | 5 | 5 |
| | S | | 89.0 | 84.5 | 86.6 | 91.9 |
| | | sd | 14.0 | 31.6 | 12.4 | 84.0 |
| | | n | 3 | 5 | 3 | 5 |
| | | | | | | |
| | | | | | | |
| NS=nonsupplemented exercise test; S=B-6 supplemented exercise | | | | | | |
| Pre Weight=before exhaustive exercise; Post Weight=after exhaustive exercise | | | | | | |
| Time=duration of exercise to exhaustion | | | | | | |

# DISCUSSION

This study was undertaken to determine the relationship between vitamin B-6 supplementation and the changes in plasma catecholamine concentrations during exhaustive endurance exercise. Previous exercise studies have examined the response of plasma catecholamines during exercise (Hartley et al., 1972a & b; Galbo et al., 1975; Rogers et al., 1991; Young et al., 1992; Hagberg et al., 1979; Hall et al., 1983; O'Brien et al., 1993; Friedmann and Kindermann, 1989; Farrell et al., 1987; Nieman et al., 1993). Research has also examined the response of plasma PLP during exercise in vitamin B-6 supplemented and nonsupplemented states (deVos et al., 1983; Manore et al, 1987a; Manore and Leklem, 1988; Dunton et al, 1992; Leklem and Shultz, 1983). The present study was the first to examine both the plasma PLP response and the plasma catecholamine response to endurance exercise in the vitamin B-6 supplemented and nonsupplemented conditions.

The primary strength of this study compared to previous research on catecholamines and exercise lies in the strict control of the subjects' dietary intake. Subjects consumed a controlled diet for six days prior to each exercise test, wherein protein and micronutrients involved in catecholamine synthesis (e.g., vitamin B-6, ascorbic acid, niacin) were held constant. Kilocalorie intake was adjusted with simple carbohydrate or fat, and the amount of energy consumed in the two dietary periods did not differ significantly from one another. Subjects were non-smokers and abstained from caffeine the day before and the day of the exercise tests. Most of the previous studies of catecholamines and exercise that reported absolute catecholamine measurements have called for a graded workload to maximal effort (Hartley et al., 1972a; Galbo et al., 1975; Rogers et al., 1991; Young et al., 1992). Two studies that examined plasma catecholamine responses during prolonged submaximal exercise (Hartley et al, 1972b; Galbo et al., 1975) are widely referred to in the catecholamine and exercise literature. However, as previously discussed, the Hartley et al. (1972b) study had some methodological problems that make this author question the accuracy of the reported catecholamine measurements. The current study used an exhaustive, submaximal exercise protocol with the goal of elucidating the response of plasma catecholamines under these conditions. In addition, the investigators in this study used well-accepted methods of analyses with good standardization and quality control.

The hypothesis of this research relied on the role of PLP in catecholamine synthesis (Berne and Levy, 1988; Zubay, 1988). Hence, vitamin B-6 supplementation

over time would be expected to increase the availability of PLP to DOPA decarboxylase in the tissues, as would the elevated rise in plasma PLP seen during exercise after supplementation (deVos, 1982; Manore et al., 1987a; Manore and Leklem, 1988; Dunton et al., 1992). In addition, it has been proposed that an acute injection of PL stimulates the release of plasma catecholamines in rats (Lau-Cam, 1991). This suggests that the rise in plasma PLP concentration during exercise enhances catecholamine release, and a greater rise in plasma PLP levels during exercise with supplementation compared to nonsupplementation would augment this effect. Further objectives of the study attempted to determine if the hypothesized increase in plasma catecholamine concentrations with PN supplementation would, in turn, influence fuel use and heart rate during exercise, and oxygen consumption at rest.

This discussion will attempt to elucidate any difference in the response of various plasma constituents and physiological parameters between the vitamin B-6 supplemented state and the nonsupplemented state. Toward this end, comparisons of these responses during nonsupplementation and supplementation at specific time points and comparisons of the pattern of response between each condition across time will be covered.

Where percent differences between values of a given plasma constituent were small, it was suspected that the observed difference might be a result of plasma volume changes and not actual changes in the concentrations of a plasma contituent. In this case, observed values were compared to predicted values based soley on plasma volume changes. Predicted values for 60 minutes of exercise, post-exercise and post60 were calculated by dividing the pre-exercise value by the volume adjusted for the plasma volume changes at the specific time point. For example, the calculated percent plasma volume change post-exercise in the supplemented trial was -15.4%. Plasma epinephrine concentration was reported in pmol/mL. Taking into account the percent plasma volume change, the adjusted volume would be 0.84 mL [1 mL - (1 mL x 0.15)]. Dividing the pre-exercise plasma epinephrine value from the supplemented period by the adjusted volume provides the predicted plasma epinephrine concentration at post-exercise caused by plasma volume change (0.35 pmol/0.84 mL=0.41 pmol/mL). This value can be compared to the observed value to provide an indication of the actual change in the concentration of a plasma constituent. In this example, the observed plasma epinephrine value at post-exercise during supplementation was 0.63 pmol/mL so it is not likely that the entire increase in concentration at post-exercise above rest was caused by plasma volume changes.

Predictions of plasma concentrations of variables from plasma volume changes may only be used as a guideline since they are based on measurements and calculations

that may contain error. Further, corrections for plasma volume changes were not made or examined in the exercise literature reviewed for this discussion. Quantitative percent PVC results from the two calculation methods (Dill and Costill, 1974; Van Beaumont, 1972) varied somewhat, and the difference in percent PVC between the trials at post-exercise only achieved significance with the Dill and Costill method. The calculation methods are based on differing assumptions regarding RBC mass and it is unclear which assumption is more accurate. Aside from calculation methods, error in percent PVC may be introduced from variability in Hb and Hct measurements, upon which the percent PVC calculations are based. In particular, although mean Hct and Hb values were higher during S versus NS at Dx, post-exercise and post60, individual subjects did not all experience greater observed plasma volume changes during the supplemented trial.

As with time point comparisons of percent PVC between the trials, error may be introduced to comparisons of percent PVC over time in each trial due to variations in Hb and Hct measurements and standard deviations in mean calculations. For example, Hb and Hct levels would not be expected to change during rest. However, some measured individual Hct and Hb values changed (2-3%) from pre1 to pre2 in both trials. These differences in experimental variation between the trials are small, but must be taken into account when viewing plasma volume changes, particularly since percent PVC calculations are based on mean pre-exercise values.

Predictions of mean norepinephrine and epinephrine values based on mean pre-exercise values appear to be erroneous, as complete pre-exercise data were not detected for either catecholamine. However, calculating predicted values for each individual based on his plasma volume changes did not alter the direction of change in plasma catecholamine concentration between the trials. Plasma volume changes may have influenced the magnitude of percent differences, but this author cannot quantitate these differences.

For all plasma variables examined, it is problematic to compare mean plasma volume changes from Dx (n=3) to post-exercise (n=5). The mean percent PVC for each trial would indicate a decrease in plasma volume between these points. However, the three individuals who attained the 60-minute exercise point actually showed an increased plasma volume from Dx to post-exercise in both trials. Hence, plasma volume changes could not account for the observed changes in plasma substrate concentrations between these time points.

Given the above complications involved with predicting plasma substrate concentration changes based soley upon calculated plasma volume changes, this author has chosen to refer to plasma volume changes as a possible influence on observed results when applicable rather than report all predicted values calculated. It is useful to look at

plasma volume changes when comparing the changes in a particular plasma substrate concentration between the trials. However, plasma volume changes are irrelevant when comparing the pattern of two different plasma constituents, as the predictions assume plasma volume changes similarly effect the observed concentrations of all substances in the plasma.

As expected, plasma PLP was significantly higher (80%) with vitamin B-6 supplementation compared to nonsupplementation at all time points measured (i.e., pre-exercise, 60 minutes of exercise, post-exercise and 60 minutes post-exercise). Dunton et al (1992) reported similar results for a previous study in this laboratory that followed basically the same protocol as the present study. Absolute PLP values during the supplemented period were higher at all time points in the Dunton study (e.g., 199 nmol/L vs 173 nmol/L pre-exercise; 261 nmol/L vs 188 nmol/L at 60 minutes of exercise). The pattern of plasma PLP change during exercise in the present study concurred with the Dunton et al. results: PLP concentrations increased from pre-exercise to 60 minutes into exercise then began to decline, with post60 PLP levels attaining lower levels than resting values. The basic differences in PLP response between the studies involves: a plasma PLP increase of about 5% more in the Dunton study versus the current research after supplementation at all time points, and a greater fluctuation in plasma PLP concentration during supplementation across time and less fluctuation during nonsupplementation across time in the Dunton research than in the current study.

It was expected that with vitamin B-6 supplementation, the plasma catecholamine concentrations would be higher at all time points, especially during exercise, than in the nonsupplemented state. Comparison of plasma norepinephrine concentrations between the trials at pre-exercise and post-exercise (exhaustion), but not 60 minutes of exercise or post60, may support the hypothesis of vitamin B-6 supplementation enhancing norepinephrine synthesis. The mean resting plasma norepinephrine level was lower (13%) during the supplemented compared to the nonsupplemented period, but two of the three individuals for whom paired resting levels were measurable actually showed higher resting plasma norepinephrine concentrations with supplementation. This suggests vitamin B-6 increased basal norepinephrine synthesis in these two individuals (subj2 and subj4). At post-exercise, three of five individuals, including two who attained the 60-minute exercise point, had a higher plasma norepinephrine concentration after supplementation. This suggests vitamin B-6 supplementation enhanced the plasma norepinephrine response during exhaustive exercise. However, duration of exercise must be considered and will be discussed below.

Like the norepinephrine results, pre-exercise plasma epinephrine comparisons between the trials suggest that vitamin B-6 supplementation enhanced basal epinephrine synthesis. The mean resting plasma epinephrine (pre2, n=4) was higher (30%) during the supplemented compared to the nonsupplemented period, and three of the four subjects with paired data for pre2 followed this trend. Post60 plasma epinephrine comparisons between the trials suggest vitamin B-6 supplementation enhanced the plasma epinephrine response during recovery from exhaustive exercise. The mean post60 plasma epinephrine concentration (n=4) was higher (15%) during the supplemented compared to the nonsupplemented trial, and three of four individuals followed this trend. Comparison of plasma epinephrine concentrations between the trials at 60 minutes of exercise and post-exercise do not support the theory of vitamin B-6 supplementation enhancing the plasma epinephrine response during exercise. At Dx, there was no consistent trend in individual plasma epinephrine response between the trials; at post-exercise, three of five individuals actually had lower plasma epinephrine concentrations while two had higher plasma epinephrine concentrations during the supplemented versus the nonsupplemented trial.

Data generated in another portion of this study (Virk, 1994) showed that plasma tyrosine tended to be lower at all time points during the supplemented versus the nonsupplemented trial. The post60 data resulted in a statistically significant difference between the two trials. Recall that tyrosine is the precursor to dopamine synthesis. Lower plasma tyrosine levels during the vitamin B-6 trial may indicate greater synthesis of catecholamines in the supplemented state (Benedict et al., 1983). Indeed, plasma epinephrine concentration was higher at post60 during supplementation, although the difference did not achieve statistical significance.

Duration of exercise seemed to have a more decisive influence on post-exercise and post60 plasma norepinephrine and epinephrine levels than the state of supplementation. At all individual post-exercise points in both trials but one, the individual plasma norepinephrine concentration after exercise was higher when the duration of exercise was greater, regardless of the trial. One subject (subject 1) did show an increased plasma norepinephrine concentration at exhaustion during the supplemented trial compared to the nonsupplemented trial (4.71 pmol/mL vs 4.27 pmol/mL), even though he exercised longer during the nonsupplemented trial (130 min. vs 110 min.). The direction of change in plasma epinephrine concentration between the trials at post-exercise coincided with the longest duration of exercise regardless of the supplementation status (i.e., higher plasma epinephrine was seen with greater duration of exercise). At post60, all five subjects had a greater level of plasma norepinephrine after exercise of the longest duration, regardless of supplementation status. Three of four subjects with paired data at post60 had higher

plasma epinephrine levels when the duration of exercise was longer, regardless of supplementation status. One subject (subject 1) did show a somewhat higher (5%) plasma epinephrine concentration at post60 during the supplemented trial compared to the nonsupplemented trial, even though he exercised longer during the nonsupplemented trial.

In addition to the confounding variable of exercise duration, post-exercise plasma catecholamine measurements also may be problematic since the metabolic clearance rate of plasma norepinephrine is reported to be 2.0 to 4.0 L/minute, and the metabolic clearance rate of plasma epinephrine is reported to be 3.5 to 6.0 L/minute (Berne and Levy, 1988). Further, plasma norepinephrine levels (Young et al., 1992; Hagberg et al., 1979) and plasma epinephrine levels (Young et al., 1992) have been found to increase in the minute following exercise. Hence, timing of the blood draw immediately after exercise plays a crucial role in obtaining consistent catecholamine measurements. The elapsed time after exercise before the post-exercise blood draw in the current research was not tightly controlled, but taken quickly after the subject dismounted from the cycle.

The mean resting plasma norepinephrine levels reported in this study both before and after PN supplementation are somewhat lower than the values reported by other exercise researchers (Galbo et al., 1975; Rogers et al., 1991; Young et al., 1992; Hagberg et al., 1979) for young men: resting mean plasma norepinephrine ranged from 0.69 pmol/mL to 0.88 pmol/mL in this study, but from 1.4 pmol/mL to 3.3 pmol/mL in the aforementioned studies, excluding Hartley et al (1972a & b) (Table 18). Other researchers have reported resting plasma norepinephrine concentrations of 1.0 to 2.0 pmol/mL (Trendelenburg and Weiner, 1989; Landsberg and Young, 1992; Berne and Levy, 1988). Only one subject showed resting norepinephrine levels in the range reported by other researchers: Subject 1 had resting norepinephrine levels in the nonsupplemented trial of 1.14 pmol/mL and 1.71 pmol/mL. Peak plasma norepinephrine levels achieved in this study are also lower than plasma norepinephrine levels reached at 40 minutes of treadmill exercise at 76% $VO_2$ max (Galbo et al., 1975), at the end of submaximal exercise to exhaustion (Galbo et al., 1987) and at the end of maximal exercise (Rogers et al., 1991; Young et al., 1992; Hagberg et at., 1979; Galbo et al., 1975). Post60 plasma norepinephrine values cannot be compared to the literature as no reliable studies have reported absolute norepinephrine values for this time point. However, plasma norepinephrine concentrations of 6.3 pmol/mL (Young et al., 1992) 10 minutes after maximal exercise, 2.3 pmol/mL (Galbo et al., 1975) 30 minutes after maximal exercise and 2.9 pmol/mL (Galbo et al., 1975) 30 minutes after exhaustive exercise have been reported. The mean post60 plasma norepinephrine concentrations in the current study were 1.16 pmol/mL and 1.25 pmol/mL after the nonsupplemented and supplemented

# Table 18. Plasma Catecholamine Concentrations (pmol/mL)-Literature Values

| Reference | n | VO2 max (ml/kg/min) | age (years) | mode | type | duration (min.) | intensity (% VO2max) | norepinephrine concentration pre | during | end | post |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Hartley, 1972a | 7 | 47.9 | 20-24 | cycle | graded | 21 | 42-98% VO2max | 8.8 | 10.6-13.5 | 20(max) | 10(post60) |
| Hartley, 1972b | 7 | 47.9 | 20-24 | cycle | exhaustive | 96 | 73% | 12.0 | 17.1(40 min.) | 17.7 | 11.2(post60) |
| Galbo, 1975 | 8 | 54.7 | 20-28 | run | graded | 24 | 47-100% | 2.3 | 2.6-7.6 | 13(max) | 2.3(post30) |
| Galbo, 1975 | 8 | 54.7 | 20-28 | run | exhaustive | 81 | 76% | 2.0 | 8.5(40 min.) | 8.9 | 2.9(post30) |
| Rogers, 1991 | 10 | 55.2 | 33±7 | cycle & run | graded | | 52-100% | 3.3 | 5.3(4 min.) | 34.5(max) | |
| Young, 1992 | 13 | | 24-34 | run | graded | 14 | | 2.9 | | 16.5(max) | 6.3(post10) |
| Hagberg, 1979 | 5 | 52.4 | 28-34 | cycle | graded | 5 | 100% | 1.4 | | 23.5 | |
| | | | | | | | | epinephrine concentration | | | |
| Hartley, 1972a | | | | | | | | 0.11-0.16 | 0.38-0.60 | 2.3(max) | 0.5 (post60) |
| Hartley, 1972b | | | | | | | | 1.1-0.55 | 1.6(40 min.) | 4.4 | 0.11 (post60) |
| Galbo, 1975 | | | | | | | | 0.38 | 0.38-0.66 | 2.3(max) | 0.38 (post30) |
| Galbo, 1975 | | | | | | | | 0.27 | 1.31(40 min.) | 1.8 | 0.6 (post30) |
| Rogers, 1991 | | | | | | | | 0.49 | 0.93(4 min) | 5.6(max) | |
| Young, 1992 | | | | | | | | 0.34 | | 2.7(max) | 0.56(post10) |

All subjects were male. Mode=exercise mode: run=treadmill, cycle=cycle ergometer. Type=exercise type. Pre=before exercise; During=during exercise; end=at exhaustion or maximal effort; post=during recovery (time noted)

trials, respectively. These values appear reasonable compared to the literature, provided that plasma norepinephrine levels continue to decline from 30 minutes of recovery to 60 minutes. The post60 plasma norepinephrine levels in this study were elevated above resting values, but were within the range of resting values reported by other researchers (Trendelenburg and Weiner, 1989; Landsberg and Young, 1992; Berne and Levy, 1988).

The mean resting plasma epinephrine levels reported in this study are in line with the values reported by other exercise researchers (Galbo et al., 1975; Rogers et al., 1991; Young et al., 1992) for young men: resting mean plasma epinephrine ranged from 0.26 pmol/mL to 0.37 pmol/mL in this study, and from 0.27 pmol/mL to 0.49 pmol/mL in the aforementioned studies, excluding Hartely et al (1972a & b) (Table 18). Other researchers have reported resting plasma epinephrine concentrations of 0.10 to 0.25 pmol/mL (Trendelenburg and Weiner, 1989; Landsberg and Young, 1992; Berne and Levy, 1988). Peak plasma epinephrine levels achieved in this study are lower than plasma epinephrine levels reached at 40 minutes of treadmill exercise at 76% $VO_2$ max (Galbo et al., 1975), at the end of submaximal exercise to exhaustion (Galbo et al, 1975) and at the end of maximal exercise (Rogers et al., 1991; Young et al., 1992; Galbo et al., 1975). The post-30 value reported by Galbo et al. (1975) after exhaustive exercise (0.60 pmol/mL) is similar to the mean peak plasma epinephrine level found in the current study at exhaustion, which were 0.62 pmol/mL and 0.63 pmol/mL during the nonsupplemented and supplemented trials, respectively. Post60 plasma epinephrine values cannot be compared to the literature, as again no reliable studies have reported absolute epinephrine values for this time point. However, plasma epinephrine concentrations of 0.56 pmol/mL (Young et al., 1992) 10 minutes after maximal exercise, 0.38 pmol/mL (Galbo et al., 1975) 30 minutes after maximal exercise and 0.6 pmol/mL (Galbo et al., 1975) 30 minutes after exhaustive exercise have been reported. The mean post60 plasma epinephrine concentrations in the current study were similar to post-30 values seen after maximal exercise (Galbo et al., 1975): the mean post60 plasma epinephrine concentrations in the current study were 0.30 pmol/mL (n=5) and 0.34 pmol/mL (n=4) after the nonsupplemented and supplemented trials, respectively.

The overall lower plasma norepinephrine values and lower exercising plasma epinephrine values in this study compared to the other exercise studies cited were not likely due to training status, age, gender, site of blood collection or time of day as these variables were the same or similar between the studies. Research has also indicated that the methods of analyzing plasma norepinephrine show reasonable agreement (Hjemdahl et al., 1979; Galbo et al., 1981). The anxiety level of the subjects, body position during blood sampling and dietary control could have contributed to the disparity in results. During

exercise, the intensity and duration of the effort are known to influence the magnitude of the rise in plasma catecholamines. This would explain the differing plasma norepinephrine and epinephrine responses between the current study and studies with a graded exercise protocol to maximal effort (Galbo et al., 1975; Rogers et al., 1991; Young et al., 1992). Only one study (Galbo et al., 1975) provided an exercise protocol of similar intensity and duration to the current study. Galbo et al. (1975) exercised eight men (age 20-28, $VO_2$ max=54.7 ml $O_2$/kg/min.) at 76% of $VO_2$ max to exhaustion. Recall that in the current study, five men (age 21-37, $VO_2$ max=53.9 ml/kg/min.) exercised at 75% of $VO_2$ max to exhaustion. Referring to Table 18, Galbo et al. (1975) found mean plasma norepinephrine levels roughly twice those of the current study during exercise (40 minutes) and exhaustion. Galbo et al. found mean plasma epinephrine levels roughly twice those of the current study during exercise (40 minutes) and about three times those of the current study at exhaustion. Duration of exercise was 10-15 minutes longer in the current study versus the Galbo et al. research. The Galbo et al. (1975) protocol involved treadmill exercise, 10-minute rest breaks every 20 minutes and unlimited mineral water; the current study used the cycle ergometer, allowed no resting and restricted fluids. It would be expected that the combination of longer exercise duration and no breaks in the current study would result in higher plasma norepinephrine levels than in the Galbo et al. (1975) study. It is likely that the lower plasma norepinephrine levels in men at rest and during exercise in the current study compared to the literature is attributable to problems associated with the catecholamine analysis method used in the current study or the possible loss of sample from prolonged storage. Detection of pre-exercise and post60 plasma catecholamines was problematic: some norepinephrine chromatographic peaks were obscured by an interfering substance in the plasma and some plasma epinephrine levels were below the detection limit of the system. Therefore, some data for pre-exercise plasma norepinephrine and epinephrine and post60 plasma epinephrine levels is missing. There was not enough sample collected to perform duplicate analyses, which could have helped ascertain precision and provide more complete data. Assay variation was not inconsequential, and sensitivity of the detection system decreased as plasma norepinephrine concentrations became smaller, as evidenced by greater coefficients of variation for controls from resting volunteers than from exercising volunteers. With regard to storage, the samples in this study were stored with 5mM glutathione at -70°C for 18 months. This is longer than has been reported in the literature so plasma levels of norepinephrine and epinephrine may have declined. The longest storage time noted in the literature is one year (Goldstein, 1986), in which untreated plasma stored at -70°C showed no decline in plasma norepinephrine or epinephrine. In contrast to the resting plasma norepinephrine values and

the exercising plasma norepinephrine and epinephrine values, resting plasma epinephrine values were not lower than the literature values even though all samples were subject to the same handling and processing procedures. Hence, it is possible that plasma epinephrine was more stable than plasma norepinephrine and individual variation in plasma epinephrine response during exercise, rather than loss of epinephrine, contributed to the differences in plasma epinephrine response during exercise between the current study and the Galbo et al. (1975) research. It would be useful if there were more studies using a submaximal exercise protocol to exhaustion that reported absolute plasma epinephrine values.

Although the absolute norepinephrine values reported in this study appear lower than previously reported values, the general pattern of plasma norepinephrine response to exercise corresponds to other research that found between a two-fold and 10-fold increase in plasma norepinephrine during exercise in young men, with peak values occurring at exhaustion or maximal effort and a decline during recovery (Hartley et al., 1972 a & b; Galbo et al., 1975; Hall et al., 1983; O'Brien et al., 1993; Farrell et al, 1987; Friedmann and Kindermann, 1989). In both the supplemented and nonsupplemented exercise tests, plasma norepinephrine concentrations were significantly higher at 60 minutes of exercise and post-exercise relative to resting values, and significantly lower 60 minutes after exercise relative to 60 minutes of exercise and post-exercise.

With regard to recovery from exercise, the literature reports elevated plasma norepinephrine levels relative to resting levels 10 minutes (O'Brien et al., 1993), 20 minutes (Hall et al., 1983) and 30 minutes (Galbo et al., 1975) after exhaustive exercise and a return to baseline 30 minutes (Farrell et al., 1987) and 60 minutes after exhaustive exercise (Hartley et al., 1972b). In the present study, plasma norepinephrine levels were elevated in all subjects 60 minutes after exercise relative to resting values (nonsignificant difference) in both the supplemented (n=3) and nonsupplemented trials (n=5). The difference in magnitude of the mean elevation may support the role of vitamin B-6 in plasma norepinephrine response during recovery. However, two of three subjects showed a greater elevation after exercise of the longest duration, regardless of supplementation status.

Mean resting plasma norepinephrine values increased from 0.5 hr. pre-exercise (pre1) to shortly before exercise (pre2), regardless of supplementation status (n=3). However, the magnitude of the increase in plasma norepinephrine concentrations during rest was greater during NS compared to S. This pattern reflects the data from three individuals: A significant change in plasma norepinephrine during rest may have been achieved with a greater sample size. The rise in plasma norepinephrine during rest may be partially explained by SNS stimulation in an anticipatory response to the impending

exercise trial (Mason et al., 1973; McArdle et al, 1986) or anxiety (Callingham, 1975). It is plausible that subjects experienced more anxiety prior to the first exercise test (nonsupplemented) than to the second exercise test (supplemented), accounting for the greater increase in plasma norepinephrine during rest in the nonsupplemented trial compared to the supplemented trial.

The general pattern of plasma epinephrine response to exercise corresponded to other research that found between a two-fold and 10-fold increase in plasma epinephrine during exercise in young men, with peak values occurring at exhaustion or maximal effort and a decline during recovery (Hartley et al., 1972a; Galbo et al., 1975; Hall et al., 1983; Nieman et al., 1993; O'Brien et al., 1993; Farrell et al, 1987; Friedmann and Kindermann, 1989). In contrast to changes in plasma norepinephrine levels, there were no significant changes in mean plasma epinephrine concentration over time for either the nonsupplemented or the supplemented trial. The greater magnitude of plasma epinephrine increase during exercise in the nonsupplemented trial versus the supplemented trial does not appear to support the role of vitamin B-6 in enhancing epinephrine release.

With regard to recovery from exercise, the literature reports elevated plasma epinephrine levels relative to resting levels 10 minutes (O'Brien et al., 1993; Young et al., 1992), 20 minutes (Hall et al., 1983) and 30 minutes (Galbo et al., 1975) after exhaustive exercise and a return to baseline 30 minutes (Farrell et al., 1987) and 60 minutes after prolonged exercise (Nieman et al., 1993). The data suggests a difference in the plasma epinephrine concentration pattern during recovery between the trials. Mean (n=4) plasma epinephrine levels in the current study were slightly depressed at post60 relative to resting values during the supplemented trial and somewhat elevated at post60 relative to resting values during the nonsupplemented trial. However, individual plasma epinephrine concentrations at post60 relative to pre2 suggest that plasma epinephrine concentrations were higher during recovery relative to rest with vitamin B-6 supplementation versus nonsupplementation. Further, three individuals (subj1, 4 and 6) displayed a change in plasma epinephrine response at post60 relative to rest from the nonsupplemented to the supplemented condition. It is possible that the duration of exercise played a role in this change in plasma epinephrine response between the trials: the trial in which each subject had an elevated plasma epinephrine concentration at post60 relative to rest was also the trial in which he exercised the longest. However, the fourth individual (subj2) had a lower plasma epinephrine concentration at post60 versus rest in both trials, even though his duration of exercise was much shorter during the supplemented trial versus the nonsupplemented trial.

From the data available, a rise in resting plasma epinephrine from pre1 to pre2 was realized in both the nonsupplemented (n=2) and supplemented (n=3) states. There may have been a greater rise during rest in the nonsupplemented condition compared to the supplemented condition, but it is difficult to determine this effect with a sample size of two. A rise in plasma epinephrine during rest may be explained by stimulation of the sympathoadrenal system in response to anxiety (Callingham, 1975).

This study hypothesized that vitamin B-6 supplementation would increase the plasma catecholamine response to exercise and the greater catecholamine levels would, in turn, influence fuel substrate levels in the plasma. Based on the role of the catecholamines in gluconeogenesis and glycogenolysis, plasma glucose levels were expected to be higher during exercise in the vitamin B-6 supplemented condition. The catecholamines stimulate gluconeogenesis and glycogenolysis in the liver, which leads to glucose production and subsequent release to plasma. The catecholamines also stimulate muscle glycogenolysis and inhibit glucose uptake into muscle, both of which could lead to higher plasma glucose levels (Landsberg and Young, 1992; Vranic et al., 1984). However, there were no significant differences in plasma glucose levels between the vitamin B-6 supplemented and nonsupplemented trials at any of the time points examined. In fact, mean plasma glucose levels showed little change between the two trials. In a previous study of the same design in this lab, Virk (1992) also did not find any significant differences in plasma glucose between the supplemented and nonsupplemented tests at any of the time points examined. The mean plasma glucose levels are in the range reported by Virk (1992) and Hofmann et al. (1991) during rest, exercise and recovery (Table 19).

Although the absolute numbers are not widely disparate, the pattern of plasma glucose response during exercise in the supplemented and nonsupplemented conditions varied somewhat between the current study and other research (Hofmann et al, 1991; Virk, 1992). The current study found no significant changes in plasma glucose over time in either of the trials and individual values revealed no consistent pattern of plasma glucose response. Furthermore, predicted values for 60 minutes of exercise, post-exercise and post60 based on plasma volume changes indicated that observed values were likely a sole result of plasma volume changes in each trial. Virk (1992) found significant changes in plasma glucose over time during the nonsupplemented but not the supplemented trial. During nonsupplementation, Virk found peak plasma glucose levels at 60 minutes of exercise that were significantly higher than post-exercise and post60 plasma glucose levels that were significantly lower than pre-exercise values. In a review of plasma fuel levels during rest, exercise and recovery, Virk (1992) has noted that plasma glucose concentrations change little compared to other substrates and are also variable, particularly

# Table 19. Plasma Fuel Concentrations-Literature Values

| Reference | n | VO2 max (ml/kg/min) | age (years) | mode | type | duration (min.) | intensity (% VO2max) | glucose concentration (mmol/L) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | pre | during | end | post30 | post60 |
| Galbo, 1975 | 8 | 54.7 | 20-28 | run | exhaustive | 81 | 76% | 5.6 | 5.8(40 min) | 5.6 | 5.0 | |
| Hofmann, 1991 | 6 | 56 | 26-36 | cycle | prolonged | 120 | 63 | 5.2 | 5.5 | 5.2 | 5.1 | 5.1 |
| Gleeson, 1986 | 10 | 46.7 | 32±3 | run | exhaustive | 96 | 73 | 5.5 | 5.0 | 5.0 | 5.5(post15) | |
| Ravussin, 1986 | 6 | 50 | 23-35 | cycle | prolonged | 150 | 47 | | | | | |
| Virk, 1992 | 6 | 59.6 | 19-37 | cycle | exhaustive | 120 | 64-75 | 4.75 | 4.89 | 4.21 | 4.12 | 4.08 |
| Virk, 1992* | 6 | 59.6 | 19-37 | cycle | exhaustive | 124 | 64-75 | 4.7 | 4.65 | 4.63 | 4.24 | 4.31 |
| | | | | | | | | lactate concentration (mmol/L) | | | | |
| Galbo, 1975 | | | | | | | | 0.6 | 2.7(20 min) 1.7(40 min) | 2.0 | 1.2 | |
| Hofmann, 1991 | | | | | | | | 1.4 | 2.5 | 2.7 | 1.3 | 1.4 |
| Gleeson, 1986 | | | | | | | | 0.54-0.51 | 3.1(15min) 2.1(60 min) | 2.5 | 1.5(post15) | |
| Virk, 1992 | | | | | | | | 1.6 | 4.17 | 4.36 | 2.76 | 2.26 |
| Virk, 1992* | | | | | | | | 1.32 | 3.9 | 3.72 | 2.18 | 1.73 |
| | | | | | | | | glycerol concentration (mmol/L) | | | | |
| Gleeson, 1986 | | | | | | | | 0.45 | 0.13(40 min) 0.15(60 min) | 0.29 | 0.23(post15) | |
| Ravussin, 1986 | | | | | | | | 0.12 | 0.21 | 0.58 | | |
| Virk, 1992 | | | | | | | | 0.15 | 0.36 | 0.57 | 0.34 | 0.29 |
| Virk, 1992* | | | | | | | | 0.18 | 0.33 | 0.64 | 0.45 | 0.33 |
| | | | | | | | | free fatty acid concentration (mmol/L) | | | | |
| Galbo, 1975 | | | | | | | | 0.57 | 1.3(40 min) | 1.81 | 1.72 | |
| Hofmann, 1991 | | | | | | | | 0.40 | 0.50 | 1.1 | 1.2 | 1.1 |
| Ravussin, 1986 | | | | | | | | 0.52 | 0.49 | 1.4 | | |
| Virk, 1992 | | | | | | | | 0.61 | 0.73 | 1.57 | 1.85 | 1.49 |
| Virk, 1992* | | | | | | | | 0.47 | 0.55 | 1.59 | 1.48 | 1.26 |

All subjects were male. Mode=exercise mode: run=treadmill, cycle=cycle ergometer. Type=exercise type. Pre=before exercise; During=60 min. of exercise unless otherwise noted; end=final exercising value; post30=30 min. after end exercise (unless otherwise noted); post60=60 min. of recovery.

Virk, 1992*=subjects supplemented with vitamin B-6

one hour after exercise. Indeed, during supplementation and nonsupplementation in the current study and during supplementation in the Virk research, no significant change in plasma glucose was seen between any time points.

Plasma lactate levels also can be examined as an indicator of carbohydrate utilization during exercise. The catecholamines stimulate glycogenolysis in working muscle, which leads to lactate production and subsequent release into the circulation. With vitamin B-6 supplementation, it was expected that plasma lactate levels would be higher during exercise than in the nonsupplemented state. There were no significant differences in mean plasma lactate levels between the nonsupplemented and supplemented tests at any of the time points studied. However, plasma lactate concentrations at pre-exercise and at 60 minutes of exercise were higher during the supplemented versus the nonsupplemented trial, supporting the hypothesis of vitamin B-6 supplementation enhancing carbohydrate utilization. Higher pre-exercise plasma lactate concentrations suggest that vitamin B-6 supplementation enhanced basal lactate production. All three subjects had higher plasma lactate concentrations at 60 minutes of exercise during the supplemented versus the nonsupplemented trial. This suggests vitamin B-6 enhanced glycogen use in muscle. However, three of five subjects had lower plasma lactate concentrations at post-exercise and post60 during the supplemented versus the nonsupplemented trial. These directional changes held true when the absolute values were compared to predicted values based on plasma volume changes. Virk (1992) has suggested that lactate is utilized more extensively as a substrate for gluconeogenesis in the liver after supplementation, accounting for the lower lactate level post-exercise and post60 during the supplemented versus the nonsupplemented trial. However, determining the effect of PN supplementation on plasma lactate levels at post-exercise and post60 is complicated by the differing durations of exercise attained by each subject between the trials. All subjects had lower plasma lactate levels at post-exercise and three of five subjects had lower plasma lactate levels at post60 when exercise duration was shorter regardless of the trial.

In the previous study of the same design, Virk (1992) also found no significant differences in plasma lactate values between the nonsupplemented and the supplemented trials. In agreement with the current study, Virk found lower mean plasma lactate concentrations at post-exercise during the supplemented versus the nonsupplemented trial. Individual directional changes in plasma lactate levels at post-exercise and post60 in the Virk research did not appear to be dependent upon the duration of exercise. This may indicate a true effect of vitamin B-6 supplementation on plasma lactate levels at exhaustion. In addition, deVos (1982) reported an increase in plasma lactate concentration after 50 minutes of cycling in men supplemented with vitamin B-6 versus controls. In contrast to the current

study, Virk (1992) found lower mean plasma lactate concentrations pre-exercise and during exercise after supplementation relative to nonsupplementation. The absolute plasma lactate values during the supplemented trial in the current study were lower than the values obtained by Virk at all time points except at 60 minutes of exercise. Mean plasma lactate values in the current study compared to the Virk research were: 1.17 mmol/L and 1.32 mmol/L pre-exercise, 3.92 mmol/L and 3.90 mmol/L at 60 minutes of exercise, 2.83 mmol/L and 3.72 mmol/L post-exercise, and 1.21 and 1.73 mmol/L post60, respectively. The consistently longer exercise times attained by individuals in the Virk (1992) study (124.9 ± 37.4 min. vs 91.9 ± 83.9 min.) compared to the current study and the somewhat higher level of training in the former (59.6 mlO$_2$/kg/min. vs 53.0 mlO$_2$/kg/min.) may have partially accounted for the higher plasma lactate concentrations post-exercise and post60 during the Virk study. The difference in pre-exercise values between the studies may result from timing of the blood draws. The pre-exercise value in the current study is a mean calculated from the means of two resting samples, pre1 (1.12 mmol/L) and pre2 (1.23 mmol/L). Each blood sample was drawn after 0.5 hr. rest; pre2 was drawn about 10 minutes prior to exercise. Note that pre2 was 10% higher (not statistically significant) than pre1; this may indicate some pre-exercise glycogen mobilization. In the Virk (1992) study, only one resting sample was drawn from each subject after 10-15 minutes of rest, about 10 minutes prior to exercise. In the Virk study, the pre-exercise plasma lactate value is more similar to the pre2 value in the current study than to the pre1 value. The Virk pre-exercise value may be higher than the pre2 value in the current study due to the shorter resting time prior to blood draw in the Virk protocol, which may have provided less time to clear lactate from the plasma after subjects travelled to the lab. Further, posture-induced plasma volume changes have been found to take 20 minutes to dissipate (Hagan et al., 1978).

During nonsupplementation, the absolute mean plasma lactate concentrations in the current study were lower than the plasma lactate concentrations found by Virk (1992) in subjects at pre-exercise (1.07 mmol/L vs 1.60 mmol/L), 60 minutes of exercise (2.64 mmol/L vs 4.17 mmol/L), post-exercise (3.01 mmol/L vs 4.36 mmol/L) and 60 minutes after exercise (1.21 mmol/L vs 2.26 mmol/L). Referring to Table 19, the plasma lactate concentrations reported by Virk (1992) are also somewhat higher than the values reported by Gleeson et al.(1986) and Hofmann et al. (1991) in men, while the plasma lactate concentrations reported in the present study are comparable to these studies. The lower plasma lactate level during exercise and at exhaustion in the Galbo et al. (1975) research relative to the other studies is likely due to the 10-minute rest breaks allowed subjects every 20 minutes of exercise. This protocol would, in effect, decrease the overall intensity of the workout, resulting in lower plasma lactate levels. Resting plasma lactate levels

varied among the studies, ranging from 0.54 mmol/L (Gleeson et al., 1986) to 1.6 mmol/L (Virk, 1992). As discussed above, timing of pre-exercise blood draws may effect resting plasma lactate measurements. Differing results among the studies also could have arisen from variations in training status of the subjects, exercise intensity and duration, and diet. However, the exercise protocol and dietary control were the same in the Virk (1992) study and the present study. As during the supplemented trial, subjects attained consistently longer exercise times in the Virk study (120.6 ± 16.7 min. vs 94.8 ± 42.9 min.) and had a higher level of fitness compared to subjects in the current study. These differences may have partially accounted for the higher plasma lactate concentrations at 60 minutes of exercise, post-exercise and post60 during the Virk study. As with any comparison of research, biochemical individuality and methodology always introduce the chance for variability in results.

In addition to examining directional changes in plasma lactate concentrations at specific time points after PN supplementation, the pattern of plasma lactate response over time in each test may reflect the effects of PN supplementation on carbohydrate use. The trend in plasma lactate differed between the nonsupplemented and supplemented trials, suggesting a change in fuel use. Plasma lactate increased significantly above resting values at 60 minutes of exercise in both trials. The magnitude of the change may support the role of vitamin B-6 in enhancing glycogen use: plasma lactate was elevated 188% during the supplemented trial compared to 123% during the nonsupplemented trial at 60 minutes of exercise relative to resting values (n=3). The data appear to suggest a change in plasma lactate response from 60 minutes of exercise to post-exercise between the trials. Between these time points, plasma lactate increased (nonsignificant) during the nonsupplemented trial but decreased significantly during the supplemented trial. The data also suggest a change in plasma lactate response between the trials during recovery from exercise. At post60 in the nonsupplemented trial, the plasma lactate level was elevated above the mean pre-exercise value. Plasma volume changes could not account for the percent difference (13%). Although the mean plasma lactate concentration at post60 during the supplemented trial was elevated above resting (3%), plasma volume changes indicate there was no difference or that post60 values may have been slightly depressed relative to resting values. The decrease in plasma lactate from 60 minutes to post-exercise and at post60 versus rest in the supplemented state may indicate that lactate was being taken up and used more extensively as a substrate for gluconeogenesis in the liver after 60 minutes of exercise and through recovery during the supplemented trial. Unfortunately, increases in plasma glucose levels between these time points during the supplemented versus the nonsupplemented trial were not observed to lend support for this theory.

In addition to exercise and recovery, plasma lactate appeared to increase more during rest in the nonsupplemented trial versus the supplemented trial. From pre1 to pre2, the plasma lactate concentration increased in three of five subjects during nonsupplementation, and decreased or showed no change in three of five subjects during supplementation. However, all subjects but one (subj1) had an increase or decrease in plasma lactate during rest regardless of supplementation status. This may suggest that vitamin B-6 supplementation influenced the pattern of plasma lactate response during rest in subject one. In all subjects, a change in plasma lactate during rest may reflect an anticipatory response to exercise that is greater in some individuals than others. This response is likely mediated by catecholamines via sympathoadrenal activity.

To summarize the differences in plasma lactate response over time between the trials: plasma lactate showed a greater magnitude of increase from resting to 60 minutes of exercise during the supplemented trial; plasma lactate decreased significantly from 60 minutes of exercise to post-exercise in the supplemented trial but increased between these points during the nonsupplemented trial; plasma lactate level at post60 was elevated above resting levels during the nonsupplemented but not supplemented trial.

Virk (1992) also found significant changes in the plasma lactate concentrations over time in both the nonsupplemented and supplemented trials. In agreement with the current study, Virk found an increase in plasma lactate concentration from 60 minutes of exercise to post-exercise during the nonsupplemented trial but a decrease in plasma lactate concentration between these points during the supplemented condition. Virk found plasma lactate concentrations at post60 that were elevated above resting values in both trials 30-40%, with significance being reached for the supplemented trial. In contrast, the current study found an elevation (not significant) at post60 relative to resting only after the nonsupplemented trial. Combining the results from the two studies indicates that PN supplemention may effect a change in the plasma lactate response between 60 minutes of exercise and exhaustion. Studies by other researchers in nonsupplemented men provide additional support for this effect. All the studies listed in Table 19 show a higher plasma lactate value at exhaustion relative to 60 minutes of exercise or to 40 minutes of exercise. Note that Galbo et al. (1975) and Gleeson et al. (1986) found plasma lactate levels earlier in exercise than 60 minutes that were higher than the plasma lactate levels at exhaustion. Research in marathon runners (O'Brien et al., 1993) has also reported a higher plasma lactate concentration at the end of a marathon relative to 60 minutes of exercise, but fluctuations in plasma lactate levels throughout exercise. It would be helpful to have more blood samples drawn during exercise to elucidate the effects of PN supplementation on lactate response between time points.

Comparing changes in the plasma catecholamine concentrations and the plasma lactate concentrations may help discern any relation between their responses to PN supplementation. Correlations between plasma epinephrine and plasma lactate at 60 minutes of exercise and post-exercise were not strong for either trial. Correlations between plasma lactate and plasma norepinephrine at 60 minutes of exercise were positive (r=0.82) during the nonsupplemented trial, but negative during the supplemented trial (r=-0.11). Positive relationships were found at post-exercise between plasma lactate and plasma norepinephrine, with higher correlations occurring during the supplemented versus the nonsupplemented trials. This relationship held true whether data were used from all five subjects or from the three subjects who attained the 60-minute exercise point. A strong correlation (r=.993) occurred at post-exercise during the supplemented trial using the data from the three subjects who exercised longer than 60 minutes. Correlations between plasma lactate and the catecholamines pre-exercise were not significant, even though the majority of subjects had higher plasma norepinephrine, epinephrine and lactate concentrations pre-exercise during supplementation versus nonsupplementation. At post60, plasma norepinephrine and epinephrine were elevated above resting levels more during the supplemented trial versus the nonsupplemented trial, but plasma lactate was elevated above resting levels more during the nonsupplemented versus the supplemented trial. However, individual responses with regard to plasma epinephrine and lactate were variable at post60 relative to pre-exercise making it difficult to determine the trend for any one of these plasma contituents, let alone determine a relationship between them. Nevertheless, using the data from the three subjects who attained the 60-minute exercise point revealed a strong correlation at post60 between plasma lactate and plasma norepinephrine (r=.946) and between plasma lactate and plasma epinephrine (r=.999) during the nonsupplemented but not the supplemented trial.

In addition to their role in carbohydrate metabolism, the catecholamines stimulate catabolism of triglycerides in adipose tissue and in working muscle, aiding in mobilization of free fatty acids (FFA) and glycerol (Wahrenberg et al., 1987; Stankiewicz-Choroszucha and Gorski, 1978). Increased catecholamine levels could, therefore, result in higher concentrations of plasma FFA and glycerol. However, researchers have previously hypothesized that PN supplementation directly increases carbohydrate metabolism and, consequently, indirectly decreases fat metabolism (Virk, 1992, Manore and Leklem, 1988). In support of this hypothesis, lower resting FFA concentrations in humans have been reported after PN supplementation (Virk, 1992; Manore and Leklem, 1988). An objective of the current study was to examine the response of plasma FFA and plasma glycerol before and after supplementation. FFAs are the preferred fuel for cardiac muscle

and are burned for energy in working muscle. Plasma glycerol is used as an indicator of fat mobilization from adipose tissue and may also be used for gluconeogenesis in the liver. There were no significant differences in mean plasma FFA or plasma glycerol concentrations between the nonsupplemented and supplemented tests at any of the time points studies. However, mean plasma FFA and plasma glycerol concentrations were lower at 60 minutes of exercise, exhaustion and post60 during the supplemented versus the nonsupplemented trial. These findings lend support to the theory of decreased fat utilization after PN supplementation. In contrast, directional changes in pre-exercise plasma FFA and plasma glycerol were at odds to one another: pre-exercise FFA concentration was lower, but the mean pre-exercise glycerol concentration was higher during the supplemented versus the nonsupplemented trial. These directional changes held true when the absolute values were compared to predicted values based on plasma volume changes. As with plasma lactate, determining the effect of PN supplementation on plasma FFA and glycerol levels at post-exercise is complicated by the differing durations of exercise attained by each subject between the trials. All five subjects had a lower plasma FFA concentration and all but one subject (subject 4) had a lower plasma glycerol concentration at post-exercise and post60 when exercise duration was shorter regardless of the trial.

In the previous study of the same design, Virk (1992) also found no significant differences between the trials with regard to plasma glycerol values at any time point or plasma FFA values at any time point except pre-exercise. He found a significantly lower plasma FFA concentration pre-exercise after PN supplementation. Virk found lower mean plasma FFA concentrations at 60 minutes of exercise and post60 after supplementation as did the present study, but found a minor increase (1.4%) in plasma FFA concentration at exhaustion. Like the present study, Virk found a higher mean plasma glycerol concentration pre-exercise and a lower mean plasma glycerol concentration at 60 minutes of exercise during the supplemented versus the nonsupplemented trial. In contrast to the present study, Virk found higher mean plasma glycerol levels at the end of exercise and recovery during the supplemented compared to the nonsupplemented trial. Virk suggested that the higher plasma glycerol and slightly higher FFA concentration at exhaustion during the supplemented trial indicated a decreased utilization of plasma FFA after supplementation. He supported this idea with R value data that were consistently lower during the nonsupplemented versus the supplemented trial, indicating greater fat use relative to carbohydrate use during the nonsupplemented state. Although higher plasma FFA concentrations can indicate less utilization of FFA, lower plasma FFA concentrations do not necessarily indicate greater utilization of FFA. Lower plasma FFA may indicate

less mobilization of FFA, which is reflected in lower plasma glycerol levels. This appeared to be the case in the Virk study at 60 minutes of exercise during the supplemented trial: since both plasma FFA and plasma glycerol concentrations were lower, presumably mobilization, and subsequently utilization, was decreased. During recovery in the Virk study, plasma glycerol levels were higher but plasma FFA levels were lower after supplementation. This suggests that FFA mobilization and uptake increased. Unfortunately, there was no R value data during recovery to support this effect. Contrary to the Virk (1992) results, directional changes in plasma FFA matched directional changes in plasma glycerol at all time points except pre-exercise; they were both lower during the supplemented versus the nonsupplemented trial. This may be interpreted to mean there was less FFA mobilization as well as utilization during exercise and recovery in the supplemented trial. The R values provide support for decreased fat utilization during exercise in the supplemented trial: overall R values were lower during the nonsupplemented test versus the supplemented test, indicating a decrease in fat use after supplementation. Note that the sample size used to calculate R values changed over time during both trials. The increased pre-exercise plasma glycerol concentration and decreased pre-exercise plasma FFA concentration in both trials after supplementation suggests greater mobilization and use of FFA at rest. However, resting R values were higher during the supplemented versus the nonsupplemented trial, indicating less fat use relative to carbohydrate after supplementation.

The absolute plasma glycerol values during the supplemented trial in the current study were slightly higher pre-exercise and at 60 minutes of exercise, and lower at exhaustion and post60 than the values obtained by Virk (Table 19). With nonsupplementation, the absolute mean plasma glycerol concentrations in the current study were somewhat higher than the plasma glycerol concentrations found by Virk (1992) at all time points and somewhat higher than values from other research in Table 19. The absolute plasma FFA values in the current study were lower than the values obtained by Virk (1992) at all time points during both trials. The FFA values reported by Virk are slightly higher than most of the values reported by other researchers (Table 19). The higher plasma FFA levels during exercise and at exhaustion in the Galbo et al. (1975) research relative to the other studies is likely due to the 10-minute rest breaks allowed subjects every 20 minutes of exercise. This protocol would, in effect, decrease the intensity of the workout, resulting in greater fat use relative to glycogen, also reflected in the lower plasma lactate levels. The exercise protocol and dietary control were the same in the Virk (1992) study and the present study. The higher plasma FFA concentrations during the Virk study versus the current study may have been influenced by the

consistently longer exercise times attained by individuals in the Virk study (124.9 ± 37.4 min. vs 91.9 ± 83.9 min.) compared to the current study and the somewhat higher level of training in the former (59.6 mlO$_2$/kg/min. vs 53.0 mlO$_2$/kg/min.). The difference in pre-exercise values between the studies may result from timing of the blood draws, as discussed with regard to pre-exercise plasma lactate levels.

In addition to the directional changes in plasma glycerol and FFA between the nonsupplemented and supplemented trials, the pattern of plasma glycerol and FFA response over time in each test helps support the role of PN supplementation in decreasing fat utilization relative to carbohydrate use during exercise. The pattern of plasma glycerol and FFA response over time was the same in both trials, except pre-exercise, but the magnitude of the changes differed significantly. These differences between the trials indicate greater fat mobilization and use during the nonsupplemented trial: plasma glycerol showed a significant increase from resting to 60 minutes of exercise during the nonsupplemented but not the supplemented trial; plasma glycerol and plasma FFA increased significantly from 60 minutes to post-exercise during the nonsupplemented trial but not the supplemented trial; and the magnitude of increase in plasma glycerol and plasma FFA at post-exercise relative to pre-exercise was greater during the nonsupplemented trial.

Virk (1992) also found significant changes in plasma glycerol and FFA concentrations over time in both the nonsupplemented and supplemented trials. As in the current study, Virk found a greater magnitude of plasma glycerol increase from pre-exercise to 60 minutes of exercise during the nonsupplemented versus the supplemented trial; significant increases in plasma glycerol from pre-exercise to post-exercise in both trials, with a greater magnitude of change occurring in the nonsupplemented trial; and a greater magnitude of plasma glycerol elevation above resting values at post60 during the nonsupplemented versus the supplemented trials, providing support for the role of PN supplementation in fat mobilization (i.e., less mobilization with supplementation). In contrast to the present study, Virk found a greater magnitude of increase in plasma FFA concentration from pre-exercise to post-exercise during the supplemented versus the nonsupplemented trial; an increase of greater magnitude from 60 mninutes of exercise to post-exercise during the supplemented trial; and a greater magnitude of plasma FFA elevation above resting values at post60 during the supplemented versus the nonsupplemented trials. The contrary patterns of plasma FFA response between the current study and the Virk study do not necessarily support the role of PN supplementation in decreasing fat utilization. Virk interpreted the greater plasma FFA responses during the supplemented trial to indicate less utilization of FFA and supported this theory by citing the

higher R values that were attained during the supplemented versus the nonsupplemented trials. The current study found a decreased plasma FFA response and plasma glycerol response with supplementation, but also found higher (not significant) R values during the supplemented versus the nonsupplemented trials. This suggests a closer pairing of FFA mobilization and utilization than that of the Virk study, which would be more physiologically advantageous in terms of energy efficiency. Combining the results from the two studies indicates that PN supplemention may decrease fat mobilization and use during exercise and recovery as well as at rest.

The respiratory exchange ratios (R) provide support for an alteration in substrate use after PN supplementation. Based on previous exercise and vitamin B-6 research (Virk, 1992; Campuzano, 1988), it was expected that R values during exercise would increase after PN supplementation. Although there were no significant differences in R values between the trials, overall R values were higher throughout rest and exercise during the supplemented versus the nonsupplemented trial. This indicates that carbohydrate utilization relative to fat use was greater during the supplemented period versus the nonsupplemented period. At rest during the nonsupplemented trial, carbohydrate provided about 70.8% of the energy expenditure and fat provided about 29.2% of the energy expenditure; at rest during the supplemented trial, carbohydrate provided about 80.7% of the energy expenditure and fat provided about 19.3% of the energy expenditure. Virk (1992) also found overall higher R values (not significant) during exercise during the supplemented versus the nonsupplemented trial. The Virk research and the current study found no significant changes in R value over time in either trial, but R values generally decreased over time in both tests. During nonsupplemented exercise, carbohydrate provided 77.4% to 60.8% of total kcal expenditure and fat provided 22.6% to 39.2% of total kcal expenditure. During supplemented exercise, carbohydrate provided 77.4% to 67.5% of total kcal expenditure and fat provided 22.6% to 32.5% of total kcal expenditure.

Combining the plasma lactate, glycerol and FFA results with the R values and exercise durations provides some support for the role of PN supplementation in altering fuel metabolism during exhaustive exercise. Specifically, carbohydrate use increased and fat use decreased with supplementation. Although there were no significant differences in the concentrations of fuels between the periods, some consistent trends were noted. During the supplemented period, pre-exercise lactate values were higher while plasma FFA levels were lower than during the nonsupplemented trial. At 60 minutes of exercise, plasma lactate values were higher while plasma glycerol and plasma FFA were lower than during the supplemented trial. The pre-exercise and 60-minute results indicate a greater

use of carbohydrate (glycogen) relative to fat during PN supplementation. At exhaustion, plasma lactate, glycerol and FFA were lower during the supplemented period compared to the nonsupplemented period, but directional changes in these plasma constituents appeared to be dependent upon the duration of exercise and may not truly reflect the effect of PN supplementation. At post60, plasma lactate was unchanged, or lower if plasma volume change is considered, and plasma glycerol and plasma FFA were lower during the supplemented versus the nonsupplemented trial. However, directional changes in plasma glycerol and plasma FFA levels at post60 seemed to be dependent upon duration of exercise. Patterns of change in each fuel over time within the trials support the role of PN supplementation in increasing carbohydrate utilization during exhaustive exercise. From pre-exercise to 60 minutes of exercise, plasma lactate increased more while plasma glycerol and FFA increased less during the supplemented versus the nonsupplemented trial. This suggests a greater use of glycogen relative to fat up to 60 minutes after PN supplementation. From 60 minutes of exercise to exhaustion, plasma glycerol and FFA increased less during the supplemented versus the nonsupplemented trial. This indicates a decreased use of fat during exhaustive exercise after PN supplementation. Plasma lactate showed a different pattern between 60 minutes of exercise and post-exercise between the two trials. Plasma lactate increased from 60 minutes to post-exercise during the nonsupplemented trial, but decreased between these points during the supplemented trial. This does not appear to support the idea of greater glycogen use with PN supplementation. However, it has been suggested that gluconeogenesis in the liver is increased with PN supplementation (Virk, 1992). If plasma lactate was used as a substrate for gluconeogenesis, its disappearance from the plasma during the supplemented trial would be explained. Virk (1992) found increased plasma glucose levels to support this theory, but the results from the present study do not support this theory. Nevertheless, when R values are considered in addition to the plasma fuel responses, the results indicate that carbohydrate use relative to fat use is increased with PN supplementation.

During exercise, the catecholamines stimulate an increase in heart rate and oxygen consumption (Landsberg and Young, 1983b, 1992). Previous research (Virk, 1992) found significantly lower heart rates at several time points during exercise and lower resting heart rates (nonsignificant) after PN supplementation versus nonsupplementation. Virk also found lower mean oxygen consumption (nonsignificant) at all time points measured during exercise in the supplemented versus the nonsupplemented trial. Researchers have also found higher R values and lower plasma FFA at rest after PN supplementation (Virk, 1992; Campuzano, 1988). The ß-blocker propranolol has been shown to decrease resting and exercising heart rate and oxygen consumption in trained

men (Tremblay et al., 1992; Christensen et al., 1975; Galbo et al., 1976). Propranolol administration also produced lower plasma FFA and a higher R value in trained men (Tremblay et al., 1992). Based on these findings, it was theorized that PLP may function like a ß-adrenoreceptor blocker. As such, PN supplementation would result in a lower heart rate and lower oxygen consumption at rest and during exercise compared to nonsupplementation. As previously discussed, plasma FFA was lower and R value was higher during supplementation compared to nonsupplementation. However, the data provide no evidence to support the theory that this level of PN supplementation influenced heart rate at rest or heart rate and oxygen consumption during exercise. The data do indicate a possible influence of PN supplementation on resting oxygen consumption. The lower heart rate results (rest and exercise) and lower oxygen consumption results (exercise only) reported by Virk (1992) with PN supplementation were not duplicated in this study. There were no significant differences in mean heart rates between the two tests at any of the time points recorded. Furthermore, there was no consistent pattern of increase or decrease in heart rate during the supplemented trial compared to the nonsupplemented trial during rest or exercise. In contrast, Virk (1992) found consistently lower individual heart rates in all six subjects during the supplemented versus the nonsupplemented trial. The present study found a significant increase in heart rates above resting levels during exercise in both trials and the pattern of change was similar between the trials. Virk found a significant change in heart rate over time only during the nonsupplemented trial, indicating consistently greater magnitudes of heart rate change among subjects over time during the nonsupplemented trial versus the supplemented trial. Virk found lower oxygen consumption during exercise after supplementation, whereas the present study found overall higher oxygen consumption during exercise in the supplemented versus the nonsupplemented condition. The increase in oxygen consumption was statistically significant at only one time point (10 minutes). Individual exercising oxygen consumption was higher in four of five subjects during the supplemented trial, while one subject had no change between the trials. The mean exercising oxygen consumption observed by Virk (1992), on the other hand, appeared to be affected by data from only two of six subjects: two subjects showed a decrease in oxygen consumption after supplementation but four showed no change between the trials. Hence, the majority of individuals from each study showed either an increase in oxygen consumption or no change in oxygen consumption during exercise with PN supplementation as opposed to nonsupplementation. The magnitude of the increase in oxygen consumption from rest to 10 minutes of exercise in the present study provides further support for a greater oxygen consumption response after PN supplementation. A significant increase from rest to 10 minutes of exercise was found

in both trials, but the increase was greater during the supplemented versus the nonsupplemented trial (786% vs 632%). Virk did not measure resting oxygen consumption, so no comparison to the change in oxygen consumption from rest to exercise can be made. The disparity between the Virk (1992) results and the current study results may arise from the greater plasma PLP response during exercise after supplementation in the former, suggesting a possible role for PLP in heart rate and oxygen consumption down-regulation. The decreased heart rates and oxygen consumption observed by Virk (1992) after supplementation may reflect the greater differences in plasma PLP between the trials and greater changes in plasma PLP concentration over time during the supplemented trial compared to the current study. The Virk results may also reflect a training effect, as the supplemented trial was conducted 23 days later than the nonsupplemented trial. Methodology, such as small deviations in workload setting, and sample size may have contributed to the differing results.

This study sought to determine if PN supplementation would enhance the increase in plasma norepinephrine and plasma epinephrine during exhaustive submaximal exercise. No significant differences in plasma norepinephrine or plasma epinephrine concentrations were found when comparing the nonsupplemented to the supplemented conditions at rest, during exercise, at exhaustion or during recovery. Concentrations of both catecholamines appeared to be higher during rest in the supplemented versus nonsupplemented condition, although complete data were not obtained. Difficulty interpreting the data arose from individual variation, plasma volume changes, missed data points due to the catecholamine assay procedure and the failure of two subjects to attain the 60-minute exercise point, assay variation, differing exercise durations of individuals between the trials and the overall small sample size. Hence, no consistent trends in plasma catecholamine response to PN supplementation during exhaustive exercise were observed. The results do not indicate that the pattern of catecholamine response to exercise may be altered with PN supplementation.

This thesis hypothesized that the anticipated increase in plasma catecholamine response during PN-supplemented exercise would influence fuel use, heart rate and oxygen consumption. Evidence has been presented in this discussion to support the role of PN supplementation in increasing the use of carbohydrate (glycogen) relative to fat during rest and exercise. This thesis has suggested that higher catecholamine concentrations at rest during the supplemented trial may have contributed to the higher lactate and glycerol but lower FFA concentrations at rest after supplementation. No other time point data showed a consistent increase or decrease in the fuel and catecholamine concentrations. Since no significant change in plasma catecholamine concentration was

found between the trials at any specific time point, directional changes in plasma fuels cannot be attributed to changes in plasma catecholamine concentrations. Changes in fuel use with supplementation may have been influenced by an increase in catecholamine concentrations within the tissues, but invasive procedures to obtain tissue samples would not have been humane nor desirable. With regard to heart rate and oxygen consumption, this study provides no evidence to support the findings of Virk (1992), who found decreased heart rates and oxygen consumption with PN supplementation. Since plasma catecholamine levels did not appear to be altered with PN supplementation in this study, a relationship between plasma catecholamines and heart rate or oxygen consumption cannot be elucidated. This thesis hypothesized that heart rate and oxygen consumption would be lower after PN supplementation. The results did not provide any compelling evidence that PN supplementation decreases heart rate or exercising oxygen consumption. The results do indicate that resting oxygen consumption was lower after PN supplementation, although this finding was not statistically significant. It is not likely that the lower resting oxygen consumption was due to a training effect since heart rate was not lower during the second (supplemented) trial. Exercising oxygen consumption increased substantially after PN supplementation, reaching significance at one time point and approaching significance at several others. This may suggest that the decreased resting oxygen consumption observed during the supplemented period may have been the result of individual variation. It is possible that subjects were calmer before the second trial, producing a somewhat lower resting oxygen consumption. This idea is supported by the response of resting plasma catecholamines, which were observed to increase more from pre1 to pre2 during the first (nonsupplemented) trial compared to the second (supplemented) trial.

Although this study did not provide any compelling evidence that 20 mg/d PN supplementation alters plasma catecholamine levels, this does not rule out the possibility that PN supplementation may effect catecholamine synthesis. Plasma catecholamine concentrations may have been altered, but not sufficiently to cause a consistent pattern of change among the subjects. Plasma PLP concentrations showed a greater increase with supplementation during the previous study in this laboratory (Dunton, 1992) than in the present study. Plasma catecholamines may also have increased more in that study and contributed more to fuel modifications, such as the enhanced glucose production that was noted during the supplemented period. Plasma catecholamines in the present study may have increased substantially with PN supplementation but were inadequately detected due to prolonged storage of sample or methodological problems. If plasma catecholamine concentrations were enhanced, their actions could have contributed to the observed modifications in fuel use. Plasma catecholamines may not have increased with PN

supplementation, but catecholamine levels in tissues, such as the brain and heart, may have increased. Obviously, increased concentrations of catecholamines in the tissues could not be assessed in this study. If catecholamine concentrations were increased in the SNS, however, spillover of norepinephrine would be expected to increase plasma norepinephrine levels (Leuenberger, 1993). Many other factors aside from the action of catecholamines regulate fuel use during exercise. The action of other hormones, particularly insulin, glucagon and cortisol, influence fuel use and plasma glucose homeostasis (Vranic et al., 1984; Chasiotis et al., 1982). It has also been hypothesized that PLP *per se* influences fuel modification (Virk, 1992; Leklem, 1988b).

Recommendations for further research in the area of vitamin B-6 supplementation and plasma catecholamine concentrations include: a larger sample size of at least eight to 10 subjects; greater quantities of plasma, allowing triplicate determinations of at least 1.0 mL plasma each; maximum storage time of sample not to exceed one year. Exercise of greater intensity might produce more dramatic plasma catecholamine responses. Regardless of the intensity, duration of exercise should be standardized so that the effect of PN supplementation on plasma catecholamines and fuels would not be confounded by differing exercise durations between trials. Jeukendrup et al (1996) have reported poor reproducibility of a cycle ergometer test at 75% of maximal workload (Wmax) to exhaustion (CV=26.6%) compared to timed trials (CV=3.35% and 3.49%). However, Burnham et al (1995) have reported good reproducibility for time to fatigue in well-trained cyclists riding their own bicycles at 75-80% $VO_2$ max to exhaustion (R=0.897, SEM=5.4 minutes). Hence, it may be of benefit to test subjects on their own bicycles mounted on trainers. Timing of the post-exercise blood draw and drawing of an additional post-exercise sample (e.g., with subject remaining on the bike) would help improve the accuracy of comparisons made between the trials at the end of exercise. Subjects should be encouraged to hydrate themselves well the day before the exercise trials, and allowance of greater water consumption during exercise should be researched in attempts to avoid dehydration and possibly reduce plasma volume changes. Finally, a greater level of vitamin B-6 supplementation might produce more pronounced effects on catecholamine synthesis.

# SUMMARY AND CONCLUSIONS

This study hypothesized that oral vitamin B-6 supplementation (20 mg/d PN-HCl) would enhance the increase in plasma catecholamine concentration that is stimulated by exhaustive endurance exercise. The objectives of the study were: 1) to examine the change in plasma catecholamines produced by exhaustive endurance exercise in trained male cyclists during the vitamin B-6 supplemented and nonsupplemented states, 2) to analyze the relationship between the rise in plasma catecholamines and fuel use during exhaustive endurance exercise in the vitamin B-6 supplemented and nonsupplemented trials, and 3) to evaluate heart rate changes during exhaustive endurance exercise and resting metabolic rate (RMR), indicated by oxygen consumption, in the vitamin B-6 supplemented and nonsupplemented condition.

Five trained men (age=18-35 years; $VO_2max$=53 ml $O_2$/kg/min.) participated in two controlled dietary periods that were identical except for the addition of 20 mg/d PN supplementation during the second period. On the seventh morning of each period, fasted subjects exercised to exhaustion on a cycle ergometer at 74.5% ± 7.8 $VO_2max$. Blood was drawn pre-exercise (twice), 60 minutes into exercise, immediately post-exercise and 60 minutes post-exercise. Plasma was analyzed for norepinephrine, epinephrine, glucose, pyridoxal 5'-phosphate (PLP), lactic acid, glycerol and free fatty acids (FFA). Heart rate and oxygen consumption were measured pre-exercise and at 10-minute intervals during exercise.

The data were analyzed by standard statistical techniques; null hypotheses were rejected at the 0.05 level of significance. Paired t-tests were used to determine if a difference existed in a given variable between the exercise tests at each time point. ANOVA was used to determine if changes occurred over time within each test for each variable studied. A 95% confidence interval (CI) using the Bonferroni method was used for the multiple range tests to determine between which pairs of time points significant differences existed.

Plasma PLP was significantly higher during the supplemented versus the nonsupplemented trial at all time points. There was no significant change in plasma PLP over time in either trial. However, the magnitude of the increase in plasma PLP concentration from rest to 60 minutes of exercise and the magnitude of the decrease in plasma PLP concentration from 60 minutes of exercise to post60 was greater during the nonsupplemented compared to the supplemented test. There were no statistically significant differences in plasma catecholamine concentrations or plasma fuel

concentrations between the nonsupplemented and supplemented trials at any of the time points examined. However, plasma glycerol and plasma FFA concentrations were consistently lower during exercise and recovery in the supplemented versus the nonsupplemented trial. At rest, mean plasma epinephrine, lactate and glycerol concentrations were higher while mean FFA concentration was lower during the supplemented versus the nonsupplemented trial. In addition, pre-exercise plasma norepinephrine concentration was higher during the supplemented versus the nonsupplemented condition in two of three individuals. There were significant changes in the concentration of plasma norepinephrine, lactic acid, glycerol and FFA over time in both trials. There was a nonsignificant rise in mean plasma epinephrine concentration during nonsupplementation and a nonsignificant decrease during supplementation from 60 minutes of exercise to post-exercise. In addition, two of three individuals had a nonsignificant rise in plasma norepinephrine concentration during nonsupplementation and a nonsignificant decrease during supplementation between these time points. Concentrations of mean plasma norepinephrine and epinephrine increased during rest in both trials, with a greater increase occurring during the nonsupplemented trial (nonsignificant). The magnitude of the significant elevation in mean plasma lactate at 60 minutes of exercise relative to pre-exercise was greater during the supplemented trial versus the nonsupplemented trial. There was also a significant decrease in mean plasma lactate concentration from 60 minutes of exercise to post-exercise during the supplemented trial, and a nonsignificant increase in plasma lactate between these time points during the nonsupplemented trial. The magnitude of the significant rise in plasma glycerol and plasma FFA from pre-exercise to post-exercise was greater during the nonsupplemented versus the supplemented trial. There was also a significant increase in mean plasma glycerol and plasma FFA concentrations from 60 minutes of exercise to post-exercise during the nonsupplemented trial, but not during the supplemented trial. Mean respiratory exchange ratios (R) were higher during the supplemented trial compared to the nonsupplemented trial, but the differences did not attain statistical significance. There were no significant differences in mean exercise times to exhaustion or heart rates between the trials. Overall mean oxygen consumption was higher during the supplemented versus the nonsupplemented trial and the difference attained significance at one time point (10 min.). There was one significant increase in the mean percent plasma volume change (PVC) from pre- to post-exercise in the supplemented compared to the nonsupplemented trial, and the percent PVC increased significantly during the supplemented but not the nonsupplemented trial.

In conclusion, the results suggest that 20 mg/d of vitamin B-6 supplementation effects a change in fuel utilization, with a greater reliance on carbohydrate relative to fat utilization, and an increase in exercising oxygen consumption. However, the results do not provide any significant evidence that B-6 supplementation influences plasma catecholamine concentrations. As such, the effects of catecholamines on fuel utilization, heart rate or oxygen consumption at rest or during submaximal exercise to exhaustion under conditions of PN supplementation cannot be determined. The results do suggest a modification in the pattern of plasma catecholamine and plasma substrate response during supplemented exercise, but a relationship between the plasma catecholamine and plasma substrate patterns cannot be verified from the data in this study.

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# APPENDIX

**Table A.1. Individual Plasma Volume Changes (%)**

| Subject | Test | *Dill and Costill* | | | *Van Beaumont* | | |
|---------|------|--------|------|--------|--------|------|--------|
|         |      | During | Post | Post60 | During | Post | Post60 |
| 1 | NS | -5.11 | -2.16 | 1.75 | -1.99 | 3.06 | 6.22 |
|   | S | -13.17 | -10.56 | -5.51 | -8.22 | -9.09 | -4.456 |
| 2 | NS |  | -16.76 | 0.77 |  | -13.99 | 7.366 |
|   | S |  | -23.57 | -4.47 |  | -22.81 | -1.555 |
| 4 | NS |  | -19.29 | -11.68 |  | -18.40 | -11.54 |
|   | S |  | -19.89 | -8.89 |  | -18.27 | -5.913 |
| 5 | NS | -11.11 | -10.03 | -0.49 | -8.61 | -7.69 | 4.088 |
|   | S | -12.30 | -10.44 | 2.77 | -9.53 | -7.70 | 5.127 |
| 6 | NS | -10.90 | -8.32 | -7.20 | -10.17 | -1.55 | -2.55 |
|   | S | -16.15 | -16.06 | -7.33 | -13.23 | -11.47 | -3.998 |

Percent changes are relative to the mean of two pre-exercise values. Dill and Costill & Van Beaumont refer to methods of calculating PVC.
NS=nonsupplemented exercise test to exhaustion; S=B-6 supplemented exercise test to exhaustion
During=1.0 hour into exercise; Post=immediately after exercise; Post60=60 min after exercise

## Table A.2. Individual Hemoglobin (g/dl) Changes

| Subject | Test | Pre1 | Pre2 | During | Post | Post60 |
|---------|------|------|------|--------|------|--------|
| 1 | NS | 14.93 | 15.16 | 15.70 | 15.59 | 15.20 |
|   | S  | 14.77 | 14.71 | 16.32 | 15.77 | 15.28 |
| 2 | NS | 14.85 | 14.54 |       | 16.45 | 15.04 |
|   | S  | 13.78 | 13.71 |       | 16.03 | 14.29 |
| 4 | NS | 14.17 | 14.57 |       | 16.29 | 15.44 |
|   | S  | 15.53 | 15.31 |       | 17.56 | 16.48 |
| 5 | NS | 16.08 | 15.84 | 17.17 | 17.05 | 16.35 |
|   | S  | 17.07 | 17.06 | 18.47 | 18.28 | 17.02 |
| 6 | NS | 14.02 | 14.23 | 15.13 | 15.30 | 15.05 |
|   | S  | 14.26 | 14.25 | 15.96 | 16.09 | 15.11 |

Individual values are means of samples analyzed in triplicate.
NS=nonsupplemented exercise test to exhaustion; S=B-6 supplemented exercise test to exhaustion
Pre1=0.5 hr before exercise; Pre2=before exercise; During=1.0 hour into exercise; Post=immediately after exercise; Post60=60 min after exercise

**Table A.3.  Individual Hematocrit (%) Changes**

| Subject | Test | Pre1 | Pre2 | During | Post | Post60 | |
|---------|------|------|------|--------|------|--------|--|
| 1 | NS | 46.25 | 47.75 | 47.50 | 46.25 | 45.50 | |
|   | S  | 44.75 | 45.00 | 47.00 | 47.25 | 46.00 | |
| 2 | NS | 45.00 | 44.50 |        | 48.50 | 43.00 | |
|   | S  | 41.25 | 41.00 |        | 47.50 | 41.50 | |
| 4 | NS | 41.00 | 41.50 |        | 46.25 | 44.25 | |
|   | S  | 43.00 | 43.00 |        | 48.00 | 44.50 | |
| 5 | NS | 48.50 | 48.50 | 50.75  | 50.50 | 47.50 | |
|   | S  | 50.50 | 50.50 | 53.00  | 52.50 | 49.25 | |
| 6 | NS | 41.75 | 42.00 | 44.50  | 42.25 | 42.50 | |
|   | S  | 43.00 | 42.00 | 46.00  | 45.50 | 43.50 | |

Individual values are means of samples analyzed in duplicate.  NS=nonsupplemented exercise test to exhaustion;
S=B-6 supplemented exercise test to exhaustion.  Pre1=0.5 hr before exercise; Pre2=before exercise;
During=1.0 hour into exercise; Post=immediately after exercise; Post60=60 min after exercise

# GENERAL INSTRUCTIONS FOR SUMMER 1992 STUDY

## COLLECTION OF URINE:

1. Collect all urine in containers provided (24 hr. urine collection). You will receive clean urine containers each morning of each feeding period, and during the 4 day 24 hr. diet period (day 16-19).

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

3. Each collection day:

Urine collections will be made on a 24 hr. basis. You will collect, for example, from 6:45 am. on one day through the same time the next day. 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. THIS IS CRUCIAL TO THIS STUDY!

4. Urine will be collected starting with breakfast on the day you start on the diet study. Empty your bladder upon rising and begin collection from then on. Return urine samples daily at any time convenient for you to room 106, Milam 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, and record the loss in your daily log. The urine collections are a very critical part of this study.

7. Try to drink the same amount of fluids each day.

## OTHER:

1. Eat all food given to you during each controlled dietary period (days 1-9 and 25-34). It is our goal to maintain your body weight and adjustments in calorie intake can be made, so please let us know if you are receiving too much or too little food.

2. Record all activities every day. A journal will be provided for you at breakfast to fill in for the previous day's activities.

3. No alcoholic beverages, including beer and wine, are to be consumed during the study. Caffeine containing foods/beverages will not be allowed the day before, the day of or the day after each exercise period.

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

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

DEPARTMENT OF NUTRITION & FOOD MANAGEMENT
OREGON STATE UNIVERSITY
6/92
INFORMED CONSENT

The purpose of this study is to examine alterations in energy metabolism during exhaustive exercise by studying growth hormone and vitamin B-6 changes, in the vitamin B-6 supplemented and the unsupplemented states.

This investigation will involve 8 male, trained cyclists. The study will last 34 days. I understand that my participation is voluntary and that I am free to leave this experiment at any time. All information obtained from me will be confidential. My data will be identified by code number. The only persons who will have access to my data are the principal investigator, the medical technologist and the graduate students who are assigned to the research.

## I. PRE-STUDY TESTING:

1) <u>VO$_2$ MAX TEST-</u> This test will be used to determine maximal aerobic capacity. Each exercise to exhaustion test will be performed at 75% of this VO$_2$ max level. This test will be conducted 1-2 weeks prior to the start of period I (controlled metabolic period) I understand that I will:

(A) cycle on a bike ergometer (stationary bike) for approximately 20 minutes at increasing resistance (2 minute intervals) until my VO$_2$ level does not rise with increasing workload or I request to stop
(B) be allowed a 2-5 minute warm-up period on the bike prior to the test
(C) be monitored for heart rate and normal cardiac function by electrocardiogram by trained personnel 5 minutes prior to, at 2 minutes intervals during and at 3, 6 and 9 minutes following the max test.
(D) not be penalized if I request to stop the test. I understand that the test of VO$_2$ max has a chance of precipitating a cardiac event (such as abnormal heart rhythms) or even death. However, the possibility of such an occurrence is very slight (less than 1 in 10,000) since I am in good physical condition with no known symptoms of heart disease.

2) <u>PRELIMINARY EXERCISE TEST-</u> This test is meant to aid in the accurate setting of the workload and to familiarize myself with the testing equipment. This test will be conducted after the VO$_2$ max test and 1-7 days prior to the start of period 1 (controlled metabolic period). I understand that I will:

(A) be monitored by EKG, and will wear the gas collection equipment, continuously during the preliminary test ride
(B) be allowed a 2-5 minute warm-up period on the bike prior to the preliminary test

(C) have the workloads gradually increased, at 1-2 minute intervals until 75% of my $VO_2$ max is attained (This will take 10-15 minutes).

(D) ride for 10 minutes at 75 % $VO_2$ max to familiarize myself with the exhaustive test conditions.

(E) be allowed a cool down period before coming off the bike.

## II. EXERCISE TO EXHAUSTION TESTS: I understand that:

(A) I will exercise to exhaustion (that point where I am unable to maintain a preset pedaling speed which is 75% of my $VO_2$ max) on the ergometer on 2 separate occasions (day 7 and day 31).

(B) The investigator will stop the study when exhaustion occurs

(C) I will be able to stop the exercise test at any time. The exercise test will be stopped prior to exhaustion if indicated by the American College of Sports Medicine guidelines for stopping an exercise test (see criteria for stopping an exercise test).

(D) I will not be able to keep track of time during the exercise test, but will try to cycle as long as possible during each test.

(E) I will be allowed a 2-5 minute warm-up period before the start of each test.

(F) I will be monitored by electrocardiogram for normal cardiac functioning for approximately 1 minute prior to each gas collection (at 10 minute intervals)

(G) I will not be allowed to eat anything after 7 pm the day before each exercise test. I can consume water during the 12 hours of fasting (7 pm-7 am) and recognize the importance of consuming at least 2 glasses (20 oz.) of water 1 hour prior to reporting for this test.

(H) I will be allowed 100 ml of water after the exercise blood sample is drawn. Otherwise, I may rinse my mouth out with water during the exercise session, but not swallow. I understand that a feeling of discomfort, fatigue and/or lightheadedness may develop. If signs of dehydration develop, I will be given water.

(I) I will keep my daily exercise training level constant throughout the study.

## III. DIET: I understand that I will:

(A) eat only the metabolic diet provided by the Department of Nutrition and Food Management at Oregon State University on days 1-9 and 25-33

(B) eat my own food on days 10-24

(C) keep a 4 day 24 hour dietary intake record on days 16-19

(D) not take any vitamin, mineral or nutritional supplement other than the supplement provided throughout the study

(E) not take any vitamin, or other nutritional supplement except what is provided me (one capsule per day containing either sugar and citric acid or pyridoxine (vitamin B-6) throughout the 34 days)

(F) not know when I am receiving the vitamin B-6 supplement until the study is over (day 34)

(G) not consume alcoholic beverages during the study

(H) avoid caffeine containing items the day prior to, the day of and the day after each exercise test

(I) inform the principle investigator (Dr. Leklem) if any prescription or non-prescription drugs are taken

4) URINE: I understand that I will:

(A) collect 24-hour urine specimens days 1-9 and days 25-33 as per the General Instructions form
(B) bring the 24-hour urine specimens to the lab each morning
(C) report any accidental loss of urine to the investigators

5) DAILY LOG: I understand that I will:

(A) record my daily activities, including exercise and sleep, in a log book during days 1-9, 16-19 and 25-33.

6) BLOOD: I understand that:

(A) I will have 20 ml (about 1.5 tablespoons) of blood drawn prior to the start of the study before breakfast
(B) I will have 20 ml of blood drawn 5 times (120 ml total; about 9 tablespoons) during each exercise to exhaustion session (two at 0 hours, 1 during the exercise test, one 1-2 minutes after exercise, and one 1 hour after exercise). When blood is donated (ie-Red Cross), 500 ml are taken without adverse effects
(C) I may develop a bruise, which will go away, at the site where blood is drawn
(D) I will have 20 ml of blood drawn before breakfast on July 30
(E) I will have all blood drawn from an arm vein in the arm by a Medical Technologist
(F) There is a minimal risk of infection when blood is drawn. Sterile, disposable needles and sterile procedures will be used to minimize this risk.
(G) As an evaluation of any adverse effects of blood loss, my blood will be analyzed for hemoglobin, hematocrit and proteins which bind iron. If any of these fall below normal values, I will be given and iron supplement (35 mg/d) for the remainder of the study.
(H) Persons at increased risk for hepatitis B or HIV (commonly called AIDS) should not donate blood or any other body fluids and therefore should not participate in this preliminary investigation (screening) since the donation of blood is required to determine my eligibility. Persons at increased risk include men who have had sexual contact with another man since 1977, persons who have used intravenous drugs, and persons who have had sexual contact with either a member of one of these groups, a person who has AIDS, or a person who is HIV infected.

7) EXPIRATORY GASES: I understand:

(A) I will breath into a gas collection devise (by wearing a mouthpiece and nose clip) during the $VO_2$ max test and each exercise to exhaustion test
(B) expiratory gas data will be collected every 30 seconds during the max test and for 3 minutes (at 10 minute intervals) during each exercise to exhaustion test

8) SWEAT: I understand that:

(A) I will have samples of my sweat collected onto filterpaper from my back during each exhaustive exercise test at 10 minute intervals

9) BENEFITS/RISKS:

I will incur no medical of health risks from participating in this research. I will receive:
(A) $50 ($3/day) from Oregon State University at the end of the study
(B) the results of my blood values, body composition and fitness values obtained during this study
(C) 18 days of meals at no cost

It is important to note that strict daily adherence to the requirements of this project is necessary to my participation in this study. Failure to do so may result in the discontinuance of my participation as a subject. Examples include not showing up for meals, not adhering strictly to the diet, failure to report loss of urine to a member of the research staff.

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

All of my questions have been answered to my satisfaction. If I have additional questions at any time I will call Dr. James E. Leklem 737-3561.

_____          _____
subject signature                                    date

_____          _____
address                                                  phone #

_____
witness

_____
Principal Investigator

NUTRITION & EXERCISE STUDY-SUMMER 92    NAME_____
NUTRITION & FOOD MGMT.
OREGON STATE UNIV.          DATE_____

## DAILY ACTIVITY SHEET

Record all activity for the previous day and length spent in each, perceived intensity (1 = minimal effort required, 10 = maximal effort)

| ACTIVITY | TIME (min,hrs) | INTENSITY | TIME OF DAY* |
|---|---|---|---|
| SLEEPING | | | |
| SITTING | | | |
| WALKING | | | |
| PHYSICAL WORK | | | |
| OTHER: | | | |
| | | | |
| SPORTS: | | | |
| | | | |
| | | | |
| | | | |

* M = morning, A = afternoon, E = evening, L = late night/early morning

**BEVERAGES:**
RECORD ALL "FREE" FOODS IN EXACT AMOUNTS USED.  INDICATE TYPE USED (DECAF, DIET, REGULAR)

Coffee/Tea (cups)_____
Pop (cans,oz.)_____
candy/sugar_____
Other (water, electrolyte replacement)_____

**GENERAL HEALTH:**
How do you feel today? Excellent____ Good____ Fair____ Poor____

**MEDICATIONS:**
Any medications? (aspirin, etc.)_____

**UNUSUAL EVENTS:** (injuries, exams)_____

**TURNED IN URINE:** yes  no    **WEIGHT TODAY:**_____