AN ABSTRACT OF THE THESIS OF

Nancy J. Dunton for the degree of Doctor of Philosophy in Nutrition and Food Management presented on November 4, 1994. Title: The Effect of Exhaustive Endurance Exercise and Vitamin B-6 Supplementation on Vitamin B-6 Metabolism and Growth Hormone in Men.

Abstract approved: _____

James E. Leklem

Trained male cyclists (6 in study 1, 5 in study 2) cycled to exhaustion (EXH) at 75% of VO_2 max twice; once in the non-supplemented (NS) state and once in the vitamin B-6 (B-6)(20 mg PN) supplemented (S) state. The diet contained 2.3 mg B-6 in study 1 and 1.9 mg B-6 in study 2. Urine was collected during each dietary period. During each exercise (EX) test, blood was drawn prior to (PRE), one hour during (DX), immediately after (POST) and one hour after (POST 60) EX and sweat was collected.

Compared to baseline (PRE) levels, plasma pyridoxal 5'-phosphate (PLP) and vitamin B-6 (PB-6) concentrations increased at DX, decreased at POST, and decreased below PRE at POST 60 in the NS and S states. EX to EXH in the S state resulted in a greater increase in PLP DX in study 1 (31% increase vs. 16%) and PB-6 in study 2 (25% increase vs. 11%) as compared to the NS state. Red blood cell (RBC) PLP significantly increased from POST to POST 60 in the S state in study 2.

The excretion of urinary 4-pyridoxic acid (4-PA) and urinary B-6 (UB-6) was not significantly altered by EX to EXH. The mean excretion of 4-PA was significantly greater in the NS state in study 2 (7.98 ±1.83 mmol/d) as compared to the excretion in study 1 (6.20 ±0.93 mmol/d), whereas the excretion was significantly greater in the S state in study 1 (92.2 ±8.69 mmol/d) compared to the excretion in study 2 (82.7 ±6.16 mmol/d). The

percent of B-6 intake excreted as UB-6 (6% in study 1 and 10% in study 2) was significantly different between the studies in the NS state.

Vitamin B-6 supplementation did not significantly alter the rise in growth hormone (hGH) concentration seen with EX to EXH. The loss of B-6 in sweat with EX to EXH was not altered by B-6 supplementation. The loss of B-6 in sweat ranged from 0.0011 mmol to 0.0039 mmol.

Therefore, EX to EXH in the B-6 S state resulted in a greater increase in plasma PLP and PB-6 DX as compared to the NS state. The decrease in PB-6 and PLP at POST 60 in the S state coincided with a significant increase in RBC PLP, suggesting the movement of B-6 from the plasma into the RBC at POST 60. EX to EXH and B-6 supplementation did not alter the excretion of 4-PA or UB-6 suggesting that B-6 metabolism was unchanged. The loss of B-6 in sweat was comparable to previously reported values and was not altered by B-6 supplementation. B-6 supplementation did not alter the changes in hGH resulting from EX to EXH alone.

The Effect of Exhaustive Endurance Exercise and Vitamin B-6 Supplementation on Vitamin B-6 Metabolism and Growth Hormone in Men

by

Nancy J. Dunton

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Doctor of Philosophy

Completed November 4, 1994
Commencement June 1995

Doctor of Philosophy thesis of Nancy J. Dunton presented on November 4, 1994

APPROVED:

Major Professor, representing Nutrition and Food Management

Chair of Department of Nutrition and Food Management

Dean of Graduate School

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

Nancy J. Dunton, Author

ACKNOWLEDGMENTS

I would like to thank the people who have helped me grow personally and professionally through this Ph.D. process:

Mom and Dad. Your unconditional love and support has given me the confidence to go out and accomplish my dreams.

My Sister, Leslie and Family. For your loving support.

Chris Andersen. You have been my main support person through this whole process. You were always there to listen and provide advice and support, which meant a lot to me. I know it wasn't always easy. I appreciate your belief in me. You are a very special person.

Dr. Jim Leklem. Thank you for your guidance and support. You have provided me with some memorable experiences and a new perspective on science.

Dr. Margy Woodburn. You believed all the students were special and were always there to listen.

Ricky Virk. You have been a friend and colleague since day 1 and have shared the joys and frustrations along the way.

Fellow Graduate Students. We supported each other through some trying times.

Karin Hardin and Jim Riddlington. You always kept me on track. Your friendship means a lot to me.

Ginny Lesser. Your unending help with my statistics was a lifesaver.

John Lawrence. Your e-mail support helped me preservere.

Running Buddies. Without the constant bantering and critical evaluation on long runs, I would never have developed the rebuttal skills necessary in life. You guys kept my life in balance and provided perspective. How can I ever thank you?

Bryan Andersen. Thank you for making life fun and keeping things in perspective.

Kenai. Last but not least, my cat, who was there every minute of the way.

TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

TABLE OF CONTENTS (Continued)

TABLE OF CONTENTS (Continued)

LIST OF FIGURES

LIST OF TABLES

LIST OF TABLES (Continued)

LIST OF TABLES (Continued)

LIST OF APPENDICES

LIST OF APPENDIX FIGURES

LIST OF APPENDIX TABLES

LIST OF APPENDIX TABLES (Continued)

The Effect of Exhaustive Endurance Exercise and Vitamin B-6 Supplementation on Vitamin B-6 Metabolism and Growth Hormone in Men

INTRODUCTION

Athletes today are constantly searching for a means to ensure peak performance. Often, dietary manipulations are involved. Specific nutrients may be consumed for a specific purpose. The nutrient requirements of athletes, above and beyond those currently recommended to the general population, may be unknown in many cases. Scientific investigation allows for the systematic study of the effects of exercise on nutrient metabolism. Knowledge of these effects are needed to make nutrient recommendations to athletes.

Vitamin B-6, known to be involved in energy producing pathways, has been of interest to athletes for some time. The type of demands placed on the energy producing pathways (i.e., the type of exercise) can influence the changes in vitamin B-6 metabolism. Exercise of varying durations and intensities has been shown to affect the metabolism of vitamin B-6 in men (Leklem and Shultz, 1983; Hatcher et al., 1982; Hofmann et al., 1991). A significant increase in the plasma pyridoxal 5'-phosphate (PLP) concentration has been found during exercise by Leklem and Shultz (1983), Hatcher et al. (1982) and Hofmann et al. (1991) even after considering the changes in plasma volume. The origin of this PLP remains controversial. PLP is stored in the muscle with the enzyme glycogen phosphorylase. Black and co-workers (1977, 1978) have determined that muscle glycogen phosphorylase can be a reservoir for vitamin B-6 during conditions of starvation. Therefore, Leklem and Shultz (1983) have hypothesized that exercise mimics an acute starvation, thereby promoting the release of PLP from the muscle into the plasma. A possible reason for the release of PLP into the plasma may be to assist in gluconeogenic pathways. Vitamin B-6 supplementation may actually increase the rate of glycogenolysis (Leklem, 1985) during exercise due to its association with glycogen phosphorylase.

Assuming that PLP is released from the muscle during exercise to participate in gluconeogenesis (possibly in the liver), the intensity and duration of the exercise becomes important. A high intensity, short duration activity will utilize primarily carbohydrate for energy, whereas a low intensity, long duration activity will draw upon fat and glycogen reserves (McArdle et al., 1986). In activities exceeding two hours in duration, muscle glycogen stores can become depleted (Coyle et al., 1985). Previous studies examining vitamin B-6 metabolism and exercise in men have examined a defined period of exercise (i.e., a 4500 meter run (Leklem and Shultz, 1983), a total of 21 minutes of cycling on a cycle ergometer (Munoz, 1982), 50 minutes of cycling on a cycle ergometer (Hatcher, 1983) and 120 minutes of treadmill running (Hofmann et al., 1991). No studies to date have examined the effect of exhaustive exercise on vitamin B-6 metabolism in men. Based on previous exercise and supplemental vitamin B-6 studies of shorter duration, enhanced glycogenolysis during exhaustive endurance exercise could result in early fatigue as compared with the non-supplemented condition.

The regulation of energy sources during exercise is also under hormonal control. Growth hormone (hGH) is an anabolic polypeptide hormone that influences carbohydrate, protein and fat metabolism. Recent interest in growth hormone originates from its ability to promote muscle mass, a role of interest to athletes. Growth hormone has been shown to increase several fold during exercise compared to resting conditions (Hartley et al., 1972; Raynaud et al., 1983; Lassare et al., 1974). A link between vitamin B-6, hGH and exercise has been documented in the literature. Supplemental (provided intravenously) vitamin B-6 has been shown to enhance the rise in hGH concentration typically occurring with exercise (Moretti et al., 1982; Delitala et al., 1976). To date, no studies involving oral vitamin B-6 supplementation, plasma hGH concentration and exhaustive exercise have been conducted. The ability to manipulate plasma hGH concentrations not only by exercise, but also by vitamin B-6 supplementation, may have practical applications for those seeking to improve muscle mass (i.e., athletes and the elderly).

Hypothesis

Oral vitamin B-6 supplementation and exhaustive endurance exercise will alter vitamin B-6 metabolism and increase the exercise-induced rise in plasma growth hormone concentration in men. The plasma pyridoxal 5'-phosphate concentration, plasma growth hormone concentration and urinary 4-pyridoxic acid excretion will increase more with exhaustive exercise in the vitamin B-6 supplemented state than in the non-supplemented state.

Objectives

The purpose of this project was to examine the effect of exhaustive endurance exercise and vitamin B-6 supplementation on vitamin B-6 metabolism and growth hormone concentrations in men under controlled conditions. Specifically, our purpose was:

1) To determine if exhaustive endurance exercise resulted in significant changes in plasma pyridoxal 5'-phosphate and vitamin B-6 concentrations compared to resting conditions, and to monitor these changes at exhaustion and during recovery.

2) To determine if the magnitude of change in plasma vitamin B-6 indices was greater with exhaustive exercise in the vitamin B-6 supplemented state compared to the non-supplemented state.

3) To determine if the percent of vitamin B-6 intake excreted as urinary vitamin B-6 and 4-pyridoxic acid was greater with exhaustive exercise in the vitamin B-6 supplemented state as compared to the non-supplemented state and to monitor the excretion during the days following exercise.

4) To determine if plasma growth hormone concentration significantly increased with exhaustive exercise in the vitamin B-6 supplemented state compared to the non-supplemented state.

5) To quantify the loss of vitamin B-6 in sweat during exhaustive endurance exercise with and without vitamin B-6 supplementation.

LITERATURE REVIEW

Vitamin B-6

History

The discovery of each individual B-complex vitamin took place during the early part of the 20th century. The isolation of vitamin B-6 (B-6) was pursued as a result of studies on riboflavin. Goldberger and Lillie (1926) observed that a type of dermatitis occurred in rats fed a diet free of vitamin B-2 (riboflavin or lactoflavin). The dermatitis did not respond to the administration of purified vitamin B-2. The new compound ($C_3H_{11}O_3N$), found in association with riboflavin, was isolated by Odake in 1931 from rice bran but was not recognized as vitamin B-6 until 1934. Paul Gyorgy (1934) was the first to demonstrate that there was a difference between the rat pellagra preventive factor and vitamin B-2. He termed this factor vitamin B-6. Vitamin B-6 was found in association with vitamin B-2 in liver, yeast and other B-vitamin sources. Four years later, Gyorgy (1938) and Lepkovsky (1938) reported the isolation of pure crystalline vitamin B-6. Similarly, three other researchers also isolated vitamin B-6 in 1938 (Keresztesy and Stevens, 1938; Kuhn and Wendt, 1938; Ichiba and Michi, 1938). The chemical structure and synthesis of vitamin B-6 was determined a year later by Kuhn et al.(1939) and by Harris and Folkers (1939). Vitamin B-6 was found to be a pyridine derivative known chemically as 3-hydroxy-4,5-dihydroxymethyl-2-methylpyridine.

An extension of early animal research led to the documentation of the essentiality of vitamin B-6 to humans. The vitamin B-6 deficient state was chosen as a model to determine essentiality. Spies et al. (1939) was the first to report symptoms of weakness, irritability, abdominal pain, difficulty walking, insomnia and nervousness in undernourished patients eating a diet low in vitamin B-6 content. The administration of 50 mg vitamin B-6 alleviated the symptoms in as little as 24 hours. Snyderman et al. (1953) observed that infants given a

diet void of vitamin B-6 for 2 1/2 months presented with convulsive disorders. The administration of 50 mg vitamin B-6 immediately halted the seizures. Likewise, infants receiving a formula low in pyridoxine (60 μg/L) were found to have convulsive seizures which ceased with the inclusion of vitamin B-6 in the formula (Coursin, 1954). Diets low in vitamin B-6 have been shown to produce clinical symptoms such as abnormal electroencephalogram pattern in infants, convulsions in infants, stomatitis, cheilosis, glossitis, irritability, depression and confusion (Leklem, 1988). The alleviation of deficiency symptoms by the administration of vitamin B-6 establishes that vitamin B-6 is an essential nutrient in human nutrition.

Structures and Chemistry

Vitamin B-6 is the recommended generic term for all 3-hydroxy-2-methylpyridine derivatives. Confusion over the terminology of vitamin B-6 dates back to 1934, when Gyorgy first used the term "vitamin B-6" to describe pyridoxine (PN), the alcohol form. The terms "vitamin B-6" and "pyridoxine" became interchangeable. Today, the term vitamin B-6 describes the three primary forms of B-6 (pyridoxine (PN), pyridoxal (PL) and pyridoxamine (PM)), and their 5'-phosphate forms (pyridoxine-5'-phosphate (PNP), pyridoxal-5'-phosphate (PLP), and pyridoxamine-5'-phosphate (PMP)). In addition to the primary and phosphorylated forms of vitamin B-6, foods contain the glycosylated form of B-6, 5'-O-(ß-D-glycopyranosyl)pyridoxine (PN-glucoside). The 5'-phosphate forms are the main forms of vitamin B-6 found in organs and tissues. The form most often found in vitamin B-6 supplements is pyridoxine hydrochloride, due to its stability and ease of incorporation into products (Bauernfeind and Miller, 1978). The chemical structure of vitamin B-6 is depicted in Figure 1. The side chain at the fourth carbon in the pyridinium ring determines the primary form of vitamin B-6 (PN, PL or PM). The fifth carbon in the pyridinium ring is the site of phosphorylation and glycosidic linkage. The end-product of

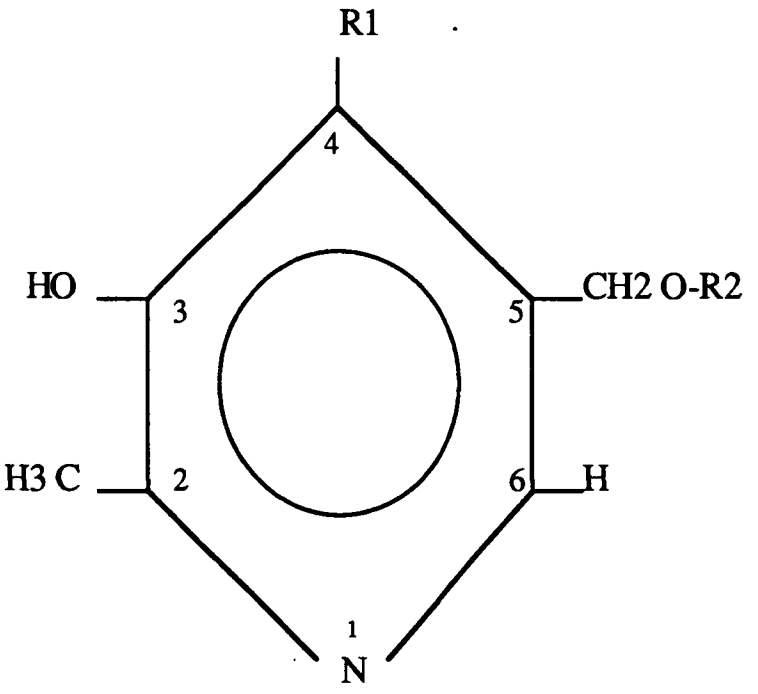

Figure 1. Structure of the B-6 vitamers (Adapted from Leklem, 1991)

B-6 metabolism, 4-PA, is similar in structure to the B-6 vitamers except that it has a carboxyl group at the fourth carbon.

Snell (1944a, 1944b) was the first to demonstrate a role for vitamin B-6 in transamination reactions and to show nonenzymatic catalysis of transamination by PL and PM. The major metabolically active coenzyme form of vitamin B-6 is PLP. In most cases, PLP is bound to enzymes via the formation of a Schiff base with the ε-amino group of a lysine residue (Hughes et al., 1962). The mechanism of action of PLP developed from studies involving trivalent metals complexed with PL. A PL-metal complex was found to nonenzymatically catalyze most of the amino acid reactions catalyzed by enzymes containing PLP (Braunstein and Shemyakin, 1953; Metzler et al., 1954). In an enzymatic reaction, the amino group of a substrate forms a Schiff base with the cofactor PLP associated with that enzyme. The strong electron-attracting nature of the pyridine ring of PLP withdraws electrons from either the R group, the hydrogen or the carboxyl group of

the substrate α-carbon to form a quinoinoid structure. The enzymatic reactions catalyzed by PLP can be categorized as 1) reactions involving α-carbon 2) reactions involving β-carbon or 3) reactions involving the γ-carbon. A list of these reactions is found in Table 1. The

Table 1. Enzymatic reactions catalyzed by pyridoxal 5'-phosphate.

Type of Reaction	Reaction or Enzyme
α-carbon reactions	
Transamination	Alanine ↔ pyruvate + PMP
Racemization	D-amino acid ↔ L-amino acid
Decarboxylation	5-OH Tryptophan → 5–OH tryptamine + CO_2
Oxidative deamination	Histamine → imidazole-4-acetaldehyde + NH_4^+
Loss of the side chain	THF + Serine → glycine + N5, 10-methylene THF
β-carbon reactions	
Replacement (exchange)	Cysteine synthetase
Elimination	Serine and threonine dehydratase
γ-carbon reactions	
Replacement (exchange)	Cystathionine → cysteine + homoserine
Elimination	Homocysteine desulfhydrase
Cleavage	Kynurenine → anthranilic acid

Adapted from: Leklem, J.E., 1991.

chemical properties of certain constituents of PLP help to define the properties of the molecule as a whole. As described by Leussing (1986), the 2-methyl group acts to bring the pK_a of the ring pyridine proton closer to the biological range; the phenoxide oxygen contributes to the stability of the ammonium Schiff base, facilitating the expulsion of a nucleophile at the 4-carbon position; the 5'-phosphate serves as an anchor for the coenzyme in a polar pocket directed away from the aldimine, thus preventing hemiacetal formation and the drain of electron density from the ring; and the protonated pyridine nitrogen aids in delocalizing the negative charge and regulating the pK_a of the 3-hydroxyl group.

The physical and chemical properties of vitamin B-6 help explain its function and are important when handling the vitamin. While vitamin B-6 is generally considered a labile nutrient, much of its stability is pH dependent. In solution, vitamin B-6 is stable under acidic conditions and labile under alkaline condition. The forms of vitamin B-6 are sensitive to light to varying degrees depending upon pH. Likewise, the three forms of vitamin B-6 are heat-stable under acidic conditions and heat labile under alkaline conditions. Vitamin B-6 is soluble in water, but not in organic solvents.

Food Sources

Vitamin B-6 is taken into the body through both food and supplemental sources. Foods contain the primary forms (PN, PL and PM), the phosphorylated forms (PNP, PLP and PMP) and the glycosylated form (PN-glucoside) in varying amounts. Other conjugated forms of vitamin B-6 have also been detected in foods. The phosphorylated forms of vitamin B-6 appear to be the primary form found in foods. Kant and Block (1990), using NHANES II data, documented that animal foods, cold cereals and potato products ranked highest as major sources of vitamin B-6 for adults aged 19-74 years.

Animal foods contain primarily PL, the majority of which is present as PLP. Leklem (1991) has compiled a list of selected foods from Orr (1969) and other sources, including analyses done at Oregon State University describing the vitamin B-6 content and percentage of the three vitamers (PL,PN and PM) contained in each. The percent of vitamin B-6 present in animal foods as PL is generally 50-85%. Excellent animal sources of vitamin B-6 include chicken breast, halibut and tuna. Meat, poultry and fish contain between 0.170-0.683 mg vitamin B-6/100 g edible portion, whereas milk and dairy products contain only 0.010-0.110 mg vitamin B-6/100g edible portion. Although dairy products contain a high percent of their vitamin B-6 content as PL, they are considered to be a poor source of vitamin B-6. Certain specific animal foods, such as pork, salmon and cheddar cheese contain a high percent (84-89%) of their vitamin B-6 content as PM.

In general, plant foods contain more PN and PM (or their respective phosphorylated forms) than PL. Examples of plant foods high in vitamin B-6 include: vegetables such as spinach, potatoes, and cauliflower; legumes such as white beans, lentils, soybeans and peanut butter; nuts such as filberts and walnuts; cereals and grains such as brown rice, barley, wheat products and cornmeal; and fruits such as bananas, avocado, raisins and apricots. The amount of vitamin B-6 in 100 grams of edible portion of plant foods ranges from 0.019 mg for peaches to 3.515 mg for rice bran (Leklem, 1988). PN-glucoside, the glycosylated form of pyridoxine, occurs in foods of plant origin. This compound can comprise 5-80% of the total vitamin B-6 content found in fruits and vegetables (Gregory and Ink, 1987). Kabir et al. (1983b) examined the PN-glucoside content of 22 foods. Grains and legumes contained 6-57% of their vitamin B-6 content as PN-glucoside. The highest PN-glucoside content in fruits was found in orange juice, which contained 47% of its total B-6 as PN-glucoside. Raw carrots contained the highest amount of PN-glucoside of any raw vegetable (51%). Processing increased the PN-glucoside content of vegetables such as broccoli and cauliflower compared to the raw form. The authors concluded that the enzyme β-glucosidase in the raw vegetables was denatured during processing thus lowering the PN-glucoside content. Animal foods were found to contain no measurable amount of PN-glucoside.

The vitamin B-6 content of a food can be influenced by processing and storage. Losses of vitamin B-6, the interconversion to another form of vitamin B-6 or the conversion to a compound not previously present have been shown to occur in a variety of foods (Lushbough et al., 1959; Richardson et al., 1961; Augustin et al., 1980, 1981; Gregory and Kirk, 1978). The vitamin B-6 content of milk and milk products has been studied extensively . Woodring and Storvick (1960) have reviewed the effect of food processing on the vitamin B-6 content of milk and milk products. Vitamin B-6 losses ranged from 0-70% during the storage of heat treated milk. Part of this loss could be accounted for by the formation of a compound not previously present, bis-4-pyridoxyl-

disulfide (Woodring and Storvick, 1960). Bernhart et al. (1960) examined the B-6 content of milk before and after heat sterilization, and were able to document the conversion of PL to PM. The vitamin B-6 content was not changed, but the proportion of vitamers comprising the B-6 value was altered. Experiments with foods other than milk have shown that vitamin B-6 added to flour which is baked into bread is stable (DeRitter, 1976; Perera et al., 1979).

A loss of vitamin B-6 activity and resistance to hydrolysis has been observed during processing and storage by Gregory and Kirk (1977, 1978). They found a decrease in the binding of PL and PLP to the ε-amino groups of protein or peptide lysyl residues in thermally processed and low-moisture stored foods. There is evidence in rats that the formation of ε-pyridoxyllysine bound to dietary protein has anti-vitamin B-6 activity (Gregory, 1980). Interactions between nutrients in foods may limit the availability of one or more of these nutrients. The incubation of PN with foods high in vitamin C has resulted in the conversion of PN to 6-hydroxypyridoxine (Tadera et al., 1986). Implications of this knowledge to food processing and fortification is unknown.

Absorption

Vitamin B-6 enters the body via the gastrointestinal tract. Once ingested, vitamin B-6 is either absorbed from the small intestine or is excreted in the feces. Researchers have examined which forms of vitamin B-6 are absorbed and how the absorption process occurs. Factors which can influence the extent of vitamin B-6 absorption and utilization will be addressed under the topic of bioavailability.

Investigators have used the animal model extensively in studying the absorption of vitamin B-6. Evidence to date indicates that the majority of vitamin B-6 is absorbed as PN, PL and PM (Henderson, 1985). Henderson (1985) summarized rat perfusion studies from Mehansho et al. (1979), Hamm et al. (1979) and Buss et al. (1980). In one study, either a low physiological dose (20 nmol) of labeled vitamin B-6 (PN, PL, PLP, PM or PMP) or a

high dose (200 nmol) of the same labeled vitamin B-6 vitamer was infused. Henderson (1985) concluded that the major forms of B-6 absorbed at the low dose were the free forms (i.e.- if PLP was the substrate, PL was the primary vitamer absorbed). At the high dose, the form absorbed corresponded to the form perfused. Therefore, the phosphorylated forms of vitamin B-6 were able to cross the mucosal membrane if the dose given was high. The contribution of the phosphorylated B-6 forms to overall B-6 absorption was minimal. Middleton (1984), using in vitro techniques, also found that PN, PL and PM are the primary forms of B-6 which cross the mucosal membrane. The major difference between the absorption of PL, PN and PM was in the transport from the mucosal cell to the plasma. PL appeared to be transported at the greatest rate (24% vs 3% for PM)(Mehansho et al., 1979; Hamm et al., 1979). In humans, the absorption of the B-6 vitamers occurs in descending order: PL>PM>PN (Henderson, 1984).

Our diet contains both the free and phosphorylated forms of vitamin B-6. Hydrolysis of the phosphorylated forms is therefore necessary prior to absorption. Under normal conditions, the phosphorylated forms are hydrolyzed by alkaline phosphatase in the brush border of the small intestine (Mehansho et al., 1979; Middleton, 1979, 1982; Hamm et al., 1979). Once the forms are hydrolyzed, the mechanism of absorption becomes important.

Numerous studies examining the mechanism of absorption were conducted in rats by Middleton (1977, 1981,1979). The results of these studies indicate that the B-6 vitamers (PL, PN and PM) cross the intestinal lumen by a nonsaturable, passive diffusion process. A linear relationship has been found between the PN dose and the amount of PN transported to the mucosal cells and basolateral membranes in rats (Buss et al., 1980). No evidence of saturability was evident, even at a dose 10,000 fold greater than the initial dose (20 nmol). Similar experiments using PL and PM were reported (Mehansho et al., 1979; Hamm et al., 1979). A linear relationship between B-6 absorption and 4-PA excretion (Booth and Brain, 1962; Johansson et al., 1966) implies absorption via a passive, non-

saturable process. Middleton (1985) has recently raised concerns that there may be a saturable component to the absorption of vitamin B-6. Using an in vivo perfused intestinal segment model, he found that the intestinal uptake of PN decreased from the proximal to the distal small intestine, indicating that there may be a saturable process which is greatest in the duodenum.

Bioavailability

The proportion of a nutrient present in a food that is both absorbed and available to cells is known as bioavailability. Consumption of a diet containing vitamin B-6 sources does not ensure complete utilization of that nutrient by cells. Incomplete absorption of vitamin B-6 from dietary sources and microbiological synthesis of B-6 in the gut will result in the presence of vitamin B-6 in the feces. Vitamin B-6 which is absorbed and/or metabolized by the body will appear in the urine as 4-PA and the vitamin B-6 vitamers. Indices such as urinary 4-PA and total vitamin B-6, fecal vitamin B-6, and plasma PLP provide an estimate of the amount of vitamin B-6 which is absorbed and utilized by cells.

A variety of techniques have been used to study the bioavailability of vitamin B-6. Rat bioassays measure the vitamin B-6 content of a food by monitoring animal growth. Sarma et al. (1947) was one of the first to suggest a wide variation of vitamin B-6 bioavailability based on a comparison of results from rat bioassays and Saccharomyces uvarum methods. The researchers found a 5 to 211% range of bioavailability relative to PN for the grain and plant products analyzed. There appears to be a poor correlation between estimates of biological availability of vitamin B-6 derived from growth studies and plasma PLP concentrations, questioning the appropriateness of animal growth studies for determining the bioavailability of vitamin B-6 from a diet (Gregory and Litherland, 1986). Another technique used to determine vitamin B-6 bioavailability involves feeding animals radiolabelled forms of vitamin B-6 mixed with a selected diet. The quantity of the isotope detected in the feces, urine, liver, blood and carcass provides information about the

bioavailability of the radiolabelled vitamers (Johansson and Tiselius, 1973). The intestinal perfusion technique, used in both animals and humans, involves the infusion of a test substance at some point in the jejunum, the aspiration of the luminal contents at some distal point and the analysis of the luminal contents. A direct in vivo measurement of the selected uptake of vitamin B-6 is possible by this method. Using an intestinal perfusion technique, Nelson et al. (1977) suggested that a low-molecular-weight binder might be responsible for the reduced absorption (50% compared to crystalline PN) of vitamin B-6 from orange juice infused into the jejunum in humans.

An early study in humans, suggesting that some of the vitamin B-6 in the diet was not absorbed, was conducted by Harding et al.(1959). In their study, 9 men developed a mild B-6 deficiency while receiving canned combat rations (1.9 mg of vitamin B-6/day), which had been stored at 100° F for 20 months. The majority of vitamin B-6 bioavailability studies in humans involve metabolic studies which examine intake and output. Human metabolic vitamin B-6 studies provide specific quantities of vitamin B-6 to subjects in a controlled environment. Blood, urine and fecal samples are collected and analyzed to assess the metabolism and excretion of vitamin B-6 and its metabolites.

A series of human metabolic studies have examined vitamin B-6 bioavailability and fiber content of the diet. Leklem et al. (1980) compared the bioavailability of vitamin B-6 from wheat bread versus white bread in men. Each bread contained 75% of the 1.6 mg of vitamin B-6 provided to each subject through diet. Subjects received each bread for one week. Urinary and fecal data indicated that vitamin B-6 was 5-10% less available from whole wheat bread than from the white bread (Leklem et al., 1980). The researchers concluded that dietary fiber may slow the contact of nutrients with the mucosa and therefore limit absorption and utilization. To examine this point, Lindberg et al. (1983) studied the bioavailability of vitamin B-6 from a diet containing 1.7 mg B-6/day with or without 15 g of wheat bran/day. The addition of wheat bran to the diet resulted in an increase in fecal B-6 excretion, a decrease in urinary 4-PA excretion and a decrease in plasma PLP levels. The

addition of wheat bran to the diet therefore resulted in reduced vitamin B-6 bioavailability. A study in women to test the effect of the bran type on B-6 bioavailability was conducted by Kies et al. (1984). A diet containing either 20 g of wheat, rice or corn bran was provided in bread and was given for one week. Using only urinary B-6 excretion as a measure of vitamin B-6 bioavailability, these researchers calculated total dietary vitamin B-6 bioavailability to be 60-65% when all types of bran were fed. Foods representing the typical American diet have been estimated, using a dose-response curve based on plasma PLP and urinary B-6 concentration, to be 71-79% available with respect to vitamin B-6 (Tarr et al., 1981). Studies in animals using an intestinal perfusion technique have been unable to find an effect of purified dietary fiber on PN bioavailability in bioassays (Gregory and Litherland, 1986; Nguyen et al., 1983). A food component other than fiber may be influencing vitamin B-6 bioavailability. Today, it is generally accepted that this dietary component is PN-glucoside.

Original work in Leklem's lab helped to identify PN-glucoside as a primary factor influencing vitamin B-6 bioavailability. Kabir et al.(1983a) examined the bioavailability of vitamin B-6 (1.6 mg/day) from a diet where 50% of the vitamin B-6 content was provided by tuna, whole wheat bread or peanut butter. Urine was analyzed for 4-PA excretion, feces were analyzed for total vitamin B-6 excretion and plasma was analyzed for PLP concentration. Assuming the vitamin B-6 content of tuna was 100% available, they calculated the bioavailability of wheat bread and peanut butter to be 75% and 63% respectively. Regression analysis of the vitamin B-6 bioavailability and the PN-glucoside content of these foods (Kabir et al., 1983b) resulted in a significant relationship between the PN-glucoside content and B-6 bioavailability. Subsequent studies by Leklem have confirmed the relationship between PN-glucoside content of a diet and vitamin B-6 bioavailability (Shultz and Leklem, 1987; Hansen et al., 1992). Similarly, studies in rats using purified PN-glucoside have found low (20-30%) vitamin B-6 bioavailability (Ink et al., 1986; Trumbo and Gregory, 1988, 1989, Trumbo et al. 1988).

Controversy exists in the literature as to the bioavailability of PN-glucoside. In rats, the hydrolysis of the glycosidic bond in vivo appears to be the limiting step in the utilization of PN-glucoside, rather than intestinal absorption (Ink et al., 1986; Trumbo and Gregory, 1988, Trumbo et al. 1988). Tsuji et al. (1977) reported that synthetic PN-glucoside exhibited vitamin B-6 activity which was essentially equivalent to that of PN when administered orally or intravenously to rats, as indicated by the tryptophan load test and the activity of various PLP-dependent enzymes. Gregory et al. (1991) has used stable isotopes to demonstrate that the incomplete bioavailability of PN-glucoside is substantially higher in humans than previously found in rats (Ink et al., 1986; Trumbo and Gregory 1988, 1989, Trumbo et al., 1988). The mean bioavailability of orally administered deuterated PN-glucoside (9.6 mmol PN) was shown by Gregory et al. (1991) to be 58%, relative to free PN. In summary, factors such as the amount of PN-glucoside present in a food, the diet composition, and the degree of food processing, storage and cooking all influence the absorption of vitamin B-6 by the body.

Metabolism and Interconversions

Several organs throughout the body are involved in the metabolism and interconversion of vitamin B-6. As stated previously, the primary forms of vitamin B-6 which are absorbed (PL, PN and PM) are the primary forms leaving the intestinal cell for the portal circulation (Henderson, 1985).While the intestinal cell can interconvert the three primary forms of vitamin B-6, the significance of this pathway appears minimal (Henderson, 1985). Metabolic trapping can occur within the intestinal cell by the action of cytoplasmic pyridoxal kinase (Middleton, 1984). The subsequent dephosphorylation enables the vitamer to leave the intestinal basolateral membrane by a passive diffusion process (Henderson, 1985).

Liver

The liver is the primary organ responsible for the metabolism and interconversion of the three primary forms of vitamin B-6 (Lumeng and Li, 1980; Lumeng et al., 1974a). A major function of the liver with regard to B-6 metabolism is to supply the body with PLP, the active coenzyme form of vitamin B-6. Conversion of PL, PN and PM to PLP is therefore important. Figure 2 depicts the metabolic interconversions of the three primary

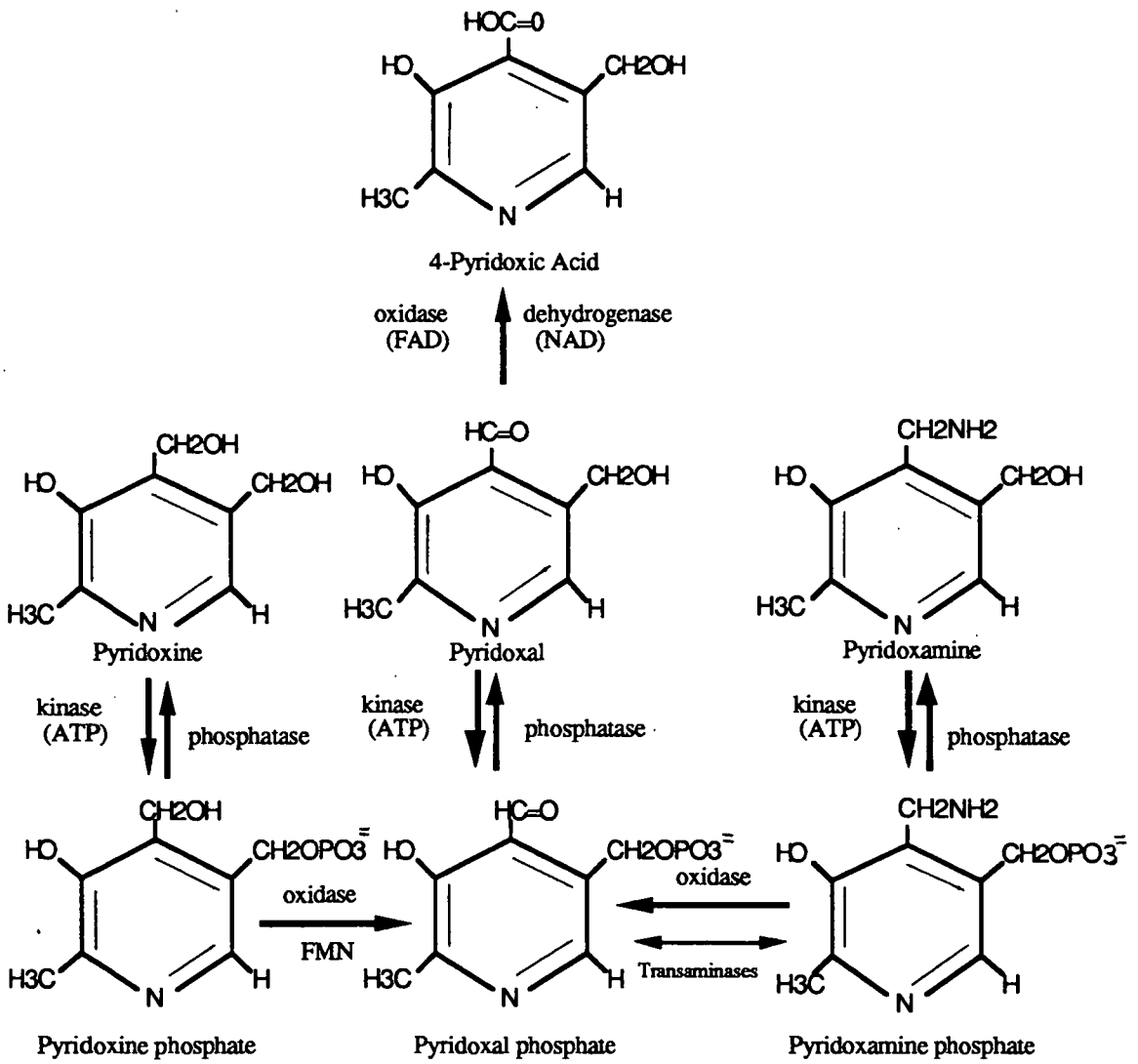

Figure 2. Metabolic interconversions of the forms of vitamin B-6. (Adapted from Leklem, 1988)

forms of B-6 in the liver. All three forms can be phosphorylated at the 5' position by pyridoxal kinase (EC2.7.1.35). Pyridoxal kinase requires zinc and ATP for phosphorylation. Phosphorylation of the primary B-6 vitamers serves as a form of metabolic trapping within the liver. The resulting forms of vitamin B-6 are PNP, PLP and PMP. PNP and PMP can be converted to PLP by a flavin mononucleotide (FMN) requiring pyridoxine (pyridoxamine) 5'-phosphate oxidase (Wada and Snell, 1961) (EC 1.4.3.5). The three phosphorylated forms of vitamin B-6 (PNP, PLP and PMP) can be dephosphorylated to their original forms (PN, PL and PM) via a phosphatase enzyme. Studies by Li et al. (1974) and Lumeng and Li (1975) in rats concluded that the phosphatase involved was probably the membrane-bound alkaline phosphatase. Merrill et al. (1984) confirmed the presence of alkaline phosphatase in human liver. Liver PL (from dephosphorylation of PLP or from PL in circulation) can be irreversibly converted to 4-pyridoxic acid by either a FAD-dependent aldehyde oxidase or a NAD-dependent aldehyde dehydrogenase. In human liver, only the activity of aldehyde oxidase has been detected (Merrill et al., 1984). 4-pyridoxic acid is released from the liver and is excreted in the urine. The majority of consumed vitamin B-6 is excreted as 4-PA (Brown et al., 1975; Wozenski et al., 1980; Shultz and Leklem, 1981)

The liver enzymes that participate in the interconversion of the B-6 vitamers are involved in regulating the rate of vitamin B-6 metabolism. The first quantitative analyses of the enzymes responsible for metabolizing vitamin B-6 in the human liver were conducted by Merrill et al. (1984) and subsequently updated in 1990 (Merrill and Henderson, 1990). The rates of phosphorylation of PN and PL were greater than the dephosphorylation. Under conditions approximating those in vivo (pH=7), the activity of PL kinase was 11 nmol/min/g liver whereas the activity of PLP phosphatase was found to be approximately 2 nmol/min/g liver tissue (Fonda, 1987). The rate of oxidation of PL to 4-PA was similar to that for phosphorylation (approximately 16.5 nmol/min/g liver). Therefore, a substantial portion of PL was rapidly converted to 4-PA. Studies in humans by Wozenski et al. (1980)

demonstrated that a 19.45 μmole dose of PL produced a rapid rise in urinary 4-PA (from 0.2 μmoles/hr to over 1.6 μmoles/hr) by 3 hours. The metabolism of PL to 4-PA may prevent large amounts of the highly reactive PLP from accumulating (Leklem, 1991). The phosphorylation of PN and PM to the 5'-phosphates was somewhat slower than the conversion of PNP and PMP to PLP via the oxidase. PNP(PMP) oxidase is known to be sensitive to product inhibition, therefore excess PLP could slow the conversion of PMP and PNP to PLP and effectively reduce the waste of PLP through excessive production.

Describing vitamin B-6 metabolism based solely on liver enzyme activity may lead to erroneous results. Wozenski et al. (1980) demonstrated that equimolar doses of PL, PN or PM (19.45 μmoles) produced lower urinary B-6, 4-PA and plasma PLP levels when PM was given compared to PL or PN, indicating either slower absorption and/or metabolism of PM. Similarly, Merrill et al.(1984) found a sixfold increase in the enzyme activity of PNP(PMP) oxidase with PNP as the substrate compared to PMP. Transaminases, which use PMP as a cofactor to produce PLP, may influence the enzyme activity of PMP oxidase in the conversion of PMP to PLP. This would explain the difference in vitamin B-6 vitamer metabolism despite similar enzyme systems. Competition between enzymes for substrate, product inhibition of the enzymes involved in the conversion of the vitamin B-6 vitamers and the binding of product (i.e., PLP) to proteins may help explain the differences seen between the metabolism of the vitamin B-6 vitamers.

Plasma

The PLP formed in the liver can participate in numerous enzymatic reactions within the liver or can be released into circulation (Lumeng et al., 1974a). The plasma is involved with the transport of the B-6 vitamers to cells. Under fasting conditions, PL and PLP account for 70-90% of the total B-6 vitamers in circulation, with PLP comprising 50-75% of the total amount (Leklem, 1991). PN, PMP and PM are the next most abundant forms of vitamin B-6 in circulation, respectively. PNP is essentially absent from fasting plasma.

Phosphorylated forms typically do not cross cell membranes in most tissues. However, the liver is able to release PLP directly into circulation without dephosphorylation. The majority of PLP present in circulation is bound to the protein albumin through the formation of a Schiff base. The site where the initial binding of PLP to albumin occurs has not yet been established (liver vs. plasma). Albumin acts to protect PLP from hydrolysis while in transit to cells. Studies with PLP and albumin in bovine (Dempsey and Christensen, 1962) and human (Lumeng et al., 1974a) serum have shown the tight binding of PLP to albumin and the weak binding to globulin. PL is only partially bound to albumin in circulation; the majority is found as the free form. The phosphatases which are bound to cellular membranes are thought to hydrolyze the PLP and allow for the entry of PL into the cell. Animal tracer studies by Coburn et al. (1992) indicated no difference in the uptake of PL and PLP from the plasma by cells. In cells, PL is rephosphorylated to PLP and bound to enzymes, effectively trapping or storing the vitamin (Lumeng and Li, 1980; Li et al., 1974).

Red Blood Cells

Red blood cells (RBCs) are actively involved in vitamin B-6 metabolism and transport. While the interconversion of the vitamin B-6 vitamers is known to occur in RBCs, the physiological implications of these conversions are uncertain. The RBC has been viewed both as a "factory" for the production of PL and as a "reservoir" for maintaining a constant PL ratio between red cells and plasma (Anderson, 1980).

The metabolism of the vitamin B-6 vitamers in red blood cells has been systematically studied in vitro and verified in vivo. RBC's readily take up PL and PN from the circulation by simple diffusion (Mehansho and Henderson, 1980). PM is also taken up by red blood cells, but at a considerably slower rate than PN or PL. To study the rate of uptake of B-6 into RBCs, Yamada and Tsuji (1968) incubated red blood cells with 1 μg/mL of PL, PN or PM. After 30 minutes, approximately 0.42, 0.28 and 0.18 μg/mL of

PL, PN and PM, respectively, were present in the RBCs, indicating an uptake primarily of PL and PN. Red blood cells incubated with increasing amounts of PN (25-1000 ng/mL) took up 40-50% of the PN by 1 minute, indicating rapid uptake of PN (Anderson, 1980). Only when the concentration of PN was reduced by conversion to PL and PLP could more PN be taken up by the red cell (Anderson, 1980). The incubation of blood with >2000 ng/mL PN produced a consistent rate of conversion to PL and PLP with increasing amounts of PN (Anderson, 1980). Similarly, the incubation of blood with 500 ng/mL of PL for 1 minute rapidly produced a RBC PL: plasma PL ratio of 2:1. The ratio was maintained over the course of the 2 hour study (Anderson et al., 1971). Since the majority of PL in circulation is present as PLP bound to albumin, the uptake of PLP into RBCs was examined. Anderson et al.(1971) incubated whole blood with 200-2000 ng/mL of PLP and found no significant uptake of PLP by the red cell. However, the incubation of whole blood with 500 ng/mL of PNP did result in an uptake of PNP into the red cell. While the uptake of PNP by RBCs can occur with large doses, this may be of limited physiological significance.

The interconversion of the B-6 vitamers within RBCs has been studied extensively. The findings of Anderson et al.(1971) suggest that the PN (and PL) taken up by the RBC is converted to PLP and then to PL, which is released into the plasma. The proposed pathway is similar to that seen in the liver (Wada et al., 1959). The presence of RBC PL kinase and PNP(PMP) oxidase support the existence of this pathway (Hamfelt, 1967; Yamada and Tsuji, 1968; Lumeng et al., 1974a). Likewise, the PM taken up by RBCs can result in the formation of PLP, but at a much slower rate than PN or PL (Hamfelt, 1967; Yamada and Tsuji, 1968). Anderson et al. (1989) observed that an intravenous injection of 48.6 or 118 μmol of PN in healthy female subjects resulted in 19.6% and 11.2% of the dose, respectively, taken up by the RBCs after just 1 minute. By 3 minutes, similar amounts of PN had cleared from the RBCs for either dose (4.59 and 4.30 μmol, respectively). A small percent (1.8% and 1%, respectively) of the PN which disappeared

from the RBC by 3 minutes could be accounted for by conversion to PLP and PL within RBCs. However, the conversion of PN to PLP and PL rose steadily for 10 minutes after each dose. Therefore, the RBC rapidly takes up PN from circulation, converts a portion to PLP and PL and also releases PN back into circulation. The amount of PN leaving the RBC and entering the plasma within minutes after injection led the authors to hypothesize that the RBC may be transporting PN to other metabolic sites. These results may be important in vitamin B-6 supplementation, which is typically as PN-HCL.

The functional significance of the conversion of the vitamin B-6 vitamers to PLP and PL in red blood cells has not yet been established. The red blood cell may play a role in the production of PL for tissues lacking in the oxidase enzyme, such as muscle. The availability of PL and PLP for cofactor activities may be limited in muscle without such a conversion. In the RBC, the nonspecific binding of PL and PLP to hemoglobin may account for the limitation in net cofactor activity (Solomon, 1982).

In red blood cells, PL and PLP are bound to hemoglobin at distinct sites on the hemoglobin molecule (Fonda and Harker, 1982; Ink et al., 1982). PL is bound to the α-chain of hemoglobin and PLP is bound to the β-chain of hemoglobin (Ink et al., 1982). PL is bound tighter to hemoglobin in the RBC than to albumin in the plasma (Ink and Henderson, 1984a), resulting in a concentration of PL in RBCs 4-5 times higher than in plasma (Ink and Henderson, 1984b). Within the RBC, PLP is bound more tightly to hemoglobin than is PL (Benesch et al., 1973). PLP binds to the same site on the hemoglobin molecule as 2,3-diphosphoglycerate (DPG) (a compound produced in erythrocytes during anaerobic glycolysis) and ATP. Both DPG and ATP lower the oxygen affinity of hemoglobin and facilitate oxygen unloading (Benesch et al., 1969,1972; Suzue and Tachibana, 1970; Perutz, 1970; Gibson, 1970). PLP has also been shown to have this property (Chanutin and Curnish, 1967; Benesch et al., 1969; Maeda et al., 1976). The binding of PL to the α-chain of hemoglobin increases the oxygen binding capacity, whereas the binding of PLP to the β-chain of hemoglobin lowers oxygen binding affinity

(Kark et al., 1982; Benesch et al., 1977; Maeda et al., 1976). Unfortunately, therapeutic doses of PN have not been found to lower oxygen affinity in vivo (Anderson, 1980). Anderson (1980) believes that the high concentration of DPG (several thousand times more than PLP) in the RBC competes for the binding of PLP to hemoglobin, resulting in more PLP available to be dephosphorylated by the phosphatase and reducing the oxygen lowering capacity of PLP. The binding characteristics of PLP and PL may be of physiological significance in sickle cell anemia (Reynolds and Natta, 1985). The functional significance of the binding of PLP to the hemoglobin molecule at physiological levels is unknown.

Storage

Excessive intakes of water-soluble vitamins are thought to be excreted, not stored by the body. Vitamin B-6 appears to be an exception. The concept of storage compartments for vitamin B-6 in the body has helped describe the turnover and storage of this vitamin. Isotopic studies by Johansson et al.(1966) in the rat promoted the idea of a two compartment model of vitamin B-6 storage and turnover. A larger pool with a slower vitamin B-6 turnover was believed to be in equilibrium with a smaller pool with a more rapid vitamin B-6 turnover. An application of this model was a study by Coburn et al. (1984) using the long-term administration of labeled pyridoxine. The predicted intake of vitamin B-6 appeared too high for a normal diet (18 μmol/day), based on the two compartment model of vitamin B-6 storage. Subsequent long-term tracer studies by Coburn et al. in rats (1989b), guinea pigs (1984) and swine (1985) were unable to predict body pools using the two compartment model. Coburn believed a slower, third storage compartment must exist. Animals raised on labeled PN were able to produce uniform labeling of body vitamin B-6 stores after 140 days, suggesting the existence of a third, very slow B-6 turnover compartment (Coburn et al., 1989a). Predictions of total body vitamin B-6 content (15 nmol/g) from these animal studies have been verified in humans using

muscle biopsies (Coburn et al., 1988). Further examination of vitamin B-6 pools in rats using the Simulation, Analysis and Modeling program found that even 75 pools were insufficient to describe all the vitamin B-6 vitamers in every tissue (Coburn and Townsend, 1989).

The major vitamin B-6 pool in the body is in the muscle. Although the liver contains the greatest concentration of vitamin B-6, the muscle, by sheer mass, represents the largest storage site (Li and Lumeng, 1981, Coburn et al., 1991). Glycogen phosphorylase, the enzyme responsible for the breakdown of glycogen to glucose in muscle and liver, is the major storage site for PLP in muscle (Coburn et al., 1988; Palm et al., 1990; Cori and Illingsworth, 1957; Black et al., 1977,1978). Sixty percent of the vitamin B-6 present in rat muscle and 75-96% of that present in mouse muscle is associated with glycogen phosphorylase (Krebs and Fischer, 1964). Black et al. (1977) found an increase in muscle glycogen phosphorylase content and PLP levels of skeletal muscle in growing rats fed a diet high in vitamin B-6 (70 mg of vitamin B-6/kg of diet) relative to a control situation. These rats were then fed a diet deficient in vitamin B-6 for 8 weeks. The muscle glycogen phosphorylase content and muscle PLP did not decrease until a caloric deficit was also present (Black et al., 1978). These researchers assumed that a decrease in glycogen phosphorylase content was concomitant with the release of PLP from the enzyme. They concluded that the B-6 stored in muscle was not utilized in times of low vitamin B-6 intake unless a caloric deficit also existed. The marked increase in muscle PLP and glycogen phosphorylase content during vitamin B-6 supplementation may have been due in part to the rapid growth the rats were experiencing. The findings by Black et al. (1977,1978) supported previous research that muscle is a storage site for vitamin B-6. A recent study in rats fed excess PN(1400 mg/kg diet) found a statistically significant increase in gastrocnemius muscle glycogen phosphorylase *a* activity (Schaeffer et al., 1989). Studies examining human muscle under variable conditions of vitamin B-6 nutriture have been limited. Coburn et al. (1991) has studied the vitamin B-6 content of human muscles in

response to a low B-6 diet (1.76 μmol/d) for 6 weeks followed by a self-selected diet supplemented with 0.98 mmol pyridoxine HCL/day. Although statistical significance was not attained, this level of vitamin B-6 supplementation produced a 19% increase in muscle vitamin B-6 PLP from baseline levels. The vitamin B-6 depletion diet did not alter muscle B-6 from baseline levels, similar to the findings in rats by Black et al. (1978). A significant decrease in muscle glycogen phosphorylase activity (Coburn et al., 1991) from baseline levels during the B-6 supplementation phase was evident. The decrease could have been due to an increase in physical activity by the subjects. No mention of controlling for physical activity was made in this paper. Therefore, as was seen in rats, vitamin B-6 supplementation in humans can increase PLP storage in muscle. The physiological significance of this has yet to be established.

Excretion

Vitamins are excreted in the urine, feces, sweat and other bodily fluids. Knowledge of the excretion of vitamin B-6 provides insight into the metabolism of vitamin B-6. Studies of vitamin B-6 metabolism have examined the excretion of urinary 4-pyridoxic acid, urinary vitamin B-6 and fecal vitamin B-6. Recently, HPLC techniques have been used to measure the excretion of individual vitamin B-6 vitamers in urine. Few studies have dealt with the excretion of vitamin B-6 in sweat.

Urine

A majority of studies examining vitamin B-6 metabolism measure urinary excretion of the irreversible end-product, 4-pyridoxic acid. Urinary 4-PA excretion is considered to be a short term, direct indicator of vitamin B-6 status. The production of 4-PA by the human liver was first examined by Merrill et al. (1984). They found that PL was rapidly oxidized to 4-PA in the liver (28.1 ±19.8 nmol/minute per gram of tissue) via aldehyde

oxidase. The conversion of the B-6 vitamers to PL from PLP in the liver appears to be the major control point regulating 4-PA production (Leklem, 1990). The contribution of other tissues to urinary 4-PA excretion is not known. Reddy et al. (1958) found that urinary 4-pyridoxic acid excretion accounted for only half of the vitamin B-6 intake of four subjects consuming a normal diet containing 7.3 μM vitamin B-6/day for six days but accounted for approximately all of the intake when a semisynthetic diet (14.6 μM vitamin B-6/day) was used. The semisynthetic diet may have been more bioavailable than the normal diet, therefore, bioavailability may be an issue in this study. A study by Lui et al.(1985) using daily vitamin B-6 intakes of 12 μmol orally or 121.6 μmol intravenously recovered 85-90% as urinary 4-PA. Urinary 4-PA excretion accounted for the majority of vitamin B-6 intake, regardless of route of administration. The major excretory route for vitamin B-6 in humans is therefore through urinary 4-PA excretion.

The amount of 4-PA excreted in the urine changes rapidly with intake. The rapid excretion of urinary 4-PA with increasing vitamin B-6 dose has been documented using timed urine collections. Wozenski et al. (1980) gave 5 men incremental doses of PN-HCL similar to the amount found in diet and supplements (0, 0.5, 1,2,4,10 mg PN-HCL). The rate of 4-PA excretion was maximal during the first three hours after the doses. As the PN dose increased, the mean 4-PA excretion increased from 5.4 μmol/24 hr to 19 μmol/24 hr. The percent of the PN dose recovered as 4-PA decreased from 84.8% to 34.5% with increasing dose. These authors suggested that as PN dose increased, either more PN was retained in the body or some was excreted as a metabolite which was not measured, such as 5-phosphate-4-pyridoxic acid. Recent studies by Mahuren et al. (1991) indicate that 5-pyridoxic acid and 5-pyridoxic acid lactone can comprise 10-20% of the urinary vitamin B-6 metabolites in an individual who consumed 823 mg vitamin B-6/day for approximately one year. Supplemental doses of PN (10, 25, 50 and 100 mg) given to women (Ubbink et al., 1987) likewise produced a constant fraction of the dose excreted as urinary 4-PA. However, when 100 mg. of PN was given, a higher percent of the dose was excreted as 4-

PA than was excreted with the lower doses (60% vs 43%). Ubbink et al. (1987) also analyzed urinary PN and PL excretion using HPLC. They found an increase in the percent of PN excreted in the urine (3.2% to 9.3%) with an increase in PN dose, but a relatively constant excretion of PL (<2%). The binding of PL to albumin or renal reabsorption may account for the low levels of PL urinary excretion. The relatively low excretion of PN indicates effective metabolism of PN.

The form of vitamin B-6 ingested influences B-6 metabolism and therefore 4-PA excretion. Studies have shown that the ingestion of PL results in a greater conversion to 4-PA than the ingestion of either PN or PM (Wozenski et al., 1980; Leklem, 1988). A diet rich in PL (found primarily in animal products) would presumably result in a greater proportion of the total vitamin B-6 content converted to 4-PA than a diet containing primarily PN or PM (found in vegetable sources). As mentioned previously, the bioavailability of vitamin B-6 from a diet containing plant foods is limited by the presence of PN-glucoside. A decreased excretion of 4-PA from diets containing PN-glucoside versus the control condition has been documented (Gregory et al., 1991; Kabir et al., 1983a).

Dietary factors other than B-6 intake have been shown to influence 4-PA excretion. The effect of protein intake on vitamin B-6 metabolism has been studied by Miller et al.(1985). A semipurified diet containing increasing amounts of protein (0.5, 1.0 and 2.0 g/kg body weight/day) and a constant vitamin B-6 intake (1.6 mg/d) was fed to eight men for 15 days each. As dietary protein content increased, urinary 4-PA excretion decreased (from approximately 4.5 µmol/24 hr to 2.8 µmol/24 hr, respectively). A high protein diet appears to promote the retention of vitamin B-6 in the body, presumably for the increased catabolism of amino acids. PL kinase, involved in the conversion of PL, PM and PN to the phosphorylated forms, is a zinc requiring enzyme. Therefore, low zinc status could impair PL kinase activity and, ultimately, the formation of 4-PA. Riboflavin is a cofactor for PNP

oxidase and aldehyde oxidase. Low intakes of riboflavin could limit the conversion of the vitamin B-6 vitamers to 4-PA, lowering urinary 4-PA levels.

Another urinary measure used to monitor vitamin B-6 metabolism is urinary vitamin B-6 (UB-6). Urinary vitamin B-6(UB-6) measures all forms of vitamin B-6, including the phosphorylated and glycosylated forms, excreted in the urine. Although urinary vitamin B-6 is considered to be a direct indicator of vitamin B-6 status, the excretion of vitamin B-6 in the urine accounts for only 8-10% of the daily intake (Leklem, 1990). Only a few of the researchers mentioned above who measured 4-PA have also measured UB-6 (Miller et al., 1985; Kabir et al., 1983a; Wozenski et al., 1980; Leklem, 1988). Typical urinary excretion concentrations of vitamin B-6 with a vitamin B-6 intake of 1.5-2.3 mg/d ranges from 0.8 to 1.1 μmol/d. Protein intake has not been shown to affect urinary vitamin B-6 excretion to the same extent as 4-PA excretion (Miller et al., 1985). Urinary vitamin B-6 is considered to be a sensitive indicator of vitamin B-6 status only when B-6 intake is very low (Kelsay et al., 1968).

Feces

Fecal vitamin B-6 excretion appears to be a minor route for vitamin B-6 excretion. The amount of fecal vitamin B-6 is related to the composition of the diet, specifically, the presence of PN-glucoside. Single-dose tracer studies in humans indicate that fecal vitamin B-6 accounts for less than 3% of the original dose (Tillotson et al., 1966). Fecal vitamin B-6 content is presumed to originate from unabsorbed dietary vitamin B-6 and intestinal microbial synthesis. The measurement of fecal vitamin B-6 may be useful in studies examining vitamin B-6 bioavailability. However, the contribution of intestinal microflora to the fecal vitamin B-6 content confounds the usefulness of fecal vitamin B-6 as a status indicator. A study by Kabir et al. (1983a) found an inverse relationship between the percent of urinary vitamin B-6 and 4-PA excreted and the percent of fecal vitamin B-6 with the consumption of whole wheat bread and peanut butter as compared to tuna. Fecal vitamin B-

6 excretion for tuna, whole wheat bread and peanut butter ranged from 2.72 µmol/d for adjustment period to 4.42 µmol/d for the peanut butter dietary period. Tuna and whole wheat bread contributed 3.08 and 3.80 µmol/d to the fecal vitamin B-6 excretion, respectively. The effect of wheat bran on the bioavailability of vitamin B-6 in 10 men was studied by Lindberg et al. (1983). The fecal vitamin B-6 content of a constant diet with or without the addition of 15 g of wheat bran was examined. The diet with the addition of bran contained 1.69 mg vitamin B-6 and the diet without wheat bran contained 1.66 mg vitamin B-6. Lindberg et al. found a consistently higher excretion of fecal vitamin B-6 during the last nine days of each 18 day period containing the wheat bran than without the bran indicating less bioavailability of vitamin B-6 in the diet when bran was added (3.97 and 3.72 µmol/24 hours versus 3.42 µmol/24 hours in group 1 and 3.58 versus 3.33 or 3.36 µmol/24 hours in group 2). Fecal vitamin B-6 content was similarly used by Leklem et al. (1980) to examine the bioavailability of vitamin B-6 from wheat bread in humans. Nine men consumed either wheat bread, white bread or white bread fortified with vitamin B-6 for a one week period. The subjects maintained a daily vitamin B-6 intake of 1.5 mg/day during each period from diet, bread and an oral supplement. The fecal vitamin B-6 content was similar when the white bread and white bread with the added vitamin B-6 were fed (17.7 and 18.3 µmol/week) compared to the wheat bread (28.4 µmol/week). Similarly, wheat bran appeared to decrease the vitamin B-6 bioavailability.

Sweat

Sweat is an often neglected source of vitamin loss. Although the loss of electrolytes in sweat can be considerable, it is generally accepted that losses of vitamins due to exercise or thermally induced sweating are minimal and rarely result in a significant vitamin deficiency (Brotherhood, 1984). The exact composition of human sweat has not yet been agreed upon in the literature. The primary component in sweat is water, followed by sodium and chloride, potassium, calcium and magnesium (Verde et al., 1982). The

amounts vary depending upon the testing conditions and collection methods. Tanaka et al. (1990) found that the electrolytes in sweat samples (sodium, potassium and chloride) can serve as a useful indice of exercise intensity. The most recent quantification of vitamin B-6 (PL) in sweat dates back to 1945 (0.002-0.049 μmol/100 mL; n=3), at which time pyridoxal was known only as "pseudopyridoxine" (Johnson et al., 1945). Pseudopyridoxine was determined with Streptococcus lactis R with the medium of Luckey et al. (1944) for the folic acid assay with the omission of pyridoxine and the addition of folic acid. Sweat PN was analyzed by a modified method of Atkin et al. (1943) using Saccharomyces carlsbergensis. The complete forms of vitamin B-6 were not yet fully understood. At that time, the sum of pyridoxal, 4-PA and pyridoxine was considered to be the total amount lost in sweat. Today, the complete forms of vitamin B-6 metabolites have been identified and newer methods of detection have been developed, yet the analysis of vitamin B-6 in sweat has not been repeated.

Functions

The active coenzyme form of vitamin B-6, PLP, is involved in numerous cellular processes and enzymatic reactions throughout the body. The involvement of PLP at the cellular level ultimately affects the functioning of bodily systems. The systems and functions influenced by PLP include gluconeogenesis, niacin formation, immune function, nervous system function, hormone modulation and red cell formation and metabolism . A majority of the metabolic processes vitamin B-6 is involved in are related to the role of PLP in amino acid metabolism. The involvement of PLP in amino acid, carbohydrate and lipid metabolism means PLP is ultimately involved in energy regulation. The major functions of PLP will be reviewed.

Gluconeogenesis

Our body maintains glucose supplies during times of inadequate carbohydrate intake by the pathways of gluconeogenesis. Gluconeogenesis is responsible for converting non-carbohydrates to glucose. Pyridoxal 5'-phosphate is involved in gluconeogenesis through its role as a cofactor for transamination reactions (Sauberlich, 1968). The production of glucose via glycogenolysis also involves PLP as a cofactor for glycogen phosphorylase (Krebs and Fisher, 1964). The association of PLP with glycogen phosphorylase has been mentioned previously. As a coenzyme for glycogen phosphorylase, PLP does not function as a classical Schiff base, but is bound as a Schiff base with the phosphate group probably involved in its actual coenzymatic role (Helmreich and Klein, 1980). The release of PLP from the muscle appears to occur when there is a need for gluconeogenesis. Rose et al. (1975) studied women consuming a diet low in vitamin B-6 content (0.2 mg B-6 per day) versus an adequate diet (1.8 mg vitamin B-6 per day) and found no significant alteration of fasting plasma glucose concentrations, but did find an impaired glucose tolerance with the diet low in vitamin B-6. While glucose production may not be influenced by a low vitamin B-6 intake, glucose metabolism probably is affected.

Erythrocyte Function

Vitamin B-6 provides a metabolic function within red blood cells. Erythrocyte transaminases (or aminotransferases) require PLP, bound as a Schiff base, to function. Transaminases generally remove the α-amino group from an amino acid and transfer it to an α-keto acid, forming the corresponding amino acid plus the α-keto derivative (Mathews and van Holde, 1990). Both erythrocyte aspartate (EAST) and alanine (EALT) aminotransferases require PLP for activity, and are therefore used as long-term indirect indicators of vitamin B-6 status (Sauberlich, 1981; Sauberlich et al., 1974). EALT

catalyzes the transfer of an amino group to pyruvate from alanine and EAST catalyzes the transfer of an amino group to oxaloacetate from aspartate.

PLP is also a cofactor for δ-aminolevulinic acid synthetase (Kikuchi et al., 1958), the enzyme that catalyzes the condensation between glycine and succinyl-CoA to δ-aminolevulinic acid which is a precursor in heme synthesis (Bottomley, 1983). Numerous reports in humans have documented anemias which are responsive to vitamin B-6 (Harris et al., 1956; Horrigan and Harris, 1968). A defect in δ-aminolevulinic acid synthetase known as sideroblastic anemia does not always respond to pyridoxine (Pasanen et al., 1982), suggesting a complex involvement of vitamin B-6 in anemia.

Niacin Formation

PLP is involved in the conversion of tryptophan to niacin in the liver (Brown, 1985). Vitamin B-6 functions as a coenzyme in the conversion of kynurenine to kynurenic acid, kynurenine to anthranilic acid, 3-hydroxy-kynurenine to xanthurenic acid and 3-hydroxy-kynurenine to 3-hydroxy-anthranilic acid. Kynureninase, the enzyme responsible for the conversion of 3-hydroxykynurenine to 3-hydroxyanthranilic acid, is the only PLP-dependent enzyme in the direct conversion of tryptophan to niacin. In a vitamin B-6 deficiency, this enzyme is not functional. The effect of a vitamin B-6 deficiency on the conversion of tryptophan to niacin has been examined by Leklem et al. (1975) in 15 oral contraceptive users. After 4 weeks of a low vitamin B-6 diet (0.19 mg PN/day), the total excretion of two metabolites of niacin (N'-methylnicotinamide and N'-methyl-2-pyridone-5-carboxamide) following a 2g L-tryptophan load test was approximately half that of when subjects received a PN-HCL supplement in addition to the diet of 0.8 or 2.0 mg vitamin B-6 per day. Therefore, the physiological significance of a diet low in vitamin B-6 is to moderately decrease the formation of niacin from tryptophan.

Lipid Metabolism

The involvement of vitamin B-6 in lipid metabolism to date remains controversial. A link between fat metabolism and vitamin B-6 was first suggested by Birch (1938). Subsequent studies in rats found that a vitamin B-6 deficiency resulted in a decrease in body fats (McHenry and Gauvin, 1938) and liver lipid levels (Audet and Lupien, 1974). Fat synthesis (primarily triglycerides) in the vitamin B-6 deficient rats, compared to the adequate vitamin B-6 condition, has been found to be greater (Sabo et al., 1971), normal (Desikachar and McHenry, 1954; Angel, 1975) or depressed (Angel and Song, 1973). Differences between the studies may be due in part to the meal pattern used to feed the animals (Angel and Song, 1973).

The metabolism of lipids may be impaired by vitamin B-6 deficiency. Studies in rats have shown an impairment in the conversion of linoleic to arachidonic acid (Witten and Holman, 1952; Cunnane et al., 1984; Delmore and Lupien, 1976). A study in humans of vitamin B-6 deficiency and fatty acid metabolism by Mueller and Iacono (1963) found only minor changes in fatty acid levels in plasma and erythrocytes, but interpreted the fatty acid pattern to support the findings of Witten and Holman (1952). Based on the observations of Delmore and Lupien (1976), the decrease in arachidonic acid in liver phospholipids and the increase in linoleic acid in vitamin B-6 deficient rats were theorized to originate from a decrease in phosphatidylcholine via methylation of phosphoethanolamine. Loo and Smith (1986) found the levels of S-adenosylmethionine (SAM) in the livers of vitamin B-6 deficient rats were nearly five times higher than in livers of pair-fed animals. The change in SAM is secondary to the inhibition of the catabolism of homocysteine, a PLP-dependent process. The negative feedback of SAM on the conversion of phosphatidyl ethanolamine to phosphatidyl choline may explain the changes seen in fatty acid metabolism (Leklem, 1991). A link between vitamin B-6, arachidonic acid and cholesterol metabolism remains controversial. Fincnam et al. (1987) have found a significant positive correlation between plasma PLP and HDL cholesterol, and a negative correlation with total cholesterol and LDL

cholesterol in monkeys fed an atherogenic Western diet and a "prudent" Western diet. A reduction in serum cholesterol, mainly in LDL cholesterol, of 0.8 mmol/L was seen in 34 subjects given 10 mg pyridoxine in a multivitamin (Serfontein and Ubbink, 1988). A primary effect of vitamin B-6 on lipid metabolism is in the synthesis of carnitine from lysine (Cunnane et al., 1984). Carnitine is a carrier molecule needed to move fatty acyl units into the mitochondria for oxidation. The indirect effect of vitamin B-6 on phospholipid and fatty acid metabolism and on the synthesis of carnitine appear to be the primary involvement of vitamin B-6 on lipid and fatty acid metabolism. Cho and Leklem (1990) found a decrease in total and free carnitine in the plasma, liver skeletal muscle, heart muscle and urine in rats fed a vitamin B-6 deficient diet for six weeks, and an increase in total and free carnitine concentrations after two weeks of repletion with 5.7 mg PM/kg. The researchers concluded that vitamin B-6 is required in the synthesis of carnitine.

Immune Function

Vitamin B-6 may also be involved in immune function. PLP serves as a cofactor for serine transhydroxymethylase (Schirch and Jenkins, 1964) which is an important enzyme in 1-carbon metabolism. The proper functioning of 1-carbon metabolism is required for normal nucleic acid synthesis. Therefore, adequate supplies of PLP may be needed for proper immune system functioning. Indeed, studies by Talbott et al. (1987) found impaired immune systems in 11 elderly females (who typically have low vitamin B-6 intakes). The subject's immune system improved (as evidenced by an improved lymphocyte response) with 50 mg pyridoxine per day for two months. Animal studies likewise support the need for vitamin B-6 in maintaining immune system functioning. A vitamin B-6 deficiency has been shown to adversely affect lymphocyte production and antibody response to antigens in animals (van den Berg et al., 1988; Chandra and Puri, 1985; Cheslock and McCully, 1960). Meydani et al. (1991) studied the effect of vitamin B-6 deficiency on immune status

in eight healthy elderly. They found that a vitamin B-6 deficiency (3 μg/kg/day for 20 days) impaired in vitro indices of cell-mediated immunity.

Hormone Modulation

The involvement of vitamin B-6 with hormone modulation has recently been investigated in steroid hormones. PLP reacts with lysine residues on the steroid receptor to form a Schiff base at two distinct areas: one at the steroid binding site on the receptor and one at the DNA binding domain on the receptor. The result of this interaction is a decreased expression of the steroid (Allgood and Cidlowski, 1991). In other words, the steroid action is depressed in the presence of PLP (Litwack et al, 1985). Physiological levels of PLP have been shown to react reversibly with receptors of estrogen, androgen, progesterone and glucocorticoids (Muldoon and Cidlowski, 1980; Hiipakka and Liao, 1980; Nishigori et al., 1978; DiSorbo et al., 1980). However, definitive studies in humans examining the physiological significance of this interaction have not yet been documented. The known involvement of vitamin B-6 in the immune and reproductive systems would suggest the involvement of vitamin B-6 in the hormones regulating these systems.

PLP is a cofactor for dopa-decarboxylase, the enzyme required for the conversion of L-dopa to dopamine. Dopamine is a precursor to growth hormone releasing hormone, a compound that stimulates the release of human growth hormone (hGH). The involvement of vitamin B-6 with hGH production and release will be discussed in detail later.

Nervous System

PLP is involved in the production of serotonin, a neurotransmitter, from tryptophan. PLP is also a cofactor for the neurotransmitters dopamine, norepinephrine, histamine, and aminobutyric acid (Dakshinamurti, 1982). The role of vitamin B-6 in the nervous system is well documented in both human and animal studies. As stated

previously, infants fed a formula where the vitamin B-6 has been destroyed during processing developed convulsions and abnormal EEG tracings (Coursin, 1954, 1969). In adults with a vitamin B-6 deficiency and high protein intake (100 g/day), abnormal EEG's have been documented (Canham et al., 1969). A long-term B-6 deficiency appears to be needed to alter EEG readings in adults (Leklem, 1991). Studies conducted during the development of the brain in rats have documented the role of vitamin B-6 in the nervous system. Myelination is reduced in the progeny of severely B-6 deficient female rats (Morre et al., 1978). Chang et al. (1981) observed that the total length of Purkinje cell dendrites was reduced in the offspring of rats with vitamin B-6 deficiency. Specific amino acid levels in certain areas of the brain are also altered with vitamin B-6 deficiency (Wasynczuk et al., 1983a). Glycine, leucine, isoleucine, valine and cystathionine were elevated, whereas alanine and serine levels were reduced. Groziak et al. (1984) have shown that a vitamin B-6 deficiency effects areas such as the corpus striatum and cerebellum more than the spinal cord and hypothalamus, indicating some degree of metabolic trapping of PLP in the spinal cord and hypothalamus.

Status Indicators

The importance of vitamin B-6 to human nutrition has been established. Our increasing knowledge of vitamin B-6 intake through nutritional surveys and nutrient databases has necessitated the establishment of accepted indicators of vitamin B-6 status.

Vitamin B-6 status indicators can be classified as either direct or indirect. Leklem (1990) has reviewed the current vitamin B-6 status indicators and has suggested values for adequate status (see Table 2). Direct indicators of vitamin B-6 status include the measurement of plasma PLP, plasma PL, plasma vitamin B-6, and erythrocyte PLP in the blood or 4-PA and vitamin B-6 in the urine. These include the major vitamin B-6 vitamers and their metabolic end-products. Indirect indicators of vitamin B-6 status include erythrocyte alanine and aspartic transaminase indexes and urinary xanthurenic acid (after a

Table 2. Indices for evaluating vitamin B-6 status and suggested values for adequate status in adults.

Indices	Suggested value for adequate status
Direct	
Blood	
Plasma pyridoxal 5'-phosphate	>30 nmol/L
Plasma pyridoxal	NV
Plasma total vitamin B-6	>40 nmol/L
Erythrocyte pyridoxal 5'-phosphate	NV
Urine	
4-Pyridoxic acid	>3.0 µmol/d
Total vitamin B-6	>0.5 µmol/d
Indirect	
Blood	
Erythrocyte alanine transaminase index	<1.25
Erythrocyte aspartic transaminase index	<1.80
Urine	
2g Tryptophan load; xanthurenic acid	<65 µmol/d
3g Methionine load; cystathionine	<350 µmol/d
Diet intake	
Vitamin B-6 intake, weekly average	>1.2-1.5 mg/d
Vitamin B-6: protein ratio	>0.020
Other	
Electroencephalogram pattern	NV

NV= no value established; limited data available
Adapted from: Leklem, 1990.

2g tryptophan load) and cystathionine (after a 3g methionine load), and oxalate excretion. These indirect measures focus on metabolites of metabolic pathways in which PLP is required or on the activities of PLP dependent enzymes. Dietary intake of vitamin B-6, by itself, appears to be a poor indicator of vitamin B-6 status. Leklem (1990) feels that a minimum of three indices should be measured to adequately assess vitamin B-6 status.

These include a direct indicator, such as plasma PLP, a short term indicator such as urinary 4-PA and an indirect indicator such as erythrocyte transaminase.

Plasma PLP is considered to be one of the most reliable B-6 status indicators (Leklem, 1990). In rats, plasma and tissue levels of PLP are highly correlated (Pogell, 1958; Lumeng et al., 1985). In humans, plasma PLP is significantly correlated with dietary B-6 intake (Shultz and Leklem, 1981). Therefore, it follows that dietary vitamin B-6 intake is probably correlated with tissue PLP levels. Protein intake, in addition to vitamin B-6 intake, has been shown to influence the fasting plasma PLP level (Shultz and Leklem, 1981). Miller et al. (1985) have found an inverse relationship between protein intake and plasma PLP and vitamin B-6 concentration in men fed 0.5 to 2g protein/kg/day. Plasma PLP values reported from numerous studies in the literature range from 27 to 75 nmol/L for males and 26 to 93 for females (Leklem, 1991). However, this may not represent the normal range, as intake was either controlled or not measured in some of the studies. Plasma PLP, aside from being influenced by vitamin B-6 and protein intake, is influenced by age (Rose et al., 1976; Lee and Leklem, 1985; Hamfelt and Soderhjelm, 1988), exercise (Leklem, 1985) and pregnancy (Lumeng et al., 1974b). Rose et al. (1976) found a general decline in plasma PLP levels with age, although the mechanism remains illusive. Exercise acutely increased plasma PLP levels (Leklem and Shultz, 1983; Hatcher et al., 1982), and pregnancy lowered plasma PLP levels (Barnard et al., 1987).

Since plasma PLP crosses cell membranes as PL, plasma PL has been proposed as an indicator of vitamin B-6 status. Plasma PL comprises 8-30% of total plasma vitamin B-6 (Leklem, 1990, 1988). To date, the extent that PL can be used as a valid indicator of vitamin B-6 status remains unanswered. However, the measurement of plasma PL is still encouraged. Plasma vitamin B-6 can be used, along with plasma PLP, to calculate the amount of plasma PL by difference, since plasma PLP and PL represent approximately 90% of the total plasma vitamin B-6 concentration.

Changes in vitamin B-6 intake are rapidly reflected in urinary 4-PA excretion (Brown et al., 1975; Leklem, 1988). There is an inverse relationship between protein intake and excretion of 4-PA (Miller et al., 1985). Knowledge of protein intake is therefore important when evaluating this status indicator also. Urinary vitamin B-6 is not felt to be a good indicator of vitamin B-6 status (Leklem, 1988). Incomplete urine collections can compound the error associated with the detection of UB-6.

Recommended Dietary Intake

Recommendations to the general public for safe and adequate levels of vitamin B-6 intake have been set forth in the recommended dietary allowances (RDAs) (National Research Council, 1989). Levels for adults, pregnancy and lactation, infants and children have been established. Biological indicators of deficiency are examined in an effort to establish a level of vitamin B-6 intake compatible with health for the majority of people. The 1989 RDA level for adult men is 2.0 mg/day and 1.6 mg/day for women. This is based on the upper boundary of protein intake for men (126 g/day) and women (100 g/day). A vitamin B-6 to protein ratio of 0.016 mg/g protein appears to produce acceptable vitamin B-6 status indices in adults of both sexes (National Research Council, 1989).

Exercise Physiology and Fuels

Our body utilizes energy to maintain daily functioning and to respond to physiologic demands. Food is an external source of potential energy for our bodies. We consume, digest, absorb and transport energy producing nutrients (carbohydrate, protein and fat) to the cells of our body. Together with oxygen, these nutrients provide a constant supply of energy. At the cellular level, enzymes and coenzymes participate in the transfer of this energy from food to a common "currency", adenosine triphosphate or ATP. Muscle transforms chemical energy into mechanical energy by means of ATP hydrolysis. The

complex machinery of muscle energy metabolism is designed to sustain the ATP concentration through various chemical processes that rephosphorylate ADP. The energy consumed from the diet will influence the energy supply available to cells to produce the ATP needed to perform exercise. The mix of dietary nutrients, the intensity and duration of exercise and the availability of oxygen to cells will all influence the fuel source and metabolic pathways used to supply energy to exercising muscle.

Aerobic and Anaerobic Exercise

The regulation of energy metabolism in the body is a sensitive mechanism. When exercise is initiated, the body must rely on available ATP. The body maintains only small amounts of ATP at one time (approximately 5 mmoles of ATP per kg muscle or a total of 3 ounces) (McArdle et al., 1986). A need for energy rapidly alters the concentration of ATP within the cell, which stimulates the recycling and production of ATP from energy substrates in an efficient, responsive manner. The enzyme ATPase catalyzes the hydrolysis of ATP to ADP with the release of 7.3 kcals of free energy per mole (McArdle et al., 1986). The release of energy from ATP does not require oxygen, therefore the ATP stored in cells provides an immediate source of energy when exercise begins. The ADP molecule which is produced is unable to cross cell membranes, therefore the resynthesis of ATP within cells is necessary. Cells also contain another high energy compound, creatine phosphate (CP) in a concentration three to five times greater than ATP. Creatine phosphate interacts with ADP to regenerate ATP and hence is called an energy reservoir. ATP and CP are only able to provide energy for a few seconds during maximal exercise (Bergstrom et al., 1971). Energy systems known as glycolysis (the breakdown of glucose for energy) and glycogenolysis (the breakdown of glycogen for energy) are utilized as exercise continues. Glucose from the blood or that within cells can be broken down in the cytoplasm to two pyruvate molecules. Likewise, glycogen, a glucose polymer stored in muscle cells, can also be broken down to two pyruvate molecules. Anaerobic glycolysis

yields a net of 2 ATP from the breakdown of glucose and anaerobic glycogenolysis yields 3 ATP from glycogen.

Exercise intensity will determine the availability of oxygen to cells, and the further progression of energy production. If the oxygen supply to cells is insufficient, as would occur with high intensity exercise, pyruvate combines with nicotinamide-adenine-dinucleotide (NAD) and excess hydrogen to form lactic acid. This reaction is catalyzed by the enzyme lactic dehydrogenase. The production of lactic acid is important in many respects. Lactic acid produced via glycolysis or glycogenolysis yields energy in the absence of oxygen (thus the term anaerobic), provides a temporary storage site for hydrogen, removes end-products of anaerobic glycolysis from the muscle to the blood and produces glucose and muscle glycogen via the Cori cycle once sufficient oxygen is provided.

As oxygen becomes increasingly available to cells, pyruvate (a three carbon compound) enters the mitochondria and forms acetyl-CoA (a two carbon compound) after the release of CO_2. This reaction appears to be irreversible. Acetyl-CoA enters the tricarboxlic acid cycle (TCA) by combining with oxaloacetate to form citrate. Fatty acid β-oxidation also produces acetyl-CoA. In this manner, fat enters the TCA cycle to produce energy aerobically. As exercise persists, fatty acids from stored adipose tissue are released into circulation and taken up by exercising muscle cells. The efficiency of the utilization of both fat and carbohydrate is approximately 40% (McArdle et al., 1986). Protein can be broken down to amino acids and enter the TCA cycle and is recognized as an important source of energy during exercise. Nitrogen must first be removed from the protein by the process of deamination. The production of energy for exercising muscles occurs primarily by the metabolism of carbohydrate and fat. The sequence of reactions that occur once acetyl-CoA enters the TCA cycle yields CO_2 and hydrogen atoms. These hydrogens progress through the electron transport chain in the mitochondria and ultimately combine with oxygen to form water. The requirement for oxygen in this final stage of energy transfer defines these reactions as aerobic (oxygen requiring). The energy produced from

aerobic mitochondrial oxidation yields 36 ATP. Therefore, the sum of anaerobic and aerobic metabolism of a molecule of glycogen yields 39 ATP. The complete oxidation of a fatty acid such as palmitate (C_{16}) yields 129 ATP, a concentrated source of energy.

Oxygen Consumption

As exercise begins, the body's oxygen requirement exceeds the ability to take in and deliver oxygen via the respiratory and circulatory systems. This lag time during the first 2-3 minutes of exercise where oxygen need exceeds supply has been termed the oxygen deficit. Not enough oxygen is supplied to meet energy demands, therefore anaerobic metabolism is initiated. The continuation of exercise at a submaximal level of intensity results in a steady state condition where the oxygen uptake equals the oxygen requirement of the tissues. Eventually, physiological responses to exercise such as dehydration, an elevation in body temperature and an increase in the utilization of free fatty acids (which require more oxygen per unit of energy yield than carbohydrates) contribute to fatigue and exercise ceases. If exercise intensity is maximal and adequate oxygen is not consumed to meet needs, there is a greater reliance on anaerobic metabolism and lactic acid accumulates. The resulting acidosis influences muscle tissues, respiration and other functions to contribute to fatigue. Following maximal exercise, there is a gradual recovery period, known as the oxygen debt, during which the lactic acid which has accumulated during exercise is transported primarily to the liver for the synthesis of glucose and glycogen via the Cori cycle and ATP and CP stores are replenished.

Oxygen uptake and utilization during exercise influence exercise tolerance and fuel utilization (Taylor et al., 1955). Robinson et al. (1937) were the first to identify maximal oxygen uptake as an important determinant of exercise performance. Factors which limit the maximal oxygen uptake during exercise include the delivery of oxygen centrally (which depends upon cardiac output and arterial oxygen content) and the peripheral extraction of oxygen at the tissue level. Combining the central and peripheral factors that influence VO_2

max led to the development of the Fick equation (VO_2 = Q(a-v diff); VO_2= oxygen uptake, Q= cardiac output, (a-v diff)= arteriovenous oxygen difference). VO_2 max therefore refers to an individual's maximal ability to take up oxygen. While a high VO_2 max means an individual has a high capacity for oxygen uptake, a high VO_2 max does not guarantee top performance. Performance also depends on technique, tactics, motivation, training and experience.

The measurement of VO_2 max is done through a VO_2 max test. The measurement of VO_2 max occurs under maximal effort exercise and is usually corrected for body weight (ml/kg/min.) to compare between individuals. A graded exercise test on either a treadmill or cycle ergometer are typically used to measure and individual's VO_2 max. The workload is systematically increased every few minutes until the subject is unable to continue or until a plateau in O_2 uptake occurs. At this point, known as VO_2 max, a further increase in workload will not result in an increase in oxygen uptake. The body has reached its maximal capacity to deliver oxygen to the muscles. The testing method may influence the resulting max VO_2. The VO_2 max has been reported to be 5-7% higher using a treadmill test compared to the cycle ergometer (Hermansen, 1973). Reasons for the higher VO_2 max with treadmill testing may include the involvement of a greater number of muscles and therefore greater oxygen demand during running as compared to cycling. A localization of muscle fatigue during cycling may also contribute to the early cessation of the test. Although some have found a difference in VO_2 max between testing methods (Hermansen and Saltin, 1969; Rowell, 1974; Hermansen, 1973), others have been unable to find a consistent difference (Astrand and Saltin, 1961). An individual's VO_2 max is dependent upon heredity, training state, gender, body composition and age (McArdle et al., 1986). Factors such as heredity, gender and age are not able to be manipulated by the researcher, but can be controlled within a study. Natural endowment or hereditary factors appear to be responsible for approximately 70% of an individual's VO_2 max (Bouchard and Malina, 1983). A gender difference is also evident. Males have been found to have a 15-30%

greater VO_2 max than females (Hermansen and Anderson, 1965). The degree to which age influences VO_2 max is controversial. The loss of muscle tissue resulting from aging can account for the decline of VO_2 max accompanying aging (Fleg and Lakatta, 1988). Current evidence indicates that the level of habitual exercise may be more important than age per se (Drinkwater et al., 1975; Upton et al., 1984; Vaccaro et al., 1981). The level of training is a controllable factor influencing aerobic capacity. Improvements in aerobic capacity with training generally range between 6-20%. Approximately 69% of the differences in max VO_2 scores among individuals can be explained by differences in body weight, 4% by differences in height and 1% by variations in lean body weight (Wyndham and Hegns, 1969). Some of the highest VO_2 max values reported so far are in male and female cross-country skiers (7.4 L/min. and 4.5 L/min. respectively) (Astrand and Rodahl, 1986). Typical max O_2 uptake in moderately well-trained males aged 20-30 are approximately 3-3.5 L/min. (Astrand and Christensen, 1964). A standard deviation of approximately 3% in VO_2 max has been found with repeated testing on a given individual (Astrand and Rodahl, 1986).

Fuel Sources

In the resting state, metabolic requirements are met by a combination of carbohydrate (42%), fat (41%) and protein (17%) (Knoebel, 1971). During exercise >60-65% VO_2 max and of short duration (< 60 minutes), carbohydrates provide the major energy source, although fats are also utilized. After approximately 60-120 minutes of continuous exercise, depending upon the level of exercise intensity, muscle glycogen is largely depleted and fat becomes the major energy source (Astrand and Rodahl, 1986; Coyle et al., 1986; Stanley et al., 1988). Current evidence suggests that certain amino acids, such as alanine and leucine are utilized for energy during exercise (Brooks, 1980; Lemon et al., 1979, 1980b, 1982; White and Brooks, 1981). Factors such as high exercise

intensity, long duration exercise, endurance training, decreased carbohydrate availability, and perhaps even the exercise environment appear to promote a greater amino acid oxidation (Lemon, 1987). The specific contributions to fuel utilization during exercise by carbohydrate, fat and protein will be discussed in the following sections.

Carbohydrates

Carbohydrate sources play a significant role in energy production during endurance exercise. Carbohydrates are stored as glycogen in the muscle (approximately 350 g) and liver (80-90 g) (Felig and Wahren, 1975). Blood glucose (approximately 20 g) also provides an energy source during exercise. From an endurance study involving treadmill marathon runners, O'Brien et al. (1993) concluded that carbohydrate, not fat, was the primary fuel during marathon running based on the examination of plasma glucose, free fatty acids, glycerol, lactate and respiratory gas exchange ratios. A direct relationship between carbohydrate oxidation and running speed was evident. The runners completed the race in less than 2 hours and 45 minutes at 73.3% VO_2 max indicating a high level of training and effort. The involvement of carbohydrate as an energy source during endurance exercise has similarly been demonstrated through the examination of muscle glycogen samples. Muscle glycogen content, as accessed by needle-biopsy samples, was found to drop approximately 75 mmol/kg/wet muscle during 90 minutes of exercise at 77% VO_2 max (Hermansen et al., 1967). Measurement of the carbohydrate involvement in energy production during exercise can also be accomplished through measuring the ratio of CO_2 produced to O_2 consumed. This ratio is known as the respiratory quotient (RQ)(Lewis and Gutin, 1973). Christensen and Hanson (1939) were some of the first to examine the participation of carbohydrate and fat in energy metabolism on the basis of the RQ during physical exercise of varying intensities. An RQ of 1.00 indicates that all of the oxygen consumed is used to oxidize the carbon in the carbohydrate molecule to CO_2, whereas an RQ value of 0.70 indicates that fat is the primary energy source (McArdle et al., 1986). The

RQ, in combination with techniques such as muscle biopsies and isotopically labeled substrate studies, provide critical information regarding carbohydrate utilization during exercise.

Factors which influence the extent of carbohydrate utilization during exercise include the type of exercise (intermittent vs. continuous), the state of training, prior diet (high fat vs. high carbohydrate) and state of health (i.e., the presence of a pathological condition) (Astrand and Rodahl, 1986). Rodahl et al. (1964) found a greater reliance on carbohydrate as a fuel source with short high intensity (10 minutes at an O_2 uptake of 2.4-2.7 liters/min) cycling compared with continuous cycling for 60 minutes at a moderate intensity (150 watts) as evidenced by elevated plasma lactate concentrations and depressed plasma free fatty acid concentrations. Based on a lower respiratory exchange ratio, blood lactate level, and net muscle glycogenolysis at given absolute work loads before and after training, endurance training may result in a shift in fuel utilization towards lipid dependence during sustained exercise (Gollnick, 1985). Prior diet has been demonstrated to influence muscle glycogen content. A regime known as glycogen loading is utilized by athletes to supercompensate muscle glycogen stores (Sherman, 1983). The technique involves the intense training for five or six days prior to competition, and then tapering while maintaining a high carbohydrate diet (>600 g/day). Such a regime can increase muscle glycogen stores 20-40% or more above normal concentrations (Sherman, 1983). Approximately six hours prior to competition, a high carbohydrate low fat meal consisting of 100-200 g carbohydrate will ensure that glycogen stores are saturated (Coyle et al., 1985). A 45% carbohydrate diet supplemented with a 1.1 g carbohydrate/kg body weight beverage (75g carbohydrate) one hour prior to cycling at 70% VO_2 max for 90 minutes was found to improve cycling performance 12.5%, presumably by enhancing carbohydrate oxidation (Sherman et al., 1991). The feeding of carbohydrate during exercise has also been shown to improve performance by extending the time to exhaustion. Coyle et al. (1986) exercised trained cyclists to exhaustion at 70-74% of VO_2 max and found a similar

depletion of muscle glycogen with and without ingestion of a 2g/kg glucose polymer drink. However, the athletes who consumed the drink were able to exercise longer. This study by Coyle indicates that highly trained endurance athletes are capable of oxidizing carbohydrate at relatively high rates from sources other than muscle glycogen (i.e., blood glucose) to prolong time to fatigue (Coyle et al., 1986).

The rate of breakdown of muscle glycogen is dependent on the activity of glycogen phosphorylase, which is present in two forms: the phosphorylase *a* form and the phosphorylase *b* form. Taylor et al. (1972) found that active men exercised to exhaustion on a bike ergometer at 70% VO_2 max had higher phosphorylase *a* activity (the enzyme responsible for the degradation of glycogen in aerobically exercised muscle) than untrained men exercised at the same level. The increase in phosphorylase *a* activity is suggestive of an increase in the concentration of muscle phosphorylase content and a rapid catabolism of skeletal muscle glycogen with exercise. The rate of muscle glycogen resynthesis following exhaustive exercise appears to be related to the muscle glycogen synthetase activity and carbohydrate content of the diet (Costill and Miller, 1980). The depletion of muscle glycogen through exercise results in a marked elevation of glycogen synthetase activity which subsequently facilitates muscle glycogen storage.

Lactic Acid

Lactic acid was once viewed as a metabolic waste product. Currently, lactic acid is viewed (1) as an energy source, (2) as a means of disposing of dietary carbohydrate and (3) as a building block for blood glucose and liver glycogen (Brooks, 1987). Two hypotheses are responsible for the positive view of the role of lactic acid in energy metabolism: the Glucose Paradox (Foster, 1984; Newgard et al., 1983) and the Lactic Acid Shuttle (Brooks, 1985, 1987).

The rate of appearance of lactic acid in the blood is a reflection of the rate of production and release of lactic acid and the rate of removal from the blood. The production

of lactic acid by the muscles is not limited to the anaerobic condition; lactic acid is formed and removed continuously and frequently at rapid rates, even at rest when adequate oxygenation of muscle occurs (Connett et al., 1984). The Glucose Paradox, developed by McGarry and associates (Newgard et al., 1983), describes the indirect route of liver glycogen production. Dietary carbohydrate is digested, absorbed and transported as glucose, not to the liver but directly to muscles where lactic acid is formed. The lactic acid that is formed travels back into circulation and reaches the liver to form glycogen. The use of isotopic tracers identified the role of lactic acid as an oxidizable fuel source both at rest and during exercise (Brooks et al., 1973; Brooks and Gaesser, 1980; Donovan and Brooks, 1983; Newgard et al., 1983; Stanley et al., 1985). The Lactic Acid Shuttle hypothesis explains how lactic acid production and removal are balanced during exercise and how this balance affects blood lactic acid concentrations (Brooks and Fahey, 1986). Lactic acid, formed at sites where glycogen and glucose are broken down at high rates, can reach sites where lactic acid can be used as fuel or can serve as a source for gluconeogenesis or glycogen resynthesis (Stanley et al., 1986). The level of blood lactic acid during exercise appears to depend primarily upon the duration and intensity of the exercise. The rapid rate of glycolysis during the initial phases of exercise results in an increase in blood lactic acid concentrations. During steady state submaximal exercise, the oxidative energy systems in the mitochondria of the muscles are activated, the need for rapid glycolysis declines and blood lactic acid concentrations decline (Brooks, 1986). Therefore, the lactic acid which is formed in active muscles during exercise can be effectively used for energy and for restoration of muscle and liver glycogen.

Fats

Fat provides a virtually unlimited supply of energy to the body. The energy reserve stored in fat has been estimated to be a minimum of 100,000 kcals or 15,000g of fat (Cahill and Owen, 1965). Therefore, even the leanest athlete possesses ample fat stores to fuel

hours of exercise. Fat is stored primarily as triglyceride, the primary form of dietary fat. Triglycerides are composed of a glycerol "backbone" and fatty acids attached through esterification. Triglycerides must be hydrolyzed to glycerol and free fatty acids (FFA) in order to be utilized for energy during exercise. The hydrolysis of triglycerides during exercise is under both hormonal and nervous system control (Bjorntorp, 1981; Pequignot et al., 1980). The resulting glycerol and free fatty acids both serve as important fuel sources during exercise.

The extent to which fat is utilized as an energy source during exercise is dependent upon level of training, prior diet, exercise intensity and exercise duration. Issekutz et al. (1963) studied the effect of the ingestion of 100g carbohydrate prior to 30 minutes of cycling at a low intensity (300 kpm/min) during one of four controlled dietary periods (a high protein diet; a low protein; a high fat, high carbohydrate diet, or a low protein, low fat diet). They found that, compared to the fasted state, the ingestion of carbohydrate decreased the utilization of fat as an energy source as evidenced by higher respiratory quotients (0.87 compared to 0.80). Issekutz et al. concluded that the carbohydrate content of the diet, not the level of fat, determined the utilization of fat for energy during exercise. Hormones such as catecholamines and growth hormone are elevated during exercise and may influence the metabolism of fat as an energy source. Growth hormone is discussed in detail separately. The catecholamines are stimulators of FFA mobilization and utilization (Issekutz, 1964). Physical training has similarly been demonstrated to increase fat utilization during exercise in dogs (Issekutz et al., 1965). Training enhances the oxygen supply to the active muscle, therefore aerobic metabolism can be maintained. Less lactic acid, which inhibits the release of FFA from adipose tissue, is produced at a given workload with training. The resulting fall in plasma FFA concentration has been documented in trained exercised dogs compared to the untrained condition, indicating a decreased rate of fat utilization (Issekutz et al., 1965). Training likewise results in a more efficient lipid mobilization process in adipose tissues and an increase in the concentration of

aerobic enzymes in muscle responsible for lipid oxidation (Holloszy, 1982). Similarly, studies in humans indicate that training enhances the utilization of fat as an energy source (Holloszy and Coyle, 1984).

The influence of exercise intensity on fat utilization relates to the availability of oxygen. The combustion of fat during exercise requires oxygen. When exercise first begins, oxygen consumption does not meet metabolic needs, therefore carbohydrate, creatine phosphate and ATP are used for energy. There is an initial uptake of FFA by muscles and plasma FFA concentrations fall (Hagenfeldt, 1979). The subsequent stimulation of adipose lipase by norepinephrine results in the mobilization of FFA and glycerol (Ahlborg and Felig, 1982). Except for the initial lag period described above, the mobilization of FFA continues at a level four to six times greater than basal levels during submaximal exercise (Hagenfeldt, 1979). The reliance of fat metabolism on oxygen uptake led to the relationship between FFA utilization and VO_2 max. At rest and during light prolonged exercise, FFA supplies over 90% of the oxidative needs of the muscle. As intensity increases and the oxygen supply is not adequate to the muscles, the reliance on fat for energy declines and the use of carbohydrate increases. At exercise intensities of 70-80% VO_2 max, carbohydrate is preferred to fat 70:30 (Costill and Miller, 1980). Caffeine may improve exercise performance by sparing muscle glycogen and enhancing the utilization of FFAs as an energy source. Current evidence suggests that caffeine may improve performance during prolonged, moderate intensity exercise (> 30 minutes, 75-80% of VO_2 max) (Dodd et al., 1993). In contrast, studies involving high intensity, short term exercise and caffeine ingestion do not consistently produce enhanced performance (Williams, 1991). Growing evidence suggests that inhibition of adenosine receptors is one of the most important mechanisms to explain the physiological effects of caffeine (Dodd et al., 1993).

Protein

Protein has not traditionally been considered an important energy source during exercise. This belief has been based primarily on 24-hour urinary nitrogen excretion values (Astrand and Rodahl, 1986; Young et al., 1966). In studies examining both sweat and urea nitrogen losses during endurance exercise, the evidence suggests that protein losses can range from 5.8 g/hour under conditions of carbohydrate loading to 13.7 g/hour under conditions of carbohydrate depletion (Lemon and Mullin, 1980). Protein catabolism generally involves the removal of nitrogen from amino acids by a process known as deamination. The transfer of nitrogen from a specific amino acid to another compound is known as transamination. Pyridoxal 5'-phosphate serves as a cofactor for transamination reactions. The carbon skeletons remaining from the deamination and transamination processes are degraded further through energy metabolism.

Evidence for protein catabolism during exercise is provided by an increase in serum free tyrosine levels during prolonged exercise (Haralambie and Berg, 1976). Since muscle has no tyrosine deaminase activity, the majority of tyrosine release appears to originate from the muscle (Ahlborg et al., 1974). Using a muscle perfusion system in rats, Dohm et al. (1980) demonstrated a decrease in protein synthesis and an increase in protein degradation following either a 1-3 hour treadmill run or a one hour swim to exhaustion. The response of protein metabolism to exercise depends upon the duration of the exercise (sprint vs. endurance), the type of skeletal muscle fibers recruited, the specific muscle fractions and the type of measurement (Wenger et al., 1981; Wilkinson and Wenger, 1979). Cerny (1975) observed a 20% rise in serum urea and uric acid (a protein breakdown product) only when the exercise (cycling at 60-65% VO_2 max) duration exceeded one hour.

Amino acids are also degraded during endurance exercise. In vitro experiments have demonstrated that skeletal muscle, as well as the liver, has the capacity to oxidize amino acids during exercise (Adibi et al., 1974; Beatty et al., 1974; Buse et al., 1975;

Dohm et al., 1976, 1977; Manchester, 1965; Odessey and Goldberg, 1972). The branch chain amino acids are felt to be the primary amino acids involved, since the α-keto acid dehydrogenases are found predominantly in the muscle (Miller, 1962). The use of radioactive tracers has enabled investigators to access amino acid oxidation in vivo by measuring $^{14}CO_2$ production. The injection of radiolabelled leucine (Askew et al.,1979; Dohm et al., 1977; White and Brooks, 1981), glutamate (Askew et al., 1979), and alanine (White and Brooks, 1981) or the ingestion of leucine (Lemon et al., 1980a, 1980b) have resulted in an increased $^{14}CO_2$ production during exercise. The evidence to date strongly suggests that both protein and amino acids contribute more to exercise metabolism than is generally assumed.

Currently, the RDA for protein is 0.8 g/kg body weight (Recommended Dietary Allowances, 1989). A recent study involving runners who consumed the RDA for protein found a negative nitrogen balance during an exercise test day, indicating that body protein was lost (Friedman and Lemon, 1989). When the diet was 70% higher in protein, there was retention of nitrogen. Carbohydrate and total calories were adequate during both exercise tests (75 minutes at 72% VO_2 max)(Friedman and Lemon, 1989). Based on nitrogen balance studies in body builders, the estimated RDA for protein in body builders may be as high as 112% of the current RDA (Tarnopolsky et al., 1988).

Exhaustive Exercise

The factors limiting endurance exercise capacity have been intensely studied for more than a century now, yet the mechanisms involved still remain a mystery. Fatigue, the end-point of exhaustive exercise, has several definitions. A standard physiological definition of fatigue is the inability to sustain a specified force or work rate (Edwards, 1981). Fatigue can be broadly categorized as subjective or objective. Objective muscle fatigue is primarily caused by biochemical and physiological changes that take place inside the muscle itself (Layzer, 1990). This type of fatigue results from a decline of the force-

generating capacity of the muscle. Exercise capacity may also be limited by subjective fatigue, which is defined as the unpleasant physical sensations that result indirectly from metabolic changes taking place in the working muscles. Bodily discomfort impairs the willingness to continue the exercise. Therefore, our ability to participate in prolonged endurance exercise has both a physiological and a psychological component. There seems to be a consensus that the development of fatigue is a multifactorial process.

Dehydration can negatively impact endurance exercise and decrease the time to exhaustion (Staff and Nilsson, 1971). Sawka (1992) has summarized the effects of dehydration on performance and has found that a critical water deficit of 3% can reduce maximal aerobic power in a temperate environment. A reduction in body weight of as little as 1-2 % may represent a deterioration in physical performance (Pitts et al., 1956; Adolph, 1947; Ladell, 1955; Saltin, 1964). Therefore, a simple method of determining the extent of dehydration is needed. The method most often employed is to weigh the subjects prior to and after the exercise and calculate the percent loss of body weight. A 4% reduction in body weight has been equated with a 16-18% reduction in plasma volume (Costill and Fink, 1974). Dehydration is associated with a decrease in stroke volume during exercise and a concomitant increase in heart rate and body temperature during submaximal exercise. A critical water deficit of 1% of body weight can elevate core body temperature during exercise (Ekblom et al., 1970). The performance of well-trained athletes appears to be less affected by dehydration than the performance of untrained subjects (Buskirk et al., 1958; Saltin, 1964). An important consideration when estimating hypohydration is that the metabolism of large amounts of glycogen (to CO_2 and water) can underestimate the extent of dehydration resulting from exercise. The American College of Sports Medicine recommends 4-8 ounces of fluid every 15-20 minutes during exercise (American College of Sports Medicine Position Stand, 1985). The thirst mechanism is often not adequate to compensate for the fluid loss.

The level of prior training can influence the time to exhaustion. A well-trained athlete can maintain a higher work rate than an untrained subject. Training improves the oxygen transport efficiency and substrate utilization in muscles. Marathon runners have been found to run for hours at 70-80% VO_2 max with little or no increase in blood lactate concentration (Costill, 1970; Maron et al., 1976). The endurance trained athlete may have an enhanced ability to utilize fatty acids as a substrate, thus sparing glycogen.

Fatigue may vary greatly between individuals for apparently unknown reasons. The K^+/Na^+ ratios across the muscle cell membrane could be disturbed during prolonged exercise and the enzyme systems may be altered. There may be reduced enzyme synthesis and/or enhanced loss of enzyme proteins. There may also be diurnal (Rodahl et al., 1976) and seasonal variations (Erikssen and Rodahl, 1979) contributing to physical performance. The motivation of the subject and susceptibility to boredom, which are difficult to measure, may also factor into defining the point of exhaustion.

Fluid Volume Shifts

The shifts in plasma volume that occur with exercise are not simply a loss of fluid through sweat, but a complex interaction of several factors. The body adapts to the stress of exercise by producing water via the oxidation of carbohydrates and the shifting of fluid between the plasma, interstitial and intracellular compartments. Costill et al.(1976) examined the effect of three bouts of cycling (70% VO_2 max in the heat) on fluid loss in the body. A 6% loss of body weight equated to an 11% loss of plasma fluid, a 39% loss of interstitial fluid and a 50% loss of intracellular fluid. A 2% loss of body weight resulted in a 10% loss of plasma, a 60% loss of interstitial fluid and a 30% loss of intracellular fluid. The fluid shift among the body compartments was very different depending upon the extent of dehydration. When body water losses are minimal, the extracellular compartment accounts for the majority of the water deficit. However, when body water losses increase, a greater percentage of the water deficit comes from the intracellular compartment.

The loss of fluid during exercise is dependent upon the osmotic and oncotic pressures within the body, the initial level of hydration, the environmental temperature, the level of conditioning of the individual and the intensity of the exercise. Plasma proteins such as albumin contribute to the oncotic pressure in the plasma. During prolonged submaximal exercise (30 minutes at 32% VO_2 max followed by 45 minutes at 64% VO_2 max), van Beaumont et al. (1972) found a net gain in plasma protein as measured by radiolabelled albumin. Plasma albumin contributed to the stabilization of the vascular volume. Exercise has been associated with a net shift of fluid into the active muscles. The shift appears to be a result of the tissue hyperosmolality and increased capillary hydrostatic pressure (Mellander et al., 1967; Lundvall et al., 1972). There is evidence that the loss of plasma volume as a result of exercise is directly related to the work intensity from rest through 60% VO_2 max (Wilkerson et al., 1977). Others have been unable to demonstrate a reduction of plasma volume during exercise despite a reduction in body weight. Sawka et al. (1980) showed that during a 100 minute treadmill run, the plasma volume remained stable while the body weight decreased 4%. Similarly, Kolka et al.(1982) reported a 7% reduction in body weight during a marathon run but a stable plasma volume. Reasons for the stable plasma volume may include the production of metabolic water from the breakdown of glycogen (Pivarnik et al., 1984) or the redistribution of water from inactive skeletal muscle (Sawka, 1988). The influx of plasma proteins may also play a role. The endocrine system is also involved in the redistribution of water into the intravascular spaces during exercise. Plasma angiotensin and vasopressin have been shown to increase in relation to exercise intensity (Convertino et al., 1981, 1983) and magnitude of water deficit (Brandenberger et al., 1986; Francesconi et al., 1983). Angiotensin produces vasoconstriction and acts on the brain to increase the thirst mechanism and vasopressin release. Angiotensin also stimulates the adrenal cortex to secrete aldosterone, which results in sodium retention in the kidney. Vasopressin similarly acts on the kidney to retain water. The result is an increase in extracellular fluid volume (Ganong, 1979). As the exercise

intensity increases and dehydration develops, these endocrine hormones are released into the plasma.

Several measurement techniques have been developed to account for the factors that affect plasma volume during exercise. The measurement of hematocrit, hemoglobin and plasma proteins before and after exercise have resulted in the development of formulas to determine the extent of the plasma volume shift. The calculation of the plasma volume shift is important to know, since other blood constituents are expressed in terms of plasma concentration. van Beaumont (1972) measured only hematocrit changes in the blood with exercise to determine plasma volume shifts, whereas Dill and Costill (1974) utilized both the hemoglobin and hematocrit changes before and after exercise to determine plasma volume. van Beaumont et al. (1972) later concluded that the calculation of plasma volume changes using hematocrit changes by themselves underestimate the changes in plasma volume. Dill and Costill (1974) were able to demonstrate a loss of red blood cell mass as a result of dehydration which was highly correlated to the increase in plasma osmolality.

Vitamin B-6 and Exercise

Physical activity provides a metabolic stress which alters the steady state condition of the body. There is an increased need for energy by the exercising body. The production of this energy, through a system of metabolic pathways, requires PLP as a cofactor for some of the enzymes that are involved. PLP is involved as a cofactor for transaminase enzymes in gluconeogenesis and for glycogen phosphorylase in glycogenolysis. The influence of exercise on vitamin B-6 metabolism is an active area of research. The current vitamin B-6 literature indicates that there is an increase in plasma PLP during and immediately following exercise, but uncertainty as to its origin. The rise in plasma PLP as a result of exercise may ultimately be involved in fuel utilization. The extent of the involvement of PLP during exercise may rely on several factors, such as the level of training, amount of glycogen stored (i.e.-prior diet), amount of PLP stored, intensity and

duration of the exercise and availability of a carbohydrate source. An outcome of the research on exercise and vitamin B-6 metabolism may be the finding of an increased requirement for athletes.

Studies conducted at Oregon State University under the direction of Dr. James Leklem, were some of the first to address the vitamin B-6 and exercise issue. Several of these studies examined the effect of varying types and durations of exercise on plasma PLP levels. An early study by Leklem and Shultz (1983) examined the effect of three 1500 meter runs (a total of 4500 meters) on plasma PLP levels in seven, adolescent, male runners. All meals the day of and day after the second run were provided to the subjects so that the analysis of urine for 4-PA could be interpreted. All other meals and exercise intensity were not controlled. Subjects completed each 1500 meter run in 4-5 minutes at a heart rate of 150 to 165 beats per minute. The plasma PLP concentrations significantly increased by 7.7, 12.5 and 18.3 nM after each individual 1500 meter run. Plasma vitamin B-6 concentration also increased following exercise. The origin of the plasma PLP was not determined. This study demonstrated for the first time that plasma PLP concentrations are increased by exercise. Another running study by Leklem (1985), consisting of twice the distance (9000 meters), likewise resulted in elevated plasma PLP levels immediately post-exercise (mean increase in PLP of 14.7 nM). To explain this phenomenon, Leklem theorized that exercise provides the stimulus for a cascade of events that ultimately results in the release of intact muscle PLP into the blood system for utilization by the liver in gluconeogenesis. Vitamin B-6 is stored in muscle with the enzyme glycogen phosphorylase. The need for energy during exercise promotes the conversion of glycogen to glucose-1-phosphate and ultimately to pyruvate. Pyruvate produces variable amounts of lactic acid, depending upon characteristics of the individual and the exercise conditions. As exercise progresses, lactic acid accumulates and muscle pH can fall to 6.5-6.6 (Sahlin et al., 1978). Leklem theorized that the low muscle pH alters the PLP-glycogen phosphorylase attraction, and results in PLP entering the plasma. The plasma PLP travels

to the liver, where it is available to participate in gluconeogenesis. The outcome is that the exercising muscle is supplied with a source of energy. The release of PLP from muscle in rats (Black et al. 1977,1978) has been documented to occur only when a caloric deficit is also present. Exercise has been viewed as an acute form of starvation or caloric deficit (Lemon and Nagle, 1981). Therefore, Leklem feels that exercise results in a flux of PLP from the muscle into the plasma via the mechanism described above. An important component of his theory is the influence of supplemental vitamin B-6 on the mechanism. An increase in vitamin B-6 intake is felt to increase the amount of muscle glycogen phosphorylase and PLP in the muscle. An increase in muscle glycogen phosphorylase activity has been documented in rats (Schaeffer et al., 1989), but not in humans (Coburn et al., 1991) as a result of supplemental vitamin B-6 sources. When muscle glycogen phosphorylase and PLP content are elevated, exercise may increase the rate of muscle glycogen utilization and result in more PLP released into the plasma than would typically ocurr with exercise alone. To date, all evidence in support of this theory has been indirect.

To test the theory that exercise results in a flux of PLP into the plasma for the purpose of altering fuel utilization, studies were conducted where the carbohydrate content of the diet and vitamin B-6 intakes were controlled. Leklem (1985) assumed that there would be less reliance on gluconeogenesis as an energy source (and less need for PLP by the liver) if muscle glycogen concentrations were maintained through a high carbohydrate diet. Indeed, he found a drop in plasma PLP (18-21%) after 5 hours of an oral glucose load of one gram D-glucose/kg body weight (Leklem and Hollenbeck, 1990). The ingestion of glucose during exercise would therefore seem to lessen the need for PLP by the liver for gluconeogenesis. During a 2 hour treadmill run at 60-65% VO_2 max., Hofmann et al. (1991) found that there was no significant difference between the rise in PLP during exercise seen either with the consumption of water or glucose polymer beverage in trained, male runners. Plasma PLP increased with exercise (19-26%) as previously documented by Leklem, but the ingestion of the carbohydrate beverage versus water made no difference on

the magnitude of the increase. These findings do not support the assumption that the release of PLP from the muscle is for the purpose of gluconeogenesis in the liver. Hofmann et al. (1991) believed that the majority of the rise in plasma PLP originates in the liver and is going to the muscle to be used for PLP-dependent reactions.

Supplemental vitamin B-6, which may increase muscle glycogen phosphorylase concentrations, could result in a rapid turnover of muscle glycogen during exercise. We would expect to find 1) a shift in fuel source to carbohydrates, 2) less reliance on plasma free fatty acids (FFA) as an energy source, and 3) higher plasma lactic acid concentrations. Manore et al. (1987) examined the relationship between two levels of carbohydrate intake (49% and 64%) and two levels of vitamin B-6 intake (2.3 and 10.4 mg/day) on fuel substrates (glucose, FFA and lactate), considering training, age and vitamin B-6 supplementation of women exercised for 20 minutes on a bike ergometer at 80% of VO_2 max. A total of 15 women were fed four diets varying in carbohydrate and vitamin B-6 content over a seven week period. The subjects were exercised at the end of each dietary period. Blood was drawn pre, post, post-30 and post-60 minutes of exercise and analyzed for plasma PLP, glucose, FFA and lactate. Plasma PLP levels increased pre-exercise to post-exercise in all groups; 8-11 nmol/l in the unsupplemented state and 14-21 nmol/l in the B-6 supplemented state. The largest increase, in each case, occurred with the high carbohydrate diet, with or without vitamin B-6 supplementation. These researchers also found that a high carbohydrate diet and/or supplemental vitamin B-6, depressed circulating FFA levels during 20 minutes of cycling at 80% of VO_2 max. Also, 10.4 mg of B-6 alone were found to depress circulating levels of FFAs during exercise. These findings appear to support Leklem's theory that elevated levels of PLP are able to alter energy substrate utilization during exercise. The fact that lactate levels were similar for all groups and diets was unexpected in light of findings by other researchers that supplemental vitamin B-6 increases lactate levels (Lawrence et al, 1975, Leklem, 1985). Hatcher et al. (1982) conducted a similar study in men. The exercise protocol consisted of 50 minutes of cycling;

30 minutes at 60% of VO_2 max, 15 minutes at 80% VO_2 max and 5 minutes at 90% VO_2 max. Three levels of carbohydrate intake were provided over a one week period (40%, 11% and 71% CHO) either with or without a vitamin B-6 supplement (8 mg pyridoxine-HCL). The low carbohydrate diet in the presence of the B-6 supplementation produced the greatest increase in mean plasma PLP levels of the conditions studied. These results appear to indicate that low glycogen stores result in a need to redistribute PLP from the muscle to the liver for utilization in gluconeogenesis.

Examination of R values during exercise can also provide support for the metabolism of carbohydrate during exercise. Campuzano (1988) measured R values as part of a study to determine if PN supplementation affected fuel metabolism. Trained males were exercised twice on a cycle ergometer at 72% VO_2 max for one hour. The exercise tests occurred during a controlled dietary period. Subjects received either 20 mg PN or a placebo capsule six days prior to the exercise tests. Vitamin B-6 supplementation was provided the day of exercise and the day following exercise. Resting R values were higher with vitamin B-6 supplementation compared to the placebo group (0.86 versus 0.80) suggesting that the subjects receiving the B-6 supplement had an increase in carbohydrate metabolism. Conversely, fat utilization decreased from 45 to 36% with B-6 supplementation. Again, these results appear to support the hypothesis that B-6 supplementation may stimulate glycogen utilization. A recent study by Sampson et al. (1993) questioned the relationship between the rise in plasma PLP with exercise and the involvement in fuel utilization. They hypothesized that if PLP is involved with fuel utilization during exercise, the intensity of the exercise should alter the magnitude and rate of rise. Nine subjects were tested twice at both 60% and 80% of VO_2 max on a cycle ergometer. Plasma was collected at minute 0, 5, 10 and 20 of exercise using an indwelling catheter and analyzed for vitamin B-6 by HPLC. After adjusting the values for changes in plasma volume, these researchers found that plasma PLP rose significantly within 5 minutes of the onset of exercise to concentrations that were maintained throughout the

exercise. Exercise intensity did not alter the rise. The plasma 4-PA increased throughout the exercise irregardless of exercise intensity. These results seem to indicate that the increase in plasma PLP that accompanies exercise of moderate to severe intensity is not related to fuel use during exercise. This study did not control dietary intake prior to the exercise, did not examine urinary indicators of vitamin B-6 metabolism, and did not measure other indicators of fuel use, such as plasma FFAs, glucose or lactate, so it is difficult to conclude that fuel use has not been altered.

The effect of the shift in plasma PLP with exercise on vitamin B-6 metabolism and requirements has not been answered. The examination of the excretion of vitamin B-6 and its metabolite (4-PA) provides a measure of the extent of vitamin B-6 metabolism with exercise. Research in rats and humans has documented an increase in the excretion of 4-PA with exercise (Efremor and Zaburkin, 1972; Borisov, 1977). Dreon and Butterfield (1986) compared vitamin B-6 utilization in trained male runners and inactive controls and found that baseline 4-PA excretion was lower in trained runners than in controls. Either the trained runners were retaining more vitamin B-6 than the untrained controls, or training increased the requirement for vitamin B-6. These data are not conclusive, since the excretion of other vitamin B-6 metabolites was not assessed. A methionine challenge resulted in an increased excretion of 4-PA in the runners, indicating that they may have a liable pool of vitamin B-6 which can be mobilized under conditions of increased need. Munoz (1982) examined the effects of exercise on vitamin B-6 metabolism in four groups of subjects, both trained and untrained. The subjects were exercised either on a bicycle ergometer, by running three 1500 meter intervals or both. Blood was collected before exercise, immediately after exercise and 30 minutes into recovery and was analyzed for plasma PLP, PB-6 and glucose. Twenty-four hour urine samples were collected the day before and the day of exercise and were analyzed for UB-6, 4-PA, creatinine and urea nitrogen. The bike ergometer exercise had a significant effect on the plasma PLP concentration of the trained and untrained groups and PB-6 concentration of the college

group following exercise. The 30 minute post-exercise plasma PLP concentrations were significantly lower for the trained group. The runs likewise resulted in significantly higher plasma PLP and PB-6 immediately post-exercise compared to resting concentrations. Urinary vitamin B-6 and 4-PA were not significantly altered by either exercise. Therefore, exercise resulted in an redistribution of vitamin B-6 without the apparent increase in vitamin B-6 metabolism. Manore et al. (1987) found that 4-PA excretion increased significantly from the pre-exercise day to the day of exercise irregardless of the carbohydrate content of the diet, age of the subjects or degree of training. The finding from Manore et al. (1987) suggests that exercise accelerates vitamin B-6 metabolism and excretion. Rokitzki et al. (1994) examined vitamin B-6 status in trained endurance runners before and after a marathon and found a two-fold increase in the serum vitamin B-6 concentration post-exercise and an increased excretion of 4-PA (3.68 μmol.L more) two hours after the race. The loss of vitamin B-6 due to the race was calculated to be 1 mg.

Lawrence et al. (1975) developed a study to address the question of diet and performance. They examined the effects of vitamin E (900 IU α-tocopherol) on swimming performance but also administered vitamin B-6 (51 mg PN-HCL/day) for no stated reason. Seventy-two male and female swimmers were given tablets containing either vitamin E, vitamin B-6 or a placebo for 6 months during their training season. The researchers hypothesized that vitamin E would decrease oxygen debt, so lactic acid levels were monitored following exercise. They found that 51 mg of vitamin B-6 did not enhance swimming endurance. However, what this study did find was that subjects taking the vitamin B-6 supplement showed significantly higher lactic acid levels than either the vitamin E or placebo groups after a continuous 15 minute swim. This may be indicative of enhanced carbohydrate metabolism, in accordance with the hypothesis proposed by Leklem (1988). A recent study by Singh et al. (1992) examined the influence on blood and urinary levels of chronic (12 weeks) vitamin B-6 supplementation (135 mg/day) in the form of a high-potency multivitamin-mineral supplement in active men. Plasma B-6 rose to 960

nmol/L and urinary vitamin B-6 rose to approximately 8 μmol/day by 12 weeks of supplementation. The data imply that a new steady state had been reached and vitamin B-6 was provided in excess of need, even for active adults.

A complete picture of the flux of vitamin B-6 during and following exercise of varying intensities and durations is not yet established. There appears to be agreement in the literature that plasma PLP levels increase during and immediately following exercise. However, there is still uncertainty as to the origin of this PLP and its role in energy metabolism. The long term implications of this flux in vitamin B-6 pools throughout the body and possible acceleration of vitamin B-6 metabolism on vitamin B-6 status remain to be determined. Some contradictory findings need further clarification before an accurate picture can be developed. Complicating the picture is the influence of exercise on hormonal responses. Exercise is a known stimulant for growth hormone release. The involvement of vitamin B-6 in the synthesis of growth hormone releasing hormone may mean that exercise increases the need for vitamin B-6 in the body. Conversely, supplemental vitamin B-6 may enhance the synthesis of growth hormone releasing hormone and result in a greater release into the plasma during exercise. The anabolic effects of plasma growth hormone could be beneficial to athletes and older adults alike. The interaction between exercise, vitamin B-6 and plasma growth hormone will be discussed in detail later.

Growth Hormone

Human growth hormone (hGH, or somatomedin) is an anabolic polypeptide hormone that affects all body systems and plays an important role in muscle growth. Although hGH is of prime importance during growth and development, the regulation of daily metabolism is also an important function. Growth hormone (GH) influences carbohydrate, protein and fat metabolism. Recent interest in this hormone originates from its ability to promote muscle mass, a role which could benefit athletes and older adults alike.

History

Questions were raised in the mid-1930's as to the existence of the anterior hypophyseal growth hormone (Bates et al., 1935; Schooley et al., 1938). Final proof for the existence of growth hormone came with its separation and isolation by Li et al.(1945). These researchers isolated growth hormone from the anterior lobe of an ox pituitary and were able to demonstrate growth and an increase in epiphyseal cartilage cells of the tibia of hypophysectomized rats. In man, Beck et al. (1957) was the first to demonstrate activity of purified monkey and human pituitary growth extracts.

Production and Release

Human growth hormone is composed of 191 amino acids in a single chain weighing 22 kDa and containing two intrachain disulfide bonds between cystine residues at positions 53 and 165 and between 182 and 189. The production and secretion of hGH occurs in the somatotropic cells of the anterior pituitary gland. Animal studies indicate that it is the anterior median eminence, central median eminence and pituitary stalk which mediate reflex hGH discharge (Abrams et al., 1966). While the hGH molecule has a high degree of heterogeneity in pituitary extracts and plasma (Baulieu and Kelly, 1990), the 22 kDa form accounts for the majority of the 5 to 10 mg of hGH found in the human pituitary.

The secretion of hGH from the pituitary gland is regulated by several factors (see Table 3). In the past, hGH secretion was viewed as the result of the simplistic interchange between two controlling neurohormones: growth-hormone-releasing hormone (GHRH), which stimulates the release of hGH, and somatostatin, which inhibits its release. Current research indicates that factors such as diet, insulin-like growth factor 1(IGF-1), gender, age, exercise and body composition affect the secretion of hGH from the pituitary to some extent. Each factor mentioned will be discussed in detail below.

At the level of the hypothalamus, GHRH specifically stimulates the secretion of hGH. GHRH was first identified as a hGH releasing factor from a patient who experienced clinical signs of excess hGH production (acromegaly, the abnormal enlargement of the facial features, hands and feet) from a pancreatic tumor. The tumor was found to secrete a compound, GHRH, which was later isolated, sequenced and synthesized in 1982 by two independent groups, Rivier et al. (1982) and Guillemin et al. (1982). There are several forms of GHRH (37, 40 and 44 amino acid residues). All have been shown to be equipotent in stimulating the release of hGH from the pituitary (Wehrenberg et al., 1982). The stimulatory effect of GHRH on hGH release has been shown to occur via stimulation of cyclic AMP (cAMP) production, a second messenger in the cell (Cronin et al., 1983; Belezikjian and Vale, 1983).

Growth hormone secretion is inhibited by somatostatin, a neurohormone released from the hypothalamus. Somatostatin exists in the 14 amino acid form or the 28 amino acid N-terminally extended form. Somatostatin is so widely distributed and conserved in the animal kingdom that it is even found in protozoa. The mechanism of action of somatostatin is believed to be downstream from cAMP in target tissues where somatostatin acts to inhibit hGH secretion by reducing membrane permeability to calcium (Reichlin, 1983a, 1983b).

The interplay between GHRH and somatostatin results in the release of hGH from the pituitary in a pulsatile manner throughout a 24-hour period. Current hGH detection techniques (IRMA) indicate that the secretion of hGH throughout the day is oscillatory rather than episodic (Winer et al., 1990). The relatively short half-life of hGH (17-45 minutes) contributes to the fluctuations observed in the detection of hGH (Cameron et al., 1969). A description of normal plasma hGH concentrations will be discussed under normal values. Several additional factors which will be discussed later also affect the secretion of hGH from the pituitary.

Feedback regulation of hGH secretion occurs by several mechanisms (see Fig 3). Factors that influence the secretion of hGH (such as stress, exercise, nutritional status and pharmacological factors; see Table 3) can have marked effects on circulating hGH concentrations. These regulators will be discussed in detail later. Circulating hGH exerts negative feedback on its own secretion by stimulating the release of somatostatin from the hypothalamus and stimulating the release of insulin-like growth factor I (IGF-1 or somatomedin C) from the liver. IGF-1 stimulates somatostatin release and inhibits the release of hGH secretion from the pituitary (Reichlin, 1983a, 1983b).

Table 3. Factors affecting hGH secretion in humans.

Increase	Decrease
Physiological:	
Exercise	cold
Sleep	rising blood sugar
Stress (physical, psychological, heat)	
Hypoglycemia (falling blood sugar)	
Pharmacological:	
Hormones- GHRH, estrogen, glucagon, vasopressin	somatostatin, progesterone, glucocorticoids
Neurotransmitters and analogues- adrenaline, propranolol, levodopa, clonidine, serotonin, bromocriptine	phentolamine, isoproterenol, methysergide, cyproheptadine, atropine
Amino Acids (arginine)	
Other (apomorphine)	chlorpromazine, imipramine, morphine, theophylline

Adapted from: Macintyre, 1987.

Transport

Once in the plasma, approximately half of plasma hGH (45%) is attached to a high affinity, low capacity binding protein (growth hormone binding protein, GHBP) (Baumann et al., 1988; Tar et al., 1990). The hGH binding protein is identical to the extracellular domain of the GH receptor on the liver membrane (Baumann et al., 1988). The exact source of GHBP production is currently undetermined. The liver may be the source. The

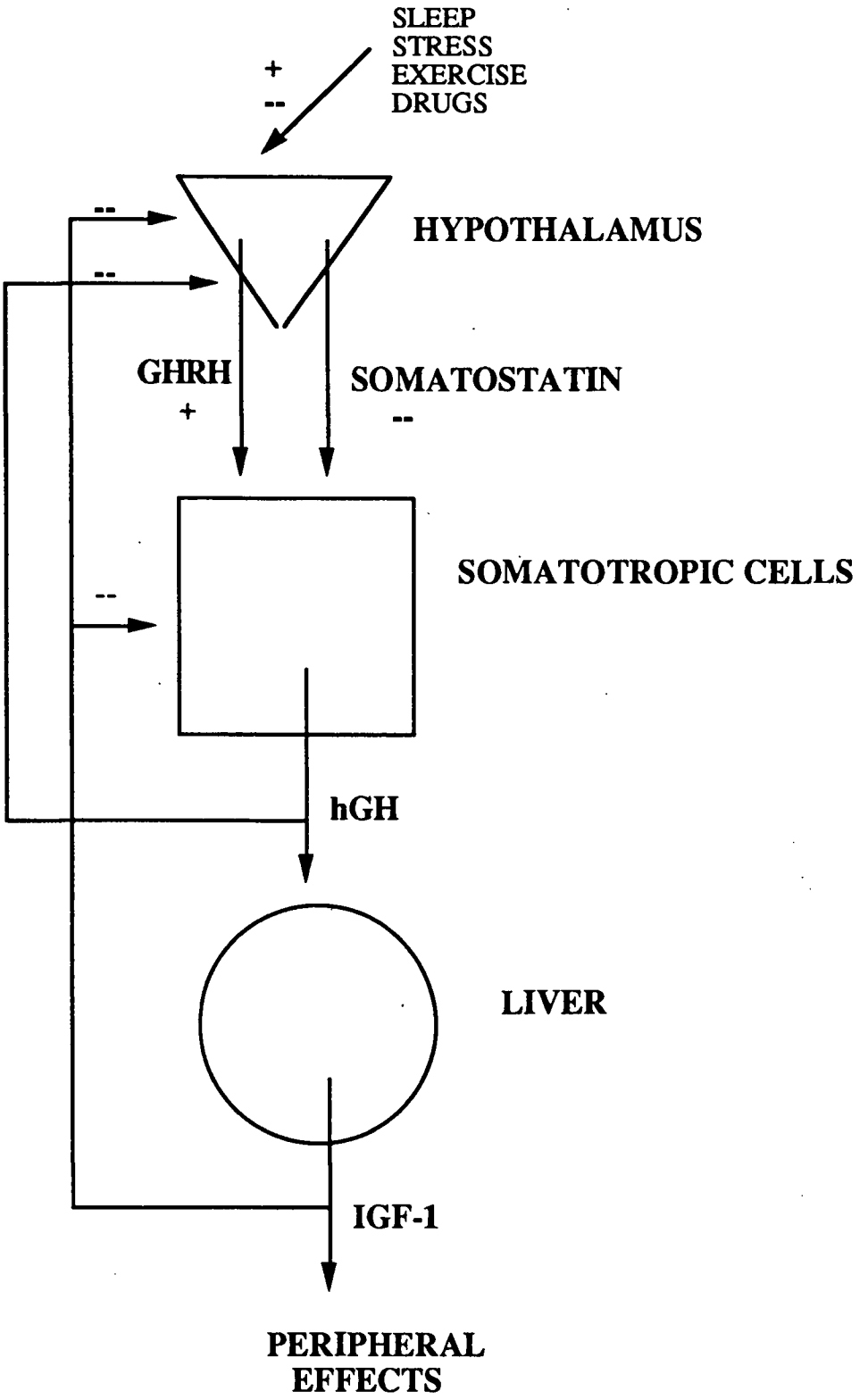

Figure 3. Principle central and peripheral regulatory factors affecting hGH secretion (Adapted from Baulieu and Kelly, 1990)

level of GHBP is believed to parallel the level of liver membrane growth hormone receptors. The plasma hGH protein activity in the plasma has been shown to remain constant throughout the day (19-24% bound fraction of hGH based on 100 μL plasma) (Snow et al., 1990). Recently, a second lower affinity GHBP has been identified in human plasma which appears to be unrelated to the hGH receptor. The low affinity binding protein is (1) structurally distinct from the high affinity binding protein, (2) has a low affinity for hGH but has a high binding capacity and (3) contributes about 10-15% to the overall bound hGH (Baumann and Shaw, 1990).

Target Tissues and Receptors

Improved detection techniques and the advent of mRNA transcripts have resulted in the identification of several hGH binding sites. Protein hormones typically bind to receptors, located on membrane surfaces. The interaction of the hormone with the receptor produces a change (chemical and/or physical) that results in a sequence of events specific to that hormone at that site. In mammals, GH receptors are found in a variety of organ and cell types including the liver, adipose tissue, lymphatic and immune cells, intestine, heart, kidney, lung, pancreas, brain, cartilage, skeletal muscle, corpus luteum and testis (Kelly et al., 1991). Studies involving defective hGH binding sites have confirmed the importance of hGH binding sites. A defect in the hGH receptor gene has been identified in the autosomal recessive disorder known as Laron type dwarfism (Golde et al., 1980). These patients have severe growth failure and low circulating levels of IGF-1 despite elevated levels of biologically active hGH.

Various conditions can alter growth hormone receptor number. A decrease in hGH receptors in the liver has been shown to occur with chronic renal failure, fasting and streptozotocin-induced diabetes (Finidori et al., 1980; Postel-Vinay et al., 1982; Baxter et al., 1980). Down-regulation of hGH receptors in lymphocytes and liver can also result from growth hormone alone. In this situation, hGH provides a negative feedback

mechanism. Estrogen has been shown to increase the binding of hGH to the liver 2-fold following puberty in female rats, whereas no change has been observed in males (Maes et al., 1983). Pregnancy results in a 10-fold increase in GH binding to liver membranes in rats (Hughes et al., 1985). Obesity has also been found to result in elevated GH receptor number. Obese rats have more GH receptors than normal weight rats and are hyperresponsive to GH (Landron et al., 1989).

The mechanism of action of hGH after it binds to the GH receptor is currently an area of intensive research. In vitro evidence to date in both animal and human studies suggests that the GH receptor may utilize a tyrosine kinase second messenger (Silva et al., 1993; Wang et al., 1992, 1993).

Functions

As its name implies, hGH is involved in the stimulation of skeletal and soft tissue growth. It is directly involved in 1) insulin-like growth factor (IGF-1) production by the liver and other cells (skeletal muscle, bone, heart and kidney), 2) protein synthesis, 3) amino acid transport, 4) lipolysis and 5) carbohydrate metabolism. Indirectly, hGH acts via IGFs to influence chondrogenesis, skeletal growth, protein synthesis and general cell growth. The contribution of hGH to the regulation of metabolism in adulthood has been researched for over 60 years, yet many functions and mechanisms remain illusive.

Amino acid uptake by cells is enhanced by growth hormone. The use of radioactively labeled, nonmetabolizable amino acids has shown that hGH can stimulate amino acid transfer from the extracellular to the intracellular compartment (Noall et al., 1957). Growth hormone can increase the intracellular transport of neutral and basic amino acids into cells (Ganong, 1979). The reason for the enhanced uptake of amino acids into cells by hGH may be for protein synthesis. Turner et al. (1976) proposed that the role of GH in protein metabolism is to sustain cellular protein synthesis when there is a decrease in the level of substrate amino acids (i.e.- short term fast or inadequate intake).

There is clear evidence that hGH promotes protein synthesis. In studies on hGH deficient subjects, improvement in nitrogen metabolism and lean body mass have been observed when exogenous hGH was given. Henneman et al. (1960) found that when growth hormone deficient adults were given daily exogenous GH injections, nitrogen retention was achieved within one month. This anabolic effect halted when the exogenous GH was removed. Salomon et al. (1989) observed that exogenous GH given to GH deficient subjects produced an increase in lean body mass of 10.8% over a 6 month period. Likewise, the use of human growth hormone to improve nitrogen retention after surgery (Ward et al., 1987) and in the hyponitrogenous state (Lundberg et al., 1991) have been shown to be clinically relevant. The elevation of forearm hGH levels by selective GH infusion has been shown to stimulate forearm skeletal muscle protein synthesis within 6 hours after beginning the infusion (Fryburg et al., 1991). Protein synthesis is impaired in muscle from hypophysectomized animals and is stimulated by either the administration of GH in vivo or by inclusion of GH in the incubation medium in vitro (Kostyo and Knobil, 1959; Manchester and Young, 1961).

Another hormone which plays an integral role in protein metabolism is insulin. Insulin is produced and secreted by the beta cells of the pancreas. Insulin's role in protein metabolism, like that of hGH, is anabolic. Insulin enhances the uptake of amino acids into muscle and diminishes net muscle protein catabolism by suppressing proteolysis without affecting protein synthesis (Gelfand and Barrett, 1987; Fryburg et al., 1990). Although insulin and hGH perform a similar role with respect to protein metabolism, hGH results in insulin resistance. This would appear to be counterproductive to protein synthesis. The reason for this occurrence remains poorly understood.

HGH and insulin act in an opposite manner to maintain blood glucose concentrations. In response to low blood glucose, hGH is secreted and hepatic glucose output is increased (Kostyo and Regan, 1976). In healthy subjects, the rate of secretion of hGH is stimulated by hypoglycemia, by intracellular deprivation of glucose produced from

deoxyglucose, by a rapid fall in blood sugar in the absence of hypoglycemia, by prolonged fasting and by muscular exercise (Roth et al., 1963). During starvation, hypoglycemia can be prevented in hGH deficient adults through the use of hGH therapy (Merimee et al., 1971). The result is an increase in blood glucose concentration. Elevated blood glucose concentrations are a stimulus for insulin secretion. Insulin results in the suppression of endogenous glucose production and the stimulation of peripheral glucose uptake. The result is a fall in blood glucose concentration. The effect of hGH on plasma glucose concentration, which is in opposition to the effect of insulin, is known as the anti-insulin-like effect of hGH.

The anti-insulin-like effects of GH are evident 2-4 hours following exogenous GH administration. Bratusch-Marrain et al.(1982) found that at least 2 hours were needed for the insulin antagonistic effects of a physiological amount of hGH (2 μg/kg/hr) to develop. These effects include the inhibition of glucose utilization (glucose intolerance) and the stimulation of lipolysis. The anti-insulin-like effects of hGH are reversed in hGH deficient individuals. In the hGH deficient state, children and animals manifest decreased hepatic glucose production, decreased fasting glucose concentrations, impaired glucose tolerance, decreased insulin secretion and increased insulin sensitivity in vivo (DeBodo and Altszuler, 1958; Altszuler, 1974; Lippe et al., 1981; Merimee et al., 1971; Grunt et al., 1967; Costin et al., 1972; Bougneres et al., 1985; Gold, 1968). When hGH-deficient children are given hGH, fasting glucose production increases (Lippe et al., 1981; Merimee et al., 1971; Bougneres et al., 1985). Long term administration of GH to GH deficient animals has been shown to raise fasting glucose concentrations, further impair glucose tolerance in spite of increasing insulin secretion and abolish the enhanced insulin sensitivity by decreasing glucose utilization and increasing hepatic glucose production (DeBodo and Altszuler, 1958; Altszuler, 1974). In intact animals, increased fasting glucose and insulin concentrations occur within the first few hours of exogenous GH administration (Steele, 1968; Hart et al., 1984; Campbell and Rastogi, 1969, Campbell et al., 1978; Morgan et al., 1975; Pierluissi

and Campbell, 1980, 1981; Dunbar et al., 1985). A similar lag time for physiological amounts of endogenous GH has been observed in vivo as was observed in vitro (Yalow et al., 1969; Schnure et al., 1971). The physiological importance of the anti-insulin-like effects of hGH appears to be to spare muscle protein as a fuel source, maintain glucose stores and utilize fat as an energy source.

Although the ability of hGH to produce carbohydrate intolerance has been known for years (Houssay, 1942), a mechanism to explain such an occurrence is not completely understood. Butler et al. (1991) designed a study to address the mechanism of action of the carbohydrate intolerance associated with hGH. They concluded that hGH increased post-prandial glucose concentrations by decreasing the uptake of glucose by tissues and by increased hepatic gluconeogenesis. Murray et al.(1990) likewise addressed the issue of a mechanism for the carbohydrate intolerance attributable to hGH and found that the administration of exogenous hGH results in hyperglycemia from a combined decrease in peripheral utilization of glucose and an increase in hepatic production via gluconeogenesis . Antagonism of the action of insulin by hGH is probably due to a post-receptor mechanism, as insulin has been shown to bind normally to its receptors (Bratusch-Marrain et al., 1982; Rizza et al., 1982). Perhaps the binding of hGH to its receptor negatively effects the post-receptor actions of insulin. The net effect of hGH on carbohydrate metabolism is to increase blood glucose levels and decrease glucose uptake by tissues such as muscle.

In contrast to the anti-insulin-like effects discussed above, growth hormone has also been found to produce insulin-like effects. Growth hormone has been also shown to increase glucose utilization, produce antilipolysis, increase the oxidation of leucine and enhance the transport of amino acids; all insulin-like effects. Acute in vivo insulin-like effects have been demonstrated in hypophysectomized dogs (Steele, 1986; Sirek et al., 1969), rats (DeBodo and Altszuler, 1958; Hart et al., 1984; Batchelor et al., 1976) and in hGH-deficient kids (Frohman et al., 1967). Glucose uptake, oxidation and conversion to glycogen has been shown to occur initially when GH is given to hypophysectomized rats

(Goodman, 1967; Bolodia and Young, 1967; Ahren et al., 1970; Rillema and Kostyo, 1971). There appears to be an initial uptake of glucose and conversion to either glycogen or fat in vitro. Mahler and Szabo (1969) suggested that the insulin-like effects of GH result from the slowed degradation of insulin. However, because the insulin-like effects of hGH were not suppressed by the addition of anti-insulin serum (Froesch et al., 1963), the source of the insulin-like effects in muscle and adipose tissue was investigated. Compounds known as insulin-like growth factors I and II were identified as the factors responsible for the insulin-like effects attributed to hGH (Rinderknecht and Humbel, 1978a, 1978b).

HGH stimulates the release of IGF from the liver. IGF-I and II are homologous peptides structurally related to insulin (Blundell et al., 1978). Greater than 90% of the total IGF is secreted by the liver (Froesch et al., 1985) and travels in the plasma bound to specific carrier proteins (Zapf et al., 1975). Unlike hGH, the concentration of IGF does not undergo diurnal variations and is very stable in any given subject under constant nutritional and endocrinological conditions (Froesch et al., 1985). The major regulatory factors of the IGF concentrations in the serum are hGH levels (Zapf et al., 1981), nutrition, adequate insulin secretion and probably thyroid function (Foesch et al., 1985). Only IGF-I is absolutely dependent on hGH secretion (Zapf et al., 1981). IGF elicits all the classical insulin effects on target tissues; the stimulation of glucose transport, lipid, glycogen and protein synthesis. Lipolysis is inhibited by IGF in a manner similar to insulin. Therefore, the insulin-like effects resulting from hGH secretion may actually result from the actions of IGF-I.

The effect of hGH on insulin has also been studied. Exogenous hGH has been shown to increase the secretion of insulin into the blood. Stein et al. (1962) observed that 10 mg of hGH given to hypopituitary patients along with a glucose load, resulted in a plasma insulin and insulin-like activity 200-400% greater than that observed before hGH treatment. Frohman et al. (1967) likewise found that hGH deficient subjects secreted more

insulin (113%) when 1 mg IV hGH was given prior to a glucose load compared to the control condition without hGH injection (13.5%). Paradoxically, while hGH appears to stimulate the release of insulin, hGH also results in insulin resistance (Altszuler et al., 1961; Schalch and Kipnis, 1965; Zierler and Rabinowitz, 1963). The physiological effectiveness of insulin is impaired. Blood glucose levels remain elevated despite elevated insulin levels. In effect, hGH has created a carbohydrate intolerance not much different from that seen in diabetes. Hence, hGH is said to possess a "diabetogenic effect".

Growth hormone is also involved in regulating energy metabolism through its effect on lipid metabolism. The secretion of hGH (1) promotes the release of FFAs and glycerol from adipose tissues, (2) increases circulating levels of FFAs and (3) causes an increase in the oxidation of FFAs in the liver (Murray et al., 1990). Grunt et al. (1967) studied the acute effects of exogenous hGH administration on serum FFA levels in normal and hypopituitary children and found a significant increase in plasma FFA levels in both groups receiving exogenous hGH (2 mg IV). The eight hour fasting value in children with idiopathic hypopituitary was significantly greater (658 ±65 μEq/L) than normal children (411 ±65 μEq/L) in Grunt's study. Salomon et al. (1989) found a 20% reduction in body fat mass in hGH deficient subjects given 0.07 U per kg body weight hGH therapy for 6 months. Bak et al. (1991) infused healthy subjects with 45 ng/kg/min hGH and found that lipid oxidation contributed 71.7±5.6% of the nonprotein energy expenditure compared with 48.7±5.2% during the control infusion. Therefore, the effect of hGH on lipid metabolism is to enhance the release and oxidation of FFAs as an energy source.

In summary, the net result of the modulation of carbohydrate, protein and lipid metabolism by hGH is to enhance muscle mass, conserve glucose stores and utilize fat stores as an energy source. The anabolic, lipolytic and insulin antagonist properties may serve to promote optimal nutrient utilization during periods of deprivation.

In addition to influencing metabolism, growth hormone affects body composition. Recombinant hGH given to hGH deficient adults has been shown to increase lean tissue

and skeletal muscle mass and decrease fat mass (Cuneo et al., 1991a, 1991b; Salomon et al., 1989). The increase in limb skeletal muscle mass as revealed by radiographs shows that this process occurs rapidly, especially during the first month of treatment in hGH deficient children given recombinant hGH (Tanner et al., 1977). Well-trained adults given supraphysiological doses (8 mg per week) of exogenous hGH were able to increase fat-free weight, decrease percent body fat and increase the fat-free weight:fat weight ratio more than by exercise alone (Christ et al., 1988). This level was considered supraphysiological since spontaneous release of human hGH during a 24-hour period is purportedly 0.68 mg (4.8 mg/wk) in men and 0.79 mg (5.5 mg/week) in women (Thompson et al., 1972).

Lean body mass is an important determinant of exercise capacity (Fleg and Lakatta, 1988; Shepard et al., 1988; VonDoebln, 1956). Cuneo et al. (1991b) concluded from studies on hGH deficient adults given exogenous hGH (0.07 U/kg body weight for 6 months) that hGH treatment improves and normalizes maximal exercise performance and improves submaximal exercise performance. These changes are related to increases in lean body mass. Limb girdle muscle strength was increased by the same amount of exogenous GH provided to GH deficient adults, but an increase in strength in other muscle groups was unable to be detected, indicating the need for studies involving a larger sample size (Cuneo et al., 1991a). Although muscle mass increases with exogenous hGH treatment, a change in overall strength has not yet been documented.

Normal Values

Resting plasma hGH concentration oscillates throughout the day. On average, resting values are less than 5 ng/ml in humans with a range (including nighttime) of 3-13 ng/mL (Baulieu and Kelly, 1990; Winer et al., 1990). Winer et al. (1990) detected fluctuations in plasma hGH concentration throughout the day in men of between 40 ng/L and 7000 ng/L. Daily plasma hGH concentration in 9 men (age 26 ±4 years) varied from <1 ng/mL to approximately 4 ng/mL during the day and then rose to approximately 14

ng/mL during the night (Corpas et al., 1993). It has been known for some time that the largest hGH concentrations occur at night during slow wave sleep (stages 3 and 4) (Van Cauter et al., 1992; Holl et al., 1991; Hunter et al., 1966; Takahashi et al., 1968). The greatest elevation of plasma hGH concentration has been shown to occurr during the first half of sleep (to approximately 20,000 ng/L)(Winer et al., 1990).

A difference in hGH concentration has been found between genders. Although an identical number of peaks and valleys occur in hGH secretion in men as in women (an average of 13 pulses/day), women have higher overall hGH levels (1.49 ng/mL vs. 1.03 ng/mL), higher peak amplitudes (3.38 ng/mL vs. 1.64 ng/mL) and higher valleys (0.54 ng/mL vs. 0.39 ng/mL), respectively (Winer et al., 1990).

Plasma growth hormone concentrations have been examined in the fasted state. Ho et al.(1988) measured plasma hGH concentration in six men aged 21-36. Morning plasma hGH values (before 10 AM) were 10 ng/mL or less. Four of the six subjects had fasting morning values less than 5 ng/mL. Twenty minute blood samplings were obtained throughout the fast. Only water and a vitamin supplement were provided. Daily plasma hGH concentrations during day one of the fast (including nighttime) were 7.3 ±0.6 ng/mL and during day 5 of the fast were 9.9 ±0.7 ng/mL. Fasting progressively increased the hGH pulse frequency and maximal pulse amplitude. Hunter and Greenwood (1964) examined plasma hGH concentrations in 6 healthy men after an overnight fast and reported a mean of 2.7 ±3.1 ng/mL. In two of the subjects measured on two separate occasions, the fasting values ranged between 1.2 and 7.7 ng/mL Hunter and Greenwood (1964) concluded that an overnight fast stimulated plasma growth hormone secretion but to varying degrees between individuals. Similar values for an overnight fast have been reported elsewhere in the literature (Kelijman and Frohman, 1988; Holl et al., 1991).

When hGH concentrations are reported, the value is indicative of the rate of secretion, rate of excretion, release from a binding site and hemoconcentration (Shepard and Sidney, 1975). Factors which elevate hGH concentration include exercise and stress,

and those which depress hGH concentration include obesity, depression and hyperglycemia (Corpas et al., 1993). Additional factors which alter plasma hGH concentration will be discussed in detail below.

Regulators

Human growth hormone is an extremely sensitive hormone which is affected by numerous external and internal stimuli. Some factors are uncontrollable (i.e., gender, age, time of day) whereas others are under the control of the individual to some degree (i.e., diet composition, physical and psychological stress, medications, body composition, exercise and level of fitness). A combination of these controllable and uncontrollable variables results in a given growth hormone plasma value at a particular point in time. Knowledge of the factors affecting plasma hGH values is necessary when interpreting a hGH value.

Exercise is a potent stimulus for hGH release. The type, intensity and duration of the exercise will affect the hGH response to exercise (see Table 4). Previous exercise studies have demonstrated variable and inconsistent effects of different types of exercise on plasma hGH concentration (Karagiorgos et al., 1979; Kindermann et al., 1982; Kuoppasalmi et al., 1976; Lassare et al., 1974; Shepard and Sidney, 1975). Raynaud et al. (1983) studied the hGH response of subjects to different types of work and found that there was a significant intra-subject consistency to a given type of exercise over time, but a high variability between subjects. Intermittent exercise (a total of one hour of exercise at 60% VO_2 max separated into 30 minute intervals) was found to result in higher maximal hGH concentrations than continuous exercise (mean of 46.8 ±23.3 ng/mL versus 19 ±11.3 ng/mL) (Raynaud et al., 1983) even when the total amount of work was equal (Karagiorgos et al., 1979; Vanhelder et al., 1984). Arm work has been shown to result in a greater hGH response than leg work at equivalent oxygen uptake (Kozlowski et al., 1983).

The growth hormone release resulting from cycle ergometry has been found to be proportional to the intensity of the work (Sutton and Lazarus, 1976). Elevated hGH

Table 4. Exercise and growth hormone studies in men.

Reference	(n)	age(yrs)	type	duration (minutes)	intensity of exercise (EX)	hGH concentration	time measured
Karagiorgos et al. (1979)	10	21-30	cycling	40 min.	(1) 45% VO_2 max continuous (2) 90% VO_2 max intermittent	(1) 12.1 ng/mL (2) 9.7 ng/mL	during EX
Rennie et al. (1974)	4 (trained) 5 (untrained)	23-31 24-32	cycling	20 min.	(1) 1070 kpm/min (2) 715 kpm/min	(1) 27 ng/mL (2) 33 ng/mL	end of EX
Kaciuba-Usilko et al. (1992)	10	22 ±1.4	cycling	120 min., 4 equal parts, 30 min. rest between each	50% VO_2 max	(1) ≈10 ng/mL (2) ≈12 ng/mL (3) ≈13 ng/mL (4) ≈17 ng/mL	end of each bout
Scavo et al. (1991)	19	35 ±0.8	running	1/2 marathon (93 min) marathon (189 min)	subject's choice (race pace)	(1) 1/2 marathon-8 nmol/mL (2) marathon-6 nmol/mL	end of race
Naveri et al. (1985)	11	20-28	cycling	(1) 10 min. (2) 10 min. (3) 5-7 min.	(1) 63% VO_2 max (2) 86% VO_2 max (3) 100% VO_2 max	(1) 10 ng/mL (2) 21 ng/mL (3) 41 ng/mL	end of EX
Lassare et al. (1974)	10	20-47	cycling	60 min.	45-71% VO_2 max	5-40 ng/mL	end of EX
Hagen et al. (1972)	5	18-28	cycling	30 min.	500-750 kg/min	15 ng/mL	during EX
Sutton (1978)	8	23-29	cycling	20 min.	750 kpm/min: (1) 35% VO_2 max (2) 85% VO_2 max	 (1) 4 ng/mL (2) 13 ng/mL	after EX

Table 4. Exercise and growth hormone studies in men (continued).

Reference	(n)	age (yrs)	type	duration (minutes)	intensity of exercise (EX)	hGH concentration	time measured
Hartley et al. (1972)	7	20-24	cycling	20 min. bouts w/ 10 min. rest to exhaustion	≈75% VO_2 max	Pretraining: 13.2 ng/mL 3.7 ng/mL Trained: 13.6 ng/mL 7.0 ng/mL	40 min into EX 60 min into recovery 40 min into EX 60 min into recovery
Raynaud et al. (1983)	9	20-47	cycling	(1) 60 min submax, 20 min ex (2) 30 min (3) 60 min: continuous or 30 sec ex/rest	(1) 60% VO_2 max (2) 80% VO_2 max (3) 80% VO_2 max (continuous and intermittent)	(1) 15-30 ng/mL (2) 10-70 ng/mL (3) 5-30 ng/mL (continuous) 25-74 ng/mL (intermittent)	end of EX in all cases

secretions may result after 30-60 minutes of exercise at a work load equal to only 10-15% of VO_2 max (Galbo, 1981). Some studies have shown that hGH response is greatest with moderate exercise (75% of VO_2 max) and decreases towards basal levels with maximal exercise (Hartley et al., 1972; Hartley, 1975), whereas others have failed to show any relationship between exercise intensity and the magnitude of hGH response (Tatar et al., 1984). Felsing et al. (1992) found an elevation in hGH concentration (7.7 ±2.4 ng/mL above baseline concentrations) after 10 minutes of high intensity cycling (above lactate threshold) in men, whereas there was no increase in hGH concentration above baseline after cycling at an intensity below the lactate threshold. Hartley et al. (1972) found that hGH concentration increased from a mean of 3.1 ±0.8 ng/mL at rest to 13.2 ±2.4 ng/mL after 40 minutes of cycling at 73% VO_2 max but decreased to 7.7 ng/mL just prior to exhaustion. A high load weight lifting protocol resulted in a greater increase in hGH concentration (to 17 ng/mL in females; 14 ng/mL in men) than a low load protocol (hGH rose to 4.5 ng/mL in women and 2 ng/mL in men) (Kraemer et al., 1991).

The release of hGH following exercise may be related to the subject's level of fitness, with a greater response generally seen in unfit than in fit subjects, even at equal workloads (Galbo, 1983). Sutton et al. (1969) saw a greater rise in hGH concentration during maximal bike ergometry in unfit males (10.8 ng/mL) as compared to fit males (5.1 ng/mL). The high hGH concentrations in the unfit group were maintained for at least 120 minutes after fatigue whereas the concentration of hGH in the fit males fell immediately and returned to baseline concentrations by 60 minutes. Kraemer et al. (1993) found that 30 minutes of treadmill running at 80% max heart rate resulted in a 2.5 fold increase (to approximately 11 ng/mL mid-exercise) in hGH concentration which was not dependent on level of training. Longitudinal training studies have shown that the hGH response to an equivalent intensity of exercise is lower following training (Hartley, 1975; Tatar et al., 1984). The levels of hGH remained elevated until exhaustion while performing a moderate load in the trained subjects, whereas the untrained subject's hGH levels declined prior to

exhaustion. Conversely, Rolandi et al. (1985) found hGH to be increased equally in both athletes and non-athletes exercised to exhaustion. Luger et al. (1992) observed that the exercise-stimulated peak level of plasma hGH concentration was similar among untrained (6.1 ±2.0 ng/mL), moderately trained (8.1 ±3.6 ng/mL) and highly trained runners (11.5 ±4.3 ng/mL), when based on a percent VO_2 max. The maximum hGH response occurred at 70% VO_2 max in all groups. These findings indicate that training may result in a similar hormonal response to higher absolute workloads. The authors state that lactate may play a role in the exercise-induced hGH response.

Body composition is another factor regulating the amount of hGH released, both on a daily basis and in response to exercise. Body fat acts to inhibit hGH secretion. At a given age, endogenous hGH release is inversely related to the degree of adiposity (Rudman et al., 1981). Obese individuals are less responsive to hGH rises with exercise than their lean counterparts (Daugheday, 1985; Galbo, 1983; Merimee, 1979; Refetoff et al., 1979). Some studies which examined fit and unfit individuals failed to weight match the unfit subjects to the fit subjects, inadvertently including some obese subjects in the unfit group and possibly skewing the results. Obese subjects are able to raise their plasma hGH levels, however, if subjected to strenuous exercise (Schwartz et al., 1969).

Diet composition and caloric content can influence the concentration of plasma hGH. A diet low in calories has been shown to elevate plasma hGH concentrations. Plasma hGH concentrations have been measured during fasting (Roth et al., 1963; Copeland et al., 1980; Hayek and Peake, 1981), anorexia nervosa (Alvarez et al., 1972), kwashiorkor and marasmus (Pimstone et al., 1968), and in all cases have been found to be elevated. An increase in plasma hGH responsiveness to GHRH after as little as 24 hours of fasting has been documented in both obese and nonobese men and women . Plasma hGH responses to GHRH in normal weight subjects were significantly higher after fasting 24 hours and 72 hours than after an overnight fast (Kelijman and Frohman, 1988). Glucose ingestion has been found to reduce the hGH elevation resulting from mild exercise (Shepard and Sidney,

1975). McMurray et al. (1991) examined the combined effects of caloric restriction and diet composition (high carbohydrate-75% versus normal carbohydrate-50%) on the hGH response to 8 minutes of treadmill running at 85% VO_2 max and found that with caloric restriction and normal carbohydrate intake, hGH concentration five minutes post-exercise was 5.48 ng/mL whereas with caloric restriction and a high carbohydrate diet, the hGH concentration was 3.34 ng/mL. High carbohydrate diets have been shown to reduce the hGH response to exercise (Galbo, 1981; Quirion et al., 1988). Quirion et al. (1988) found that hGH concentrations following sub-maximal exercise (30 minutes of cycling at 75% VO_2 max) with a high carbohydrate diet (68% of total kcal) 48 hours prior to exercise were 8.33 ng/mL compared to 13.9 ng/mL of hGH following exercise on a high fat diet (57% of total kcal). Dietary protein seems to have a variable effect on plasma hGH levels. The rise in hGH produced by an overnight fast can be lowered by 50% within 1-2 hours after the fast is terminated by feeding 50 g of protein (Hunter and Greenwood, 1964). Conversely, amino acids such as arginine are known stimulators of hGH secretion. Arginine appears to be a potent hGH stimulator when given in doses of 250 mg/kg (Campistron, 1980). The ingestion of 1.2 g of l-arginine and 1.2 g of l-lysine resulted in a 7-fold increase in serum hGH concentration within 90 minutes (Isidori et al., 1981). The combination of dietary protein and fat can also increase the hGH response to exercise (Casaneuva et al., 1984; Casaneuva et al., 1981; Galbo et al., 1979). Dietary fat alone acts to inhibit hGH release. The IV injection of fat (Intralipid) with heparin by Cryer et al. (1972) into baboons resulted in a rapid fall in serum hGH concentration. Similarly, Blackard and Hull (1971) found a 91% decline in the hGH induced rise (from either an arginine or insulin stimulus) by 90 mL of oral Lipomul (a fat source) in females.

Additional factors also influence the response of hGH to exercise. Environmental temperature during exercise can be a stimulus for the release of hGH. Exercise in a warm environment has been shown to result in a greater hGH response than the same exercise in the cold (Buckler, 1973; Christensen et al., 1984; Frewin et al., 1976). Exposure to heat

alone, in the fasting state and in the absence of exercise, has resulted in a hGH surge (18.3 ng/mL; range of 7-44 ng/mL) greater in magnitude than that resulting during exercise (cycling at 450 kpm/min for 40 min; 8.4 ng/mL; range of 0.4-34 ng/mL) (Christensen et al., 1984). Both conditions evoked the same degree of elevation in body temperature. These authors concluded that exercise per se does not stimulate GH-secretion; temperature may provide the stimulus. Cold ambient conditions have resulted in almost complete inhibition of hGH secretion in response to exercise (Christensen et al., 1984; Frewin et al., 1976).

Age and gender are important mediators of the response of hGH to exercise. At rest, women exhibit significantly higher pre-exercise hGH concentrations compared to men (8.0 ng/mL vs. 1.5 ng/mL (n=8), Kraemer et al., 1991). At rest, Bunt et al. (1986) found higher resting hGH values in female runners (3.1 ng/mL) and female controls (2.4 ng/mL) than in either male runners (1.7 ng/mL) or male controls (1.2 ng/mL) (n=7 for each group). Exercise has been found to elevate hGH concentrations more in women than men. The gender difference in hGH concentration with exercise may be due to estrogen levels, the level of fitness or the degree of physiological stress associated with the exercise (Shephard and Sidney, 1975; Galbo, 1983). Bunt et al. (1986) found higher hGH concentrations in the female control group (n=7) compared to the male controls (n=7) during running at 60% VO_2 max until 60 minutes when both sexes had similar values (11 ng/ml). However, the mean peak hGH concentration in female runners was 15.6 ng/mL whereas in male runners, the peak concentration was 16.1 ng/mL. Males had a significantly greater percent increase in hGH than the females, due to the lower resting values.

After the age of 30, the secretion of hGH by the pituitary gland tends to decrease in both sexes (Rudman et al., 1981, Rudman, 1985, Finkelstein et al., 1972). Along with the decrease in hGH secretion is a decrease in lean body mass and concomitant increase in fat stores with age. Dudl et al. (1973) were unable to find a significant correlation with age and a decrease of hGH in men when body composition was accounted for; a decrease in lean

body mass and an increase in fat mass was observed. The mechanism of the age-associated decline of hGH may be related to a decrease in GHRH or an increase in somatostatin tone. However, to date, the accurate measurement of peripheral blood GHRH or hypothalmic somatostatin in peripheral blood is not available (Corpas et al., 1993). Fleg and Lakatta (1988) believe that a large portion of the age-associated decline in VO_2 max seen in both sexes in non-endurance trained individuals is explained by the loss of muscle with aging and not age per se. The stimulation of hGH by exercise and the resulting anabolic actions of the hormone may be of interest to the older active adult who wants to maintain muscle mass and/or improve athletic performance.

Psychological stress may increase resting hGH levels, but this appears difficult to prove. In rats, Coiro et al. (1981) studied the stress response invoked in vitro by epinephrine and norepinephrine and found a rapid responsiveness of tissues (loss of refractoriness) to hGH. Malarkey et al.(1991) examined the effect of academic stress and of season on 24-hour concentrations of hGH and concluded that academic stress among the 37 medical students did not significantly increase hGH concentrations. However, hGH concentrations were significantly higher in the fall than in the spring. Conversely, Greenwood (1966) found that a saline injection given to a medical student (n=1) who was told he had been given a large dose of insulin and would experience a severe hypoglycemic reaction resulted in an increase in plasma hGH concentration. A control group, without prior suggestion, did not result in an elevation in hGH concentration. The number of subjects in this study was small and this should be considered when interpreting the results. In student pilots undergoing a flight training program, a significant elevation of hGH from a mean of 1 ng/mL to 5 ng/mL occurred after a flight training program (Biselli et al., 1993). The physical stress of the training program could account for part of the elevation in hGH. The influence of psychological stress on hGH levels in marathon runners was examined 48 hours prior to the event and 1 hour prior to the event. On both occasions, subjects were in the rested and post-absorptive state. Growth hormone levels

increased significantly 1 hour prior to the event as opposed to 48 hours prior, indicating that the anticipatory stress produced an increase in plasma GH levels (Scavo et al., 1991). The involvement of psychological stress on the level of hGH remains unclear.

In summary, exercise is a stimulus for hGH secretion. Several studies have documented the elevation in plasma hGH concentration as a result of varying exercise treatments in men (see Table 4). Numerous factors mentioned above can confound the degree of elevation in plasma hGH resulting from exercise. A highly controlled study, accounting for as many variables known to influence plasma hGH concentration as possible, is therefore optimal when studing plasma hGH changes during exercise.

Vitamin B-6, Exercise and Growth Hormone

Pyridoxal 5'-phosphate is a cofactor for the apoenzyme dopa-decarboxylase, which is involved in the conversion of L-dopa to dopamine in neuronal and adrenal cells (Murray et al., 1990). L-tyrosine is the precursor for L-dopa and dopamine is the precursor for growth hormone releasing hormone. One study has examined the effect of vitamin B-6 supplementation on plasma growth hormone concentration and found that vitamin B-6 influences the production and secretion of plasma growth hormone (hGH) concentration (Delitala et al., 1976). Although conflicting results have been found as will be discussed below, supplemental vitamin B-6 has been shown to produce an elevation of plasma hGH concentration (Tolis et al., 1976; Mims et al., 1975). As previously discussed, exercise can also elevate plasma hGH concentrations. The next logical step was to examine the combined effect of exercise and vitamin B-6 supplementation on plasma hGH concentrations. To date, only a small group of researchers have examined this relationship. A link between vitamin B-6 supplementation, exercise and elevated plasma hGH concentrations would be beneficial in conditions where muscle wasting is a problem (i.e., aging, illness, impaired mobility).

Studies examining the effect of vitamin B-6 supplementation on plasma growth hormone concentration originated with vitamin B-6 deficient animals. In 1965, Huber and Gershoff demonstrated that vitamin B-6 deficient rats have less biologically active growth hormone in their pituitaries than was found in the pituitaries of control rats. Makris and Gershoff (1973) and Jain (1987) likewise found depressed circulating hGH concentrations in vitamin B-6 deficient rats. A portion of the low hGH concentrations was believed to be due to decreased food intake by the hGH deficient rats.

Pyridoxine administration has been reported to influence hGH release in man. Delitala et al. (1976) demonstrated the hypothalamic-dopaminergic effect of pyridoxine by injecting healthy volunteers (n=8) with a single dose (300 mg IV) of pyridoxine and found a statistically significant rise in plasma hGH concentrations to approximately 7 ng/mL by 120 minutes. In contrast to the stimulatory effect on hGH release, pyridoxine has also been shown to inhibit the stimulatory effect of exogenous L-dopa on plasma hGH. Mims et al. (1975) administered one gram of L-dopa to 12 male subjects and examined plasma growth hormone response. A mean plasma growth hormone concentration of 34 ng/mL by 60 minutes after the ingestion resulted. When a total of 200 g pyridoxine was infused on a separate occasion one hour before and 30 minutes after repeating the L-dopa dose, the mean peak hGH response decreased to 9 ng/mL by 90 minutes. Vitamin B-6 either blunted or completely prevented the hGH response to L-dopa. As an explanation for these controversial results, Rose (1978) proposed that the high concentration of pyridoxal 5'-phosphate stimulated dopa decarboxylase activity in the liver, so that it was rapidly converted to dopamine, which is unable to cross the blood-brain barrier. Less L-dopa would be available for the intraneural production of dopamine and plasma hGH concentration would fall.

Tolis et al. (1976) injected galactorrhea-amenorrhea women (n=9), who had a normal hGH reserve, with a 300 mg IV bolus of pyridoxine and measured serum hGH concentrations every 30 minutes for 3 hours. Two baseline blood samples were collected

prior to the injection. No consistent changes in growth hormone concentration from baseline were observed. Likewise, Delitala et al. (1978) found a paradoxical suppression of hGH secretion by the injection of 100 mg of pyridoxine in 28 full term newborns. Based on previous studies and the involvement of vitamin B-6 in the production of dopamine, these researchers expected hGH concentrations to either rise or remain unchanged. The unexpected results were attributed to the immature hypothalamic-pituitary system in infants.

The conflicting studies concerning the effect of pyridoxine supplementation and hGH release could have arisen from differences in the amounts of available tyrosine within the brain. While the stimulation of aromatic L-amino acid decarboxylase by pyridoxine has been shown to increase the metabolism of exogenous L-dopa to dopamine, it is less certain that this would result in enhanced dopamine production from the amino acid precursor tyrosine. In animal tissues, the hydroxylation of tyrosine to L-dopa has been shown to be the rate limiting step in catecholamine production (a metabolite of dopamine) (Udenfriend, 1964). In humans, there may be a different control point for dopamine production. Robins et al. (1967) has reported that the decarboxylation of 5-hydroxytryptophan (5-HT), not the hydroxylation reaction, is the rate limiting step in 5-HT synthesis in the brain. Since the synthesis of dopamine and 5-HT are dependent on a common enzyme, the decarboxylase may also be a rate limiting enzyme in the synthesis of dopamine. PLP, the cofactor for dopa decarboxylase, would then be an important factor in the synthesis of dopamine and growth hormone releasing hormone.

All of the aforementioned studies dealing with vitamin B-6 supplementation and plasma hGH concentration have dealt with an acute injection of massive doses of pyridoxine. Any stimulation of brain decarboxylase would result primarily from the activation of the existing apoenzyme. However, the oral supplementation of vitamin B-6 (200-600 mg/day) over a 94 day period in women with galactorrhea-amenorrhea may have resulted in the cofactor induction of enzyme synthesis (Greengard and Gordon, 1963; Holten et al., 1967).

The combination of pyridoxine administration and exercise on plasma hGH concentration was studied by Moretti et al. (1982). On two separate occasions, six healthy subjects exercised on a bike ergometer (80% of maximum heart rate; about 170 beats per minute) for eight minutes either with or without the simultaneous intravenous infusion of pyridoxine (600 mg) in a single blind design. Pre-exercise plasma hGH concentrations were 15% and 27% higher during the pyridoxine infusion 20 minutes prior and immediately prior to the start of the exercise respectively. They found significantly higher plasma hGH concentrations 60 minutes after exercise when pyridoxine was infused compared to saline (17 ng/mL vs 6 ng/mL). Peak plasma hGH concentrations (81 ng/mL) occurred 15 minutes after exercise stopped with the pyridoxine infusion. The results of this study indicate that an acute injection of vitamin B-6 (approximately 300 times the RDA) appears to stimulate growth hormone release above that typically seen with exercise. No studies examining oral vitamin B-6 supplementation, exercise and plasma growth hormone have been reported to date.

METHODS

Overview

Two similar but separate studies were conducted during the fall of 1990 (study 1) and the summer of 1992 (study 2). Following the completion of study 1, a freezer malfunction resulted in the unfortunate loss of samples to be used in several planned assays (plasma hGH, red blood cell PLP, plasma vitamin B-6, plasma albumin, plasma alkaline phosphatase). Therefore, study 2 was developed as a repeat of study 1. Study 2 was modified to strengthen the study design yet allow for the combination of data from both studies. A description of the modifications made in study 2 will be described below. In studies 1 and 2, trained cyclists were exercised to exhaustion twice; once in the non-supplemented state (NS) and once in the vitamin B-6 supplemented state (S) on a cycle ergometer at 75% of VO_2 max. Subjects consumed a controlled, nutritionally adequate diet for several days prior to and following each exercise test. The exercise tests were separated in time by a minimum of 23 days to allow blood levels of vitamin B-6 to stabilize prior to exercise test 2. Subjects followed the timeline consistent with the study they participated in (study 1 or study 2; see Figure 4). Subjects were familiarized with the experimental protocol and signed the informed consent forms. Studies 1 and 2 were approved by the Oregon State University Committee for Protection of Human Subjects.

Experimental Design

Study 1

Each subject progressed through the 32 day study protocol (see Figure 4). A staggered start design, in which two subjects per day began the study, was required in

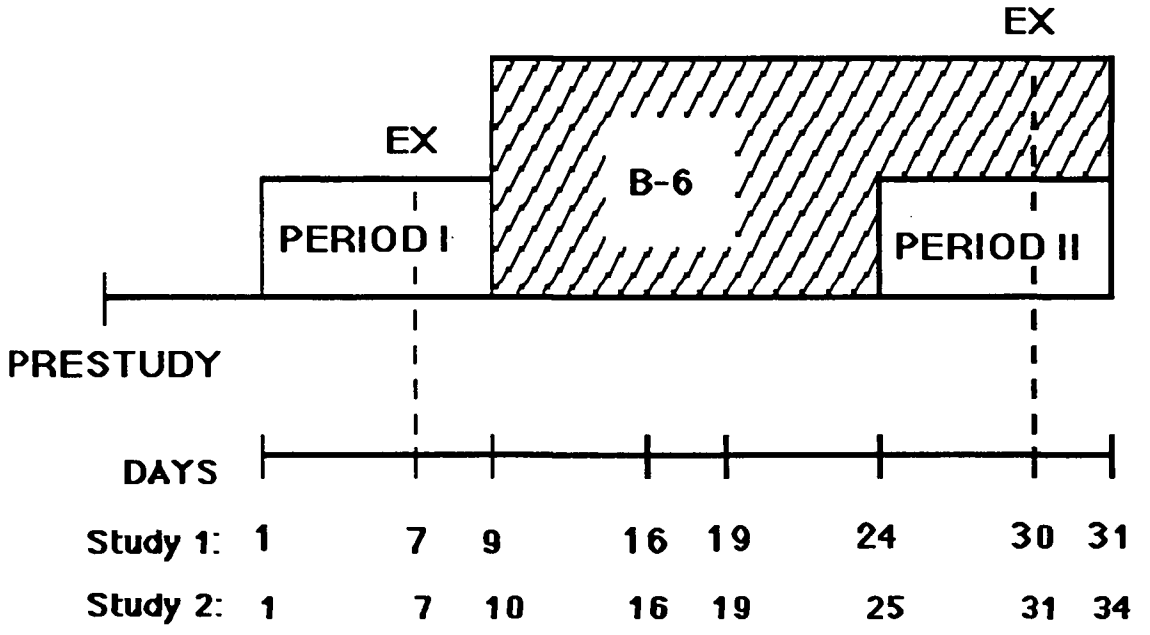

Figure 4. Timeline of study 1 and study 2.

order to conduct the lengthy exhaustive exercise tests. The first four days of each experimental period were designed to adapt subjects to the diet. Continuation of the controlled diet for one day after each exercise test was designed to assess post-exercise metabolic changes. A placebo capsule was provided to each subject during experimental period 1 in a single blind design. A vitamin B-6 supplement (20 mg (117.9 μmol) PN) was provided to subjects from day nine through day 31. Each subject served as his own control. Since several weeks are required to normalize vitamin B-6 levels in the body following B-6 supplementation (Johansson et al., 1966; Shane, 1978), all subjects rode to exhaustion first in the NS state and then in the S state. Prestudy testing included a VO_2 max test to assess maximal oxygen consumption. Each exhaustive exercise test was conducted at 64-75% of VO_2 max.

Midstudy testing included underwater weighing to assess the percentage of fat-free mass and the completion of three consecutive 24-hour dietary records to assess usual intake

of all nutrients, but especially kilocalories, protein and vitamin B-6. The food consumed during the midstudy period was self-selected.

Study 2

Study 2 was similar to study 1 in experimental design (see Figure 4). Differences between the two studies included the study length and experimental period length. The study 2 length was 34 days long vs. 32 days for study 1. The experimental periods in study 2 were lengthened to nine days instead of eight days. The extra day was added to the end of each experimental period to better assess metabolic changes which might have occurred post-exhaustive exercise. Each exhaustive exercise test occurred the morning of day seven in each experimental period of both studies.

Prestudy testing differed between study 1 and 2. Study 2 included a training ride prior to the start of the study to familiarize subjects with the exhaustive exercise test procedure and equipment and to eliminate any training effect. This training ride also allowed for accurate setting of the workload at a calculated 75% of VO_2 max. The training ride was approximately 30 minutes in duration.

During the midstudy period, four 24 hour dietary records were kept by subjects (vs. three during study 1) to better understand the subject's usual dietary intake.

Subjects

Study 1

Seven healthy, trained male cyclists were recruited from the Oregon State University campus and surrounding community. Women were not included in this study because of the potential hormonal influence on vitamin B-6 metabolism which would have

necessitated a study of longer duration (Leklem, 1991). A blood chemistry screening was done prestudy to evaluate health status.

Physical characteristics of the subjects are given in Appendix Table B.1. The level of training was determined prestudy by using the "Adapted guidelines from the American College of Sports Medicine (1986) for VO_2 max testing" (Appendix C). Subjects were asked to maintain this level of training throughout the study to minimize any training effect. Criteria for entry into the study included:

1. a wellness panel with lipid profile within the normal range (Good Samaritan Hospital, Corvallis, Oregon)
2. a normal xylose absorption test to assess carbohydrate absorption (i.e., excrete > 1.2 g in five hour for a 5 g intake)
3. no medical conditions known to interfere with vitamin B-6 metabolism (health history questionnaire)
4. completion of an exercise history questionnaire
5. a minimum of 120 minutes of aerobic activity (preferably cycling) in a minimum of 3 days per week
6. a normal ECG at rest and at maximal heart rate
7. a blood pressure of 120/80 or less at rest
8. no vitamin supplementation for at least four weeks prior to beginning the study
9. no nicotine use for at least one year or use of drugs known to interfere with vitamin B-6 metabolism or methodology (health history questionnaire)
10. completion of a VO_2 max test to assess fitness level

Study 2

Subject selection for study 2 was identical to study 1 except that the no xylose absorption test was conducted. Five trained male cyclists participated in Study 2. Physical characteristics of the subjects are given in Appendix Table B.1. One subject was common

to both study 1 and study 2. Two subjects in study 2 were consuming a vitamin supplement containing vitamin B-6 at least two weeks prior to the start of the study. Study 1 was conducted in the fall whereas study 2 was conducted in the summer.

Diet

Study 1

There were two 8-day controlled dietary periods (day 1-8 and day 23-31). The midstudy diet was self-selected. The diet contained 3476 kcal; 62% of kcal as carbohydrate, 17% of kcal as protein and 20% of kcal as fat. These foods were divided among three meals and one bedtime snack. The "Foods consumed daily for both the vitamin B-6 supplemented and non-supplemented diets (study 1)" are found in Appendix C. The diet met at least 100% of the Recommended Dietary Allowances (RDAs)(1989) for all nutrients for men 18-35 years of age. The NS controlled dietary period provided 2.31 mg of vitamin B-6 from food sources (based on food analyses) and the S controlled dietary period provided 2.32 mg of B-6. Following experimental period I (day 9), 20 mg/day of PN (in the hydrochloride form; individually weighed and placed in a gelatin capsule) were provided to each subject for the remainder of the study (days 9-31). The total vitamin B-6 intake during experimental period II was 22.3 mg/day.

All food served in this study was purchased in bulk from the same lot when possible. The tuna was thoroughly mixed, aliquoted into portions and frozen for daily use. The fresh produce was purchased on a weekly basis to ensure freshness and nutrient stability. Each food item on the menu was weighed to the nearest 0.1 g prior to being served to the subject to assure uniform nutrient intake. All meals were prepared and served at the Oregon State University Metabolic Kitchen. Subjects were instructed to eat all food and beverage provided and to rinse their bowls and utensils thoroughly with redistilled water after eating. The vitamin B-6 content of the diet was determined in our laboratory by

Karin Hardin using a microbiological assay (Miller and Edwards, 1981) of aliquots of food composites. One food composite from each experimental period was analyzed for vitamin B-6 content. This was done to determine the actual amount of vitamin B-6 the subjects received and to assess if storage and food preparation were altering the vitamin content of the food. Meals were prepared one meal in advance and refrigerated. No alcoholic beverages were allowed throughout the study and no caffeinated beverages were allowed the day before, the day of or the day after the exercise tests. The 24-hour diet records collected midstudy were analyzed for nutrient composition using Food Processor II Nutrient Analysis System, version 3.04 Plus (ESHA Research, 1990).

Study 2

The "Foods consumed daily for both the vitamin B-6 supplemented and non-supplemented diets (study 2)" are found in Appendix C. Differences in the diet in Study 2 included the substitution of Life™ cereal for shredded wheat, and salad dressing for low-calorie mayonnaise. Portion sizes were slightly altered in some cases to provide realistic serving sizes and improve the palatability of the diet. The diet contained 3569 kcals; 60% from carbohydrate, 17% from protein and 23% from fat. The vitamin B-6 intake from foods, assessed by microbiological assay, was 1.89 mg for period one and 1.92 mg for period two. Two food composites from each experimental period were analyzed for vitamin B-6 content. A placebo capsule was given to each subject from day 1-9 and 20 mg PN (in the hydrochloride form; individually weighed and placed in a gelatin capsule) were provided on days 10-33. Total vitamin B-6 intake during period II was 21.9 mg per day.

™ Trademark for Life cereal; Quaker Oats Co., Chicago, IL.

Exercise Procedure

Study 1

Prior to the start of the study, subjects completed a VO_2 max test. The VO_2 max protocol consisted of increasing the workload 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 exercise sessions. All exercise testing was completed on the same Monark cycle ergometer (Quinton Instruments). A tension belt on the cycle was used to alter the resistance.

Two identical exercise to exhaustion sessions were conducted at the Human Performance Lab on the Oregon State University campus: the first session was on the morning of day 7 (NS) and the second was on the morning of day 30 (S). A maximum of two subjects were tested per day. The "Information sheet for the endurance exercise test" is shown in Appendix C. The pre-exercise rest period included a resting blood pressure, measurement of body weight, the placement of ECG electrodes and an ECG tracing, placement of the sweat collector (NS exercise test only), adjustment of the bike seat height, and a resting blood draw. Sweat collectors were pre-made onto a glass plate using surgical tape, plastic wrap and glue prior to the start of the exercise test. A piece of plastic tubing with perforations was inserted into the sweat collector and a syringe at the other end of the tubing allowed for the collection of sweat. Each subject had their back cleaned with alcohol prior to the start of the exercise test. The sweat collector was adhered with surgical tape to the upper left hand corner of the back. Sweat was collected as sample accumulated in the sweat collector. Sweat samples were placed in 1 mL plastic vials and frozen for later analysis. The cyclists were exercised to exhaustion at 64-75% of their maximum aerobic capacity at 80 RPM (revolutions per minute). A brief warm-up period (approximately five

minutes) preceded each exhaustive ride. During this time, the workload was set. The length of time of the warm-up was constant for both exhaustive rides.

Difficulty in adhering the sweat collector that was used in the NS exercise test to the subject's back and collecting an adequate sweat sample for analysis prompted a change in sample collection for the S exercise test. Prior to the start of exercise, while the subjects were resting, each subject had the upper 3/4 of their back (the site for sweat collection) cleaned with alcohol swabs. The back was allowed to air dry. Pre-weighed pieces of Whatman 18.5 cm #1 filter paper and pre-weighed plastic storage containers were used to collect the sweat samples. As the subjects began to exercise, sweat samples were collected directly onto the filter paper between minute 3-4 of each 10 minute period. The upper portion of the back was lightly dabbed with one piece of preweighed filter paper at a time to absorb the sweat. The sweat sample was then immediately transferred to a pre-weighed plastic storage container. The number of pieces of filter paper collected was dependent upon the rate of sweat production of the individual. As many pieces of filter paper were collected as possible during each collection time. The container with the sample was weighed, the lid sealed with tape and frozen at -40° C for later analysis.

Subjects were tested in the fasting condition and were not allowed to exercise the day before, the day of or the day after the exhaustive exercise test in an effort to eliminate confounding variables. No fluids were provided to the cyclists during the exercise test to prevent dilutional effects in blood constituents. However, after the post-exhaustion blood draw, 100 mL of room temperature water were given to each subject. Climatic conditions in the Human Performance Lab were controlled to 20-24° C and < 60% humidity. A fan was provided to the exercising subjects at their request to aid in evaporative cooling. The subjects were weighed without clothes by a male volunteer before and after each exercise test.

During each exercise test, the subject's heart rate and ECG were monitored at ten minute intervals (with a Quinton Instruments ECG, Model 630 A) using three limb leads.

Respiratory gases were collected for two minutes at 10 minute intervals to calculate R values and measure oxygen consumption (Applied Electrochemistry; S-3A oxygen analyzer; Beckman, LB-2 carbon dioxide analyzer; Parkinson Cowan dry gas meter). The cycle rate was decreased for two minutes or less at 60 minutes to facilitate blood collection. The rate of perceived exertion (RPE) was asked of subjects at intervals to help identify the progression to exhaustion. Exhaustion was defined as the inability of the subject to maintain a cycle rate of 80±5 rpm for a total of 20 seconds. Most subjects were able to define exhaustion on their own without using this criteria. Subjects were not coached or encouraged to continue during the tests. Time (clock time and time to exhaustion) was concealed from the subjects in both exhaustive tests.

Following the cessation of exercise (i.e., exhaustion), the subject's heart rate was monitored for 3 minutes and an ending ECG was taken. Subjects rested quietly in a chair for the next hour. Following this period, a final body weight was obtained and the subjects were allowed to eat and drink.

Study 2

The "Endurance ride protocol in study 2" (Appendix C) was similar to study 1. Subjects were exercised to exhaustion on the same Monark cycle ergometer (Quinton Instruments) used in study 1. New monitoring equipment used in study 2 included a Sensormedics (Marquette Electronics) ECG monitor and Sensormedics 2900 Metabolic Cart.

The pre-exercise rest period was lengthened to provide two resting blood draws. The resting blood draws established baseline values for growth hormone and catecholamine analyses. The catecholamine analysis was completed by another graduate student in conjunction with this study. Subjects arrived at the Human Performance Lab at OSU with minimal exertion. They rested for a total of one hour before the start of the exhaustive exercise test. During this time, ECG electrodes were applied to the subject, the subject's

body weight was recorded, and the bike seat height was adjusted. A resting heart rate and ECG was taken prior to the start of the test. Sweat collection in both the NS and S state was identical to the filter paper method described in study 1.

The warm-up period and setting of the workload were identical to study 1. Once the VO_2 was set at 75% of max, subjects exercised to exhaustion. The revolutions per minute (RPM) were maintained at 80. The exhaustive exercise test conditions were identical between the NS and S states. Room temperature water (120 mL) was provided to the subjects following the one hour blood draw during each exercise test. Another 120 mL of water were given to the subjects following the post exercise blood draw. This is more fluid than was provided to the subjects in study 1.

Daily Procedures

Study 1 and Study 2

Subjects followed a number of daily procedures to assure compliance with the metabolic study and monitor health and activity patterns. A daily log was kept by each subject during the experimental periods (NS and S) . Subjects recorded information on: completeness of intake, food items consumed to adjust weight, beverage consumption, medications taken, overall health, accuracy of daily urine collections, daily body weight and the amount, type and perceived intensity of daily exercise. Subjects were required to return any unused portions of margarine and salad dressing, which were provided to increase calories for weight maintenance. Thus, accurate energy intakes could be calculated. During the mid-study period, subjects monitored their foods consumed, body weights, overall health and exercise. Food consumption was monitored mid-study using 24-hour dietary records. Subjects were required to record all foods and beverages consumed and the amounts.

Blood and Urine Collections

Study 1

Blood was collected prior to entry into the study, midstudy and on the exhaustive exercise test days (day 7 and day 30) according to the schedule below. A total of 20 mL of blood was collected during each collection, at the following times:

1) prestudy

2) exhaustive exercise tests:

resting:	(PRE; 5-10 min. prior to the start of the exercise test)
during exercise:	(DX; 60 minutes into the ride)
post exercise:	(POST; 1-2 minutes post-exhaustion)
	(POST 30; 30 minutes into recovery)
	(POST 60; 60 minutes into recovery)

3) mid-study

All samples were collected in the fasting condition. Heparinized evacuated blood tubes were used for collection and all samples were kept on ice until centrifuged. The plasma portion was extracted and stored at -40° C for later analysis. The red blood cells were washed three times with 0.9% saline and stored at -40° C.

Complete 24-hour urine collections were kept by the subjects during each day of the experimental periods and for each of the three days during the dietary recall period mid-study. All 24-hour collections were kept in plastic urine bottles, which contained approximately 10 mL of toluene as a preservative. Each morning, all daily samples were thoroughly mixed, portioned into small bottles, and frozen at -10° C for subsequent analysis. The pH readings were also taken and recorded for each daily urine sample.

Study 2

Blood was collected prestudy, the day of the exhaustive exercise tests and midstudy, as in study 1. However, the exercise test blood schedule was different from study 1. On the exhaustive exercise test days, blood was collected during study 2 at:

1) resting: (PRE 30; 30 minutes into the pretest rest period)
(PRE 60; 60 minutes into the pretest rest period)
2) during exercise: (DX; 60 minutes into the exercise test)
3) post exercise: (POST; 1-2 minutes post exhaustion)
(POST 60; 60 minutes into the recovery period)

These changes were made to establish a stable resting baseline value for the analysis of growth hormone. The 30 minute recovery blood draw was eliminated, since study 1 did not find a significant difference in vitamin B-6 indices in samples collected between 30 and 60 minutes of recovery.

Urine was collected during each day of the experimental periods and for each of the four days mid-study during which the 24-hour dietary recalls were kept. Collection and processing of the urine were identical to study 1.

Biochemical Analyses

The analysis of the blood and urine samples from study 1 (those not destroyed when the freezer malfunctioned) and study 2 will be described below. The analyses were chosen to assess the change in vitamin B-6 and growth hormone concentrations before, during and after exhaustive endurance exercise in the non-supplemented and vitamin B-6 supplemented states.

Vitamin B-6

Plasma and red blood cell PLP were analyzed using a modified method of Chabner and Livingston (1970). Plasma PLP samples were analyzed in duplicate and counted in a Beckman LS 5000 TD liquid scintillation counter. Counts were compared to a standard curve to calculate the nanomolar concentration. The inter-assay coefficient of variation (CV) was 12.9% (mean control value = 50.9 nmol/L) for the 6/4/91 control pool (n=21), 5.5% (mean control value = 82.7 nmol/L) for the 10/1/91 pool (n=7) and was 3.1% (mean control value = 35.0 nmol/L) for the 8/21/92 pool (n=12). The 6/4/91 control pool was used for the study 1 PLP analyses, and the 8/21/92 control pool was used for the study 2 PLP analyses.

The red blood cell PLP method of analysis was similar to the modified plasma PLP method (Chabner and Livingston, 1970) described above but there were some differences. The thawed red blood cell samples were kept on ice prior to the deproteinization step rather than at room temperature to prevent the conversion of PLP to other forms. The protein precipitation step involved different amounts of water (2 mL), sample (0.25 mL) and 75% TCA (0.50 mL). The dilution factor for the RBCs and water was 1:9. The dilution factor used for the RBC PLP assay was 101.2 as compared with a dilution factor of 48 for the plasma PLP assay. The centrifuged samples were decanted and washed with water-saturated ether 3 times. The ether layer was extracted each time under suction. From this point on, the RBC PLP analysis was identical to the modified method of Chabner and Livingston (1970). The inter-assay CV was 23.5% (mean control value on a vitamin B-6 supplemented sample was 1240 nmol/L) (n=9).

Urinary 4-PA was analyzed using a modified high performance liquid chromatography (HPLC) procedure of Gregory and Kirk (1979). Urine samples and controls were thawed, mixed and a sample was centrifuged. In a separate tube, the centrifuged urine (0.05% of the total urine volume), 0.2 mL of a pyridoxamine solution (0.1 mg/mL) and nanopure H_2O were combined to total 5 mL. The samples were vortexed

and filtered prior to filling sample vials. Samples from the S state were diluted with H_20 1:5 prior to sample preparation. A Beckman HPLC with a 0.034M, pH 2.2 phosphate-methanol mobile phase buffer (1.25% acetonitrile, 5% methanol) was used to analyze samples. A reverse phase column (Econsil C18 10μ; 250 mm x 4.6 mm) was used to separate the 4-PA. Samples were read on a Perkin Elmer fluorometer at 320 nm (excitation) and 425 nm (emission). Peak areas were printed on a Hewlett-Packard Integrator (3390A). The inter-assay CV was 3.6% (mean control value = 8.01 μmol/day) (n=33) and the intra-assay CV was 2.3% ±2.0 (n=11).

Plasma (study 2 only), sweat, dietary (using the AOAC method with chromatography step) and urinary vitamin B-6 were analyzed using a microbiological method of Miller and Edwards (1981). Sample preparation differed depending upon the type of sample to be analyzed. All samples were analyzed in duplicate. For plasma, 2 mL of sample were combined with 10 mL of 10% TCA to deproteinize the sample. The sample was mixed, centrifuged and decanted three times prior to autoclaving. The pH of the sample was adjusted to 4.5. The sample was diluted with nanopure H_20 to a final volume of 40 mL. For plant food, 2 g of sample were combined with 200 mL 0.44 N HCL and autoclaved for 2 hours. The pH of the sample was adjusted to 4.5. The sample was brought to a final volume of 250 mL, filtered and frozen for later analysis. For animal foods, 0.055 N HCL was added to 2 g of sample and autoclaved for 5 hours. The pH was adjusted to 4.5 and the final volume brought up to 250 mL. For urine, 10 mL of sample and 50 mL 0.01 N HCL were combined and autoclaved for 30 minutes to hydrolyze the sample. The pH of the sample was adjusted to 4.5. The sample was diluted to a final volume of 100 mL, mixed and filtered.

The inter-assay CV for plasma B-6 (study 2) was 12.6 % (n=4); no inter-assay CV could be calculated for the study 1 urinary B-6 since no controls were run; the inter-assay CV for the study 2 urinary B-6 was 12.8% (n=6). No food vitamin B-6 CV was calculated since no control was assayed in study 1, and only 1 control was assayed in study 2.

A procedure to extract sweat from filter paper was developed. Initial experiments to develop a sweat extraction procedure were conducted in 1991 following study 1. A series of extractions using controls (synthetic sweat on filter paper) and blanks (blank filter paper) were analyzed for vitamin B-6. The goal was to develop a technique to maximize the recovery of a known amount of vitamin B-6 from filter paper and assess the contribution of other factors to the final B-6 value. Synthetic sweat, containing a known amount of vitamin B-6, served as a control. The synthetic sweat contained 993.4 mg NaCl, 932.5 mg KCl, 957.15 mg Cl, 150.2 mg urea, 78.6 mg arginine-HCL and 7.5 mL of a 1.5 ng/mL PN-HCL solution in a total of 500 mL H_2O (pH 4.5). A sample of the synthetic sweat (2.7 mL) was pipetted onto a piece of Whatman 18.5 cm filter paper #1 and frozen in a manner similar to the sweat samples. Four pieces of the frozen control filter paper were thawed per control extraction. Various methods of washing the control and blank samples were tested. Examples included 1) mixing 1 cm pieces of the sample filter paper with 150 mL of 0.01N HCL for approximately 20 minutes to form a slurry, filtering the slurry through a glass filter under suction and washing the slurry two more times with 75 mL 0.01N HCL 2) placing two halves of the sample filter paper in a ceramic filter at one time and washing them three times with 25 mL 0.01N HCL under suction, or 3) layering 16 sample filter paper quarters into a ceramic filter and washing them four times with 60 mL 0.01N HCL under suction. The control and blank filtrates were evaporated under suction at 48-50°C to a final volume of approximately 30 mL using a rotary flash-evaporator (Buchler Instruments). An identical amount of synthetic sweat was also combined with 260 mL of 0.01 N HCL directly into a 1000 mL round distillation flask and evaporated under suction in a manner similar to the control and blank filtrates, eliminating the filter paper variable.

The results of these studies indicated an involvement of the filter paper and possibly other variables (i.e., the water) in the final value for vitamin B-6. Recoveries were 117% and 104%. The extraction and analysis of sweat blanks indicated there was 0.14-0.32 ng/mL of vitamin B-6. The source of this interference was unknown. The remainder of the

B-6 present was comparable to the condensed synthetic sweat sample which was not placed on filter paper. Slightly more synthetic sweat was extracted from the filter paper when the samples were soaked in 0.01N HCL prior to filtering; however, the blank vitamin B-6 value was also higher using this technique. The glass and ceramic filter appeared to produce similar values for the blank. Based on these limited experiments, the study 1 sweat samples were extracted from the filter paper using the ceramic filter, two filter paper halves at a time. The two halves were washed three times with 20 mL 0.01N HCL under suction. The filtrate was evaporated under suction to a final volume of 25 mL and the samples were frozen for later analysis.

The collection of sweat samples from study 2 prompted further experimentation with the sweat extraction procedure to determine the origin of the blank vitamin B-6 observed in 1991. The linearity of the blank value measured as B-6 was examined by washing either one, three or six pieces of blank filter paper with 0.01 N HCL (160 mL/piece). The filter paper pieces were either quartered and washed in a ceramic filter under suction (2 x 20 mL 0.01N HCL/quarter) one quarter at a time, or soaked in 200 mL 0.01N HCL to form a slurry and filtered. The slurry extracts were filtered under suction through a five inch ceramic filter lined with an acid-washed piece of filter paper. A blank consisting of three pieces of filter paper was also extracted using H_2O instead of HCL as above. An additional sample of 160 mL 0.01N HCL was passed through the ceramic filter and analyzed. The results indicated that the blank values resulting from the quartered pieces of filter paper were linear, whereas the values for the slurry extracts were not linear. Extracting three blank pieces of filter paper with either H_2O or HCL did not influence the resulting blank value. The sample containing filtered HCL only resulted in a blank value approximately half of that seen when one piece of chopped filter paper was extracted, indicating some involvement of the water to the final apparent B-6 value.

Experiment 2 was developed to examine the contribution of the filter paper and extraction water to the final vitamin B-6 value. First, the contribution of the water used in

the wash was tested. A total of 300 mL of 0.01 N HCL made with nanopure water was condensed to 40 mL, the pH was adjusted to 4.5 and the sample was frozen for later analysis. Another sample of 35 mL 0.01N HCL made with the same water as above had the pH adjusted to 4.5 and the final volume adjusted to 40 mL. The sample was frozen for later analysis. To test the contribution of the paper to the growth of B-6 in the assay, one, three and nine pieces of quartered filter paper were washed with a constant volume of 0.01N HCL (300 mL) in a ceramic filter under suction. The extract was condensed, the pH adjusted to 4.5 and the final volume adjusted to 40 mL. The sample was frozen for later analysis. To examine the amount of sample recovery, a known amount of PN (0.3, 0.45 or 0.6 ng) was placed on three pieces of filter paper and frozen for later extraction. The recovery samples were cut into quarters and washed with a total of 300 mL of 0.01 N HCL. The extracts were condensed, the pH was adjusted to 4.5 and the sample was frozen for later analysis. The water and filter paper appeared to make a negligible contribution to the vitamin B-6 assay total vitamin B-6 concentration (0.1 ng/mL each). The assay readings for the water and filter paper fell at the low end of the total B-6 assay curve, where interpretation of the curve is inaccurate. Improvement in recovery technique was recommended as a result of this experiment.

A third and final experiment was conducted to improve the percent recovery from the filter paper of a known amount of vitamin B-6. Three identical recoveries (three pieces of paper containing 2 ng/mL PN each) were extracted. The recovery filter papers were added to 200 mL 0.01N HCL and stirred for 20 minutes to form a slurry. The slurry was filtered under suction in a ceramic filter lined with an acid-washed piece of filter paper and was washed three times with 50 mL 0.01 N HCL. The recoveries were distilled to a final volume of 45 mL, the sample pH was adjusted to 4.5 and the sample was frozen for later analysis. The recovery values were 99%, 102% and 99%. A follow-up experiment involved the extraction of synthetic sweat (2 mL x 6 pieces of filter paper) prepared without

vitamin B-6 in a manner similar to above. No detectable vitamin B-6 was found in this follow-up experiment.

From these preliminary experiments, the following sweat extraction procedure was developed. Prior to the start of each extraction, 2000 mL of 0.01N HCL (using nanopure H_2O) were prepared. All work was done under yellow lights. Frozen samples were thawed at room temperature in their storage containers. The number of frozen sweat samples combined into one batch were based on the expected mg B-6/g sweat (Johnson et al., 1945) and the detection limits of the microbiological vitamin B-6 assay. The thawed samples were transferred directly to a 1000 mL beaker using a plastic stir stick. Two-hundred mL of 0.01 N HCL were added to the beaker. A portion of the 200 mL 0.01 N HCL was used to rinse the sample storage container. The sample and 200 mL of 0.01 N HCL in the beaker were mixed manually with the plastic stir stick until the filter paper broke up (approximately 10 minutes). The mixture was spread evenly into a porcelain filter containing one piece of acid-washed filter paper. The mixture was washed three times with 50 mL of 0.01 N HCL under suction. The 1000 mL extraction beaker was rinsed with one of these three washes. The final wash was allowed to sit without suction for approximately 15 seconds before the final suction. The extract was carefully transferred to a 1000 mL round flask in two batches for evaporation under suction. Three glass beads were added to the round flask to prevent spattering. The extract was evaporated to approximately 30 mL, transferred to a 100 mL beaker, the pH adjusted to 4.5 and the extract was brought to a final volume of 30-50 mL in a graduated cylinder. The extracted sample was then frozen in a plastic vial for later analysis. The intra-assay CV for study 1 B-6 sweat extraction assay was 4.6% (n=2). All sweat samples from study 2 were analyzed in one assay with one control, therefore, no CV could be calculated.

Growth Hormone

Plasma hGH was analyzed using an Allegro hGH IRA kit from Nichols Lab (San Juan Campistrano, CA). The samples were analyzed in duplicate and read on a gamma counter for 10 minutes at a 1% error. The values were calculated from a standard curve. The intra-assay CV was 24.3% (mean control value=0.0004 nmol/ml) (n=3). The control values fell below the lowest point on the standard curve, where interpretation is inaccurate. Nichols Labs reported an intra-assay CV of 4.2% for a control value of 0.0083 nmol/ml.

Other

Hemoglobin and hematocrit measurements were made in triplicate on all blood samples immediately following drawing. The hematocrit was measured by the microhematocrit method and the hemoglobin by the cyanomethemoglobin method. The hemoglobin and hematocrit from two separate blood samples were used to calculate plasma volume changes by the Dill and Costill (1974) and Van Beaumont (1972) methods according to the following formulas:

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

$$\% \text{ change} = [(100/100\text{-}Hct_1)\ 100\ (Hct_1\text{-}Hct_2) / Hct_2 \quad \text{(van Beaumont, 1972)}$$

Hb_1= initial hemoglobin conc., Hb_2= final hemoglobin conc.

Hct_1= initial hematocrit %; Hct_2= final hematocrit %

Creatinine was analyzed in duplicate with assistance from Ricky Virk using an automated procedure (Pino et al., 1965) and the values were used to access completeness of urine collections. The intra-assay CV for study 1 was 7.8 % (n= 2). No inter-assay CV

was calculated, since all samples were assayed in one assay. The intra-assay CV for study 2 was 2.8% (n=10) and the inter-assay CV was 2.4% (n=13).

Urinary urea nitrogen was analyzed using the method of Georgia (1974). The intra-assay CV for study 1 was 0.9% (n=2) and was 0.6% for study 2 (n=2). The samples in the respective studies were analyzed at one time.

Plasma albumin was analyzed in duplicate in study 2 using the method of Doumas and Biggs (1972) in our laboratory by Jim Ridlington. The intra-assay CV was 5.3 % (n=6). All study 2 alkaline phosphatase analyses were done at one time in duplicate by Jim Ridlington using the procedure of Roy (1970). The intra-assay CV was 7.8 % (n=4).

In conjunction with these studies, other analyses were conducted by graduate students working on the project. Plasma glucose in study 1 and 2 was auto-analyzed by Ricky Virk using the glucose oxidase method of Trinder (1969). Plasma free fatty acids were measured by Ricky Virk in study 1, and Ricky Virk and Jenny Young in study 2 using a colorimetric method (Falholt et al., 1973). Plasma lactic acid was analyzed in duplicate in study 1 and 2 by Ricky Virk spectrophotometrically (Henry, 1968). A kit from Sigma Chemical Co. was used.

Statistical Analyses

The data from study 1 and study 2 have been combined where possible to increase the statistical power of the experiment. The SAS statistical analysis software package (version 6.04) was used to analyze all data (SAS Institute Users Guide, Cary NC). Significance for the analyses was set at $p \leq 0.05$. Data were first checked for normality using the univariate procedure. Normally distributed data were analyzed using the GLM (general linear model) procedure which relies on normality and performs an analysis of variance (ANOVA). Any significant differences between means were tested using the Bonferroni t-test. Data which were not normally distributed were first log transformed to try to achieve normality. If the data were non-normal after the transformation, a non-

parametric analysis (NPAR1WAY) was used. Spearman correlations were performed on plasma, urine, sweat, plasma volume and study characteristics (kcal consumed, time to exhaustion, weight changes during the exercise test and daily exercise) both within and between variables. The Spearman correlation (a non-parametric analysis) was chosen because only five data points (the PRE values in study 2; NS and S states each evaluated separately) for nine variables were examined and normality could not be assumed. The r values were calculated using the SAS statistical package which ranks the data. The p values, approximated in SAS, were taken from the two-tailed significance level Spearman rank correlation coefficient table in Snedecor and Cochran (1980).

RESULTS

The results discussed below are from study 1 (1990) and study 2 (1992) vitamin B-6 and exercise studies. Data from studies 1 and 2 were combined to increase the sample size if no significant differences were found between specific data for the two studies. The change in blood vitamin B-6 measures, plasma hGH concentration and urinary vitamin B-6 measures before, during and after exhaustive exercise were used to assess the metabolic changes in vitamin B-6 status and growth hormone over time. The extent to which vitamin B-6 supplementation affected vitamin B-6 status and hGH concentration with exhaustive exercise was also analyzed.

Subject Characteristics

The subjects in study 1 and study 2 had similar physical characteristics (Table 5). Subjects in study 2 had a slightly higher mean VO_2 max (2.3%), and had approximately 12% greater mean body weight at the start of the study than the weight for the subjects in study 1. Although the subjects in study 2 were larger in body size (height and weight), the mean percent body fat of the subjects was identical between the two studies (13%), indicating a greater lean body mass in the subjects in study 2. The mean body weights did not change significantly during the course of either metabolic period (NS and S states). At the start of the NS period, the subjects in study 1 had a mean body weight of 76 ±4 kg and the subjects in study 2 had a mean weight of 85 ±12 kg. At the end of the NS period, the subjects in study 1 had a mean body weight of 75 ±5 kg and the subjects in study 2 had a mean body weight of 86 ±12 kg. At the start of the vitamin B-6 supplemented period, the subjects in study 1 had a mean body weight of 75 ±4 kg and the subjects in study 2 had a mean weight of 85 ±13 kg. By the end of the vitamin B-6 supplemented period, the subjects in study 1 had a mean body weight of 75 ±4 kg and the subjects in study 2 had a mean body weight of 85 ±13 kg.

Table 5. Mean age, body weight (start of study and mid-study), weight, height, percent body fat and VO_2 max of cyclists in study 1 and study 2.

	Study 1 (n=6)	Study 2 (n=5)
Age (years)	26 ±7+	29 ±7
Body weight (kg)- start of study	76 ±4	85 ±12
Body weight (kg)- mid-study	74 ±4	83 ±12
Height (cm)	179 ±3	185 ±6
Body fat (%)[1]	13 ±3	13 ±7
VO_2 max (L/min)[2]	4.4 ±0.5	4.5 ±0.3
(mL/kg/min)	60 ±8	55 ±6

+ Mean ± standard deviation
1 Body fat percentages were determined hydrostatically (mid-study)
2 VO_2 max was determined on a cycle ergometer (pre-study)

Exercise Tests

The mean time to exhaustion for study 1 and study 2 did not differ significantly between the NS and S state (Table 6). In the NS state, mean time to exhaustion was 120.6 ±16.7 minutes in study 1 and 94.8 ±42.9 minutes in study 2. Study 1 (NS state) represents a 27% longer time to exhaustion than study 2. In the S state, the mean time to exhaustion in study 1 was 36% longer than the time to exhaustion in study 2 (124.9 ±37.4 min vs 91.9 ±63.8 min). All subjects (except one in the S state) in study 1 cycled for at least 60 minutes before reaching exhaustion. The subject not cycling for 60 minutes rode for a total of 37

Table 6. Mean time to exhaustion, percent of VO_2 max exercised throughout each exhaustive exercise test, VO_2, rating of perceived exertion (RPE) at the last collection point and heart rate prior to exercise (PRE) and at the last sampling point prior to the end of exercise (END) in the non-supplemented (NS) and vitamin B-6 supplemented (S) state in study 1 and study 2.

		Study 1		Study 2	
		NS	S	NS	S
Time to exhaustion (min)		120.6+	124.9	94.8	91.9
		±16.7	±37.4	±42.9	±63.8
% of VO_2 max		71.5	69.0	73.9	75.1
		±2.0	±1.4	±5.2	±10.5
VO_2 (mL/kg/min)		31.5	30.6	39.9	42.4
		±4.0	±3.3	±5.0	±5.6
RPE		19.0	16.0	16.5	15.9
		±1.3	±2.1	±1.2	±2.1
Heart rate[1] (beats/min)	PRE	66.5[a]	64.0[a]	57.4[a]	58.0[a]
		±10.1	±10.3	±12.3	±15.0
	END	170.5[b]	166.7[b]	162.2[b]	162.8[b]
		±6.6	±13.6	±15.7	±17.7

+Mean ±standard deviation

[1] Values followed by a different letter denote a significant difference ($P<0.05$) between the PRE and the END heart rates in the NS and S conditions in study 1 and study 2.

minutes. In study 2, two subjects did not ride for at least 60 minutes in both the NS and S states. The first subject rode for 53 minutes in the NS state and for only 22 minutes in the vitamin B-6 supplemented state. The second subject rode for 52 minutes in the NS state and for 56 minutes in the vitamin B-6 supplemented state. The standard deviation in the time to exhaustion for the subjects in study 2 was large (42.9 minutes). The percent of VO_2

max at which the subjects exercised during each exercise test was 3% and 9% higher in study 2 than in study 1 in the NS and S states, respectively. The highest rating of perceived exertion (RPE) occurred in the NS state in study 1 (19.0). The other exercise tests (NS and S states) were perceived similarly by all subjects. The mean heart rates prior to exercise (PRE) were not significantly different between the NS and S states or between study 1 and study 2. Similarly, the mean heart rates at the end of exercise (END) were not significantly different between the NS and S states or between study 1 and study 2. As expected, there was a significant difference between the mean heart rates prior to exercise as compared to the end of exercise for the NS and S states in both studies.

The amount of exercise (duration times intensity) recorded in the subject's daily log books for both studies was significantly different between metabolic periods (NS vs. S condition)(Appendix Tables B.2 and B.3). The amount of exercise was approximately 30% greater during the NS period of both study 1 and study 2 than in the S period of each study. The absolute amount of exercise recorded by each subject was greater (non-significant) in study 2 (660 ±273 minutes of intensity) than in study 1 (545 ±288 minutes of intensity), indicating a higher level of exercise throughout the study periods.

Diet

The total amount of kilocalories (kcals) consumed by the subjects (Appendix Tables B.4 and B.5) were significantly different between study 1 and study 2. The difference in caloric intake was consistent between metabolic periods (NS and S states) within each study. Subjects in study 1 consumed an average of 3407 kcals (n=6), whereas subjects in study 2 consumed an average of 3845 kcals (n=5). The planned diets represent 50-52% of total calories from carbohydrate (552 g in study 1 and 542 in study 2), 17% of total calories from protein (155 g in study 1 and 154 g in study 2) and 20-23% of total calories from fat (81 g in study 1 and 93 g in study 2). The consumption of certain carbohydrate containing foods were allowed ad libitum to maintain the subject's body weight. During the vitamin B-

6 supplemented period of each study, the subjects consumed a greater (non-significant) mean amount of carbohydrate from the diet and other sources (581 ±52 g/d in study 1 and 644 ±44 g/d in study 2) than consumed during the NS period of each study (550 ±15 g/d in study 1 and 619 ±22 g/d in study 2).

The vitamin B-6 content of the diet, as analyzed by microbiological assay, was 2.31 mg during the NS period in study 1(1.30 mg from plant foods; 0.737 mg from animal foods and 0.28 mg from dairy foods) and was 2.32 mg during the S period study 1(1.30 mg from plant foods; 0.793 mg from animal foods and 0.24 mg from dairy foods). In study 2, the mean vitamin B-6 content of the diet was 1.89 mg during the NS period (1.13 mg from plant foods; 0.573 mg from animal foods and 0.277 mg from dairy foods) and 1.92 mg during the S period (1.09 mg from plant foods; 0.552 mg from animal foods and 0.29 mg from dairy foods). The differences in the vitamin B-6 content of the diets were primarily in the plant and animal foods. The mean vitamin B-6:protein ratio in study 1 was 0.0148 and was 0.0123 in study 2. During the mid-study period, the subjects in study 1 consumed a mean of 3365 ±945 kcals, 116 ±47 g protein and 3.08 ±1.52 mg vitamin B-6 on a self-selected diet. The mean vitamin B-6 to protein ratio was 0.026. Similarly, subjects in study 2 consumed a mean of 3519 ±1115 kcals, 126 ±59 g protein and 3.03 ±1.07 mg vitamin B-6 mid study, with a mean vitamin B-6:protein ratio of 0.026. The percent of calories coming from carbohydrate (CHO), protein (PRO) and fat during the mid-study period in study 1 was 61%, 14% and 25%, respectively, whereas in study 2, subjects consumed less of their total kcals from CHO and more from fat (56% CHO, 14% PRO and 31% fat). The serving sizes of several food items (orange juice, bread, raisins, mayonnaise, pears, gelatin, cheddar cheese, kidney beans, milk, peaches, turkey and grape juice) differed in study 1 and study 2 by 40 gm or less to provide realistic serving sizes and improve the palatability of the diet. The only difference in foods served was the cereal. In Study 1 shredded wheat was used, whereas in study 2 Life™ cereal was fed.

™ Trademark for Life cereal; Quaker Oats Co., Chicago, IL.

Hemoglobin and Hematocrit

The mean hemoglobin and hematocrit values for both studies are found in Table 7. The Dill and Costill method of plasma volume calculation (1974) requires the measurement of both hemoglobin and hematocrit, whereas the van Beaumont method (1972) requires only the hematocrit value. In study 1, the mean pre-study hematocrit value was 43.7 ±2.8% with a range of 39.6-47.5%. In study 2, the mean pre-study hematocrit value was 45.4 ±2.7% with a range of 42.5-49.5%. No significant differences were found in the hematocrit values between the NS and S states in either study or between study 1 and study 2. The mean resting (PRE) hematocrit value (NS and S states) was similar between study 1 and study 2 (44.5% in study 1 and 44.6% in study 2). The PRE value in study 2 represents the mean of two resting samples; one sample 30 minutes prior to exercise and one immediately prior to the start of exercise. The PRE hematocrit values (NS and S state) varied slightly more in study 2 as compared with study 1 (44.6±3.2% in study 2 with a range of 41.1-50.5 % as compared to 44.8±2.0% in study 1 with a range of 40.2-47.8 %). In study 2, the mean (NS and S) hematocrit values DX and POST were significantly greater than the mean PRE or POST 60 values ($p<0.05$). The PRE and POST 60 mean hematocrit values were not significantly different from each other.

The pre-study mean hemoglobin concentrations in study 1 and study 2 (151 ±1.0 g/L in study 1; range of 135 to 167 g/L; 154 ±1.3 g/L in study 2; range of 137 to 169 g/L) were similar. The resting (PRE) hemoglobin concentrations were also similar between study 1 and study 2 (147 g/L and 150 g/L, respectively). As was the case for the hematocrit values, there were no significant differences in hemoglobin concentration between the NS and S states or between study 1 and study 2. The mean (NS and S) DX and POST hemoglobin concentrations were significantly higher than the PRE and POST 60 hemoglobin concentrations. The mean (NS and S) hemoglobin concentrations PRE and POST 60 were not significantly different. The highest concentration of hemoglobin in the NS state (study 1) was found immediately after exercise (POST) in four of the six subjects.

Table 7. Mean hematocrit (Hct) percent and hemoglobin (Hgb) concentration before (PRE), during (DX), immediately after (POST) and one hour after (POST 60) exhaustive endurance exercise in the non-supplemented (NS) and vitamin B-6 supplemented (S) state in study 1 and study 2.

	Hct (%)			Hgb (g/L)		
Study 1	**NS**	**S**	**Mean (NS & S)[1]**	**NS**	**S**	**Mean (NS & S)[2]**
PRE[3]	44.5 ±1.5[+]	44.4 ±2.6	44.5 ±2.0	149 ±6.4	146 ±9.4	147 ±7.9[a]
DX	46.7 ±1.4	46.7 ±2.7	46.7 ±2.0	162 ±7.1	159 ±10.7	161 ±8.6[b]
POST	46.4 ±2.2	46.5 ±2.5	46.4 ±2.2	163 ±9.0	158 ±8.7	161 ±8.9[b]
POST 60	45.2 ±2.2	44.6 ±2.5	44.9 ±2.3	160 ±5.9	150 ±8.2	154 ±7.7[a]
Study 2						
PRE[4]	44.9 ±3.2	44.3 ±3.8	44.6 ±3.2[a]	149 ±6.4	150 ±12.9	150 ±9.9[a]
DX	47.6 ±3.1	48.7 ±3.8	48.1 ±3.2[b]	160 ±10.5	169 ±13.6	165 ±12.1[b]
POST	46.8 ±3.1	48.2 ±2.6	47.5 ±2.8[b]	161 ±7.0	166 ±12.5	165 ±9.4[b]
POST 60	44.6 ±2.0	45.0 ±2.9	44.8 ±2.4[a]	154 ±5.5	156 ±11.0	155 ±8.3[a]

[1] The mean hematocrit (NS and S state) in study 1 and study 2. Values followed by a different letter denote a significant difference ($p<0.05$) between time points. There was no significant difference between the NS or S states.

[2] The mean hemoglobin concentration (NS and S state) in study 1 and study 2. Values followed by a different letter denote a significant difference ($p<0.05$) between time points. There was a significant difference between the mean of all time points in the NS as compared to the mean values in S state in study 1 ($p<0.046$).

[3] PRE represents n=6; DX represents n=6 in the NS state and n=5 in the S state; POST represents n=6; POST 60 represents n=6.

[4] PRE represents n=5; DX represents n=3, POST represents n=5; POST 60 represents n=5.

[+] Mean ± standard deviation

The mean hemoglobin concentration in the vitamin B-6 supplemented state in study 1 was greatest at the exercise (DX) sample point for three of the subjects and at the post-exercise (POST) sample point for the other three subjects. In study 2, the highest mean concentration of hemoglobin occurred during exercise in both the NS and S states in two of the three subjects exercising to the DX sample point. The highest concentration of hemoglobin in those subjects exercising less than one hour occurred at the POST exercise sample point (NS and S state). The hemoglobin concentrations in the one subject common to both studies were greater before (8%), during (8%) and after exercise (8% at POST and 9% at POST 60) in the S state in study 2 as compared to study 1. For this subject, the hemoglobin concentrations in the NS state were similar for the two studies.

Plasma Volume Changes

The change in plasma volume represents a change from resting (PRE) levels (see Table 8). Mean plasma volume changes were calculated based on the method of Dill and Costill (1974) and van Beaumont (1972). Using the Dill and Costill method of calculation (1974), the plasma volume in study 1 and study 2 decreased by varying degrees during exercise (DX), immediately after exercise (POST) and one hour after exercise (POST 60) under the NS and S conditions. A decline in plasma volume represents a concentration of plasma constituents relative to a given initial volume. In study 1, the decline in mean plasma volume DX (-11.6% (NS) and -13.4% (S)) was greater than the decline POST or POST 60. There was a decrease in the mean plasma volume at POST 60 of -6.0% in the NS state and -4.4% in the S state. The changes in mean plasma volume during, immediately after and one hour after exercise in the NS state were not significantly altered by vitamin B-6 supplementation.

In study 2, the greatest percent decrease in the mean plasma volume, unlike study 1, occurred immediately after exercise in the NS and vitamin B-6 supplemented states (-11.6% and -16.1%, respectively). By one hour post-exercise, there was a decrease in the

Table 8. Mean percent change in plasma volume from resting conditions (PRE) during (DX), immediately after (POST) and one hour after (POST 60) exhaustive endurance exercise in the non-supplemented (NS) and vitamin B-6 supplemented (S) state in study 1 and study 2.

		DC[1]			VB[2]	
		NS	S	Mean (NS & S)[3]	NS	S
Study 1	DX[5]	-11.6 ±2.1[+]	-13.4 ±2.0	-12.5 ±2.1[a]	-8.4 ±1.9	-10.5 ±2.1[a]
	POST	-11.5 ±4.4	-11.6 ±2.7	-11.6 ±3.5[a]	-7.0 ±4.7	-8.4 ±2.6[a]
	POST 60	-6.0 ±5.7	-4.4 ±4.1	-4.5 ±4.7[b]	-2.6 ±5.4	-0.9 ±2.6b
Study 2	DX[6]	-9.0 ±3.4	-13.9 ±2.0	-11.5 ±3.64[a]	-6.9 ±4.3	-10.3 ±2.6[a]
	POST	-11.3 ±6.9	-16.1 ±5.8	-13.7 ±6.5[a]	-7.7 ±8.8	-13.9 ±6.5[a]
	POST 60	-3.4 ±5.8	-4.7 ±4.5	-4.1 ±5.0[b]	0.7 ±7.9	-2.2 ±4.4[b]

[1] DC refers to the Dill and Costill method of plasma volume calculation (1974).

[2] VB refers to the van Beaumont method of plasma volume calculation (1972).

[3] Mean plasma volume change (NS and S states) from PRE using the Dill and Costill method of calculation (1974). Values followed by a different letter denote a significant difference ($p<0.05$) in time points. There was no significant difference between the NS and S states.

[4] Mean plasma volume change (NS and S states) from PRE using the van Beaumont method of calculation (1972). Values followed by a different letter denote a significant difference ($p<0.05$) in time points. A significant difference was found between the mean of all time points in the NS and S state ($p<0.04$).

[5] Mean change in plasma volume from PRE in study 1 DX (n=6 in the NS state; n=5 in the S state), POST (n=6) and POST 60 (n=6).

[6] Mean change in plasma volume from PRE in study 2 DX (n=3), POST (n=5) and POST 60 (n=5).

[+]Mean ± standard deviation

mean plasma volume of -4.4% in the NS state and -4.7% in the S state. Using the Dill and Costill method (1974) of calculation of plasma volume changes, no significant differences were found when comparing the plasma volume changes for the NS and S states or comparing the values for studies 1 and 2. However, the mean plasma volume change POST 60 was significantly less than the mean plasma volume changes DX or POST. Using the van Beaumont method of calculation (1972), there were no significant differences in plasma volume changes between study 1 and study 2. However, there were significant differences in plasma volume changes between the NS and S states ($p<0.04$). In the S state, the mean plasma volume change POST 60 was significantly less than the plasma volume change DX or POST. The change in mean plasma volume at all sampling points appeared to be greater under conditions of vitamin B-6 supplementation.

The Dill and Costill (1974) formula for the calculation of plasma volume changes was used as the basis for calculating a predicted plasma albumin, plasma PLP and plasma vitamin B-6 (PB-6) concentrations for a particular time point. The predicted value is based on the assumption that the only changes in the PRE PLP, PB-6 and albumin concentration during and after exercise resulted from a change in plasma volume. Therefore, the predicted values are not a corrected concentration, but are a hypothetical concentration of the plasma (PRE) concentrations based solely on the observed changes in plasma volume during and after exercise. The measured plasma PLP, PB-6 and albumin concentrations are all expressed per liter. To calculate the predicted plasma PLP, PB-6 and albumin concentrations, the measured value (PRE) was divided into that portion of one liter remaining after adjusting for the change in plasma volume. For example, if a 10% loss of plasma volume occurred when a 30 nmol/L concentration of PRE PLP was measured, the predicted PLP concentration, based only on a change in plasma volume, would be 30 nmol/.9L or 33.3 nmol/L.

Plasma Values

Albumin

Plasma albumin was analyzed only in study 2 (see Table 9). Vitamin B-6 supplementation did not significantly effect mean plasma albumin concentrations before, during or after endurance exercise. However, endurance exercise did alter mean plasma albumin concentration in both the NS and S states. There was an increase in the mean plasma albumin concentration from resting values during exercise in both the NS and S conditions (7.4 g/L (13%) and 8.8 g/L (16%), respectively). The mean plasma albumin concentration during exercise, resulting solely from the observed decrease in plasma volume at that point, should have been 63.2 ±3.7 g/L in the NS state and 64.8 ±3.4 g/L in the S state (see Table 10). We observed a mean concentration of 62.8 ±2.3 g/L during exercise in the NS state and 63.5 ±3.4 g/L in the S state. Therefore, plasma volume changes appeared to account for the majority of the increase in plasma albumin concentration during exercise. POST exercise, the mean plasma albumin concentration decreased (from the DX sample point) -2.7 g/L (-2%) in the NS state and -1.4 g/L (-1%) in the S state. When the plasma albumin concentration from the same three individuals was compared, there was no change from DX in the NS state and a slight decrease (-1 g/L) in the S state at POST. A decrease in the mean plasma albumin concentration from the POST level occurred one hour after exercise (-6.5 g/L (-6.5%) in the NS state and -6.3 g/L (-10%) in the S state). This difference is evident when the same three individuals were compared at POST and POST 60. However, the plasma volume changes in these three individuals at POST 60 accounts for all of the measured plasma albumin concentration and suggests no true change in plasma albumin concentration at POST 60. pyridoxal 5'-phosphate (PLP) (studies 1 and 2), red blood cell PLP (RBC PLP) and plasma vitamin B-6 (PB-6) were all significantly higher with vitamin B-6 supplementation as compared to the NS condition. However, the magnitude of the rise was different.

Table 9. Mean plasma albumin concentration before (PRE), during (DX), immediately after (POST) and one hour after (POST 60) exhaustive endurance exercise in the non-supplemented (NS) and vitamin B-6 supplemented (S) state in study 2.

	NS	S	Mean (NS & S)[1]
	g/L		
PRE[2]	55.4 ±3.8+[a]	54.7 ±3.7[a]	55.1 ±3.6[a]
DX	62.8 ±2.3[b]	63.5 ±3.4[b]	63.2 ±2.7[b]
POST	61.1 ±1.5[ab]	62.6 ±2.5[b]	61.9 ±2.1[b]
POST 60	57.1 ±4.2[ab]	56.3 ±3.8[a]	56.7 ±3.8[a]

[1] Mean (NS & S) represents the mean albumin concentration in the NS and S states. Different letters denote a significant difference ($p<0.05$) between time points within each column. No significant differences were found between the albumin concentration in the NS and S states.
[2] PRE represents n=5; DX represents n=3; POST represents n=5; POST 60 represents n=5.
+Mean ± standard deviation

Table 10. Mean predicted plasma albumin concentration during (DX), immediately after (POST) and one hour after (POST 60) exhaustive endurance exercise in the non-supplemented (NS) and vitamin B-6 supplemented (S) state in study 2 corrected for the changes in plasma volume (Dill and Costill method, 1974).

	NS	S
	g/L	
DX[1]	63.2 ±3.7+	64.8 ±3.4
POST	62.6 ±3.1	65.2 ±3.0
POST 60	58.9 ±2.2	57.4 ±3.3

[1] DX represents n=3; POST represents n=5; POST 60 represents n=5.
+ Mean ± standard deviation

Plasma Vitamin B-6 Indices

As expected, vitamin B-6 supplementation resulted in a significant elevation of the vitamin B-6 indices in the blood as compared to the non-supplemented state. Plasma

between the studies. For example, in study 1 the PLP concentration was approximately 6 fold greater in the S state as compared with the NS state, whereas in study 2, the PLP concentration was approximately 4.5 fold greater.

Plasma Pyridoxal 5'-Phosphate

The pre-study plasma pyridoxal 5'-phosphate (PLP) levels ranged from 16.1 nmol/L to 46.9 nmol/L in study 1 and from 18.5 nmol/L to 209.1 nmol/L in study 2. We were aware that two subjects in study 2 had been consuming vitamin supplements prior to the start of the study. One subject with a pre-study PLP concentration of 130 nmol/L reported taking one multivitamin/day prior to the start of the study and the another subject with a pre-study PLP concentration of 209 nmol/L reported taking 500 mg vitamin C two weeks prior to the start of the study. However, based on the laboratory findings, we suspect that this subject was also taking supplemental vitamin B-6.

The mean plasma PLP concentrations for the subjects in study 1 and study 2 are reported in Table 11. Two of the six subjects in study 1 had PRE concentrations below the recommended 30 nmol/L concentration prior to the NS exercise test (Leklem, 1990). Likewise, in study 2, two of the five subjects were below the recommended value. One of the two subjects below the recommended plasma PLP concentration in study 2 was one of the same subjects that had been supplementing prior to the start of the study. The mean plasma PLP concentration as a result of the exhaustive exercise alone (NS state) will be described first, followed by the results of the combined vitamin B-6 supplementation and exercise tests (S state).

Compared to resting conditions (PRE), an increase in the mean plasma PLP concentration of 4.9 nmol/L (18.8%) occurred during exercise in study 1 in the NS state (Table 11). An increase in the plasma PLP concentration during exercise was seen for all six subjects (an increase of 1.8 to 7.5 nmol/L). However, since the variation between

Table 11. Mean plasma pyridoxal 5'-phosphate concentration before (PRE), during (DX), immediately after (POST) and one hour after (POST 60) exhaustive endurance exercise in the non-supplemented (NS) and vitamin B-6 supplemented (S) state in study 1 and study 2.

Test		Study 1(n=6)[1]	Study 2(n=3)	Study 2(n=5)
			nmol/L	
NS	PRE	31.4 ±12.5[+]	38.3 ±6.30	32.4 ±10.1
	DX	36.3 ±12.8	42.5 ±10.0	---
	POST	30.0 ±13.4	34.4 ±7.74	32.6 ±7.52
	POST 60	25.3 ±9.65	30.0 ±6.07	26.9 ±7.54
S	PRE	199 ±47.6	177 ±6.51	172 ±24.2
	DX	261 ±65.2[a]	188 ±18.0	---
	POST	201 ±39.5	171 ±10.4	179 ±28.3
	POST 60	168 ±33.0[b]	144 ±12.0	146 ±18.6

[1] PRE, POST and POST 60 represent n=6; DX represents n=6 (NS state) and n=5 (S state). Values followed by different letters denote a significant difference ($p<0.05$).
[+] Mean ± standard deviation.

values was so great, statistical significance was not obtained. There was a -6.3 nmol/L (-17%) decrease in mean plasma PLP concentration at the POST sample point compared to DX and an additional -4.7 nmol/L (-16%) decline from the mean POST concentration one hour after exercise. Similarly in study 2 (NS state), the mean PLP concentration DX was higher (4.2 nmol/L, n=3)(11%) than the mean PRE PLP concentration. A complicating factor when examining each exhaustive exercise test in study 2 was that only three of the five subjects were able to exercise to the DX blood sampling point. The two subjects who reached exhaustion prior to one hour (i.e., the POST sample point) completed each exercise test at a chronological time more comparable to the DX sample point of the other three subjects. The mean plasma PLP concentration POST and POST 60 was less than the mean PLP concentration DX. There was an -8.1 nmol/L (-19%) decrease (n=3) in the mean plasma PLP concentration between DX and POST, and there was a -4.4 nmol/L (-13%)

decrease in the mean plasma PLP concentration between POST and POST 60. When all subjects in study 2 were included, the mean POST PLP concentration was only 0.2 nmol/L higher than the resting value (PRE). Compared to the PRE concentration, a mean decline in the plasma PLP concentration of -5.5 nmol/L (-15.6%) occurred one hour after exercise (n=5).

The predicted plasma PLP concentrations during and after exercise (NS state) were calculated based on the plasma volume changes (see Table 12). In study 1, we would

Table 12. Mean predicted plasma pyridoxal 5'-phosphate concentration during (DX), immediately after (POST) and one hour after (POST 60) exhaustive endurance exercise in the non-supplemented (NS) and vitamin B-6 supplemented (S) state in study 1 and study 2 (Dill and Costill method, 1974).

Test		Study 1(n=6)[1]	Study 2(n=3)	Study 2(n=5)
NS			nmol/L	
	DX	35.5 ±14.0[+]	42.1 ±7.44	---
	POST	35.7 ±14.8	41.1 ±7.03	36.1 ±9.73
	POST 60	33.5 ±13.4	39.8 ±7.70	33.5 ±10.3
S	DX	215 ±41.9	205 ±12.5	---
	POST	226 ±54.5	202 ±14.6	206 ±35.0
	POST 60	194 ±47.1	183 ±16.4	181 ±24.9

[1] PRE, POST and POST 60 represent n=6; DX represents n=6 in NS state and n=5 in S state. [+] Mean ± standard deviation.

expect a mean plasma PLP concentration of 35.5 nmol/L during exercise if the loss of plasma volume contributed to all of the rise in plasma PLP DX. However, the measured mean plasma PLP concentration during exercise in study 1 (NS) was 36.3 nmol/L, which indicates that a small amount of PLP was entering the plasma during exercise. In study 2, there was a similar small increase in the mean measured plasma PLP concentration (n=3) above the predicted value DX (measured PLP: 42.5 nmol/L; PLP adjusted for plasma

volume(predicted value): 42.1 nmol/L). Based on the shift in plasma volume occurring POST, a plasma PLP concentration of 35.7 nmol/L and 36.1(n=5) or 41.1 (n=3) nmol/L could be expected in studies 1(n=6) and 2(n=5 or n=3), respectively. The measured plasma PLP concentration immediately after exercise was 30.0 nmol/L and 32.6(n=5) or 34.4 (n=3) nmol/L, respectively. These results suggest a loss of PLP from the plasma in both studies at POST. The loss was common to all but two subjects, whose plasma PLP concentrations increased by 2% and 13%. Both of these subjects were in study 2. Similar plasma PLP concentrations one hour after exercise (POST 60) would be expected between studies 1(n=6) and 2(n=5) based only on plasma volume changes (33.5 nmol/L in study 1 and study 2). However, the measured PLP values at POST 60 (25.3 nmol/L in study 1 and 26.9 nmol/L in study 2(n=5)) were less than the predicted values, suggesting a further loss of PLP from the plasma.

In the vitamin B-6 supplemented state, there was a rise in plasma PLP with exercise and a fall in the concentration immediately after and one hour after exercise as was seen in the NS state. A blood sample was not obtained for one subject in study 1 at the DX point in the vitamin B-6 supplemented state, and for two subjects in study 2 since they exercised for less than one hour. Of the subjects who exercised for at least one hour in both studies, all but one subject had an increase in plasma PLP concentration (an increase of 5-55%). There was no change in plasma PLP concentration in one subject. While the trend was consistent among the individuals, the variation in the values did not result in statistical significance. Considering the POST PLP sample as the chronological equivalent of the DX time point, then the two subjects in study 2 who reached exhaustion prior to one hour had a similar increase in plasma PLP during exercise. The increase in the mean plasma PLP concentration during exercise in study 1 (62 nmol/L or 28%) was greater than the mean PLP increase in study 2 (11 nmol/L or 6%)(Table 11). The predicted mean plasma PLP concentration during exercise in the vitamin B-6 supplemented state based on plasma volume changes was 215 nmol/L in study 1 and 205 nmol/L in study 2 (n=3). A greater

mean concentration of PLP was found in study 1 during exercise (261 nmol/L) whereas a lower concentration was found in study 2 during exercise (188 nmol/L). The large standard deviation in the measured mean plasma PLP values in study 1 complicates the interpretation of this data. In study 1, there was a decrease from the DX level in mean plasma PLP concentration POST exercise comparable to the rise during exercise (60 nmol/L or 23%). In study 2, the decrease in the mean PLP concentration in those subjects who were able to exercise for at least one hour (n=3) was slightly greater than the rise in PLP concentrations seen during exercise (17 nmol/L (9%) vs 11 nmol/L (6%)).

As was evident in the NS state, the decrease in the mean plasma PLP concentration between the DX and POST sample points under vitamin B-6 supplementation conditions in study 1 was greater than the decrease observed in study 2. When the changes in plasma volume POST were considered, a mean plasma PLP value of 226 nmol/L in study 1 and 206 nmol/L in study 2 (n=5) value would be expected. In both cases, the measured mean plasma PLP concentration (201 nmol/L in study 1 and 179 nmol/L in study 2) was lower than the mean predicted based only on plasma volume changes. A loss of plasma PLP POST exercise in the vitamin B-6 supplemented state was therefore evident in studies 1 and 2. The concentration difference between the actual and predicted mean concentration of plasma PLP immediately after exercise was comparable in study 1 (25 nmol/L)(n=3) and study 2 (n=5) (27 nmol/L). The mean plasma PLP concentration continued to decrease from POST to POST 60. As compared with the POST sample point, there was 16% (33 nmol/L) less plasma PLP present POST 60 in study 1, and a 16% (27 nmol/L)(n=3) or 18% (33 nmol/L)(n=5) less mean plasma PLP present POST 60 in study 2. The mean predicted plasma PLP concentration POST 60 based solely on the associated plasma volume change was 194 nmol/L in study 1 and 183 nmol/L in study 2. The measured mean PLP concentrations (168 nmol/L in study 1 and 144 nmol/L in study 2) were lower than the predicted mean values, indicating a further loss of PLP from the plasma. The plasma PLP

concentration for all subjects in both studies decreased between the POST and POST 60 sample points.

When all sample points were considered, the PLP values for study 1 and study 2 were found to be significantly different from each other under the conditions of vitamin B-6 supplementation ($p<0.009$). The subjects in study 2 had significantly lower PLP values than the subjects in study 1. The difference may be due to differences in the retention of vitamin B-6 by the body or may be due to differences in the vitamin B-6 and/or protein content of the diets used. The decrease in the mean plasma PLP concentration between the DX and POST 60 sample point in study 1 in the S state was also found to be statistically significant.

Plasma Vitamin B-6

Plasma vitamin B-6 (PB-6) was measured only in study 2 (see Table 13). The changes in plasma vitamin B-6 with exercise followed a pattern similar to the plasma PLP changes. The pre-study PB-6 values ranged between 49.9-234.9 nmol/L. The changes in PB-6 as a result of the exercise will be discussed first (NS state), followed by a discussion of the combined effect of vitamin B-6 supplementation (S state) and exercise.

There were approximately three weeks between the pre-study blood draws and the first exhaustive exercise test. At the start of the NS exercise test, the resting PB-6 values were between 34.9-79.6 nmol/L. The suggested PB-6 concentration for adequate status is >40 nmol/L (Leklem, 1990). Two subjects, the same two with PLP concentrations below 30 nmol/L, had resting PB-6 concentrations below 40 nmol/L. In the NS state, there was a 6.8 nmol/L(12%) elevation of the mean plasma PB-6 concentration during exercise (n=3) compared to resting concentrations (PRE)(Table 13). The PB-6 concentration increased DX for each of the three subjects. Considering the POST PB-6 value as the DX value for the two subjects who did not exercise to DX, a similar increase in the PB-6 concentration

Table 13. Mean plasma vitamin B-6 concentration before (PRE), during (DX), immediately after (POST) and one hour after (POST 60) exhaustive endurance exercise in the non-supplemented (NS) and vitamin B-6 supplemented (S) state in study 2.

Test		(n=3)	(n=5)
		..... nmol/L	
NS	PRE	59.9 ±17.1+	50.3 ±17.9
	DX	66.7 ±15.9	---
	POST	60.7 ±15.3	56.2 ±12.7
	POST 60	49.9 ±13.7	46.5 ±10.9
S	PRE	239 ±16.8	240 ±34.3
	DX	298 ±44.6	---
	POST	246 ±15.1	261 ±25.5
	POST 60	237 ±22.1	234 ±17.8

+Mean ± standard deviation

resulted (compared to PRE). When the plasma volume changes during exercise were considered, the predicted mean PB-6 concentration was 66.1 nmol/L (see Table 14), and the mean measured PB-6 value was 66.7 nmol/L. There was essentially no change in the PB-6 concentration during exercise when plasma volume changes were considered.

At the POST sampling point (NS state), there was a decrease in the mean PB-6 concentration equal to the rise found during exercise (6 nmol/L or 9%)(n=3). The mean plasma vitamin B-6 concentration POST, therefore, approximates the mean resting (PRE) value. A further decrease in the mean PB-6 concentration from the POST sample point was evident at one hour after exercise (10.8 nmol/L or 18%; n=3). The POST 60 mean PB-6 concentration was below the mean initial PRE concentration. This trend was consistent when all subjects were included (9.7 nmol/L or 18%)(n=5). The predicted mean PB-6 concentration at the POST sample point was similar to the mean measured value (n=5; 56.3 nmol/L vs 56.2 nmol/L). However, the mean plasma vitamin B-6 concentration one hour

Table 14. Mean predicted plasma vitamin B-6 concentration during (DX), immediately after (POST) and one hour after (POST 60) exhaustive endurance exercise in the non-supplemented (NS) and vitamin B-6 supplemented (S) state in study 2 corrected for the changes in plasma volume (Dill and Costill method, 1974).

Test		(n=3)	(n=5)
NS		nmol/L	
	DX	66.1 ±20.2+	---
	POST	64.6 ±19.5	56.3 ±17.9
	POST 60	62.6 ±20.3	53.4 ±19.0
S			
	DX	278 ±23.0	---
	POST	274 ±26.1	272 ±26.5
	POST 60	248 ±19.3	240 ±19.7

+Mean ± standard deviation

after exercise was lower than would be predicted based on the mean plasma volume changes (46.5 nmol/L compared to a predicted 53.4 nmol/L). A loss of PB-6 therefore occurred one hour after exercise in the NS state.

A similar trend, but a greater magnitude of change of PB-6 concentration, occurred with the combination of exhaustive exercise and vitamin B-6 supplementation. An increase in the mean PB-6 concentration with exercise was evident (n=3)(a mean of 59 nmol/L or 24%). Assuming that the POST sample point for the two subjects who did not exercise longer than one hour approximated the DX sample point in time, then the PB-6 concentration for these subjects similarly increased. The predicted mean PB-6 concentration DX (based on the changes in plasma volume) was 278 nmol/L. A mean PB-6 concentration of 298 nmol/L was measured DX, indicating an increase in plasma vitamin B-6 concentration. A decline in the mean PB-6 concentration occurred between the DX and POST sample points (-52 nmol/L or -17%; n=3). The magnitude of the decrease was similar to the magnitude of the increase during exercise. Accounting for the plasma volume

changes POST, a mean PB-6 concentration (n=5) of 272 nmol/L was predicted. However, a mean PB-6 concentration of 261 nmol/L was measured. Therefore a loss of PB-6 concentration was observed between the DX and POST sample points. A further decrease in the mean PB-6 concentration occurred one hour after exercise as compared to the mean PB-6 concentration at the POST sample point (-27 nmol/L decrease (-10%)(n=5); -9 nmol/L decrease (-4%)(n=3)). All subjects except one experienced a decrease in the concentration of PB-6 at POST 60. The conflicting plasma PLP and PB-6 results between the POST and POST 60 sample points for this subject (PB-6: increased from 234 to 262 nmol/L; PLP: decreased from 174 to 145 nmol/L) suggest that an error in labeling of the sample vials in which the plasma was stored may have occurred. There is no indication that the samples for the other subjects were in error. Considering the change in plasma volume, the POST 60 mean PB-6 concentration was 240 nmol/L. The mean measured value of 234 nmol/L indicates a further loss of vitamin B-6 from the plasma during the one hour of recovery.

Plasma Vitamin B-6 and PLP Difference

The relationship between the plasma PLP and plasma vitamin B-6 concentrations can only be discussed for study 2. In the NS state, a mean of 65% of the PB-6 was present as PLP at PRE, whereas in the S state, a mean of 72% of the PB-6 was present as PLP. The mean plasma PLP concentration DX in the NS and S states represented 64% of the mean plasma vitamin B-6 concentration. A greater percent of plasma vitamin B-6 concentration was present as PLP POST exercise (68%) and one hour after exercise (63%) in the S state as compared with the NS state (58% and 63%, respectively). These data suggest that a greater percent of plasma PLP is present relative to the amount of plasma vitamin B-6 following exhaustive exercise in the vitamin B-6 supplemented state than in the NS state.

A discussion of the changes in the PB-6 and plasma PLP difference before, during and after exercise is also appropriate (Table 15). Assuming that the majority of PB-6 is composed of plasma PLP and PL (Coburn and Mahuren, 1983; Lumeng et al., 1985), the difference between PB-6 and PLP provides an indication of the plasma PL ("PL") level present with exercise and vitamin B-6 supplementation. A comparison between PB-6 and PLP can only be made for study 2, since PB-6 was not analyzed in study 1. The NS state will be addressed first, followed by a discussion of the vitamin B-6 S state. For the discussion below, "PL" is therefore defined as the difference between the plasma vitamin B-6 and plasma PLP concentrations.

The PRE "PL" concentration in the NS state was 17.9 ±10.2 nmol/L, which represents 34% of the PRE PB-6 concentration. During exercise in the NS state, there was a mean measured "PL" concentration of 24.3 nmol/L(n=3), which was similar to the mean predicted PB-6 concentration DX (24.1 nmol/L). The measured "PL" concentration (n=3) POST (26.4 nmol/L) was 2.1 nmol/L higher than the mean measured value DX. And the "PL" concentration POST was 2.1 nmol/L lower than the mean predicted value (n=3; a predicted value of 28.5 nmol/L). At POST 60 (n=3) the measured "PL" concentration (19.9 nmol/L) was 1.8 nmol/L less than the mean PRE "PL" concentration. The mean predicted "PL" concentration at POST 60 (n=3; 21.0 nmol/L) was 1.1 nmol/L more than the measured "PL" concentration POST 60. Therefore, the amount of plasma "PL" present during and after exhaustive exercise in the NS state is comparable to the amount predicted based solely on plasma volume changes.

The PRE concentration of "PL" in the S state was 68.2 ±17.6 nmol/L, which represented 28% of the PRE PB-6 concentration. The concentration of "PL" PRE in the S state was 50.3 nmol/L greater than the "PL" concentration PRE in the NS state. There was a 46 nmol/L increase (74%) in the mean "PL" concentration DX in the S state as compared to PRE. In the vitamin B-6 supplemented state (n=3), the mean plasma "PL" concentration during exercise (109 nmol/L) was higher than predicted (72.8 nmol/L) based on the mean

Table 15. Mean measured and predicted difference between the plasma vitamin B-6 (PB-6) concentration and plasma pyridoxal 5'-phosphate (PLP) concentration during (DX), immediately after (POST) and one hour after (POST 60) exhaustive endurance exercise in the non-supplemented (NS) and vitamin B-6 supplemented state (S) in study 2.

	n=3								n=5							
	NS				S				NS				S			
	Measured[1]		Predicted[2]		Measured		Predicted		Measured		Predicted		Measured		Predicted	
	nmol/L								nmol/L							
PRE	21.7	±11.5[+]	-----		62.7	±17.9	-----		17.9	±10.2	-----		68.2	±17.6	-----	
DX	24.3	±8.2	24.1	±13.3	109	±52.3	72.8	±20.6	-----		-----		-----		-----	
POST	26.4	±8.4	28.5	±9.84	75.3	±13.3	86.2	±16.6	23.6	±7.4	20.2	±11.1	82.2	±19.7	82.3	±24.9
POST 60	19.9	±11.9	21.0	±13.0	93.0	±11.4	96.6	±14.8	19.5	±8.9	19.2	±11.2	87.6	±14.1	71.4	±17.5

[1] The measured mean PLP-PB-6 difference in the NS and S states, n=3 and n=5.

[2] The predicted mean PLP-PB-6 difference based on the PRE value and the change in plasma volume from PRE at each sample point in the NS and S states, n=3 and n=5. A significant difference was found between the mean of all time points in the NS and S states ($p<0.001$). No significant differences were found in the time points (PRE, DX, POST or POST 60).

[+] Mean ± standard deviation

plasma volume changes. The mean plasma "PL" concentration at the POST sample point (n=3) was 75.3 nmol/L whereas the mean predicted value was 86.2 nmol/L. These data suggest a loss of plasma "PL" concentration from DX to POST exercise when plasma volume changes are considered. The mean plasma "PL" concentration one hour after exercise was 93.0 nmol/L (n=3) whereas the mean predicted value was essentially the same (96.6 nmol/L). However, when all subjects are included (n=5), the mean measured PLP concentration at POST 60 (87.6 nmol/L) was greater than the predicted value (71.5 nmol/L).

Red Blood Cell Pyridoxal 5'-Phosphate

Pre-study red blood cell pyridoxal 5'-phosphate (RBC PLP) values ranged between 55.2 nmol/L and 218.4 nmol/L. Three values exceeded 100 nmol/L. Two of the subjects suspected to have taken vitamin supplements prior to the start of the study had RBC PLP concentrations over 100 nmol/L (218.4 and 153.8 nmol/L), as did one subject who reported he did not take supplements (128.5 nmol/L). Resting values (PRE) ranged from 51.6 nmol/L to 108 nmol/L.

Red blood cell PLP did not follow the pattern seen with plasma PLP or PB-6 in the NS or S states (see Table 16). The RBC PLP concentration did not change significantly during or immediately after exercise, but rose one hour after exercise compared to resting concentrations (8-10% in the NS state and 30-33% in the S state). The increase was significant when comparing the POST and POST 60 sample points in the S state. Plasma PLP and PB-6 were at their lowest concentrations one hour after the end of exercise whereas RBC PLP was at its highest concentration.

In the NS state, the RBC PLP concentration increased (7.3 nmol/L (14%) and 3.8 nmol/L (9%)) from PRE values in two of the three subjects exercising for at least one hour (DX). After exercise (POST), the mean RBC PLP concentration in these same three subjects increased an additional 2.7 nmol/L (4%). When comparing the values of all five

Table 16. Mean red blood cell pyridoxal 5'-phosphate concentration before (PRE), during (DX), immediately after (POST) and one hour after (POST 60) exhaustive endurance exercise in the non-supplemented (NS) and vitamin B-6 supplemented (S) state in study 2.

Test		(n=3)	(n=5)[1]
NS		.....nmol/L	
	PRE	66.8 ±36.2+	65.6 ±27.2
	DX	68.8 ±30.5	---
	POST	71.5 ±31.6	68.4 ±25.0
	POST 60	73.4 ±20.9	70.6 ±18.3
S			
	PRE	144 ±32.9	154 ±28.2
	DX	136 ±34.0	---
	POST	131 ±37.7	140 ±30.7[a]
	POST 60	191 ±40.8	200 ±32.5[b]

[1] Values followed by a different letter are significantly different ($p<0.05$)
+Mean ± standard deviation

subjects PRE to POST there was a 2.8 nmol/L (6.5%) increase in the mean RBC PLP concentration (Table 17). Assuming that the POST sample point approximated the DX sample point in time, the RBC PLP concentration for the two subjects not exercising for one hour was essentially unchanged DX. One hour after exercise, a slight increase in the mean RBC PLP concentration occurred (1.9 nmol/L or 3%) compared to POST. Compared to PRE concentrations, exhaustive exercise alone (NS state) resulted in a 13% increase in the RBC PLP concentration by one hour post-exercise (Table 17).

The combination of exhaustive exercise and vitamin B-6 supplementation produced a change in the RBC PLP concentration compared to the levels at the resting condition. The mean RBC PLP concentration at all sample points was significantly greater under conditions of vitamin B-6 supplementation compared to the NS state. The mean resting (PRE) RBC PLP concentration was over two-fold greater when a vitamin B-6 supplement was provided than the levels in the NS state (144 nmol/L compared to 66.8 nmol/L). There

Table 17. Mean percent change from resting values (PRE) in plasma pyridoxal 5'-phosphate (PLP), red blood cell pyridoxal 5'-phosphate (RBC PLP) and plasma vitamin B-6 (PB-6) in the non-supplemented (NS) and vitamin B-6 supplemented (S) state in study 1 and study 2.

	PLP (study 1)[1]	PLP (study 2)[2]	PB-6 (study 2)	RBC PLP (study 2)
NS				
DX	18.8 ±11.3[+]	10.2 ±7.9	12.3 ±8.5	6.3 ±9.7
POST	-7.3 ±14.4	5.2 ±22.7	16.3 ±21.0	6.5 ±14.3
POST 60	-18.1 ±12.4	-15.6 ±9.6	-3.5 ±20.4	13.1 ±21.1
S				
DX	28.0 ±19.7	6.6 ±6.4	24.0 ±10.4	-5.8 ±2.3
POST	3.0 ±17.6	4.6 ±12.6	10.1 ±16.0	-9.6 ±5.7
POST 60	-14.0 ±15.2	-14.6 ±8.3	-1.2 ±15.5	31.6 ±18.2

[1] DX represents n=6 (NS state) and n=5 (S state); POST and POST 60 represent n=6

[2] Mean plasma PLP, PB-6 and RBC PLP concentrations in study 2 represent n=3 (DX), n=5 (POST) and n=6 (POST 60)

[+] Mean ± standard deviation

was a decline in the mean RBC PLP concentration (8 nmol/L or 6%) from the PRE value during exercise (n=3)(Table 17). The decrease in the RBC PLP concentration DX occurred in each of the three subjects. A further mean decline of 5 nmol/L(4%) from DX concentrations was found at the POST sample point (n=3). When all five subjects were considered, there was a significant increase ($p<0.05$) in the mean RBC PLP concentration one hour after exercise in the vitamin B-6 supplemented state compared to the POST concentration (60 nmol/L or 46% increase from POST concentration). The mean RBC PLP concentration one hour after exercise was approximately 46 nmol/L (31%) above the initial PRE concentration.

Alkaline Phosphatase

The alkaline phosphatase activity was analyzed only in study 2 (see Table 18). The pre-study alkaline phosphatase activity (measured in the wellness panel at Good Samaritan Hospital, Corvallis, OR.) ranged between 0.21 and 0.26 μkat/L. Exhaustive exercise and/or vitamin B-6 supplementation did not significantly alter the activity of alkaline phosphatase. In the NS state, the slight increase in alkaline phosphatase activity from PRE levels during(3%) and after exhaustive exercise (POST)(2%) could be accounted for by the changes in plasma volume at those points (see Tables 18 and 19). Similarly, the slight increase in plasma alkaline phosphatase activity from PRE levels that resulted DX (5%) and POST (5%) could be accounted for by the changes in plasma volume at those points. The changes in measured alkaline phosphatase activity resulting from endurance exercise followed a pattern similar to the plasma PLP concentration. However, the slight changes observed in the alkaline phosphatase activity with exhaustive exercise are probably a result of the changes in plasma volume, and are not due to an increase in activity.

Table 18. Mean plasma alkaline phosphatase activity before (PRE), during (DX), immediately after (POST) and one hour after (POST 60) exhaustive endurance exercise in the non-supplemented (NS) and vitamin B-6 supplemented (S) state in study 2.

Test		(n=3)	(n=5)
N S		μkat/L	
	PRE	0.34 ±0.03+	0.30 ±0.07
	DX	0.37 ±0.03	---
	POST	0.36 ±0.04	0.35 ±0.06
	POST 60	0.34 ±0.03	0.31 ±0.07
S			
	PRE	0.36 ±0.04	0.33 ±0.07
	DX	0.41 ±0.05	---
	POST	0.41 ±0.06	0.39 ±0.08
	POST 60	0.34 ±0.02	0.33 ±0.06

+ Mean ± standard deviation

Table 19. Mean predicted plasma alkaline phosphatase activity during (DX), immediately after (POST) and one hour after (POST 60) exhaustive endurance exercise in the non-supplemented (NS) and vitamin B-6 supplemented (S) state in study 2 corrected for the changes in plasma volume (Dill and Costill method, 1974).

Test		(n=3)	(n=5)
N S		μkat/L	
	DX	0.37 ±0.03+	---
	POST	0.37 ±0.03	0.34 ±0.06
	POST 60	0.35 ±0.03	0.32 ±0.06
S			
	DX	0.42 ±0.05	---
	POST	0.41 ±0.06	0.40 ±0.09
	POST 60	0.37 ±0.04	0.35 ±0.07

+Mean ± standard deviation

Plasma Growth Hormone

Plasma growth hormone (hGH) was analyzed only in study 2 (see Table 20). In the NS state, the mean resting (PRE) hGH concentrations were 2.2 ng/mL (n=4) and 5.7 ng/mL (n=5). The resting hGH concentration in the NS state for subject 5 (19.7 ng/mL) was 4-fold greater than the normal value of <5 ng/mL given by Winer et al. (1990), therefore he was excluded from the results.

During exercise in the NS state, the mean plasma hGH concentration (n=4) increased to 28.7 ng/mL or 13-fold. The rise was statistically significant. However, by excluding subject 5, the DX sample now represents a sample size of 2. Immediately following exercise, the mean plasma hGH concentration decreased -7.9 ng/mL (-27.5%) compared to the mean DX concentration. The mean plasma hGH concentration decreased -16.2 ng/mL or -78% at the POST 60 sample point compared to the mean POST concentration (NS state). The mean plasma hGH concentration POST 60 was significantly lower than either the mean DX or POST concentration, but not significantly different from the initial mean resting value (PRE).

Compared to the plasma levels in the NS condition, the plasma hGH concentrations before, during and after exercise were not significantly affected by vitamin B-6 supplementation. Resting plasma hGH concentrations were 1.8 ng/mL lower under vitamin B-6 supplementation conditions compared to the NS state; a difference which was not statistically significant. Exercise and vitamin B-6 supplementation resulted in a significant increase of 29.6 ng/mL (67-fold) in the mean plasma hGH concentration DX compared to PRE. In the S state, the mean plasma hGH concentration was -7.5 ng/mL or -25% less at POST as compared to the DX sampling point. A further significant decrease in the plasma hGH concentration occurred at POST 60 (-16.9 ng/mL or -75%) compared to the POST or DX concentrations. The percent decrease in mean plasma hGH concentrations at POST and POST 60 was nearly the same in the NS and S states (78% in the NS state; 75% in the S state). A separate statistical analysis of the S state found a statistically significant difference

Table 20. Mean plasma growth hormone concentration before (PRE), during (DX), immediately after (POST) and one hour after (POST 60) exhaustive endurance exercise in the non-supplemented (NS) and vitamin B-6 supplemented (S) state in study 2.

	NS	S	Mean (NS & S)[1]
	ng/mL		
PRE[2]	2.20 ±3.81[a+]	0.45 ±0.27[a]	1.32 ±2.67[a]
DX	28.7 ±0.57[b]	30.0 ±11.2[b]	29.4 ±6.50[b]
POST	20.8 ±9.13[b]	22.5 ±11.0[b]	21.6 ±9.42[b]
POST 60	4.66 ±2.83[ac]	5.63 ±4.15[c]	5.15 ±3.33[a]

[1] Mean (NS & S) represents the mean plasma growth hormone concentration (NS and S states) PRE, DX, POST and POST 60. No significant difference between the NS and S states. Values followed by a different letter are significantly different ($p<0.05$) for time points within a colum.
[2] PRE, POST and POST 60 represent n=4; DX represents n=2
[+] Mean ± standard deviation

between the PRE and POST 60 sample points. The difference provided evidence for a supplement by time interaction effect. The interaction was not evident when all samples were included from both the NS and S states. The POST 60 hGH concentration in the S state was significantly higher than the initial PRE concentration. During exercise and into recovery, the mean concentration of plasma hGH was higher when vitamin B-6 supplementation was provided than without the supplementation. This difference was not statistically significant.

The shifts in plasma volume occurring DX, POST and POST 60 should be mentioned. The increase in plasma hGH concentration DX (NS and S states; n=2) was several fold greater than the initial (PRE) concentration, therefore the shift in plasma volume that occurred at this point did not have an important influence on the plasma hGH concentration.

Sweat

The mean concentration of vitamin B-6 in sweat is reported in Table 21. The three

Table 21. Mean sweat vitamin B-6 concentration during exhaustive endurance exercise in the non-supplemented (NS) and vitamin B-6 supplemented (S) state in study 1 and study 2.

		NS	S
		µmol/100 mL	
Study 1	n=5	---	0.0233 ±0.0199+
Study 2	n=3	0.0006 ±0.0037	0.0065 ±0.0051
	n=5	0.0071 ±0.0034	---

--- refers to inadequate sample collection
+ Mean ± standard deviation

subjects in study 2 with sweat samples from both the NS and S states appeared to have a greater loss of vitamin B-6 in the sweat under the conditions of vitamin B-6 supplementation (0.0065 ±0.0051 μmol/100 mL sweat compared to 0.0006 ±0.0037 μmol/100 mL sweat, respectively). The mean concentration of the sweat samples (both studies) ranged between 0.0057 μmol/100 mL in the NS state to 0.0233 μmol/100 mL in the vitamin B-6 supplemented state. The results represent the combination of several individual samples for a given individual collected throughout the course of each exercise test.

Plasma Correlations

The Spearman correlations between the resting (PRE) plasma alkaline phosphatase activity, plasma albumin, plasma PLP, plasma PB-6, plasma "PL", RBC PLP and plasma hGH concentration in study 2 in the non-supplemented state are given in Table 22. Only study 2 was analyzed since the majority of the data are from study 2. Spearman correlations do not assume that the data are normally distributed, and therefore rely on a ranking of values. As expected, in the NS state, there was a correlation ($p<0.08$; $r=0.90$) (although not at the preset level of significance, $p<0.05$) between the plasma PLP and PB-6, since PLP comprises approximately 70-90% of the PB-6 (Leklem, 1991). There was also a correlation between the plasma PLP concentration and alkaline phosphatase activity ($p<0.08$; $r=0.90$). Plasma hGH was correlated with alkaline phosphatase activity ($p<0.08$; $r=0.90$). In the vitamin B-6 supplemented state (Table 23), the plasma PLP correlated with the PB-6 ($p<0.08$; $r=0.90$). As was found in the NS state, plasma hGH correlated with alkaline phosphatase activity ($p<0.08$; $r=0.90$).

Urine Values

Twenty-four hour urine collections were obtained from each subject during each

Table 22. Spearman correlation between resting (PRE) alkaline phosphatase activity (ALK), albumin (ALB), plasma pyridoxal 5'-phosphate (PLP), plasma vitamin B-6 (PB-6), plasma pyridoxal (PB-6-PLP difference; "PL"), red blood cell PLP (RBC PLP) and plasma growth hormone (HGH) concentration in the non-supplemented state (n=5).

	ALK	ALB	PLP	PB-6	"PL"	RBC	HGH
ALK		0.30[1]	0.90 0.08[2]	0.70	0.20	0.80	0.90 0.08
ALB	0.30		0.60	0.70	0.30	-0.30	0.10
PLP	0.90 0.08	0.60		0.90 0.08	0.50	0.50	0.70
PB-6	0.70	0.70	0.90 0.08		0.70	0.30	0.60
"PL"	0.20	0.30	0.50	0.70		0.00	0.10
RBC	0.80	-0.30	0.50	0.30	0.00		0.90 0.08
HGH	0.90 0.08	0.10	0.70	0.60	0.10	0.90 0.08	

[1] Represents the Spearman correlation coefficient (r value). [2] Denotes the p value, from Snedecor and Cochran, 1980.

Table 23. Spearman correlation between resting (PRE) alkaline phosphatase activity (ALK), albumin (ALB), plasma pyridoxal 5'-phosphate (PLP), plasma vitamin B-6 (PB6), plasma pyridoxal (PB-6-PLP difference; "PL"), red blood cell PLP (RBC PLP) and plasma growth hormone (HGH) concentration in the vitamin B-6 supplemented state (n=5).

	ALK	ALB	PLP	PB-6	"PL"	RBC	HGH
ALK		0.20[1]	0.30	0.60	0.60	0.15	0.90 0.08[2]
ALB	0.20		-0.70	-0.60	-0.40	-0.87	-0.10
PLP	0.30	-0.70		0.90 0.08	0.50	0.56	0.50
PB-6	0.60	-0.60	0.90 0.08		0.80	0.67	0.70
"PL"	0.60	-0.40	0.50	0.80		0.62	0.50
RBC	0.15	-0.87	0.56	0.67	0.62		0.41
HGH	0.90 0.08	-0.10	0.50	0.70	0.50	0.41	

[1] Represents the Spearman correlation coefficient (r value).[2] Denotes the p value, from Snedecor and Cochran, 1980.

day of the NS and S metabolic periods in study 1 and study 2. All samples were analyzed for creatinine (to assess completeness of collection), urea nitrogen, 4-pyridoxic acid (4-PA) and vitamin B-6 (UB-6). The mean creatinine, urea nitrogen, 4-PA and UB-6 values represent a mean of four days prior to exercise (Prior to EX), the day of the exercise test (EX), one day after exercise (1 Day After) and, in study 2 only, two days after exercise (2 Days After). The grand means for creatinine and urea nitrogen in the NS and S state represent the mean of all values for all persons. The 4-PA collections prior to exercise represent the four consecutive days prior to exercise. Values were excluded if the creatinine concentration was greater than 10% of the mean value for a given subject, indicating an incomplete urine collection. The UB-6 collections prior to exercise represent four non-consecutive days chosen for their completeness of collection. In both cases (4-PA and UB-6), the mean concentrations during the days prior to exercise represent a baseline excretion. The mean creatinine concentrations reported below prior to exercise represent the same four consecutive days prior to exercise used for the 4-PA analysis.

Creatinine

The daily creatinine values provide an indication of the completeness of urine collection and, given a controlled dietary and exercise regime, an indication of muscle mass (see Table 24). Normal daily variation under controlled conditions, unexplained by changes in physical activity and diet, has been shown to range between 4-8% (Cryer and Sode, 1970; Greenblatt et al., 1976). No significant differences in creatinine excretion were found the days before exercise, on the exercise test day or the day (s) after exercise in either study 1 or study 2. The mean excretion of creatinine was similar in the NS and S states in study 1 (1.93 g/d). However, in study 2 there was significantly more creatinine excreted at all collection points than in study 1 (NS and S states). The difference in the subject's mean creatinine excretion may be a reflection of a difference in the subject's daily exercise, muscle mass, emotional stress, dietary protein intake or completeness of urine collections

Table 24. Urinary excretion of creatinine and urea nitrogen during the days prior to (Prior to EX), the day of (EX), one day after (1 Day After) and two days after (2 Days After) exhaustive endurance exercise in the non-supplemented (NS) and vitamin B-6 supplemented (S) state in study 1 and study 2.

		Prior to EX[1]	EX	1 Day After	2 Days After	Mean[2]
				g/day		
Creatinine						
Study 1	NS	1.92 ±0.22+	1.98 ±0.27	1.95 ±0.15	-----	1.93 ±0.22[a]
	S	1.96 ±0.15	2.02 ±0.11	1.72 ±0.51	-----	1.93 ±0.26[a]
Study 2	NS	2.08 ±0.16	2.13 ±0.18	2.10 ±0.22	2.20 ±0.22	2.11 ±0.18[b]
	S	2.25 ±0.20	2.11 ±0.11	2.33 ±0.12	2.09 ±0.19	2.22 ±0.19[c]
Urea nitrogen						
Study 1	NS	16.26 ±1.49	16.20 ±0.79	16.91±0.98	-----	16.34±1.33[d]
	S	16.24 ±1.19	16.57 ±1.20	14.89±4.13	-----	16.07±1.98[d]
Study 2	NS	15.42 ±1.52	14.79 ±1.59	14.73±0.19	14.76±1.54	15.13±1.40[e]
	S	15.76 ±3.17	14.18 ±1.54	16.30±1.77	14.77±1.26	15.48±2.64[e]

[1] Mean excretion of creatinine and urea nitrogen four days prior to(4 Days Prior), the day of(EX), one day after (1 Day After) and two days after(2 Days After) the exhaustive exercise ride in the non-supplemented (NS) and vitamin B-6 supplemented states.
[2] Mean excretion of creatinine and urea nitrogen (all values) in the NS and S states in study 1 and study 2. Different letters denote a significant difference ($p<0.02$).
+ Mean ± standard deviation

of the subjects in the studies (Heymsfield et al., 1983). In study 2, significantly more creatinine was excreted in the S state than in the NS state when all sample points were considered ($p<0.02$). The mean creatinine excretion in the S state the day after exercise in

study 1 (n=5) (1.72 ±0.51 g/d; a -15% decrease from the EX day value) and 2 days after exercise in study 2 (n=4) (2.09 ±0.19 g/d; a -10% decrease from one day post value) are due to a greater than 10% decrease in creatinine excretion in at least one individual in each study.

Urea Nitrogen

The urinary urea nitrogen excretion on the corresponding days mentioned above was measured in studies 1 and 2 (see Table 24). Although the excretion of urea nitrogen the days prior, the day of or the day (s) after the exercise test was not significantly different in the NS and S states, there was significantly more urea nitrogen excreted in study 1 (16.3 g/d in the NS state and 16.07 g/d in the S state) as compared to study 2 (15.13 g/d in the NS state and 15.48 g/d in the S state). Compared to the EX test day value, there was a decrease in the urea nitrogen excretion one day after exercise in study 1 (-10%) and two days after exercise in study 2 (-9%) in the S state.

Vitamin B-6 Measures

The excretion of 4-pyridoxic acid (4-PA) and vitamin B-6 (UB-6) in the urine and the percent of vitamin B-6 intake excreted as 4-PA and UB-6 was examined. The NS and S states were analyzed separately to determine differences between studies 1 and 2, and differences before, during or after the exhaustive exercise test. There was a significant difference between study 1 and study 2 in both the NS and S states for both 4-PA and UB-6 excretion. The changes in each variable mentioned above before, during an after exhaustive exercise in the NS state and vitamin B-6 supplemented state will be addressed separately.

4-Pyridoxic Acid

The mean excretion of 4-pyridoxic acid (4-PA) before, during and after exercise in the NS state is found in Table 25. There were no significant differences between the mean 4-PA excretion values in the NS state in study 1 or in study 2. However, the mean 4-PA value in the NS state in study 2 was significantly greater than the mean 4-PA value in study 1 (including all sample points). Subject 2 in study 2 was a significant contributor to the mean 4-PA excretion at each sampling point in the NS state. For example, 4 days prior to exercise, subject 2 had a mean 4-PA excretion of 11.2 μmol/day whereas the mean 4-PA excretion for the other subjects was 7.87 μmol/day. The individual 4-PA values 4 days prior to exercise for subject 2 varied by less than 5%. The excretion of 4-PA for subject 2 on the day of exercise was 7.54 μmol/day as compared to a mean of 7.13 μmol/day for the other subjects; 7.78 μmol/day one day after exercise as compared to a mean of 6.87 μmol/day for the other subjects and 8.58 μmol/day two days after exercise as compared with a mean of 7.60 μmol/day for the other subjects. It is difficult to comment on the reason for these differences, as subject 2 did not return his daily logbook containing a list of daily activity and uneaten food items.

The baseline (average of 4 days prior to exercise) mean 4-PA value was 6.15 μmol/d in study 1 and was 8.45 μmol/d in study 2. In study 1 (NS state), there was an 6% increase (0.39 μmol/d) in the mean excretion of 4-PA on the day of exercise compared to the mean excretion the four days prior to exercise. The mean urinary 4-PA value one day after exercise in study 1 was -5% (-0.33 μmol/d) less than the value the day of exercise. Unlike the 4-PA data of study 1, in study 2 a 13% decrease (-1.22 μmol/d) in the mean 4-PA value was found the day of exercise as compared to the mean excretion the days prior to EX. Subject 2 in study 2 who had been taking a vitamin supplement prior to the start of the study had a 32% decrease in 4-PA excretion on the day of exercise as compared to the mean 4-PA excretion four days prior to exercise. The decrease continued one day after

Table 25. Mean urinary 4-pyridoxic acid (4-PA) and urinary vitamin B-6 (UB-6) excretion during the days prior to (Prior to EX), the day of (EX), one day after (1 Day After) and two days after (2 Days After) exhaustive endurance exercise in the non-supplemented (NS) and vitamin B-6 supplemented (S) state in study 1 and study 2.

	4-PA				UB-6			
	Study 1		Study 2		Study 1		Study 2	
NS	.....µmol/day							
Prior to EX[1]	6.15	±0.92+	8.45	±2.12	0.78	±0.13	1.11	±0.27
EX	6.54	±1.11	7.33	±1.08	0.75	±0.07	1.05	±0.18
1 Day After	6.21	±0.82	7.03	±1.31	0.80	±0.11	1.05	±0.19
2 Days After	-----		7.80	±1.36	-----		1.03	±0.17
Mean (NS)	6.20	±0.93[a]	7.98	±1.83[b]	0.78	±0.13[e]	1.08	±0.23[f]
S								
Prior to EX	91.9	±9.06	81.3	±6.03	9.69	±1.33	8.87	±1.82
EX	94.9	±5.62	84.1	±7.44	9.18	±0.95	8.01	±1.07
1 Day After	90.9	±10.5	86.7	±4.04	9.49	±1.52	8.93	±1.09
2 Days After	-----		82.4	±7.00	-----		9.68	±1.87
Mean (S)	92.2	±8.69[c]	82.7	±6.16[d]	9.57	±1.29[g]	8.85	±1.64[h]

+ Mean ± standard deviation

[1]Prior to EX represents the mean 4-PA and UB-6 excretion four days prior to the exercise test (in study 1, 4-PA and UB-6 represents n=24 (NS and S states); in study 2, 4-PA represents n=19 (NS and S states) and UB-6 represents n=17 (NS state) and n=20 (S state).

EX represents the mean 4-PA and UB-6 excretion on the exercise test day (in study 1 and 2, the 4-PA and UB-6 excretion represents n=6 and n=5, respectively).

1 Day After represents the mean 4-PA and UB-6 excretion one day after the exercise test day (in study 1 (NS state) the 4-PA and UB-6 excretion represents n=5; in the S state, the 4-PA and UB-6 excretion represents n=6; in study 2 (NS and S states) the 4-PA and UB-6 excretion represent n=5).

2 Days After represents the mean 4-PA and UB-6 excretion two days after the exercise test only in study 2 (in the NS state, the 4-PA and UB-6 excretion represents n=5; in the S state, the 4-PA and UB-6 excretion represents n=4).

Different letters denote a significant difference ($p<0.05$) across columns and between supplementation states.

exercise (a 4% decrease). The excretion of 4-PA in study 2 did not increase until two days after the exercise test, when the mean value increased to 7.80 µmol/d (an 11% increase

above the 4-PA value one day after exercise). The excretion of 4-PA in study 2 remained below the baseline value (prior to EX) two days after exercise.

As expected, under the condition of vitamin B-6 supplementation, a significantly greater amount of 4-PA was excreted in study 1 as compared to the NS state. In study 1, there was a greater mean excretion of 4-PA during the days prior to exercise as compared with study 2 (91.9 μmol/d compared to 81.3 μmol/d). The difference was consistent for each of the days prior to exercise. In study 1, the mean excretion of 4-PA increased 3.0 μmol/d the day of exercise (a 3% increase) compared to the days prior to exercise. The mean 4-PA excretion one day after exercise (90.9 μmol/d) in study 1 was similar to the mean value prior to exercise (91.9 μmol/d). The mean 4-PA value one day after exercise in study 1 represented a 4% (-4 μmol/d) decrease from the mean 4-PA value on the exercise test day. In study 2, there was a slight increase in the mean 4-PA excretion on the exercise test day (2.8 %). Thus, the increase in 4-PA excretion under vitamin B-6 supplementation conditions on the exercise test day as compared to the days prior to exercise was similar for the two studies (a 3% increase and a 2.8% increase). A 3% (2.6 μmol/d) increase in the mean 4-PA value was found in study 2 one day after exercise compared to the four days prior to exercise. Two days after exercise, the mean 4-PA value (82.4 μmol/d) was 5% (4.3 μmol/d) less than the level one day after exercise; a level comparable to the four days prior to exercise (81.3 μmol/d).

Urinary Vitamin B-6

The mean excretion of urinary vitamin B-6 (UB-6) is presented in Table 25. In the NS state, the excretion of UB-6 was significantly greater in study 2 than in study 1. Conversely, under vitamin B-6 supplementation conditions, the excretion of UB-6 was significantly greater in study 1 as compared to study 2. No significant differences were found in the excretion of UB-6 the days before, the day of, or the day (s) after the exhaustive exercise test.

In study 1, the pattern of UB-6 excretion was similar for the days before, the day of and the day after exercise in the NS and S states. The day of exercise, the excretion of UB-6 was slightly less than the excretion the days prior to exercise (baseline levels)(-4% in excretion for the NS state and -5% in excretion for the S state). There was a small increase in the excretion of UB-6 (7% in the NS state (0.05 μmol/d); 3% (0.31 μmol/d) in the S state) one day after the exercise test as compared to the exercise test day, to a level comparable to the baseline excretion.

As observed in study 1, in study 2 there was a decrease in the UB-6 excretion on the day of exercise as compared to the days prior to exercise (-5% in the NS state (0.06 μmol/d) and -10% (0.86 μmol/d) in the S state). In the NS state, there was no change in the mean UB-6 excretion one day after exercise as compared to the exercise test day. A slight decrease in the UB-6 excretion was seen two days after exercise (-2%). In the S state, an 11% increase in the UB-6 excretion occurred one day after exercise and an additional 8% increase occurred two days after exercise, to a level 9% higher than baseline.

Percent of Intake Excreted as 4-PA and UB-6

In order to normalize, and thus compare, the data between the supplementation periods, the excretion of 4-PA and urinary B-6 were expressed as a percent of vitamin B-6 intake (see Table 26). The mean (NS) value represents the mean of all sample collection days in the NS state and the mean (S) represents the mean of all sample collection days in the S state. When expressed in this manner, there were significant differences between the studies in the mean (NS) and mean (S) percent of vitamin B-6 intake excreted as 4-pyridoxic acid and as UB-6. Vitamin B-6 supplementation significantly influenced the excretion of urinary 4-pyridoxic acid and UB-6 (expressed as a percent of vitamin B-6 intake) in study 1. A significantly greater percent of vitamin B-6 intake was excreted as mean (S) 4-PA and as mean (S) UB-6 in study 1 compared to the mean (NS) conditions.

Table 26. Mean percent of vitamin B-6 intake excreted as urinary 4-pyridoxic acid (4-PA) and urinary vitamin B-6 (UB-6) excretion during the days prior to (Prior to EX), the day of (EX), one day after (1 Day After) and two days after (2 Days After) exhaustive endurance exercise in the non-supplemented (NS) and vitamin B-6 supplemented (S) state in study 1 and study 2.

	% intake 4-PA[1]		% intake UB-6	
	Study 1	Study 2	Study 1	Study 2
NS	.....% of intake			
Prior to EX[2]	45.0 ±6.8[+]	75.5 ±19.0	5.7 ±1.1	9.9 ±2.4
EX	48.1 ±8.2	65.5 ±9.6	5.5 ±0.5	9.0 ±1.4
1 Day After	45.6 ±6.1	62.8 ±11.7	5.9 ±0.8	9.4 ±1.7
2 Days After	-----	69.6 ±12.2	-----	9.2 ±1.5
Mean (NS)	45.6 ±6.9[a]	71.3 ±16.4[b]	5.7 ±1.0[d]	9.6 ±2.0[f]
S				
Prior to EX	70.0 ±6.6	62.8 ±4.7	7.4 ±1.0	6.5 ±2.1
EX	71.9 ±4.3	65.0 ±5.7	7.0 ±0.7	6.2 ±0.8
1 Day After	69.0 ±7.9	67.0 ±3.1	7.2 ±1.2	6.9 ±0.8
2 Days After	-----	63.7 ±5.4	-----	7.5 ±1.4
Mean (S)	69.9 ±6.6[b]	63.9 ±4.8[b]	7.3 ±1.0[e]	6.6 ±1.7[e]

[+] Mean ± standard deviation

[1]Prior to EX represents the mean percent(%) of intake excreted as 4-PA and UB-6 four days prior to the exercise test (in study 1, % intake 4-PA and % intake UB-6 represents n=24 (NS and S states); in study 2, % intake 4-PA represents n=19 (NS and S states) and % intake UB-6 represents n=17 (NS state) and n=20 (S state).

EX represents the mean % intake excreted as 4-PA and UB-6 on the exercise test day (in study 1 and 2, the % intake 4-PA and % intake UB-6 excretion represents n=6 and n=5, respectively).

1 Day After represents the mean % intake excreted as 4-PA and UB-6 one day after the exercise test day (in study 1(NS state), the % intake 4-PA and % intake UB-6 represent n=5; in the S state, the % intake 4-PA and % intake UB-6 represent n=6; in study 2 (NS and S states) the % intake 4-PA and % intake UB-6 represent n=5).

2 Days After represents the mean % intake excreted as 4-PA and UB-6 two days after the exercise test only in study 2 (in the NS state, the % intake 4-PA and % intake UB-6 represent n=5; in the S state, the % intake 4-PA and % intake UB-6 represent n=4).

Different letters denote a significant difference ($p<0.05$) across columns and between supplementation states.

In study 2, the opposite effect was observed. Vitamin B-6 supplementation produced no significant change in the mean (S) excretion of the percent of intake excreted as 4-PA (compared to the NS state) during exercise and resulted in a significant mean (S) decrease in the percent of intake excreted as urinary vitamin B-6 compared to the mean (NS) value. A non-significant decline in the mean (S) urinary 4-PA excretion (expressed as a percent of intake) occurred in study 2 as compared with the mean (NS) 4-PA value.

Plasma and Urine Spearman Correlations

The only relevant urine correlation, considering all collection days, was between the 4-PA and UB-6 excretion ($p<0.05$). In study 1(NS state), there was a significant correlation between the plasma PLP concentration at DX (n=6) and the UB-6 excretion on the day of exercise (EX)($r=0.80$; $p=0.06$). A significant correlation between the plasma PLP concentration one hour after exercise and the UB-6 concentration on the exercise test day was found in study 1 (S state)($r=-0.99$; $p<0.01$). In study 2 (NS state), the plasma PLP concentration at POST 60 and the urinary 4-PA on the exercise test day (EX) were significantly correlated ($r=0.90$; $p<0.04$). The physiological significance of these findings is unclear. No other significant correlations of plasma and urine variables were found in study 1 or study 2 at other time points. Sweat vitamin B-6 and the daily exercise amount did not correlate with any of the plasma or urine variables.

DISCUSSION

The focus of this study was to examine the changes in vitamin B-6 metabolism and growth hormone concentration as a result of submaximal exhaustive endurance exercise and oral vitamin B-6 supplementation. The evidence suggests that exhaustive endurance exercise and vitamin B-6 supplementation alters vitamin B-6 metabolism. Knowledge of a change in vitamin B-6 metabolism as a result of exercise is important when recommending an appropriate vitamin B-6 intake to athletes. The changes in plasma growth hormone concentration with exhaustive exercise and an oral vitamin B-6 supplement suggest a possible role for vitamin B-6 in muscle anabolism, an important concern for athletes and others alike. A discussion of the changes in vitamin B-6 metabolism and plasma growth hormone concentration will be addressed below.

Vitamin B-6 Metabolism

Plasma and urinary measures were used to assess the changes in vitamin B-6 metabolism as a result of exhaustive exercise and vitamin B-6 supplementation. The evidence suggests that the concentration of PLP in the plasma and RBC is altered by vitamin B-6 supplementation and exhaustive exercise. The urinary data (4-PA and UB-6) also suggests that vitamin B-6 metabolism is altered under the conditions mentioned above. Each blood and urinary vitamin B-6 indice will be discussed below in an effort to explain the role the changes in vitamin B-6 metabolism play in endurance exercise.

Blood

The changes in plasma volume as a result of exercise can impact the interpretation of the observed changes in plasma PLP, plasma vitamin B-6, plasma albumin and plasma alkaline phosphatase activity. Small changes in these plasma indices may be due in a large

part to changes in plasma volume alone. A change in plasma volume is not simply due to a loss of water from the plasma. Forces such as osmotic and oncotic pressures dictate the directional flow of plasma volume during exercise (McArdle et al., 1986). Shifts occur back and forth between the plasma and the extracellular spaces. Plasma proteins such as albumin contribute in large part to the observed changes in plasma volume. The small changes in albumin concentration with exhaustive exercise in the present study can be explained based on the change in plasma volume.

The methods used to evaluate changes in plasma vitamin B-6 indices based on changes in plasma volume were the Dill and Costill method (1974) and the van Beaumont method (1972) of plasma volume calculation. The van Beaumont method uses hematocrit to calculate plasma volume changes whereas the Dill and Costill method uses both hematocrit and hemoglobin. The van Beaumont method makes the assumption that the volume of circulating red blood cells is constant and that the relationship between the venous hematocrit and the whole body hematocrit does not change with exercise or dehydration. While both methods of plasma volume calculation were used to examine the changes in plasma volume that result from exhaustive exercise in these studies, we focused primarily on the plasma volume changes calculated with the Dill and Costill method. Costill et al. (1974) have found a shrinkage of red blood cells as a result of dehydration (a 2-4% loss of body weight) violating an assumption of the van Beaumont method. The Dill and Costill method of plasma volume calculation was used to calculate the predicted concentrations of the plasma PLP, plasma vitamin B-6, the PLP and PB-6 difference, albumin and alkaline phosphatase activity. The method of adjusting plasma concentrations based on a shift in plasma volume should be viewed as a conservative approach to a complex issue.

An additional factor in evaluating data on plasma volume changes is the potential error associated with the measurement of hemoglobin (hgb) and hematocrit (hct) and their contribution to the total error in the calculation of the change in plasma volume and ultimately, to the predicted plasma parameters. Harrison et al. (1981) have estimated that

there can be a ±0.5 unit error in the hematocrit measurement and a ±0.15g/100 mL error in the hemoglobin measurement. Assuming that these errors could have occurred in the present data, a PRE and POST hematocrit and hemoglobin concentration was used from a subject in study 2 to calculate the extent of the assay error in the plasma volume calculation. Since the error in the hemoglobin is so slight, only hematocrit concentrations were examined. Using the Dill and Costill method of calculation (1974), a 5.6% decrease in plasma volume was found for this subject. The greatest variation in the plasma volume calculation would result from an erroneously high percent of one of the hematocrit values and an erroneously low concentration of the other hematocrit value. If the PRE hematocrit value were erroneously elevated, there would be a 7.9% decrease in plasma volume. Whereas if an erroneously high POST hematocrit value were used, there would be a 3.3% decrease in plasma volume. The resulting plasma volume change at a given sampling point could therefore vary by approximately 2% in either direction. Therefore, plasma values based on the changes in plasma volume need to be interpreted with caution.

Dehydration increases plasma osmolality and decreases blood volume. Even though the subjects in study 1 received 100 mL of water after the POST blood draw and 120 mL after DX and 120 mL after POST in study 2, the mean plasma volume changes at one hour after exhaustion still indicated a loss of plasma volume. Physiological consequences of dehydration are an increase in heart rate to dissipate the heat produced from exercise (McArdle et al., 1986) and a decrease in exercise capacity. An 8% increase in the mean heart rate (beats per minute) in the last 10 minutes of exercise as compared to the mean heart rate during exercise suggests that the subjects may have been dehydrated at the end of exercise. A decrease in exercise capacity has been shown to occur with a marginal decrease in body water (1-2% loss of body weight)(Armstrong et al., 1986; Caldwell et al., 1984). Two of the five subjects in study 2 exceeded a 3% loss of body weight as a result of the exhaustive exercise test. However, these two subjects did not have the greatest change in plasma volume, indicating that plasma volume changes are not always directly related to the

loss of body water. Although we were unable to definitively diagnose our subjects as dehydrated based on the data collected, dehydration could have contributed to the fatigue of the subjects (Sawka, 1992; Pitts et al., 1956; Saltin, 1964) and resulted in part to the cessation of exercise.

Pyridoxal 5'-Phosphate

Plasma pyridoxal 5'-phosphate (PLP) is the major form of vitamin B-6 found in the blood and is a major indicator of vitamin B-6 status (Leklem, 1988; Leklem, 1990; Lumeng et al, 1974a; Merrill et al., 1984). Dietary vitamin B-6 intake is a primary factor influencing vitamin B-6 status. The pre-study plasma PLP concentrations suggest that most of the subjects entered both studies in good vitamin B-6 status and remained in good status at the start of the first exercise (NS) test. The low pre-study plasma PLP concentration in one subject in study 1 (16.1 nmol/L) and one subject in study 2 (18.5 nmol/L) was less than the recommended >30 nmol/L concentration (Leklem, 1990). These low pre-study PLP concentrations may be reflective of prior dietary intake, as plasma PLP is significantly correlated with dietary vitamin B-6 intake (Shultz and Leklem, 1981). The PRE PLP concentration in the NS state was below 30 nmol/L in two of the six subjects in study 1 and was equal to or below 30 nmol/L in two subjects in study 2. The relative resting (or baseline) concentration of PLP is important to consider when examining the potential for change in a given value with exercise. Hofmann et al. (1991) noted that subjects with a higher pre-exercise PLP concentration experienced a greater absolute elevation in plasma PLP than did subjects with concentrations in the normal range, but offered no explanation for this finding. Some of the subjects in the Hofmann et al. (1991) study were supplementing with vitamins, whereas others were not. The potential for change in plasma PLP concentration may be greater with a greater concentration of PLP relative to a lower concentration. Plasma PLP concentration provides an available supply to tissues such as the liver to participate in gluconeogenesis and to muscles for glycogenolysis and

transamination reactions. Perhaps there is a tighter binding of plasma to albumin at low concentrations or there is greater membrane alkaline phosphatase activity at higher PLP concentrations. Although the evidence suggests that tissue-nonspecific alkaline phosphatase functions physiologically as an ecto-PLP phosphatase (Whyte et al., 1985, 1988), there are no documented reports of increased alkaline phosphatase activity with higher plasma PLP concentrations.

Exhaustive exercise (i.e., NS state) resulted in an increase (19% in study 1 and 10% (n=3) in study 2) in the mean plasma PLP concentration during exercise and a decrease in the mean PLP concentration (17% in study 1 and 19% in study 2) after exercise (POST) to approximately resting (PRE) concentrations. These findings support our hypothesis that exhaustive endurance exercise results in a change in the plasma PLP concentration compared to resting conditions. Even though the increase in plasma PLP was not of statistical significance, all subjects had an increase in plasma PLP concentration. An increase in the plasma PLP concentration during exercise suggests that vitamin B-6 metabolism may have been altered. The majority of previous exercise and vitamin B-6 studies have examined plasma PLP prior to and after exercise (Leklem and Shultz, 1983; Manore, 1985; Rokitzki et al., 1994; Munoz, 1982). However, studies by Sampson et al., (1993), Hofmann et al. (1991) and Hatcher (1983) have found an increase of plasma PLP during exercise as compared to before exercise. Hatcher (1983) found a 14% increase from resting conditions in the mean plasma PLP concentration approximately 45 minutes during exercise in subjects consuming a normal carbohydrate diet (40%) and a high carbohydrate diet (71%) and exercising at 80% of max heart rate. Crozier et al. (1994) noted a significant increase of approximately 12% in the plasma PLP concentration within the first five minutes of exercise at both 60% and 85% of VO_2 max. Hofmann et al. (1991) found a 24% increase in plasma PLP from resting concentrations one hour into a two-hour treadmill run. However, Hofmann did not find any significant changes in plasma volume with exercise and therefore did not consider plasma volume changes as we did in the present

study. The 16% and 11% increase in plasma PLP concentration observed from resting conditions in the present study (study 1 and study 2, respectively) are consistent with these findings. The mean plasma PLP concentration one hour after exercise (POST 60) was lower than the initial mean PRE concentration (19% lower in study 1 and 17% (n=5) and 22% (n=3) lower in study 2). The decrease in plasma PLP at POST 60 (as compared with PRE concentrations) could not be completely accounted for by the change in plasma volume. Our data suggest that PLP leaves the plasma during recovery from exhaustive exercise. Previous studies have found a decrease in plasma PLP concentrations during recovery, but to a lesser extent than the present study. Manore (1985) found a 6% and 2% decrease in plasma PLP at POST 60 (from PRE concentrations) in women exercising on a cycle ergometer at 80% VO_2 max for 20 minutes when a medium carbohydrate (49% carbohydrate) and high carbohydrate (64%) diet was consumed, respectively. Hatcher (1983) observed a 4% and 9% decrease in plasma PLP concentrations from PRE to POST 60 after 50 minutes of cycling when a 40% carbohydrate diet was fed and observed a 5% decrease when a 71% carbohydrate diet was fed. Hofmann et al. (1991) and Munoz (1982) found only a 2% decline in the mean plasma PLP concentration from PRE levels at POST 60.

The reason for these changes in vitamin B-6 metabolism with exercise have been hypothesized (Leklem, 1988; Hofmann et al., 1991; Crozier et al., 1994) but remain controversial. Hofmann et al. (1991) suggested that the rise in plasma PLP during exercise may reflect an interorgan transport of PLP from the liver to the working muscle where it is involved in PLP-dependent reactions. Leklem (1988) has suggested just the opposite; a transport of PLP from the muscle to the liver. Leklem hypothesized that the rise in plasma PLP during exercise is a means of redistribution of vitamin B-6 from storage reservoirs in the muscle to the blood for transport to tissues. The muscle glycogen phosphorylase content of muscle (and therefore vitamin B-6 content of the muscle) has been shown to decrease only when there is a concurrent caloric deficit, not only a vitamin B-6 deficiency.

Exercise has been proposed as an acute form of caloric deprivation (Black et al., 1978). The breakdown of muscle glycogen for energy via glycogen phosphorylase during exercise with a resulting increase in muscle lactic acid concentration and drop in muscle pH favors the release of PLP from the muscle into the blood for redistribution. The liver is one tissue that requires PLP as a cofactor in gluconeogenesis and glyconeogenesis. The body relies on the production of glucose by the liver to maintain blood glucose levels (Ahlborg and Felig, 1982; Lemon and Nagle, 1981). Therefore, the redistribution of PLP from the muscle to the liver would ensure the production of glucose for continued activity.

The hypothesis that exhaustive endurance exercise in combination with oral vitamin B-6 supplementation (20 mg PN), would result in a greater rise in plasma PLP concentration during exercise and a greater decrease in plasma PLP concentration after exercise than in the non-supplemented state was tested. In study 1, there was a 28% rise in the mean plasma PLP concentration between the PRE and DX sample points (compared to a 19% rise in the NS state) and a 23% decrease in the mean plasma PLP concentration between the DX and POST sample points (compared to a 17% decrease in the NS state). The decrease in plasma PLP concentration from DX at POST 60 was significant. These findings are in agreement with the vitamin B-6 and exercise study in men by Hatcher et al. (1982). She found an 11% rise in the plasma PLP concentration between the PRE and DX sample points and a 15% decrease in the plasma PLP concentration between the DX and POST sample points (as compared with a 6% increase and 7% decrease, respectively in the NS state) under conditions of a low carbohydrate diet (11% CHO) and 8 mg/d of vitamin B-6 (a total intake of vitamin B-6/day of approximately 10 mg).

A difference between the studies was evident by examining the plasma PLP concentrations. When plasma volume changes were considered, there was essentially no change in the mean plasma PLP concentration (from PRE concentrations; 42.5 nmol/L measured DX in the NS state and 42.1 nmol/L predicted DX; see table 12) during exercise in the NS state in study 2, and there may even have been a loss of plasma PLP in the

vitamin B-6 supplemented conditions. Since these observations are consistent between the NS and S states, the subjects in study 2 appear to be retaining more PLP in the muscle during exercise than those subjects in study 1.

The retention of PLP by the muscle in the NS and S states in the subjects in study 2 may be a function of the level of training or amount of fat-free mass. The data suggest that the subjects in study 2 may have been more highly trained and had a greater fat-free mass than the subjects in study 1. The mean body weight of the subjects in study 2 (Table 5) was 12% greater than the mean body weight of the subjects in study 1, but the mean percent body fat (13%) was comparable in the studies. Therefore, the subjects in study 2 appeared to have a greater amount of fat-free mass than the subjects in study 1. The level of training of the subjects in study 2 may also have been higher than the subjects in study 1, as indicated by a slightly greater mean VO_2 max (2.3%). However, when the VO_2 max was expressed per kilogram of body weight, the difference disappeared. The one subject common to both studies exercised for a total of 190.3 minutes in the S state in study 2 at a higher oxygen consumption than during the S state in study 1 (160.5 minutes). These results suggest that this subject was in better physical condition in study 2 as compared with study 1. The exercise reported in the daily logs (minutes of intensity) was greater in study 2 (660 ±273) as compared with study 1 (545 ±288). While this measure is partially subjective, these data suggest that the subjects in study 2 were training longer and/or at a greater intensity on a regular basis than the subjects in study 1 and may have been more highly trained.

Another difference in the study 1 and study 2 which could influence the plasma PLP concentrations was a difference in the vitamin B-6 content of the diets. The diets were planned to provide approximately 2.3 mg of vitamin B-6 per day in 3500 kcals. The vitamin B-6 content of the diet in study 1 was higher (2.31 mg in the NS state and 2.32 mg in the S state) than of the diet in study 2 (1.89 mg in the NS state and 1.92 mg in the S state). The difference in the vitamin B-6 content of the diets was primarily in the animal and

plant vitamin B-6 sources. There was a difference between the brands of turkey used in the studies which may have contributed to the difference. The amounts of the foods used in the studies differed slightly. Life™ cereal was substituted (in study 2) for the shredded wheat in study 1. Previous bioavailability studies (Bills, 1991) suggest that shredded wheat may influence the bioavailability of vitamin B-6. The contribution of shredded wheat and Life cereal to the vitamin B-6 content of the diets was 5.5% and 4.7%, respectively. Therefore, even though twenty-eight percent of the vitamin B-6 content of shredded wheat is glycosylated vitamin B-6 (Leklem, 1991), the contribution of the cereal to the total vitamin B-6 content of the diet was probably of minimal significance unless shredded wheat also decreased the bioavailability of vitamin B-6 from other foods.

The diets in study 1 and study 2 provided less than the recommended vitamin B-6 to protein ratio (a ratio of 0.0148 mg/g in study 1 and a ratio of 0.0123 mg/g in study 2). These ratios are based on the protein analysis of the Food Processor software program. If the protein content of the diet was less in study 2 than reported, the higher B-6 to protein ratio could account for the differences in the urinary excretion of 4-PA and UB-6 discussed later. A vitamin B-6:protein ratio of 0.016 mg/g has been recommended by the National Research Council (1989) to ensure acceptable vitamin B-6 status in adults. However, acceptable vitamin B-6 status indicators (plasma PLP, urinary 4-PA and vitamin B-6 excretion) have been reported with ratios of 0.0125 to 0.015 mg/g (Shultz and Leklem, 1981). Even though this observation was in women, our data suggests that the vitamin B-6 to protein ratio was adequate to maintain health.

A high carbohydrate diet and vitamin B-6 supplementation has been shown to blunt the rise in plasma PLP associated with exercise (Manore et al., 1987; Hatcher, 1983). The greater carbohydrate consumption (approximately 11%) by the subjects in study 2 in the S state (a mean of 619 g/d in the S state compared to 550 g/d in study 1) may have suppressed the release of PLP from the muscle during exercise (Manore et al., 1987;

™ Trademark for Life cereal; Quaker Oats Co., Chicago, IL.

Hatcher, 1983) and accounted for some of the difference in plasma PLP concentration between the studies in the S state.

A difference in plasma PLP concentrations based on the level of training has been observed previously. Leklem observed little change in plasma PLP concentrations during exercise in highly trained (perhaps over-trained) athletes (unpublished observations). The changes in plasma PLP in study 2 (S state) were essentially flat, suggesting a retention of vitamin B-6 in storage pools, such as the muscle. Dreon and Butterfield (1986) likewise found a retention of vitamin B-6 by the body in male subjects consuming 4-4.5 mg vitamin B-6 per day and running either 5 or 10 miles per day for 29 days.

Plasma Vitamin B-6

The changes in the mean plasma vitamin B-6 (PB-6) concentration as a result of exhaustive exercise and vitamin B-6 supplementation are similar to those of plasma PLP. This is not suprising, since PB-6 is composed primarily of PLP and PL (Leklem, 1988, 1990). The PB-6 and PLP difference therefore provides an estimate of the plasma PL concentration (defined here as "PL").

The data suggest (n=3) that exhaustive exercise (NS state) resulted in an increase (11%) in the mean PB-6 concentration during exercise from resting levels, a decrease (-9%) in the PB-6 concentration after exercise (POST) to approximately resting levels and a further decrease (-17%) at POST 60. The mean POST 60 PB-6 concentration represented a -4% decrease in the PB-6 concentration from resting levels. Vitamin B-6 supplementation resulted in a greater magnitude change in the mean PB-6 concentration from resting levels DX (25%) and POST(-17%) than was found with exhaustive exercise alone.

In the NS state, the 12% increase in mean plasma "PL" was comparable to the rise in PB-6 and PLP at that point (11% for both). However, at POST, the mean plasma "PL" concentration increased 8% from DX, whereas the mean PLP and PB-6 concentrations decreased (-19% for the PLP and -9% for the PB-6). Therefore, the data suggest an

increase in plasma "PL" concentration after exercise. By one-hour of recovery (POST 60), the plasma PLP, PB-6 and "PL" are all less than the POST concentrations. In a two-hour running study by Hofmann et al. (1991) at 60-65% VO_2 max, plasma PL concentrations were measured by HPLC method at similar time intervals as our study. The mean PL concentration at PRE (29.1 ±10.0 nmol/L) and DX (30.5 ±8.0 nmol/L) were comparable to our values. However, we observed less mean plasma "PL" at POST 60 (19.9 nmol/L) than was found in their study at POST 60 (29.6 nmol/L).

In the vitamin B-6 supplemented state, the rise in the PB-6 concentration from PRE levels was greater at DX (24%) as compared to the NS state (12%), suggesting that exhaustive exercise and vitamin B-6 supplementation enhanced the change in vitamin B-6 metabolism typically seen during exercise. Hatcher (1983) reported an 8% increase in the PB-6 concentration in four trained cyclists on a 71% carbohydrate diet 45 minutes into exercise (compared to before exercise) without vitamin B-6 supplementation, and a 10% increase in the PB-6 concentration during exercise with vitamin B-6 (8 mg/d) supplementation. Our data suggest that a greater increase in plasma vitamin B-6 (either PLP and/or plasma PL) occur during exercise under vitamin B-6 supplementation conditions.

The source of the additional "PL" during exercise in the vitamin B-6 supplemented state remains an issue. The rise in the plasma "PL" concentration during exercise in the vitamin B-6 supplemented state may indicate a movement of PL into the plasma, a dephosphorylation of existing plasma PLP by alkaline phosphatase or a decreased uptake of plasma PL by cells. If the dephosphorylation of plasma PLP by alkaline phosphatase was contributing to the plasma "PL" concentration during exercise in the vitamin B-6 supplemented state, we would expect a significant correlation between alkaline phosphatase activity and "PL". However, no such correlation was found between these two variables in either the NS or vitamin B-6 supplemented states. The plasma alkaline phosphatase activity is probably a reflection of tissue alkaline phosphatase activity responsible for the dephosphorylation of PLP to PL (Coburn and Whyte, 1988).

The majority of plasma PLP is carried through the bloodstream bound to albumin to protect it while in transit (Anderson et al., 1974). Even though there was essentially no change in plasma albumin concentration during exercise (NS and S states) when plasma volume changes were considered, the albumin concentration present was probably adequate to transport the additional plasma PLP concentration (10% additional mean plasma PLP from resting concentrations in the NS state; 7% additional in the S state) due to its high binding capacity (>600-700 nmol/L)(Vander et al., 1985). There may be an indication that the red blood cell is contributing to a portion of the PL to the plasma during exercise. Evidence for this observation will be discussed below.

Red Blood Cell PLP

To date, no exercise studies have examined red blood cell (RBC) PLP changes with exercise in men. Thus, it is not possible to make comparisons with other studies. The change in RBC PLP concentration did not follow the trends observed with the plasma PLP and PB-6 concentrations before, during and after exercise. Two distinctly different patterns emerged in the NS and S states. In the NS state, there was a gradual increase in the mean RBC PLP concentration(n=3) during and after exercise (66.8 nmol/L PRE, 68.8 nmol/L DX, 71.5 nmol/L POST and 73.4 nmol/L POST 60)(see Table 16). This trend was evident when all samples (n=5 for POST and POST 60) were included. The change in RBC PLP concentration at POST 60 in the NS state represented a 13% rise above resting (PRE) concentrations (n=5). The gradual increase in RBC PLP concentration during and following exhaustive exercise (NS state) may represent an uptake of PLP precursors by the RBC. Plasma PLP is bound tightly to albumin, making it an unlikely source of uptake by the RBC. Present evidence suggests that plasma PL is readily taken up by RBCs both in vitro and in vivo (Anderson, 1980). PL is converted in the RBC to PLP since the necessary oxidase is present (Anderson, 1980). Cell surface alkaline phosphatase activity may play a role in the dephosphorylation of plasma PLP to PL for uptake by the RBC (Fedde and

Whyte, 1990). PL is converted in the RBC to PLP since the necessary oxidase is present (Anderson, 1980). The physiological significance of the RBC as a reservoir for PL is unknown. RBC PLP may be important for oxygen transport, since RBC PLP has been shown to facilitate oxygen unloading in vitro (Benesch et al., 1969; Chanutin and Curnish, 1967).

The data suggest that vitamin B-6 supplementation altered the uptake of vitamin B-6 into the RBC. In contrast to the NS state, there was a decrease in the mean RBC PLP concentration (from PRE levels) DX and POST, and a 32% increase (n=5) in the RBC PLP concentration at POST 60. The slight decrease in RBC PLP concentration (from PRE) during exercise in the vitamin B-6 supplemented state, while not significant, may account for some of the increase in the plasma "PL" concentration. Anderson (1980) described the mechanism of conversion of PLP in the RBC via the phosphatase to PL which is then released into the plasma. The marked shrinkage of RBC volume due to dehydration resulting from exercise (Costill, 1974) should have produced an increase in the DX RBC PLP concentration instead of a decrease. Therefore, the loss of RBC PLP during exercise in the S state may actually have been greater than was measured. In addition to releasing PL into the plasma, the RBC may also deliver PL to cells that require PLP as a coenzyme for gluconeogenic pathways, such as the liver and/or muscle. Vitamin B-6 supplementation appeared to result in an increased uptake of PL by the RBC after exercise. The increase in mean RBC PLP concentration (46%) from the POST sampling point one hour after exercise corresponds to the decrease in mean plasma PLP concentration (-16% (n=3)). Plasma PLP probably does not account for all of the increase in RBC PLP following exercise. Therefore, plasma PL may account for a portion of the rise in RBC PLP observed one hour after exhaustive exercise in the vitamin B-6 supplemented state. There is an indication that the RBC may also function to transport amino acids to tissues independent of the plasma amino acids (Felig et al., 1973). The passage of the RBC through the narrow capillaries may facilitate the exchange of amino acids to cells for energy production and

protein synthesis. The RBC therefore may function to facilitate gluconeogenesis by providing both substrate and the cofactor (PL which can be phosphorylated to PLP) to tissues for energy production. RBC amino acids were not analyzed in study 2.

Urine

Urinary creatinine and urea nitrogen excretion was used as a measure of completeness of collection. Both should remain constant from day to day within a given individual if diet and exercise are controlled. The dietary intake of our subjects in both studies was controlled, however, the daily exercise, which was supposed to be constant day to day, was controlled by the subjects. There was an indication, from examining the individual creatinine and urea nitrogen data, that daily collections may not have been complete, especially during the day(s) after exercise in both studies. Motivation may have been a problem or a lack of understanding of the importance of the urine collections. These factors should be considered when evaluating the urinary 4-PA and UB-6. The slightly greater consistent excretion of creatinine in study 2 (as compared with study 1) may indicate a greater fat-free mass (Heymsfield et al., 1983) in these subjects or that the subjects in study 2 were exercising more on a consistent basis than the subjects in study 1 (Hobson, 1939; Srivastava et al., 1957). The body composition and VO_2 max data support the idea that the subjects in study 2 may have had a greater fat-free mass and been more highly trained.

4-Pyridoxic Acid

The excretion of 4-pyridoxic acid (4-PA) is a reflection of vitamin B-6 intake and metabolism. The variability in the individual 4-PA, creatinine and urea nitrogen data makes the interpretation of the urinary 4-PA results difficult. The data suggest incomplete collections in at least one individual from each study. Given these constraints, there were

no significant differences in the excretion of 4-PA before, during or after exhaustive exercise in either the NS or S states in study 1 or study 2, suggesting no change in vitamin B-6 metabolism with exercise.

There was a significant difference in the overall excretion of 4-PA in study 1 (6.20 μmol/d) and study 2 (7.98 μmol/d) in the NS state. This difference was more pronounced when expressed as a percent of vitamin B-6 intake (45.6% in study 1 and 71.3% in study 2). A difference in the mean vitamin B-6 content of the diets (2.3 mg/d in study 1 and 1.9 mg/d in study 2), difference in daily exercise, and individual variation may account for part of these differences. The 4-PA excretion (as a percent of vitamin B-6 intake) in study 2 was higher (63-75%) at all sample points (NS and S states) than most percentages previously reported in men (44% during a dietary period containing 1.55 mg of vitamin B-6, Leklem et al., 1980; 53% during a dietary period containing 1.55 mg of vitamin B-6, Kabir et al., 1983b and 45% during a dietary period containing 4.2 ±0.4 mg of vitamin B-6, Dreon and Butterfield, 1986). The difference in the percent of vitamin B-6 intake excreted as 4-PA in the present study as compared to the previously reported percentages in men may reflect differences in the amount of dietary vitamin B-6, the amount of dietary protein and/or the amount of exercise the subjects received.

Based solely on the difference in the vitamin B-6 content of the diets in the present study, we would expect a greater excretion of 4-PA throughout the NS period in study 1 instead of in study 2 (Wozenski et al., 1980). However, the requirement for vitamin B-6 is also related to the level of dietary protein (Miller and Linkswiler, 1967; Miller et al., 1985; Leklem et al., 1991). Even though the computer analysis of the protein content of the diet in study 1 (155 g/d) and study 2 (154 g/d) was similar, there was an indication form Kjeldahl analysis of the protein content of the diet in study 1 (139 g/d) that the subjects received less protein than planned. The subjects in study 1 therefore consumed a vitamin B-6:protein ratio of 0.0165 and 1.83 g protein/kg body weight. A low protein diet (0.5 g/kg) in men has been shown to increase the excretion of 4-PA (to approximately 4.5 μmol/d) as

compared with a high protein diet (2 g/kg; approximately 2.8 μmol/d) when the vitamin B-6 content of the diet is kept constant (1.6 mg/d)(Miller et al., 1985). Based on the mean excretion of 4-PA throughout the NS state in study 2 in comparison to study 1, it is reasonable to assume that the protein content of the diet (study 2) was less than planned. If the protein content of the diets in study 1 and 2 were comparable (i.e., 139 g/d), a difference in the mean excretion of 4-PA could still have existed in the studies due to the 12% difference in the subject's mean body weight. A difference in mean body weight would mean a difference in the grams of protein per kg body weight consumed. The differences in overall body weights, fat-free mass and 4-PA excretion between the studies may suggest a relationship between 4-PA excretion and fat-free mass, since muscle is one site of transamination reactions involving PLP and contains glycogen phosphorylase which requires PLP as a cofactor.

There is a suggestion, based on body weight and percent body fat, that the subjects in study 2 had a greater amount of fat-free mass than the subjects in study 1. Intuitively, a greater amount of fat-free mass, which includes muscle, would seem to require and possibly metabolize more vitamin B-6 through participation in daily muscle metabolism reactions such as transferases, transamination, decarboxylation and cleavage. However, documentation linking vitamin B-6 metabolism to fat-free mass are lacking. The significantly greater amount of daily activity recorded in the log books for the subjects in study 2 suggests that vitamin B-6 metabolism may have been greater at all sample collection points for the subjects in study 2 as compared to the subjects in study 1. Rokitzki et al. (1994) compared the excretion of 4-PA/g creatinine in trained marathon runners to untrained individuals and found a greater basal excretion of 4-PA in the marathon racers (4.48 μmol/g creatinine as compared to 2.69 μmol/g creatinine). These findings are in line with the mean 4-PA excretion/g creatinine calculated for study 2 (3.78 μmol/g creatinine) in the NS state as compared with the value for study 1 (3.21 μmol/g creatinine) and provides support for a difference in the level of training between the subjects in studies 1 and 2. A

difference in the excretion of 4-PA due to level of training has also been demonstrated in women by Manore et al. (1987) who found a consistently greater excretion of 4-PA in young trained women in the NS state (7.36 μmol/d for 12 days) as compared with the young untrained group (a mean of 6.56 μmol/d for 10 days). Both Manore et al. (1987) and Hatcher et al. (1982) have shown that an acute bout of cycling at approximately 75% VO_2 max will increase the excretion of 4-PA (5-8% in women; Manore et al. (1987); 25% in men; Hatcher et al. (1982)) as compared to the day prior to exercise. Therefore, exercise appears to increase vitamin B-6 metabolism and may explain some of the differences in the 4-PA excretion between the studies.

Under vitamin B-6 supplementation conditions, the differences in the overall mean percent of intake excreted as 4-PA in study 1 and study 2 disappeared. One explanation for this may be that the vitamin B-6 intake (diet plus supplement) was approximately 10-fold greater than the RDA, therefore, any differences in the vitamin B-6 intake in relation to protein intake were small. The differences in the subjects' daily exercise amount and perhaps level of training should have resulted in a greater excretion of 4-PA by the study 2 subjects than the study 1 subjects. However, the subjects in study 2 excreted significantly less mean 4-PA than the subjects in study 1 under vitamin B-6 supplementation conditions. This difference may be reflective of the 2% difference in vitamin B-6 intake between the two studies (diet plus supplement) and/or may represent a retention of vitamin B-6 by the subjects in study 2. Dreon and Butterfield (1986) observed a significant decrease in 4-PA excretion in trained men consuming 4.2 mg of vitamin B-6 and running either 5-10 miles/d for a 29 day period as compared with and untrained group.

The differences in the excretion of 4-PA observed in study 1 and study 2 probably reflect the combination of differences in the subjects, differences in the subjects' level of activity and fat-free mass, and differences in the vitamin B-6 and protein content of the diets.

Urinary Vitamin B-6

The excretion of urinary vitamin B-6 (UB-6) contributed far less as a percent of vitamin B-6 intake than urinary 4-PA (see Table 26). The percent of vitamin B-6 intake excreted as urinary vitamin B-6 in study 1 (NS state) was slightly lower (mean of 5.7%) than the 8-10% of intake typically excreted with an intake of 2 mg vitamin B-6 per day whereas the percent of vitamin B-6 intake excreted as UB-6 in study 2 was consistent with this estimate (a mean of 9.6%)(Leklem, 1988).

Although there were no significant changes in the excretion of UB-6 during the days before, the day of and the day(s) after exercise, the significant differences between the NS and S states within a study and between study 1 and study 2 were consistent with the mean excretion of 4-PA. Exhaustive exercise did not appear to significantly alter the excretion of UB-6, even though a decrease in the excretion of UB-6 was found for both studies in the NS and S states. There may be an increased retention of the forms of vitamin B-6 typically excreted in the urine as UB-6 on the exercise test day, perhaps for the purpose of maintaining energy producing pathways for exercise. The concentration of UB-6 was consistently greater during the days before, during and after exhaustive exercise in study 2 (a mean of 1.08 μmol/d) compared to study 1 (a mean of 0.78 μmol/d) in the NS state even though the dietary vitamin B-6 content was greater in study 1. These findings are in line with the differences noted with the excretion of 4-PA in study 1 and study 2, and suggests a difference in the protein content of the diet or activity level of the subjects as previously noted. There was consistently greater UB-6 excretion in the S state in study 1 (a mean of 9.57 μmol/d) as compared to study 2 (a mean of 8.71 μmol/d). These results are consistent with those of Munoz (1982) who observed a mean excretion of UB-6 the day before and day of cycling of 1.01 μmol/d in trained college athletes as compared with a mean excretion of 0.76 μmol/d the day before exercise in untrained subjects.

The mid-study analysis of urinary 4-PA and UB-6 provided an indication of compliance in taking the vitamin B-6 supplement. There was an indication from study 1

that one subject did not consume the vitamin B-6 supplement on one day mid-study and no indication that any supplements were missed in study 2. In study 2, one subject did not provide urine samples mid-study, therefore we were unable to assess his compliance in taking the vitamin B-6 supplement.

Sweat

Sweat is a form of body temperature regulation initiated when the body becomes overheated. Both fluid and nutrients can be lost in sweat. The loss of vitamin B-6 through sweat has seldom been measured. The last reported value for vitamin B-6 in sweat (0.0024 to 0.0010 μmol/100 mL) dates back to 1945 when Johnson et al. analyzed complete eight hour skin excretions using a slight modification of the Saccharomyces carlsbergenesis method of Atkin et al. (1943). At this time, all of the actual forms of vitamin B-6 had not been determined. The amount of vitamin B-6 lost through sweat during exercise provides an indication of the metabolism of vitamin B-6 in athletes. The loss of vitamin B-6 we observed from sweat in the NS state during exercise (0.0006 μmol/100 mL, n=3; 0.0071 μmol/100 mL, n=5) was comparable to the previously reported values. The concentration of vitamin B-6 in sweat in the S state represents two different methods of extraction. Although the sweat samples in study 1 and study 2 were collected onto filter paper, the extraction methods were slightly different, which could account for a portion of the 3-fold difference between the values. Vitamin B-6 supplementation did not significantly influence the amount of vitamin B-6 lost in sweat (0.0065 μmol/100 mL, n=3, in study 2). During endurance exercise, sweat rates of 1.5 L/hr are common under normal conditions (Brotherhood, 1984). Assuming that, of the body weight loss during exercise, 3 g are lost per minute as respiration and the remainder is lost as sweat during exercise (Mitchell et al., 1972), the mean sweat concentration collected in study 1 (S state) represents a mean total loss of vitamin B-6 from the body through sweat of 0.0039 μmol. In study 2, the loss of vitamin B-6 in the sweat in the NS state represents a mean loss of 0.0011 μmol and

represents a mean loss of 0.0008 μmol in the vitamin B-6 supplemented state. The total loss of vitamin B-6 through sweat appeared to be minimal even under the conditions of exercise and vitamin B-6 supplementation. The individual differences between the loss of vitamin B-6 in sweat (a range of 0.0042 μmol/100 mL to 0.0485 μmol/100 mL) could be due to differences in the sweat rates or to contamination during analysis.

The sweat extraction process appeared to introduce error into the final sweat vitamin B-6 concentration. A series of repeated trials testing the water and filter paper used to collect and extract the sweat revealed that the filter paper stimulated the growth of the yeast in the vitamin B-6 assay. An unidentified compound in the filter paper may have been extracted along with the sweat. This compound either stimulated the growth of the yeast in the microbiological vitamin B-6 assay or may have killed some of the yeast in the assay, thereby providing the remaining yeast with a nutrient-rich medium for growth. In either case, an inconsistent source of error was introduced into the assay procedure. Individual differences in the sweat rate were found in both studies. A greater sweat rate, as indicated by the loss of body weight, could indicate a greater excretion of vitamin B-6 in the sweat. However, we found no significant correlation between the amount of weight loss as a result of exercise and the sweat vitamin B-6 content.

Correlations between the PRE plasma values in the NS state and correlations between the plasma values in the vitamin B-6 supplemented state were determined. As expected, the plasma PLP and the PB-6 significantly correlated in both the NS state and in the S states, since the plasma PLP (and PL) comprise approximately 90% of the PB-6 (Leklem, 1990). The significant correlation between plasma PLP and alkaline phosphatase in the NS state suggests a relationship between the alkaline phosphatase and PLP. Whyte et al. (1985) found markedly increased circulating concentrations of plasma PLP (a mean of 1174 nM) in patients with clinical hypophosphatasia, an inborn error of metabolism characterized by a deficient activity of tissue non-specific alkaline phosphatase. Conversely, a study in children (Reynolds et al., 1991) with familial hypophosphatemic

rickets, a disease characterized by elevated alkaline phosphatase activity, found undetectable concentrations of plasma PLP (<0.2 nmol/L) in 15 of 31 kids. These data demonstrate an inverse relationship between alkaline phosphatase and PLP. Anderson (1980) likewise has reported an inverse relationship between alkaline phosphatase activity and plasma PLP concentration. The relationship appears to be independent of the intracellular PLP concentration and metabolic functioning (Whyte et al., 1985; Reynolds et al., 1991). Therefore, the relationship found in this study between PLP and alkaline phosphatase is probably an inverse relationship.

In summary, exhaustive exercise and 20 mg of vitamin B-6 altered vitamin B-6 metabolism such that there was an increase of PLP into the plasma during exercise and out of the plasma by one hour of recovery. These changes could not be accounted for entirely by a change in plasma volume. The PLP concentration in the red blood cell significantly increased by one hour after exercise, indicating an uptake of PLP precursors, presumably PL. The data suggest a movement of plasma PL into the red blood cell by one hour of recovery following exhaustive exercise in the S state in trained men. The alkaline phosphatase activity suggests that the RBC PL did not originate from the plasma PLP. There is reason to believe exhaustive exercise does not significantly increase the excretion of vitamin B-6 as 4-PA. Training and/or dietary components such as the amount of protein may result in a consistently greater excretion of 4-PA on a daily basis. The excretion of vitamin B-6 through sweat does not appear to be influenced by vitamin B-6 supplementation and appears to vary between individuals.

Plasma Growth Hormone

Plasma growth hormone concentration has been reported to change as a result of exercise of varying duration and intensity (Karagiorgos et al., 1979; Lassare et al., 1974; Raynaud et al., 1983). In the present study, exhaustive exercise resulted in a significant increase in the mean plasma hGH concentration from resting levels during (22-fold) and

after (a 16-fold increase at POST) exercise and resulted in a significant decrease in the mean plasma hGH concentration at POST 60 (approximately 6-fold from DX). The mean plasma hGH concentration during exercise is in agreement with previously reported plasma hGH concentrations during exercise (a range of 10-40 ng/mL; Hartley, 1975; Rolandi et al., 1985; Naveri, 1985). The mean hGH concentration before during and after exercise (NS and S states) excluded subject 5, who had a resting (PRE) hGH concentration in the first exercise test (NS state; 19.7 ng/mL) that was greater than normal resting hGH concentrations (<5 ng/mL; (Winer et al., 1990). The decision to exclude subject 5 in the growth hormone results was made based on the knowledge that this subject was extremely apprehensive about having his blood drawn, and that there is an indication that stress can increase plasma growth hormone concentrations (Greenwood, 1966; Biselli et al., 1993; Scavo et al., 1991). The plasma hGH concentration for subject 5 (NS state) was essentially unchanged between the PRE and DX sampling points. The plasma hGH concentration at POST and POST 60 for subject 5 was less than the PRE concentration (3.4 ng/mL less at POST and 17.6 ng/mL less at POST 60). Therefore, it appeared that the blood drawing procedure resulted in a stressful situation for subject 5. The anticipatory stress of blood drawing appeared to contribute to subject 5's high PRE hGH concentration. Excluding the PRE value for subject 5, his other hGH concentrations during and after exercise (DX, POST and POST 60) were in-line with the values found for the other subjects. Exercise seemed to lessen this subject's stress level.

Vitamin B-6 supplementation did not significantly affect the changes in plasma hGH concentration observed with exhaustive exercise alone. However, as compared to the plasma hGH concentration in the NS state, the mean plasma hGH concentrations were consistently higher in the vitamin B-6 supplemented state at the DX (1.3 ng/mL), POST (1.7 ng/mL) and POST 60 (1.0 ng/mL) sampling points. The plasma hGH concentration for subject 5 in the S state changed in a manner similar to the other subjects (an increase of 10.9 ng/mL from PRE to DX, an increase of 16.0 ng/mL from PRE to POST and a

decrease of 1.21 ng/mL from PRE to POST 60). The data do not support the hypothesis that the combination of exhaustive exercise and vitamin B-6 supplementation results in a significant increase in plasma hGH concentration above that typically found with exercise. Previous studies have found a higher resting hGH concentration when vitamin B-6 was infusioned as compared to the control condition. The plasma hGH concentration during resting conditions with a 600 mg of pyridoxine infusion was found by Moretti et al. (1982) to be 2.6 ng/mL higher than the plasma hGH concentration during resting conditions with a saline infusion. A 300 mg pyridoxine infusion given to eight volunteers (four females and four males) after an overnight fast similarly resulted in a rise in the resting hGH concentration to 7 ng/mL after 120 minutes (Delitala et al., 1976). These findings are in contrast to the lower resting hGH concentrations we observed in the vitamin B-6 supplemented state (1.75 ng/mL less). There may have been less test anxiety during the second exhaustive exercise ride resulting in a lower mean PRE plasma hGH concentration as compared to the first exhaustive exercise test ride (NS state). The higher mean increase in plasma hGH concentration at POST 60 (as compared to PRE) under oral vitamin B-6 supplementation conditions (5.2 ng/mL as compared to a 2.46 ng/mL mean rise in the NS state) was less than the increase POST 60 (12.5 ng/mL as compared to 4.2 ng/mL in the control condition) previously reported by Moretti et al. (1982) for six subjects following 8 minutes of cycle ergometry (80% of max heart rate) and a 600 mg infusion of pyridoxine. In the present study, the 20 mg PN oral dose provided may not have been large enough to cause a change in plasma hGH concentration reported previously by Moretti et al. (1982).

Fuels

The exhaustive exercise component of these studies was included as a means of depleting muscle glycogen and producing fatigue. In accordance with the theory set forth by Leklem (1985), supplemental vitamin B-6 was hypothesized to accelerate glycogen depletion and result in early fatigue (compared to exercise in the non-supplemented

condition). An increase in plasma lactate concentration and decrease in plasma free fatty acid (FFA) concentration may result as a consequence. The effect of vitamin B-6 supplementation on fuel substrates during exercise has been examined in previous studies (Leklem, 1985; Manore and Leklem, 1988) and is being examined by another graduate student in conjunction with this thesis project. The data suggest the enhanced utilization of carbohydrate as an energy source during exhaustive exercise with vitamin B-6 supplementation. Plasma lactate concentrations during exercise in study 2 (analyzed by Ricky Virk) were greater before, during and after exhaustive exercise in the S state compared to the NS state. In the vitamin B-6 supplemented state, there was a significant increase in the lactate concentrations DX (compared to PRE) and at POST (compared to PRE). These findings agree with those of Manore and Leklem (1988) who found the highest plasma lactate concentrations with exercise and vitamin B-6 supplementation as opposed to the NS state. The higher respiratory exchange ratios in the vitamin B-6 supplemented state also indicate that carbohydrate was utilized as an energy source. The physiological significance of these findings are unclear, since the time to exhaustion did not vary significantly between the NS and S states.

The plasma FFA concentrations during exercise indicate less utilization of fat as an energy source (as compared with exercise in the NS state) in the vitamin B-6 supplemented state in study 2. The plasma FFA concentrations before, during and after exhaustive exercise in the S state (study 2) were lower than the plasma FFA concentrations during exercise in the NS state. These differences were not found to be statistically significant. The plasma FFA concentrations in study 2 during exercise (S state) are in agreement with those of Manore and Leklem (1988) in women exercising for 20 minutes at 80% VO_2 max with 8 mg/d of vitamin B-6. The findings in study 2 suggest that vitamin B-6 supplementation altered fuel utilization in a manner consistent with the hypothesis of Leklem (1985)

Opposite findings regarding fuel utilization during exhaustive exercise and vitamin B-6 supplementation were observed in study 1. There was a greater reliance on fats as an energy source and less reliance on carbohydrates in the vitamin B-6 supplemented state. The plasma FFA concentrations in study 1 were significantly lower in the NS state as compared to the S state. The difference in the carbohydrate intake of the subjects between the studies and between the NS and S states were probably not significant enough to account for the observed difference in fuel utilization since the subjects were exercised in the fasted state.

The purpose of exercising the subjects to exhaustion was to deplete muscle glycogen and determine whether the involvement of supplemental vitamin B-6 influenced muscle glycogen depletion. However, factors other than glycogen depletion can result in the cessation of exercise due to fatigue. Fatigue has been defined as an increase in the perception of effort necessary to exert a desired force, and the eventual inability to produce this force (Enoka and Stuart, 1985). This definition implies both a psychological and physiological component to fatigue. The subjects in both studies defined fatigue on their own as the inability to maintain a pedaling rate of 80 rpm at a workload of 75% VO_2 max. Subjects commented that their legs felt "heavy" and were "burning" at the point of fatigue.

One subject in study 2 (subject 2) lasted only 22 minutes during exhaustive exercise with vitamin B-6 supplementation as compared to 53 minutes without the supplement. The shorter time to exhaustion in the S state appeared to support the finding that vitamin B-6 supplementation accelerated glycogen depletion, resulting in early fatigue. He complained of feeling "off" from the start of exercise and never recovered. We were unable to support the hypothesis that vitamin B-6 supplementation results in early fatigue based only on the time to exhaustion. Factors such as the amount of sleep, prior exercise (this subject competed in a strenuous mountain bike race two days prior to the exercise test in the S state) or a shift in sleep pattern may have contributed to his early fatigue. The amount of sleep a person obtains prior to an endurance event could effect how tolerant they are of

physical fatigue. We were unable to analyze this subject's sleep patterns, as he did not return his daily log book.

Under the circumstances present when study 1 and study 2 were conducted, an excellent level of control of the diet and exercise components of these studies was maintained. A study of this magnitude requires consistent attention to detail by a variety of people. As with any human study, error is inevitable. Even the best intentioned subject will fail to collect all his/her urine and may forget to eat some food. The reliance on mechanical instruments also is not without error. Metabolic carts and freezers will malfunction. The uncontrollable factors, present in all studies, represent the greatest challenge in providing high quality research projects. Studies reported in the literature seldom control both diet and exercise when measuring plasma growth hormone changes to the extent this project did. The study design and physical facilities available to conduct these experiments were some of its greatest strengths.

SUMMARY AND CONCLUSIONS

The purpose of this project was to examine the effect of exhaustive endurance exercise and vitamin B-6 supplementation on vitamin B-6 metabolism and plasma growth hormone in men under controlled conditions. It was hypothesized that 20 mg of pyridoxine in combination with exhaustive endurance cycling would alter plasma and urinary vitamin B-6 indices and plasma growth hormone concentration more than exhaustive endurance cycling alone. Specifically, the objectives were to: 1) determine if exhaustive endurance exercise would result in significant changes in plasma pyridoxal 5'-phosphate and vitamin B-6 concentrations compared to resting conditions, and to monitor these changes at exhaustion and during recovery 2) to determine if the magnitude of change in plasma vitamin B-6 indices was greater with exhaustive exercise in the vitamin B-6 supplemented state compared to the non-supplemented state 3) to determine if the excretion of urinary vitamin B-6 and 4-pyridoxic acid was greater with exhaustive exercise in the vitamin B-6 supplemented state compared to the non-supplemented state and to monitor the excretion during the days following exercise 4) to determine if plasma growth hormone concentration significantly increased with exhaustive exercise in the vitamin B-6 supplemented state compared to the non-supplemented state, and finally 5) to quantify the loss of vitamin B-6 in sweat during exhaustive endurance exercise with and without vitamin B-6 supplementation.

Two similar but separate studies were conducted. Both studies examined trained male cyclists who exercised to exhaustion twice at approximately 75% of a predetermined VO_2 max. The first exhaustive exercise test for all subjects occurred under controlled dietary conditions without vitamin B-6 supplementation. The second exhaustive exercise test similarly occurred under controlled dietary conditions but with the addition of a 20 mg PN (as the hydrochloride) oral supplement. Subject consumed a controlled diet for six days prior to each exhaustive exercise test in both studies and for one day after exercise in

study 1 and two days after exercise in study 2. The changes in vitamin B-6 metabolism and growth hormone concentration were assessed through the collection and analysis of blood, urine and sweat. Blood was analyzed for vitamin B-6, plasma PLP, red blood cell PLP, albumin, alkaline phosphatase and plasma growth hormone. Urine was analyzed for creatinine, urea nitrogen, vitamin B-6 and 4-pyridoxic acid. Sweat was analyzed for vitamin B-6 concentration.

The SAS statistical package was used to analyze the data from both studies. Specific statistical tests included the analysis of variance for unequal sample sizes (ANOVA), Bonferroni t-tests, Wilcoxon rank-sum tests and Spearman correlation coefficients (r). Null hypotheses were rejected at the 0.05 level of significance.

In the NS state, the mean plasma vitamin B-6 (PB-6) and PLP concentration increased during exhaustive exercise, decreased to approximately resting concentrations post-exercise (POST) and continued to decrease below resting concentrations by one hour post-exercise (POST 60). Even though this trend was evident for the PLP and PB-6, no significant differences before, during or after exercise were found. The changes in plasma volume during and after exercise appeared to only partially account for the plasma PLP and PB-6 concentrations. Red blood cell PLP similarly was not significantly affected by exhaustive exercise alone.

In the vitamin B-6 supplemented state, the plasma PLP, RBC PLP and PB-6 concentrations before, during and after exercise were significantly greater than in the non-supplemented state. Exhaustive exercise and vitamin B-6 supplementation resulted in an increase in plasma PLP and PB-6 concentrations during exercise, a decrease to approximately resting concentrations after exercise (POST) and a further decrease below the initial resting values POST 60. In study 1, the mean plasma PLP concentration significantly decreased between the exercise (DX) and one hour post-exercise (POST 60) sample points (36%). In study 2, a similar mean decrease in plasma PLP was found between the DX and POST 60 sample points (23%). However, due to the differences in

sample size at each sample point (n=5 in study 1 DX and n=3 DX in study 2; n=6 POST 60 in study 1 and n=5 POST 60 in study 2) statistical significance was not attained. The mean plasma vitamin B-6 concentration similarly decreased (20%) between the exercise and one hour post-exercise time points, however, the decrease was not significantly significant ($p<0.05$). The mean concentration of RBC PLP decreased from resting concentrations during and after exercise (POST), but significantly increased POST 60 as compared to the POST sample point (n=5).

The mean plasma growth hormone concentration was significantly higher during and immediately after exercise compared to either the mean resting or mean POST 60 hGH concentration. Vitamin B-6 supplementation did not significantly increase plasma hGH concentrations above that resulting from exhaustive exercise alone (NS state). However, the mean plasma hGH concentrations with exercise and vitamin B-6 supplementation were (5%) higher during exercise (DX), (8%) higher after exhaustive exercise (POST) and 20% higher one hour after exercise (POST 60) as compared to the NS state. The mean plasma hGH concentration (NS and S states) DX and POST were significantly greater than either the mean resting or mean POST 60 plasma hGH concentration. In the vitamin B-6 supplemented state, the mean POST 60 plasma hGH concentration was significantly elevated above the mean resting plasma concentration.

The effect of exhaustive exercise and vitamin B-6 supplementation on vitamin B-6 metabolism is difficult to interpret between study 1 and study 2. Exhaustive exercise did not significantly affect the excretion of 4-PA or UB-6 before, during or after the exercise test in either the NS or S states. There was a significant difference in the mean 4-PA excretion between the NS and S states in study 1 and study 2. In the NS state, a significantly greater (29%) mean excretion of 4-PA was found in study 2 as compared to study 1. However, in the vitamin B-6 supplemented state, a significantly greater mean excretion of 4-PA was evident in study 1 as compared to study 2 (11%). The urinary vitamin B-6 data paralleled the trends in the 4-PA excretion.

When expressed as a percent of intake, the mean excretion of 4-PA in study 1 was significantly greater (53% greater) in the S state as compared to the NS state. In study 2, the mean percent of intake excreted as 4-PA not significantly different between the NS and S states, but was 12% greater in the NS state as compared to the S state. While the trends in the mean vitamin B-6 excretion (expressed as a percent of intake) were similar to the percent of intake excreted as 4-PA, the statistical significance of the percent of intake excreted as UB-6 did not parallel the 4-PA data. The mean excretion of UB-6 as a percent of intake was significantly greater (68%) in study 2 (as compared to study 1) during the NS state and was significantly greater (11%) in study 1 (as compared to study 2) during the S state. The percent of intake excreted as UB-6 in study 1 was significantly greater in the S state (28%)(compared to the NS state), whereas the percent of intake excreted as UB-6 in study 2 was significantly greater (45%) in the NS state (as compared to the S state).

There were no significant differences in the vitamin B-6 content of sweat collected throughout the exercise tests in study 1 and study 2 or between the NS and vitamin B-6 supplemented states. Changes in sweat vitamin B-6 content at specific collection points could not be assessed in this study due to the small sample size. There was also no significant difference in the time to exhaustion of the endurance ride with or without vitamin B-6 supplementation.

Other significant findings ($p<0.05$) were found in this study. The subjects in study 2 consumed more energy as compared to the subjects in study 1 and the subjects consumed more carbohydrate in the vitamin B-6 supplemented state in both studies as compared to the NS state. The subjects had a significantly ($p<0.05$) higher levels of daily exercise during the NS period in each study as compared to the vitamin B-6 supplemented period. The subjects exercised a greater amount on a consistent basis in study 2 vs study 1. The subjects in study 2 had a greater amount of fat-free mass than the subjects in study 1.

In conclusion, exhaustive endurance cycling and 20 mg of oral pyridoxine appeared to significantly alter plasma PLP, RBC PLP and PB-6 concentrations in men more than

with exhaustive exercise and no vitamin B-6 supplement. The differences in the excretion of urinary 4-PA and UB-6 between the study years remains a unexplained. In recognition of the small sample size in each study, differences between the study protocols (i.e., dietary vitamin B-6 content and/or dietary protein), individual variations (such as body composition, level of training) or bioavailability may have influenced the response to vitamin B-6 supplementation and exhaustive exercise.

Oral vitamin B-6 supplementation and exhaustive exercise did not appear to significantly alter plasma hGH concentration above that typically resulting from exhaustive exercise. However, the non-significant elevation of the plasma hGH concentration during and after exercise with vitamin B-6 supplementation warrants further examination. The loss of vitamin B-6 through sweat did not appear to be influenced by vitamin B-6 supplementation and is not a major route of vitamin B-6 loss in athletes.

Further research on effects of exercise and vitamin B-6 supplementation on vitamin B-6 metabolism helps to answer the commonly asked question "Do athletes need to consume more vitamins?" There is no indication at this time that vitamin B-6 is needed by athletes in amounts above those normally consumed through diet. While the metabolism of vitamin B-6 may be influenced by exercise, the practical application of this information remains unknown.

BIBLIOGRAPHY

Abrams, R.L., Parker, M.L., Blanco, S., Reichlin, S. and Daughaday, W.H. (1966) Hypothalmic regulation of growth hormone secretion. *Endocrinol* 78: 605-613.

Adamafio, N.A. and Ng, F.M. (1984) Effects of growth hormone on lipogenesis and glucose oxidation in genetically GH-deficient mice. *Mol Cell Endocrinol* 37: 241-244.

Adibi, S.A., Krzysik, B.A., Morse, E.L., Amin, P.N., Allen, E.R. (1974) Oxidative energy metabolism in the skeletal muscle: biochemical and ultrastructural evidence for adaptive changes. *J Lab Clin Med* 83: 548-562.

Adolph, E.F. (1947) Physiology of Man in the Desert. Interscience: New York, pgs. 33-76.

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

Ahlborg, G., Felig, P., Hagenfelt, L., Hendler, R., Wahren, J. (1974) Substrate turnover during prolonged exercise in man. *J Clin Invest* 53: 1080-1090.

Ahren, K., Hjalmarson, A., Isaksson, O. (1970) Failure of growth hormone to exert an acute inhibitory effect on glucose uptake in the rat diaphragm. *Acta Physiol Scand* 78: 574-576.

Allgood, V.E. and Cidlowski, J.A. (1991) Novel role for vitamin B-6 in steroid hormone action: a link between nutrition and the endocrine system. *J Nutr Biochem* 2: 523-534.

Altszuler, N. (1974) Actions of growth hormone and carbohydrate metabolism In: Knobil, E., Sawyer, W.H. (eds.) Handbook of Physiology, American Physiological Society: Washington D.C., pgs. 233-252.

Altszuler, N. Dunn, A., Steele, R., Bishop, S., deBodo, R.C. (1961) Influence of cortisone on the effects of growth hormone on carbohydrate metabolism. *Fed Proc* 20:188 (abstract).

Alvarez, L.C., Dimas, C.A., Castro, A., Rossman, L.G., Vanderlaan, E.F., Vanderlaan, W.P. (1972) Growth hormone in malnutrition. *J Clin Endocrinol* 34: 400-409.

American College of Sports Medicine. (1986) Guidelines for exercise testing and prescription. 3rd Ed., Lea and Febiger: Philadelphia, PA, pgs. 18-21.

American College of Sports Medicine Position Stand: The prevention of thermal injuries during distance running. (1985) National Center: Indianapolis, IN.

Anderson, B.B. (1980) Red Cell Metabolism of Vitamin B-6. In: Tryfiates, G.P., ed., Vitamin B-6 Metabolism and Role in Growth, Food & Nutr Press Inc.: Westport, CT., pgs. 53-83.

Anderson, B.B., Fulford-Jones, C.E., Child, J.A., Beard, M.E., Bateman, C.J.T. (1971) Conversion of Vitamin B-6 compounds to active forms in the red blood cell. *J Clin Invest* 50: 1901-1909.

Anderson, B.B., Newmark, P.A., Rawlins, M., Green, R. (1974) Plasma binding of vitamin B-6 compounds. *Nature* 250: 502-504.

Anderson, B.B., Perry, G.M., Clements, J.E., Greany, M.F. (1989) Rapid uptake and clearance of pyridoxine by red blood cells in vivo. *Am J Clin Nutr* 50: 1059-1063.

Angel, J.F. (1975) Lipogenesis by hepatic and adipose tissues from meal-fed pyridoxine-deprived rats. *Nutr Rept Int* 11: 369-378.

Angel, J.F.and Mellor, R.M. (1974) Glycogenesis and gluconeogenesis in meal-fed pyridoxine-deprived rats. *Nutr Rep Int* 9: 97-107.

Angel, J.F.and Song, G-W. (1973) Lipogenesis in pyridoxine-deficient nibbling and meal-fed rats. *Nutr Rept Int* 8: 393-403.

Armstrong, L.E., Hubbard, R.W., Jones, B.H., Daniels, J.T. (1986) Preparing Alberto Salazar for the heat of the 1984 Olympic marathon. *Phys Sportsmed* 14: 73-81.

Askew, E.W., Klain, G.J., Lowder, J.F., Jr., Wise, W.R., Jr. (1979) Influence of exercise on amino acid mobilization and oxidation. *Med Sci Sports* 11: 106 (abstract).

Astrand, P.-O. and Christensen, E.H. (1964) Aerobic work capacity In: F.Dickens, E. Neil, and W.F. Widdas (eds.), Oxygen in the Animal Organism. Pergamon Press, New York, pg. 295.

Astrand, P.-O. and Rodahl, K.(eds.) (1986) Textbook of Work Physiology. McGraw-Hill Pub. Co.:New York, pgs. 523-566.

Astrand, P.-O. and Saltin, B. (1961) Maximal oxygen uptake and heart rate in various types of muscular activity. *J Appl Physiol* 16: 977-981.

Astrand, P.-O. and Saltin, B. (1964) Plasma and red cell volume after prolonged severe exercise. *J Appl Physiol* 19: 829-832.

Atkin, L., Schultz, A.S., Williams, W.L., Frey, C.N. (1943) Yeast micorbiological methods for determination of vitamins: Pyridoxine. *Ind Eng Chem* 15: 141-144.

Audet, A. and Lupien, P.J. (1974) Triglyceride metabolism in pyridoxine-deficient rats. *J Nutr* 104: 91-100.

Augustin, J., Marousek, I., Tholen, L.A., Artz, W.E., Swanson, B.G. (1981) Retention of some water-soluble vitamins during home preparation of commercially frozen potato products. *J Food Sci* 46: 1697-1700.

Augustin, J., Marousek, I., Tholen, L.A., Bertelli, B. (1980) Vitamin retention in cooked, chilled and reheated potatoes. *J Food Sci* 45: 814-816.

Bak, J.F., Moller, N., Schmitz, O. (1991) Effects of growth hormone on fuel utilization and muscle glycogen synthase activity in normal humans. *Am J Physiol* 260: E736-E742.

Barnard, H.C., deKock, J.J., Vermaak, W.J.H., Potgieter, G.M. (1987) A new perspective in the assessment of vitamin B-6 nutritional status during pregnancy in humans. *J Nutr* 117: 1303-1306.

Batchelor, B.R., Penner, J., Hirsch, J., Stein, J.S. (1976) Effects of hypophysectomy and acute growth hormone treatment upon glucose metabolism in adipose tissues and isolated adipocytes of rats. *Horm Metab Res* 8: 24-32.

Bates, R.W., Laanes, T., Riddle, O. (1935) *Proc Soc Exp Biol and Med* 33: 446.

Bauernfeind, J.C. and Miller, O.N. (1978) Vitamin B-6: nutritional and pharmaceutical usage, stability, bioavailability, antagonist, and safety In: Human Vitamin B-6 Requirements. National Acad of Science: Washington DC, pgs.78-110.

Baulieu, E.E. and Kelly, P.A.(eds.) (1990) Growth Hormone and Prolactin. In: Hormones: From Molecules to Disease. Hermann Publishers & Chapman & Hall: New York, pgs. 191-207.

Baumann, G. and Shaw, M.A. (1990) A second, lower affinity growth hormone-binding protein in human plasma. *J Clin Endocrinol Metab* 70: 680-686.

Baumann, G., Shaw, M.A., Buchanan, T.A. (1988) In vivo kinetics of a covalent growth hormone-binding protein complex. *Metabolism* 38: 330-333.

Baxter, R.C., Bryson, J.M., Turtle, J.R. (1980) Somatogenic receptors of rat liver: regulation by insulin. *Endocrinol* 107: 1176-1181.

Beatty, C.H., Curtis, S., Young, M.K., Bocek, R.M. (1974) Oxidation of amino acids by red and white muscle fiber groups. *Am J Physiol* 277: 268-272.

Beck, J.C., McGarry, E.E., Dyrenfurth, I., Venning, E.H. (1957) Metabolic effects of human and monkey growth hormone in man. *Science* 125: 884.

Belezikjian, L.M. and Vale, W.W. (1983) Stimulation of adenosine 3',5'-monophosphate production by growth hormone-releasing factor and its inhibition by somatostatin in anterior pituitary cells in vitro. *Endocrinol* 113: 1726-1731.

Benesch, R., Benesch, R.E., Edalji, R., Suzuki, T. (1977) 5'-Deoxypyridoxal as a potential anti-sickling agent. *Proc Natl Acad Sci USA* 74: 1721-1723.

Benesch, R.E., Benesch, R., Renthal, R.D., Maeda, N. (1972) Affinity labeling of the polyphosphate binding site of hemoglobin. *Biochem* 11: 3576-3582.

Benesch, R.E., Benesch, R., Yu, C.I. (1969) The effect of pyridoxal phosphate on the oxygenation of hemoglobin. *Fed Proc* 28: 604 (abstract).

Benesch, R., Young, S., Suzuki, T., Bauer, C., Benesch, R. (1973) Pyridoxal compounds as specific reagents for the α and β N-termini of hemoglobin. *Proc Nat Acad Sci USA* 70: 2595-2599.

Bergstrom, J.R., Harris, R.C., Hultman, E., Nordensjo, L.O. (1971) Energy rich phosphagens in dynamic and static work. In: B.Pernon and B. Saltin (eds.). Muscle metabolism during exercise, Plenum Pub. Co.: New York, pg. 341.

Bergstrom, J., Hermansen, L., Hultman, E., Saltin, B. (1967) Diet, muscle glycogen and physical performance. *Acta Physiol Scand* 71: 140-150.

Bernhart, F.W., D'Amato, E., Tomarelli, R.M. (1960) The vitamin B-6 activity of heat-sterilized milk. *Arch Biochem Biophys* 88: 267-269.

Bills, N.D. (1991) In vivo and in vitro determination of the bioavailability of vitamin B-6 from plant foods containing pyridoxine glucoside. Ph.D. Thesis, Dept. Nutrition and Food Management, Oregon State University, Corvallis, OR.

Biselli, R., Farrace, S., D'Amelio, R., Fattorossi, A. (1993) Influence of stress on lymphocyte subset distribution- A flow cytometric study in young student pilots. *Aviation, Space and Envir Med* 64: 116-120.

Bjorntrop, P. (1981) Effect of exercise and training on fat metabolism in normal and obese man. *Med Sport* 13: 63-65.

Black, A.L., Guirard, B.M., Snell, E.E. (1977) Increased muscle phosphorylase in rats fed high levels of vitamin B-6. *J Nutr* 107: 1962-1968.

Black, A.L., Guirard, B.M., Snell, E.E. (1978) The behavior of muscle phosphorylase as a reservoir for vitamin B-6 in the rat. *J Nutr* 108: 670-677.

Blackard, W.G. and Hull, E.W. (1971) Effect of lipids on growth hormone secretion in humans. *J Clin Invest* 50: 1439-1443.

Blundell, T.L., Bedarkar, S., Rinderknecht, E., Humbel, R.E. (1978) Insulin-like growth factors: A model for tertiary structure accounting for immunoreactivity and receptor binding. *Proc Natl Acad Sci USA* 75: 180-184.

Boisvert, P., Brisson, G.R., Peronnet, P. (1993) Effect of plasma prolactin on sweat rate and sweat composition during exercise in men. *Am J Physiol* 264: F816-F820.

Bolodia, G. and Young, F.G. (1967) Growth hormone and carbohydrate metabolism in vitro. *Nature* 215: 960-961.

Booth, C.C. and Brain, M.C. (1962) The absorption of tritium-labeled pyridoxine hydrochloride in the rat. *J Physiol* 164: 282-294.

Borisov, I.M. (1977) Pyridoxine allowances of students in a physical culture institute. *Voprosy Pitaniya* 36: 48-52.

Bosron, W.F., Veitch, R.L., Lumeng, L., Li, T-K. (1978) Subcellular localization and identification of pyridoxal 5'-phosphate-binding proteins in rat liver. *J Biol Chem* 253: 1488-1492.

Bottomley, S.S. (1983) Iron and vitamin B-6 metabolism in the sideroblastic anemias. In: Nutrition in Hematology. Lindenbaum (ed.), Churchill Livingston: New York, pgs. 203-223.

Bouchard, C. and Malina, R.M. (1983) Genetics of Physical Fitness and Motor Performance. *Ex and Sport Sci Rev* 11: 306-339.

Bougneres, P.F., Artavia-Loria, E., Ferre, P., Chaussain, J.L., Job, J.C. (1985) Effects of hypopituitarism and growth hormone replacement therapy on the production and utilization of glucose in childhood. *J Clin Endocrinol Metab* 61: 1152-1157.

Brandenberger, G., Candas, V., Follenius, M., Libert, J.P., Kahn, J.M. (1986) Vascular fluid shifts and endocrine responses to exercise in the heat. *Eur J Appl Physiol* 55: 123-129.

Bratusch-Marrain, P.R., Smith, D., DeFronzo, R.A. (1982) The effect of growth hormone on glucose metabolism and insulin secretion in man. *J Clin Endocrinol* Metab 55: 973-982.

Braunstein, A.E. and Shemyakin, M.M. (1953) A theory of amino acid metabolic processes catalyzed by pyridoxal-dependent enzymes. *Biokhimiya* 18: 393-411.

Brisson, G.R., Boisvert, P., Peronnet, F., Perrault, H., Boisvert, D., Lafond, J.S. (1991) A simple and disposable sweat collector. *Eur J Appl Physiol* 63: 269-272.

Brooks, G.A. (1980) Muscle, blood and liver alanine and leucine levels in rats during rest and two intensities of treadmill running. *Med Sci Sports Exercise* 12: 101 (abstract).

Brooks, G.A. (1985) Lactate: glycolytic end product and oxidative substrate during exercise in mammals- the lactate shuttle. In: Comparative Physiol and Biochem-Current Topics and Trends, Vol. A. Respiration-Metabolism-Circulation R.Gilles (ed.), Springer Verlag, pgs. 208-218.

Brooks, G.A. (1986) The lactate shuttle during exercise and recovery. *Med Sci Sports Exerc* 18: 360-368.

Brooks, G.A. (1987) Lactate production during exercise: oxidative substrate versus fatigue agent. In: Exercise-Benefits, Limits and Adaptations, C. Macleod, R. Maughn, M. Nimo, T. Reilly, and C. Williams (eds.), E. & R.N. Spon., Ltd.: London, pgs. 144-158.

Brooks, G.A., Brauner, K.E., Cassens, R.G. (1973) Glycogen synthesis and metabolism of lactic acid after exercise. *Am J Physiol* 224: 1162-1166.

Brooks, G.A. and Fahey, T.D. (1986) Fundamentals of Human Performance. Macmillan Publishing Co.: New York, pgs. 34-55.

Brooks, G.A. and Gaesser, G.A. (1980) End points of lactate and glucose metabolism after exhausting exercise. *J Appl Physiol* 49: 1057-1069.

Brotherhood, J.R. (1984) Nutrition and sports performance. *Sports Med* 1: 350-389.

Brown, R.R. (1985) The tryptophan load test as an index of vitamin B-6 nutrition. In: Methods in vitamin B-6 nutrition. Leklem, J.E. and Reynolds, R.D. (eds.), Plenum Press: New York, pgs. 321-340.

Brown, R.R., Rose, D.P., Leklem, J.E., Linkswiler, H. and Anand, R. (1975) Urinary 4-pyridoxic acid, plasma pyridoxal phosphate, and erythrocyte aminotransferase levels in oral contraceptive users receiving controlled intakes of vitamin B-6. *Am J Clin Nutr* 28: 10-19.

Brusilow, S.W. (1965) An anaerobic sweat collection technique. *J Lab Clin Med* 65: 513-517.

Buckler, J.M. (1973) The relationship between changes in plasma growth hormone levels and body temperature occurring with exercise in man. *Biomedicine* 19: 193-197.

Bunt, J.C., Boileau, R.A., Bahr, J.M., Nelson, R.A. (1986) Sex and training differences in human growth hormone levels during prolonged exercise. *J Appl Physiol* 61: 1796-1801.

Buse, M.G., Jursinic, S., Reid, S.S. (1975) Regulation of branched chain amino acid oxidation in isolated muscle, nerves and aortas of rat. *Biochem J* 148: 363-374.

Buskirk, E.R., Iampietro, P.F., Bass, D.E. (1958) Work performance after dehydration: effect of physical conditioning and heat acclimation. *J Appl Physiol* 12: 189-194.

Buss, D.B., Hamm, M.W., Mehansho, H., Henderson, L.M. (1980) Transport and metabolism of pyridoxine in the perfused small intestine and the hind limb of the rat. *J Nutr* 110: 1655-1663.

Butler, P., Kryshak, E., Rizza, R. (1991) Mechanism of growth hormone-induced postprandial carbohydrate intolerance in humans. *Am J Physiol* 260: E513-E520.

Cahill, G.R. and Owen, O.E. (1965) In: Carbohydrate Metabolism and Its Disorders. F. Dickens, Randle, P.J., Whelan, W.J. (eds.), vol. 1, Academic Press: New York, pgs. 497-522.

Caldwell, J.E., Ahonen, E., Nousiainen, U. (1984) Differential effects of sauna diuretic-, and exercise-induced hypohydration. *J Appl Physiol* 57: 1018-1023.

Cameron, D.P., Burger, H.G., Catt, K.J., Doig, A. (1969) Metabolic clearance rate of radioiodinated human growth hormone in man. *J Clin Invest* 48: 1600-1608.

Campbell, J., Pierluissi, J., Green, G.R. (1978) Somatotrophic diabetes: insulin release responses to arginine and glucagon in dogs. *Diabetologia* 15: 205-212.

Campbell, J. and Rastogi, K.S. (1969) Actions of growth hormone: enhancement of insulin utilization with inhibition of insulin effect on blood glucose in dogs. *Metabolism* 18: 930-944.

Campistron, G. (1980) Approche pharmacologique de l'arginine et de l'acide aspartique Etude Pharmacocinetique et Pharmacologique. These No. 112. Faculte des Sciences Pharmaceutiqule, Toulouse.

Campuzano, G. (1988) Effect of Vitamin B-6 supplementation before strenuous exercise on restoration of plasma urea and ammonia levels. M.S. Thesis, Dept. Foods and Nutr., Oregon State University.

Canham, J.E., Baker, E.M., Harding, R.S., Sauberlich, H.E., Plough, I.C. (1969) Dietary protein-its relationship to vitamin B-6 requirements and function. *Ann N.Y. Acad Sci* 166: 16-29.

Casanueva, F.F., Villanueva, L., Cabranes, J.A., Cabezas-Cerrato, J., Fernandes-Cruz, A. (1984) Cholinergic mediation of growth hormone secretion elicited by arginine, clonidine and physical exercise in man. *J Clin Endocrinol Metab* 59: 526-530.

Casanueva, F.F., Villanueva, L., Penalva, A., Villa, T., Cabezas-Cerrato, J. (1981) Free fatty acid inhibition of exercise-induced growth hormone secretion. *Hormone Metab Res* 13: 348-350.

Catalioto, R.M., Ailhaud, G., Negrel, R. (1990) Diacylglycerol production induced by growth hormone in OB 1771 preadipocytes arise from phosphatidylcholine breakdown. *Biochem Biophys Res Commun* 173: 840-848.

Cerny, F.J. (1975) Protein metabolism during two hour ergometer exercise. In: Metabolic Adaptations to Prolonged Physical Exercise. H. Howald and J.R. Poortmans (eds.) Birkauser Verlag:Basal, pgs. 232-237.

Chabner, B. and Livingston, D. (1970) A simple enzymic assay for pyridoxal phosphate. *Anal Biochem* 34: 413-425.

Chandra, R.K. and Puri, S. (1985) Vitamin B-6 modulation of immune responses and infection. In: Reynolds, R.D., Leklem, J.E.(eds.) Vitamin B-6: Its Role in Health and Disease. A.R. Liss: New York, pgs. 163-175.

Chang, S.J., Kirksey, A., Morre, D.M. (1981) Effects of vitamin B-6 deficiency on morphological changes in dendritic trees of purkinje cells in developing cerebellum of rats. *J Nutr* 111: 848-857.

Chanutin, A. and Curnish, R.R. (1967) Effect of organic and inorganic phosphates on the oxygen equilibrium of human erythrocytes. *Arch Biochem Biophys* 121: 96-102.

Cheng, J.S. and Kalant, N. (1970) Effects of insulin and growth hormone on the flux rates of plasma glucose and plasma free fatty acids in man. *J Clin Endocrinol* 31: 647-653.

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

Cho, Y.-O. and Leklem, J.E. (1990) In vivo evidence for a vitamin B-6 requirement in carnitine synthesis. *J Nutr* 120: 258-265.

Christ, D.M., Peake, G.T., Egan, P.A., Waters, D.L. (1988) Body composition response to exogenous growth hormone during training in highly conditioned adults. *J Appl Physiol* 65: 579-584.

Christensen, E.H. and Hanson, O. (1939) Arbeitsfahigkeit und Ermundung. *Skand Arch Physiol* 81: 160-171.

Christensen, S.E., Jorgenson, O.L., Moller, N., Orskov, H. (1984) Characterization of growth hormone release in response to external heating: comparison to exercise induced release. *Acta Endocrinol* 107: 295-301.

Clemmons, D.R., Snyder, D.K., Williams, R., Underwood, L.E. (1987) Growth hormone administration conserves lean body mass during dietary restriction in obese subjects. *J Clin Endocrinol Metab* 64: 878-883.

Coburn, S.P., Lewis, D.L., Fink, W.J., Mahuren, J.D., Schaltenbrand, W.E., Costill, D.L. (1988) Estimation of human vitamin B-6 pools through muscle biopsies. *Am J Clin Nutr* 48: 291-294.

Coburn, S.P. and Mahuren, J.D. (1983) A versatile cation-exchange procedure for measuring the seven major forms of vitamin B-6 in biological samples. *Anal Biochem* 129: 310-317.

Coburn, S.P., Mahuren, J.D., Erbelding, W.F., Townsend, D.W., Hachey, D.L., Klein, P.D. (1984) Measurement of vitamin B-6 kinetics in vivo using chronic administration of labelled pyridoxine. In: Chemical & Biological Aspects of Vitamin B-6 Catalysis. Evangelopoulos (ed.), Part A, New York, pgs.43-54.

Coburn, S.P., Mahuren, J.D., Kennedy, M.S., Schaltenbrand, W.E., Townsend, D.W. (1992) Metabolism of [^{14}C]- and [^{32}P] pyridoxal 5'-phosphate and [^{3}H] pyridoxal administered intravenously to pigs and goats. *J Nutr* 122: 393-401.

Coburn, S.P., Mahuren, J.D., Schaltenbrand, W.E., Sampson, D.A., O'Connor, D.K., Synder, D.L., Wostmann, B.S. (1989a) B-6 vitamer content of rat tissues measured by isotope tracer and chromatographic methods. *Biofactors* 4: 307-312.

Coburn, S.P., Mahuren, J.D., Szadkowska, Z., Schaltenbrand, W.E., Townsend, D.W. (1985) Kinetics of vitamin B-6 metabolism examined in miniature swine by continuous administration of labelled pyridoxine. In: Proceedings of the 1985 Conference on Mathematical Models in Experimental Nutrition. N.L. Canolty and T.P. Cain (eds.), University of Georgia, Athens, GA., 99-111.

Coburn, S.P., Mahuren, J.D., Wostmann, B.S., Snyder, D.L., Townsend, D.W. (1989b) Role of intestinal microflora in the metabolism of vitamin B-6 and 4'-deoxypyridoxine examined using germ-free guinea pigs and rats. J Nutr 119: 181-188.

Coburn, S.P. and Townsend, D.W. (1989) Modelling vitamin B-6 metabolism in rodents. *In Vivo* 3: 215-224.

Coburn, S.P. and Whyte, M.P. (1988) Role of phosphatases in the regulation of vitamin B-6 metabolism in hypophosphatasia and other disorders. In: Clinical and Physiological Applications of Vitamin B-6. New York: Liss Inc., 65-93.

Coburn, S.P., Ziegler, P.J., Costill, D.L., Mahuren, J.D., Fink, W.J., Schaltenbrand, W.E., Pauly, T.A., Pearson, D.R., Conn, P.S., Guilarte, T.R. (1991) Response of vitamin B-6 content of muscle to changes in vitamin B-6 intake in men. *Am J Clin Nutr* 53: 1436-1442.

Coiro, V., Grichting, G., Goodman, M. (1981) Induction of insulin-like responses to growth hormone by stress. *Endocrinol* 109: 2213-2219.

Cole, D.E. and Boucher, M.J. (1986) Use of a new sample-collection device (macroduct) in anion analysis of human sweat. *Clin Chem* 32: 1375-1378.

Connett, R.J., Gaueski, T.E.J., Honig, G.R. (1984) Lactate accumulation in fully aerobic, working dog gracilis muscle. *Am J Physiol* 246: H120-H128.

Convertino, V.A., Keil, L.C., Bernauer, E.M., Greenleaf, J.E. (1981) Plasma volume, osmolality, vasopressin, and renin activity during graded exercise in man. *J Appl Physiol* 50: 123-128.

Convertino, V.A., Keil, L.C., Greenleaf, J.E. (1983) Plasma volume, renin and vasopressin responses to graded exercise after training. *J Appl Physiol* 54: 508-514.

Copeland, K.C., Underwood, L.E., VanWyk, J.J. (1980) Induction of immunoreactive somatomedin C in human serum by growth hormone: dose-response relationships and effect on chromatographic profiles. *J Clin Endocrinol Metab* 50: 690-697.

Cori, C.F. and Illingsworth, B. (1957) The prosthetic group of phosphorylase. *Proc Natl Acad Sci* 43: 547-552.

Corpas, E., Harman, S.M., Blackman, M.R. (1993) Human growth hormone and human aging. *Endocrinol Reviews* 14: 20-39.

Costill, D.L. (1970) Metabolic response during distance running. *J Appl Physiol* 28: 251-255.

Costill, D.L., Branam, L., Eddy, D., Fink, W. (1974) Alterations in red cell volume following exercise and dehydration. *J Appl Physiol* 37: 912-916.

Costill, D.L., Cote, R., Fink, W. (1976) Muscle water and electrolytes following varied levels of dehydration in man. *J Appl Physiol* 40: 6-11.

Costill, D.L., Dalsky, G.P., Fink, W.J. (1978) Effects of caffeine ingestion on metabolism and exercise performance. *Med Sci Sports* 10: 155-158.

Costill, D.L and Fink, W.J. (1974) Plasma volume changes following exercise and thermal dehydration. *J Appl Physiol* 37: 521-525.

Costill, D.L. and Miller, J.M. (1980) Nutrition and endurance sports: carbohydrate and fluid balance. *Internat J Sports Med* 1: 2-14.

Costill, D.L., Sherman, W.M., Fink, W.J., Maresh, C., Witten, M., Miller, J.M. (1981) The role of dietary carbohydrate in muscle glycogen resynthesis after strenuous running. *Am J Clin Nutr* 34: 1831-1836.

Costill, D.L. and Sparks, K. (1973) Rapid fluid replacement following thermal dehydration. *J App Physiol* 34: 299-303.

Costin, G., Kogut, M.D., Frasier, S.D. (1972) Effect of low-dose human growth hormone on carbohydrate metabolism in children with hypopituitarism. *J Pediatr* 80: 796-803.

Coursin, D.B. (1954) Convulsive seizures in infants with pyridoxine deficient diets. *JAMA* 154: 406-408.

Coursin, D.B. (1969) Vitamin B-6 and brain function in animals and man. *Ann N.Y. Acad Sci* 166: 7-15.

Coyle, E.F., Coggan, A.R., Hemmert, M.K., Ivy, J.L. (1986) Muscle glycogen utilization during prolonged strenuous exercise when fed carbohydrate. *J Appl Physiol* 61: 165-172.

Coyle, E.F., Coggan, A.R., Hemmert, M.K., Lowe, R.C., Walters, T.J. (1985) Substrate usage during prolonged exercise following a pre-exercise meal. *J Appl Physiol* 59: 429-433.

Cronin, M.J., Rogol, A.D., MacLeod, R.M., et al. (1983) Biological activity of a growth hormone-releasing factor secreted by a human tumor. *Am J Physiol* 244: E346-E353.

Crozier, P.G., Cordain, L., Sampson, D.A. (1994) Exercise-induced changes in plasma vitamin B-6 concentrations do not vary with exercise intensity. *Am J Clin Nutr* 60: 552-558.

Cryer, P.E., Coran, A.G., Keenan, B.S., Sode, J. (1972) Cessation of growth hormone secretion associated with acute elevation of the serum-free fatty acid concentration. *Metabolism* 21: 867-873.

Cryer, P.E. and Sode, J. (1970) Variation in urinary creatinine excretion and its relationship to measurement of urinary 17-hydroxy-corticosteroids. *Clin Chem* 16: 1012-15.

Cuneo, R.C., Salomon, F., Wiles, C.M., Hesp, R., Sonksen, P.H. (1991a) Growth hormone treatment in growth hormone-deficient adults I. Effects on muscle mass & strength. *J Appl Physiol* 70: 688-694.

Cuneo, R.C., Salomon, F., Wiles, C.M., Hesp, R., Sonksen, P.H. (1991b) Growth hormone treatment in growth hormone-deficient adults II. Effects on exercise performance. *J Appl Physiol* 70: 695-700.

Cunnane, S.C., Manku, M.S., Horrobin, D.F. (1984) Accumulation of linoleic and γ-linolenic acids in tissue lipids of pyridoxine-deficient rats. *J Nutr* 114: 1754-1761.

Dakshinamurti, K. (1982) Neurobiology of pyridoxine. In: Draper, H.H.(eds.) Advances in Nutritional Research. vol. 4. Plenum Press: New York, pgs. 143-179.

Dallman, P.R. (1984) Diagnosis of anemia and iron deficency- analytic and biological variations of laboratory tests. *Am J Clin Nutr* 39: 937-941.

Daugheday, W.H. (1985) The anterior pituitary. In: Williams Textbook of Endocrinology Wilson & Foster (eds.) W.B. Saunders: Philadelphia, pgs. 577-611.

Davis, J.A., Vodak, P., Wilmore, J.H., Vodak, J., Kurtz, P. (1976) Anaerobic threshold and maximal aerobic power for three modes of exercise. *J Appl Physiol* 41: 544-550.

DeBodo, R.C. and Altszuler, N. (1958) Insulin hypersensitivity and physiological insulin antagonists. *Physiol Rev* 38: 389-445.

Delitala, G., Masala, A., Alagna, S., Devilla, L. (1976) Effect of pyridoxine on human hypophyseal trophic hormone release: A possible stimulation of hypothalamic dopaminergic pathway. *J Clin Endocrinol Metab* 42: 603-606.

Delitala, G., Meloni, T., Masala, A., Alagna, S., Devilla, L., Costa, R. (1978) Action of somatostatin, levodopa and pyridoxine on growth hormone (GH) secretion in newborn infants. *Biomed* 29: 13-15.

Delmore, C.B. and Lupien, P.J. (1976) The effect of vitamin B-6 deficiency on the fatty acid composition of the major phospholipids in the rat. *J Nutr* 106: 169-180.

Dempsey, W.B. and Christensen, H.N. (1962) The specific binding of pyridoxal 5'-phosphate to bovine plasma albumin. *J Biol Chem* 237: 1113-1120.

DeRitter, E. (1976) Stability characteristics of vitamins in processed foods. *Food Tech* (Jan): 48-54.

Desikachar, H.S.R. and McHenry, E.W. (1954) Some effects of vitamin B6 deficiency on fat metabolism in the rat. *Biochem J* 56: 544-547.

DeVos, A., Leklem, J.E., Campbell, D.E. (1982) Carbohydrate loading, vitamin B-6 supplementation and fuel metabolism during exercise in man. *Med Sci Sports Exer* 14: 137 (abstract).

Dill, D.B. and Costill, D.L. (1974) Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. *J Appl Physiol* 37: 247-248.

DiSorbo, D.M., Phelps, D.S., Ohl, V.S., Litwack, G. (1980) Pyridoxine deficiency influences the behavior of the glucocorticoid receptor complex. *J Biol Chem* 255: 3866-3870.

Dodd, S.L., Herb, R.A., Powers, S.K. (1993) Caffeine and exercise performance: An update. *Sports Med* 15: 14-23.

Doglio, A., Dani, C., Grimaldi, P., Ailhaud, G. (1989) Growth hormone stimulates c-fos gene expression via protein kinase C without increasing inositol lipid turnover. *Proc Natl Acad Sci USA* 86: 1148-1152.

Dohm, G.L., Brown, W.E., Barakat, H.A. (1976) Leucine oxidation in rat muscle, heart and liver homogenates. *Biochem Med* 15: 306-310.

Dohm, G.L., Hecker, A.L., Brown, W.E., et al. (1977) Adaptation of protein metabolism to endurance training. *Biochem J* 164: 705-708.

Dohm, G.L., Kasperek, G.J., Tapscott, E.B., Beecher, G.R. (1980) Effect of exercise on synthesis and degradation of muscle protein. *Biochem J* 188: 255-262.

Donovan, C.M. and Brooks, G.A. (1983) Training affects lactate clearance, not lactate production. *Am J Physiol* 244: E83-E92.

Doumas, B.T. and Biggs, H.G. (1972) Determination of serum albumin. In: Standard Methods of Clinical Chemistry. vol. 7, Cooper, G.R. (Ed.), Academic Press: New York, pg. 175.

Dreon, D.M. and Butterfield, G.E. (1986) Vitamin B-6 utilization in active and inactive young men. *Am J Clin Nutr* 43: 816-824.

Drinkwater, B.L., Horvath, S.M., Wells, C.L. (1975) Aerobic power of females, ages 10-68. *J Gerontol* 30: 385-394.

Dudl, R.J., Ensinck, J.W., Palmer, H.E., Williams, R.H. (1973) Effect of age on growth hormone secretion in man. *J Clin Endo Metab* 37: 11-16.

Dunbar, J.C., Brown, P., Dixon, S. (1985) Increased glucagon receptors in chronically hypersomatotrophic and hyperglucagonemic rats. *Proc Soc Exp Biol Med* 179: 32-37.

Edwards, R.H.T. (1981) Human muscle function and fatigue. In: Human Muscle Function and Fatigue. Porter, R. & Whelan J (eds.), CIBA Foundation Symposium 82, Pitman Medical: London, pgs. 1-18.

Efremor, V.V. and Zaburkin, E.M. (1972) Metabolism of pyridoxine and nicotinic acid during physical stress of prolonged swimming in experimental animals. *Voprosy Pitaniya* 31: 39-43.

Eichner, E.R. (1986) The anemias of athletes. *Phys Sports Med* 14: 122-130.

Ekblom, B., Greenleaf, C.J., Greenleaf, J.E., Hermansen, L. (1970) Temperature regulation during exercise dehydration in man. *Acta Physiol Scand* 79: 475-583.

Enoka, R.M. and Stuart, D.G. (1985) The contribution of neuroscience to exercise studies. *Fed Proc* 44: 2279-2285.

Erikssen, J. and Rodahl, K. (1979) Seasonal variation in work performance and heart rate response to exercise. *Eur J Appl Physiol* 42: 133-140.

Falholt, K., Lund, B., Falholt, W. (1973) An easy colorimetric micromethod for routine determination of free fatty acids in plasma. *Clin Chim Acta* 46: 105-111.

Fedde, K.N. and Whyte, M.P. (1990) Alkaline phosphatase (tissue-nonspecific isoenzyme) is a phosphoethanolamine and pyridoxal-5'-phosphate ectophosphatase: normal and hypophosphatasia fibrablast study. *Am J Hum Genet* 47: 767-775.

Felig, P., Marliss, E.B., Cahill, G.F. (1971) Metabolic response to human growth hormone during prolonged starvation. *J Clin Invest* 50: 411-421.

Felig, P. and Wahren, J. (1975) Fuel homeostasis in exercise. *N Eng J Med* 293: 1078-1084.

Felig, P., Wahren, J., Raf, L. (1973) Evidence of inter-organ amino acid transport by blood cells in humans. *Proc Nat Acad Sci USA* 70: 1775-1779.

Felsing, N.E., Brasel, J.A., Cooper, D.M. (1992) Effect of low and high intensity exercise on circulating growth hormone in men. *J Clin Endocrinol Metab* 75: 157-162.

Fincnam, J.E., Faber, M., Weight, M.J., Labadarious, D., Taljaard, J.J.F., Steytler, J.G., Jacobs, P., Kritchevsky, D. (1987) Diets realistic for westernized people significantly effect lipoproteins, calcium, zinc, vitamin C, vitamin E, vitamin B-6 and hematology in vervet monkeys. *Atherosclerosis* 66: 191-203.

Fineberg, S.E. and Merimee, T.J. (1974) Acute metabolic effects of human growth hormone. *Diabetes* 23: 499-504.

Finidori, J., Postel-Viney, M.C., Kleinknecht, C. (1980) Lactogenic and somatotrophic binding sites in liver membranes of rats with renal insufficiency. *Endocrinol* 106: 1960-1965.

Finkelstein, J.W., Roffwarg, H.P., Boyar, R.M., Kream, J., Hellman, L. (1972) Age-related change in 24 hour spontaneous secretion of growth hormone. *J Clin Endocrinol Metab* 35: 665-670.

Fishman, W. and Ghosh, N. (1967) Isoenzymes of human alkaline phosphatase. *Adv Clin Chem* 10: 255.

Fleg, J.L. and Lakatta, E.G. (1988) Role of muscle loss in the age-associated reduction in VO_2 max. *J Appl Physiol* 65: 1147-1151.

Fonda, M.L. (1987) Partial purification and characterization of vitamin B_6-P phosphatases from human erythrocytes. In: Biochemistry of Vitamin B-6. Proceedings of the 7th International Congress on Chemistry and Biological Aspects of Vitamin B-6 Catalysis. Korpela, T.K. and Christen, P. (eds.), vol. 2: 399-402.

Fonda, M.L. and Harker, C.W. (1982) Metabolism of pyridoxine and protein binding of the metabolites in human erythrocytes. *Am J Clin Nutr* 35: 1391-1399.

Food Processor II computer software program, v. 3.04 plus, (1990) ESHA research, Salem OR.

Foster, D.W. (1984) From glycogen to ketones-and back. *Diabetes* 33: 1188-1199.

Francesconi, R.P., Sawka, M.N., Pandolf, K.B. (1983) Hypohydration and heat acclimation: plasma renin and aldosterone during exercise. *J Appl Physiol* 55: 1790-1794.

Frewin, D.B., Frantz, A.G., Downey, J.A. (1976) T.he effect of ambient temperature on the growth hormone and prolactin response to exercise. *Austr J Exp Biol* 54: 97-101.

Friedman, J.E. and Lemon, P.W.R. (1989) Effect of chronic endurance exercise on retention of dietary protein. *Int J Sports Med* 10: 118-123.

Froesch, E.R., Burgi, H., Ramseier, E. B., Bally, P., Labhart, A. (1963) Antibody-suppressible and nonsuppressible insulin-like activities in human serum and their physiologic significance. An insulin assay with adipose tissue of increased precision and specificity. *J Clin Invest* 42: 1816-1834.

Froesch, E.R., Schmid, C., Schwander, J., Zapf, J. (1985) Actions of insulin-like growth factors. *Ann Rev Physiol* 47: 443-467.

Frohman, L.A., MacGillivray, M.H., Aceto, T. (1967) Acute effects of human growth hormone on insulin secretion and glucose utilization in normal and growth hormone deficient subjects. *J Clin Endocrinol* 27: 561-567.

Fryburg, D.A., Barrett, E.J., Louard, R.J., Gelfand, R.A. (1990) Effect of starvation on human muscle protein metabolism and its response to insulin. *Am J Physiol* 259: E477-E482.

Fryburg, D.A., Gelfand, R.A., Barrett, E.J. (1991) Growth hormone acutely stimulates forearm muscle protein synthesis in normal humans. *Am J Physiol* 260: E499-E504.

Galbo, H. (1981) Endocrinology and metabolism in exercise. *Int J Sports Med* 2: 203-211.

Galbo, H. (1983) Hormonal and Metabolic Adaptations to Exercise. Georg Thieme Verlag: New York, pgs. 1-116.

Galbo, H., Holst, J.J., Christensen, N.J. (1979) The effect of different diets and of insulin on the hormonal response to prolonged exercise. *Acta Physiol Scand* 107: 19-32.

Ganong, W.F. (1979) Review of Medical Physiology. Lange Medical Publishing: Los Altos, CA , pg. 179.

Gause, I., Eden, S., Jansson, J.O., Isaksson, O. (1983) Effects of in vivo administration of antiserum to rat growth hormone on body growth and insulin responsiveness in adipose tissue. *Endocrinol* 112: 1559-1566.

Gelfand, R.A. and Barrett, E.J. (1987) Effect of physiologic hyperinsulinemia on skeletal muscle protein synthesis and breakdown in man. *J Clin Invest* 80: 1-6.

Georgia, J.D. (1974) Non-protein nitrogenous constituents. In: Henry, R.J., Cannon, D.C., Winkleman, J.W. (eds.) Clinical chemistry: principles and technics. 2nd Ed., Harper and Row: New York, pgs. 504-563.

Gibson, Q.H. (1970) Organic phosphates and ligand binding in hemoglobin. *Biochem Biophys Res Commm* 40: 1319-1324.

Gibson, T.E. and Shelley, W.B. (1948) Sexual and racial differences in the response of sweat glands to acetyl-choline and pilocarpine. *J Invest Dermatol* 11: 137-142.

Gold, H., Spector, S., Samaan, N.A., Pearson, O.H. (1968) Effect of growth hormone on carbohydrate metabolism in hypopituitary dwarfs. *Metabolism* 17: 74-83.

Goldberger, J. and Lillie, R.D.(1926) Note on experimental pellegralike condition in albino rat. *Publ Health Reports* pgs.1-5.

Golde, D.W., Bersch, N., Kaplan, S. A., Rimoin, D.L., Li, C.H. (1980) Peripheral unresponsiveness to human growth hormone in Laron dwarfism. *New Eng J Med* 303: 1156-1159.

Gollnick, P.D. (1985) Metabolism of substrates: energy substrate metabolism during exercise and as modified by training. *Fed Proc* 44: 353-357.

Goodman, H.M and Coiro, V. (1981b) Effects of growth hormone on adipose tissue of weanling rats. *Endocrinol* 109: 2046-2053.

Goodman, H.M. (1967) Effects of growth hormone on glucose utilization in diaphram muscle in the absence of increased lipolysis. *Endocrinol* 81: 1099-1103.

Goodman, H.M. and Coiro, V. (1981a) Induction of desensitivity to the insulin-like action of growth hormone in normal rat adipose tissue. *Endocrinol* 108: 113-119.

Gordon, R.S., Thompson, R.H., Thrasher, D., Benson, J. (1976) Genesis of the sweat:plasma urea concentration gradient. *J Invest Dermatol* 66: 218-221.

Grasbeck, R., Nyberg, W., Reizenstein, P. (1958) Biliary and fecal vitamin B_{12} excretion in man. An isotopic study. *Proc Soc Exp Biol Med* 97: 780-784.

Green, R., Jacobsen, D.W., Van Tonder, S.V., Kew, M.C, Metz, J. (1981) Enterohepatic circulation of cobalamin in the nonhuman primate. *Gastroenterology* 81: 773-776.

Greenblatt, D.J., Rausil, B.J., Harmatz, J.S., Smith, T.W., Duhme, D.W., Koch-Weser, J. (1976) Variability of 24-hour urinary creatinine excretion by normal subjects. *J Clin Pharmacol* 16:321-8.

Greengard, O. and Gordon, M. (1963) The cofactor-mediated regulation of apoenzyme levels in animal tissues. *J Biol Chem* 238: 3708-3710.

Greenhaff, P.L. and Clough, P.J. (1989) Predictors of sweat loss in man during prolonged exercise. *Eur J Appl Physiol* 58: 348-352.

Greenhaff, P.L., Gleeson, M., Maughan, R.J. (1988) The effects of diet on muscle pH and metabolism during high intensity exercise. *Eur J Appl Physiol* 57: 531-539.

Greenwood, F.C. (1966) Growth hormone secretion in response to stress in man. *Nature* 210: 540-541.

Gregory J.F. and Litherland, S.A. (1986) Efficacy of the rat bioassay for determination of biologically available vitamin B-6. *J Nutr* 116: 87-97.

Gregory, J.F. (1980) Effects of ε-pyridoxyllysine bound ot dietary protein on the vitamin B-6 status of rats. *J Nutr* 110: 995-1005.

Gregory, J.F. and Ink, S.L. (1987) Identification and quantification of pyridoxine-β-glucoside as a major form of vitamin B-6 in plant-derived foods. *J Agr Food Chem* 35: 76-82.

Gregory, J.F. and Kirk, J.R. (1977) Interaction of pyridoxal and pyridoxal phosphate with peptides in a model food system during thermal processing. *J Food Sci* 42: 1554-1561.

Gregory, J.F. and Kirk, J.R. (1978) Assessment of roasting effects on vitamin B-6 stability and bioavailability in dehydrated food systems. *J Food Sci* 43: 1585-1589.

Gregory, J.F. and Kirk, J.R. (1979) Determination of urinary 4-pyridoxic acid using high performance liquid chromatography. *Am J Clin Nutr* 32: 879-883.

Gregory, J.F. and Kirk, J.R. (1981) The bioavailability of vitamin B-6 in foods. *Nutr Rev* 39: 1-13.

Gregory, J.F., Trumbo, P.R., Bailey, L.B., Toth, J.P., Baumgartner, T.G., Cerda, J.J. (1991) Bioavailability of pyridoxine -5'-b-D-glucoside determined in humans by stable-isotopic methods. *J Nutr* 121: 177-186.

Grichting, G., Levy, L.K., Goodman, H.M. (1983) Relationship between binding and biological effects of human growth hormone in rat adipocytes. *Endocrinol* 113: 1111-1120.

Groziak, S., Kirksey, A., Hamaker, B. (1984) Effect of maternal vitamin B-6 restriction on pyridoxal phosphate concentrations in developing regions of the central nervous system in rats. *J Nutr* 114: 727-732.

Grunt, J.A., Crigler Jr., J.F., Slone, D., Soeldner, J.S. (1967) Changes in serum insulin, blood sugar, and free fatty acid levels four hours after administration of human growth hormone to fasting children with short stature. *Yale J Biol Med* 40: 68-74.

Guillermin, R., Brazeau, P., Bohlen, P., Esch, F., Ling, N., Wehrenberg, W.B. (1982) Growth hormone-releasing factor from a human pancreatic tumor that caused actomegaly. *Science* 218: 585-587.

Gyorgy, P. (1934) Vitamin B-2 and the pellegra-like dermatitis in rats. *Nature* 133: 498-499.

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

Hagen, T.C., Lawrence, A.M., Kirsteins, L. (1972) Autoregulation of growth hormone secretion in normal subjects. *Metabolism* 21: 603-610.

Hagenfeldt, L. (1979) Metabolism of free fatty acids and ketone bodies during exercise in normal and diabetic man. *Diabetes* 28: 66-70.

Hamfelt, A. (1967) Pyridoxal kinase activity in blood cells. *Clin Chim Acta* 16: 7-18.

Hamfelt, A. and Soderhjelm, L. (1988) Vitamin B-6 and aging. In: Leklem, J.E., Reynolds, R.D., eds. Clinical and physiological applications of vitamin B-6. A.R. Liss, New York, 95-107.

Hamm, M.W., Mehansho, H., Henderson, L.M. (1979) Transport and metabolism of pyridoxamine and pyridoxamine phosphate in the small intestine of the rat. *J Nutr* 109: 1552-1559.

Hamm, M.W., Mehansho, H., Henderson, L.M. (1980) Management of pyridoxine and pyridoxal in the isolated kidney in the rat. *J Nutr* 110: 1597-1609.

Hansen, C., Leklem, J., Hardin, K., Miller, L., Chen, W. (1992) Effect of feeding diets of high and low pyridoxine glucoside content on vitamin B-6 status of women. *FASEB J* 6: A1374.

Haralambie, G. and Berg, A. (1976) Serum urea and amino nitrogen changes with exercise duration. *Eur J Appl Physiol* 36: 39-48.

Harding, R.S., Plough, I.S., Friedemann, T.E. (1959) The effect of storage on the vitamin B-6 content of packaged army ration with a note on the human requirement for the vitamin. *J Nutr* 68: 323-331.

Harris, S.A. and Folkers, K. (1939) Synthesis of vitamin B-6. *J Am Chem Soc* 61: 1245-1247.

Harris, J.W., Wittington, R.M., Weisman, R. Jr., Horrigan, D.L. (1956) Pyridoxine responive anemia in the human adult. *Proc Soc Exp Biol Med* 91: 427-432.

Harrison, M.H., Edwards, R.J., Graveney, M.J., Cochran, L.A., Davies, J.A. (1981) Blood volume and plasma protein responses to heat acclimatization in humans. *J Appl Physiol: Respirat Environ Ex Phys* 50: 597-604.

Harrison, M.H., Graveney, M.J., Cochran, A. (1982) Some sources of error in the calculation of relative change in plasma volume. *Eur J Appl Physiol* 50: 13-21.

Hart, I.C., Chadwick, P.M.E., Boon, T.C., Langley, K.E., Rudman, C., Souza, L.M. (1984) A comparison of the growth-promoting, lipolytic, diabetogenic and immunological properties of pituitary and recombinant-DNA-derived bovine growth hormone (somatotropin). *Biochem J* 224: 93-100.

Hartley, L.H. (1975) Growth hormone and catecholamine response to exercise in relation to physical training. *Med Sci Sports* 7: 34-36.

Hartley, L.H., Mason, J.W., Hogan, R.P., Jones, L.G., Kotchen, T.A., Mougey, E.H., Wheery, F.E., Pennington, L.L., Ricketts, P.T. (1972) Multiple hormonal responses to graded exercise in relation to physical training. *J Appl Physiol* 33: 602-606.

Hatcher, L.F. (1983) Influence of controlled strenuous exercise on vitamin B-6 metabolism in man: Effects of carbohydrate depletion-repletion diets and vitamin B-6 supplements. M.S. Thesis, Dept of Foods and Nutrition, Oregon State University.

Hatcher, L.F., Leklem, J.E., Campbell, D.E. (1982) Altered vitamin B-6 metabolism during exercise in man: effect of carbohydrate modified diets and vitamin B-6 supplements. *Med Sci Sports Exerc* 14: 112 (abstract).

Hayek, A. and Peake, G.T. (1981) Growth and somatomedin-C responses to growth hormone in dwarfed children. *J Pediatr* 99: 868-872.

Helmreich, E.J. and Klein, H.W. (1980) The role of pyridoxal phosphate in the catalysis of glycogen phosphorylase. *Agnew Chem Int Ed Engl* 19: 441-445.

Henderson, L.M. (1984) Vitamin B-6. In: Present Knowledge in Nutrition. The Nutrition Foundation Inc.:Washington, D.C., pgs. 303-317.

Henderson, L.M. (1985) Intestinal absorption of B-6 vitamers. In: Reynolds, R.D., Leklem, J.E., (eds.) Vitamin B-6: Its Role in Health and Disease. Alan R.Liss: New York 25-33.

Henneman, D.H., Forbes, A.P., Molawer, M., Dempsey, E.F., Carroll, E.L. (1960) Effects of human growth hormone in man. *J Clin Invest* 39: 1223-1238.

Henry, R.J. (1968) Clinical Chemistry- Principles and Technics. Harper and Row: New York, pgs. 664-666.

Hermansen, L.H. (1973) Oxygen transport during exercise in human subjects. *Acta Physiol Scand* (supp. 399), 11-104.

Hermansen, L. and Anderson, L. (1965) Aerobic work capacity in young Norwegian men and women. *J Appl Physiol* 20: 425-431.

Hermansen, L.H., Hultman, E., Saltin, B. (1967) Muscle glycogen during prolonged severe exercise. *Acta Physiol Scand* 71: 129-139.

Hermansen, L.H. and Saltin, B. (1969) Oxygen uptake during maximal treadmill and bicycle exercise. *J Appl Physiol* 26: 31-37.

Heymsfield, S.B., Arieaga, C., McManus, C., Smith, J., Moffitt, S. (1983) Measurement of muscle mass in humans: validity of the 24-hour urinary creatinine method. *Am J Clin Nutr* 37: 478-494.

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

Ho, K.Y., Veldhuis, J.D., Johnson, M.L., Furlanetto, R., Evans, W.S., Alberti, K.G.M.M., Thorner, M.O. (1988) Fasting enhances growth hormone secretion and amplifies the complex rhythms of growth hormone secretion in man. *Am J Clin Invest* 81: 968-975.

Hobson, W. (1939) Urinary output of creatine and creatinine associated with physical exertion and its relationship to carbohydrate metabolism. *Biochem* J 33: 1425-1431.

Hofmann, A., Reynolds, R.D., Smoak, B.L., Villanueva, V.G., Deuster, P.A. (1991) Plasma pyridoxal and pyridoxal 5'-phosphate concentrations in response to ingestion of water or glucose polymer during a 2-h run. *Am J Clin Nutr* 53: 84-89.

Holl, R.W., Hartman, M.L., Veldhuis, J.D., Taylor, W.M., Thorner, M.O. (1991) Thirty-second sampling of plasma growth hormone in man: correlation with sleep stages. *J Clin Endocrinol Metab* 72: 854-861.

Holloszy, J. (1982) Muscle metabolism during exercise. *Arch Phys Med Rehabil* 63: 213-234.

Holloszy, J.O. and Coyle E.F. (1984) Adaptations of skeletal muscle to endurance exercise. *J Appl Physiol* 56: 831-838.

Holten, D., Wicks, W.D., Kenney, F.T. (1967) Studies on the role of vitamin B-6 derivatives in regulating tyrosine a-ketoglutarate transaminase activity in vitro and in vivo. *J Biol Chem* 242: 1053-1059.

Horrigan, D.L. and Harris, J.W. (1968) Pyridoxine responsive anemia in man. *Vitam Horm* 26: 549-568.

Houssay, B.A. (1942) Advancement of knowledge of the role of the hypophysis in carbohydrate metabolism during the last twenty-five years. *Endocrinol* 30: 884-887.

Huber, A.M. and Gershoff, S.N. (1965) Some effects of vitamin B-6 deficiency on rat pituitary glands. *J Nutr* 87: 407-411.

Hughes, J.P., Elsholtz, H.P., Friesen, H.G. (1985) Growth hormone and prolactin receptors. In: Posner, B.I. (ed) Polypeptide Hormone Receptors. Marcel Dekker, New York, p. 157.

Hughes, R.C., Jenkins, W.T., Fischer, E.H. (1962) The site of binding of pyridoxal-5'-phosphate to heart glutamic-aspartic transaminase. *Proc Natl Acad Sci USA* 48: 1615-1618.

Hunter, W.M., Friend, J.A.R., Strong, J.A. (1966) The diurnal pattern of plasma growth hormone concentration in adults. *J Endocrinol* 34: 139-146.

Hunter, W.M and Greenwood, F.C. (1964) Studies of human pituitary growth hormone. *Brit Med J* 1: 804-807.

Ichiba, A. and Michi, K. (1938) Isolation of vitamin B-6. *Soc. Papers Inst. Phys. Chem Res.* (Tokyo), 34: 623-626.

Ink, S.L., Gregory, J.F. and Sartain, D.B. (1986) Determination of pyridoxine β-glucoside bioavailability using intrinsic and extrinsic labeling in the rat. *J Agric Food Chem* 34: 857-862.

Ink, S.L. and Henderson, L.M. (1984a) Vitamin B-6 metabolism. *Ann Rev Nutr* 4: 445-470.

Ink, S.L. and Henderson, L.M. (1984b) Effect of binding to hemoglobin and albumin on pyridoxal transport and metabolism. *J Biol Chem* 259: 5833-5837.

Ink, S.L., Mehansho, H., Henderson, L.M. (1982) The binding of pyridoxal to hemoglobin. *J Biol Chem* 257 4753-4757.

Isidori, A., Lomonaco, A., Cappa, M. (1981) A study fo growth hormone release in man after oral administration of amino acids. *Current Medical Research and Opinion* 7: 475-481.

Issekutz, B., Jr. (1964) Effect of exercise on the metabolism of plasma free fatty acids. In: K. Rodahl and B. Issekutz, Jr. (eds.), Fat as a Tissue Chapter 11, McGraw-Hill Book Company, New York, pgs.1-428.

Issekutz, B., Jr., Birkhead, N.C., Rodahl, K. (1963) Effect of diet on work metabolism. *J Nutr* 79: 109-115.

Issekutz, B., Jr., Miller, H.I., Paul, P., Rodahl, K. (1965) Aerobic work capacity and plasma FFA turnover. *J Appl Physiol* 20: 293-296.

Jain, G.C. (1987) Feeding response of control and vitamin B-6 deficient rats on the release of growth hormone. *Nutr Reports Intern* 36: 57-63.

Johansson, S., Lindstedt, S., Register, U. (1966) Metabolism of labeled pyridoxine in the rat. *Am J Physiol* 210: 1086-1096.

Johansson, S. and Tiselius, H.G. (1973) Metabolism of trituim and ^{14}C-labeled pyridoxine. *Scand J Clin Lab Invest* 32: 9-14.

Johnson, B.C., Hamilton, T.S., Mitchell, H.H. (1945) The excretion of pyridoxine, pseudopyridoxine , and 4-pyridoxic acid in the urine and sweat of normal individuals. *J Biol Chem* 158: 619-623.

Kabir, H., Leklem, J., Miller, L.T. (1983a) Comparative vitamin B-6 bioavailability from tuna, whole wheat bread and peanut butter in humans. *J Nutr* 113: 2412-2420.

Kabir, H., Leklem, J.E., Miller, L.T. (1983b) Relationship of the glycosylated vitamin B-6 content of foods of vitamin B-6 bioavailability in humans. *Nutr Rept Int* 28: 709-716.

Kabir, H., Leklem, J., Miller, L.T. (1983c) Measurement of glycosylated vitamin B-6 in foods. *J Food Sci* 48:1422-1425.

Kaciuba-Uscilko, H., Kruk, B., Szczypaczewska, M., Opaszowski, B., Stupnicka, E., Bicz, B., Nazar, K. (1992) Metabolic, body temperature and hormonal responses to repeated periods of prolonged cycle-ergometer exercise. *Eur J Appl Physiol* 64: 26-31.

Kant, A.K. and Block, G. (1990) Dietary vitamin B-6 intake and food sources in the US population: NHANES II, 1976-1980. *Am J Clin Nutr* 52: 707-716.

Karagiorgos, A., Garcia, J.F., Brooks, G.A. (1979) Growth hormone response to continuous and intermittent exercise. *Med Sci Sport* 11: 302-307.

Kark, J.A., Bongiovanni, R., Hicks, C.U., Tarassof, G., Hannah, J.S., Yoshida, G.Y. (1982) Modification of intracellular hemoglobin with pyridoxal and pyridoxal 5'-phosphate. *Blood Cells* 8: 299-314.

Kelijman, M. and Frohman, L.A. (1988) Enhanced growth hormone (GH) responsiveness to GH-releasing hormone after dietary manipulation in obese and nonobese subjects. *J Clin Endocrinol Metab* 66: 489-494.

Kelly, P.A., Djiane, J., Postel-Vinay, M.C., Edery, M. (1991) The prolactin/growth hormone receptor family. *Endocrine Reviews* 12: 235-251.

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

Keresztesy, J.C. and Stevens, J.R. (1938) Crystalline vitamin B-6. *Proc Soc Exp Biol and Med* 38: 64-65.

Kies, C., Kan, S., Fox, H.M. (1984) Vitamin B-6 availability from wheat, rice, corn brans for humans. *Nutr Repts Int* 30: 483-491.

Kikuchi,G., Kumar, A., Talmage, P. (1958) The enzymatic synthesis of δ-aminolevulinic acid. *J Biol Chem* 233: 1214-1219.

Kindermann, W., Schnabel, A., Schmitt, W.M., Biro, G., Cassens, J., Weber, F. (1982) Catecholamines, growth hormone, cortisol, insulin and sex hormones in anaerobic and aerobic exercise. *Eur J Appl Physiol* 49: 389-399.

Kirchgessner, M. and Kosters, W.W. (1977) Effect of storage on the vitamin B-6 activity of foods. *Z. Lebensm. Unters. Forgch.* 164: 15-16.

Knoebel, L.K. (1971) Energy metabolism. In: Physiology, E.E. Seldurt (ed). Boston: Little, Brown and Co., 635-650.

Kolka, M.A., Stephenson, L.A., Wilkerson, J.E. (1982) Erythrocyte indices during a competitive marathon. *J Appl Physiol* 52:168-172.

Kostyo, J.L. and Knobil, E. (1959) *Endocrinol* 65: 395-401.

Kostyo, J.L. and Reagan, C.R. (1976) The biology of growth hormone. *Pharmacology and Therapeutics* 2: 591-604.

Kozlowski, S., Chwalbinska-Moneta, J., Vigas, M., Kaciuba-Uscilko, H., Nazar, K. (1983) Greater serum GH response to arm than to leg exercise performed at equivalent oxygen uptake. *Eur J Appl Physiol* 52: 132-135.

Kraemer, R.R., Blair, M.S., McCaferty, R., Castracane, V.D. (1993) Running-induced alterations in growth hormone, prolactin, triiodothyronine, and thyroxine Concentrations in trained and untrained men and women. *Res Quart Exer Sport* 64: 69-74.

Kraemer, W.J., Gordon, S.E., Fleck, S.J., Marchitelli, L.J., Mello, R., Dziados, J.E., Friedl, K., Harman, E., Maresh, C., Fry, A.C. (1991) Endogenous anabolic hormonal and growth factor responses to heavy resistance exercise in males and females. *Int J Sports Med* 12: 228-235.

Krebs, E.G. and Fischer, E.H. (1964) Phosphorylase and related enzymes of glycogen metabolism. In: Harris, R.S., Wool, I.G., Lovaine, J.A. (eds.) Vitamins and Hormones, vol. 22 Academic Press: New York, pgs. 399-410.

Kuhn, R. and Wendt, G. (1938) Uber das antidermatitische Vitamin der Hefe. *Ber. Deut. Chem. Ges.* 71B: 780-782.

Kuhn, R., Westpahl, K., Wendt, G. and Westphal, O. (1939) Synthesis of adermin. *Naturwissenschaften* 27: 469-470.

Kuoppasalmi, K., Haveri, H., Rehunen, S., Harkonen, M., Adlercreutz, H. (1976) Effect of strenuous anaerobic running exercise on plasma growth hormone, cortisol, LH, testosterone, androstenedione, estrone and estradiol. *J of Steroid Biochem* 7: 823-829.

Kurtz, D.J., Levy, H., Kanfer, J.N. (1972) Cerebral lipids and amino acids in the vitamin B-6 deficient suckling rat. *J Nutr* 102: 291-298.

Ladell, W.S.S. (1955) The effects of water and salt intake upon the performance of men working in hot and humid environments. *J Physiol (London)* 127: 11-46.

Landron, D., Guerre-Millo, M., Postel-Vinay, M.C., Lavau, M. (1989) Relationship between increased binding and insulin-like effects of human growth hormone in adipocytes from young fa/fa rats. *Endocrinol* 124: 2305-2313.

Lassarre, C., Girard, F., Durand, J., Raynaud, J. (1974) Kinetics of human growth hormone during submaximal exercise. *J of Appl Physiol* 37: 826-830.

Lawrence, J., Smith, J., Bower, R., Riehl, W. (1975) The effect of α-Tocopherol (Vitamin E) and pyridoxine HCL (Vitamin B_6) on the swimming endurance of trained swimmers. *J Am Coll Health Assoc* 23: 219-222.

Layzer, R.B. (1990) Muscle metabolism during fatigue and work. *Bailliere's Clinical Endo and Metab* 4: 441-459.

Lee, C.M. and Leklem, J.E. (1985) Differences in vitamin B-6 status indicator responses between young and middle-aged women fed constant diets with two levels of vitamin B-6. *Am J Clin Nutr* 42: 226-234.

Leklem, J.E. (1985) Physical activity and vitamin B-6 metabolism in men and women: interrelationship with fuel needs. In: Reynolds, R.D., Leklem, J.E., eds., Vitamin B-6: its role in health and disease. A.R. Liss, New York, 221-241.

Leklem, J.E. (1988) Vitamin B-6 metabolism and function in humans. In: Leklem, J.E., Reynolds, R.D. eds., Clinical and Physiological Applications of Vitamin B-6. New York: Liss Inc., 3-23.

Leklem, J.E. (1990) Vitamin B-6: A status report. *J Nutr* 120: 1503-1507.

Leklem, J.E. (1991) Vitamin B-6. In: Handbook of Vitamins, 2nd Ed., Machlin, L.J. Ed., Marcel Dekker, Inc, N.Y. pp.341-392.

Leklem, J.E., Brown, R.R., Rose, D.P., Linkswiler, H., Arend, R.A. (1975) Metabolism of tryptophan and niacin in oral contraceptive users receiving controlled intakes of vitamin B-6. *Am J Clin Nutr* 28: 146-156.

Leklem, J., Hansen, C., Miller, L. (1991) Effect of three levels of protein on vitamin B-6 status of adult women. *FASEB J* 5: A557 (Abstract).

Leklem, J.E. and Hollenbeck, C.B. (1990) Acute ingestion of glucose decreases plasma pyridoxal 5'-phosphate and total vitamin B-6 concentration. *Am J Clin Nutr* 51: 832-836.

Leklem, J.E., Miller, L.T., Perera, A.D., Peffers, D.E. (1980) Bioavailability of vitamin B-6 from wheat bread in humans. *J Nutr* 110: 1819-1828.

Leklem, J.E. and Reynolds, R.D. (1981) Recommendations for status assessment of vitamin B-6. In: Leklem, J.E., Reynolds, R.D.(eds.) Methods in Vitamin B-6 Nutrition. Plenum Press: New York, pgs. 389-392.

Leklem, J.E. and Shultz, T.D. (1983) Increased plasma pyridoxal 5-phosphate and vitamin B-6 in male adolescents after a 4500-meter run. *Am J Clin Nutr* 38: 541-548.

Lemon, P.W.R. (1983) A simple and inexpensive method for making sweat collection capsules. *Res Q Exercise Sport* 54: 299-301.

Lemon, P.W.R. (1987) Protein and exercise: update 1987. *Med Sci Sports Exer* 19 (No. 5 Suppl), S179-S190.

Lemon, P.W.R., Benevenga, N.J., Nagle, F.J., Mullin, J.P. (1979) In vivo leucine oxidation in the rat. *Can J Appl Sport Sci* 4: 248 (abstract).

Lemon, P.W.R. and Mullin, J.P. (1980) The effect of initial muscle glycogen levels on protein catabolism during exercise. *J Appl Physiol* 48: 624-629.

Lemon, P.W.R., Mullin, J.P., Nagle, F.J., Benevenga, N.J. (1980b) Effect of daily exercise and food intake on leucine oxidation. *Can J Appl Sport Sci* 5(4):xi (abstract).

Lemon, P. and Nagle, F. (1981) Effects of exercise on protein and amino acid metabolism. *Med Sci Sport s Exer* 13: 141-149.

Lemon, P.W.R., Nagle, F.J., Benevenga, N.J., Mullin, J.P. (1980a) In vivo leucine oxidation during exercise. *Med Sci Sport s Exer* 12: 133 (abstract).

Lemon, P.W.R., Nagle, F.J., Mullin, J.P., Benevenga, N.J. (1982) In vivo leucine oxidation at rest and two intensities of exericise. *J Appl Physiol* 53: 947-954.

Lepkovsky, S. (1938) Crystalline Factor I. *Science* 87: 169-170.

Leussing, D.L. (1986) Model reactions. In: Dolphin, D., Poulson, R., Avramovic, O. eds. Coenzymes and Cofactors vol. 1, Vitamin B-6 pyridoxal phosphate. John Wiley & Sons, New York, 69-115.

Lewis, S. and Gutin, B. (1973) Nutrition and endurance. *Am J Clin Nutr* 26: 1011-1014.

Li, C.H., Evans, H.M. and Simpson M.E. (1945) Isolation and properties of the anterior hypophyseal growth hormone. *J Biol Chem* 159: 353-366.

Li, T-K. and Lumeng, L. (1981) Plasma PLP as indicator of nutritional status: relationship to tissue vitamin B-6 content and hepatic metabolism. In: Leklem, J.E., Reynolds, R.D.(eds.) Methods in Vitamin B-6 Nutrition. Plenum Press: New York, pgs. 289-296.

Li, T-K, Lumeng, L, Veitch, R.L. (1974) Regulation of pyridoxal 5'-phosphate metabolism in liver. *Biochem Biophys Res Comm* 61: 627-634.

Lindberg, A.S., Leklem, J.E., Miller, L.T. (1983) The effect of wheat bran on the bioavailability of vitamin B-6 in young men. *J Nutr* 113: 2578-2586.

Lippe, B.M., Kaplan, S.A., Golden, M.P., Hendricks, S.A., Scott, M.L. (1981) Carbohydrate tolerance and insulin receptor binding in children with hypopituitarism: responses after acute and chronic human growth hormone administration. *J Clin Endocrinol Metab* 53: 507-513.

Litwack, G., Miller-Diener, A., DiSorbo, D.M., Schmidt, T.J. (1985) Vitamin B-6 and the glucocorticoid receptor. In: Reynolds, R.D., Leklem, J.E., eds. Vitamin B-6: its role in health and disease. A.R. Liss, New York, 177-191.

Loo, G. and Smith, J.T. (1986) Effect of pyridoxine deficiency on phospholipid methylation in rat liver microsones. *Lipids* 21: 409-412.

Luckey, T.D., Briggs, G.M. Jr., Elvehjem, C.A. (1944) The use of strptococcus lactis R for the measurement of 'folic acid'. *J Biol Chem* 152: 157-167.

Luger, A., Watschinger, B., Deuster, P., Svoboda, T., Clodi, M., Chrousos, G.P. (1992) Plasma growth hormone and prolactin responses to graded levels of acute exercise and to a lactate infusion. *Neuroendocrinol* 56: 112-117.

Lui, A., Lumeng, L., Aronoff, G.R., Li, T-K. (1985) Relationship between body store of vitamin B-6 and plasma pyridoxal-P clearance: Metabolic balance studies in humans. *J Lab Clin Med* 106: 491-497.

Lui, A., Lumeng, L., Li, T-K. (1983) Biliary excretion of ^{14}C-Labeled Vitamin B-6 in rats. *J Nutr* 113: 893-898.

Lumeng, L., Brashear, R.E., Li, T-K. (1974a) Pyridoxal 5'-phosphate in plasma: source, protein binding and cellular transport. *J Lab Clin Med* 84: 334-343.

Lumeng, L., Cleary, R.E., Li, T-K. (1974b) Effect of oral contraceptives on the plasma concentration of pyridoxal phosphate. *Am J Clin Nutr* 27: 326-333.

Lumeng, L. and Li, T-K. (1975) Characterization of the pyridoxal 5'-phosphate and pyridoxamine 5'-phosphate hydrolase activity in rat liver. *J Biol Chem* 250: 8126-8131.

Lumeng, L., and Li, T-K. (1980) Mammalian vitamin B-6 metabolism: regulatory role of protein-binding and the hydrolysis of pyridoxal 5'-phosphate in storage and transport. In: Tryfiates, G.P.(ed.) Vitamin B-6 Metabolism and Role in Growth. Food and Nutrition Press: Westport, CN, pgs. 27-51.

Lumeng, L., Li, T-K., Lui, A. (1985) The interorgan transport and metabolism of vitamin B-6. In: Vitamin B-6: its role in health and disease. Leklem, J.E. and Reynolds, R.D. (eds), pgs. 35-54.

Lundberg, S., Belfrage, M., Wernerman, J., Von der Decken, A., Thunell, S., Vinnars, E. (1991) Growth hormone improves muscle protein metabolism and whole body nitrogen economy in man during a hyponitrogenous diet. *Metabolism* 40: 315-322.

Lundvall, J., Mellander, S., Westling, H., White, T. (1972) Fluid transfer between blood and tissues during exercise. *Acta Physiol Scand* 85: 258-269.

Lushbough, C.H., Weichman, J.M., Schweigert, B.S. (1959) The retention of vitamin B-6 in meat during cooking. *J Nutr* 67: 451-459.

Macintyre, J.G. (1987) Growth hormone and athletes. *Sports Med* 4: 129-142.

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

Maes, M., De Hertogh, R., Watrin-Granger, P., Ketelslegers, J.M. (1983) Ontogeny of liver somatotropic and lactogenic sites in male and female rats. *Endocrinol* 113: 1325-1332.

Mahler, R.J. and Szabo, O. (1969) The acute insulin synergistic activity of growth hormone. I. Inhibition by chronic growth hormone administration. *Horm Metab Res* 1: 26-31.

Mahuren, J.D., Pauly, T.A., Coburn, S.P. (1991) Identification of 5-pyridoxic acid and 5-pyridoxic acid lactone as metabolites of vitamin B-6 in humans. *J Nutr Biochem* 2: 449-53.

Main, K., Kastrup, K.W., Skakkebaek, N.E. (1991) Reduced sweating in Laron's dwarfism. *Arch Dis Child* 65: 1380.

Makris, A. and Gershoff, S.N. (1973) Growth hormone levels in vitamin B-6 deficient rats. *Horm Metab Res* 5: 457-461.

Malarkey, W.B., Hall, J.C., Pearl, D.K., Kiecolt-Glaser, J.K., Glaser, R. (1991) The influence of academic stress and season on 24-hour concentrations of growth hormone and prolactin. *J Clin Endocrinol Metab* 73: 1089-1092.

Manchester, K.L. (1965) Oxidation of amino acids by isolated rat diaphragm and the influence of insulin. *Biochim Biophys Acta* 100: 295-298.

Manchester, K.L. and Young, F.G. (1961) Insulin and protein metabolism. *Vitam & Hormones* 19: 95-132.

Manore, M.M. (1985) The effect of two carbohydrate diets and vitamin B-6 on vitamin B-6 and fuel metabolism and cardiac function during exercise. Ph.D. Thesis, Dept. Foods and Nutrition, Oregon State Univ.

Manore, M.M. and Leklem, J.E. (1988) Effect of carbohydrate and vitamin B_6 on fuel substrates during exercise in women. *Med Sci Sports Exerc* 20: 233-241.

Manore, M.M, Leklem, J.E., Walter, M.C. (1987) Vitamin B-6 metabolism as affected by exercise in trained and untrained women fed diets differing in carbohydrate and vitamin B-6 content. *Am J Clin Nutr* 46: 995-1004.

Maron, M., Horvath, S.M., Wilderson, J.E., Gliner, J.A. (1976) Oxygen uptake measurements during competitive marathon running. *J Appl Physiol* 40: 836-838.

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

Mathews, C.K. and van Holde, K.E.(1990) Metabolism of Nitrogenous compounds. In: Biochemistry, Benjamin/Cummings Pub. Co, pgs.680-693.

Maughan, R.J., Williams, C., Campbell, D.M., Hepburn, D. (1978) Fat and carbohydrate metabolism during low intensity exercise: effects of the availability of muscle glycogen. *Eur J Appl Physiol* 39: 7-16.

McArdle, W.D., Katch, F.I., Katch, V.L. (eds.)(1986) Exercise Physiology. Energy, Nutrition, and Human Performance. Second ed., Lea & Febiger: Philadelphia, pgs.69-400.

McCance, R.A. (1938) Individual variations in response to high temperatures and to the production of experimental salt deficiency. *Lancet* 2: 190-191.

McCance, R.A. and Purohit, G. (1969) Ethnic differences in the response of the sweat glands to pilocarpine. *Nature* 221: 378-379.

McCormick, D. (1988) Vitamin B-6. In: Shils, M.E. and Young, V.R., eds. Modern Nutrition in Health and Disease, 7th Ed., Lea & Febiger, Philadelphia, pp. 376-382.

McHenry, E.W. and Gauvin, G. (1938) The B vitamins and fat metabolism. I. Effects of thiamine, riboflavin and rice polish concentrate upon body fat. *J Biol Chem* 125: 653-660.

McMurray, R.G., Proctor, C.R., Wilson, W.L. (1991) Effect of caloric deficit and dietary manipulation on aerobic and anaerobic exercise. *Int J Sports Med* 12: 167-172.

Mehansho, H., Hamm, M.W., Henderson, L.M. (1979) Transport and metabolism of pyridoxal and pyridoxal phosphate in the small intestine of the rat. *J Nutr* 109: 1542-1551.

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

Mellander, S., Johansson, B., Gray, S., Johsson, O., Lundvall, J., Ljung, B. (1967) The effects of hyperosmolarity on intact and isolated vascular smooth muscle: Possible role in exercise hyperemia. *Angiologica* 4: 310-322.

Merimee, T.J. (1979) Growth hormone: secretion and action. In: Endocrinology. DeGrppt et al. (eds.), Grune & Stratton: New York, pgs. 123-132.

Merimee, T.J., Felig, P., Marliss, E., Fireberg, S.E., Cahill, G.F. (1971) Glucose and lipid homeostasis in the absence of human growth hormone. *J Clin Invest* 50: 574-582.

Merrill, A.H. and Henderson, J.M. (1990) Vitamin B-6 metabolism by human liver. *Ann NY Acad Sci* 585: 110-117.

Merrill, A.H., Henderson, J.M., Wang, E., McDonald, B.W., Millikan, W.J. (1984) Metabolism of vitamin B-6 by human liver. *J Nutr* 114: 1664-1674.

Metcalfe, P., Johnston, D.G., Nosadini, R., Orksov, H., Alberti, K.G.M.M. (1981) Metabolic effects of acute and prolonged growth hormone excess in normal and insulin-deficient man. *Diabetologia* 20: 123-128.

Metzler, D., Ikawa, M., Snell, E.E. (1954) A general mechanism for vitamin B_6-catalyzed reactions. *J Am Chem Soc* 76: 648.

Meydani, S.W., Ribaya-Mercado, J.D., Russell, R.M., Sahyoun, N., Morrow, P.D., Gershoff, S.N. (1991) Vitamin B-6 deficiency impairs interleukin 2 production and lymphocyte proliferation in elderly adults. *Am J Clin Nutr* 53: 1275-1280.

Michelson, T.C. and Hagerman, F.C. (1982) Anaerobic threshold measurements of elite oarsmen. *Med Sci Sports Exerc* 14: 440-444.

Middleton, H.M. (1977) Uptake of pyridoxine hydrochloride by rat jejunal mucosa in vitro. *J Nutr* 107: 126-131.

Middleton, H.M. (1979) Intestinal absorption of pyridoxal 5'-phosphate: disappearance from perfused segments of rat jejunum in vivo. *J Nutr* 109: 975-981.

Middleton, H.M. (1981) Transmural absorption of pyridoxine HCL in vitro in the rat jejunum. *Proc Soc Exp Biol Med* 167: 519-524.

Middleton, H.M. (1982) Characterization of pyridoxal 5'-phosphate disappearance from in vivo perfused segements of rat jejunum. *J Nutr* 112: 269-275.

Middleton, H.M. (1984) Transport and metabolism of water-soluble vitamins in intestine and kidney. In: Rose et al. Symposium Report, Fed Proc 43: 2423-2429.

Middleton, H.M. (1985) Uptake of pyridoxine by in vivo perfused segments of rat small intestine: A possible role for intracellular vitamin metabolism. *J Nutr* 115: 1079-1088.

Miller, L.L. (1962) The role of the liver and the non-hepatic tissues in the regulation of free amino acid levels in the blood. In: Amino Acid Pools, J.T. Holden (eds.). Amsterdam: Elsevier, pgs. 708-721.

Miller, L.T. and Edwards, M. (1981) Microbiological assay of vitamin B-6 in blood and urine. In: Methods in vitamin B-6 nutrition. Leklem, J.E. and Reynolds, R.D. (eds.), Plenum Press: New York, pgs. 45-55.

Miller, L.T., Leklem, J.E., Shultz, T.D. (1985) The effect of dietary protein on the metabolism of vitamin B-6 in humans. *J Nutr* 115: 1663-1672.

Miller, L.T., Linkswiler, H. (1967) Effect of protein intake on the development of abnormal tryptophan metabolism by men during vitamin B-6 depletion. *J Nutr* 93: 53-59.

Mims, R.B., Scott, C.L., Modebe, O., Bethune, J.E. (1975) Inhibition of L-dopa-induced growth hormone stimulation by pyridoxine and chlorpromazine. *J Clin Endocrinol* 40: 256-259.

Mitchell, J.W., Nadel, E.R., Stolwijk, J.A. (1972) Respiratory weight loss during exercise. *J Appl Physiol* 32: 474-476.

Moretti, C., Fabbri, A., Gnessi, L., Bonifacio, V., Fraioli, F. (1982) Pyridoxine suppresses the rise in prolactin and increases the rise in growth hormone induced by exercise. *N Eng J Med* 307: 444-445.

Morgan, M., Altszuler, N., Rathgeb, I. (1975) Failure of injected growth hormone to increase plasma glucose and insulin concentration in the puppy. *Endocrinol* 96: 538-539.

Morre, D.M., Kirksey, A., Das, G.D. (1978) Effects of vitamin B-6 on the developing central nervous system of the rat myelination. *J Nutr* 108: 1260-1265.

Mueller, J.F. and Iacono, J.M. (1963) Effect of desoxypyridoxine-induced vitamin B-6 deficiency on polyunsaturated fatty acid metabolism in human beings. *Am J Clin Nutr* 12: 358-367.

Muldoon, T.G. and Cidlowski, J.A. (1980) Specific modification of rat uterine estrogen receptor by pyridoxal 5'-phosphate. *J Biol Chem* 255: 3100-3107.

Munoz, K.D. (1982) The influence of exercise on vitamin B-6 metabolism. M.S. Thesis, Department of Foods and Nutrition, Oregon State University.

Murray, R.K., Granner, D.K., Mayes, P.A., Rodwell, V.W.(eds.)(1990) Harper's Biochemistry. Appleton & Lange: Connecticut, pgs. 481-482.

National Research Council, Recommended Dietary Allowances. (1989) 10th ed., National Academy Press: Washington D.C., pgs. 142-150.

Naveri, H. (1985) Blood hormone and metabolite levels during graded cycle ergometer exercise. *Scand J Clin Lab Invest* 45: 599-603.

Nelson, E.W., Burgin, C.W., Cerda, J.J. (1977) Characterization of food binding of vitamin B-6 in orange juice. *J Nutr* 107: 2128-2134.

Newgard, C.B., Hirsch, L.J., Foster, D.W., McGarry, J.D. (1983) Studies on the mechanism by which exogenous glucose is converted into liver glycogen in the rat. A direct or indirect pathway. *J Biol Chem* 258: 8046-8052.

Nguyen, L.B., Gregory, J.F., Cerda, J.J. (1983) Effect of dietary fiber on absorption of B-6 vitamers in a rat jejunal perfusion study. *Proc Soc Exp Biol Med* 173: 568-573.

Nishigori, H., Moudgil, V.K., Taft, D. (1978) Inactivation of avian progesterone receptor binding to ATP-sepharose by pyridoxal 5'-phosphate. *Biochem Biophys Res Comm* 80: 112-118.

Noall, M.W., Riggs, T.R., Walker, L.M., Christensen, H.N. (1957) Endocrine control of amino acid transfer. Distribution of an unmetabolizable amino acid. *Science* 126: 1002-1005.

Nylin, G. (1947) The effect of heavy muscular work on the volume of circulatory red corpuscles in man. *Am J Physiol* 149: 180-184.

O'Brien, M.J., Viguie, C.A., Mazzeo, R.S., Brooks, G.A. (1993) Carbohydrate dependence during marathon running. *Med Sci Sports Exerc* 25: 1009-1017.

Odake, S. (1931) Isolation of oryzanin crystals (antineuritic vitamin) from rice polishings. I. Report. *Proc Imp Acad (Tokyo)* 7: 102-105.

Odessey, R.and Goldberg, A.L. (1972) Oxidation of leucine by rat skeletal muscle. Am *J Physiol* 223: 1376-1383.

Orr, M.L. (1969) Panthothenic Acid, Vitamin B-6 and Vitamin B-12 in Foods. Home Economics Research Report, No. 36, U.S. Dept. of Agriculture, Washington, D.C.

Page, C.O. Jr. and Remington, J.S. (1967) Immunologic studies in normal huiman sweat. *J Lab Clin Med* 69: 634-650.

Palm, D., Klein, H.W., Schinzel, R., Buehner, M., Helmreich, E.J.M. (1990) The role of pyridoxal-5'-phosphate in glycogen phosphorylase catalysis. *Biochem* 29: 1099-1107.

Pansky, B.(1975) Dynamic Anatomy and Physiology. Macmillan Pub. Co.: New York, pg. 301.

Pasanen, A.V.O., Salmi, M., Tenhunen, R., Vuopio, P. (1982) Haem synthesis during pyridoxine therapy in two families with different types of hereditary sideroblastic anemia. *Ann Clin Res* 14: 61-65.

Pedersen, S.A., Welling, K., Michaelsen, K.F., Jorgensen, J.O., Christiansen, J.S., Skakkebaek, N.E. (1989) Reduced sweating in adults with growth hormone deficiency. *Lancet* 2: 681-682.

Pequignot, J.M., Peyrin, L., Peres, G. (1980) Catacholamine-fuel interrelationships during exercise in fasting men. *J Appl Physiol: Respirat Environ Exercise Physiol* 48:109-113.

Perera, A.D., Leklem, J.E., Miller, L.T. (1979) Stability of vitamin B-6 during bread making and storage of bread and flour. *Cereal Chem* 56: 577-580.

Perutz, M.F. (1970) The Bohr effect and combinations with organic phosphates. *Nature* 228: 734-739.

Phillips, M.R., Vandervoort, R., Becker, C.E. (1977) Long-term sweat collection using salt-impregnated pads. *J Invest Dermatol* 68: 221-224.

Pierluissi, J. and Campbell, J. (1980) Metasomatotrophic diabetes and its induction: basal insulin secretion and insulin release responses to glucose, glucagon, arginine and meals. *Diabetologia* 18: 223-228.

Pierluissi, J. and Campbell, J. (1981) Growth hormone and metasomatotrophic diabetes: effects on insulin and proinsulin of serum and pancreas in dogs. *Diabetologia* 21: 558-562.

Pietz, J., Benninger, C., Schafer, H., Sontheimer, D., Mittermaier, G., Rating, D. Treatment of infantile spasms with high-dosage vitamin B-6. (1993) *Epilepsia* 34: 757-763.

Pilardeau, P.A., Chalumeau, M.T., Harichaux, P., Vasseur, P., Vaysse, J., Garnier, M. (1988) Effect of physical training on exercise induced sweating in men. *J Sports Med* 28: 176-180.

Pimstone, B.L., Barbezat, G., Hansen, J.D.L., Murray, P. (1968) Studies on growth hormone secretion in protein-calorie malnutrition. *Am J Clin Nutr* 21: 482-487.

Pino, S., Benotti, J., Gardyna, H. (1965) An automated method for urine creatinine which does not require a dialyzer module. *Clin Chem* 11: 664-666.

Pitts, G.C., Johnson, R.E., Consolazio, F.C. (1944) Work in the heat as affected by intake of water, salt and glucose. *Am J Physiol* 142: 253-259.

Pivarnik, J.M., Leeds, E.M., Wilkerson, J.E. (1984) Effects of endurance exercise on metabolic water production and plasma volume. *J Appl Physiol* 56: 613-618.

Pogell, B. (1958) Enzymatic oxidation of pyridoxamine phosphate to pyridoxal phosphate in rabbit liver. *J Biol Chem* 232: 761-776.

Poortmans, J.R. (1968) Influence of physical exercise on proteins in biological fluids. In: Biochemistry of Exercise, Proc of the First Symposium of Exercise, J. Poortmans (ed.), pgs. 312-327.

Poortmans, J.R. (1970) Serum protein determination during short exhaustive physical activity. *J Appl Physiol* 30: 190-192.

Postel-Vinay, M.C., Cohen-Tangui, E., Charrier, J. (1982) Growth hormone receptors in rat liver membranes: effects of fasting and refeeding and correlation with plasma somatomedin activity. *Mol Cell Endocrinol* 28: 657-669.

Quirion, A., Brission, G., DeCarufel, D., Laurencelle, L., Therminarias, A., Vogelarere, P. (1988) Influence of exercise and dietary modification on plasma human growth hormone, insulin and FFA. *J Sports Med Phys Fitness* 28: 352-353.

Rabinowitz, J. and Snell, E.E. (1949) Vitamin B_6 group: Urin.ary excretion of pyridoxal, pyridoxamine, pyridoxine and pyridoxic acid in human subjects *Proc Soc Exp Biol Med* 70: 235-240.

Raynaud, J., Capderou, A., Martineaud, J., Bordachar, J., Durand, J. (1983) Intersubject variability in growth hormone time course during different types of work. *J Appl Phys; Resp Envir Exer Phys* 55: 1683-1687.

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

Reddy, S.K., Reynolds, M.S., Price, J.M. (1958) The determination of 4-pyridoxic acid in human urine. *J Biol Chem* 233: 691-696.

Refetoff, S., Frank, P.H., Roubebush, C., DeGroot, L.J. (1979) Evaluation of pituitary function. In: Endocrinology DeGroot et al. (eds.) Grune & Stratton: New York, pgs. 175-214.

Reichlin, S. (1983a) Somatostatin (first of two parts). *N Engl J Med* 309: 1495-1501.

Reichlin, S. (1983b) Somatostatin (second of two parts). *N Eng J Med* 309: 1556-1563.

Rennie, M.J., Jennett, S., Johnson, R.H. (1974) The metabolic effects of strenuous exercise: a comparison between untrained subjects and racing cyclists. *Q J Exp Physiol* 59: 201-212.

Reynolds, R.D., Lorenc, R.S., Wieczorek, E., Pronicka, E. (1991) Extremely low serum pyridoxal 5'-phosphate in children with familial hypophosphatemic rickets. *Am J Clin Nutr* 53: 698-701.

Reynolds, R.D. and Natta, C.L. (1985) Vitamin B-6 and sickle cell anemia. In: Vitamin B-6: its role in health and disease. Reynolds, R.D. and Leklem, J.L. (eds.), A.R. Liss: New York, pgs. 301-306.

Richardson, L.R., Wilkes, S., Ritchey, S.J. (1961) Comparative vitamin B-6 activity of frozen, irradiated and heat-processed foods. *J Nutr* 73: 363-368.

Rillema, J.A. and Kostyo, J.L. (1971) Studies on the delayed action of growth hormone on the metabolism of the rat diaphragm. *Endocrinol* 88: 240-248.

Rinderknecht, E. and Humbel, R.E. (1978a) The amino acid sequence of human insulin-like growth factor I and its structural honology with proinsulin. *J Biol Chem* 253: 2769-2776.

Rinderknecht, E. and Humbel, R.E. (1978b) Primary structure of human insulin-like growth factor II. *FEBS Lett* 89: 283-286.

Rivier, J., Speiss, J., Thorner, M., Vale, W. (1982) Characterization of a growth hormone-releasing factor from a human pancreatic islet tumour. *Nature* 300: 276-278.

Rizza, R.A., Mandarino, L.J., Gerich, J.E. (1982) Effects of growth hormone on insulin action in man: mechanisms of insulin resistance, impaired suppression of glucose production, and impaired stimulation of glucose utilization. *Diabetes* 31: 662-669.

Robins, E., Robins, J.M., Croninger, A.B., Moses, S.G., Spencer, S.J., Hudgens, R.W. (1967) *Biochem Med* 1: 240.

Robinson, S., Edwards, H.T., Dill, D.B. (1937) New records in human power. *Science* 85:409-410.

Robinson, S. and Robinson, A.H.(1954) Chemical composition of sweat. *Physiol Rev* 34: 202-220.

Rodahl, K., Miller, H.I, Issekutz, B.Jr. (1964) Plasma free fatty acids in exercise. *J Appl Physiol* 19: 489-492.

Rodahl, A., O'Brien, M., Firth, R.G.R. (1976) Dirunal variation in performance of competetive swimmers. *J Sports Med Phys Fitness* 16: 72-76.

Rogers, S.A. and Hammerman, M.R. (1989) Growth hormone activates phospholipase C in tubular basolateral membranes from canine kidney. *Proc Natl Acad Sci USA* 86: 6363-6366.

Rokitzki, L., Sagredos, A.N., Reub, F., Buchner, M., Keul, J. (1994) Acute changes in vitamin B-6 status in endurance athletes before and after a marathon. *Int J Sport Nutr* 4: 154-165.

Rolandi, E., Reggiani, E., Franceschini, R., Bavastro, G., Messina, V. (1985) Comparison of pituitary responses to physical exercise in athletes and sedentary subjects. *Hormone Res* 21: 209-213.

Rose, C.S., Gyorgy, P., Butler, M., Andres, R., Norris, A.H., Shock, N.W., Tobin, J., Brin, M., Spiegel, H. (1976) Age differences in vitamin B-6 status of 617 men *Am J Clin Nutr* 29: 847-853.

Rose, D.P. (1978) The interactions between vitamin B-6 and hormones. *Vitam Horm* 36: 53-99.

Rose, D.P., Leklem, J.E., Brown, R.R., Linkswiler, H.M. (1975) Effect of oral contraceptives and vitamin B-6 deficiency on carbohydrate metabolism. *Am J Clin Nutr* 28: 872-878.

Roth, J, Glick, S.M., Yalow, R.S., Berson, S.A. (1963) Secretion of human growth hormone: physiologic and experimental modification. *Metabolism* 12: 577-579.

Rowell, L.B. (1974) Human cardiovascular adjustments to exercise and thermal stress. *Physiol Rev* 54: 75-159.

Roy, A.V. (1970) Rapid method for determining alkaline phosphatase activity in serum with thymolphthalein monophosphate. *Clin Chem* 16: 431-436.

Rudman, D. (1985) Growth hormone, body composition and aging. *J Am Geriat Soc* 33: 800-807.

Rudman, D., Kutner, M.H., Rogers, C.M., Lubin, M.F., Alexander-Fleming, G., Bain, R.P. (1981) Impaired growth hormone secretion in the adult population. *J Clin Invest* 67: 1361-1369.

Sabo, D.J., Francesconi, R.P., Gershoff, S.N. (1971) Effect of vitamin B-6 deficiency on tissue dehydrogenases and fat synthesis in rats. *J Nutr* 101: 29-34.

Salomon, F., Cuneo, R.C., Hesp, R., Inst, M., Sonksen, P.H. (1989) The effects of treatment with recombinant human growth hormone on body composition and metabolism in adults with growth hormone deficiency. *N Eng J Med* 321: 1797-1803.

Saltin, B. (1964) Aerobic and anaerobic work capacity after dehydration. *J Appl Physiol* 19: 1114-1118.

Sahlin, K., Alvestrand, A., Brandt, R., Hultman. (1978) Intravascular pH and bicarbonate concentration in human muscle during recovery from exercise. *J Appl Physiol* 45: 474.

Sampson, D.A., Crozier, P.G., Gotshall, R.W., Cordain, L. (1993) Pyridoxal phosphate in plasma does not vary with exercise intensity. *FASEB J* 7: A728 (abstract 4203).

Sarma, P.S., Snell, E.E., Elvehjem, C.A. (1947) The bioassay of vitamin B-6 in natural materials. *J Nutr* 33: 121-128.

SAS Institute Users Guide (1987), Cary, N.C., version 6.04.

Sauberlich, H.E. (1968) Section IX. Biochemical systems and biochemical detection of deficiency. In: The vitamins: chemistry, physiology, pathology, assay. Sebrell, W.H. and Harris, R.S. (eds.), Acad Press: New York, pgs. 44-80.

Sauberlich, H.E. (1981) Vitamin B-6 status assessment: past and present. In: Methods in vitamin B-6 nutrition. Leklem, J.E. and Reynolds, R.D. (eds.), Plenum Press: New York, pgs. 203-239.

Sauberlich, H.E., Dowdy, R.P., Skala, J.H. (1974) Lab tests for assessment of nutritional status. CRC Press: Cleveland, OH, pgs. 37-49.

Sawka, M.N. (1988) Body fluid responses and hypohydration during exercise-heat stress. In: Human Performance Physiology and Environmental Medicine at Terrestrial Extremes, K.B. Pandolf, M.N. Sawka and R.R. Gonzalez (eds.). Indianapolis:Benchmark Press, pgs. 227-266.

Sawka, M.N. (1992) Physiological consequences of hypohydration: exercise performance and thermoregulation. *Med Sci Sports Exerc* 24: 657-670.

Sawka, M.N., Knowlton, R.G., Glaser, R.G. (1980) Body temperature, respiration and acid-base equilibrium during prolonged running. *Med Sci Sports Exerc* 12: 370-374.

Scavo, D., Barletta, C., Vagiri, D., Letizia, C. (1991) Adrenocorticotropic hormone, beta-endorphin, cortisol, growth hormone and prolactin circulating levels in nineteen athletes before and after half-marathon and marathon. *Sports Med Phys Fitness* 31: 401-406.

Schaeffer, M.C., Sampson, D.A., Skala, J.H., Gietzen, D.W., Grier, R.E. (1989) Evaluation of vitamin B-6 status and function of rats fed excess pyridoxine. *J Nutr* 119: 1392-1398.

Schalch, D.S. and Kipnis, D.M. (1965) Abnormalities in carbohydrate tolerance associated with elevated plasma nonesterified fatty acids. *J Clin Invest* 44: 2010-2020.

Schirch, L. and Jenkins, W.T. (1964) Serine transhydroxymethylase. *J Biol Chem* 239: 3797-3800.

Schnure, J.J., Raskin, P., Lipman, R.L. (1971) Growth hormone secretion during sleep: impairment in glucose tolerance and nonsuppressibility by hyperglycemia. *J Clin Endocrinol Metab* 33: 234-241.

Schooley, J.P., Riddle, O., Bates. (1938) Analysis of pituitary support of growth of body and viscera in pigeons. *Anat Rec* 72(supp.): 90 (abstract).

Schwartz, F., ter Haar, D.J., van Riet, H.G., Thijssen, J.H.H. (1969) Response of growth hormone, free fatty acids, blood sugar and insulin to exercise in obese patients and normal subjects. *Metabolism* 18: 1013-1020.

Schwartz, I.L., Thaysen, J.H., Dole, V.P. (1953) Urea excretion in human sweat as a tracer for movement of water within the secreting gland. *J Exper Med* 97: 429-438.

Schwartz, J. and Eden, S. (1985) Acute growth hormone deficiency rapidly alters glucose metabolism in rat adipocytes. Relation to insulin responses and binding. *Endocrinol* 116: 1806-1812.

Senay, L.C. Jr. (1970) Movement of water, protein and crystalloids between vascular and extravascular compartments in heat-exposed men during dehydration and following limited relief of dehydration. *J Physiol(London)* 210: 617-635.

Senay, L.C. Jr. and Christensen, M.L. (1965) Changes in blood plasma during progressive dehydration. *J Appl Physiol* 20: 1136-1140.

Serfontein, W.J. and Ubbink, J.B. (1988) Vitamin B-6 and myocardial infarction. In: Leklem, J.E., Reynolds, R.D. (eds.) Clinical and Physiological Applications of Vitamin B-6. A.R. Liss: New York, pgs. 201-217.

Shephard, R., Bouhlel, E., Vandewalle, H., Monod, H. (1988) Muscle mass as a factor limiting physical work. *J Lab Clin Med* 64: 1472-1479.

Shephard, R.J. and Sidney, K.H. (1975) Effects of physical exercise on plasma growth hormone and cortisol levels in human subjects. *Exer Sport Sci Rev* 3: 1-30.

Sherman, W.M. (1983) Carbohydrates, muscle glycogen, and muscle glycogen supercompensation. In: Ergogenic Aids in Sports. M.H. Williams (Eds.). Champaign, Illinois: Human Kinetics Publishers, pgs. 3-26.

Sherman, W.M., Peden, M.C., Wright, D.A. (1991) Carbohydrate feedings one hour before exercise improves cycling performance. *Am J Clin Nutr* 54: 866-870.

Shultz, T. and Leklem, J. (1981) Urinary 4-pyridoxic acid, urinary vitamin B-6 and plasma pyridoxal phosphate as measures of vitamin B-6 status and dietary intake of adults. In: Leklem, J.E., Reynolds, R.D.(eds.), Methods in vitamin B-6 nutrition. New York, NY: Plenum Press, 297-320.

Shultz, T.D. and Leklem, J.E. (1987) Vitamin B-6 status and bioavailability in vegetarian women. *Am J Clin Nutr* 46: 647-651.

Silva, C.M., Webe, M.J., Thorner, M.O. (1993) Stimulation of tyrosine phosphorylation in human cells by activation of growth hormone receptor. *Endocrinol* 132: 101-108.

Singh, A., Moses, F.M., Deuster, P.A. (1992) Vitamin and mineral status in physically active men: effects of a high-potency supplement. *Am J Clin Nutr* 55: 1-7.

Sirek, A., Sirek, O.V., Niki, H., Przybylska, K. (1969) The effect of dihydroergotamine on growth hormone-induced lipolysis in dogs. *Horm Metab Res* 1: 276-281.

Snedecor, G.W. and Cochran, W.G. (1980) Statistical Methods. 7th Ed., Iowa State University Press, pg. 478.

Snell, E.E. (1944a) The vitamin B-6 group. I. Formation of additional members from pyridoxine and evidence concerning their structure. *J Am Chem Soc* 66: 2082-2088.

Snell, E.E. (1944b) The vitamin activities of pyridoxal and pyridoxamine. *J Biol Chem* 154: 313-314.

Snell, E.E. (1981) Vitamin B-6 analysis: some historical aspects. In: J.E. Leklem and R.D. Reynolds, (eds.), Methods in Vitamin B-6 Nutrition, Plenum, New York, pp. 1-19.

Snell, E.E., Guirard, B.M., Williams, R.J. (1942) Occurrence in natural products of a physiologically active metabolite of pyridoxine. *J Biol Chem* 143: 519-530.

Snell, E.E. and Haskell, B.E.(1971) In: Comprehensive Biochemistry. Florkin, M. and Stotz, E.H. (eds.), Elsevier, Amsterdam, 21: 47.

Snow, K.J., Shaw, M.A., Winer, L.M., Baumann, G. (1990) Diurnal pattern of plasma growth hormone-binding protein in man. *J Clin Endocrinol Metab* 70: 417-420.

Snyder, D.K., Clemmons, D.R., Underwood, L.E. (1988) Treatment of obese, diet-restricted subjects with growth hormone for 11 weeks: Effects on anabolism, lipolysis and body composition. *J Clin Endocrinol Metab* 67: 54-61.

Snyderman, S.E., Holt, L.E., Carretero, R., Jacobs, K. (1953) Pyridoxine deficiency in the human infant. *Am J Clin Nutr* 1: 200-207.

Solomon, L.R. (1982) Vitamin B-6 Metabolism in Human Red Cells: Limitations in Cofactor Activities of Pyridoxal and Pyridoxal 5'-Phosphate. *Enzyme* 28: 242-250.

Sonntag, W.E., Rorman, L.J., Miki, N., Meites, J. (1982) Growth hormone secretion and neuroendocrine regulation. In: Handbook of Endocrinology. Gass, G.H. and Kaplan, H.M.. CRC Press, Boca Raton, Florida, pg.35-59.

Spies, T.D., Bean, W.B., Ashe, W.F.(1939) A note on the use of vitamin B-6 in human nutrition. *JAMA* 112: 2414-2415.

Srivastava, S.S., Mani, K.V., Soni, C.M., Bhati, J. (1957) Effect of muscular exercises on urinary excretion of creatine and creatinine. *Ind J Med Res* 55: 953-960.

Staff, P.H. and Nilsson, S. (1971) Fluid and glucose ingestion during prolonged severe physical activity. *Tidsskrift for Den Norske Laegeforening* 16: 1235.

Stanley, W.C., Gertz, E.W., Wisneski, J.A., Morris, D.L., Neese, R.A., Brooks, G.A. (1985) Systemic lactate kinetics during graded exercise in man. *Am J Physiol* 249: E595-E602.

Stanley, W.C., Gertz, E.W., Wisneski, J.A., Neese, R.A., Morris, D.L., Brooks, G.A. (1986) Lactate extraction during net lactate release by the exercising legs of man. *J Appl Physiol* 60: 1116-1120.

Stanley, W.C., Wisneski, J.A., Gertz, E.W., Neese, R.A., Brooks, G.A. (1988) Glucose and lactate interrelations during moderate-intensity exercise in humans. *Metabolism* 37: 165-172.

Steele, R. (1986) The effects of growth hormone on carbohydrate and lipid metabolism in the dog. *Ann NY Acad Sci* 148: 441-458.

Stein, M., Dipnis, D.M., Daughaday, W.H. (1962) The effect of growth hormone on plasma insulin dynamics in man. *J Lab Clin Med* 60: 1022 (abstract).

Steinberg, S.E., Campbell, C.L, Hillman, R.S. (1979) Kinetics of the normal folate enterohepatic cycle. *J Clin Invest* 64: 83-88.

Stryer, L. (1981) Biochemistry. W.H. Freeman & Co.: San Francisco, CA, pgs. 408-420.

Sutton, J.R. (1978) Hormonal and metabolic responses to exercise in subjects of high and low work capacities. *Med Sci Sports* 10: 1-6.

Sutton, J.R. and Lazarus, L. (1976) Growth hormone in exercise: comparison of physiological and pharmacological stimuli. *J of Appl Physiol* 41: 523-527.

Sutton, J.R., Young, J.D., Lazarus, L., Hickie, J.B., Maksvytis, J. (1969) The hormonal response to physical exercise. *Aust Ann Med* 18: 84-90.

Suzue, R. and Tachibana, M. (1970) The uptake of pyridoxal phosphate by human red blood cells. *J Vitam* 16: 164-171.

Sygusch, J., Madsen, N.B., Kasvinsky, P.J., Fletterick, R.J. (1977) Location of pyridoxal phosphate in glycogen phosphorylase a. *Proc Natl Acad Sci USA* 74: 4757-61.

Tadera, K., Arima, M., Yoshino, S., Yagi, F., Kobayashi, A. (1986) Conversion of pyridoxine into 6-hydroxypyridoxine by food components, especially ascorbic acid. *J Nutr Sci Vitaminol* 32: 267-277.

Takagi, M., Fudui, T., Shimoura, S. (1982) catalytic mechanism of glycogen phosphorylase: pyridoxal (5') diphospho (1) -alpha-D-glucose as a transition-state analogue. *Proc Natl Acad Sci USA* 79: 3716-3719.

Takahashi, Y., Kipnis, M., Daughaday, W.H. (1968) Growth hormone secretion during sleep. *J Clin Invest* 47: 2079-2090.

Talbott, M.C., Miller, L.T., Kerkvliet, N.I. (1987) Pyridoxine supplementation: effect on lymphocyte responses in elderly persons. *Am J Clin Nutr* 46: 659-664.

Tanaka, H., Osaka, Y., Chikamori, K., Yamashita, S., Yamaguchi, H., Miyamoto, H. (1990) Dependence on exercise intensity of changes in electrolyte secretion from the skin sampled by a simple method. *Eur J Appl Physiol* 60: 407-411.

Tanner, J.M., Hughes, P.C.R., Whitehouse, R.H. (1977) Comparative rapidity of response of height, limb muscle and limb fat to treatment with human growth hormone in patients with and without growth hormone deficiency. *Acta Endocrinol* 84: 681-696.

Tar, A., Hocquette, J.F., Souberbielle, J.C., Clot, J.P., Brauner, R., Postel-Vinay, M.C. (1990) Evaluation of the growth hormone-binding protein in human plasma using high pressure liquid chromatography gel filtration. *J Clin Endocrinol Metab* 71: 1202-1207.

Tarnopolsky, M.A., MacDougall, J.D., Atkinson, S.A. (1988) Influence of protein intake and training status on nitrogen balance and lean mass. *J Appl Physiol* 64: 187-193.

Tarr, J.B., Tamura, T., Stokstud, E.L.R.(1981) Availability of vitamin B-6 and patothenate in an average diet in man. *Am J Clin Nutr* 34: 1328-1337.

Tartar, P., Kozlowski, S., Vigas, M., Nazar, K., Kvetnansky, R. (1984) Endocrine response to physical efforts with equivalent total workloads but different intensities in man. *Endocrinologia Experimentalis* 18: 233-239.

Taylor, A.W., Booth, M.A., Rao, S. (1972) Human skeletal muscle phosphorylase activities with exercise and training. *Can J Physiol Pharmacol* 50: 1038-1042.

Taylor, H.L., Buskirk, L.E., Henschel, A. (1955) Maximal oxygen intake as an objective measure of cardio-respiratory performance. *J Appl Physiol* 8: 73-80.

Thompson, R.G., Rodriquez, A., Kowarski, A., Blizzard, R.M. (1972) Growth hormone: metabolic clearance rates, integrated concentrations and production rates in normal adults and the effect of prednisone. *J Clin Invest* 51: 3193-3199.

Tillotson, J.A., Sauberlich, H.E., Baker, E.M., Canham, J.E. (1966) Use of carbon-14 labeled vitamins in human nutrition studies: Pyridoxine. *Proc 7th Internat Congr Nutr* 5: 554-557.

Titani, K., Koide, A., Hermann, J., Ericsson, L.H., Kumar, S., Wade, R.D., Walsh, K.A., Neurath, H., Fischer, E.H. (1977) Complete amino acid sequence of rabbit muscle glycogen phosphorylase. *Proc Natl Acad Sci USA* 74: 4762-4766.

Tolis, G., Laliberte, R., Guyda, H., Naftolin, F. (1976) Ineffectiveness of pyridoxine to alter secretion of growth hormone and prolactin and absence of therapeutic effects on glactorrhea-amenorrhea syndromes. *J Clin Endocrinol Metab* 44: 1197-1199.

Trinder, P. (1969) Determination of blood glucose using an oxidase-peroxidase system with a non-carcinogenic chromagen. *J Clin Path* 22: 158-161.

Trumbo, P.R. and Gregory, J.F. (1988) Metabolic utilization of pyridoxine-β-glucoside in rats: influence of vitamin B-6 status and route of administration. *J Nutr* 118: 1336-1342.

Trumbo, P.R. and Gregory, J.F. (1989) The fate of dietary pyridoxine-β-glucoside in the lactating rat. *J Nutr* 119: 36-39.

Trumbo, P.R., Gregory, J.F. and Sartain, D.B. (1988) Incomplete utilization of pyridoxine-b-glucoside as vitamin B-6 in the rat. *J Nutr* 118: 170-175.

Tsuji, H., Okada, J., Iwami, K., Yasumoto, K., Mitsuda, H. (1977) Availability of vitamin B-6 and small intestinal absorption of pyridoxine-β-D-glucoside in rats. *Vitamins* 51: 153-159.

Turner, M.R., Reeds, P.J., Munday, K.A. (1976) Action of growth hormone in vitro on the net uptake and incorporation into protein of amino acids in muscle from intact rabbits given protein-deficient diets. *Br J Nutr* 35: 1-10.

Ubbink, J.B., Serfontein, W.J., Becker, P.J., de Villiers, L.S. (1987) Effect of different levels of oral pyridoxine supplementation on plasma pyridoxal-5'-phosphate and pyridoxal levels and urinary vitamin B-6 excretion. *Am J Clin Nutr* 46: 78-85.

Undenfriend, S. (1964) Amino acid decarboxylation steps in the biosynthesis of norepinephrine, serotonin and histamine. *Vitam Horm (N.Y.)* 22:445-450.

Upton, S.J., Hagen, R.D., Lease, B., Rosentswieg, J., Gettman, L.R., Duncan, J.J. (1984) Comparative physiological profiles among young and middle-aged female distance runners. *Med Sci Sports Exerc* 16: 67-71.

Uyttendaele, K., DeGroote, M., Blaton, U., Peeters, H. (1977) Analysis of the proteins in sweat and urine by agarose-gel isotachophoresis. *J Chromatogr* 132: 261-266.

Vaccaro, P., Morris, A.F., Clarke, D.H. (1981) Physiological characteristics of masters female distance runners. *Phys Sportsmed* 9: 105-108.

Van Cauter, E., Kerkhofs, M., Caufriez, A., Van Onderbergen, A., Thorner, M.O., Copinschi, G. (1992) A quantitative estimation of growth hormone secretion in

normal man: reproducibility and relation to sleep and time of day. *J Clin Endocrinol Metab* 74: 1441-1450.

van Beaumont, W. (1972) Evaluation of hemoconcentration from hematocrit measurements. *J Appl Physiol* 32: 712-713.

van Beaumont, W., Greenleaf, J.E., Juhos, L. (1972) Disproportional changes in hematocrit, plasma volume and proteins during exercise and bed rest. *J Appl Physiol* 33:55-61.

van den Berg, H., Mulder, J., Spanhaak, S., van Dokkum, W., Ockhuizen, T. (1988) The influence of marginal vitamin B-6 status on immunological indicies. In: Leklem, J.E., Reynolds, R.D. (eds.) Clinical and Physiological Applications of Vitamin B-6. A.R. Liss: New York, pgs.147-155.

Vander, A.J., Sherman, J.H., Luciano, D.S. (1985) Human Physiology: The Mechanisms of Body Function. McGraw-Hill Pub.: New York, pg. 304.

Vanhelder, W.P., Goode, R.C., Radomski, M.W. (1984) Effect of anaerobic and aerobic exercise of equal duration and work expenditure on plasma growth hormone levels. *Eur J Appl Physiol* 52: 255-257.

Verde, T., Roy, J., Shephard, P.C., Moore, R. (1982) Sweat composition in exercise and in heat. *J. Appl. Physiol: Respirat.Environ Exercise Physiol* 53: 1540-1545.

Virk, R. (1991) The effect of vitamin B-6 supplementation on fuel utilization during exhaustive exercise. Masters Thesis. Oregon State University. Department of Nutrition and Food Management. Corvallis, Oregon.

VonDoebln, W. (1956) Maximal oxygen intake, body size and total haemoglobin in normal man. *Acta Physiol Scand* 38: 193-199.

Wada, H., Morisue, T., Nishimuru, Y, Morino, Y., Sakamoto, Y., Ichihara, K. (1959) Enzymatic studies on pyridoxine metabolism. *Proc Jap Acad* 35: 299-304.

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

Wang, X., Muller, C., Norstedt, G., Carter, Su.C. (1993) Growth hormone-promotied tyrosyl phosphorylation of a 121-kDa growth hormone receptor-associated protein. *J Biol Chem* 268: 3573-3579.

Wang, X., Uhler, M.D., Billestrup, N., Norstedt, G., Talamantes, F., Nielsen, J.H., Carter, Su.C. (1992) Evidence for association of the cloned liver growth hormone receptor with a tyrosine kinase. *J Biol Chem* 267: 17390-17396.

Ward, H.C., Halliday, D., Sim, A.J. (1987) Protein and energy metabolism with biosynthetic human growth hormone after gastrointestinal surgery. *Ann Surg* 206: 56-61.

Wasserman, K., Whipp, B.J., Koyal, S.N., W.L. Bexter (1973) Anaerobic threshold and respiratory gas exchange during exercise. *J Appl Physiol* 35: 236-243.

Wasynczuk, A., Kirksey, A., Morre, D.M. (1983a) Effect of maternal vitamin B-6 deficiency on specific regions of developing rat brain: amino acid metabolism. *J Nutr* 113: 735-745.

Wasynczuk, A., Kirksey, A., Morre, D.M. (1983b) Effects of vitamin B-6 deficiency on specific regions of developing rat brain: the extrapyramidal motor system. *J Nutr* 113: 746-754.

Webster, S.F., Rutt, R.A., Weltman, A. (1988) Effects of typical dehydration practices on performance. *Med Sci Sports Exer* 20: S20 (abstract #117).

Wehrenberg, W.B., Ling, N., Brazeau, P., et al. (1982) Somatocrinin, growth hormone releasing factor, stimulates secretion of growth hormone in anesthetized rats. *Biochem Biophys Res Commun* 109: 382-387.

Wenger, H.A., Wilkinson, J.G., Dallaire, J. (1981) Uptake of ^{3}H-leucine into different fractions of rat skeletal muscle following acute endurance and sprint exercise. *Eur J Appl Physiol.* 47: 83-92.

White, T.P. and Brooks, G.A. (1981) (u-^{14}C) glucose, -alanine and -leucine oxidation in rats at rest and two intensities of running. *Am J Physiol* 240: E155-E165.

Whyte, M.P., Mauren, J.D., Fedde, K.N., Cole, F.S., McCabe, E.R.B., Coburn, S.P. (1988) Perinatal hypophosphatasia: tissue levels of vitamin B-6 are unremarkable despite markedly increased circulating concentrations of pyridoxal 5'-phosphate (evidence for an ectoenzyme role for tissue nonspecific alkaline phosphatase) *J Clin Invest* 81: 1234-1239.

Whyte, M.P., Mauren, J.D., Vrabel, L.A., Coburn, S.P. (1985) Markedly increased circulating pyridoxal 5'-phosphate levels in hypophosphatasia. *J Clin Invest* 76: 752-756.

Wilkerson, J.E., Gutin, B., Horvath, S.M. (1977) Exercise-induced changes in blood, red cell, and plasma volumes in man. *Med Sci in Sports* 9: 155-158.

Wilkinson, J.G. and Wenger, H.A. (1979) ^{14}C-uridine uptake into skeletal muscle RNA after acute endurance and sprint exercise. *Can J Appl Sport Sci* 4: 243 (abstract).

Williams, J.H. (1991) Caffeine, neuromuscular function and high-intensity exercise performance. *J Sports Med Phys Fitness* 31: 481-489.

Winer, L.M., Shaw, M.A. and Baumann, G. (1990) Basal plasma growth hormone levels in man: new evidence for rhythmicity of growth hormone secretion. *J Clin Endocrinol Metab* 70: 1678-1686.

Witten, P.W. and Holman, R.T. (1952) Polyethenoid fatty acid metabolism. VI. Effect of pyridoxine on essential fatty acid conversions. *Arc Biochem Biophys* 41: 266-273.

Woodring, M.J. and Storvick, C.A. (1960) Vitamin B-6 in milk: review of literature. *J Assoc Off Agric Chem* 43: 63-80.

Wozenski, J.R., Leklem, J.E., Miller, L. (1980) The metabolism of small doses of vitamin B-6 in man. *J Nutr* 110: 275-285.

Wyndham, C.H. and Hegns, A.J.A. (1969) Determinants of oxygen consumption and maximum oxygen intake of caucasians and Bantu males. *Int J Angew Physiol* 27: 51.

Yalow, R.S., Goldsmith, S.J., Berson, S.A. (1969) Influence of physiologic flucuations in plasma growth hormone on glucose tolerance. *Diabetes* 18: 402-408.

Yamada, K. and Tsuji, M. (1968) Transport of vitamin B-6 in human erythrocytes. *J Vitaminol* 14: 282-284.

Yip, R., Johnson, C., Dallman, P.R. (1984) Age-related changes in laboratory values used in the diagnosis of anemia and iron deficiency. *Am J Clin Nutr* 39: 427-436.

Young, D.R., Pelligra, R., Adachi, R.R. (1966) Serum glucose and free fatty acids in man during prolonged exercise. *J Appl Physiol* 21:1047-1052.

Zapf, J., Waldvogel, M., Froesch, E.R. (1975) Binding of nonsuppressible insulin-like activity to human serum: Evidence for a carrier protein. *Arch Biochem Biophys* 168: 638-645.

Zapf, J., Walter, H., Froesch, E.R. (1981) Radioimmunological determination of insulin-like growth factors I and II in normal subjects and in patients with growth hormone disorders and extrapancreatic tumor hypoglycemia. *J Clin Invest* 68: 1321-1330.

Zierler, K.L. and Rabinowitz, D. (1963) Roles of insulin and growth hormone, based on studies of forearm metabolism in man. *Medicine* 42: 385.

APPENDICES

APPENDIX A

Figure A.1. Health/diet history

Dept. Nutrition & Food Mgmt.
Oregon State University
Today's Date_____

Project Name_____
Project Dates_____
Code #_____

I. DEMOGRAPHIC DATA

Age:____ Date of Birth:_____ Birthplace:_____
Sex:____
Present Residence:_____
Present Employment:_____
Race(circle one):a. American Indian b. Black
c. Caucasian d. Hispanic
e. Chinese f. Japanese
g. Polynesian/Pacific Islander h. Other Asian(specify)_____
i. Other(specify)_____
Marital Status (circle one): a. Single b. Married c. Divorced/Separated d. Widowed
How many people live in your household?_____
Do you have any children?___Yes ___No If yes, give ages_____

II. FEMALES:

Menstrual & Reproductive History

When did your last menstrual period begin?_____
Do you have regular menstrual periods? ____Yes ____No
How long is your menstrual cycle?_____
Do you have problems with your menstruation? ____Yes ____No If yes, please explain:

Are you pregnant? ____Yes ____No Breastfeeding? ____Yes ____No
Have you ever been pregnant? ____Yes ____No If yes, how many times? ____
How many children have you carried?_____
Please check if you have had any of the following complications of pregnancy:
___1. hyperemesis gravidarum (morning sickness)
___2. pre-eclampsia or eclampsia (toxemia)
___3. high blood pressure
___4. severe edema (swelling of the legs and feet)
___5. numbness and tingling in your hands, wrists, or arms
___6. gestational diabetes
___7. premature birth(s)-please indicate gestational age of infant(s)_____
___8. kidney or bladder infections
___9. premature rupture of membranes
___10.small for date infant (less than 5 lbs at term)

III. SUBJECT CHARACTERISTICS

Height/Weight: Height_____ Present weight:_____
Most ever Weighed_____ What year?_____
Length of time you have maintained current weight_____

IV. DIETARY HISTORY

Dieting: Are you currently following a special diet?_____
If yes, for what purpose? (check as many as apply)
___1. weight loss ___2. weight gain ___3. control serum lipids
___4. diabetes ___5. kidney failure ___6. ulcers
___7. diverticulitis ___8. allergies ___9. heart trouble
___10. pregnancy ___11. high blood pressure ___12. breastfeeding
___13. other (specify):_____

If you are following a diet, was it prescribed by a doctor/dietitian/nurse?_____
If you are following a diet, what kind is it?(check as many as apply)
___1. lowfat ___2. low protein ___3. high protein ___4. low salt
___5. low carbohydrate ___6. low sugar ___7. low calorie ___8. high calorie
___9. low cholesterol ___10. bland diet ___11.other(specify):_____
If you are currently consuming a special diet, for how long have you been consuming this diet?_____

If you are dieting, is your dieting associated with any commercial weight loss program?_____ If yes, please specify:

Are you a vegetarian?_____ If yes, circle the type of vegetarian diet you follow:
a. ovo-lacto b. ovo c. lacto d. vegan

Supplementation:
Do you take vitamins? (circle one): a. yes,daily b. yes, frequently(3-6 times/week)
c. often (once or twice/week) d. occasionally (less than once/week) e. never

If yes, what type, how much, and for how long have you taken them?

Type	Amount/day	Length taken

Do you take any other nutritional supplements (such as iron, calcium, other minerals, amino acids, fiber, supplement drinks (such as Ensure, etc.) ___Yes ___No

Type	Amount/day	Length taken

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

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

V.HABITS

A. Smoking:

1) Do you currently smoke? ___Yes ___No If yes, please check what you smoke, and how much per day: cigarettes_____ # per day_____
cigars_____ # per day_____
pipe_____ # loads per day_____
At what age did you start smoking?_____

2) If you do not currently smoke, did you ever smoke? ____Yes ____No
If yes, at what age did you start?_____ When did you quit?_____
Have you quit other times?____Yes ____No
If you quit, please check below what you did smoke, and how much per day:
cigarettes_____ # per day_____
cigars_____ # per day_____
pipe_____ # loads per day_____

3) Does anyone else in your household smoke? ____Yes ____No If yes, what and and how much? cigarettes_____ # per day_____
cigars_____ # per day_____
pipe_____ # loads per day_____

B. Alcohol:

1) Do you drink alcoholic beverages? ____Yes ____No If yes, how many times do you drink per month?_____ If yes, what do you drink and how many drinks do you consume each time you drink? Beer____ # at one time____
Wine_____ # at one time____ ; Liquor____ # at one time_____ ; Other____
per time_____

C. Caffeine:

1) Do you drink beverages containing caffeine? ____Yes ____No
If yes, which and how much of the following do you drink? Coffee_____
Tea_____ Soda_____
2) Do you drink any decaffeinated or caffeine-free beverages? ___Yes ___No
If yes, which of the following beverages do you drink, and how much?
Coffee_____ Tea_____ Soda_____

D. Diet Soda Pop and other Sugarless Beverages

1)Do you drink any beverages containing artificial sweeteners? ____Yes ____No
If yes, what do you drink and how many drinks (ounces, servings) per day?

E. Exercise:

Are you currently involved in a regular exercise program? ____Yes ____No If yes, describe:

Type of exercise # minutes(continuous) Distance covered(repetitions) # days/wk

Do you monitor your heart rate during exercise? ____Yes ____No If yes, what heart rate do you try to maintain while exercising?_____

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

VI.MEDICAL HISTORY:

Have you ever had a glucose tolerance test? ____Yes ____No If yes, please explain when, why and the results:

Have you ever had a stress electrocardiogram? ____Yes ____No If yes, please explain when, why and the results:

Have you ever had any health risk screening tests, such as serum cholesterol, blood glucose or blood pressure? ____Yes ____No If yes, please explain what tests you had, and what the results and recommendations you received:

A. Clinical History (Check any condition for which you have been diagnosed and give age at diagnosis):

	Diagnosis	Age at Diagnosis
___	1. acquired immunodeficiency syndrome (AIDS)	
___	2. diabetes	
___	3. hypoglycemia	
___	4. hypothyroidism	
___	5. hyperthyroidism	
___	6. goiter	
___	7. osteoporosis	
___	8. hepatitis	

Age at Diagnosis

___ 9. cirrhosis_____
___10. kidney stones_____
___11. nephritis_____
___12. cystitis_____
___13. high blood pressure_____
___14.angina_____
___15.ulcer_____
___16.pancreatitis_____
___17.ulcerative colitis_____
___18.recurring gastritis_____
___19.allergies/hayfever_____
___20.hypoadrenalism(Addison's Disease)_____
___21. spastic colon/diverticulitis_____
___22.carpal tunnel syndrome_____
___23.rheumatoid arthritis_____
___24.systemic lupus erythematosus_____
___25.mental depression requiring regular medication_____
___26.asthma_____
___27.insomnia requiring frequent medication_____
___28.emphysema_____
___29.heart problems_____
___30.cancer(specify)_____
___31.chronic infection(specify)_____
___32.tuberculosis_____
___33.chronic headache or other pain(specify)_____
___34.hereditary condition(specify)_____
___35.premenstrual syndrome_____
___36.other condition(specify)_____

Comments:

Are you currently suffering from any cold, flu, or allergy symptoms? ___Yes ___No
If yes, please specify:

Do any of your first-degree relatives (mother,father,brother,sister,son,daughter) have any of the following conditions? ___Yes ___No If yes, indicate which condition and their relationship to you: 1)diabetes 2)heart disease before age 60 3)cancer before age 60 4)high blood pressure before age 60 5)allergies

Have you ever had a nerve conduction/muscle stimulation study? ___Yes ___No If yes, when, for what reason and what were the results?

Have you ever had any other special diagnostic tests (such as special X-ray studies or a CAT-scan) ___Yes ___No If yes, please specify:

B. Surgical History (Please specify any type of surgery you have had and the date and age when it occurred):

Operation	Age or Year

C. Medication History (Check any which you take on a regular basis and when and how often):

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

How long did you fast prior to having your blood drawn? _____hrs.

Comments:

Checked by: Date:

Figure A.2.

MAX VO2 DATA FORM

Name_____ Date_____

Weight_____ Age_____ Max HR_____bpm

Warm-up_____kg _____rpm_____watts_____min

Min (*)	kg	rpm	watts	HR bpm	BP mm/Hg	RPE	Notes
0-1							
1-2							
2-3							
3-4							
4-5							
5-6							
6-7							
7-8							
8-9							
9-10							
10-11							
11-12							
12-13							
13-14							
14-15							

* real clock time on monitor when starting the exercise portion of data collection

Comments:

Figure A.3.

PRACTICE RIDE DATA FORM

Name_____ Date_____ Weight_____
Peak max HR_____ VO_2 max_____ Target VO_2_____

Warm-up_____kg _____min _____rpm; starting workload_____kg

Time	kg	HR	VO_2	VO_2	VO_2	RPE
0-1						
1-2						
2-3						
3-4						
4-5						
5-6						
6-7						
7-8						
8-9						
9-10						
10-11						
11-12						
12-13						
13-14						
14-15						
15-16						
16-17						
17-18						
18-19						
19-20						

Time = minutes
VO_2 data = 20 second measurements

Figure A.4

ENDURANCE TEST DATA SHEET

Name_____
Date_____
Test number_____

min	VO_2 R	VO_2 R	VO_2 R	VO_2 R	VO_2 R	VO_2 R	VO_2 R	VO_2 R	VO_2 R	RPE
0-10										
10-20										
20-30										
30-40										
40-50										
50-60										
60-70										
70-80										
80-90										
90-100										
100-110										
110-120										
120-130										
130-140										

Table B.1. Physical and exercise characteristics of the subjects in study 1 and study 2.

Study	Subject	Age (yrs)	Body Weight (kg)	Height (cm)	% Body Fat	VO_2 Max (L/min)	VO_2 Max (mL/kg/min)	Time to Exhaustion (min) NS	Time to Exhaustion (min) S
1	1	34	78.3	178	9.5	4.68	59.7	131.8	160.5
	2	19	75.6	178	16.4	4.86	69.5	91.7	54.0
	3	37	74.1	183	12.0	4.53	61.4	120.4	149.4
	4	21	70.5	176	12.1	4.09	59.0	114.9	126.3
	5	22	71.4	181	9.5	3.61	45.6	140.3	134.5
	6	25	79.1	180	15.8	4.64	61.6	124.8	124.5
2	1	25	79.7	189	5.0	4.86	61.0	130.5	110.3
	2	27	73.9	183	11.8	4.65	62.9	53.7	22.3
	3	37	104.4	191	23.8	4.55	43.6	52.3	55.8
	4	21	74.6	188	9.7	4.28	57.4	92.0	80.9
	5	36	82.3	177	13.5	4.21	51.1	146.1	190.3

Study 1 refers to the 1990 vitamin B-6 and exercise study; Study 2 refers to the 1992 vitamin B-6 and exercise study.
% Body fat was measured hydrostatically mid-study. VO_2 max was measured on a cycle ergometer pre-study.
NS refers to the exercise test without vitamin B-6 supplementation; S refers to the exercise test under vitamin B-6 supplementation conditions.

Table B.2. Individual activity logs (intensity x duration) (study 1).

Subject	NS	10/15/90	10/16	10/17	10/18	10/19	10/20	10/21	10/22	10/23	10/24	10/25	10/26
	S	11/8/90	11/9	11/10	11/11	11/12	11/13	11/14	11/15	11/16	11/17	11/18	11/19
1	NS	540	240	788	300	450	1755	---	EX				
	S	300	---	854	2880	240	---	EX					
2	NS				405	765	840	735	270	---	EX		
	S					420	455	720	---	EX	300		
3	NS				705	1778	473	645	1844	---	EX		
	S			1440	500	668	390	355	240	EX	110		
4	NS					195	720	480	920	720	645	EX	720
	S				720	425	840	366	900	540	EX	660	
5	NS					690	2430	540	180	60	60	EX	---
	S				458	120	90	210	237	60	EX	180	
6	NS			1740	960	1800	1560	---	360	EX	960	540	EX
	S		540	900	120	480	210	360	EX	840			

Values represent daily activity (intensity (1-10) x duration (minutes)). NS refers to exercise test 1 in non-supplemented state; S refers to exercise test 2 in the vitamin B-6 supplemented state

Table B.3. Individual activity logs (intensity x duration) (study 2).

Subject	NS	7/14/92	7/15	7/16	7/17	7/18	7/19	7/20	7/21	7/22	7/23	7/24			
	S	8/7/92	8/8	8/9	8/10	8/11	8/12	8/13	8/14	8/15	8/16	8/17	8/18	8/19	8/20
1	NS	1110	780	2130	960	---	---	EX	1090	330					
	S	1515	255	360	1410	1320	---	EX	495	480	---	---	---	270	690
3	NS	---	---	---	800	---	600	---	EX	---	---	---			
	S	---	---	900	900	---	690	---	---	1050	300	EX	---	---	---
4	NS	---	---	1020	540	1020	1080	1080	---	EX	720	---			
	S	---	---	---	---	---	---	960	---	960	---	EX	---	1080	---
5	NS	---	---	1200	---	900	1260	825	---	EX	---	1080			
	S	---	---	---	---	---	1080	720	---	---	1260	---	EX	---	---

Values represent daily activity (intensity (1-10) x duration (minutes)). NS refers to exercise test 1 in non-supplemented state; S refers to exercise test 2 in the vitamin B-6 supplemented state

Table B.4. Individual kilocalories consumed (study 1).

Subject	NS	10/16/90	10/17	10/18	10/19	10/20	10/21	10/22	10/23	10/24	10/25	10/26
	S	11/8/90	11/9	11/10	11/11	11/12	11/13	11/14	11/15	11/16	11/17	11/18
1	NS	3626	3554	3554	3554	3476	3554	3616	3554			
	S	3726	3903	4406	4390	4349	3476	3476	3476			
2	NS			3633	3605	3515	3763	3756	3665	3570	3661	
	S			3476	3615	3722	3680	3695	3818	3637	3733	3792
3	NS			3707	3808	3640	3975	3731	3845	3950	3806	
	S			3823	3773	3755	3398	3897	3728	3958	3684	
4	NS				3976	3641	3630	3639	3684	3732	3733	3826
	S				3579	3732	3572	3554	3566	3499	3648	3546
5	NS				3647	3647	3945	3813	3865	3734	3850	3676
	S				3789	4155	3655	4153	3619	3963	3593	4143
6	NS		3665	3714	3631	3611	3777	3651	3600	3704	3603	3591
	S		3767	3774	3722	3780	3728	3623	3933	3717		

Values represent kilocalories consumed from the diet and other sources (margarine, salad dressing, candy, pop));
NS refers to exercise test 1 in the non-supplemented state; S refers to exercise test 2 in the B-6 supplemented state

Table B.5. Individual kilocalories consumed (study 2).

Subject	NS	7/14/92	7/15	7/16	7/17	7/18	7/19	7/20	7/21	7/22	7/23	7/24				
	S	8/7/92	8/8	8/9	8/10	8/11	8/12	8/13	8/14	8/15	8/16	8/17	8/18	8/19	8/20	8/21
								kcal								
1	NS	3629	3758	4197	4074	4246	3836	3811	4199	3744	---	---				
	S	4154	4881	4632	4400	4456	3994	3739	4885	3701	4707	4768	3624	4116	4009	---
2	NS	---	3652	3690	3749	3876	3913	3752	3760	3955	3544	---				
	S	3427	3505	3466	3505	3466	3544	3466	3544	3544	3778	3544	3544	3466	3505	3505
3	NS	---	3655	3534	3862	3854	3717	4023	3694	4185	3817	---				
	S	---	3395	3820	3854	3851	3853	3847	4211	3851	3693	3854	3853	3609	3518	---
4	NS	---	---	3753	3991	3794	4238	4289	3884	4007	3870	3934				
	S	---	---	---	---	3847	3847	4029	4224	4066	3788	4029	4066	4045	---	---
5	NS	---	---	3506	3751	3898	3898	3898	3751	3604	3310	3898				
	S	---	---	---	---	---	3898	3898	3898	3898	3898	3310	3310	3310	3310	---

Values represent kilocalories consumed from metabolic diet and other calorie sources (margarine, salad dressing, candy, pop))
NS refers to exercise test 1 in the non-supplemented state; S refers to exercise test 2 in the vitamin B-6 supplemented state

Table B.6. Individual hemoglobin concentration (study 1).

Subject	Pre Study	Test	PRE	DX	POST	POST 60
				gm/L		
1	135	NS	150	159	165	152
		S	133	148	149	139
2	156	NS	147	165	161	161
		S	150	----	158	151
3	151	NS	144	157	157	156
		S	145	155	160	
4	149	NS	143	157	160	156
		S	143	159	159	150
5	167	NS	161	176	180	166
		S	161	177	173	162
6	151	NS	150	161	156	150
		S	142	157	152	145

Test refers to the two exhaustive exercise rides (non-supplemented(NS) and vitamin B-6 supplemented(S))
PRE refers to the mean value prior to exercise ; DX refers to 60 minutes during exercise; POST refers to immediately after exercise and POST 60 refers to 60 minutes after exercise

Table B.7. Individual hemoglobin concentration (study 2).

Subject	Pre Study	Test	PRE	DX	POST	POST 60
				g/L		
1	152	NS	150	157	156	152
		S	147	163	158	153
2	137	NS	147	---	165	150
		S	138	---	160	143
3	163	NS	144	---	163	154
		S	154	---	176	165
4	169	NS	160	172	171	164
		S	171	185	183	170
5	148	NS	141	151	153	151
		S	143	160	161	151

Test refers to the two exhaustive exercise rides (non-supplemented(NS) and vitamin B-6 supplemented(S))
PRE refers to the mean value prior to exercise (time 0 and 30 minutes of rest), DX refers to 60 minutes during exercise, POST refers to immediately after exercise and POST 60 refers to 60 minutes after exercise

Table B.8. Individual hematocrit (study 1).

Subject	Pre Study	Test	PRE	DX	POST	POST 60
				%		
1	39.6	NS	43.2	45.5	45.5	44.0
		S	40.2	43.5	43.3	40.7
2	45.8	NS	45.5	48.5	48.0	47.3
		S	46.2		48.5	46.5
3	44.5	NS	44.3	46.5	44.5	46.5
		S	44.3	46.3	46.5	45.5
4	43.5	NS	43.5	45.7	46.5	44.0
		S	44.2	46.5	46.3	44.5
5	47.5	NS	47.0	48.5	49.7	47.5
		S	47.8	51.0	50.0	47.5
6	41.4	NS	43.7	45.5	44.0	42.0
		S	43.5	46.3	44.5	42.8

Test refers to the two exhaustive exercise rides (non-supplemented(NS) and vitamin B-6 supplemented(S))
PRE refers to the mean value prior to exercise, DX refers to 60 minutes during exercise, POST refers to immediately after exercise and POST 60 refers to 60 minutes after exercise

Table B.9. Individual hematocrit (study 2).

Subject	Pre Study	Test	PRE	DX	POST	POST 60
				%		
1	46.0	NS	47.0	47.5	46.3	45.5
		S	44.9	47.0	47.5	46.0
2	42.5	NS	44.8	---	48.5	43.0
		S	41.1	---	47.5	41.5
3	45.5	NS	41.3	---	46.5	44.3
		S	43.0	---	48.0	44.5
4	49.5	NS	48.5	50.8	50.5	47.5
		S	50.5	53.0	52.5	49.3
5	43.5	NS	41.9	44.5	42.3	42.5
		S	42.5	46.0	45.5	43.5

Test refers to the two exhaustive exercise rides (non-supplemented(NS) and vitamin B-6 supplemented(S))
PRE refers to the mean value prior to exercise (time 0 and 30 minutes of rest), DX refers to 60 minutes during exercise, POST refers to immediately after exercise and POST 60 refers to 60 minutes after exercise

Table B.10. Individual plasma volume changes (study 1).

		DC			VB		
Subject	Test	DX	POST	POST 60	DX	POST	POST 60
			%			%	
1	NS	-9.99	-13.3	-2.95	-9.01	-9.01	-3.32
	S	-14.9	-15.1	-5.25	-12.8	-12.2	-2.05
2	NS	-9.65	-4.37	2.95	-7.18	-1.37	7.02
	S	-14.1	-7.94	-1.39	-10.8	-3.98	2.77
3	NS	-11.5	-8.36	-11.3	-8.38	-0.69	-8.38
	S	-10.0	-13.3	---	-7.75	-8.38	-4.62
4	NS	-15.4	-12.6	-11.5	-11.4	-9.56	-7.09
	S	---	-9.26	-1.62	---	-8.96	-1.36
5	NS	-12.5	-15.4	-9.15	-8.37	-11.4	-2.01
	S	-13.9	-13.4	-5.24	-9.01	-8.39	-1.37
6	NS	-10.9	-15.2	-4.04	-5.84	-10.1	-1.99
	S	-14.2	-10.8	0.45	-11.9	-8.32	1.33

All values are % changes relative to a pre-exercise value. DC refers to the percent plasma volume change calculated by the Dill and Costill method (1974). VB refers to the percent plasma volume change calculated by the van Beaumont method(1972)
DX refers to 60 minutes during exercise, POST refers to immediately after exercise and POST 60 refers to 60 minutes after exercise
Test refers to the two exhaustive exercise rides (NS refers to test 1 in the non-supplemented state, S refers to test 2 in the vitamin B-6 supplemented state)

Table B.11. Individual plasma volume changes (study 2).

		DC			VB		
Subject	Test	DX	POST	POST 60	DX	POST	POST 60
			%			%	
1	NS	-5.11	-2.16	1.75	-1.86	3.06	6.22
	S	-13.2	-10.6	-5.51	-8.22	-9.09	-4.46
2	NS	---	-16.8	0.77	---	-14.0	7.37
	S	---	-23.6	-4.47	---	-22.8	-1.56
3	NS	---	-19.3	-11.7	---	-18.4	-11.5
	S	---	-19.9	-8.89	---	-18.3	-5.91
4	NS	-11.1	-10.0	-0.49	-8.61	-7.69	4.09
	S	-12.3	-10.4	2.77	-9.53	-7.70	5.13
5	NS	-10.9	-8.32	-7.20	-10.2	-1.55	-2.55
	S	-16.2	-16.1	-7.33	-13.23	-11.5	-4.00

All values are % changes relative to a pre-exercise value
DC refers to the percent plasma volume change calculated by the Dill and Costill method (1974); VB refers to the percent plasma volume change calculated by the van Beaumont method (1972)
DX refers to 60 minutes during exercise, POST refers to immediately after exercise and POST 60 refers to 60 minutes after exercise
Test refers to the two exhaustive exercise rides (NS refers to test 1 in the non-supplemented state and S refers to test 2 in the vitamin B-6 supplemented state).

Table B.12. Individual plasma albumin concentration (study 2).

Subject	Test	PRE	DX	POST	POST 60
			g/L		
1	(NS)	59.3	63.8	62.5	61.7
	(S)	57.5	64.5	63.5	60.0
2	(NS)	53.1	---	60.3	50.6
	(S)	50.8	---	59.2	52.0
3	(NS)	51.5	---	59.0	56.9
	(S)	55.1	---	65.9	56.3
4	(NS)	59.7	64.5	62.5	59.9
	(S)	59.0	66.3	63.2	60.1
5	(NS)	53.5	60.2	61.3	56.5
	(S)	51.0	59.7	61.1	53.0

Test refers to the two exhaustive exercise rides in the non-supplemented(NS) and vitamin B-6 supplemented(S)).
PRE refers to the mean value prior to exercise (time 0 and 30 minutes of rest), DX refers to 60 minutes during exercise, POST refers to immediately after exercise and POST 60 refers to 60 minutes after exercise.

Table B.13. Individual plasma pyridoxal 5'-phosphate concentration (study 1).

Test	Subject	Pre Study	PRE	DX	POST	POST 60
				nmol/L		
NS	1	37.8	43.1	44.9	39.7	28.2
	2	20.8	20.7	24.6	21.7	20.9
	3	46.9	36.7	41.9	35.1	27.3
	4	44.9	31.9	38.1	30.2	26.1
	5	16.1	12.6	17.5	8.2	9.9
	6	44.7	43.5	51.0	45.1	39.3
S	1		203	252	177	145
	2		163	---	177	150
	3		249	262	225	171
	4		263	367	265	230
	5		164	190	157	141
	6		152	236	206	168

Test refers to the two exhaustive exercise rides (non-supplemented(NS) and vitamin B-6 supplemented(S)).
Pre Study refers to prior to the start of the study, PRE refers to prior to exercise, DX refers to 60 minutes during exercise, POST refers to immediately after exercise and POST 60 refers to 60 minutes after exercise.

Table B.14. Individual plasma pyridoxal 5'-phosphate concentration (study 2).

Test	Subject	Pre Study	PRE	DX	POST	POST 60
				nmol/L		
NS	1	59.4	37.0	38.7	30.0	29.4
	2	209	29.4	---	36.4	28.7
	3	18.5	17.6	---	23.5	15.9
	4	49.5	32.7	34.9	29.8	24.3
	5	130	45.1	53.8	43.3	36.4
S	1		177	193	174	156
	2		198	---	227	173
	3		133	---	157	126
	4		170	169	179	144
	5		183	204	159	132

Test refers to the two exhaustive exercise rides (non-supplemented(NS) and vitamin B-6 supplemented(S)).
Pre Study refers to prior to the start of the study, PRE refers to the mean value prior to exercise (time 0 and 30 minutes of rest), DX refers to 60 minutes during exercise, POST refers to immediately after exercise and POST 60- 60 minutes after exercise.

Table B.15. Individual plasma total vitamin B-6 concentration (study 2).

Test	Subject	Pre Study	PRE	DX	POST	POST 60
				nmol/L		
NS	1	90.7	49.3	53.7	47.5	35.5
	2	235	36.8	---	52.8	43.7
	3	49.9	34.9	---	46.1	39.0
	4	61.9	50.9	62.1	57.2	51.4
	5	208	79.6	84.4	77.5	62.8
S	1		220	247	234	262
	2		288	---	299	240
	3		196	---	270	218
	4		248	327	263	229
	5		250	320	241	220

Test refers to the two exhaustive exercise rides (non-supplemented(NS) and vitamin B-6 supplemented(S)).
Pre Study-prior to the start of the study; PRE refers to the mean value prior to exercise (time 0 and 30 minutes of rest),
DX refers to 60 minutes during exercise, POST refers to immediately after exercise and POST 60 refers to 60 minutes after exercise.

Table B.16. Individual plasma pyridoxal 5'-phosphate: plasma vitamin B-6 ratio (study 2).

Test	Subject	PRE	DX	POST	POST 60
NS	1	0.76	0.72	0.63	0.83
	2	0.82	---	0.69	0.66
	3	0.51	---	0.51	0.41
	4	0.65	0.56	0.52	0.47
	5	0.57	0.64	0.56	0.58
S	1	0.81	0.78	0.74	0.60
	2	0.69	---	0.76	0.72
	3	0.68	---	0.58	0.58
	4	0.72	0.52	0.68	0.63
	5	0.73	0.64	0.66	0.60

Test refers to the two exhaustive exercise rides (non-supplemented(NS) and vitamin B-6 supplemented(S)).
PRE refers to the mean value prior to exercise (time 0 and 30 minutes of rest), DX refers to 60 minutes during exercise, POST refers to immediately after exercise and POST-60 refers to 60 minutes after exercise.

Table B.17. Individual red blood cell pyridoxal 5'-phosphate concentration (study 2).

Test	Subject	Pre Study	PRE	DX	POST	POST 60
				nmol/L		
NS	1	55.2	52.2	59.5	53.7	65.9
	2	218	75.9	---	77.6	80.5
	3	86.2	51.6	---	49.5	52.0
	4	129	40.2	44.0	52.9	57.3
	5	154	108	103	108	97.1
S	1		114	107	92.6	177
	2		179	---	166	201
	3		159	---	141	227
	4		138	127	132	159
	5		179	173	168	237

Test refers to the two exhaustive exercise rides (non-supplemented(NS) and vitamin B-6 supplemented(S)).
Pre Study refers to prior to the start of the study, PRE refers to the mean value prior to exercise (time 0 and 30 minutes of rest), DX refers to 60 minutes during exercise, POST refers to immediately after exercise and POST-60 refers to 60 minutes after exercise.

Table B.18. Individual plasma alkaline phosphatase activity (study 2).

Subject	Test	Pre Study	PRE	DX	POST	POST 60
				µKat/L		
1	(NS)	0.25	0.35	0.38	0.36	0.37
	(S)		0.31	0.35	0.35	0.33
2	(NS)	0.25	0.31	---	0.39	0.34
	(S)		0.37	---	0.45	0.40
3	(NS)	0.21	0.19	---	0.26	0.19
	(S)		0.22	---	0.26	0.23
4	(NS)	0.26	0.31	0.33	0.33	0.31
	(S)		0.39	0.43	0.42	0.36
5	(NS)	0.24	0.36	0.39	0.40	0.35
	(S)		0.38	0.44	0.46	0.34

Test refers to the two exhaustive exercise rides (non-supplemented(NS) and vitamin B-6 supplemented(S)). Pre Study refers to the pre-study blood sample from the wellness panel (Good Samaritan Hospital, Corvallis, OR), PRE refers to the mean value prior to exercise (time 0 and 30 minutes of rest), DX refers to 60 minutes during exercise, POST refers to immediately after exercise and POST 60 refers to 60 minutes after exercise.

Table B.19. Individual plasma growth hormone concentration (study 2).

Subject	Test	PRE	DX	POST	POST 60
			ng/mL		
1	(NS)	0.48	28.3	22.1	6.28
	(S)	0.34	22.1	17.9	3.93
2	(NS)	7.91	---	19.1	5.08
	(S)	0.63	---	22.9	8.21
3	(NS)	0.06	---	9.94	0.54
	(S)	0.12	---	11.6	0.63
4	(NS)	0.34	29.1	32.1	6.73
	(S)	0.71	37.9	37.5	9.76
5	(NS)	19.7	19.0	16.3	2.15
	(S)	2.52	13.4	18.5	1.31

Test refers to the two exhaustive exercise rides (non-supplemented(NS) and vitamin B-6 supplemented(S)).
PRE refers to the mean value prior to exercise (time 0 and 30 minutes of rest), DX refers to 60 minutes during exercise, POST refers to immediately after exercise and POST 60 refers to 60 minutes after exercise.

Table B.20. Individual sweat total vitamin B-6 concentration (study 1 and study 2).

	Study 1		Study 2	
Subject	NS	S	NS	S
		nmol /g sweat		
1	-----	0.0485	0.0099	0.0125
2	-----	0.0397	0.0080	-----
3	-----	0.0063	0.0041	0.0042
4	-----	0.0042	0.0103	-----
5	-----	0.0180	0.0030	0.0031

The subjects in study 1 and study 2 refer to different individuals.
NS refers to the exhaustive exercise ride in the non-supplemented state; S refers to the exhaustive exercise ride in the vitamin B-6 supplemented state.
----- indicates that inadequate sample was collected for analysis.

Table B.21. Individual urinary creatinine excretion (study 1).

Subject	Test	Days Prior to EX				EX test	1 Day Post
		4	3	2	1		
				g/d			
1	NS	1.32	1.71	1.92	2.12	1.54	-----
2		1.89	1.83	1.74	1.95	2.25	1.87
3		1.87	1.95	1.81	1.82	1.81	1.81
4		1.79	1.63	1.83	1.81	1.96	1.83
5		2.25	2.11	2.17	2.39	2.25	2.13
6		2.01	2.04	1.95	2.07	2.04	2.09
1	S	2.00	1.69	2.15	2.22	2.12	1.29
2		1.83	1.83	1.89	1.90	1.90	0.97
3		1.88	1.93	----	1.98	1.91	1.89
4		1.72	1.87	1.80	1.89	1.94	1.71
5		2.09	2.18	2.18	2.10	2.08	2.16
6		1.94	2.14	2.04	1.93	2.15	2.31

Test refers to exercise test (non-supplemented state(NS) and vitamin B-6 supplemented(S) state). Days prior to EX represents 4 days prior to the exercise test day, EX test refers to the exercise test day, and 1 day post refers to 1 day after the exercise test . ---- means that data was omitted due to incomplete urine collections.

Table B.22. Individual urinary creatinine excretion (study 2).

Subject	Test	Days Prior to EX				EX test	Days after EX	
		4	3	2	1		1	2
					g/d			
1	NS	1.95	1.90	1.90	2.21	2.15	2.23	2.13
2		1.97	2.03	2.10	1.95	1.83	1.83	2.02
3		-----	2.35	2.16	2.39	2.28	2.27	2.36
4		2.04	2.09	1.88	1.86	2.28	1.89	1.99
5		2.28	2.12	2.27	2.11	2.11	2.29	2.48
1	S	2.13	2.47	2.22	2.29	2.05	2.47	1.87
2		2.20	2.20	-----	2.05	2.09	2.22	NC
3		2.20	2.48	2.48	2.46	2.05	2.42	2.03
4		2.19	2.14	1.88	2.19	2.30	2.20	2.11
5		2.45	2.61	1.96	2.30	2.04	2.33	2.33

Test refers to exercise test (non-supplemented state(NS) and vitamin B-6 supplemented(S) state)
Days prior to EX represents 4 days prior to the exercise test day; EX test refers to the exercise test day, days after EX refers to 2 days after the exercise test day.
--- means value omitted due to incomplete collection
NC means sample not collected

Table B.23. Individual urea nitrogen excretion (study 1).

Subject	Test	Days Prior to EX				EX test	1 Day Post
		4	3	2	1		
				g/d			
1	NS	13.09	16.10	17.47	19.70	15.89	-----
2		16.73	16.11	15.60	15.95	15.66	15.74
3		16.81	15.19	14.92	17.69	16.41	16.33
4		13.81	14.01	16.75	15.65	17.27	17.47
5		18.18	16.61	15.38	17.60	16.82	16.77
6		15.85	17.85	16.08	17.14	15.14	18.26
1	S	16.64	16.37	16.25	16.66	16.90	10.94
2		15.22	15.22	15.01	14.97	14.97	8.63
3		16.51	17.76	----	18.26	15.46	17.62
4		13.94	16.02	16.62	16.55	18.10	15.83
5		14.56	16.57	16.57	16.38	16.44	18.71
6		19.23	16.94	16.47	15.27	17.54	17.62

Test refers to exercise test (non-supplemented state(NS) and vitamin B-6 supplemented(S) state). Days prior to EX represents 4 days prior to the exercise test day, EX test refers to the exercise test day, 1 day post refers to 1 day after the exercise test . ---- means that data was omitted due to incomplete urine collections.

Table B.24. Individual urea nitrogen excretion (study 2).

Subject	Test	Days Prior to EX				EX test	Days after EX	
		4	3	2	1		1	2
					g/d			
1	NS	14.04	15.70	15.70	13.65	15.59	14.88	12.44
2		14.14	16.37	15.75	15.54	12.78	14.82	15.36
3		-----	16.10	14.29	16.17	14.38	14.70	15.40
4		16.38	16.94	13.73	14.13	17.00	14.82	16.46
5		17.24	13.61	19.37	14.18	14.18	14.41	14.21
1	S	15.02	16.27	14.69	15.52	14.82	19.17	14.00
2		15.05	15.28	-----	15.96	13.58	16.04	NC
3		14.94	14.69	14.69	12.17	11.76	14.63	13.72
4		25.18	8.21	17.06	14.81	15.47	15.14	14.81
5		17.57	18.63	16.72	16.97	15.27	16.53	16.53

Test refers to exercise test (non-supplemented state(NS) and vitamin B-6 supplemented(S) state)
Days prior to EX represents 4 days prior to the exercise test day; EX test refers to the exercise test day, days after EX refers to 2 days after the exercise test day.
--- means value omitted due to incomplete collection
NC means sample not collected

Table B.25. Individual urinary 4-pyridoxic acid excretion (study 1).

Subject	Test	Days Prior to EX					
		4	3	2	1	EX	1 Day Post
				µmol/day			
1	NS	4.33	5.70	5.56	6.34	4.71	---
2		7.15	7.15	5.43	7.53	6.18	6.71
3		6.96	6.8	6.97	8.14	7.78	6.76
4		5.02	5.36	6.03	5.58	7.55	5.51
5		5.51	5.04	5.00	5.63	6.23	5.13
6		6.41	6.42	6.16	6.69	6.78	6.92
1	S	89.6	84.9	108	87.0	97.3	81.8
2		99.5	99.5	102	92.2	92.2	77.1
3		90.1	97.3	---	93.4	89.2	88.1
4		83.1	96.9	96.0	95.0	103	96.0
5		62.6	91.3	91.3	91.0	98.4	97.4
6		97.6	98.5	83.6	82.8	89.0	105

Test refers to exercise test (non-supplemented state(NS) and vitamin B-6 supplemented(S) state).
Days prior to EX represents 4 days prior to the exercise test day, EX test refers to the exercise test day, 1 day post refers to 1 day after the exercise test .
--- means that data was omitted due to incomplete urine collections.

Table B.26. Individual urinary 4-pyridoxic acid excretion (study 2).

Subject	Test	Days Prior to EX					Days after EX	
		4	3	2	1	EX	1	2
					µmol/day			
1	NS	9.65	8.74	8.74	7.75	8.37	8.48	9.25
2		11.6	11.4	11.4	10.4	7.66	7.66	8.58
3		---	6.99	6.30	6.17	5.85	6.29	7.09
4		6.01	6.71	5.13	5.77	6.60	5.16	5.80
5		10.1	10.9	8.62	8.18	8.18	7.56	8.27
1	S	85.2	89.2	71.9	73.7	79.5	83.3	73.5
2		79.2	80.9	---	87.8	96.7	92.0	NC
3		76.5	84.9	84.9	81.3	77.8	82.7	80.7
4		91.6	90.1	81.5	82.1	83.5	85.9	85.7
5		83.4	72.9	78.3	74.7	82.9	89.7	89.7

Test refers to exercise test (non-supplemented state(NS) and vitamin B-6 supplemented(S) state).
Days prior to EX represents 4 days prior to the exercise test day, EX refers to the exercise test day, days after EX refers to 2 days after the exercise test day.
--- means value omitted due to incomplete collection.
NC means sample not collected.

Table B.27. Individual urinary total vitamin B-6 excretion (study 1).

Subject	Test	Days Prior to EX					
		4	3	2	1	EX	1 Day Post
				µmol/d			
1	NS	0.82	0.90	1.04	1.16	0.83	---
2		0.86	0.85	0.70	0.87	0.66	0.85
3		0.73	0.59	0.60	0.75	0.73	0.71
4		0.66	0.51	0.64	0.71	0.72	0.72
5		0.73	0.69	0.65	0.80	0.72	0.73
6		0.89	0.83	0.85	0.98	0.85	0.97
1	S	12.3	10.3	12.0	11.7	9.82	10.3
2		9.34	9.34	9.53	9.00	9.00	7.79
3		10.1	8.72	9.60	9.94	8.48	8.58
4		10.2	7.46	10.7	9.16	8.06	8.53
5		7.71	9.98	9.98	10.2	10.7	9.71
6		7.87	10.5	7.00	10.0	9.01	12.0

Test refers to exercise test (non-supplemented state and vitamin B-6 supplemented state). Days prior to EX represents 4 days prior to the exercise test day (not consecutive), EX refers to the exercise test day, 1 Day Post refers to 1 day after the exercise test day. --- means that values were omitted due to incomplete urine collection.

Table B.28. Individual urinary total vitamin B-6 excretion (study 2).

Subject	Test	Days Prior to EX					Days after EX	
		4	3	2	1	EX	1	2
				µmol/d				
1	NS	---	1.43	1.34	1.10	1.21	1.14	1.12
2		1.04	1.14	1.37	1.10	0.86	1.02	1.04
3		---	1.08	1.04	1.21	1.10	1.13	1.03
4		0.70	0.81	0.69	0.66	0.85	0.73	0.75
5		---	1.56	1.39	1.23	1.23	1.22	1.20
1	S	10.7	8.71	10.8	9.61	8.78	9.49	11.68
2		6.90	7.03	-----	4.30	6.67	9.12	---
3		12.3	10.1	10.7	9.80	8.44	8.21	9.51
4		7.57	8.78	8.51	9.93	9.08	7.52	7.22
5		7.37	7.97	8.35	9.12	7.08	10.3	10.3

Test refers to exercise test (non-supplemented state and vitamin B-6 supplemented state). Days prior to EX represents 4 days prior to the exercise test day (not consecutive), EX refers to the exercise test day, Days after EX refers to 2 days after the exercise test day. ---- means that value omitted due to incomplete collections.

Table B.29. Individual chemistry screen with lipid profile (study 1).

Test	Subject values						Normal values	
	1	2	3	4	5	6		
glucose	95	85	94	92	88	89	70-105	mg/dL
urea nitrogen	20	14	13	14	10	20	6-19	mg/dL
creatinine	1.2	1.3	1.2	1.3	1.2	1.3	0.6-1.4	mg/dL
sodium	139	139	143	137	142	137	135-148	mEq/L
potassium	4.0	4.8	5.2	4.2	3.6	3.5	3.5-5.3	mEq/L
chloride	105	101	106	101	102	101	100-112	mEq/L
calcium	8.7	9.5	9.2	9.5	9.2	9.8	8.4-10.2	mg/dL
phosphorus	3.6	3.3	4.0	3.4	2.6	3.3	2.7-4.5	mg/dL
CPK	127	67	90	201	110	82	24-195	U/L
SGOT	25	18	32	19	19	20	0-37	U/L
lactic dehydrogenase	175	119	169	150	153	121	118-242	U/L
SGPT	18	15	46	24	21	13	0-41	U/L
alkaline phosphatase	151	146	167	196	156	157	115-282	U/L
gamma GT	11	13	16	13	13	17	11-51	U/L
total bilirubin	0.3	0.5	0.6	0.4	0.6	0.7	0.0-1.0	mg/dL
direct bilirubin	0.1	0.1	0.1	0.1	0.1	0.1	0.0-0.3	mg/dL
triglyceride	92	93	103	128	152	58	20-250	mg/dL
cholesterol	129	153	196	175	202	138	100-180	mg/dL
HDL cholesterol	53	36	52	45	33	40	35-100	mg/dL
LDL cholesterol	58	98	128	104	139	86	65-130	mg/dL
VLDL cholesterol	18	19	21	26	30	12	4-50	mg/dL
uric acid	5.6	4.8	4.7	4.2	4.1	6.5	3.4-7.0	mg/dL
total protein	6.4	7.2	7.1	7.2	7.7	7.1	6.5-8.0	gm/dL
albumin	4.0	4.2	4.3	4.3	4.7	4.6	3.4-5.0	gm/dL
globulin	2.4	3.0	2.8	2.9	3.0	2.5	2.3-3.5	gm/dL
A/G ratio	1.7	1.4	1.5	1.5	1.6	1.8	1.0-2.0	ratio
osmolality	271	268	276	265	273	267	260-278	mM/L
BUN/creatinine	17	11	11	11	8	15	6-30	ratio

Table B.30. Individual chemistry screen with lipid profile (study 2).

Test	Subject values						
	1	2	3	4	5	Normal values	
glucose	94	87	88	93	81	70-105	mg/dL
urea nitrogen	19	14	15	18	23	6-19	mg/dL
creatinine	1.2	1.1	1.4	1.2	1.3	0.6-1.4	mg/dL
sodium	143	142	140	143	142	135-148	mEq/L
potassium	4.0	4.1	5.0	3.5	4.5	3.5-5.3	mEq/L
chloride	102	103	99	102	105	100-112	mEq/L
calcium	9.1	8.8	9.2	9.0	9.3	8.4-10.2	mg/dL
phosphorus	3.1	3.2	2.8	3.1	4.2	2.7-4.5	mg/dL
CPK	545	162	40	105	180	24-195	U/L
SGOT	43	31	32	18	29	0-37	U/L
lactic dehydrogenase	247	183	250	154	133	118-242	U/L
SGPT	34	22	71	17	18	0-41	U/L
alkaline phosphatase	148	147	128	158	142	115-282	U/L
gamma GT	21	10	32	11	9	11-51	U/L
total bilirubin	0.8	0.6	0.5	1.2	0.6	0.0-1.0	mg/dL
direct bilirubin	0.1	0.1	0.1	0.2	0.1	0.0-0.8	mg/dL
triglyceride	80	57	103	103	132	20-250	mg/dL
cholesterol	170	139	172	126	165	100-199	mg/dL
HDL cholesterol	50	51	37	42	58	35-100	mg/dL
LDL cholesterol	104	77	114	63	81	65-130	mg/dL
VLDL cholesterol	16	11	21	21	26	4-50	mg/dL
uric acid	4.7	6.4	6.6	6.9	7.9	3.4-7.0	mg/dL
total protein	7.2	6.6	7.5	7.1	6.5	6.5-8.0	gm/dL
albumin	4.6	4.1	3.9	4.6	4.1	3.4-5.0	gm/dL
globulin	2.6	2.5	3.6	2.5	2.4	2.3-3.5	gm/dL
A/G ratio	1.8	1.6	1.1	1.8	1.7	1.0-2.0	ratio
osmolality	278	274	271	278	277	260-278	mM/L
BUN/creatinine	16	13	11	15	18	6-30	ratio

APPENDIX C

Foods consumed daily for both the vitamin B-6 supplemented and non-supplemented diets (study 1).

Breakfast:
- 240 gm orange juice, reconstituted
- 220 gm 1% milk
- 50 gm shredded wheat cereal
- 50 gm whole wheat bread
- 30 gm raisins
- 20 gm margarine (ad lib)
- 15 gm white sugar
- 3 ea. jelly packets

Lunch: Tuna Salad Sandwich:
- 60 gm tuna, water-packed,drained
- 60 gm whole wheat bread
- 30 gm low-calorie mayonnaise
- 20 gm egg white, cooked
- 15 gm dill pickle relish
- 10 gm iceberg lettuce

- 240 gm apple juice
- 160 gm pears, lite syrup
- 70 gm raw carrot
- 40 gm vanilla wafers
- 17 gm gelatin mixed with 240 gm Kool-Aid™

Dinner: Salad:
- 50 gm iceberg lettuce, chopped
- 40 gm cheddar cheese
- 40 gm kidney beans, drained
- 20 gm french dressing (ad lib)
- 15 gm red cabbage, chopped
- 10 gm raw carrot, grated

- 240 gm 1% milk
- 200 gm green beans, drained
- 150 gm peaches, lite syrup
- 100 gm vanilla lowfat frozen yogurt
- 120 gm turkey
- 70 gm white rice, dry
- 25 gm whole wheat bread
- 17 gm gelatin mixed with 240 gm Kool-Aid™

Snack:
- 240 gm grape juice
- 50 gm graham crackers

™ Trademark for Kool-Aid; Kraft-General Foods, Waukegan, IL.

Foods consumed daily for both the vitamin B-6 supplemented and non-supplemented diets (study 2).

Breakfast:
- 220 gm 1% milk
- 200 gm orange juice, reconstituted
- 200 gm pears, lite syrup
- 60 gm Life cereal™
- 60 gm whole wheat bread
- 50 gm raisins
- 20 gm margarine*

Lunch:
- 240 gm apple juice

Tuna Salad Sandwich:
- 60 gm tuna, water-packed,drained
- 60 gm whole wheat bread
- 35 gm low-calorie salad dressing
- 20 gm egg white, cooked
- 15 gm dill pickle relish
- 10 gm iceberg lettuce

Salad:
- 80 gm iceberg lettuce
- 50 gm kidney beans, drained
- 20 gm french dressing*
- 15 gm red cabbage, chopped
- 10 gm carrot, grated

- 12 gm gelatin mixed with 240 gm Kool-Aid™
- 10 each vanilla wafers

Dinner:
- 220 gm 1% milk
- 200 gm green beans, drained
- 200 gm peaches, lite syrup
- 120 gm vanilla lowfat frozen yogurt
- 110 gm turkey
- 70 gm white rice
- 70 gm carrot sticks
- 50 gm cheddar cheese
- 30 gm whole wheat bread
- 12 gm gelatin mixed with 240 gm Kool-Aid™

Snack:
- 200 gm grape juice
- 50 gm graham crackers

Beverages: regular or decaffeinated coffee and tea, regular and diet sodas

* Included ad lib to maintain subjects body weight

™ Trademark for Life Cereal; Quaker Oats Co., Chicago, IL.
Trademark for Kool-Aid; Kraft-General Foods, Waukegan, IL.

Adapted guidelines from the American College of Sports Medicine (1986) for VO_2 max testing.

Pre-testing procedures:

1) Subjects were weighed, the bike seat height was adjusted and 5 lead ECG electrodes were placed on each subject (RA, LA, RL, LL, V5).

2) The procedure was explained to each subject. The subject was seated on the bike. The subject was hooked up to the ECG monitoring equipment. The heart monitor was placed around the subject's chest. Three resting blood pressures were taken with a blood pressure cuff and three were taken from the ECG. The metabolic cart gas collection headgear was fitted to the subject. A noseclip was placed on subject's nose. Respiratory gasses were collected on each subject for phase I, II and III.

Phase I:

1) Phase I consisted of collecting respiratory gases for two minute in the rested state to establish a baseline.

Phase II:

1) Phase II consisted of a two minute warm-up period with minimal resistance at 80 revolutions per minute (rpm).

Phase III (the Max test):

1) The subject begin cycling at 80 rpm with the workload set at 80 watts for 1 minute.

2) After one minute, the workload was increased 30 watts each minute until the subject was unable to maintain 80 rpm or requested to end the test. Heart rate and rating of perceived exertion is recorded each minute. Blood pressure was recorded one minute 30 seconds into the test and every two minutes after that using an automatic blood pressure cuff.

3) After the test, subjects were allowed to pedal with minimal resistance for three minutes to recover. Heart rate, blood pressure and respiratory gasses were monitored during this period.

INFORMATION SHEET
ENDURANCE EXERCISE TEST

1) DO NOT EAT ANYTHING AFTER 8 PM THE PREVIOUS EVENING. It is important to drink 2 10 oz. glasses of water the morning of your endurance ride before you report.

2) Report to the Human Performance Lab (Women's Building, Rm. 19) for your endurance ride at your assigned time.

3) Bring: your urine bottles from the previous day, the same clothes you did your max test in, your own pedals (if you performed your max test with them), some good reading material, clothes to change into after the test. We will provide a shower area and towels.

4) Day of test procedure:
   a) report to the Human performance lab at _____.
   b) you will be weighed without clothes (don't worry, Ricky will do).
   c) rest for 10 minutes
   d) have first blood sample drawn (resting sample).
   e) have ECG hooked up (as was done in Max test)
   f) resting blood pressure will be read (blood pressures during this test will be less frequent than the max test, so we will not need to hook up the automatic blood pressure cuff).
   g) we will set the seat height and put on your pedals (if using your own)
   h) sweat collector will be taped to upper corner of your shoulder
   i) you will sit stationary on the bike for 3 minutes with the mouthpiece in to establish a baseline. The mouthpiece will remain in for the next 10-15 minutes.
   j) you will warm-up for a total of 5 minutes.
   k) we will gradually increase the workload on the bike until you are at 75% of the VO2 max level obtained from your max test.
   k) you will ride at 80 rpm, as you did in the max test, as long as you can. You will go off and on the mouthpiece at intervals throughout the test.

5) Criteria for stopping the endurance ride:
   a) maintaining less than 80 rpm for more than 15 sec
   b) you request to stop
   c) if the data we are monitoring dictates we stop the test

NOTE We ask that you wear no watches. We will inform you of your time at the completion of the entire study (after your second exercise test).

6) Blood drawing:
   a) the second blood sample will be drawn during the ride. While the blood is being drawn, your work load will be decreased.
   b) the third sample will be drawn 1-2 minutes after you finish the endurance ride.
   c) the fourth sample will be drawn 30 minutes after you finish.
   d) the last sample will be drawn 1 hour after you finish.

****NOTE*** Please do not lay down during this time. Breakfast will be available immediately after the last blood sample is drawn (1 hour after you finish the ride).
*** A controlled amount of water will be given at intervals during the ride.

Endurance ride protocol in study 2.

Pre-test:

1) Subjects arrived at the Human Performance Lab with minimal exertion. Body weights were recorded. Subjects rested quietly in a separate room for 30 minutes.

2) A blood sample was collected (PRE).

3) Subjects continued resting for an additional 25 minutes before another blood sample was collected (PRE).

4) Subjects return to the Human Performance Lab. Three ECG electrodes are placed on the subjects. The subjects entire back was cleaned with alcohol swabs in preparation for sweat collection. The bike seat height was adjusted.

5) Subjects were seated on the bike and hooked up to the ECG monitoring equipment. The gas collection equipment was placed on the subject's head and adjusted. A nose clip was placed on the subject's nose. Three minutes of resting respiratory gases were collected.

Endurance Test:

1) There was a 2-5 minute warm-up period at 80 rpm and a workload of 1 kpm.

2) The workload was set at 75% of the pre-determined VO_2 max and remained at this intensity for the remainder of the test.

3) Data was collected during each 10 minute interval of the test as follows:

at 2 minutes, an RPE was obtained from the subject

at 3-5 minutes, sweat was collected from the subject's back on filter paper

at 5 minutes, an ECG reading was taken for 10-15 seconds

at 8-10 minutes, respiratory gasses were collected

4) One hour into the endurance ride, the workload was decreased for 1-2 minutes to allow for the collection of blood. 120 mL of water was provided to the subjects after the blood collection in study 2. The workload was reset at 75% of VO_2 max and the subjects continued to cycle until exhaustion.

GENERAL INSTRUCTIONS FOR FALL 1990 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 3 day 24 hr. diet recall period (day 14-16).

2. Label all containers carefully and clearly with your initials 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 anount of fluids each day.

OTHER:

1. Eat all food given to you during each controlled dietary period (days 1-8 and 24-31). It is our goal to maintain your body weight and adjustments in caloric 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.