AN ABSTRACT OF THE THESIS OF

Ricky S. Virk for the degree of Master of Science in Nutrition and Food Management presented on March 6, 1992.

Title: The Effect of Vitamin B-6 Supplementation on Fuel Utilization During Exhaustive Endurance Exercise in Men

Abstract approved: _____
James E. Leklem

Previous studies concerned with vitamin B-6 and exercise suggest that vitamin B-6 supplementation may alter fuel metabolism during exercise through the vitamin's role in glycogenolysis and gluconeogenesis. The effect of vitamin B-6 supplementation on fuel utilization was tested in six men (age: 26 ± 7; VO_2: 59.6 ± 7.9 ml/kg/min) during submaximal endurance exercise to exhaustion on a cycle ergometer. Subjects were exercised to exhaustion twice at 64-75% VO_2 max in a fasted condition on the seventh morning of two seprate eight day controlled diet periods. The first exercise test (T1) occurred following a control or non-supplemented (NS) diet (i.e. 2.3-2.4 mg B-6/d), and the second exercise test (T2) occurred following a vitamin B-6 supplemented (S) diet (i.e. 2.3-2.4 mg B-6/d + 20 mg PN/d). Blood was drawn pre, during (i.e. 60 minutes into exercise), post, post-30, and post-60 minutes of exercise, and analyzed for plasma glucose, lactic acid, glycerol, and free fatty acids (FFA). Expired

air was collected for two minutes at 10 minute intervals during T1 and T2. Lower plasma FFA concentrations at pre-exercise in T2 (S) compared to T1 (NS) were the only significant ($p<0.05$) differences in plasma substrates between T1 and T2. Respiratory exchange ratios (R) were not statistically different between T1 and T2, however T2 maintained higher R values during exercise compared to T1. Heart rates were on average 6-10 bts/min slower during exercise in T2 compared to T1, which was statistically significant ($p<0.05$) at multiple time points. Plasma volume showed a significant decrease with exercise, however no difference was observed between T1 and T2. No significant difference was observed in the exercise time to exhaustion between T1 (2:00:37) and T2 (2:04:51). These results indicate that supplementation with 20 mg/d of vitamin B-6 does not significantly affect fuel utilization during submaximal endurance exercise to exhaustion.

The Effect of Vitamin B-6 Supplementation on Fuel Utilization During Exhaustive Endurance Exercise in Men

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDIX TABLES

The Effect of Vitamin B-6 Supplementation on Fuel Utilization During Exhaustive Endurance Exercise in Men

INTRODUCTION

With all the hype surrounding proper nutrition and exercise these days, supplementation of certain macro and micro nutrients has become a fad with many performance conscious athletes. Years of scientific scrutiny have revealed the importance of high carbohydrate diets for endurance performance (Bergstrom et al. 1967, O'Keefe et al. 1989), whereas excessive intakes of fats (Ivy et al. 1980, Decombez et al. 1983) and proteins (Consolazio 1975) are of no benefit to endurance athletes. The knowledge that vitamins are required to perform metabolic functions in the body, such as the oxidation of metabolic fuels for energy, has led many athletes to believe that high intakes can maximize performance. However, no conclusive evidence exists to suggest that vitamin supplements improve performance in nutritionally adequate individuals.

Vitamin B-6, as pyridoxal 5'-phosphate (PLP), is important in providing glucose to working muscles. The vitamin serves as a cofactor in two major energy producing pathways, glycogenolysis and gluconeogenesis. A unique property of vitamin B-6, as one of the water soluble vitamins, is the possibility of increased body stores with high intakes (Black et al. 1977). Under such conditions of

supplementation, the rate of glycogenolysis may actually increase (Leklem 1985) during exercise due to vitamin B-6's role in glycogen breakdown. Previous research (Lawrence et al. 1977, deVos et al. 1983, Manore and Leklem 1987, Campuzano 1988) suggests that vitamin B-6 may actually alter fuel utilization during exercise. The primary fuel sources are carbohydrates and fats. However, depending upon the conditions, secondary sources like protein, lactate, and glycerol may contribute significantly to energy production. If in fact vitamin B-6 does stimulate carbohydrate metabolism, then it can be placed in a category of one of many dietary factors which affect substrate metabolism (Krogh and Lindhard 1920, Berglund and Hemmingsson 1982). Other well acknowledged non-dietary fuel modifiers include: 1) exercise intensity (Chauveau 1896, Astrand et al. 1963, Saltin and Karlsson 1971, Hultman 1979), 2) exercise duration (Edwards et al. 1934, Christensen and Hansen 1939), 3) training state of individuals (Gollnick et al. 1973, Holloszy and Booth 1976, Saltin et al. 1976, Hendriksson 1977, Inger 1979, Sherman et al. 1981, Holloszy and Coyle 1984, Hurley et al. 1986), 4) and gender (Tarnopolsky et al. 1990).

Since all men/women are not truly created equal (i.e. metabolically), endeavors in human research may provide confounding results. The intricate complexity of the body and diversity between individuals makes for variable responses to environmental stimuli (i.e. exercise), which can be difficult

to explain and understand. A simple example helps clarify this issue. Vitamin B-6 is known to have a function in both glycogenolysis and gluconeogenesis. In light of this knowledge one might speculate that altering an individual's vitamin B-6 status may affect plasma glucose levels. If supplementation of vitamin B-6 results in a more rapid breakdown of muscle glycogen, then this fuel source should become depleted sooner than under conditions of no supplementation. Hence, later in exercise more of a demand would be placed on blood borne substrates to provide energy to working muscles. In this situation plasma glucose utilization may increase, and therefore lower glucose concentrations in the plasma. In contrast, a second scenario also appears possible. Supplemental vitamin B-6 may enhance gluconeogenesis to such a degree that in the later periods of exercise plasma glucose may not decrease at all, but increase. Thus, an important question arises. Which pathway does vitamin B-6 influence to a greater extent, glycogenolysis or gluconeogenesis? In addition, how does vitamin B-6's effect upon these pathways alter the utilization of other fuel sources? Fortunately, the process of experimentation allows us to investigate such scientific questions/curiosities by developing hypotheses and testing them.

Hypothesis

Supplemental vitamin B-6 can alter plasma fuel substrates during submaximal exhaustive endurance exercise, and increase the utilization of muscle glycogen for energy. Plasma glucose and lactic acid levels increase, while plasma glycerol and free fatty acids (FFA) decrease. A subsequent rise in the respiratory exchange ratio (R) occurs.

Objectives

The primary objective was to better understand the interrelationship between vitamin B-6 and the utilization of fuels during exhaustive endurance exercise (and short term recovery) by controlling diet and supplementation with vitamin B-6. Specific objectives were as follows:

1. To determine if plasma glucose and lactic acid levels were higher during exercise with supplemental vitamin B-6, and to monitor their levels at exhaustion and during recovery.
2. To determine if plasma glycerol and FFA levels were lower during exercise with supplemental vitamin B-6, and to monitor their levels at exhaustion and during recovery.
3. To determine if R values were higher during exercise with supplemental vitamin B-6.
4. To determine if exercise times to exhaustion decreased

with supplemental vitamin B-6.

LITERATURE REVIEW

Vitamin B-6

History

The discovery of vitamin B-6 dates back to 1934, when P. Gyorgy found that a specific vitamin factor prevented dermatitis in rats (Gyorgy 1934). In only four additional years, a crystalline form of vitamin B-6 had been isolated. Chemically, the compound was named 3-hydroxy-4,5-bis-(hydroxymethyl)-2-methylpyridine (Harris and Folkers 1939), and soon adopted a simplified name, "pyridoxine". Today this name applies to a particular form of the vitamin, not the entire group. During the 1940's experiments with microorganisms helped to identify other forms of vitamin B-6 (Snell et al. 1942). In addition, this decade brought about the discovery of "pyridoxal phosphate", the active coenzyme form of vitamin B-6. In 1951 the exact structure of the coenzyme was recognized as pyridoxal 5'-phosphate.

Structures, Chemistry

Vitamin B-6 exists in three primary forms (i.e. pyridoxine, PN; pyridoxamine, PM; pyridoxal, PL), with each also existing in the phosphorylated form (PNP, PMP, PLP). A fourth less well recognized form, 5-O-(B-D-glycopyranosyl) also exists. All three vitamers are derivatives of 3-

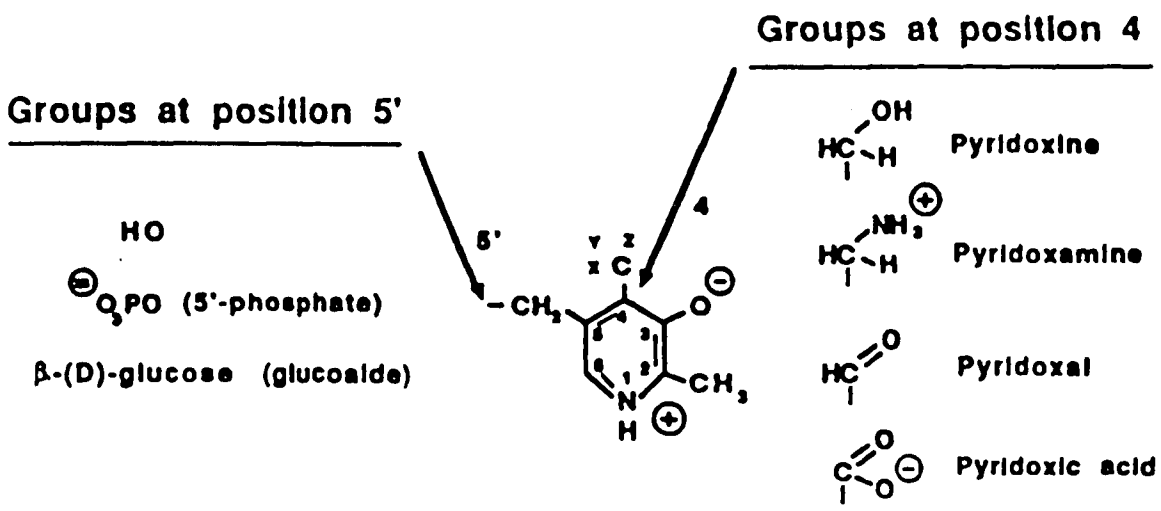
hydroxy-2-methyl-pyridine. The side chain at position four on the pyridinium ring determines the primary form, depending on whether the R group is a hydroxymethyl, aminomethyl, or formyl. If there is a carboxyl group at carbon four, the derivative is known as four-pyridoxic acid (4-PA). Position five is the site of phosphorylation and glucoside linkage (See Figure 1).

In tissues and organs PN, PM, and PL exist primarily as 5'-phosphates, and only PLP and PMP function as coenzymes. The hydrochloride salt of pyridoxine (PN-HCl) is the form used in dietary supplementation. However, quantities of PN (i.e. non-glycosylated PN) are extremely small in most natural foodstuffs compared to other forms of vitamin B-6 (Coffen 1984). All the collective forms of vitamin B-6 are water soluble. Vitamin B-6 is stable in acidic aqueous solutions, but at neutral and alkaline pH the vitamin is destroyed. In addition, light exposure and high temperatures are destructive to vitamin B-6.

Food Sources, Bioavailability

Vitamin B-6 is found in a wide variety of foods (See Table 1). Animal foods tend to contain larger proportions of PM and PL (phosophorylated) than plants, which are higher in PN and PM. Additionally, some plants contain relatively high levels of the glucopyranosyl form. Evidence suggests that the bioavailability of this glucoside of pyridoxine is lower than

Figure 1. Chemical structures of vitamin B-6



Taken from Merrill and Burnham 1988

Table 1. Vitamin B-6 and glycosylated vitamin B-6 levels in selected foods

Food	Vitamin B-6 mg/100g[b]	Glycosylated Vitamin B-6 mg/100g[b]
Vegetables		
Carrots, canned	0.064	0.055
carrots, raw		
Cauliflower, frozen	0.084	0.069
Broccoli, frozen	0.119	0.078
Spinach, frozen	0.208	0.104
Cabbage, raw	0.140	0.065
Sprouts, alfalfa	0.250	0.105
Potatoes, cooked	0.394	0.165
Potatoes, dried	0.884	0.286
Beets, canned	0.018	0.005
Yams, canned	0.067	0.007
Beans/Legumes		
Soybeans, cooked	0.627	0.357
Beans, navy, cooked	0.381	0.159
Beans, lima, frozen	0.106	0.039
Peas, frozen	0.122	0.018
Peanut butter	0.302	0.054
Beans, garbanzo	0.653	0.111
Lentils	0.289	0.134
Animal products		
Beef, ground, cooked	0.263	n.d.[c]
Tuna, canned	0.316	n.d.
Chicken breast, raw	0.700	n.d.
Milk, skim	0.005	n.d.
Nuts/Seeds		
Walnuts	0.535	0.038
Filberts	0.587	0.026
Cashews, raw	0.351	0.046
Sunflower seeds	0.997	0.355
Almonds	0.086	-0-
Fruits		
Orange juice, frozen concentrate	0.165	0.078
Orange juice, fresh	0.043	0.016
Tomato juice, canned	0.097	0.045
Blueberries, frozen	0.046	0.019
Banana	0.313	0.010
Banana, dried chips	0.271	0.024
Pineapple, canned	0.079	0.017
Peaches, canned	0.009	0.002
Apricots, dried	0.206	0.036
Avocado	0.443	0.015
Raisins, seedless	0.230	0.154
Cereals/Grains		
Wheat bran	0.903	0.326
Shredded wheat, cereal	0.313	0.087
Rice, brown	0.237	0.055
Rice, bran	3.515	0.153
Rice, white	0.076	0.015
Rice cereal, puffed	0.098	0.007
Rice cereal, fortified	3.635	0.382

Taken from Leklem 1989 (unpublished)

the phosphorylated or non-phosphorylated forms, but only marginally (Shultz and Leklem 1987). The glycosylated form is absorbed (Kabir et al. 1983), but not fully utilized (Trumbo et al. 1988). Meats are a rich source of vitamin B-6 and contain no pyridoxine B-glucoside. Fruits and vegetables also contain significant amounts of vitamin B-6, while milk is relatively low in content. In lesser developed nations, where food availability is reduced, grains (i.e. wheat) and legumes can contribute large percentages of vitamin B-6 intake.

In the processing of wheat as much as 85% of vitamin B-6 is lost, making white bread a much poorer source of vitamin B-6 than whole wheat bread (Kies et al. 1984). However, 15 g of wheat bran in the diet reduces vitamin B-6 bioavailability by up to 17% (Lindberg et al. 1983). With respect to bread consumption, possibly, white bread supplemented with pyridoxine hydrochloride will achieve maximal bioavailability compared to non-supplemented whole wheat bread (Leklem et al. 1980). Foods representing the "average" American diet are estimated to be 71-79% bioavailable (Tarr et al. 1981). In addition to processing, heating of food causes loss of vitamin B-6. For example, in the toasting of bread 50-70% losses are incurred (Gregory and Kirk 1981). In dried foods and feeds, losses of between 25-85% in vitamin B-6 have been observed between 10-40 months of storage (Kirchgessner and Kosters 1977).

Recommended Daily Allowances

The current RDA for vitamin B-6, which deliberately includes a large safety factor, is 1.6 and 2.0 mg/d for women and men, respectively (NRC 10th edition). The RDA for infants begins at 0.3 mg/d and increases to 1-2 mg/d from childhood to adolescence. Pregnancy adds 0.6 mg/d and lactation adds 0.5 mg/d for women. These figures should cover all aspects of potential vitamin loss from decreased bioavilabilty.

Further work is required to assess whether physical activity leads to an increased requirement for vitamin B-6. Limited data is available regarding this issue, however some evidence does suggest a need to establish an RDA of vitamin B-6 for active persons (Dreon and Butterfield 1986, Manore et al. 1987). Listed in Table 2 are additional factors that may affect vitamin B-6 requirements. Of the dietary factors listed, protein has received the most attention. As protein intake rises, so does the need for vitamin B-6 (Miller et al. 1985). Therefore, a vitamin B-6 to protein ratio has been proposed (Dietary Standard Canada).

Absorption, Transport, Metabolism

If not absorbed, all nutrients ingested in the diet will pass through the body in feces, non-utilized. Once absorbed, transport via the circulatory system provides a means of nutrient distribution for subsequent metabolism and storage. Finally, excesses of nutrients must be dealt with, either by

Table 2. Factors that may affect vitamin B-6 requirements of an individual

A. DIETARY—BIOAVAILABILITY
   1. Physical structure of foods consumed
   2. Forms of vitamin B_6 in foods
   3. Binding of the various forms to macromolecules in foods
B. DEFECTS IN TRANSPORT TO TISSUES AND CELLS
   1. Impaired gastrointestinal absorption
   2. Impaired transport
C. PHYSIOLOGICAL/BIOCHEMICAL
   1. Protein intake
   2. Increased catabolism
   3. Growth
   4. Sex differences
   5. Aging process
   6. Physical activity
   7. Nutrient interaction—riboflavin, carbohydrate
D. GENETIC
   1. Defects in apoenzymes requiring pyridoxal 5'-phosphate
   2. Altered levels of apoenzymes requiring pyridoxal 5'-phosphate

Taken from Leklem* 1988

storage or by renal, fecal, and sweat excretion.

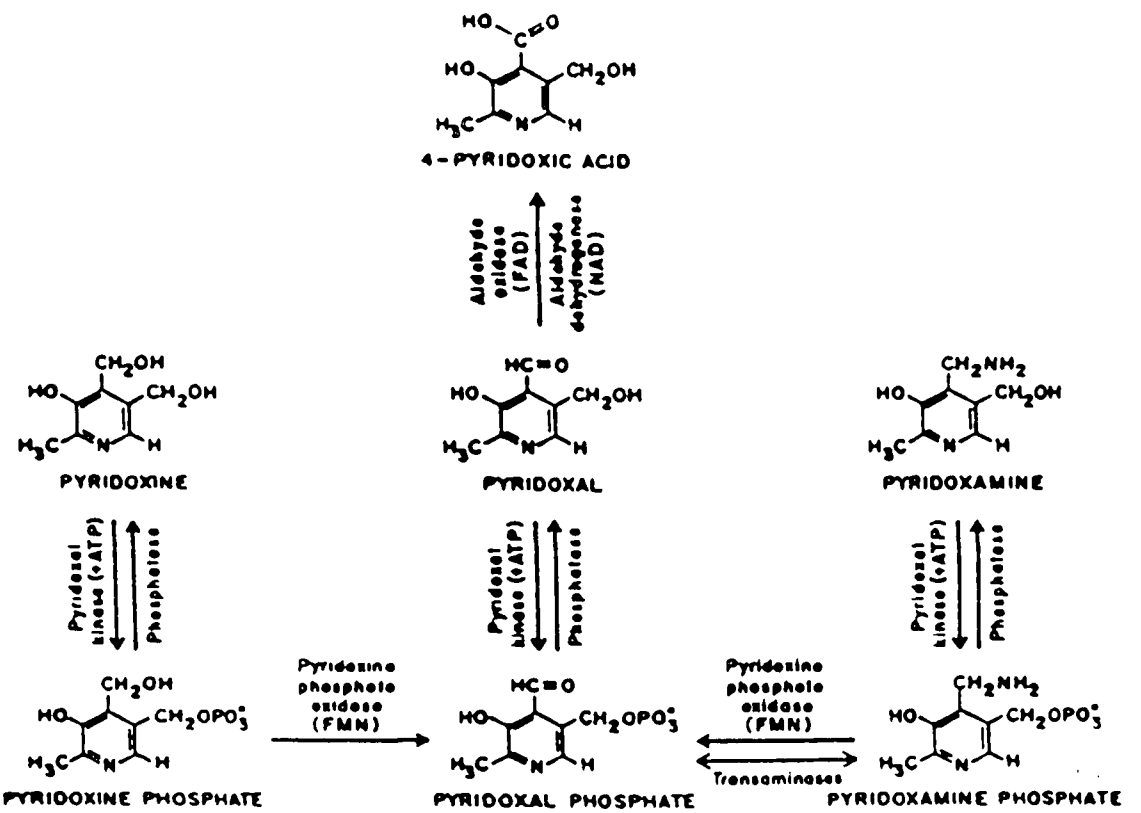
Most rat studies show that all three non-phosphorylated forms of vitamin B-6 are absorbed, primarily in the proximal jejunum by a nonsaturable process (Henderson 1985). However, some research indicates the possibility of a saturable component of uptake, especially in the duodenal section (Middleton 1985); and, if such a mechanism exists, megadoses may be wasteful. In the rat absorption has been shown to decrease from the proximal to the distal small intestine (Middleton 1985). Alkaline phosphatase is required for uptake, since phosphorylated compounds do not readily cross membranes (Middleton 1977,1978). At higher concentrations, however, there is limited absorption of phosphorylated B-6 vitamers (Henderson 1985). Once the vitamers have crossed the intestinal mucosa they must then be transported to the liver.

In the blood albumin binds PL and PLP (Dempsey and Christensen 1962). Gel filtration experiments suggest that PLP's binding affinity to albumin is greater than that of PL (Anderson et al. 1974). It appears that PL is the most important form taken into the cell (Lumeng et al. 1984). Therefore, the phosphate molecule on plasma PLP bound to albumin is first hydrolysed by alkaline phosphatase. Then the PL released from albumin easily crosses biomembranes and following uptake into cells, PL is usually rephosphorylated. Erythrocytes rapidly absorb all three forms of vitamin B-6.

In contrast to PN, the driving force behind PL uptake in the red blood cell is not coupled to rephosphorylation, but instead by an affinity to hemoglobin (Friedrich 1988). Thus, the uptake of PL and PN into erythrocytes depends upon different mechanisms.

Metabolism of vitamin B-6 occurs primarily in the liver. All three non-phosphorylated forms are taken up by passive diffusion followed by metabolic trapping through phosphorylation (Mehansho et al. 1980, Kozik and McCormick 1984). In the liver phosphorylated forms of PN and PL predominate due to pyridoxal kinase, which is higher in activity than the phosphatase (Merrill et al. 1984). Interconversion of the three vitamers is depicted in Figure 2. First, all three forms are phosphorylated at the 5'- position by pyridoxal kinase (McCormick et al. 1961). The reaction is reversible (i.e. catalyzed by a kinase or phosphatase), making it a site of regulation. Second, a flavin dependent oxidase converts PNP and PMP to PLP (Kazarinoff and McCormick 1975). This step is highly regulated by product inhibition, so high PLP concentrations limit flux through the reaction (Wada and Snell 1961, Merrill et al. 1979). Any excesses of vitamin B-6 (i.e. PL) in the liver are irreversibly converted to 4-PA by an aldehyde oxidase and/or an NAD dehydrogenase (Leklem[a] 1988). Four-pyridoxic acid is excreted renally, and on a daily basis may account for 40-60% of an adequate intake (Leklem[b] 1988). The most important forms of vitamin B-6

Figure 2. Interconversions of vitamin B-6



Taken from Leklem[4] 1988

released by the liver into plasma are PLP, PL, and 4-PA. Pyridoxine 5'-phosphate (PNP) and PMP must first be converted to PLP or PL prior to release from the liver (Lumeng et al. 1980). In addition to the liver supplying plasma with PLP, muscle reservoirs may supplement blood levels under times of a caloric deficit (Black et al. 1978).

Although PLP is the predominant form of vitamin B-6 found in the blood (i.e. 60-70%) under conditions of normal intake, other forms also exist (Shultz and Leklem 1983, Lumeng et al. 1985). Next in abundance is PL and even lower levels of PN and PM (Lumeng et al. 1985). Pyridoxamine 5'-phosphate (PMP) and PNP are extremely low and generally not detectable (Lumeng et al. 1985). Since PN and PM cannot be interconverted to PL in all tissues, especially muscle, PL and PLP are the key forms of circulating vitamin B-6. Therefore, the levels of PL and PLP are the best indicators of vitamin B-6 status (Leklem[b] 1988). The importance of PL comes from its ability to easily be supplied to tissues, while PLP must first by dephosphorylated. If the rate of dephosphorylation is sufficient, PLP could serve as a source of PL.

By using various amounts and mixtures of oral supplements, dose experiments provide a useful technique to study vitamin B-6 utilization. These studies (Lumeng et al. 1974, 1980, Wozenski et al. 1980, Ubbink et al. 1987) have quantitatively observed the changes in blood B-6 vitamers over time. For example, within one hour about 80% of an oral PN

supplement reaches the liver (Snell and Haskell 1971). Furthermore, a four to fivefold increase in plasma PLP is observed with 25 mg PN administration, two to four days after supplementation has begun (Lumeng et al. 1974).

When studying the effects of vitamin B-6 on various human metabolic conditions (i.e. exercise), data on blood and tissue levels may give insight to explain the results of individual differences. For example, two people may be ingesting equal amounts and similar forms of vitamin B-6, however their bodies may react to the vitamin differently. Absorption, metabolism, storage, and excretion may vary between persons.

Excretion

The major form of vitamin B-6 excreted in humans and animals is 4-PA. In addition to 4-PA, the three non-phosporylated forms of vitamin B-6 are also found in urine; however, their quantities are relatively small compared to 4-PA. The amount of 4-PA excreted is primarily related to vitamin B-6 intake, with higher intakes resulting in elevated excretion. Exercise can also increase 4-PA levels. Studies (Borisov 1977, Hatcher 1982, Manore et al. 1987) have shown that 4-PA is significantly increased in 24-hour urine samples of exercise test days, and to some extent may be even higher in trained versus untrained subjects (Manore et al. 1987). Thus, it appears that exercise leads to increased metabolism and excretion of vitamin B-6, and therefore revisions in the

RDA may be a necessity for active persons. The amount of 4-PA excreted appears to be independent of age, and sex differences are small. If no more than 2.96 umol/d or 2.36 umol/d of 4-PA is excreted in the urine of men and women respectively, then this indicates an inadequate supply of vitamin B-6 (Simon et al. 1982). Based on the assumption that 40-50% of the vitamin B-6 metabolized is excreted as 4-PA, then urine concentrations of this acid should be 2.96-7.09 umol/d for men and 2.36-6.50 umol/d for women (Simon et al. 1982). Often in B-6 deficiency, 4-PA is not detected in urine. Therefore, its evaluation in nutritional status is important (Snell and Haskell 1971).

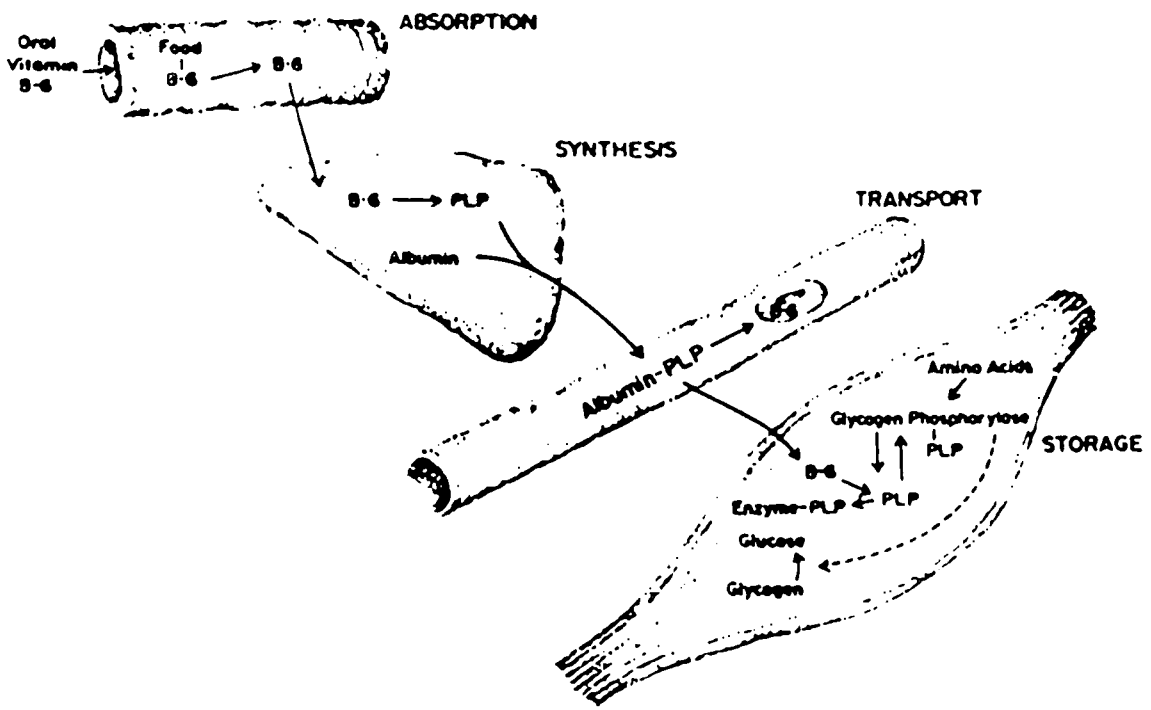
Body Pools/Stores

Generally, water soluble vitamins are not stored to any large degree in the body. Thus, large intakes are excreted in the urine. Vitamin B-6 appears to be a noteworthy exception. With large supplementary doses, the increased 4-PA excretion does not account for all the intake. Therefore, vitamin B-6 must be retained somewhere in the body or excreted as other forms or metabolic products. The total body supply of vitamin B-6 is believed to be approximately 1000 umol in a 70 kg man (Coburn et al. 1988). The highest levels of PLP in human organs are found in the liver followed by the brain, kidney, and spleen, respectively (Shin et al. 1983). In mammals PLP in the tissues is bound to proteins. Erythrocytes bind PLP

with hemoglobin, and in plasma it is bound to albumin. In rat muscles the coenzyme is found in association with glycogen phosphorylase. The binding to protein probably protects PLP from hydrolysis, and in addition serves a storage function (Fonda and Harker 1982).

The primary tissues which appear to be altered by vitamin B-6 supplementation are the blood and muscles (See Figure 3). In healthy persons receiving 100 mg PN-HCl for one to three weeks, baseline levels of total plasma vitamin B-6 increased from 114 nM before supplementation to 655 nM afterwards (Lumeng et al. 1980). The distribution of PL between erythrocytes and plasma is about 6.5:1, which somewhat reflects the fact that hemoglobin binds PL twice as strong as albumin (Friedrich 1988). Based on studies in rats and mice, belief was that muscle contained 50% of the total body pool of vitamin B-6, and as previously mentioned, probably bound to glycogen phosphorylase (Krebs and Fischer 1964). More current research puts this value at 70% (Coburn et al. 1988). Another study in rats helped confirm the muscle's role in storage (Black et al. 1977). When rats were given vitamin B-6 supplements, glycogen phosphorylase content increased relative to a control situation. Interestingly though, when the rats were made vitamin B-6 deficient the content of muscle glycogen phosphorylase did not decrease. However, a caloric deficit was found to decrease levels of the enzyme (Black et.al. 1978). Recently, a study (Coburn et al. 1991) examined if

Figure 3. The general metabolic scheme of vitamin B-6 (mouth to the muscle)



Taken from Leklem[4] 1988

similar results occur in humans. By using the technique of muscle biopsy, researchers concluded that vitamin B-6 pools in skeletal muscle are resistant to depletion with very low vitamin B-6 intakes for six weeks. In addition, the study demonstrated that with six weeks of 0.98 mmol PN-HCl/d supplementation (i.e. 166 mg/d), vitamin B-6 increases in muscle are slight. The rise is on the order of 25%. If in fact with vitamin B-6 supplements glycogen phosphorylase does increase (and hence PLP), then in accordance with vitamin B-6's biochemical functions, fuel metabolism may be affected.

Functions

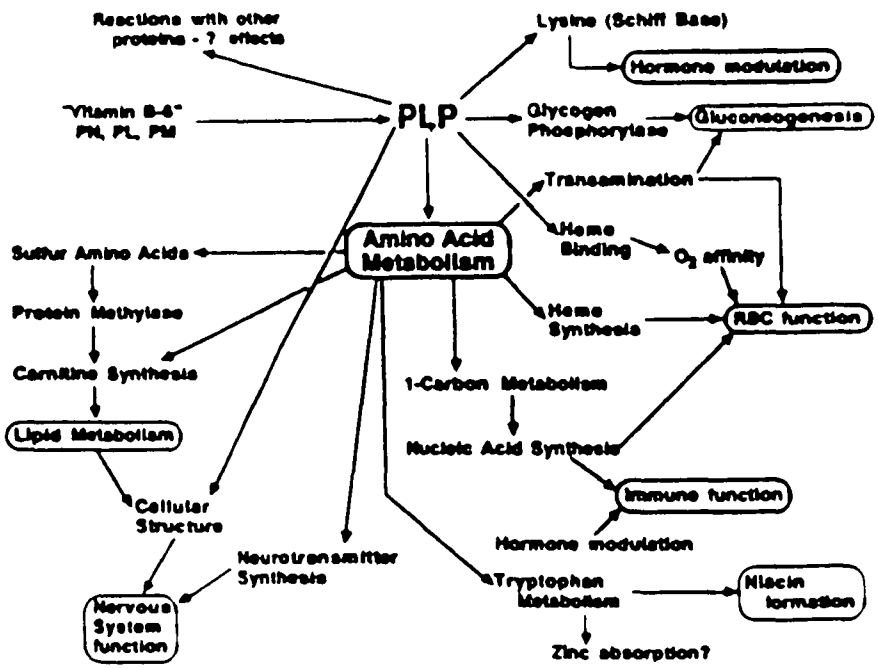
The functions of vitamin B-6 are quite extensive. Over 100 enzymes are known to require PLP as an activating coenzyme (Sauberlich 1985). The majority of PLP activated enzymes involve the formation of a Schiff base with amino acids and other nitrogen containing enzymes (Martinez-Carrion 1986). Glycogen phosphorylase, however, functions in a completely different fashion. This muscle enzyme appears to use its phosphate group as the active part of the coenzyme (Helmreich and Klein 1980, Takagi et al. 1982) instead of the aldehyde moiety, which is used in Schiff base reactions. The major types of biochemical reactions facilitated by PLP's highly reactive nature include: aminotransferase reactions, decarboxylation reactions, decarboxylation with carbon-carbon bond formation, side chain cleavage reactions, dehydratase

reactions, and racemization reactions (Merrill and Burnham 1988). The involvement of vitamin B-6 in these diverse enzymatic reactions makes it important in several cellular processes (See Figure 4). These processes include: gluconeogenesis, niacin formation, immune function, lipid metabolism, nervous system, hormone modulation, erythrocyte function, and amino acid catabolism (Leklem[b] 1988). As seen in the list carbohydrate, fat, and protein metabolism are all influenced by PLP.

Glycogenolysis involves the breakdown of liver and muscle glycogen to glucose, and vitamin B-6 is directly involved in this process by serving as a coenzyme for glycogen phosphorylase (Cori and Illingsworth 1957). This enzyme helps meet energy demands of working muscles by converting muscle glycogen to glucose-1-phosphate (Chastiotis et al. 1982). Undoubtedly, this is just one step of many in the catabolism of glycogen to glucose, but all reactions in biochemical pathways are of great importance. If glycogen phosphorylase does in fact increase with vitamin B-6 supplements (Black et al. 1977), then the rate of glycogenolysis may be increased.

Gluconeogenesis, often confused with glycogenolysis, is an entirely different metabolic process. In gluconeogenesis, non-carbohydrate precursors (i.e. amino acids, lactic acid, and glycerol) are converted to glucose in the liver. Vitamin B-6's influence in this pathway stems from PLP's role as a coenzyme in trans and deamination reactions. In the Cori-

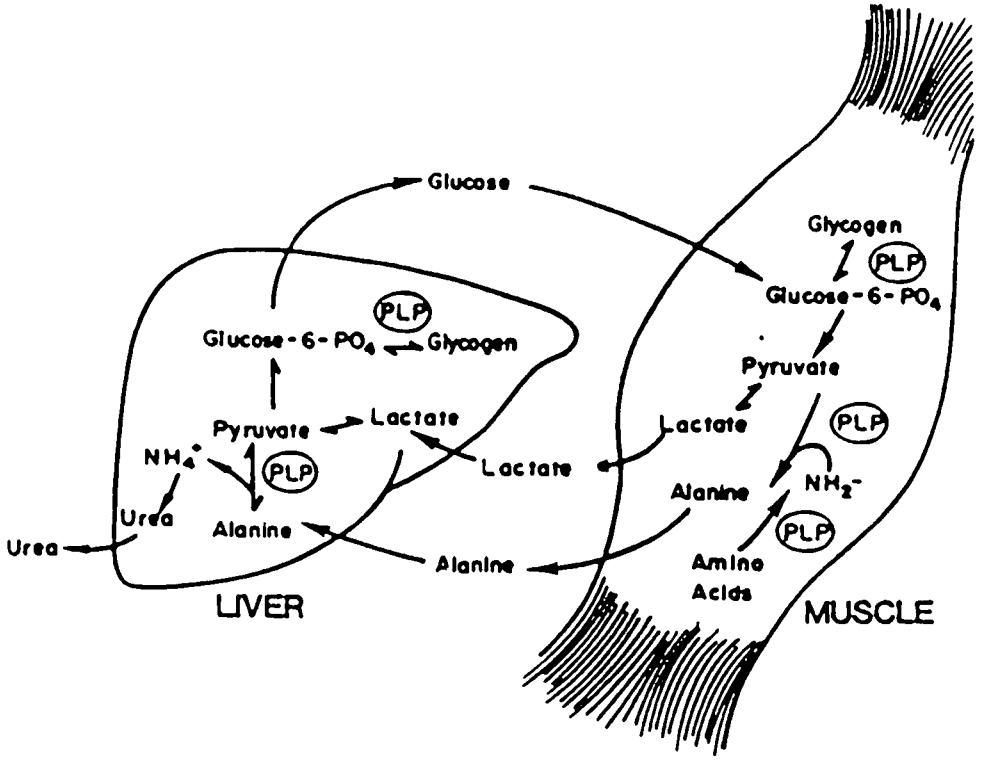
Figure 4. Functions of pyridoxal 5'-phosphate



Taken from Leklem[4] 1988

Alanine cycle, amino acids require PLP for conversion to alanine (See Figure 5). In addition, alanine aminotransferase utilizes PLP as a coenzyme for conversion of alanine to pyruvate. This reaction is reversible in both the liver and muscles, and both directions require either PLP or PMP as a coenzymes. In rats a vitamin B-6 deficiency has shown to decrease alanine transferase activity (Angel 1980). Females who were given 0.19 mg/d of vitamin B-6 for four weeks, did not show a significant change in fasting glucose concentrations (Rose et al. 1975). This suggests that low vitamin B-6 intakes may not compromise the gluconeogenic process to any large degree. However, an important question is whether supplementation of vitamin B-6 enhances the process. Unfortunately, no direct evidence answers this question.

Figure 5. Pyridoxal 5'-phosphate's involvement in the Cori-Alanine cycle



Taken from Leklem 1985

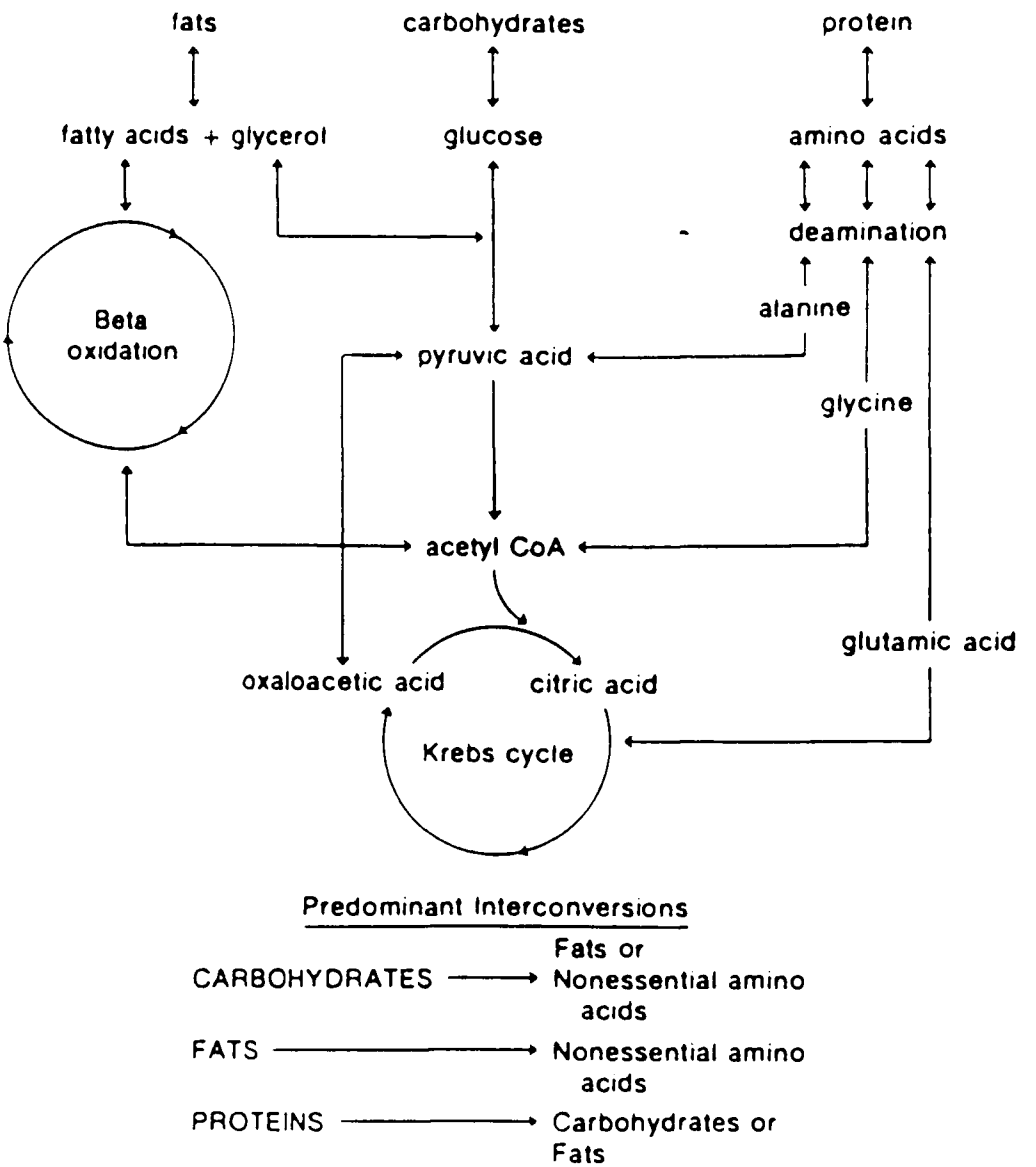
Exercise Physiology

Metabolism

Energy transfer in the body is accomplished by enzymatic catalyses of carbohydrates, fats, and proteins with the subsequent formation of adenosine triphosphate (ATP) (See Figure 6). Carbohydrates in the form of glucose are broken down through anaerobic (i.e. non-oxygen requiring) glycolysis, the Krebs Cycle, and finally the electron transport chain to yield a net of 36 ATP, whereas fats and proteins only enter at the Krebs Cycle, and due to their varying structures, produce different amounts of ATP. For example an 18 carbon fatty acid molecule will phosphorylate 147 ADP's. Following transamination selected amino acids are converted to specific Krebs cycle intermediates; and, this location in the cycle determines the amount of ATP formed. Oxidation of each mole of leucine, isoleucine, and valine (i.e. branched chain amino acids) yields between 32-42 moles of ATP (Felig and Wahren 1975).

It is within the phosphate bonds of the ATP molecule that large amounts of potential energy are contained and subsequently available for energy needs of cells. The splitting of ATP takes place whether oxygen is available or not. Hence, the term nonaerobic metabolism applies. Only about three ounces of ATP are stored within the body at any one time (McArdle 1986), or enough energy to perform maximal

Figure 6. Metabolism of fats, carbohydrates, and protein



Taken from McArdle 1986

exercise for several seconds (i.e. short sprints, heavy lifting). The ATP molecule is not supplied by blood, but must be resynthesized in each cell. This short term regeneration occurs by means of a similar cellular compound known as creatine phosphate (CP), which is maintained at cellular concentrations of three to fivefold that of ATP (McArdle 1986). By the process of phosphorylation or phosphate transfer ADP, is converted back to ATP, and the molecule is once again available for supporting muscular contraction.

ATP serves an important function in the contraction process of muscles. In brief, ATP's role is to promote sliding of the myosin and actin filaments relative to each other, which is known as the sliding filament theory, by freeing energy in ATP (Edington and Edgerton 1976). Other major components of this theory include troponin, tropomyosin, and calcium divalent cations, and due to the complexity of their interactions a review is not provided.

Oxygen Consumption

Oxygen consumption is a routine measurement performed by health professionals to aid in the quantification of various parameters, of which substrate utilization is included. Since the most basic of cellular processes require oxygen for the maintenance of life, an understanding of general energy metabolism at the level of ATP formation provides insight as to oxygen's vital role. Through multiple biochemical cycles

and pathways, oxygen is utilized to produce high energy phosphate bonds and carbon dioxide from the breakdown of foodstuffs.

Oxygen consumption, on a relative basis (i.e. ml/kg/min), is one of the most widely used measures to evaluate an individuals aerobic power. Maximum aerobic power or VO_2 max is a good indicator of the ability to resynthesize ATP aerobically, and has been described as the single best measure of cardiorespiratory fitness (MacDougall et al. 1982). Changes in this variable have been shown to be dependent upon exercise intensity, session frequency, session duration, length of training programs, and initial fitness levels of subjects (Weneger and Bell 1986).

Oxygen consumption rises rapidly during the initial minutes of exercise and eventually plateaus by the fourth minute if the intensity remains constant (McArdle 1986). In this situation one is considered to be in steady state. However, an important issue is that during the early minutes in exercise, oxygen uptake is below requirement, leading to an "oxygen deficit". During this period anaerobic processes (i.e. glycolysis) plus stored phosphates (i.e. nonaerobic) meet the energy demands (Mole et al. 1985). This so called "oxygen deficit" is repaid during recovery by an increase in the quantity of oxygen consumed above resting levels.

Open circuit spirometry is a method commonly applied for measuring oxygen consumption during exercise. The process

entails breathing ambient air, which consists of a constant composition of oxygen (i.e. 20.93%), carbon dioxide (i.e. 0.03%), and nitrogen (i.e. 79.04%). Since the body utilizes oxygen and produces carbon dioxide to perform physical work, the exhaled air contains altered concentrations of these gases. Thus, analysis of expired air reflects oxygen utilization. Not only is the procedure of measuring oxygen consumption used for exercise situations, but it is commonly used to calculate one's basal metabolic rate (BMR). However, in this situation a closed circuit spirometer is often used, which allows only the rebreathing of gas from within a prefilled container.

Calorimetry

Two methods broadly classified as direct and indirect calorimetry accurately quantify heat production or energy expenditure. Both give comparable results with the indirect method being much less expensive and relatively simple (Snellen 1980). Direct calorimetry involves placing an individual in an airtight thermally insulated living chamber and removing the body heat produced with a water cooling circuit. The difference in temperature of water entering the circuit and leaving the circuit reflects heat production. The non-invasive measurement of indirect calorimetry provides a useful tool in determining substrate utilization during rest and exercise. Following gas collection by spirometry, a

respiratory quotient (RQ) or respiratory exchange ratio (R) is calculated from oxygen consumption and carbon dioxide production to estimate the relative amounts of carbohydrate, fat, and protein combusted for human fuel metabolism. When such measures are obtained from pulmonary ventilation they are termed R and when determined from blood gas measurements across muscles or organs they are designated as RQ (Gollnick 1985). Due to differences in the chemical structures of carbohydrates, fats, and proteins varying amounts of oxygen are required for their complete oxidation. Using this principal a numerical value is assigned to each macromolecule, ranging from 0.70-1.0 (See Table 3). Thus, when carbohydrate alone serves as the fuel for the body, an equalmolar amount of carbon dioxide is produced per mole of oxygen consumed, giving a value of 1.0.

Example of glucose oxidation

$C_6H_{12}O_6 + 6O_2 \text{ ---> } 6CO_2 + 6H_2O \qquad R= 6CO_2/6O_2 = 1.0$

In the case of exclusive fat utilization, less carbon dioxide is produced for a given amount of oxygen used.

Example of palmitic acid oxidation

$C_{16}H_{32}O_2 + 23O_2 \text{ ---> } 16CO_2 + 16H_2O \qquad R= 16CO_2/23O_2 = 0.70$

When proteins are considered, the scenario becomes slightly

Table 3. Non-protein RQ's, caloric equivalents for oxygen, and the contribution of carbohydrate and fat for energy

NONPROTEIN RQ	KCAL PER LITER OXYGEN CONSUMED	PERCENTAGE KCAL DERIVED FROM		GRAMS PER LITER O2 CONSUMED	
		CARBOHYDRATE	FAT	CARBOHYDRATE	FAT
0.707	4.686	0	100	0.000	496
71	4.690	1.10	98.9	012	491
72	4.702	4.76	95.2	051	476
73	4.714	8.40	91.6	090	460
74	4.727	12.0	88.0	130	444
75	4.739	15.6	84.4	170	428
76	4.751	19.2	80.8	211	412
77	4.764	22.8	77.2	250	396
.78	4.776	26.3	73.7	290	380
79	4.788	29.9	70.1	330	363
80	4.801	33.4	66.6	371	347
.81	4.813	36.9	63.1	413	330
82	4.825	40.3	59.7	454	313
83	4.838	43.8	56.2	496	297
.84	4.850	47.2	52.8	537	280
.85	4.862	50.7	49.3	.579	263
.86	4.875	54.1	45.9	621	247
.87	4.887	57.5	42.5	663	230
.88	4.899	60.8	39.2	.705	.213
.89	4.911	64.2	35.8	.749	195
90	4.924	67.5	32.5	791	.178
.91	4.936	70.8	29.2	834	160
92	4.948	74.1	25.9	877	.143
93	4.961	77.4	22.6	921	.125
.94	4.973	80.7	19.3	964	108
95	4.985	84.0	16.0	1.008	090
96	4.998	87.2	12.8	1.052	072
97	5.010	90.4	9.58	1.097	054
98	5.022	93.6	6.37	1.142	036
.99	5.035	96.8	3.18	1.186	018
1.00	5.047	100.0	0	1.231	000

*From Zuntz, N. Pflugers Arch. Physiol., 83:557, 1901.

Taken from Zuntz 1901

more complex. Following deamination, the "keto acid" fragments are oxidized to carbon dioxide and water. The accepted value for the R of protein is 0.82 (McArdle 1986).

Rarely are either extremes of the R scale observed under normal conditions of steady state exercise, but values between 0.75-0.95 are seen, reflecting a mixture of carbohydrate and fat metabolism (Edwards et al. 1934). Only during high intensity anaerobic exercise will R values elevate to somewhere near 1.1. However, 0.70 is the bottom end of the scale and this value is commonly noted in persons of a fasted resting condition.

Plasma Volume Changes

A change in plasma volume is a common physiologic response to endurance exercise, and an accurate quantification of this value is necessary for assessing blood substrate concentrations. The two terms, hemoconcentration and hemodilution both represent the transient shifts of fluids into and out of the intravascular space. Hemoconcentration refers to the increasing concentration of a particular constituent, arising from a loss of plasma fluid. Conversely, hemodilution signifies an increase in plasma volume, which progressively decreases the concentration of solutes in the plasma. Both phenomena have been reported with exercise, and the direction and degree of change are related to heat acclimatization (Senay 1974), heat tolerance (Senay and Kok

1976), hydration states (Saltin 1966), training state (Senay 1978), and type of exercise performed (Senay et al. 1980). However, hemoconcentration generally occurs with cycle ergometry, and the magnitude is directly porportional to the intensity of exercise (Van Beaumont et al. 1981). At submaximal intensities (i.e. 70% VO_2 max), decreases of 13% in plasma volume have been observed at moderate temperatures (i.e. 26°C) (Nadel et al. 1979). Unless such changes are accounted for, concentrations of solutes in blood samples taken during exercise are relatively meaningless.

Several methods provide a measure of relative change in intravascular volume. These involve measuring hematocrit, hemoglobin, or plasma protein concentrations before and after an exercise bout. The concentration changes of the plasma constituents provide an interpretation of plasma fluid shifts. Specific equations are used for calculations. Another technique employing radioactively labeled albumin has also been used to calculate plasma volume changes in exercise (Harrison et al. 1975). Harrison et al. (1975) also employed the previous three methods mentioned; and, in conclusion rated use of hemoglobin concentrations to be the most reliable. Different indices may give different results in calculating plasma volume changes, therefore the methodology used in a given study deserves important consideration. The method of Dill and Costill (1974) determines plasma volume changes by measuring circulating hemoglobin and hematocrit before and

after exercise. In contrast, a method by Van Beaumont (1972) only measures hematocrit changes in the blood with exercise to determine plasma volume shifts.

Fuel Sources

Carbohydrates

Dietary carbohydrate plays a significant role in endurance exercise performance (Costill 1985) and has been extensively reviewed; therefore, this section will make reference to quantity, quality, timing of intake, and how they affect various metabolic sources. In particular, focus will be placed on the muscle, liver, and blood as fuel sources.

Glycogen, which is a polysaccharide composed of hundreds or thousands of glucose molecules linked together, represents a finite and limited fuel source. It is estimated that total body carbohydrate storage equals 375-475 g, of which approximately 325 g are muscle glycogen, 90-110 g are liver glycogen, and 15-20 g are blood glucose (Felig and Wahren 1975). At four kcal/g, the average person is therfore capable of 1500-2000 kcal of energy production at an R of 1.0. Data clearly show a correlation between diet, initial muscle glycogen levels, and endurance (Bergstrom et al. 1967). In subjects consuming a high carbohydrate diet (i.e. 82% of calories) muscle glycogen levels were six times greater compared to high fat diet, as determined by needle biopsy. Time to exhaustion on the high carbohydrate was nearly 200 minutes during submaximal exercise (70-80% VO_2 max), whereas when subjects consumed a high fat diet for three days, exercise could only be tolerated for about 60 minutes. A more

recent study involving trained female cyclists further confirms carbohydrates improved effects on endurance performance (O'Keefe et al. 1989). Three seprate isocaloric diets of 13%, 54%, and 72% carbohydrate resulted in times of 60, 98, and 113 minutes respectively, during cycle ergometry at 80% VO_2 max. It should be noted that these studies used a relatively short adaptation period, because investigators have shown endurance not to be impaired at slightly lower workloads (i.e. 65% VO_2 max) following adaptation to low carbohydrate diets (i.e. 20 g/d) for one month (Phinney et al. 1983). Possibly, if the workload were increased to 75-80% VO_2 max the results of Phinney et al. (1983) would differ, because glycogen depletion is best correlated with exhaustion at 75% of maximal oxygen uptake. At 50% VO_2 max, fat utilization predominates and at 100% VO_2 max, early exhaustion parallels lactate formation (Saltin and Karlsson 1971). If exertion is too low or too high, cessation of exercise may not reflect true glycogen depletion. If in fact glycogen is responsible for maximizing endurance performance at higher submaximal intensities, then it would appear advantageous to boost these levels in the liver and muscle to peak quantities prior to competition. Originally, in order to achieve this it was proposed that individuals consume a low carbohydrate diet (i.e. <10% of calories) for three days following exhaustive depletion exercise, and thereafter for three days to consume a high carbohydrate diet (i.e. >90% of calories). This

procedure, known as supercomposition, was shown to increase muscle glycogen levels by 200-300% (Hultman et al. 1971). A more recent scheme has proposed a "tapering" in exercise along with three days of 50% carbohydrate intake followed by three days of 70% carbohydrate intake to have similar results (Sherman 1983). An important consideration involving supercomposition is whether the type of carbohydrate affects resynthesis of glycogen. Complex carbohydrates and simple sugars show no difference 24 hours post-depletion exercise. However, between 24-48 hours a starch diet was significantly better in replacing muscle glycogen (Costill et al. 1981). The single most important factor in supercomposition is exercise induced depletion (Evans and Hughes 1985), which results in a marked elevation in glycogen synthase activity (Piehl 1974).

Although carbohydrate feedings in the days prior to exercise demonstrate positive effects, the intake of simple sugars within one hour of activity may ultimately be detrimental as a result of increased insulin levels, which decreases plasma glucose and free fatty acids and places a greater demand on muscle carbohydrate stores (Costill et al. 1977). However, ingestion of fructose prior to cycling at 75% VO_2 max did not induce the same results as glucose and sucrose (Costill 1985). Others have also demonstrated similar findings with fructose administration (Levine et al. 1983).

When carbohydrate supplementation is given during

exercise at 60-80% VO_2 max, fatigue, which normally occurs after two hours is delayed by 15-30 minutes (Coyle and Coggan 1984). Generally, fluids are the preferred form of supplement, and their osmolarity has a large impact on the rate of gastric emptying regardless of whether the drinks contain glucose, fructose, or sucrose. With high sugar concentrations (i.e. 150 mM) and intense exercise (i.e. 70-75% VO_2 max) solutions will be delayed in the stomach (Costill and Saltin 1974), but studies suggest that at least 50 g of glucose can be emptied during one hour of activity (Costill 1985). In conclusion, for carbohydrate feeding to be successful in prolonging exhaustion, the uptake and utilization of exogenous glucose by muscles must exceed the lost energy from a decrease in free fatty acid oxidation.

Blood glucose provides an alternate metabolic source of carbohydrate, which can only be provided by the liver due to glucose-6-phosphatase, not being present in muscle. During the early minutes of exercise, both blood glucose and glycogen serve as the primary energy sources, with blood glucose uptake by muscles increasing by ten to twentyfold above rest (Wahren et al. 1971). Likewise, high intensity exercise places large demands on plasma glucose and under such conditions may supply up to 40% of the total energy requirement (Felig and Wahren 1975). When comparing hepatic glucose output with intensity (i.e. 30-80% VO_2 max) an elevated release was noted with each respective increase in workload, and the range of increase

after 20 minutes of work was 1.5-5.5 mmol/min (Hultman 1989). A large demand on blood sugar, like that during exercise may lead to eventual hypoglycemia, which is often associated with fatigue. However, this should not be confused with muscle glycogen depletion. Non-invasive techniques (i.e. endurance times) of predicting true exhaustion can be a difficult and somewhat inaccurate process; therefore, muscle biopsies or plasma samples should be collected to obtain other possible causes of fatigue. Since endurance exercise does not require maximal exertion, blood glucose is used to lesser degree and greater emphasis is placed on muscle glycogen; however, both sources represent limited stores, so it is logical to expect an alternate fuel such as fat contributes significantly to the energy demands of prolonged exercise.

Fats

One of the most noteworthy functions of body fat is to provide a large reserve of energy. In the average college-aged male, there is sufficient fat to fuel a run from New york City to Madison, Wisconsin, in contrast to carbohydrate which provides only enough energy for a 20 mile run. Likewise, even the leanest of athletes possess vast quantities of storage fat (i.e. approximately 600,000 KJ) (Evans and Hughes 1985). The primary storage form in adipose tissue is triglyceride, accounting for more than 95% of the total. This moiety consists of a three carbon glycerol "backbone" with long chain

fatty acids esterified. Both fatty acids in a "free" form and glycerol serve as important fuels, and since their relative contributions to endurance exercise differ they will be reviewed seprately.

As moderate exercise continues, the energy derived shifts from mostly carbohydrate at the onset, to a greater shift in fat with time. Typically, graphs are used for visual aids, and the hypothetical lines representing these two fuels would mirror each other from the start to finish of submaximal events. During one to four hours of prolonged exercise, free fatty acid (FFA) uptake into muscles rises about 70%, and fats may supply nearly 80% of the total energy requirement (Ahlborg et al. 1974). The hormonal effects of decreased insulin and increased glucagon, which accompany lowered blood sugar during endurance exercise, probably stimulate this increase in fat mobilization and subsequent metabolism (Saudek and Felig 1976). It appears that mobilization from adipose tissue is the key to fat oxidation, because plasma FFA concentrations are related to utilization (Havel et al. 1966). High plasma FFA levels signify greater utilization, and conversely lower plasma concentrations relate to a decreased usage of FFA for fuel.

Researchers (Callow et al.1986) consider fats to be the preferred fuel during exercise, but feel that their contribution to ATP production via B-oxidation is rate limiting (possibly restrained by solubility), because of

inadequate plasma FFA levels. According to this scheme, they feel fat's contribution as an energy source is always at the maximum possible. However, with higher exercise intensities the required rate of ATP production cannot be met solely from fat, therefore carbohydrate must make up this discrepancy in providing energy for muscle contraction. In fact, the maximum intensity which can be sustained from fat is only 50% VO_2 max in untrained persons and roughly 65% VO_2 max in trained populations (Holloszy and Coyle 1984). A major problem arises with complete carbohydrate depletion. Citric acid cycle intermediates are needed to continue oxidative metabolism, and it is via carbohydrates (i.e. pyruvate) that oxaloacetate is formed. When the concentration of oxaloacetate in the mitochondria is low, the ability of acetyl groups combining to form citrate is limited. This can have a retarding effect on fat metabolism (Gollnick 1985). Callow et al. (1986) believed that the contribution of plasma and intramuscular triglycerides was insignificant; but, future research contradicted this thinking. In a study (Hurley et al. 1986) involving exercise training, the results showed lower plasma FFA and glycerol concentrations in the trained versus the untrained condition, suggesting decreased fat availability; but, respiratory exchange data revealed greater overall fat utilization from 35% to 57% of the total exercise energy expenditure. In addition, muscle triglyceride concentration after exercise decreased roughly twofold with training, and

this difference correlated with the total increase in fat lipolysis.

Certain food items, namely caffeinated beverages, have demonstrated positive effects in fat utilization and endurance performance. When the equivalent of two to three cups of brewed coffee (i.e. 330 mg of caffeine) was consumed one hour prior to moderately strenous exercise, subjects performance times increased from 75 to 90 minutes relative to a decaffeinated trial (Costill et al. 1978). Plasma substrate levels of FFA and glycerol indicated higher fat metabolism. Furthermore, a decrease in R values was observed, confirming increased lipid oxidation and a corresponding decrease in carbohydrate oxidation. Apparently, caffeine's mechanism of action is via inhibition of phosphodiesterase, which increases cAMP levels and epinephrine (Arogyasami et al. 1989). It should be noted that not all studies involving caffeine and endurance performance have yielded the same results. In an attempted replication of the above study, other researchers (Butts and Crowell 1985) did not find a caffeine effect similar to that of Costill et al. (1978). Apparently, the responses to caffeine ingestion vary markedly among individuals.

Protein

Proteins play several crucial roles in exercise, however their contribution to energy expenditure via amino acid

oxidation is only 5-15% of the total caloric expenditure (Lemon and Mullin 1980). Proteins are involved in 1) enzymatic pathways to form ATP, 2) oxygen delivery (i.e. myoglobin and hemoglobin), 3) muscle formation and contraction (i.e. myosin and actin), and 4) tissue repair (Stryer 1981).

The catabolism of protein first involves degradation to amino acid components. Nitrogen is then cleaved from amino acids in a process known as deamination, and excreted from the body as urea. Another process, not to be confused with deamination, is transamination. This involves the removal of nitrogen from specific amino acids and subsequent transfer to other compounds. The carbon skeletons remaining from both deamination and transamination are further degraded in energy metabolism. If an individual is excreting less nitrogen in urine, feces, and sweat than they are ingesting from protein sources then a positive balance exists. When protein intake is insufficient, negative nitrogen balance occurs. For an even more complete view of protein breakdown, the rate of leucine oxidation should be measured. For example 90% of this indispensible amino acid (i.e. leucine) may be oxidized during two hours of moderate exercise (Evans et al. 1983), regardless of any change in urea production (Wolfe et al. 1982).

The current RDA for protein is 0.8 g/kg body weight, and is an approximation based on data from relatively sedentary individuals (U.S. Food and Nutrition Board 1989). Although eating three times the recommended level of protein has been

shown to result in no enhancement of work capacity during intensive training (Consolazio et al. 1975), endurance athletes may require higher amounts than the RDA. The basis for this statement comes from a study involving well trained runners (Friedman and Lemon 1989). When the subjects consumed the RDA for protein, nitrogen balance was negative (i.e. -5.29 ± 2.58 g/d) during an exercise test day. However, following a diet 70% higher in protein, retention of nitrogen was observed (i.e. 2.41 ± 1.99 g/d). All subjects were consuming adequate carbohydrates and total calories for both tests, which were 75 minutes in duration at 72% VO_2 max. Adequate energy intake becomes very important, because in a glycogen depleted state the involvement of protein in energy production dramatically rises (Lemon and Nagle 1981).

Factors such as high exercise intensity, long duration exercise, chronic training, and decreased carbohydrate availability promote increased amino acid oxidation (Lemon 1987). It appears that certain muscle and liver proteins (i.e. amino acids) are labile. In particular the branch chain amino acids are available for energy metabolism (White and Brooks 1981, Lemon et al. 1982). Protein found in nervous and connective tissue is relatively "fixed", therefore leaving it unable for supplying fuel (McArdle 1986).

During exercise protein synthesis is depressed (Wolfe et al. 1981). This allows movement of amino acids, mostly in the form of alanine from muscle to liver where gluconeogenesis is

increased. Clearly, there is an increase in alanine output as exercise intensities progress (Felig and Wahren 1971). A model known as the alanine-glucose cycle explains how alanine indirectly serves the energy requirements of exercise Amino acids in the muscle are transaminated to form alanine. The alanine is shuttled in the blood to the liver, where it undergoes deamination to pyruvate. Finally, pyruvate is converted to glucose, which is released into the blood and taken up by muscles. The original transaminated amino acids, which synthesized alanine can be oxidized for energy within cells. The alanine-glucose cycle can supply as much as 45% of hepatic glucose output and 10-15% of the total energy requirement during four hours of continuous exercise (Felig and Wahren 1971). Physiologically, this cycle helps to prevent hypoglycemia and may ultimately prolong exhaustion in susceptible individuals.

Lactic Acid

Lactic acid (i.e. lactate) formed during glycolysis is often considered a waste product of exercise, but it actually serves as an important energy source. Two recent hypotheses, The Glucose Paradox and The Lactic Acid Shuttle, have lead to this change in belief (Newgard et al. 1983, Foster 1984, Brooks 1985,1987). The Glucose Paradox describes how glucose from digested dietary carbohydrate bypasses the liver and is taken up by muscle. The muscle can either synthesize glycogen

or produce lactic acid. If lactic acid is formed, it recirculates to the liver and stimulates glucose and glycogen formation. The Lactic Acid Shuttle holds that specific muscle fibers (i.e. FG-fast glycolytic) can form lactate, which can be combusted in adjacent muscle fibers (i.e. SO-slow oxidative), or can be released into general circulation from muscle capillaries. Humans are constantly forming lactate at rest, but continuous removal from the blood maintains low concentrations (Brooks 1988). Recall that at the onset of exercise much of the energy is supplied non-oxidatively from blood glucose and muscle glycogen. Hence, with the initiation of exercise a rise in blood lactate occurs. However, during submaximal exercise the oxidative systems in mitochondria will be activated in a few minutes, and the rapid rate of glycolysis will be diminished. In fact, the blood lactate levels may even decline (Brooks 1986). This may in part be due to an increased rate of gluconeogenesis often associated with low liver glycogen stores in the later stages of exercise. If the exercise is highly anaerobic or maximal, concentrations of lactate may rise to 10-15 mM in the plasma, a level which is associated with fatigue (Hogan and Welch 1984). High levels of lactate have been shown to decrease FFA release from adipose tissue (Bagby et al. 1978). Lactic acid begins to accumulate at about 55% of an untrained persons max capacity, and depending on the level of training, higher max capacities can be achieved before plasma lactate accumulates

(Costill et al. 1973, Davis et al. 1979). Compared to trained animals untrained animals produce similar lactate amounts, but they show greater lactate oxidation and conversion to glucose (Donovan and Brooks 1983). In a sense the removal mechanisms are superior in trained animals. At the point when removal cannot match production, one is said to be at an anaerobic threshold. Generally, the onset of blood lactate accumulation (OBLA) can be detected in several ways: 1) an increase in blood lactate concentrations, 2) a decrease in blood pH and bicarbonate, 3) an increase in R, due to an excess of CO_2 produced from the buffering of lactic acid by bicarbonate, and 4) a rapid increase in ventilation to blow off extra CO_2 (McArdle 1986). In addition to training, lactate levels can be influenced by diet. Compared to low carbohydrate diets high carbohydrate diets increase lactic acid levels at rest and during submaximal exercise (Bergstrom et al. 1967, Hermansen et al. 1967, Saltin and Hermansen 1967, Karlsson and Saltin 1971, Bergstrom and Hultman 1972).

Once lactic acid is formed and enters the circulation, its fate consists of several possibilities. In addition to being oxidized by skeletal muscles, lactate can be extracted from the blood and used as energy by the myocardium. Lactate formed by physically active muscles can also be transported in the blood to inactive muscles and utilized. Approximately 75% of lactic acid formed during aerobic exercise is used directly as an aerobic energy source (Brooks 1988). The remaining 25%

is converted to glucose by the liver and kidneys (Depocas et al. 1969, Donavan and Brooks 1983). By vascular shuttling of lactic acid, muscles depleted of glycogen can be restored during recovery of strenous exercise (Ahlborg and Felig 1982, Ahlborg et al. 1986). For example, after strenuous cycling the quadriceps would potentially deplete their carbohydrate stores. The relatively inactive arms release lactic acid, which goes to the liver and kidneys. Within these two organs, lactate is converted to glucose. Glucose is then released into the circulation and taken up by the previously active leg muscles to resynthesize glycogen. The conversion of lactic acid to glucose is generated via the Cori cycle. Simply stated, the cycle converts pyruvic acid and lactic acid formed in the muscle into glucose in the liver.

The importance of lactic acid as a fuel source can partially be explained by its molecular characteristics (Brooks 1988). Unlike other substates (e.g. glucose), lactate is relatively small and readily exchangeable. Its movement across membranes requires no cofactor (e.g. insulin) and occurs by facilitated diffusion. Lastly, in contrast to muscle which lacks the enzyme necessary to release glucose into the blood from glycogen, lactate can be made rapidly in large quantities and released into the blood. Evidence to date strongly suggests that lactate is a preferred fuel source for submaximal endurance exercise.

Glycerol

Glycerol can be utilized as a direct energy source during exercise. Following lipolysis, glycerol enters glycolysis as 3-phosphoglyceraldehyde and is degraded to pyruvic acid. Pyruvate then proceeds through the TCA cycle, and in total 22 ATP are formed from one glycerol molecule. Indirectly, glycerol contributes to energy formation by providing carbon skeletons for glucose synthesis. This gluconeogenic role becomes increasingly important during endurance exercise, when glycogen reserves become depleted or when dietary carbohydrate is restricted. In comparison to a basal resting condition, glycerol utilization increases tenfold with prolonged exercise (Ahlborg et al 1974). A study involving cycling at 73% VO_2 max looked at the effects of pre-exercise feedings of glycerol (i.e. 1g/kg body weight) on endurance times (Gleeson et al. 1986). No time difference was observed between glycerol and the placebo trial. With the ingestion of glycerol, plasma FFA was depressed, which may be a reason why no increase in endurance was noted. The benefits of glycerol as a direct energy source and as a means of gluconeogenesis during long term exercise may be balanced out by its inhibition on FFA utilization. The authors concluded that glycerol cannot be used rapidly enough as a gluconeogenic substrate to serve as a major energy source. In addition, plasma osmolality markedly increased following glycerol intake, which could have led to significant tissue dehydration; and, interestingly all

subjects complained of headaches following glycerol ingestion.

As previously mentioned, triglycerides are broken down to glycerol and FFA. It seems reasonable to expect that plasma levels of glycerol can aid in better understanding the quantities of FFA utilized. In fact, glycerol is used to estimate lipolysis in adipose tissue (Hetenyi et al. 1983). Since FFA are rapidly taken up by muscle during physical activity, their plasma levels may slightly underestimate FFA utilization. However, glycerol remains in the blood for a longer period of time than FFA, and therefore better indicates fat mobilization.

Plasma Fuel Levels

Listed in Table 4 is a quantitative summary of plasma substrate concentrations observed in five seprate studies. Glucose and lactic acid are commonly measured in the plasma to help explain carbohydrate metabolism, whereas glycerol and FFA help in an understanding of the utilization of fats for energy. In the table a brief description of the five studies is provided along with concentrations of the aforementioned fuel sources. The values reported in the table provide a possible range one might expect when performing the various assays associated with these fuels. In most cases there is relative consistency in values obtained between the studies. If, however, particular values appear significantly different in a particular study compared to other studies (e.g. Coyle et

Table 4 Plasma substrate concentrations: pre, during, and post endurance exercise

SUBSTRATE and Reference	Sub	Ex	Dur	Int	DC	Conc				
						Pre	During	Post	Post 30	Post 60
								mmol/L		
GLUCOSE										
Hofmann et al 1991	Six TM	CE	120	63	Fast	5 20	5 50	5 20	5 10	5 10
Tarnopolsky et al 1990	Six TM	TR	93	63	Fast	4 50	5 00	4 50	4 00	
Gleeson et al 1986	Six TM	CE	95.9	73	Fast	5 50	5 00	5.00	5 50	
Hurley et al 1986	Nine TM	CE	120	64	Fed					
Coyle et al 1983	Ten TM	TR	134	74	Fast	4 00	4 10	2 90		
LACTIC ACID										
Hofmann et al 1991						1 40	2 50	2 70	1 30	1 40
Gleeson et al 1986						0 50	2 10	2.50	1 60	
Hurley et al 1986						0 80	1 20	1 40		
Coyle et al 1983						0 90	3 60	2 60		
GLYCEROL										
Tarnopolsky et al 1990						0 09	0 30	0 39	0 19	
Gleeson et al 1986						0 04	0 15	0 29	0 23	
Hurley et al 1986						0 07	0 14	0 30		
Coyle et al 1983						0 07	0 24	0 40		
FREE FATTY ACIDS										
Hofmann et al. 1991						0 40	0 50	1 10	1 20	1 10
Tarnopolsky et al 1990						0 65	0 50	0 80	1 35	
Gleeson et al 1986						0 25	0 25	0 80	1 10	
Hurley et al 1986						0 50	0 65	1 00		
Coyle et al 1983						0 41	0 43	0 85		

Sub- Subjects; T = trained; M = male

Ex- Exercise type; TR = treadmill run; CE = cycle ergometer

Dur- Duration of exercise (minutes)

Int- Intensity as a % of VO2 max

DC- Dietary conditions, fast = fasting; fed = non-fasting

Conc- Concentrations of substrates in mmol/L

Pre-before exercise, During-60 min into exercise, Post-immediately after exercise; Post 15, 30 and 60 15, 30, and 60 min after exercise

al. 1983 post-glucose), then the difference may be related to the variables described (i.e. subjects, exercise, and diet) between the studies or to the methodology used.

The table shows that glucose concentrations were variable. Plasma glucose increased or decreased during exercise and was also quite variable during one hour of recovery. In general, glucose concentrations changed relatively little compared to the other three plasma substrates. Lactic acid rose throughout the duration of exercise in each study described, except for Coyle et al. (1983). The increase in lactic acid occurred predominantly within the first hour of exercise. Little change in lactic acid occurred from 60 minutes into exercise to two hours plus of exercise. In the recovery period, lactic acid concentrations decreased in the plasma. In all the studies, glycerol dramatically rose from pre-exercise to 60 minutes into exercise and from 60 minutes to the the end of exercise. Plasma levels of glycerol decreased in recovery. Similar to glucose, FFA gave slightly variable results. From pre-exercise to 60 minutes into exercise plasma FFA concentrations increased or decreased slightly. However, in all the studies the post-exercise plasma FFA concentration was significantly higher than the during (i.e. 60 minutes into exercise) or pre-concentration. Inconsistent with glycerol, plasma FFA concentrations continued to rise in the recovery period or remain elevated, but showed no signs of a significant decline.

Fuel Summary

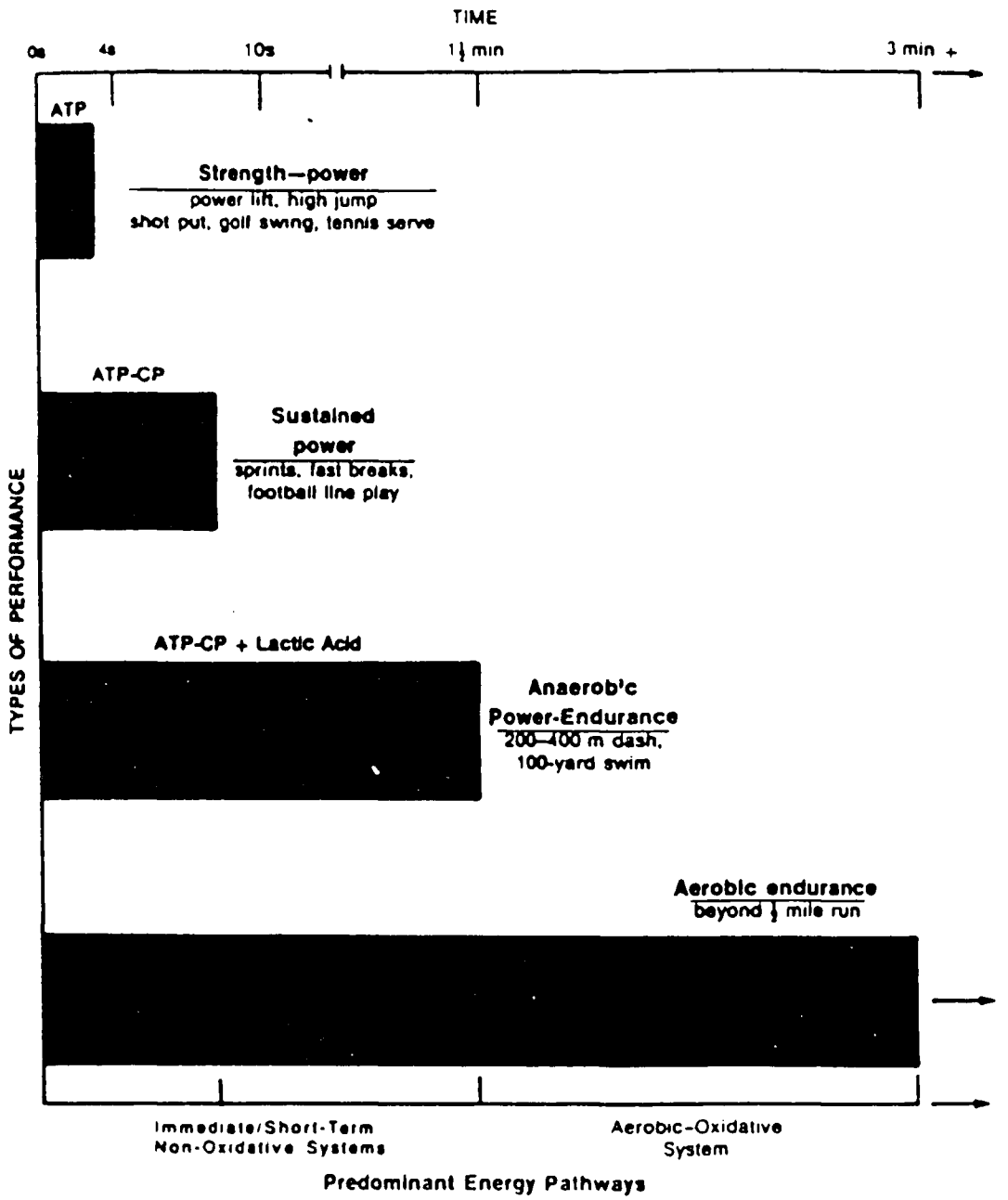
In general, human fuel utilization can be summarized as follows: Muscle cells serve to transform chemical energy into mechanical energy and heat through the use of ATP. The regeneration of ATP occurs from CP, as well as by aerobic and anaerobic metabolism, with cellular ADP levels regulating production. Since CP can only provide adequate energy for a few seconds of exercise, other substrates, primarly carbohydrate and fat must be utilized. These can either be endogenous cellular sources like glycogen and triglycerides, or can be provided from circulation as glucose and FFA. During moderate prolonged endurance activity three phases of fuel usage are observed. Early in exertion cardiac output is not directed towards muscles and inadequate oxygenation exists, which necessitates anaerobic metabolism, primarily through glycogenolysis (Rodahl et al. 1964). Additionally, the hormonal changes for exogenous fat supply have not yet taken place (Consolazio and Johnson 1972). In the second phase, a blend of carbohydrate and fat supplies energy demands of the working body. Carbohydrate stores become depleted and the level of insulin declines, whereas the level of epinephrine rises, resulting in FFA release from adipose tissue (Havel et al. 1967). Finally, in phase three glycogen may become entirely depleted, in which case fat may supply almost all the energy requirements. However, it is at this point that amino acids and other metabolites like glycerol and

lactate become important contributors through gluconeogenesis. The post-exercise recovery period is one of transition from catabolism to anabolism. Glycogen, lipid, and ultimately muscle mass must be restored. From zero to four hours after exercise at 70-75% VO_2 max on a cycle, exercised muscles show an increased rate of glucose uptake for glycogen replacement, while non-exercised muscles demonstrate insulin resistance and continue to release alanine and lactate for two hours after exercise. By 12-16 hours of recovery, subjects continue to oxidize more fat and less carbohydrate than in a resting post-absorptive state (Horton 1989). All the events described serve only as a general scheme for exercise metabolism. In the next section, variables will be discussed that can significantly alter fuel use.

Fuel Modifiers

Substrate utilization during exercise can be modified by several factors, such as intensity, duration, training, diet, and gender. Under circumstances of inadequate oxygen supply, as in high intensity exercise bouts, carbohydrate becomes the preferred fuel (See Figure 7). Carbohydrate's role in intense exercise dates back to the previous century. Measurements of R showed an increase from 0.75 at rest to 0.95 during intense exercise (Chauveau 1896). Researchers have demonstrated an exponential relationship between the rate of muscle glycogen metabolism and percentage of maximal oxygen consumption

Figure 7. Energy pathways for selected types of exercise



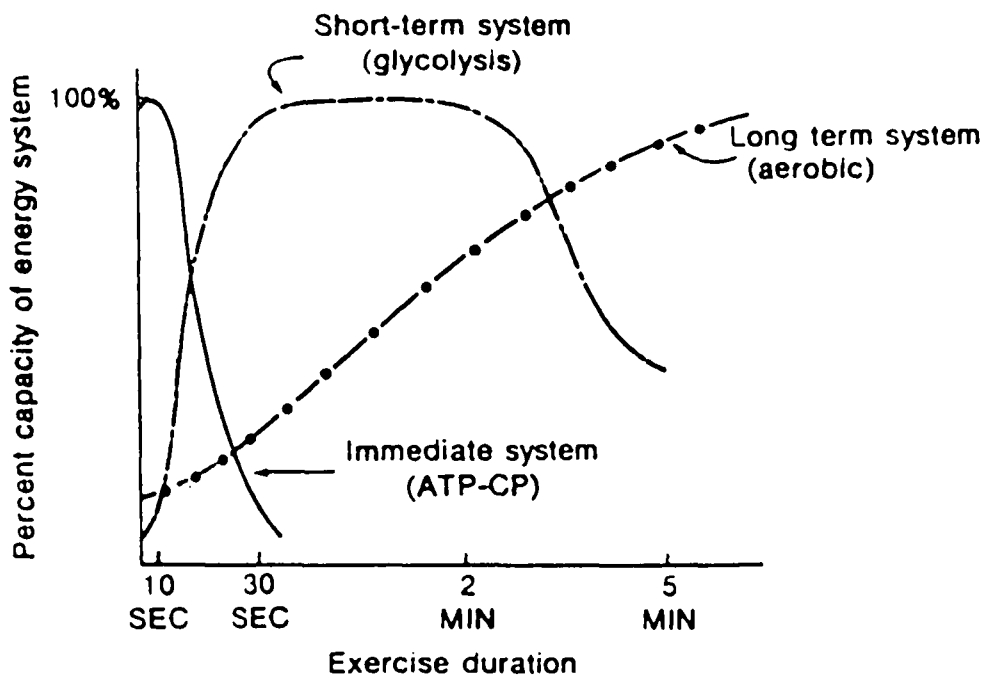
Taken from McArdle 1986

(Saltin and Karlsson 1971), and when muscle stores are undoubtedly empty, maximal oxygen uptake is only 85% of a control condition (Astrand et al. 1963). Very little carbohydrate is combusted during activities such as walking (i.e. 0.3 mmol/kg/min), however during maximal isometric contractions the demand is greatly increased (i.e. 40 mmol/kg/min) (Hultman 1979).

Duration has just the opposite effect on R data, which is indicative of greater reliance on fat (See Figure 8). At a constant workload of 60-70% VO_2 max on a treadmill, R values declined from a range of 0.93-0.97 to 0.76-0.77 during an extensive time course of seven hours. During the run carbohydrate metabolism declined from an intial value of 90% to roughly 20% (Edwards et al. 1934). Although seven hours may seem quite lengthy, this same phenomenon was observed in about half this duration (Christensen and Hansen 1939).

An individual's endurance capacity is greatly enhanced through training (See Table 4a). Two reasons for this are 1) an alteration in fuel use during submaximal exercise, and 2) an increase in total body oxygen consumption (Gollnick 1985). Training results in a more rapid shift to fat metabolism and greater overall utilization (Holloszy and Coyle 1984). In addition, trained individuals appear to store much higher quantities of muscle glycogen than their untrained counterparts (Gollnick et al. 1973, Sherman et al. 1981). Hence, by shifting to fat earlier in activity, and therefore

Figure 8. The relationship between energy pathways and exercise duration



Taken from McArdle 1986

Table 4a. Training adaptations in man

VARIABLE	UNTRAINED	TRAINED	PERCENT DIFFERENCE[a]
Glycogen, mmol·g wet muscle^{-1}	85.0	120	41
Number of mitochondria, mmol^{3}	0.59	1.20	103
Mitochondrial volume, % muscle cell	2.15	8.00	272
Resting ATP, mmol·g wet muscle^{-1}	3.0	6.0	100
Resting CP, mmol·g wet muscle^{-1}	11 0	18.0	64
Resting creatine, mmol·g wet muscle^{-1}	10.7	14 5	35
Glycolytic enzymes			
Phosphofructokinase, mmol·g wet muscle^{-1}	50.0	50.0	0
Phosphorylase, mmol·g wet muscle^{-1}	4–6	6–9	60
Aerobic enzymes			
Succinate dehydrogenase, mmol·kg wet muscle^{-1}	5–10	15–20	133
Max lactic acid, mmol·kg wet muscle^{-1}	110	150	36
Muscle fibers			
Fast twitch, %	50	20–30	−50
Slow twitch, %	50	60	20
Max stroke volume, ml·beat^{-1}	120	180	50
Max cardiac output, l·min^{-1}	20	30–40	75
Resting heart rate, beats·min^{-1}	70	40	−43
Max heart rate, beats·min^{-1}	190	180	−5
Max (a-$\bar{v}$) O_2 diff, ml·100 ml^{-1}	14.5	16.0	10
Max $\dot{V}O_2$, ml·kg^{-1}·min^{-1}	30–40	65–80	107
Heart volume, l	7.5	9.5	27
Blood volume, l	4.7	6.0	28
$\dot{V}e$ max, l·min^{-1}	110	190	73
Percent body fat	15	11	−27

Taken from McArdle 1986

sparing carbohydrate which is presumably at much higher levels, a trained individual can sustain a constant workload longer than if he/she were sedentary. Following a 12 week training program, muscle glycogen utilization decreased by 41%, whereas overall fat metabolism increased by 61% in adult males (Hurley et al. 1986).

Higher lactate levels are indicative of carbohydrate metabolism, and therefore can help identify fuel modification. Studies involving training of only one leg and exercise involving both legs showed a net release of lactate from the untrained leg with a net uptake in the trained limb (Saltin et al. 1976), and the reported RQ across the trained leg was lower (Hendriksson 1977). An explanation of the enhanced oxidative capacity of muscles with training can be traced back to basic metabolic pathways. In a previous section, energy transfer was linked to enzymatic catalyses of foodstuffs. In this respect, it might then be expected that alterations in enzymes contribute to a "training effect". In fact, within the mitochondria there is an increase of enzyme activity involved in fat oxidation (Holloszy and Booth 1976). Following training there is also an increase in capillarization of trained muscles (Ingjer 1979), which allows for greater gas exchange of oxygen and carbon dioxide. In summary, training exerts a significant influence on fuel utilization by promoting efficient oxygen delivery (i.e. increased capillarization) and enhancing the oxidative

potential (i.e. increased enzyme activity) of energy transfer.

The contribution of carbohydrate, fat, and protein to energy production is clearly influenced by diet. Early in the twentieth century researchers observed that at the same intensity of exercise and following consumption of a high fat diet, the R was below 0.80 compared to above 0.85 when subjects ate carbohydrate rich foods (Krogh and Lindhard 1920). This translates to roughly 66% and 50% of the calories derived from fat, respectively. Other research (Phinney et al. 1983) demonstrated similar results of decreased carbohydrate utilization and increased fat oxidation following adaptation to a high fat diet. Glucose oxidation decreased threefold and muscle glycogen utilization decreased fourfold during exercise after a four week ketogenic diet compared to a diet higher in carbohydrate.

Not only does the composition of foods affect fuel availability and utilization, but timing of intake can significantly alter substrate metabolism. For example, in a fasted condition gluconeogenic substrates account for a larger fraction of hepatic glucose output (Wilson et al. 1979), and total output of glucose is decreased by 65% with starvation (Hagberg et al. 1980). With the aid of naturally occuring ^{13}C-glucose isotopes an estimation of glucose metabolism can be measured. Ingestion of 100 g of glucose resulted in 88% of the total amount of glucose being oxidized during four hours of exercise at 45% VO_2 max (Laughlin and Armstrong 1983).

Another study used a similar exercise intensity, duration, and glucose load, and recovered 11% of the $^{13}CO_2$ produced from ^{13}C-glucose during the three hour rest period prior to exercise. From the start of exercise to 30 minutes into exercise, 19% of the $^{13}CO_2$ was recovered, and by one hour of exercise 68% of the $^{13}CO_2$ was recovered (Jenkins et al. 1986). These results provide evidence that fuel sources in the form of carbohydrate consumed within several hours of activity are actively utilized in energy production.

Early investigations of a gender effect on substrate utilization yielded contrasting results. However, a recent well controlled study (Tarnopolsky et al. 1990) concluded that males and females possibly rely on different energy sources during moderate intensity (i.e. 65% VO_2 max) long duration (i.e. 95 minutes) exercise. The significant findings of this study were that males maintained and excreted 30% more urea nitrogen than equally trained and nourished females, and females clearly demonstrated higher FFA oxidation and less carbohydrate and protein metabolism than males.

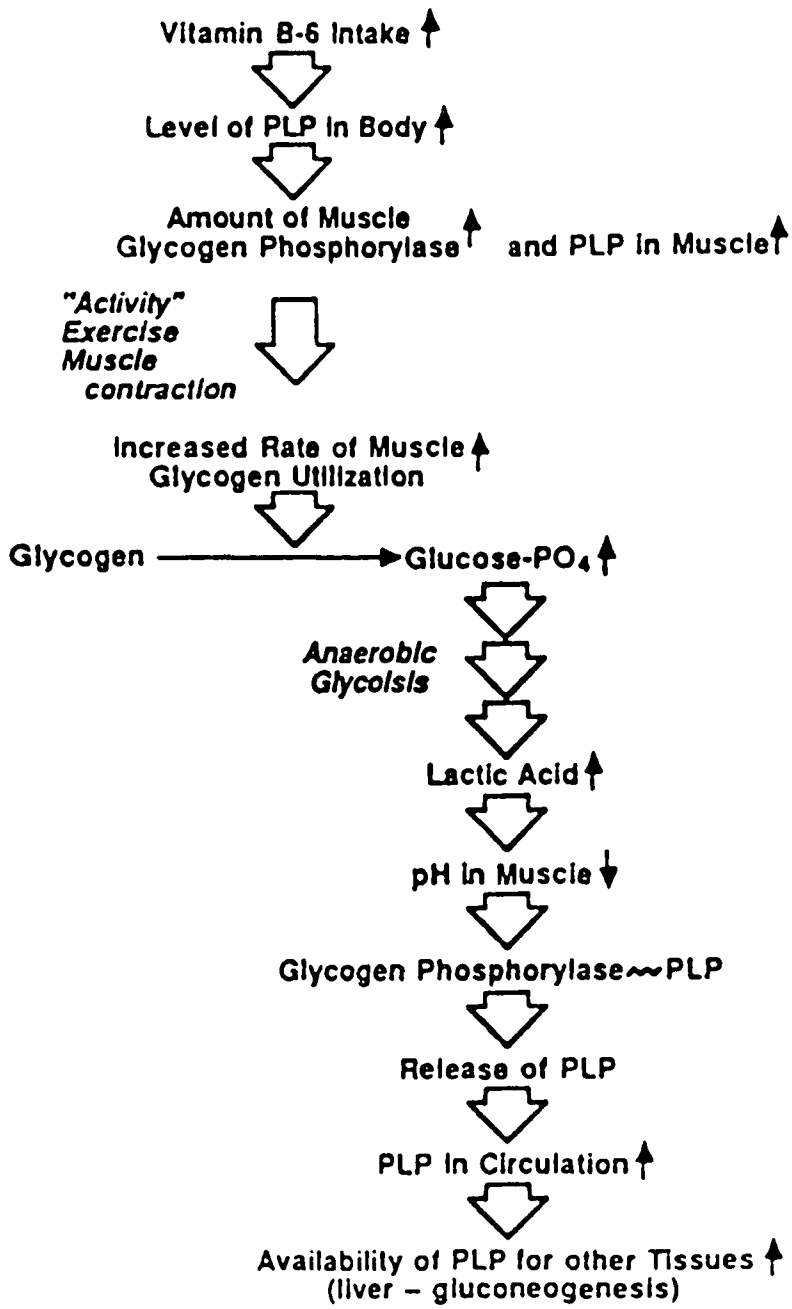
Vitamin B-6 and Exercise

Enormous amounts of research have been conducted on vitamin B-6. Likewise, the topic of exercise physiology has compiled a vast data base. However, when the two subject areas are combined, relatively little is known, particularly in relation to fuel utilization. To this date, there is no conclusive evidence to suggest that vitamin supplementation improves performance in nutritionally adequate individuals (Belko 1987); however, supplements may affect performance. There is some evidence to suggest that vitamin B-6 supplements may actually alter substrate metabolism in humans during moderate intensity exercise, and regarding vitamin B-6, "more" possibly is not better.

One of the first studies (Leklem and Shultz 1983) involving vitamin B-6 and exercise in humans was designed to determine what effect strenuous activity (i.e. running approximately three miles) would have on plasma levels of PLP. In order to study this, a group of seven, adolescent, male, runners were studied. The investigation showed for the first time, that exercise in the form of middle distance running alters plasma PLP levels. Increases of the vitamer in blood following exercise ranged from 0.35-2.98 nmol/100ml (i.e. 6.4-57.7%) for individual subjects, and these results were, in part, independent of plasma volume shifts. The rise in plasma PLP during exercise may be slightly higher in males than

females (Leklem 1985). Based on previous rat studies (Black et al. 1977, 1978), the researchers hypothesized that the increase in PLP occurred via release from glycogen phosphorylase, a potential storage site. Belief was that exercise , which has been viewed as acute starvation (Lemon and Nagle 1981), may be stimulating the same mechanism of PLP release in humans as was observed in calorie deficient rats (Black et.al. 1978). A current review on vitamin B-6 (Leklem[b] 1988) has proposed an interesting mechanism and rational for the release of PLP from muscle during exercise (See Figure 9). An increase in vitamin B-6 intake results in an increase in glycogen phosphorylase and PLP, as observed in rats. During physical activity an increase in glycogen breakdown would occur, resulting in higher muscle lactate levels. This would lower the pH to 6.5-6.6 in the muscle, and thus allow release of PLP from the enzyme. Once in the circulation PLP could travel to other tissues (i.e. liver) and aid in gluconeogenesis. Further credence can be given to the rational of this hypothesis, when one looks at just the opposite of a caloric deficit. Plasma levels of PLP have been shown to decrease by 18-21% after five hours of an oral glucose load of one gram D-glucose/kg body weight (Leklem and Hollenbeck 1990). In this fed condition, less of a reliance is placed on gluconeogenisis, so PLP might not leak from its muscle storage site. More recently, a group of researchers studied the effects of PLP concentrations in response to water

Figure 9. The exercise induced mechanism of pyridoxal 5'-phosphate release from muscles



Taken from Leklem[4] 1988

and glucose ingestion (Hoffman et al. 1991). The subjects consisted of trained males, and the exercise protocol entailed treadmill running at 65% VO_2 max for two hours. Their findings were similar in that PLP levels increased with exercise. The magnitude of increase was 19-26%, and both the water and glucose polymer gave similar results. Prior to the study, belief was that glucose ingestion would reduce the requirement for PLP by the liver (Leklem 1985). Clearly, this was not the case. In discussing the results, the authors concluded that the liver was responsible for increased plasma PLP levels, not muscle. They suggested a possibility of leakage from non-hepatic tissues, but this would account for only a fraction of the increase in plasma PLP. Possibly, the liver releases PLP during exercise to skeletal muscle, where the coenzyme is critical for PLP dependent reactions.

With the knowledge that exercise lead to changes in plasma PLP, the next line of focus shifted to fuel utilization. Less of a reliance should be placed on gluconeogenesis by increasing the carbohydrate intake, and hence muscle glycogen levels. An increase in vitamin B-6 was hypothesized to increase glycogen phosphorylase levels. Therefore, it was hypothesized that by increasing the amount of carbohydrate and vitamin B-6 in the diet, possibly a more rapid utilization of glycogen would result. To investigate this hypothesis researchers undertook a very complex study (Manore and Leklem 1987). The experiment involved 1)

examining the effects of two levels of carbohydrate on fuel substrates (i.e. glucose, FFA, and lactate) during exercise in women; and 2) determining if these fuel substrates were affected by training, age, and vitamin B-6 supplementation. Three groups of five women were fed alternately four diets varying in carbohydrate and vitamin B-6 (i.e. 2.4-10.4 mg/d) over a seven week period. At the end of each dietary phase subjects were exercised on a cycle ergometer at 80% VO_2 max for 20 minutes. Blood was drawn pre, post, post-30 and post-60 minutes of exercise and analyzed for plasma PLP, glucose, FFA, and lactate. The major findings of the study are as follows: 1) lactate was similar for groups and diets, and did increase with time, 2) FFA was variable, and ANOVA showed interactions for time x group x diet, 3) likewise, glucose was variable, and showed interactions for diet x time and time x group, 4) supplementation and/or increased carbohydrate resulted in lower FFA during exercise in all groups, and 5) PLP increased during exercise for all groups and showed a decrease from post to post-60. Interestingly, in this study lactate did not change with different diets. Some previous work in this area suggests that with supplemental vitamin B-6, lactate levels are higher (Lawrence et al. 1977, Leklem 1985). In addition, with high carbohydrate diets lactate levels are higher compared to low carbohydrate diets (Bergstrom et al. 1967, Hermansen et al. 1967, Saltin and Hermansen 1967, Karlsson and Saltin 1971, Bergstrom and Hultman 1972); but, no

such finding was observed. Possibly, the relative differences of carbohydrate in the diets were not adequate to impose changes in plasma lactate levels during exercise. An increase in carbohydrate metabolism would result in increased plasma lactate concentrations.

An increase in R values is also indicative of increased carbohydrate utilization. In a M.S. thesis project (Campuzano 1988) researchers primarily studied plasma urea and ammonia as they related to vitamin B-6 and exercise. However, they also analyzed respiratory gases 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. Each exercise test was placed in the middle of three days of a controlled diet. For six days before the exercise tests, subjects either received a placebo capsule or 20 mg PN in capsule form. In addition, capsules were also taken the day of and the day after exercise. When subjects were ingesting PN supplements, the results of gas collection indicated that carbohydrate metabolism increased from 43 to 52% of the total caloric expenditure during exercise. Fat utilization decreased from 45 to 36%. In addition, resting R values were higher with supplementation compared to the placebo treatment (i.e. 0.86 versus 0.80). These somewhat limited but important data help support the hypothesis that PN supplementation may stimulate glycogen utilization. Most of the studies up to this point were conducted at Oregon State University (OSU),

where much of the research in this field has been done; however, others have also been intrigued with the relationship of vitamin B-6 and exercise.

A study concerned with vitamin supplementation and endurance exercise performance seemed to indirectly stumble across vitamin B-6's role as a possible fuel modifier during exercise (Lawrence et al. 1977). Researchers wanted to investigate the effects of vitamin E on swimming performance, and without any real basis or hypothesis they also administered vitamin B-6. Seventy-two male and female swimmers were either given a placebo, 51 mg/d PN-HCl or 900 IU alpha tocepherol (i.e. vitamin E) for six months and swimming performance was examined. Vitamin E was hypothesized to decrease oxygen debt, and therefore the study looked at this relationship by measuring lactic acid levels following exercise. Although none of the treatments significantly affected swimming performance, subjects taking vitamin B-6 showed a slight decrease in improvement at the end of the study. In addition, this group did exhibit significantly higher lactic acid levels. Unfortunately, no explanation was provided for the elevated lactic acid levels in the vitamin B-6 group.

As stated in the vitamin B-6 section, the RDA of vitamin B-6 for physically active persons may require revision. Currently, there is no separation between athletic and sedentary populations. A very well controlled study directly

addressed the possibility of increased need for vitamin B-6 with exercise by studying B-6 utilization in active and inactive young men (Dreon and Butterfield 1986). Four trained men were confined in a metabolic unit for roughly two months, and during this period they either ran five or ten miles/day. They were compared to a free living group of inactive men, who were however, consuming the same diet as the active group (i.e. 4.2 mg B-6/d). Excretion of 4-PA was predominantly used for assessing differences in vitamin B-6 utilization. The results of decreased excretion of 4-PA in the trained subjects compared to the untrained subjects lead to an interesting hypothesis. Researchers explained the data by implying that active individuals may have a labile pool of pyridoxine, which is capable of redistribution under circumstances of increased need (i.e. exercise). In other words, exercise may actually promote storage of vitamin B-6.

To this date, little of the work on vitamin B-6 and exercise in humans has involved the vitamins role as a possible fuel modifier. After reviewing the literature, it appears that most of the research focused on the effects of exercise on vitamin B-6 metabolism. Experiments have primarily studied plasma vitamer levels and excretion products of vitamin B-6, as they relate to exercise. "Historically, nutritionists have been interested in the effects of exercise on vitamin requirements while exercise physiologists have been concerned with the effects of vitamin supplementation on

exercise performance" (Belko 1987).

Of all the multifaceted roles vitamin B-6 plays in the body, PLP's involvement as a cofactor in glycogenolysis and gluconeogesisis suggests that the vitamin is directly related to energy production. If in fact supplemental vitamin B-6 does increase the rate of glycogenolysis, as determined from increased lactate levels, then excesses may be detrimental for athletes involved in long term strenuous activity. On the other hand, insufficient vitamin B-6 may result in decreased gluconeogenesis with a subsequent inability to maintain blood glucose levels. Either scenario poses a threat to the performance conscious athlete; therefore, this study will in part investigate the effects of vitamin B-6 supplementation on fuel utilization during exhaustive endurance exercise.

METHODS

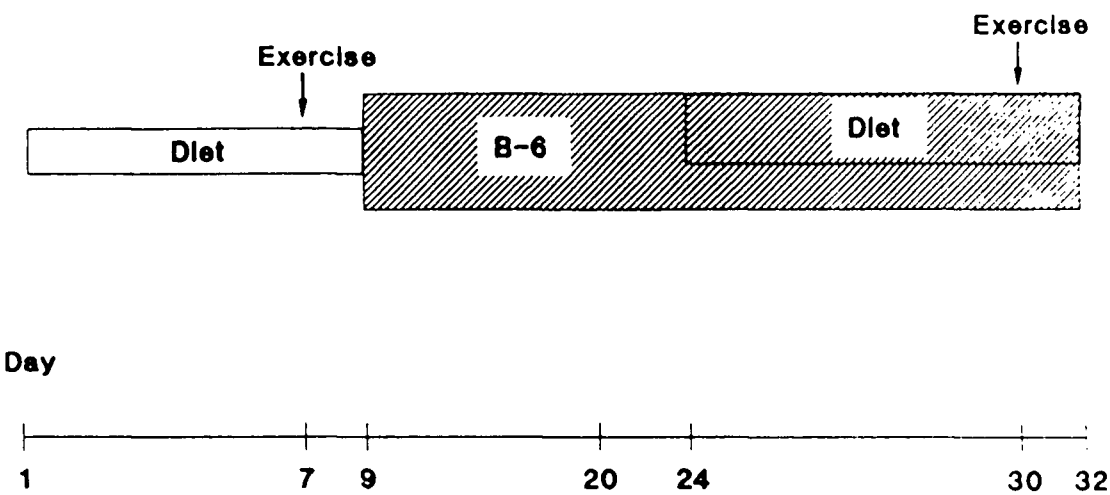
Overview

This study examined the effects of vitamin B-6 supplementation on fuel utilization during exhaustive endurance exercise. Trained cyclists exercised to exhaustion on a cycle ergometer at 75% of a predetermined VO_2 max. One exercise test occurred in an unsupplemented state and the other test in a vitamin B-6 supplemented state. For six days before and two days after each of the testing sessions, the subjects were fed a controlled diet, which was nutritionally adequate. In order to analyze any changes in fuel utilization (and vitamin B-6 metabolism), blood, urine, and respiratory gases were collected. Blood was analyzed for glucose, lactic acid, glycerol and FFA. A staggered start, in which two subjects per day began the study, was necessary in order to conduct the lengthy exhaustion tests. All subjects followed the same timeline (See Figure 10).

Subjects

Seven, healthy, trained, male cyclists volunteered to participate in the study, which was approved by the OSU human subjects committee. Training was defined as 120 minutes of aerobic activity (i.e. running, biking, swimming) per week, in

Figure 10. Timeline of the study



Taken from Nancy Dunton unpublished

a minimum of three days per week; and, it was asked that all subjects had maintained this exercise standard for at least one year. In addition, it was requested that each individual maintain his fitness regimen throughout the investigation. This was done to minimize a training effect. Prior to initiation in the study, all subjects met the following qualifications:

1. Normal health history based on a questionnaire
2. Normal blood chemistry screen
3. Normal xylose absorption test to assess carbohydrate absorption (i.e. excrete > 1.2 g in five hr for a 5 g intake)
4. Normal ECG at rest and at maximal heart rate
5. Normal blood pressure at rest (i.e. 120/80) and with exercise
6. No vitamin supplements used for at least four weeks prior to starting the study
7. No nicotine for at least one year or use of drugs known to interfere with vitamin B-6 metabolism or methodology
8. Signed an informed consent
9. Underwent a max VO_2 test to assess fitness level

Daily Procedures

A daily log was kept by each subject to assure compliance with the study and to monitor activity levels. During the dietary phases subjects recorded: completeness of food

intake, non-caloric beverages consumed, medications taken, overall health, accuracy of urine collections, daily body weights, and a complete profile of exercises performed (i.e. type, duration, intensity, RPE). During the three day self-selected dietary period subjects monitored: foods consumed, body weights, overall health, and an exercise profile.

Exercise Testing

All exercise testing was carried out in the human performance laboratory located on the OSU campus (i.e. Womens Building Room 19). A total of three exercise tests per subject were administered, and these consisted of a VO_2 max test and two endurance rides. The VO_2 max test 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 bicycle (Quinton Instruments), which used a tension belt to alter the resistance. On the morning of the seventh day of each dietary period, the cyclists exercised to exhaustion at 64-75% of their maximum aerobic capacity following a 12-14 hour fast. A brief warm up consisting of about five minutes allowed subjects to loosen up. During this

time frame the workloads were set. The length of time of the warm-up was constant for both exhaustive rides. During the test heart rate was monitored by an ECG (Quinton Instruments, 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). No fluids were allowed while cycling. However, after the post-blood draw 100 ml of water was given. Even though the lab temperature and humidity were comfortable for exercise (i.e. 20-24° C, < 60%, respectively), a fan was provided for evaporative cooling. Body weights were recorded pre and post-testing. Ratings of perceived exertion (RPE) were frequently asked of subjects, which helped identify progressive fatigue and subsequent exhaustion. Exhaustion was defined as the inability to maintain within five rpm of the initial cadence (i.e. 80 rpm) for a total of 20 seconds. However, in most subjects this criteria was unnecessary due to instant cessation of pedalling at exhaustion. During both exercise tests subjects were not aware of cycling times (i.e. clocks/watches were concealed).

Diet

Two, eight-day feeding periods involved serving meals at the Department of Nutrition and Food Management metabolic kitchen. Both dietary phases were identical in food (i.e. macronutrient) content, and were comprised of 62% carbohydrate, 21% fat, and 18% protein (i.e. approximately 3500 total kcal). Table 5 lists all foods and amounts fed daily. Foodstuffs provided 2.30-2.35 mg of vitamin B-6 for both diets. The vitamin B-6 content was determined by microbiological assay of aliquots of food composites. Phase one was supplemented with a placebo capsule, while phase two was supplemented with an additional 20 mg PN/d in similar capsule form as the placebo. The pyridoxine was fed as the hydrochloride. A single blind design was chosen. All other micronutrients met 100% of their recommended daily allowances. When possible, all foods were purchased from the same lot, and careful preparation by means of weighing foods to $\pm$ 0.1 g assured consistency in the diets. Alcoholic beverages were prohibited throughout the study, and caffeine was not allowed on the days before exercise testing. Between the two feeding periods, three 24-hour diet records were collected from subjects. These records were analyzed for nutrient composition (Food Processor II software).

Table 5. Foods consumed daily for both the vitamin B-6 supplemented and non-supplemented diets

Breakfast:	240 gm orange juice
	220 gm 1% milk
	50 gm shredded wheat cereal
	50 gm whole wheat bread
	30 gm raisins
	15 gm white sugar
	3 ea jelly packets (optional)
Lunch:	240 gm apple juice
	160 gm pears
	70 gm raw carrot
	40 gm vanilla wafers
	17 gm gelatin mixed with 240 gm "koolade"
	sandwich:
	60 gm whole wheat bread
	60 gm drained tuna, water packed
	30 gm low-calorie mayonnaise
	20 gm egg white, cooked
	15 gm dill pickle
	10 gm iceberg lettuce
Dinner:	240 gm 1% milk
	200 gm green beans
	150 gm peaches
	120 gm turkey
	100 gm frozen yogurt
	70 gm white rice, dry
	25 gm whole wheat bread
	20 gm margarine
	17 gm gelatin mixed with 240 gm "koolade"
	salad:
	50 gm iceberg lettuce
	40 gm red kidney beans
	40 gm cheddar cheese
	20 gm french dressing
	15 gm red cabbage
	10 gm raw carrot, grated
Snack:	240 gm grape juice
	50 gm graham crackers
Beverages:	regular or decaffeinated coffee and tea, regular and diet sodas

Blood and Urine Collections

Blood was drawn before the start of the experiment, on the two exercise test days (i.e. days 7,30), and once during the vitamin B-6 supplementation phase (i.e. day 21) by a registered medical technologist. Day 21 provided non-exercising blood data on vitamin B-6. This gave some indication as to whether the subjects were taking the supplement. Each blood draw required approximately 17 ml of blood in order to carry out all the analyses necessary. On exercise test days, the first blood sample taken was 5-10 minutes prior to exercise after the subject had rested for 10-15 minutes. The second sample taken was 60 minutes into exercise. At this time the workload was decreased slightly to allow for a safe blood draw. The third sample was taken one-two minutes post-exhaustion. The fourth and fifth samples were collected in the exercise recovery phase at post-30 and 60 minutes, respectively. Heparinized blood tubes were used for collection and all samples were kept on ice until centrifuged. After extraction of the plasma portion, the seprately aliquoted samples were frozen at -40°C.

Urine was collected daily for each of the two diet periods, as well as during the three days of the self-selected diet. All 24-hour collections were kept in plastic urine bottles, which contained approximately 10 ml of toluene, a preservative. Each morning without delay, all daily samples

were thoroughly mixed, portioned into small bottles, and frozen at -20°C for subsequent analysis. In addition, pH readings were obtained on each subjects daily urine sample.

Risks/Benefits

Prior to max testing all subjects were screened to aid in evaluation of obvious symptoms of cardiac disease. Trained personnel conducted the max tests, and guidelines set forth by the ACSM were followed for exercise intolerance and termination of testing. Fatalities are extremely rare in sports trained individuals, with the morbidity rate equalling about one in 10,000 tests completed.

Upon completion of the study subjects received an amount of $ 50.00 from OSU. In addition, all data compiled on individual blood chemistry values, body composition, and exercise information was provided free of charge. Each participant also received 16 days of well balanced meals, at no cost.

Analysis

Plasma glucose was measured by the glucose oxidase method (Trinder 1969). Glucose is oxidized to gluconic acid and hydrogen peroxide. The hydrogen peroxide reacts with 4-aminoantipyrine and phenol in the presence of peroxidase to

form a red color. The color intensity is measured photometrically with a Technicon Autoanalyzer System II (Alpkem) and related directly to the plasma glucose concentration. The coefficient of variation (CV) for the assay was 6.6% for control samples (n=8).

Plasma lactic acid was measured spectrophotometrically (Henry 1968). A kit from Sigma Chemical Co. was used. In the presence of lactate dehydrogenase and excess NAD, pyruvic acid is formed from lactic acid. The increased absorbance is due to NADH formation, which is a measure of the lactate concentration. A Beckman DU 40 spectrophotometer was used. The CV for the assay was 3.6% for control samples (n=12).

Plasma glycerol was measured by a modified enzymatic method (Pinter et al. 1967) for triglycerides by omitting the saponification step. Glycerol phosphate is formed from glycerol with the ultimate conversion of NADH to NAD through coupled reactions. The amount of NADH oxidized, represented by a decrease in absorbance, is equivalent to the amount of glycerol in the sample. A Beckman spectrophotometer was used. The CV at mid-assay was 5.5% for control samples (n=5). If all controls are used in the determination of a CV (n=8), then the coefficient is 18.9%. Prior to starting analysis of actual samples, dilution problems resulted in inconsistent control values. Thus, when all controls (n=8) are used in

the calculation of a CV, the CV is quite high. The dilution problems were corrected.

Plasma FFA were measured by a colorimetric method (Faholt et al. 1973). Following extraction of FFA by chloroform-heptane-methanol and phosphate buffer, the copper soaps of FFA are determined colorimetrically with diphenylcarbazide. Unfortunately, a freezer malfunction resulted in an inability to perform repeats. No CV was calculated, because all the control sample was used in fixing a problem with the color reagent in the assay.

Plasma volume changes were measured by using hemoglobin and hematocrit data from two seprate blood samples and applying the following equations:

$$\% \text{ change} = [Hb_2/Hb_1\ (1-Hct_1) - (1-Hct_2)]/(1-Hct_2)$$

(Dill and Costill 1974)

$$\% \text{ change} = [(100/100-Hct_1)\ 100](Hct_1 - Hct_2)/Hct_2$$

(Van Beaumont 1972)

Hb_1= initial hemoglobin conc.; Hb_2= final hemoglobin conc.
Hct_1= initial hematocrit %; Hct_2= final hematocrit %

Note that in the Van Beaumont method only hematocrit values are needed. A mathematical program was used on a Hewlett Packard Model 10 Calculator.

Computerized R analysis of expired air (Apple II computer, Rayfield Ltd. software 1985) during exercise assessed substrate utilization. A ratio of CO_2 production to O_2 consumption determined the relative contribution of fats and carbohydrates metabolized. In addition, the computerized printout provided VO_2 data in an absolute and relative basis.

Other analysis:

1. Hemoglobin and hematocrit were done by microhematocrit and cyanomethemoglobin methods, respectively.
2. Xylose absorption (Harris 1969) assessed proper carbohydrate absorption. This was done prior to the start of the study.
3. Creatinine was determined by an automated procedure (Pino et al. 1965) for insuring compliance of subjects urine collections.

Statistics

The data was analyzed by standard statistical methods: analysis of variance (ANOVA), students t-test, and product moment correlation coefficients (r) based on linear regression. A students t-test for paired values was used to determine if a difference existed between each exercise test, and ANOVA was used to determine if changes occurred over time for each variable studied. Null hypotheses were rejected at the 0.05 level of significance.

RESULTS

To better help understand the following results, recall that blood samples were obtained at the following time points: Pre-exercise, during-exercise (i.e. 60 minutes into exercise), post-exercise, post-30 minutes of exercise, and post-60 minutes of exercise. In addition, recall that expired air was collected at 10 minute intervals throughout both exercise tests (i.e. test-1 = non-supplemented; test-2 = vitamin B-6 supplemented). Heart rates and RPE were also taken at 10 minute intervals during each exericse test. In test-1 the mean exercise times to exhaustion were 2:00:37, and in test-2 the mean times to exhaustion were 2:04:51. Times to exhaustion will be more thouroughly presented later in the results section. Table 6 lists a description of the subjects.

All values/concentrations mentioned in the following results refer to group means unless otherwise stated.

Glucose

Listed in Table 7 are the mean plasma glucose concentrations and standard deviations for the two exercise tests. There were no significant differences between the two tests at any of the specific time points. However, there was a significant change in plasma glucose concentrations over

Table 6. Subject's characteristics (n=6)

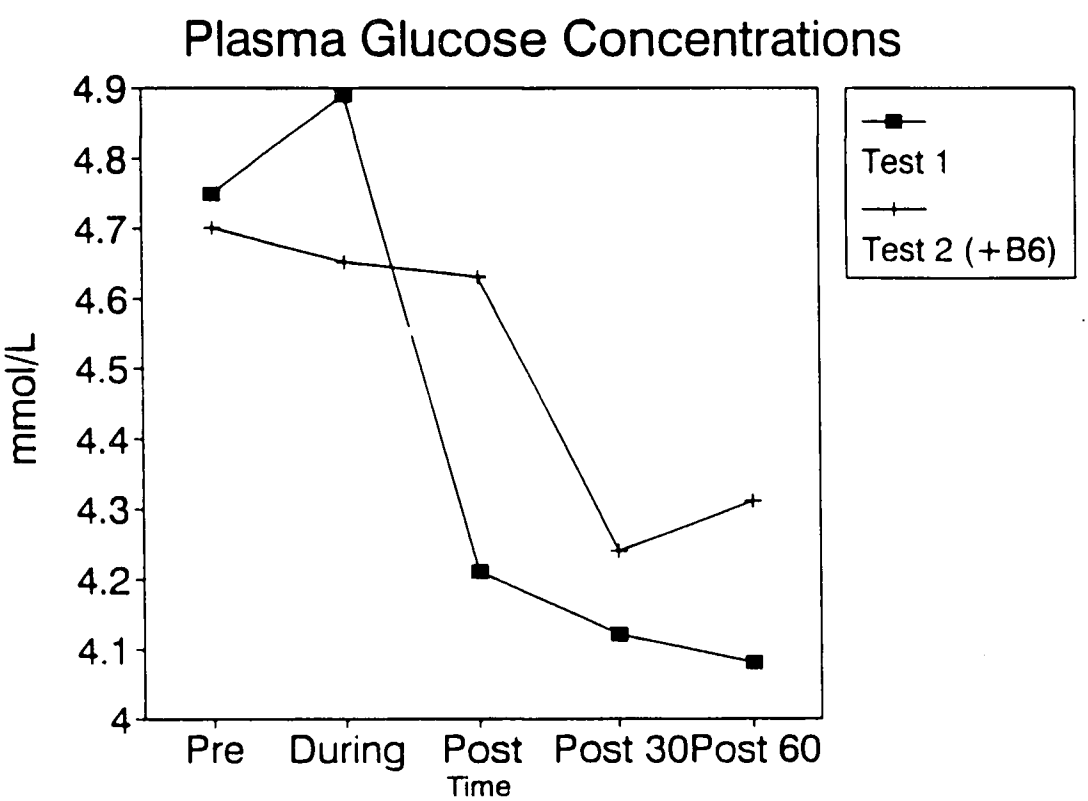
	Mean	SD	Range
Age (years)	26.3	7.4	19 - 37
Body Weight (kg)	73.6	3.5	69.3 - 79.1
Height (cm)	179	2.6	175 - 183
Body Fat (%)	12.6	3.0	9.5 - 16.4
VO2 (L/min.)	4.4	0.5	3.6 - 4.9
VO2 (ml/kg/min.)	59.6	7.9	45.3 - 69.5

Body weights depict data from body composition measures (mid-study)
Body fat percentages were measured hydrostatically (mid-study)
VO2 (max) was determined on a cycle ergometer (pre-study)

TABLE 7. Mean plasma glucose concentrations: effects of vitamin B-6 and exercise

		Pre	During	Post	Post 30	Post 60
				mmol/L		
Test 1	mean	4.75	4.89	4.21	4.12	4.08
	SD	0.79	0.63	0.42	0.29	0.31
Test 2 (+B6)	mean	4.70	4.65	4.63	4.24	4.31
	SD	0.94	0.74	0.86	0.60	0.57

All values are means and SD; n=6
Pre-before exercise; During-60 min. into exercise; Post-immediately after exercise; Post 30-30 min. after exercise; Post 60-60 min. after exercise
Test 1 refers to the non-supplemented exhaustive ride
Test 2 refers to the vitamin B-6 supplemented exhaustive ride



time for exercise test-1, but not for exercise test-2.

Plasma glucose concentrations showed different trends in response to exercise for each exercise test. The pre-exercise concentrations for both tests were almost identical. In test-1 plasma glucose increased from 4.75 mmol/L to 4.89 mmol/L at 60 minutes into exercise, before dramatically decreasing to 4.21 mmol/L within one-two minutes post-exercise. In the one-hour recovery period plasma glucose concentrations continued to decrease slightly. All three post-exercise values were significantly lower than either the pre or during exercise values in test-1 (95% LSD). Test-2 resulted in a maintenance of plasma glucose concentrations from pre-exercise to post-exercise. A decrease of only 0.05 mmol/L was observed. Post-exercise plasma glucose concentrations in test-2 were on average 10.5% higher compared to post-exercise plasma glucose concentrations of test-1, relative to their respective pre-exercise values. In test-2 a large decrease (i.e. nine percent) in plasma glucose concentration was observed during the first 30 minutes of recovery. Although such a decrease was observed, the test-2 post-30 minute value remained higher than any of the test-1 post-exercise values. Unlike test-1 which resulted in a continual decrease in plasma glucose levels throughout the recovery phase, test-2 showed a slight increase from post-30 to post-60 minutes of exercise.

In general, individual directional changes in plasma glucose concentrations at each time point varied among the

subjects. Thus, p-values never approached a significance level of 0.05.

Lactic acid

Listed in Table 8 are the mean plasma lactic acid concentrations and standard deviations for each of the two exercise tests. There were no significant differences between the two tests for any of the specific time points. However, there was a significant change in plasma lactic acid concentrations over time for both tests.

A consistent trend was observed in the mean plasma lactic acid concentrations between both exercise tests. Each plasma lactic acid concentration from test-1 was higher than the plasma lactic acid concentration from test-2 (i.e. all time points). This included the pre-exercise values of 1.60 mmol/L and 1.32 mmol/L for test-1 and test-2, respectively. In both tests plasma lactic acid concentrations increased from pre-exercise to 60 minutes into exercise. The average magnitude of increase for both tests was 2.8-fold, and both were significantly higher than their respective pre-exercise plasma lactic acid concentrations (95% CI). The only inconsistent trend in the plasma lactic acid concentrations between the tests occurred from 60 minutes into exercise to post-exercise. In test-1 plasma lactic acid continued to increase from during exercise to post-exercise, whereas in test-2 a decrease was

TABLE 8. Mean plasma lactic acid concentrations: effects of vitamin B-6 and exercise

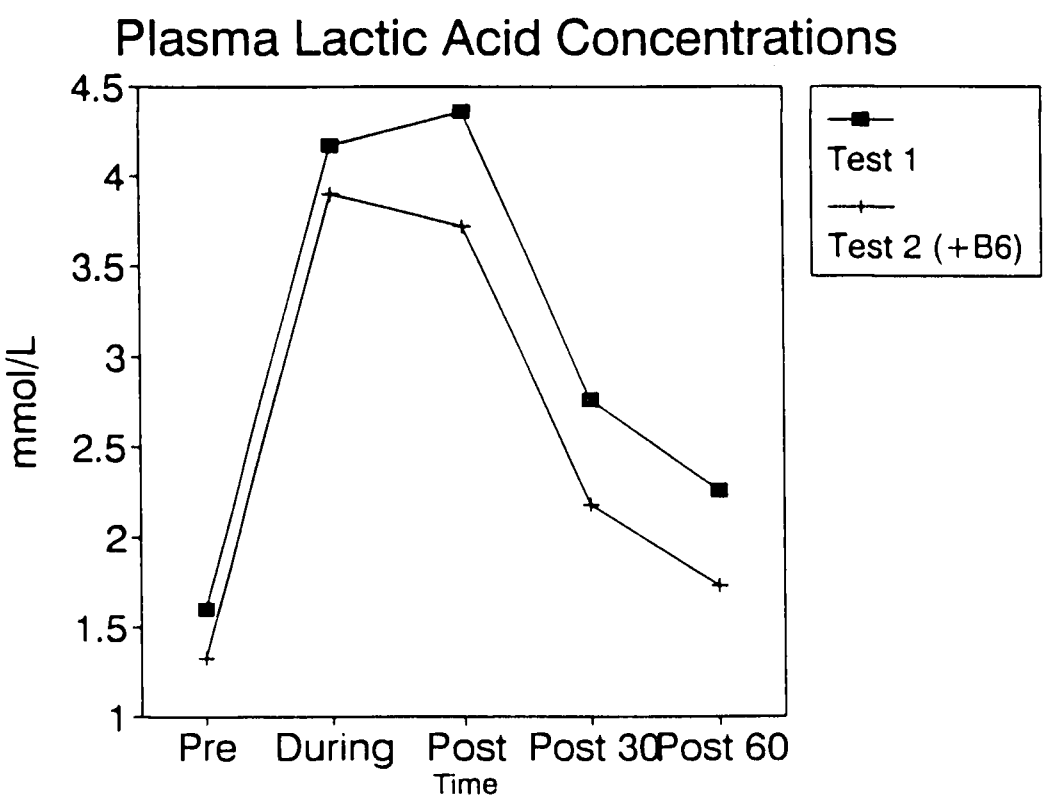
		Pre	During	Post	Post 30	Post 60
				mmol/L		
Test 1	mean	1.60	4.17	4.36	2.76	2.26
	SD	0.78	0.92	1.67	0.70	0.97
Test 2 (+B6)	mean	1.32	3.90	3.72	2.18	1.73
	SD	0.76	1.38	1.53	1.41	0.58

All values are means and SD; n=6

Pre-before exercise; During-60 min. into exercise; Post-immediately after exercise; Post 30-30 min. after exercise; Post 60-60 min. after exercise

Test 1 refers to the non-supplemented exhaustive ride

Test 2 refers to the vitamin B-6 supplemented exhaustive ride



noted between these two time points. The post-exercise lactic acid concentration in test-1 was 4.36 mmol/L compared to a lactic acid concentration of 3.72 mmol/L in test-2. During the first 30 minutes of recovery plasma lactic acid concentrations decreased markedly (i.e. 37-42%) in both tests, relative to post-exercise values. By post-60 minutes of exercise a continual decline in plasma lactic acid was observed. However, post-60 minute values were 30-40% higher than pre-exercise values. In test-2 this resulted in a significant elevation over the pre-exercise value (95% CI).

All subjects showed similar trends in individual plasma lactic acid concentrations in response to each exercise test; and, if not for a few inconsistent values, p-values may have reached significance. For example, in each of the three post-exercise blood samples five of six subjects had lower plasma lactic acid concentrations during test-2 compared to test-1. In addition, in the pre-exercise sample four out of six subjects had lower plasma lactic acid concentrations in test-2 compared to test-1, with one individual showing no change between the tests.

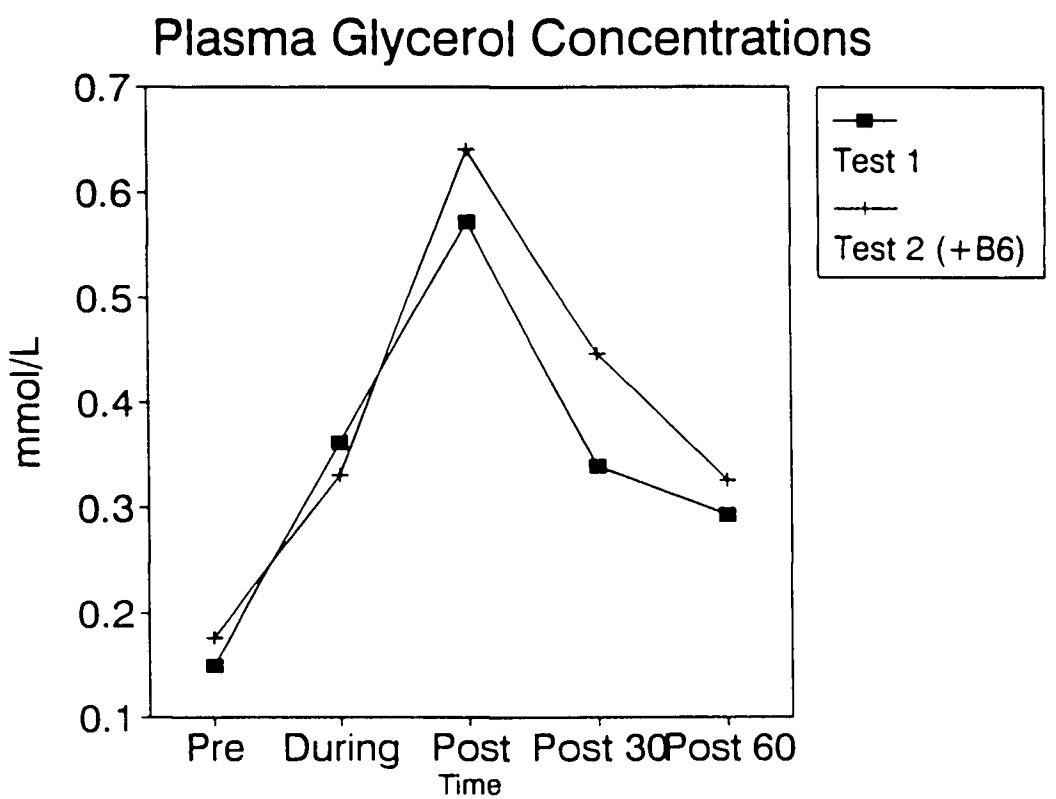
Glycerol

Listed in Table 9 are the mean plasma glycerol concentrations and standard deviations for the two exercise tests. There were no significant differences between the

TABLE 9. Mean plasma glycerol concentrations: effects of vitamin B-6 and exercise

		Pre	During	Post	Post 30	Post 60
				mmol/L		
Test 1	mean	0.15	0.36	0.57	0.34	0.29
	SD	0.08	0.10	0.18	0.18	0.15
Test 2 (+B6)	mean	0.18	0.33	0.64	0.45	0.33
	SD	0.07	0.17	0.10	0.10	0.07

All values are means and SD; n=6
Pre-before exercise; During-60 min. into exercise; Post-immediately after exercise; Post 30-30 min. after exercise; Post 60-60 min. after exercise
Test 1 refers to the non-supplemented exhaustive ride
Test 2 refers to the vitamin B-6 supplemented exhaustive ride



tests for any of the specific time points. However, there was a significant change in plasma glycerol concentrations over time for both tests.

From pre-exercise plasma glycerol concentrations of 0.15 mmol/L and 0.18 mmol/L for test-1 and test-2, respectively, an increase of approximately twofold was observed by 60 minutes into exercise for both tests; however, test-1 levels increased slightly more than test-2 levels at the during time point. By the end of exercise, plasma glycerol concentrations increased to 0.57 mmol/L in test-1 and to 0.64 mmol/L in test-2. Both post-exercise values represent significant changes over time compared to their respective pre-exercise values (95% CI). At post-30 minutes of exercise a large decrease in plasma glycerol concentrations was observed during both tests. This time point showed the largest difference between the tests. The vitamin B-6 supplemented test resulted in a 32% higher plasma glycerol concentration at post-30 minutes of exercise compared to the unsupplemented test at post-30 minutes of exercise. Plasma glycerol concentrations continued to decline by post-60 minutes of exercise. However, at this time point, the concentrations were still approximately double their respective pre-exercise concentrations.

Only the post-30 minute blood sample resulted in a trend among the subjects individual plasma glycerol concentrations. Five of six subjects had higher plasma glycerol concentrations at this time point in test-2 compared to test-1. At all other

blood sample time points, directional changes in plasma glycerol concentrations were variable among the subjects. Thus, no significance between the tests was found.

Free Fatty Acids

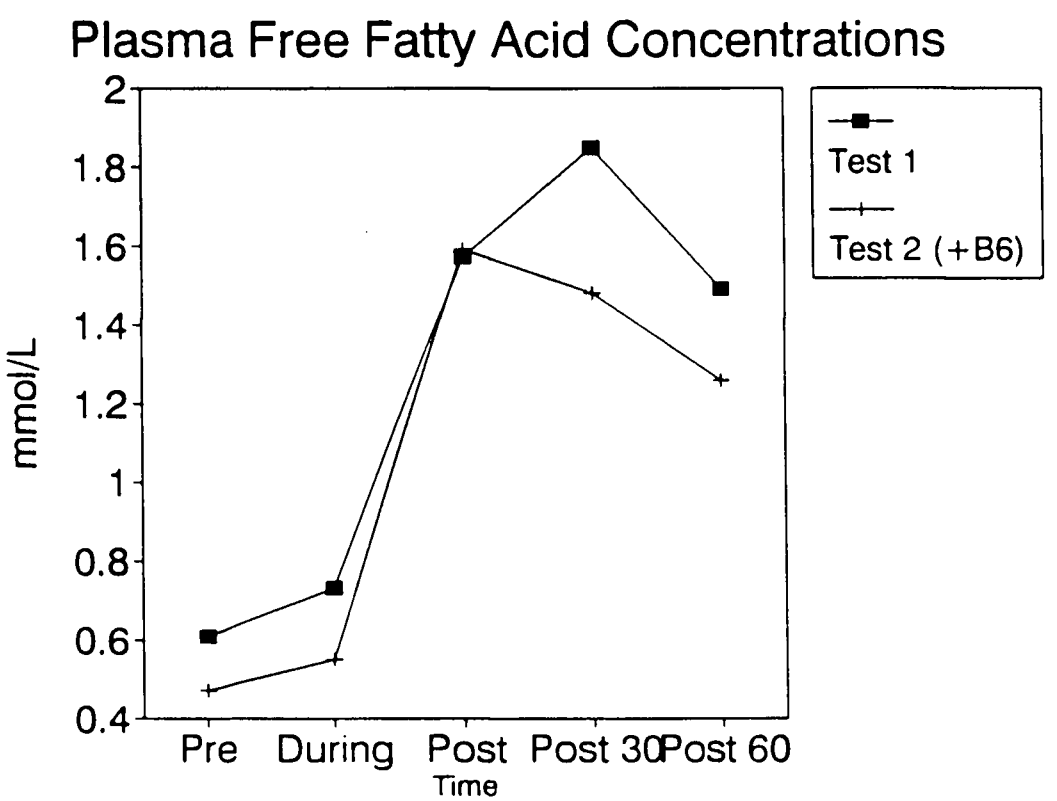
Listed in Table 10 are the mean plasma FFA concentrations and standard deviations for the two exercise tests. There was a significant difference between the tests for only one specific time point. In addition, there was a significant change in plasma FFA concentrations over time for both tests.

Pre-exercise plasma FFA concentrations were 0.61 mmol/L in test-1 and 0.47 mmol/L in test-2. Statistically, this was a significant difference ($p<0.05$). During the first 60 minutes of exercise, plasma FFA concentrations increased proportionally (i.e. 17-19%) for each of the two tests. The largest change in concentrations occurred between 60 minutes of exercise to post-exercise for both tests. In both tests almost identical post-exercise plasma FFA concentrations were observed (i.e. 1.57-1.59 mmol/L). In relation to the during time point values, test-2 increased by 74% more than test-1 at post-exercise. Regardless of a more dramatic increase of plasma FFA concentrations in test-2 compared to test-1, both post-exercise values were significantly higher than either of their respective pre or during-exercise values (95% CI). Test-1 continued to show an increase in plasma FFA

TABLE 10. Mean plasma FFA concentrations: effects of vitamin B-6 and exercise

		Pre	During	Post	Post 30	Post 60
				mmol/L		
Test 1	mean	0.61	0.73	1.57	1.85	1.49
	SD	0.20	0.25	0.49	0.55	0.48
		a				
Test 2 (+B6)	mean	0.47	0.55	1.59	1.48	1.26
	SD	0.13	0.21	0.62	0.46	0.44

All values are means and SD; n=6; (a) means different ($p<0.05$) conc. between tests
Pre-before exercise; During-60 min. into exercise; Post-immediately after exercise;
Post 30-30 min. after exercise; Post 60-60 min. after exercise
Test 1 refers to the non-supplemented exhaustive ride
Test 2 refers to the vitamin B-6 supplemented exhaustive ride



concentrations during the first 30 minute recovery period, whereas in test-2 a slight decrease in plasma FFA concentrations was observed. At this time point the absolute difference in concentrations was 0.37 mmol/L, the highest difference observed. However, as a relative difference, the pre and during-exercise values showed a greater difference. In the second 30 minute recovery period, test-1 values decreased greater than test-2 values, resulting in more similar post-60 minute plasma FFA concentrations compared to post-30 minute plasma FFA concentrations.

A trend was noted for individual plasma FFA concentrations at the during-exercise time point and at 30 and 60 minutes post-exercise. At all these three time points, only one subject deviated from the group by increasing in plasma FFA concentration in test-2 compared to test-1. In other words, five of six subjects showed a decrease in plasma FFA levels in test-2 relative to test-1 at 60 minutes into and post-30 minutes of exercise. At the post-exercise time point, directional variability among the subjects' individual plasma FFA concentrations resulted in no statistically significant differences between the two tests.

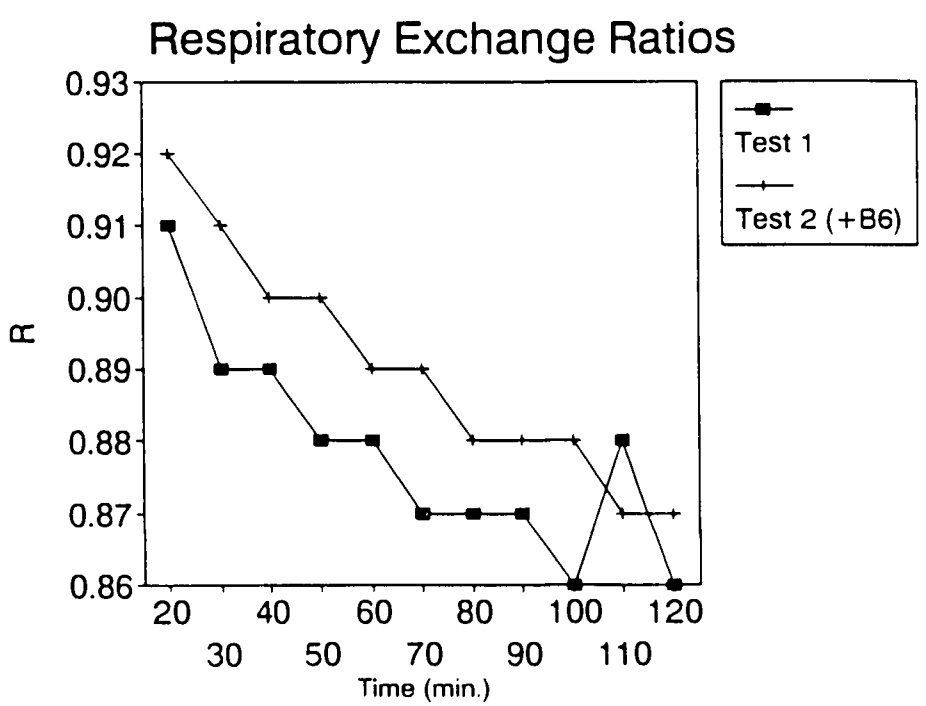
Respiratory Exchange Ratios

The respiratory exhange ratios (R) means and standard deviations for the two exercise tests are listed in Table 11.

TABLE 11. Mean respiratory exchange ratios (R): effects of vitamin B-6 and exercise

	Test 1		Test 2 (+B6)	
Time (min.)	mean	SD	mean	SD
20	0.91	0.03	0.92	0.04
30	0.89	0.03	0.91	0.04
40	0.89	0.02	0.90	0.03
50	0.88	0.02	0.90	0.04
60	0.88	0.03	0.89	0.03
70	0.87	0.03	a 0.89	0.01
80	0.87	0.03	a 0.88	0.01
90	0.87	0.02	a 0.88	0.01
100	a 0.86	0.02	a 0.88	0.01
110	a 0.88	0.02	a 0.87	0.02
120	a 0.86	0.02	a 0.87	0.02

All values are means and SD; n=6; (a) refers to n=5
Time refers to minutes of exercise
Test 1 refers to the non-supplemented exhaustive ride
Test 2 refers to the vitamin B-6 supplemented exhaustive ride



There were no significant differences between the two tests for any of the specific time points. However, there was a significant change in R values over time (i.e. two hours) for both exercise tests.

Respiratory exchange ratios were consistently higher in test-2 than in test-1. Only at 110 minutes into exercise did a reversal of this trend occur, and by 120 minutes the R values were once again higher in test-2 compared to test-1. Both tests resulted in a significant absolute decrease of 0.5 in R values after two hours of exercise (95% CI). Test-1 decreased from 0.91 to 0.86, and test-2 decreased from 0.92 to 0.87. A notable difference other than the 110 minute time point, occurred between the two tests within the first 40 minutes of exercise. Test-1 decreased by 40% of both tests absolute change (i.e. 0.5) in one-half the time of test-2. Thus, test-2 maintained higher R values earlier in exercise compared to test-1.

On an individual basis, four of six subjects followed the trend of higher test-2 mean R values compared to test-1 mean R values. Subject number four showed virtually no change, and subject number two exhibited higher R values in test-1 compared to test-2. In general, the trend among subjects was for higher R values under conditions of vitamin B-6 supplementation compared to no supplementation of vitamin B-6.

Oxygen Consumption

The percent VO_2 max means and standard deviations for both exercise tests are listed in Table 12. There were no significant differences between the two tests for any of the specific time points. In addition, there was no significant change in oxygen consumption over time for both tests.

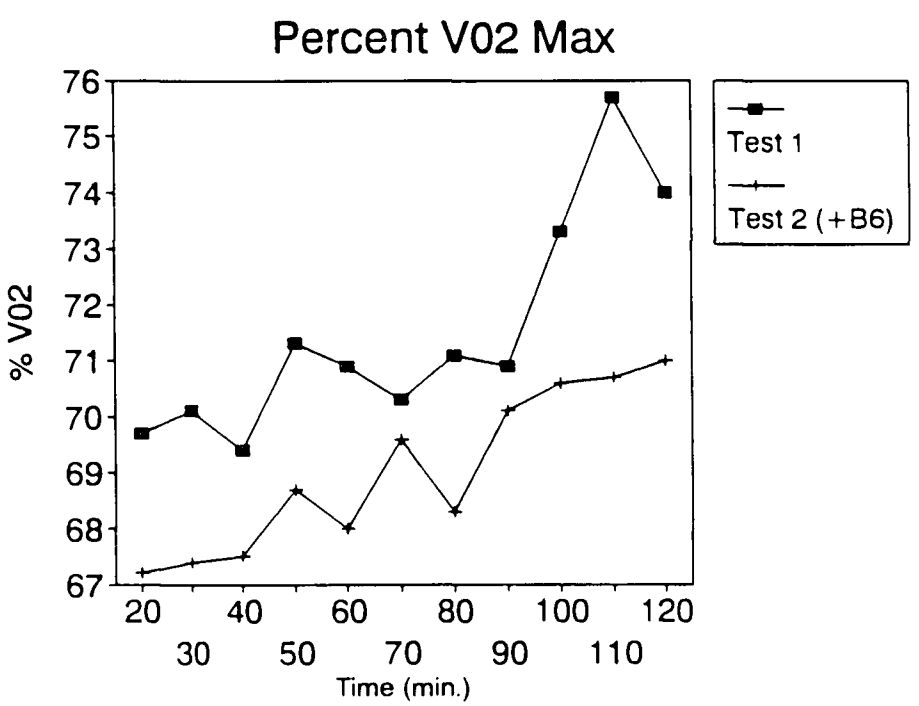
Although no significant difference was found between the tests, a trend did occur. Oxygen consumption was higher during test-1 at all time points compared to test-2. At the start of exercise, oxygen consumption was 69.7% VO_2 max in test-1 and 67.2% VO_2 max in test-2. The percent difference in oxygen consumption between the two tests narrowed between 70-90 minutes of exercise, however by 110 minutes of cycling a peak difference of five percent was observed. After 120 minutes of exercise, there was an increase in oxygen consumption of six percent in test-1 and almost four percent in test-2, relative to the 20 minute time point for both exercise tests. However, no significant change over time was found.

Individual oxygen consumption values showed that two subjects, numbers one and three, consumed approximately 8-10% more oxygen in test-1 compared to test-2, while the other four subjects showed virtually no change between the two tests (i.e. $\pm$ one percent). In four of the six subjects, there was a trend for oxygen consumption to increase from the start to

TABLE 12. Mean percent V02 max: effects of vitamin B-6 and exercise

	Test 1		Test 2 (+B6)	
Time (min.)	mean	SD	mean	SD
20	69.7	3.0	67.2	2.6
30	70.1	3.6	67.4	2.5
40	69.4	4.9	67.5	1.6
50	71.3	3.7	68.7	2.3
60	70.9	3.7	68.0	2.8
70	70.3	3.9	a 69.6	3.0
80	71.1	4.7	a 68.3	2.6
90	70.9	5.3	a 70.1	3.0
100	a 73.3	4.9	a 70.6	3.0
110	a 75.7	6.7	a 70.7	4.0
120	a 74.0	6.7	a 71.0	3.5

All values expressed are means and SD; n=6; (a) refers to n=5
Time refers to minutes of exercise
Test 1 refers to the non-supplemented exhaustive ride
Test 2 refers to the vitamin B-6 supplemented exhaustive ride



the end of exercise in both tests. In subject number three, an increase of 21.6% was observed from the start to the finish of exercise during test-2.

Heart Rates

The heart rate means and standard deviations for the two exercise tests are listed in Table 13. There were significant differences between the two tests for several specific time points. In addition, there was a significant change in heart rates over time (i.e. two hours) for exercise test-1, but not for exercise test-2.

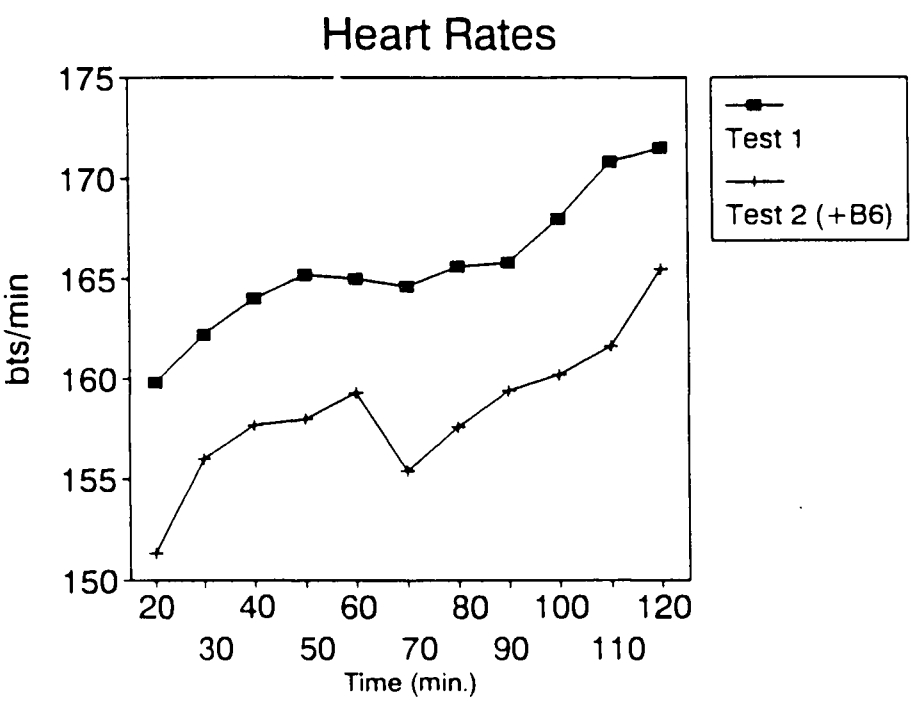
A consistent difference in heart rates between the two exercise tests was observed. At each time point in test-2 (excluding rest), the heart rates were between 6-10 bts/min slower during exercise than in test-1. The 30, 50, 70, 80, and 110 minute time points showed significantly lower heart rates in test-2 compared to test-1 ($p<0.05$). Although the absolute increase in heart rates from 20 minutes of exercise to two hours of exercise was greater in test-2 (i.e. 14 bts/min) compared to test-1 (i.e. 12 bts/min), a significant change over time was only found in test-1. Larger standard deviations in test-2 compared to test-1 appear to have led to such findings.

Individually, all six subjects maintained lower heart rates in exercise test-2 compared to test-1. At times during

TABLE 13. Mean heart rates: effects of vitamin B-6 and exercise

	Test 1		Test 2 (+B6)	
Time (min.)	mean	SD	mean	SD
rest	67.0	10.5	63.0	8.8
20	159.8	9.3	151.3	16.0
30 b	162.2	9.8	156.0	12.3
40	164.0	9.3	157.7	12.5
50 b	165.2	8.2	158.0	11.4
60	165.0	7.0	159.3	9.7
70 b	164.6	7.8	a 155.4	7.1
80 b	165.6	6.5	a 157.6	10.2
90	165.8	5.7	a 159.4	9.0
100 a	168.0	4.2	a 160.2	10.7
110 ab	170.8	5.2	a 161.6	11.8
120 a	171.5	5.5	a 165.5	11.9

All values are means and SD in beats per minute; n=6; (a) refers to n=5
Time refers to minutes of exercise; (b) means different ($p<0.05$) rates between the tests
Test 1 refers to the non-supplemented exhaustive ride
Test 2 refers to the vitamin B-6 supplemented exhaustive ride



exercise, the decrease in heart rate during test-2 was as much as 17 bts/min on an individual basis. Of the combined 63 total time point comparisons for all six subjects, only one time point for one subject resulted in a higher heart rate in test-2. The increase for this single subject's one time point was merely one beat per minute.

Ratings of Perceived Exertion

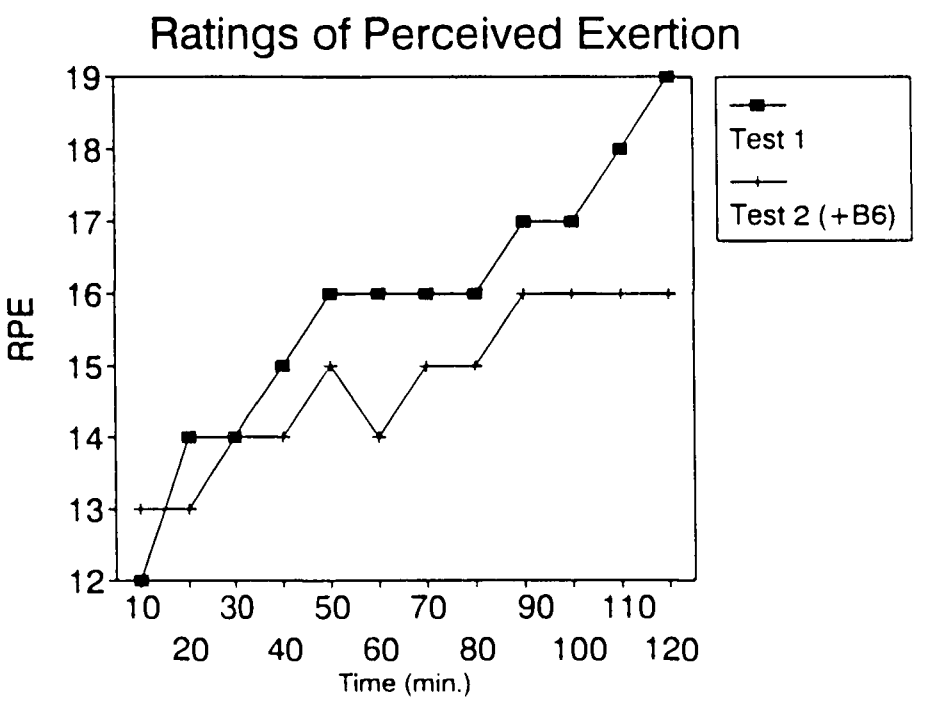
The ratings of perceived exertion (RPE) means and standard deviations for the two exercise tests are listed in Table 14. There were significant differences in RPE between the two tests at several specific time points. In addition, there was a change in RPE's over time for both exercise tests.

During the first 30 minutes of exercise no general trend in RPE was observed between the two exercise tests. However, as exercise progressed past 30 minutes, test-2 was perceived as an easier effort compared to test-1. From 90-110 minutes into exercise a significant decrease in RPE from test-1 to test-2 was observed ($p<0.05$). In test-1 RPE significantly increased from the onset of exercise to two hours of exercise. Initially, the perceived exertion was 12 (i.e. moderately hard) on a 20 point scale, and by two hours the perceived exertion was 19 (i.e. very very hard). In test-2 there was not a significant change in RPE from the start of exercise to the two hour time point. Subjects initially rated the effort

TABLE 14. Mean RPE: effects of vitamin B-6 and exercise

	Test 1		Test 2 (+B6)		
Time (min.)	mean	SD		mean	SD
10	12	1.3		13	0.5
20	14	0.7		13	0.7
30	14	0.7		14	1.1
40	15	1.0		14	1.3
50	16	1.1		15	2.2
60	16	1.2		14	1.3
70	16	1.5	a	15	0.9
80	16	1.8	a	15	1.2
90 b	17	2.0	a	16	1.4
100 ab	17	1.7	a	16	1.5
110 ab	18	1.7	a	16	1.7
120 a	19	1.3	a	16	2.1

All values are means and SD; n=6; (a) refers to n=5
Time refers to minutes of exercise; (b) means different ($p<0.05$) RPE between tests
Test 1 refers to the non-supplemented exhaustive ride
Test 2 refers to the vitamin B-6 supplemented exhaustive ride



at 13 (i.e. moderately hard), and by two hours the perceived exertion increased to 16 (i.e. hard).

After 50 minutes of exercise each subject rated test-2 to be equally hard or of lesser intensity than test-1 for all time points. From 20-50 minutes into exercise, inconsistent directional changes in RPE values between the tests were observed. For reasons which will later be discussed, test-2 appeared to be an easier effort compared to test-1 for almost all of the subjects.

Plasma Volume Changes

The plasma volume change (PVC) means and standard deviations for the two exercise tests are listed in Table 15. Two different methods (i.e. Dill and Costill, Van Beaumont) were used to calculate percent PVC relative to a pre-exercise blood sample. There were no differences in PVC between the two exercise tests at any of the specific time points for both methods. However, there was a significant change in plasma volume over time during both exercise tests, for both methods.

Although not statistically significant, both the Dill and Costill (DC) and Van Beaumont (V) methods showed a greater PVC decrease at the during and post-exercise time points in test-2 compared to test-1. On average a significant 12.5% decrease in PVC-DC occurred by 60 minutes into exercise relative to pre-exercise (95% CI). By post-exercise the decrease in PVC-

TABLE 15. Mean hematocrit, hemoglobin, and plasma volume changes: effects of vitamin B-6 and exercise

	Start	Mid-study			Pre	During	Post	Post 30	Post 60
HCT (%)									
mean	43.7	44.7	Test 1	mean	44.5	46.7	46.4	45.0	45.2
SD	2.9	1.9		SD	1.5	1.5	2.2	1.6	2.2
			Test 2 (+B6)	mean	44.4	46.7	46.5	44.8	44.6
				SD	2.6	2.7	2.5	2.2	2.5
HGB (gm/L)									
mean	151	153	Test 1	mean	149	162	163	154	157
SD	10	8		SD	6	7	9	5	6
			Test 2 (+B6)	mean	146	159	158	151	150
				SD	1	11	9	7	8
PVC-DC (%)			Test 1	mean		-11.6	-11.5	-4.2	-6.0
				SD		2.1	4.4	2.9	5.7
			Test 2 (+B6)	mean		-13.4	-11.6	-4.4	-2.6
				SD		2.0	2.7	4.1	2.5
PVC-V (%)			Test 1	mean		-8.4	-7.0	-1.9	-2.6
				SD		1.9	4.7	3.7	5.4
			Test 2 (+B6)	mean		-10.5	-8.4	-1.9	-0.9
				SD		2.1	2.6	3.0	2.6

All values are means and SD; n=6

HCT-hematocrit; HGB-hemoglobin; PVC-plasma volume change (DC-Dill and Costill method, V-VanBeaumont method)

Start-pre-study; Mid-study-during the self-selected diet; Pre-before exercise; During-60 min. into exercise; Post-immediately after exercise; Post 30-30 min. after exercise; Post 60-60 min. after exercise

Test 1 refers to the non-supplemented exhaustive ride

Test 2 refers to the vitamin B-6 supplemented exhaustive ride

DC was on average 11.6% for both exercise tests, and this was significantly different from pre-exercise values (95% CI). The V-PVC values were approximately three-four percent higher (i.e. less negative %) than the PVC-DC values at during and post-exercise, and both time points were significantly different from pre-exercise (95% CI). By post-30 minutes of exercise PVC-DC increased relative to post-exercise values to an average of -4.3% for both tests; and, the PVC-V increased relative to post-exercise values to -1.9% for both tests. In test-1 there was a decrease in plasma volume from post-30 to post-60 minutes of exercise for both methods, but only in the DC method was post-60 PVC different than pre-exercise values (95% CI). The V method consistently showed less PVC compared to the DC method at all the specific time points for both tests. In test-2 a continual increase (i.e. less negative %) in plasma volume occurred from post-30 to post 60-minutes of exercise for both methods. After one hour of recovery, neither method showed plasma volume to be fully restored, regardless of the exercise test.

A trend among individuals appeared at only one time point. Four of five subjects showed a greater decrease in PVC at the during-exercise time point in test-2 compared to test-1 for both methods. Paired data was not available for the sixth subject due to his less than 60 minute ride. However, if this subject's post-exercise value is used, which represents approximately 57 minutes into exercise, then four of six

subjects showed a greater decrease in PVC during exercise in test-2 compared to test-1. In general, all subjects varied in PVC directional response to exercise, which resulted in no significant findings between the two tests.

Diet

The eight day total calorie and carbohydrate intake means and standard deviations for all six subjects during both diet periods are listed in Table 16. As a group, no difference was found between the two dietary periods in total calorie and carbohydrate consumption. In period-1 subjects averaged consuming 3352 kcal and 550 g of carbohydrate, while in period-2 the subjects averaged 3462 kcal and 581 g of carbohydrate. The dietary vitamin B-6 content for both periods was relatively constant. Based on analysis of diet composites, a total of 2.30-2.35 mg of vitamin B-6 was consumed in both diet periods.

Subject numbers one and two did significantly increase their total calorie intake in diet period-2 compared to diet period-1 ($p<0.05$). Subject number one increased by an average of 582 kcal, and subject number two increased by and average of 101 kcal. In addition, subject number five consumed significantly different calories between the two periods ($p<0.05$), however there was a decrease of an average of 146 kcal from period-1 to period-2 for this subject. The

TABLE 16. Mean daily calorie and carbohydrate intake following the vitamin B-6 supplemented and non-supplemented diets

	D1-Kcal		D2-Kcal		D1-CHO		D2-CHO	
Subject	mean	SD	mean	SD	mean	SD	mean	SD
1	a 3227	33	3809	318	b 538	9	682	65
2	a 3309	61	3410	87	b 533	8	550	16
3	3462	116	3406	169	569	18	553	22
4	3300	85	3366	70	555	25	559	21
5	a 3387	118	3241	72	541	45	550	26
6	3426	112	3538	249	566	21	589	56

All values are means and SD; CHO=carbohydrate and values are in total grams
D1 refers to the first diet period (8-days); D2 refers to the second diet period (8-days)
Different ($p<0.05$) Kcal and CHO intakes between the periods are represented by (a) and (b)

different calorie intakes of these three subjects resulted in a significant increase in carbohydrate for subjects number one and two ($p<0.05$) between the two diet periods, but subject number five showed no change. Subject number one increased by an average of 144 g, and subject number two increased by an average of 17 g. Although the diet periods were statistically different in calories and macronutrient composition for two to three subjects, the minor changes in subject numbers two and five should not have affected their exercise tests. However, subject number one's exercise performance in test-2 may have been more influenced by dietary changes than the other five subjects.

Times to Exhaustion

The exercise times to exhaustion means and standard deviations for the two exercise tests are listed in Table 17. In addition, within the table all individual data is provided for each subject. There was no significant difference in the time to exhaustion between the tests. The group mean for test-1 was 2:00:37, and the group mean for test-2 was 2:04:51. Two subjects cycled for approximately 30 minutes longer in test-2 than in test-1, and a third subject cycled approximately 12 minutes longer in test-2. However, one subject exercised for 37 minutes less in test-2 compared to test-1. The other two subjects showed zero-five minutes of

Table 17. Exercise times to exhaustion: effects of vitamin B-6

Subject			Time (min.)
1	Test 1		131.8
	Test 2 (+B6)		160.5
2	Test 1		124.8
	Test 2 (+B6)		124.5
3	Test 1		120.4
	Test 2 (+B6)		149.4
4	Test 1		91.7
	Test 2 (+B6)		54.0
5	Test 1		114.9
	Test 2 (+B6)		126.3
6	Test 1		140.3
	Test 2 (+B6)		134.5
Test 1		mean	120.6
		SD	16.7
Test 2 (+B6)		mean	124.9
		SD	37.4

Test 1 refers to the non-supplemented exhaustive ride
Test 2 refers to the vitamin B-6 supplemented exhaustive ride

difference between the non-supplemented and supplemented exhaustive rides.

Body Weights

Pre and post-exercise mean body weights and standard deviations for the two exercise tests are listed in Table 18. There was no difference between the pre or post-exercise body weights between the tests. In addition, there was no significant change in body weight from pre to post-exercise during both tests (95% CI). During exercise a range of 1.5-2.8 kg in body weight per subject was lost. Body weights were closely monitored throughout each dietary period, and thus the subjects' weights remained consistent on a daily basis.

Table 18. Body weights before and after exhaustive endurance exercise

Subject			WT-1 kg	WT-2
1	Test 1		78.3	75.5
	Test 2 (+B6)		79.9	76.9
2	Test 1			
	Test 2 (+B6)		74.6	72.2
3	Test 1		74.1	71.5
	Test 2 (+B6)		73.9	71.2
4	Test 1		68.5	67.0
	Test 2 (+B6)		68.4	66.9
5	Test 1		69.9	68.0
	Test 2 (+B6)		71.4	69.7
6	Test 1		78.3	76.0
	Test 2 (+B6)		78.0	76.2
Test 1		mean	73.8	71.6
		SD	4.6	4.1
Test 2 (+B6)		mean	74.3	72.2
		SD	4.2	4.3

All body weights are in kilograms
WT-1 refers to the pre-exercise weight; WT-2 refers to the post-exercise weight
Test 1 refers to the non-supplemented exhaustive ride
Test 2 refers to the vitamin B-6 supplemented exhaustive ride

DISCUSSION

This study, for the first time, examined the effect of vitamin B-6 supplementation (i.e. 20 mg/d) on fuel utilization during exhaustive endurance exercise. All previous work in the area of fuels, vitamin B-6, and exercise (deVos et al. 1983, Manore and Leklem 1987, Campuzano 1988) either administered smaller supplemental doses of vitamin B-6 and/or used a shorter duration for their exercise protocols than the present study. Hopefully, by increasing the vitamin B-6 intake to higher levels than in earlier studies and extending the length of physical activity, we would enhance the possibility of determining whether vitamin B-6 does actually alter fuel metabolism during exercise. By exercising to exhaustion, a complete spectrum of exercise duration would be covered; and, therefore questions arising about the possibility of altered fuel metabolism later in exercise (i.e. > one hour) might be explained. In comparison to the above mentioned studies, the strengths of this present research came from a tight control over diet and exercise. All subjects were fed highly regulated and constant diets for six days prior to exercise testing, and the exercise sessions were performed according to each individual's VO_2 max test. All the experimental procedures and laboratory analyses performed were done by standard or well accepted methods. Therefore, in design and methodology, this study was well constructed.

Based on the knowledge of vitamin B-6's role in glycogenolysis and gluconeogenesis, this study attempted to determine if a vitamin B-6 supplemented state would result in higher plasma glucose levels than a non-supplemented state. Under conditions of supplementation, possibly muscle glycogen would be utilized to a greater extent (Manore and Leklem 1987), thereby decreasing the requirement of plasma glucose uptake into the muscles. Thus, plasma glucose concentrations would be higher with vitamin B-6 supplementation. Secondly, under conditions of supplementation gluconeogenesis may be enhanced, and therefore would help maintain plasma glucose levels via hepatic release of glucose into the blood. The mean plasma glucose results partially support these ideas. The higher plasma glucose concentrations at 60 minutes into exercise during test-1 compared to test-2 were the only time point in which test-1 means were higher than test-2 means during and after exercise. The pre-exercise levels were virtually identical, and the mean plasma glucose levels were higher in test-2 compared to test-1 at all other time points. The test-1 results showed a similar trend to that found by Coyle et al. (1983) in which plasma glucose concentrations showed an increase after 60 minutes of exercise and then a marked decrease by the end of exercise in comparison to pre-exercise levels. Eventually, blood glucose concentrations slowly fall as hepatic glucose output fails to keep pace with muscle utilization (Ahlborg and Felig 1982). During the

recovery period in test-1 a slight continual decline in plasma glucose levels was observed. Other studies have also found similar decreases during a recovery period (Tarnopolsky et al. 1990, Hofmann et al. 1991). Throughout exercise in test-2 there was a maintenance of plasma glucose concentrations. These results help support the possibilities of either decreased uptake of plasma glucose into the muscles due to an increase in muscle glycogen utilization or enhanced gluconeogenesis via hepatic release of glucose into the blood. Another possible explanation for the higher plasma glucose concentrations in exercise test-2 compared to test-1 is the possibility of enhanced glycogenolysis in the liver. However, no evidence suggests that vitamin B-6 is as abundantly stored in the liver, as large reservoirs of the vitamin are believed to be contained in muscle tissue, associated with glycogen phosphorylase (Black et al. 1977, 1978). During the initial recovery period in exercise test-2, the mean plasma glucose concentrations decreased by nine percent at post-30 minutes of exercise relative to post-exercise values. A similar change was observed by Tarnopolsky et.al. (1990).

It is known that plasma PLP levels increase with exercise (Leklem and Shultz 1983, Manore and Leklem 1987, Hoffman et al. 1991) and then decrease during the recovery from exercise (Manore and Leklem 1987, Hoffman et al. 1991). In addition, during the recovery period following exercise there is a three to fourfold higher uptake of glucose into previously worked

muscles compared to a resting state (Wahren et al. 1973). With less circulating PLP in the blood (and thus available to the liver for enhanced gluconeogenesis) and an elevated uptake of plasma glucose into muscles, a decline in plasma glucose appears possible during recovery.

No discussion on plasma glucose can be viewed as entirely complete without mentioning the neural and hormonal responses associated with hepatic glucose production. The primary regulators are the sympathetic nervous system (activated by norepeinephrine), insulin, glucagon, epinephrine, growth hormone, and cortisol (Horton 1989). Figure 4 (see pg 23) shows some of the functions PLP is involved in, and hormone modulation appears in the figure. Could vitamin B-6 somehow be affecting specific hormone levels, and therefore altering fuel substrate concentrations in the plasma during and after exercise? Unfortunately, the vitamin B-6 and exercise literature is relatively small, and hence no data answer this question.

Whenever a rise or fall in a blood constituent is monitored, plasma volume shifts may account for some, if not all, the concentration changes observed. However, plasma volume changes appear unlikely to explain any of the non-statistical differences in plasma glucose values within or between the exercise tests. Plasma glucose is only one of several possible fuel sources available to the body during exercise. If vitamin B-6 supplementation does in fact alter

fuel metabolism during exercise, then other circulating substrates in the plasma may also change in concentration.

In addition to glucose, lactic acid is another well acknowledged fuel measure of carbohydrate utilization. The byproduct lactate reflects the total glycolytic activity occurring in the body (i.e. all carbohydrate metabolism). One of the hypotheses of this research was that supplemental vitamin B-6 would increase the utilization of muscle glycogen for energy. Therefore, an objective of the study was to determine if lactate levels were higher under conditions of vitamin B-6 supplementation compared to a non-supplemented condition. The mean plasma lactic acid results were in complete contrast to the predicted objective of determining whether test-2 levels of lactate would be higher than test-1 levels. Although not statistically significant, all the test-2 plasma lactate concentrations were lower than the test-1 concentrations. Training, exercise intensity (Hurley et al. 1984), and diet (Bergstrom et al. 1967, Hermansen et al. 1967, Saltin and Hermansen 1967, Karlsson and Saltin 1971, Bergstrom and Hultman 1972) can all be reasons for different fuel (i.e. lactate) concentrations during exercise. However, in this study subjects maintained similar training patterns prior to both exercise tests. In addition, their diets were identical for six days before each exercise test, and the exercise intensities were constant for both endurance rides.

The plasma lactic acid results from the two exercise

tests suggest either a decrease in carbohydrate metabolism, or an increased clearance of lactate from the blood in test-2 compared to test-1. If a decrease in carbohydrate utilization actually occurred in test-2 compared to test-1, then additional evidence for this would be indicated by lower R values in test-2. Respiratory exchange ratios were in fact higher in test-2 than in test-1. Therefore, gas collection results provide evidence for an increased clearance or utilization of lactate from the blood. Under conditions of vitamin B-6 supplementation there may be increased conversion of lactate to pyruvate and subsequently to glycogen (the conversion of glycogen to glucose-6-phosphate is the only PLP requiring step). However, it would appear inefficient for the conversion of lactate to proceed to glycogen synthesis and then back to glucose, unless supplemental vitamin B-6 enhances the process to such a degree that excessive amounts of glucose are formed above the demands of hepatic output.

In both exercise tests, lactate concentrations increased by approximately 150-200% by 60 minutes into exercise from pre-exercise mean values. With the initiation of intense exercise, lactate removal can no longer match lactate production, and plasma lactate accumulates (Brooks 1985). In test-1 the mean plasma lactate concentrations peaked at 4.36 mmol/L, but in test-2 the observed concentrations never surpassed 3.90 mmol/L. Interestingly, on average our subjects did cycle longer in test-2 compared to test-1.

The most notable non-statistical difference in the mean plasma lactic acid concentrations between the two exercise tests occurred from 60 minutes into exercise to post-exercise. In test-1 lactate continued to increase until exhaustion, whereas in test-2 the plasma lactate concentrations actually decreased from the during exercise blood sample to the post-exercise sample. In addition, the plasma volume changes were greater in test-2 than in test-1. Therefore, the actual difference in plasma lactate concentrations between the two tests at 60 minutes into exercise would be larger, assuming that lactate does not move out of the intravascular space. Recall that lactate can be either directly utilized for energy (Brooks 1988) or can be converted to glucose/glycogen by the process of gluconeogenesis (Stanley et al. 1988). Also recall that in exercise test-1, a large decrease in plasma glucose was observed from during exercise to post-exercise, and in test-2 plasma glucose concentrations essentially remained at pre-exercise concentrations throughout exercise. The fall in plasma lactic acid levels from the during-exercise time point to post-exercise in test-2 may be related to an increased conversion of plasma lactic acid to glucose, due to vitamin B-6 supplementation stimulating gluconeogenesis. This also helps to explain the higher plasma glucose concentrations in test-2 compared to test-1. In test-1 the continual rise in plasma lactate levels may be reflective of a decreased ability to form glucose from lactate.

During the recovery period, plasma lactate concentrations decreased proportionally for both exercise tests. The greatest decrease occurred during the first 30 minutes of recovery, and by post-60 minutes of exercise the lactate levels remained 30% higher than pre-exercise concentrations. In a study by Hoffman et al. (1991), lactate levels at post-60 minutes of exercise were identical to pre-exercise levels in their study. However, the exercise workload used in their study was lower than in the present study. If vitamin B-6 supplementation was in fact responsible for the enhanced conversion of lactate to glucose, via gluconeogenesis, then it would be expected that post-exercise plasma lactate concentrations would decrease more rapidly in test-2 than in test-1. However, this was not observed. As previously discussed with the glucose results, if the decreases in plasma PLP during exercise recovery occurred here, as observed by others (Manore and Leklem 1987, Hoffman et al. 1991) then this may have influenced the conversion of lactate to glucose. With less PLP available to the liver during recovery compared to during exercise, there may be a decline in the gluconeogenic conversion of lactic acid to glucose.

The absolute mean lactate values in this study were higher than all the studies in Table 4 (see pg 52). However, the magnitude of the changes over time were within ranges seen in the five studies summarized in Table 4. The pre-exercise values of 1.32 mmol/L and 1.60 mmol/L from both tests closely

parallel the values obtained by Hoffman et al. (1991), and the during-exercise values of 3.90 mmol/L and 4.17 mmol/L are comparable to values from Coyle et al. (1983). In general, this study observed higher absolute plasma lactate concentrations than all the studies listed in Table 4. Training status, exercise intensity, and diet in our study were not significantly different in comparison to the other studies. Our higher observed plasma lactate levels may in part be due to methodology.

A common method in determining the contribution of blood borne fats to energy production involves not only the measurement of plasma FFA, but also plasma glycerol levels. The primary reason for measuring plasma glycerol is to better estimate the mobilization of FFA from adipose tissue during exercise. Therefore, in this discussion plasma glycerol concentrations will be used in conjunction with plasma FFA concentrations to help explain lipid metabolism. Emphasis will not be placed on glycerol as a direct energy source. A pre-study objective was to determine if plasma glycerol and FFA concentrations would be lower under conditions of vitamin B-6 supplementation compared to no supplementation. Supplementation was believed to directly increase carbohydrate metabolism, and therefore indirectly decrease fat utilization. The only statistically significant finding in plasma fuel substrates between the two exercise tests was in the mean pre-exercise FFA concentrations. Test-2 FFA levels were

significantly ($p<0.05$) lower compared to test-1 levels. However, mean plasma glycerol values were slightly higher in test-2 than in test-1. Manore and Leklem (1987) observed similar results in plasma FFA levels at rest, following vitamin B-6 supplementation (i.e. eight mg/d). In their study vitamin B-6 supplementation decreased plasma FFA concentrations compared to no supplementation, regardless of the subjects age, diet, and training status.

In both tests after 60 minutes of exercise, plasma FFA increased proportionally by 15-17%, and plasma glycerol levels approximately doubled. These magnitude changes are similar to those observed by Hurley et al. (1986). The higher plasma FFA values under conditions of vitamin B-6 supplementation suggest a decrease in fat utilization. However, Hurley et al. (1986) showed that with a decrease in plasma FFA, overall fat metabolism may actually increase. In this situation muscle triglyceride utilization increases, as partially reflected by lower R values. Although not statistically significant, in test-1 R values were consistently lower than in test-2. Again this suggests a decrease in overall fat metabolism during exercise in test-2. During both exercise sessions, the contribution of fats to energy expenditure increased from the during-exercise time point to post-exercise. Plasma FFA levels increased approximately threefold from the pre-exercise levels, and plasma glycerol was approximately fourfold higher at post-exercise compared to pre-exercise for both tests.

Gleeson et al. (1986) found similar increases in plasma FFA levels, and others have noted similar increases in plasma glycerol levels (Hurley et al. 1986, Tarnopolsky et al. 1990).

After 30 minutes of recovery, the plasma FFA concentrations continued to increase in test-1 (i.e. 0.28 mmol/L), but decreased in test-2 (i.e. 0.11 mmol/L). Several other studies (Gleeson et al. 1986, Tarnopolsky et al. 1990, Hoffman et al. 1991) have also observed an increase in plasma FFA concentrations from post-exercise to post-30 minutes of exercise, as we did in test-1. The relative changes in plasma FFA observed in this study virtually mimic the results of Hoffman et al. (1991) from pre-exercise to post-60 minutes of exercise. In comparison to other research, the vitamin B-6 supplemented exercise test presented unusual results by showing a decrease in plasma FFA concentrations from post-exercise to post-30 minutes of exercise. Plasma glucose and lactic acid results showed no evidence of increased carbohydrate metabolism during this 30 minute period; therefore, it would not be expected to find a decrease in fat utilization, as was observed. During both tests following one hour of recovery, plasma FFA concentrations remained approximately 150% higher than pre-exercise concentrations, and plasma glycerol was approximately double pre-exercise concentrations. During zero to four hours of recovery from high intensity (i.e. 70-75% VO_2 max) cycle exercise, there is a shift in fuel utilization away from carbohydrates to lipids

(Horton 1989). The non-statistical differences in plasma FFA concentrations between the two exercise tests are unlikely to be due to different plasma volume changes between the tests. The plasma FFA results along with respiratory exhange ratios do suggest a possible decrease in fat metabolism under conditions of vitamin B-6 supplementation.

When used in conjunction with plasma substrate levels, R values provide further evidence regarding altered fuel metabolism. The contribution of protein to energy expenditure is no more than five percent during steady state exercise (Plante and Houston 1984), therefore R values primarily represent carbohydrate and fat metabolism. High R values represent greater catbohydrate utilization, while low R values indicate greater fat utilization. A pre-exercise objective was to determine if R values would be higher during exercise following a period of vitamin B-6 supplementation compared to a non-supplemented period. As predicted, mean R values were in fact higher in a vitamin B-6 supplemented state compared to a non-supplemented state, but not statistically significant. The lack of statistical significance was due to subject number four showing no change in R values between the tests, and subject number two actually having higher R values in test-1 than test-2. For subject number two, plasma lactate was 37% higher at the during exercise time point in test-1 compared to test-2, which would support his higher observed R values in test-1. No other data appears to explain the R results for

subject number four.

Carbohydrate supplied 60% and 64% of the total exercise energy expenditure in test-1 and test-2, respectively. Campuzano (1988) found that following six days of PN supplementation (i.e. 20 mg/d) carbohydrate utilization increased from 43% to 52% of the total caloric expenditure during one hour of exercise. In the present study, the contribution of carbohydrate utilization to the total energy expenditure was 70.8% and 74.1% at the start of exercise for test-1 and test-2, respectively. By the end of exercise, carbohydrate utilization decreased to 54.1% in test-1, and 57.5% in test-2. Callow et al. (1986) observed similar results during an exhaustive marathon run. Of the total energy output, carbohydrate, lipid, and protein accounted for 59%, 40%, and 1%, respectively. In addition, carbohydrate contribution at the start of the run was between 70-80% and dropped to 50-55% by fatigue.

Muscle glycogen is the major fuel consumed during the early minutes of exercise (Rodahl et al. 1964), and the largest non-statistical difference in R values between the two tests occurred within the first 30 minutes of exercise. Hence, the higher mean R values in test-2 compared to test-1 do suggest an increase in carbohydrate metabolism, most likely from enhanced muscle glycogenolysis. If in fact carbohydrate metabolism did increase by an average of four percent in test-2 relative to test-1, and because carbohydrate is the most

important nutrient to athletic performance (Coyle 1988), how then would this translate into exercise times to exhaustion?

The major questions/curiosities of this study were to investigate 1) if vitamin B-6 supplementation would affect fuel utilization during exhaustive endurance exercise, and 2) to determine how such possible alterations in fuel metabolism would affect performance. Question two provided a practical application from this somewhat technical research. The final pre-study objective was to determine if exercise times (i.e. duration) to exhaustion would be shorter under conditions of vitamin B-6 supplementation compared to non-supplementation, due to enhanced glycogenolysis. Fatigue or exhaustion occurs when glycogen in the liver and exercising muscles is severely low (Bergstrom et al. 1967). There were no significant differences in the mean exercise times to exhaustion between the two tests. In test-1 subjects cycled for an average of 2:00:37, and in test-2 the average cycling time was 2:04:51. In reasonably well nourished individuals, fatigue from modestly intense exercise usually occurs at about two hours (McArdle 1986). Two subjects exercised approximately 30 minutes longer in test-2 than in test-1, and one subject cycled for 37 minutes shorter in test-2. The other three subjects' performance times were relatively consistent between the two exercise tests. Using exhaustion times to predict true glycogen depletion is an indirect and somewhat inaccurate process. Other non-measurable factors, such as boredom, can

definitely affect exercise performance during endurance activities. Therefore, the technique of muscle biopsies is often used to accurately quantify muscle glycogen utilization. Subject number one, who did cycle 30 minutes longer in test-2 than in test-1, did specifically mention boredom as a reason for terminating exercise prematurely in test-1. Hypothetically, let us suppose that fuel utilization accounts for 90% of an individual's exercise performance. In addition, let us suppose the remaining 10% of performance comes from non-measurable (i.e. mental/psychic) factors. Even if no change in fuel utilization occurs between two exercise trials, but an individual is three to five percent below par mentally, for whatever reason (e.g. lack of sleep), this then could translate into several minutes of decreased performance over a two to three hour exercise bout. Certainly, this is just an example, and it is only meant to clarify the limitations of predicting true glycogen depletion from exercise times to exhaustion.

Taking the four plasma substrate measurements, expired gas results, and exercise times to exhaustion into account, there is evidence for and against a vitamin B-6 effect on fuel utilization during exhaustive exercise. As for carbohydrate metabolism, the glucose results were difficult to interpret; however, the trend in decreased plasma lactic acid concentrations during exercise under conditions of vitamin B-6 supplementation favor either a decrease in carbohydrate

metabolism or enhanced conversion of lactic acid to glucose. The lower FFA concentrations and higher R values during exercise in test-2 compared to test-1 reinforce the possibility of enhanced glycogenolysis following a period of vitamin B-6 supplementation. A contradiction arises by the fact that subjects on average, exercised longer in test-2 than in test-1. With a significantly more rapid rate of carbohydrate metabolism, an obvious decrease in endurance should have been observed. Enhanced glycogenolysis may have in fact occurred, however, possibly the increase was not sufficient to translate into exercise performance. Other factors may have overshadowed the effects of vitamin B-6.

Two primary factors, which were controlled, come to mind as possible reasons for a longer exercise performance following vitamin B-6 supplementation. First, a training/order effect was possible between the two exercise tests. However, an examination of each subject's training log showed no major changes throughout the study. If any change in training occurred among the subjects, the trend was for a decrease in activity prior to test-2 relative to test-1. Subjects were requested to maintain consistent training throughout the study. An order effect could have been prevented by randomizing supplementation of vitamin B-6 between each diet period among the subjects. However, this was not practical due to the time needed to allow body pools of vitamin B-6 to return to baseline or normal values

following a period of supplementation. Mean heart rates, oxygen consumption, and RPE's all reveal a possible training effect. At multiple time points in test-2, heart rates were significantly ($p<0.05$) slower (i.e. $\bar{x}$ = 6-10 bts/min) than in test-1. Following 55 days of training, heart rates during submaximal exercise can reduce by 40 bts/min in sedentary individuals (Saltin 1969). The subjects in this study ,however, were trained (i.e. $\bar{x}$ VO_2 max = 59.6 ml/kg/min), and the time between each exercise test was only 23 days. Therefore, heart rates should not have changed significantly between the two exercise tests due to training. Vitamin B-6 supplementation is not known to have any effects on heart rates. Mean oxygen consumption was slightly lower in test-2 compared to test-1, but the difference was not statistically significant. Either a decrease in workload (i.e. intensity) or training would be responsible for a change in oxygen consumption. Small deviations in the workload settings may have resulted in particular subjects increasing and others decreasing in oxygen consumption between the exercise tests. Ratings of perceived exertion were significantly lower ($p<0.05$) at various time points during test-2 compared to test-1, particularly later in exercise. Since training imposes positive alterations in various cardiorespiratory parameters (i.e. heart rate, oxygen consumption), it therefore seems logical that one's perceived level of exertion would decrease following a period of training, provided no change in

workload. Secondly, in addition to training, observed differences in dietary intake may have contributed to longer endurance rides following vitamin B-6 supplementation. The mean carbohydrate intakes were on average 31 g higher during the supplemented diet compared to the non-supplemented diet; however, a large percentage of this number reflects significant carbohydrate increases ($p<0.05$) for only one subject. Subject number one increased his eight day mean carbohydrate intake by 144 g in diet period-2 relative to period-1. Subject-1 did in fact ride for 29 minutes longer in test-2 than in test-1. Possibly, the extra carbohydrate intake in period-2 resulted in higher glycogen stores for subject-1 than in the diet period prior to exercise test-1. Even if this subject was utilizing glycogen at a more rapid rate during test-2 than in test-1, the possibly higher glycogen stores may have masked a vitamin B-6 effect on exercise duration. Analysis of muscle biopsies would have better identified a change in muscle glycogen utilization.

Often, it is said that hindsight is "20/20". However, in looking back on this study and evaluating the given resources (i.e. budget, time, personnel), it appears that little correction would have been possible. Not to say that this research project was perfect, but ingenuity was limited by circumstances. Most limiting of all was the small subject number (n) with which we worked. Since this investigation originated from the the Department of Nutrition and Food

Management at OSU, a possible bias of mine lies in the belief that the exercise portion of the study required the most revision. Certainly, nutritionists possess an expertise in diet regulation; however, unless adequately trained, their knowledge of exercise may inhibit their strengths in nutritionl knowledge. In future studies in nutrition and exercise at OSU, more focus/understanding should be placed in the area of exercise testing. Fine tuning the exercise tests may have provided more conclusive evidence as to the effect of vitamin B-6 on fuel utilization during exhaustive exercise. From this study evidence does suggest a possible shift to increased carbohydrate and decreased fat metabolism under conditions of vitamin B-6 supplementation, however not conclusively. In the words of my major professor (Leklem[4] 1988)- "it is often distressing when researchers end their evaluations and reports with a plea for more research," however, in the field of vitamin B-6, exercise, and fuels, this is indeed necessary to draw better conclusions about a vitamin B-6 effect on fuel utilization.

SUMMARY AND CONCLUSIONS

It was hypothesized that supplemental vitamin B-6 (i.e. 20 mg/d PN-HCl) would alter plasma fuel substrates during submaximal exhaustive endurance exercise, and increase the utilization of muscle glycogen for energy. The objectives of the study were: 1) to determine if plasma glucose and lactic acid levels would be higher during exercise with supplemental vitamin B-6, 2) to determine if plasma glycerol and FFA levels would be lower during exercise with supplemental vitamin B-6, 3) to determine if R values would be higher during exercise with supplemental vitamin B-6, and 4) to determine if exercise times to exhaustion would decrease with supplemental vitamin B-6.

The effects of vitamin B-6 supplementation on fuel utilization was tested on six, trained, male cyclists. They were exercised to exhaustion twice at 64-75% of a predetermined VO_2 max. One exercise test occurred in an unsupplemented state, and the other test in a vitamin B-6 supplemented state. Subjects were fed a controlled diet for six days before and two days after each test. In order to detect any changes in fuel utilization (and vitamin B-6 metabolism), blood, urine, and respiratory gases were collected and analyzed. Blood was analyzed for glucose, lactic acid, glycerol, and FFA.

Standard statistical methods were used to analyze the

data. These included a t-test for paired values, analysis of variance (ANOVA), and correlation coefficients (r). Null hypotheses were rejected at the 0.05 level of significance.

The mean plasma glucose concentrations were not statistically different between the two tests at any of the specific sample time points. However, in the exercise test following vitamin B-6 supplementation, plasma glucose concentrations essentially remained constant, while under conditions of no supplementation, plasma glucose decreased significantly by 11% from the start to the finish of the exercise bout. Mean plasma lactic acid concentrations were lower, but not statistically different, at each specific sample time points following the vitamin B-6 supplemented period compared to the non-supplemented period. The area under the curves, which represent the lactic acid concentrations in both exercise tests, were virtually identical. Plasma lactic acid significantly increased with exercise and decreased during the recovery. The mean plasma glycerol concentrations were similar in both exercise tests. Plasma glycerol increased significantly throughout exercise and decreased during recovery. No statistical difference was found between the two tests in plasma glycerol levels. The only statistically significant ($p<0.05$) finding in the study for plasma fuel substrates was in the mean pre-exercise FFA levels. Following vitamin B-6 supplementation, FFA levels were depressed prior to exercise compared to conditions of no

supplementation. During both exercise tests, FFA increased significantly and decreased during the one-hour recovery period. Gas analysis results (i.e. R values) showed that carbohydrate utilization was on average four percent higher, but not statistically different, after the vitamin B-6 supplemented diet relative to the non-supplemented diet. The mean plasma volume changes were not statistically different between the two tests, however there was a significant decrease in plasma volume with exercise. By one hour post-exercise, plasma volume was not fully restored. At multiple time points, mean heart rates were significantly ($p<0.05$) lower (i.e. 6-10 bts/min) during exercise with supplemental vitamin B-6 compared to no supplementation. The mean RPE were significantly ($p<0.05$) lower during exercise in a vitamin B-6 supplemented state compared to a non-supplemented state, particularly later in exercise. No effect of vitamin B-6 supplementation on exercise performance times was found. The mean times to exhaustion, with and without supplemental vitamin B-6, were approximately two hours under both conditions.

In conclusion, there is evidence for and against a vitamin B-6 effect on fuel utilization during exhaustive endurance exercise. Pyridoxal 5'-phosphate's involvement in the energy deriving biochemical pathways of glycogenolysis and gluconeogenisis may be subject to individual variability. For example, supplemental vitamin B-6 may enhance glycogenolysis

and not alter gluconeogenesis in a particular individual, while in another person gluconeogenesis may increase and glycogenolysis remain constant. Vitamin B-6 supplementation appears not to have a consistent effect among individuals (i.e. trained males) on fuel utilization during submaximal exhaustive exercise.

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APPENDIX

Tables

Appendix Table 1. Individual plasma glucose concentrations

Subject	Test	Pre	During	Post	Post 30	Post 60
1 JGI	NS	5.31	5.36	3.84	4.33	4.04
	S	4.98	4.67	3.64	4.02	4.36
2 JGB	NS	3.93	4.64	4.20	3.64	3.64
	S	3.62	4.08	4.33	3.44	3.56
3 [illegible]	NS	5.53	5.62	4.47	4.33	4.49
	S	6.00	5.89	6.16	5.29	5.16
4 TDM	NS	3.64	5.18	4.56	3.89	3.82
	S	3.56		4.93	4.22	3.84
5 JRG	NS	4.78	3.87	4.60	4.36	4.18
	S	4.93	4.07	4.40	4.20	4.27
6 MCT	NS	5.31	4.69	3.56	4.16	4.31
	S	5.11	4.53	4.31	4.24	4.67

All values are in mmol/L

Test refers to the two exhaustive exercise rides (NS-Test 1, S-Test2)

NS refers to non-supplemented; S refers to supplemented with vitamin B-6

Pre-before exercise; During-60 min. into exercise; Post-immediately after exercise; Post 30-30 min. after exercise; Post 60-60 min. after exercise

Appendix Table 2. Individual plasma lactic acid concentrations

Subject		Test	Pre	During	Post	Post 30	Post 60
1	1	NS	1.32	3.77	4.91	2.50	2.12
		S	0.98	4.62	4.35	2.00	1.97
6	2	NS	2.11	4.41	3.53	1.84	1.79
		S	1.53	2.79	3.47	1.82	1.61
3	3	NS	2.55		4.71	3.98	4.17
		S	2.23	6.00	5.50	4.95	2.61
2	4	NS	2.14	2.82	2.39	2.83	1.42
		S	2.14		2.27	1.11	1.73
4	5	NS	0.95	4.56	7.20	2.79	2.14
		S	0.42	2.86	5.06	1.95	1.62
5	6	NS	0.55	5.27	3.41	2.59	1.91
		S	0.67	3.24	1.67	1.23	0.83

All values are in mmol/L

Test refers to the two exhaustive exercise rides (NS-Test 1, S-Test 2)

NS refers to non-supplemented; S refers to supplemented with vitamin B-6

Pre-before exercise; During-60 min. into exercise, Post-immediately after exercise; Post 30-30 min. after exercise; Post 60-60 min. after exercise

Appendix Table 3. Individual plasma glycerol concentrations

Subject	Test	Pre	During	Post	Post 30	Post 60
1	NS	0.126	0.433	0.651	0.404	0.326
	S	0.130	0.286	0.646	0.409	0.326
2	NS	0.143	0.350	0.505	0.236	0.223
	S	0.242	0.623	0.630	0.414	0.273
3	NS	0.128	0.298	0.505	0.327	0.314
	S	0.107	0.189	0.701	0.520	0.288
4	NS	0.102	0.395	0.436	0.201	0.213
	S	0.242		0.491	0.303	0.242
5	NS	0.092	0.203	0.426	0.193	0.116
	S	0.097	0.298	0.593	0.439	0.409
6	NS	0.302	0.487	0.911	0.664	0.567
	S	0.236	0.252	0.778	0.588	0.412

All values are in mmol/L

Test refers to the two exhaustive exercise rides (NS-Test 1, S-Test 2)

NS refers to non-supplemented; S refers to supplemented with vitamin B-6

Pre-before exercise; During-60 min. into exercise; Post-immediately after exercise; Post 30-30 min. after exercise; Post 60-60 min. after exercise

Appendix Table 4. Individual plasma free fatty acid concentrations

Subject		Test	Pre	During	Post	Post 30	Post 60
1	1	NS	0.679	1.168	1.885	2.104	2.038
		S	0.566	0.522	2.469	1.865	1.653
6	2	NS	0.675	0.777	1.580	1.660	1.165
		S	0.595	0.773	1.887	1.753	1.213
3	3	NS	0.844	0.725	1.436	2.163	
		S	0.509	0.755	1.840	1.819	1.764
2	4	NS	0.709	0.497	0.866	0.999	0.882
		S	0.520		0.736	0.652	0.600
4	5	NS	0.358	0.474	1.366	1.596	1.511
		S	0.388	0.411	1.056	1.354	1.376
5	6	NS	0.379	0.741	2.305	2.589	1.858
		S	0.249	0.292	1.532	1.426	0.942

All values are in mmol/L

Test refers to the two exhaustive exercise rides (NS-Test 1, S-Test 2)

NS refers to non-supplemented; S refers to supplemented with vitamin B-6

Pre-before exercise; During-60 min. into exercise; Post-immediately after exercise; Post 30-30 min. after exercise; Post 60-60 min. after exercise

Appendix Table 5. Individual respiratory exchange ratios (R)

			minutes													
	Subj	Test	20	30	40	50	60	70	80	90	100	110	120	130	140	150	160
	1	NS	0.905	0.890	0.880	0.870	0.855	0.855	0.855	0.850	0.840	0.875	0.850	0.865			
		S	0.920	0.905	0.890	0.890	0.885	0.895	0.870	0.875	0.875	0.860	0.865	0.860	0.850	0.850	0.865
	2	NS	0.905	0.905	0.885	0.880	0.870	0.875	0.885	0.870	0.860	0.865	0.850				
		S	0.895	0.875	0.885	0.850	0.860	0.870	0.875	0.870	0.875	0.850	0.855				
	3	NS	0.880	0.860	0.880	0.875	0.870	0.850	0.865	0.870	0.865	0.865	0.880				
		S	0.930	0.935	0.920	0.930	0.895	0.880	0.870	0.870	0.870	0.865	0.845	0.855	0.855	0.805	
	4	NS	0.875	0.855	0.860	0.850	0.875	0.855	0.855	0.865							
		S	0.865	0.865	0.860	0.865											
	5	NS	0.930	0.920	0.915	0.920	0.925	0.905	0.920	0.900	0.905	0.915					
		S	0.970	0.955	0.935	0.935	0.930	0.885	0.885	0.890	0.880	0.895	0.880				
	6	NS	0.940	0.935	0.915	0.895	0.910	0.900	0.855	0.845	0.850	0.870	0.850	0.855	0.870		
		S	0.940	0.940	0.930	0.915	0.895	0.900	0.900	0.885	0.900	0.875	0.885	0.900			

Test refers to the two exhaustive exercise rides (NS-Test 1, S-Test 2)

NS refers to non-supplemented; S refers to supplemented with vitamin B-6

Appendix Table 6. Individual oxygen consumption

			minutes													
Subj	Test	20	30	40	50	60	70	80	90	100	110	120	130	140	150	160
1	NS	3.58	3.54	3.64	3.59	3.52	3.52	3.70	3.70	3.71	4.00	3.79	3.86			
	S	3.34	3.31	3.22	3.20	3.33	3.40	3.30	3.38	3.31	3.36	3.38	3.47	3.45	3.50	3.59
2	NS	3.15	3.08	3.05	3.14	3.09	3.04	3.12	3.15	3.14	3.21	3.17				
	S	3.10	3.10	3.11	3.11	3.14	3.10	3.09	3.10	3.13	3.08	3.13				
3	NS	3.37	3.22	3.28	3.28	3.33	3.20	3.27	3.37	3.36	3.40	3.52				
	S	2.91	2.93	3.03	3.07	2.87	2.93	3.02	3.23	3.24	3.30	3.33	3.46	3.54	3.54	
4	NS	3.42	3.33	3.29	3.37	3.35	3.35	3.36	3.21							
	S	3.35	3.40	3.32	3.37											
5	NS	2.79	2.89	2.75	2.87	2.90	2.89	2.93	2.95	3.02	3.17					
	S	2.73	2.75	2.82	2.85	2.87	2.93	2.89	2.92	2.98	3.05	3.00				
6	NS	2.43	2.50	2.39	2.61	2.55	2.59	2.46	2.43	2.61	2.60	2.52	2.52	2.50		
	S	2.39	2.38	2.39	2.57	2.41	2.51	2.45	2.51	2.56	2.50	2.49	2.54			

All values are an absolute measure (L/min)

Test refers to the two exhaustive exercise rides (NS-Test 1, S-Test 2)

NS refers to non-supplemented; S refers to supplemented with vitamin B-6

Appendix Table 7. Individual heart rates

			minutes												
Subj	Test	Rest	10	20	30	40	50	60	70	80	90	100	110	120	130	140
1	NS	49	153	165	167	167	164	165	167	167	167	166	167	169	174	
	S	62	145	150	152	150	147	153	154	152	153	151	151	152	161	163
2	NS	74	169	169	172	174	174	173	173	175	174	173	176	176		
	S	63		163	165	166	164	163	165	169	170	173	175	175		
3	NS	64	150	150	150	151	152	153	152	158	162	165	167	174		
	S	50		124	142	140	145	149	148	147	149	150	154		162	167
4	NS	71	167	171	176	176	176	176	176	176	181					
	S	60	165	170	173	174	173									
5	NS	65		155	156	160	167	165	167	167	167	172	177	176		
	S	66		150	150	158	158	161	160	168	167	170	174	176		
6	NS	79	156	150	155	158	161	163	164	161	159	164	167	165	165	
	S	77	143	150	151	156	158	154	150	152	158	157	154	159	160	151

All values were obtained from an ECG (bts/min)

Test refers to the two exhaustive exercise rides (NS-Test 1, S-Test 2)

NS refers to non-supplemented; S refers to supplemented with vitamin B-6

Appendix Table 8. Individual ratings of perceived exertion (RPE)

		minutes														
Subj	Test	10	20	30	40	50	60	70	80	90	100	110	120	130	140	150
1	NS		13.0					14.0	14.0	15.0	15.0	16.0		18.0		
	S	13.0	13.0	13.5	14.0		13.0	14.0	14.0	14.0	14.0	14.0	14.0	14.5	14.5	14.5
2	NS	12.0	13.0	13.5	13.5	14.0	14.0	14.0	14.5	15.0	15.0	16.0	17.5			
	S	13.0	13.5	13.5	13.5	14.0	14.0	14.0	14.5	14.5	15.0	15.0	16.0			
3	NS	14.0	14.0	14.5	15.0	15.0	15.5	15.5	15.5	16.5	17.0	18.0	20.0			
	S	13.0	13.0	13.0	14.0	14.0	14.0	14.5	15.5	15.5	16.0	16.0	16.5	18.0	19.0	20.0
4	NS	12.0	14.0	14.0	15.0	16.0	16.5	17.0	18.5	20.0						
	S	12.0	13.0	15.0	16.0	19.0										
5	NS		14.5	15.0	16.0	16.5	17.0	17.5	18.0	18.5	19.0	20.0				
	S		14.0	14.5	16.0	16.0	16.5	16.0	17.0	17.5	18.0	18.5	19.5			
6	NS	11.0	13.0	15.0	16.0	16.5	16.5	16.5	16.5	17.0	17.0	17.5	18.0	20.0		
	S		12.0	12.0	13.0	14.0	14.0	15.0	16.0	16.0	16.5	17.0	19.0	20.0		

Test refers to the two exhaustive exercise rides (NS-Test 1, S-Test 2)
NS refers to non-supplemented; S refers to supplemented with vitamin B-6

Appendix Table 9. Individual hematocrits

Subject	Start	Mid-study	Test	Pre	During	Post	Post 30	Post 60
1	39.6	42.5	NS	43.2	45.5	45.5	45.3	44.0
			S	40.2	43.5	43.3	41.2	40.7
2	41.4	43.3	NS	43.7	45.5	44.0	43.2	42.0
			S	43.5	46.3	44.5	43.5	42.8
3	44.5	43.5	NS	44.3	46.5	44.5	45.2	46.5
			S	44.3	46.3	46.5	45.5	45.5
4	45.8	45.7	NS	45.5	48.5	48.0	45.5	47.3
			S	46.2		48.5	46.8	46.5
5	43.5	45.5	NS	43.5	45.7	46.5	43.5	44.0
			S	44.2	46.5	46.3	45.0	44.5
6	47.5	47.5	NS	47.0	48.5	49.7	47.5	47.5
			S	47.8	51.0	50.0	47.0	47.5

Start-pre-study; Mid-study-during the self-selected diet; Pre-before exercise; During-60 min. into exercise; Post-immediately after exercise; Post 30-30 min. after exercise; Post 60-60 min. after exercise
Test refers to the two exhaustive exercise rides (NS-Test 1, S-Test 2)
NS refers to non-supplemented; S refers to supplemented with vitamin B-6

Appendix Table 10. Individual hemoglobins

Subject	Start	Mid-study	Test	Pre	During	Post	Post 30	Post 60
1	135.4	145.2	NS	149.6	159.4	165.4	154.9	151.9
			S	133.2	147.8	148.5	140.9	139.4
2	150.7	150.0	NS	149.9	160.5	155.8	151.4	149.9
			S	141.7	156.7	151.2	145.4	145.4
3	150.5	149.6	NS	144.4	156.8	157.1	154.1	156.4
			S	144.5	154.8	160.2	155.5	
4	155.9	150.3	NS	147.4	164.6	160.9	154.5	160.9
			S	149.6		157.7	151.5	151.1
5	148.8	158.5	NS	142.8	156.9	159.9	148.0	155.8
			S	143.1	159.2	158.8	151.9	150.1
6	167.3	166.1	NS	161.0	175.5	180.4	163.3	166.2
			S	161.4	176.7	173.4	161.0	161.7

All values are in g/L

Start-pre-study; Mid-study-during the self-selected diet; Pre-before exercise; During-60 min. into exercise; Post-after exercise, Post 30-30 min. after exercise; Post 60-60 min. after exercise

Test refers to the two exhaustive exercise rides (NS-Test 1, S-Test 2)

Appendix Table 11. Individual plasma volume changes

		PVC-DC				PVC-V			
Subject	Test	During	Post	Post 30	Post 60	During	Post	Post 30	Post 60
1	NS	-9.99	-13.26	-7.09	-2.95	-9.01	-9.01	-8.38	-3.32
	S	-14.91	-15.05	-7.04	-5.25	-12.83	-12.23	-4.06	-2.05
2	NS	-9.65	-4.37	-0.11	2.95	-7.18	-1.37	2.06	7.02
	S	-14.10	-7.94	-2.54	-1.39	-10.81	-3.98	0	2.77
3	NS	-11.50	-8.36	-7.71	-11.27	-8.38	-0.69	-3.34	-8.38
	S	-10.01	-13.32	-9.03		-7.75	-8.38	-4.62	-4.62
4	NS	-15.38	-12.59	-4.60	-11.47	-11.35	-9.56	0	-7.09
	S		-9.26	-2.48	-1.62		-8.96	-2.66	-1.36
5	NS	-12.47	-15.44	-3.51	-9.15	-8.37	-11.41	0	-2.01
	S	-13.88	-13.39	-7.21	-5.24	-9.01	-8.39	-3.34	-1.37
6	NS	-10.86	-15.23	-2.33	-4.04	-5.84	-10.11	-1.99	-1.99
	S	-14.21	-10.79	1.84	0.45	-11.91	-8.32	3.39	1.33

All values are % changes relative to a pre-exercise value

PVC-DC refers to plasma volume changes calculated by the Dill and Costill method

PVC-V refers to plasma volume changes calculated by the Van Beaumont method

During-60 min. into exercise; Post-immediately after exercise; Post 30-30 min. after exercise; Post 60-60 min. after exercise

Test refers to the two exhaustive exercise rides (NS-Test 1, S-Test 2)

NS refers to non-supplemented; S refers to supplemented with vitamin B-6

Appendix Table 12. Individual workload settings

Subject	Kg	RPM	Watts
1	3.00	80	240
2	2.50	80	200
3	2.88	80	230
4	2.75	80	220
5	2.38	80	190
6	1.88	80	150

Workloads are for test-1 and test-2 (+B6)
Resistance in Kg x RPM = Watts

MALE CYCLIST NEEDED

VITAMIN SUPPLEMENTATION & ENDURANCE STUDY

PARTICIPANT REQUIREMENTS

18-35 years old
non-smoking
regular recreational cyclist
no-vitamin supplementation

FREE

FOOD (daily meals)
personalized results
body composition info
tests of aerobic fitness & endurance
(a $ 90.00 value)

$ 50.00 cash stipend

for more information contact
Dept. Nutrition and Food Management
Milam Hall
737-3561 or 737-0977

8-12 AM or 1-5 PM weekdays

NUTRITION & EXERCISE
SCREENING DATA

Name_____Date_____

Address_____

Phone (H)_____(W)_____Hrs:_____

Age_____ Ht._____Present Wt._____ Usual Wt_____

VITAMINS/ERGOGENIC AIDS:

yes no (if yes, how long & which ones)_____

willing to stop? yes no

ACTIVITY:

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

how long have you maintained this level of fitness?_____
are you willing to continue with this level of fitness during the 31 days of this study?_____

DIET:

Food preferences Foods will not eat (or allergies)

MEDICATIONS:

Are you currently on any medications? Yes no
please list if yes:_____

OTHER:

Able to attend 3 meals/day on campus, days 1-8, 24-31? yes no

Other helpful info?_____

(would this person be a good subject?)_____

Appointment schedule: date_____time_____with_____

interviewer initials_____

INFORMED CONSENT

TITLE: The Influence of Vitamin B-6 Supplementation and Exercise to Exhaustion on Vitamin B-6 Metabolism and Fuel Utilization

PRINCIPLE INVESTIGATOR: Jim Leklem, Ph.D.
GRADUATE STUDENT INVESTIGATORS: Nancy Dunton, R.D.,Ricki Virk

I.PURPOSE: The purpose of this research study is to (1) determine the influence supplemental vitamin B-6 has on vitamin B-6 metabolism, energy substrates and exercise capacity during exercise to exhaustion and (2) to determine the influence exercise to exhaustion has on vitamin B-6 metabolism and energy substrate utilization.

This investigation will involve 8 male, trained cyclists. The study will last 31 days (Oct.16 to the morning of Nov.16). I understand that my participation is voluntary and that I am free to leave this experiment at any time.

II.PRELIMINARY TEST: I understand that I will:
(1)complete a health history questionnaire form which will be kept confidential
(2)provide a complete list of all medications, including vitamin and other nutritional supplements taken in the last month
(3)have a sample of blood drawn (17 ml;about 1 tablespoon) to ensure normal health and vitamin B-6 status
(4)complete a xylose absorption test. This test involves drinking, prior to breakfast, a solution of water and xylose (a sugar). All urine is then collected over the next 5 hours. This test checks for normal carbohydrate absorption.
(5)have my body composition analyzed by underwater weighing
(6)keep a detailed 3 day 24-hour dietary history to determine my typical calorie and vitamin B-6 intake

III.$\dot{V}O_2$ MAX TEST: will be completed prior to the start of the study.This test will be used to determine maximal aerobic capacity. Each exercise to exhaustion test will be performed at 75% of this $\dot{V}O_2$ max level. I understand that I will:
(1)cycle on a bike ergometer (stationary bike) for approximately 20 minutes at increasing resistance (2 minute intervals) until my heart rate does not rise with increasing workload or I request to stop
(2)be allowed a 2-5 minute warm-up period on the bike prior to the test
(3)be monitored for heart rate and normal cardiac function by electrocardiogram by trained personnel 5 minutes prior to, at 2 minute intervals during and at 3, 6 and 9 minutes following the max test.
(4)not be penalized if I request to stop the test. I understand that the test of $\dot{V}O_2$ max has a chance of precipitating a cardiac event(such as abnormal heart rhythms) or even death. However, the possibility of such an occurrence is very slight(less than 1 in

10,000) since I am in good physical condition with no known symptoms of heart disease.

IV.EXERCISE TO EXHAUSTION TESTS: I understand that:
(1)I will exercise to exhaustion (that point where I am unable to maintain a preset pedalling speed which is 75% of my $\dot{V}O_2$ max) on the bike ergometer on 2 separate occasions (day 7 and day 30).
(2)The investigator will stop the study when exhaustion occurs
(3)I will be able to stop the exercise test at any time. The exercise test will be stopped prior to exhaustion if indicated by the American College of Sports Medicine guidelines for stopping an exercise test (see criteria for stopping an exercise test).
(4)I will not be able to keep track of time during the exercise test, but will try to cycle as long as possible during each test.
(5)I will be allowed a 2-5 minute warm-up period before the start of each test.
(6)I will be monitored by electrocardiogram for normal cardiac functioning 5 minutes prior to, 5 minutes during and 5 minutes following each exercise session.
(7)I will not be allowed to eat anything after 7 pm the day before each exercise test. I can consume water during the 12 hours of fasting (7pm-7am) and recognize the importance of consuming at least 2 glasses (20 oz.) of water 1 hour prior to reporting for this test.
(8)I will not be allowed water until after the last blood sample is drawn(1 hour after exercise is finished). I may rinse my mouth out with water during the exercise session, but not swallow. I understand that a feeling of discomfort, fatigue and/or lightheadedness may develop. If signs of dehydration develop, the subject will be given water.
(9)I will keep my daily exercise training level constant throughout the study.

V.DIET: I understand that I will:
(1)eat only the metabolic diet provided by the Department of Nutrition and Food Management at Oregon State University on days 1-8 and 24-32.
(2)eat my own food on days 9-23
(3)keep a 3 day 24 hour dietary intake record on days 20-22
(4)not take any vitamin, mineral or nutritional supplements other than the supplement provided throughout the study
(5)take one capsule per day containing either sugar and citric acid or pyridoxine (vitamin B-6) throughout the 31 days
(6)not know when I am receiving the vitamin B-6 supplement until the study is over (day 32)
(7)not consume alcoholic beverages during the study
(8)avoid caffeine containing items the day prior to, the day of and the day after each exercise test
(9)inform the principle investigator (Dr. Leklem) if any prescription or non-prescription drugs are taken

VI.URINE: I understand that I will:
(1)collect 24-hour urine specimens days 1-8 and days 24-32 as per the General Instructions form

(2)bring the 24-hour urine specimens to the lab each morning
(3)report any accidental loss of urine to the investigators

VII.DAILY LOG: I understand that I will:
(1)complete a daily activity form provided for me in a log book during days 1-8, 20-22 and 24-32.

VIII.BLOOD: I understand that:
(1)I will have 17 ml (about 1 tablespoon) of blood drawn prior to the start of the study before breakfast
(2)I will have 17 ml of blood drawn 5 times (85 cc total; about 6 tablespoons) during each exercise to exhaustion session (at 0 hours,1 hour during the exercise test,1-2 minutes after exercise, 30 minutes after exercise and 1 hour after exercise). When blood is donated (ie-Red Cross), 500 ml are taken without adverse effects
(3)I may develop a bruise, which will go away, at the site where blood is drawn
(4)I will have 17 ml of blood drawn before breakfast on day 21
(5)I will have all blood drawn from the antecubital vein in the arm by a Medical Technologist or Registered Nurse
(6)there is a minimal risk of infection when blood is drawn. sterile, disposable needles and sterile procedures will be used to minimize this risk.
(7)I should not donate blood of any other body fluids and therefore should not participate in this investigation if I am at increased risk for Hepatitis B or HIV (commonly called AIDS). I am at increased risk if I am a man who has had sexual contact with another man since 1977, have used intravenous drugs, am a Haitian immigrant, have had sexual contact with a member of one of these groups or have AIDS myself.

IX.EXPIRATORY GASES: I understand that:
(1) I will breath into a gas collection devise (by wearing a mouthpiece and nose clip) during the VO2 max test and each exercise to exhaustion test
(2)expiratory gas data will be collected every 30 seconds during the max test and for 2 minutes (at 10 minute intervals) during each exercise to exhaustion test

X.SWEAT: I understand that:
(1)I will have a sweat collector (see diagram) taped to my back during each exercise to exhaustion test
(2)there may be minimal skin abrasion from the placement and removal of the sweat collector

XI.BENEFITS:
(1) $50 from Oregon State University upon completion of the study
(2) the results of my blood values, body composition and fitness values obtained during this study
(3)18 days of meals at no cost

The department of Nutrition and Food Management reserves the right to remove a subject from this study if he or she is uncooperative in following the protocol of this investigation.
I have had the study explained to me and all questions answered. I give my consent to participate in this study. I may contact James E. Læklem (737-0969) concerning any questions about this research or any problems I have which I feel may be associated with participating in this study.

_____ _____
subject date

_____ _____
address phone #

_____
witness

NUTRITION & EXERCISE STUDY-FALL 90 NAME_____
NUTRITION & FOOD MGMT.
OREGON STATE UNIV. DATE_____

DAILY ACTIVITY SHEET

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

ACTIVITY	TIME SPENT(MIN,HRS.)	INTENSITY	TIME OF DAY*
Sleeping			
Sitting			
Walking			
Physical Work			
Other:			
Sports:			

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

RECORD ALL "FREE" FOODS IN EXACT AMOUNTS USED. INDICATE TYPE USED, IE.-DECAF, ETC.

Coffee/Tea (cups)_____

Pop-Regular or Decaf (cans,oz.)_____

candy/sugar_____

Other(water, electolyte replacement)_____
GENERAL HEALTH:

How do you feel today? Excellent___ Good___ Fair___ Poor___

MEDICATIONS:

Any medications?(aspirin etc,)_____

UNUSUAL EVENTS:(injuries, exams)_____

TURNED IN URINE: yes no WEIGHT TODAY:_____

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.

DATA COLLECTION

DATE: _____ Pb:___

INITIALS/COD TEMP:

TEST #:_____ %RH:

HYDROSTATIC WEIGHT:

DENSITY_____ %BF____ RV

TIME OF ARRIVAL: _____

TESTING START TIME:_____

BODY WEIGHT:

START:_____. FINISH:_____.

WEIGHT OF CLOTHES:

START:_____FINISH:_____

LAST MEAL/FOOD/DRINKS(date/time)_____

_____

TIME EKG PUT ON SUBJECT:_____

SEAT HEIGHT:_____

BIKE ERGOMETER#:_____

BLOOD PRESSURE:

BEFORE_____ DURING_____

AFTER_____

HOW DO YOU FEEL TODAY?_____

_____

NOTES/COMMENTS:_____

_____

_____