AN ABSTRACT OF THE THESIS OF

Ricky S. Virk for the degree of Doctor of Philosophy in Nutrition and Food Management presented on August 16, 1994.

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

Abstract approved: _____
James E. Leklem

Previous studies suggest that vitamin B-6 supplementation can alter fuel metabolism during exercise and plasma amino acid levels at rest. To examine the effect of vitamin B-6 supplementation on plasma fuel substrates and amino acid levels during exercise, five trained males (age: 29±7; VO_2 max: 54.7±6.2 ml/kg/min) performed two separate submaximal, endurance, exercise tests on a cycle ergometer. Subjects were exercised to exhaustion at 74.5±7.8% VO_2 max in a fasted condition on the seventh morning of two separate nine day controlled diet periods. The first exercise test (T1) occurred following a control or non-supplemented (NS) diet (i.e. 1.9 mg B-6/day), and the second exercise test (T2) occurred following a vitamin B-6 supplemented (S) diet (i.e. 1.9 mg B-6/day + 20 mg PN/day). Blood was drawn pre, during (i.e. 60 minutes into exercise), post, and post-60 minutes of exercise, and plasma was analyzed for glucose, lactic acid, glycerol, free fatty acids (FFA), and amino acids. Expired air was collected for three minutes at 10 minute intervals during both tests. Although not statistically different, there were observed trends for higher mean lactate levels and lower mean glycerol and FFA levels in T2 (S) compared to T1 (NS). Mean lactate, glycerol, and FFA concentrations all changed statistically significantly over time in both exercise tests. Mean plasma tyrosine levels were significantly lower ($p = 0.007$) at post-60 minutes of exercise and mean plasma methionine levels were significantly lower ($p = 0.03$) at post-exercise in T2 relative to T1. Of the 13 amino acids quantitated, only alanine and histidine concentrations

changed significantly over time. Although not statistically significant, mean respiratory exchange (R) values tended to be higher in T2 compared to T1. Mean oxygen consumption values were significantly higher ($p=0.02$) during the first 10 minutes of exercise and at multiple later time points showed a trend for being higher in T2 compared to T1. No statistically significant differences were observed in subjects' performance times to exhaustion between T1 (1:35:49; hr:min:sec) and T2 (1:31:56). These results indicate that vitamin B-6 supplementation can potentially alter fuel metabolism and plasma amino acid levels during exhaustive endurance exercise; however, not to such a degree that one's endurance capacity is affected.

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

by

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A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Doctor of Philosophy

Completed August 16, 1994

Commencement July, 1995

APPROVED:

Professor of Nutrition and Food Management in charge of major

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Date thesis is presented August 16, 1994

Typed by Ricky S. Virk for Ricky S. Virk

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INTRODUCTION

In hopes of gaining a competitive edge, a common misconception among endurance oriented athletes is that supplementation of various macro- and micro-nutrients benefits performance. While it is well documented that high intakes of carbohydrates can substantially enhance endurance (Bergstrom et al., 1967; Hermansen et al., 1967; Saltin and Hermansen, 1967; Karlsson and Saltin, 1971; O'Keefe et al., 1989; Williams et al., 1992; Bosch et al., 1993), high intakes of proteins (Consolazio et al., 1975) and fats (Ivy et al., 1980; Decombaz et al., 1983) appear not to benefit persons engaging in long duration exercise. As for micronutrients, no conclusive evidence to date suggests that supplementation of vitamins or minerals benefits exercise performance (Belko, 1987; Weight et al., 1988). However, supplementation may in fact affect performance from the perspective of fuel availability and utilization.

In the case of vitamin B-6, the results of previous research (primarily conducted at Oregon State University-OSU) suggest that the vitamin may in fact be categorized as a possible fuel modifier (Lawrence et al., 1975; deVos, 1982; Manore and Leklem, 1987; Campuzano, 1988; Virk et al., 1992). Based on expired gas data and plasma substrate concentrations, the results of the aforementioned studies suggest that vitamin B-6 supplementation of approximately five to ten times the recommended daily allowance (RDA) can speed the rate of carbohydrate utilization (i.e. glycogenolysis) during exercise of 65-80% VO_2 max in humans. It should be noted that all these studies involving the relationship of vitamin B-6 to fuel utilization directly addressed the possibility of enhanced glycogenolysis due to the vitamin's function as a coenzyme for glycogen phosphorylase. However, none of the research actually directly investigated the possibility of enhanced gluconeogenesis. Vitamin B-6 (i.e. pyridoxal 5'-phosphate-PLP) not only serves as a coenzyme in the conversion of glucose-1-phosphate (G-1-P) to glucose, but in addition the vitamin serves as a coenzyme for a variety of transamination

reactions. These reactions are required to convert non-glucose precursors, such as amino acids, to the readily utilizable form of energy, glucose. Thus it seems important to determine whether or not supplementation of vitamin B-6 can in fact alter the conversion of amino acids to glucose during exercise.

The primary fuel sources utilized by the exercising muscles include carbohydrates and fats. However, depending upon the specific metabolic conditions, secondary sources like amino acids, lactic acid (lactate), and glycerol may all contribute significantly to energy production. Energy metabolism/fuel utilization can be modified by several well acknowledged non-dietary factors such as: (1) exercise intensity (Chaveau, 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; Ingjer, 1979; Sherman et al., 1981; Holloszy and Coyle, 1984; Hurley et al., 1986) (4) and gender (Tarnopolsky et al., 1990). Therefore, including diet, multiple factors can affect substrate utilization during exercise with vitamin B-6 possibly being one of those factors.

With the total population of chronic exercisers seeming to be on an increase and the too often heard/read claims of vitamin supplements being falsely advertised as ergogenic aids, there is the possibility that individuals may be consuming unwarranted levels of particular vitamins. This may or may not be affecting their performance. A strong scientific verdict on vitamin B-6 is not yet available as to its effect on fuel utilization (more importantly, the exercise effect on vitamin B-6 requirements will be examined by another graduate student in this same lab). All the previous research in the area of vitamin B-6 and fuel metabolism has focused on the relative contribution of fats and carbohydrates to the total energy expenditure during exercise. In part, it is the intent of this study to investigate the role of vitamin B-6 in fuel utilization more diversely by also examining the influence of vitamin B-6 supplementation on distinct plasma amino acid concentrations pre, during, and post-exhaustive endurance exercise.

This should provide preliminary evidence as to what effect, if any, vitamin B-6 has on the ever so important metabolic pathway of gluconeogenesis during exhaustive endurance exercise.

Hypotheses

Supplemental vitamin B-6 can increase the rate of carbohydrate breakdown and carbohydrate formation during submaximal, exhaustive, endurance exercise, which is reflected in plasma amino acid levels, plasma fuel substrate levels, and respiratory exchange ratios (R).

Objectives

The primary objective is to understand better 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 are as follows:

1. To determine if selected plasma glucogenic amino acid levels (i.e. alanine, leucine, isoleucine, valine, and glycine) are higher during exercise and recovery with supplemental vitamin B-6.
2. To determine if plasma glucose levels are higher during exercise and recovery with supplemental vitamin B-6.
3. To determine if plasma lactic acid levels are lower during exercise and recovery with supplemental vitamin B-6.
4. To determine if plasma glycerol and FFA levels are lower during exercise with supplemental vitamin B-6 and to monitor their levels during recovery.
5. To determine if R values are higher during exercise with supplemental vitamin B-6.
6. To determine if vitamin B-6 supplementation has any effect on the duration of exercise performance times to exhaustion.

LITERATURE REVIEW

Vitamin B-6

History

The discovery of vitamin B-6 dates back to 1934 when Paul Gyorgy found that a specific vitamin factor prevented dermatitis in rats (Gyorgy, 1934). In only four additional years, several different research groups (Gyorgy, 1938; Lepovsky, 1938; Kuhn and Wendt, 1938; Keresztesy and Stevens, 1938; Ichiba and Michi, 1938) had isolated a crystalline form of vitamin B-6. Chemically, the compound was named 3-hydroxy-4,5-bis(hydroxymethyl)-2-methylpyridine (Harris and Folkers, 1939), and soon adopted a generic name, "pyridoxine." Today this name applies to a particular form of the vitamin, not the entire group of B-6 vitamers. During the 1940's experiments with microorganisms helped to identify other forms of vitamin B-6 (Snell et al., 1942; Snell,1981). It was also during this decade that "codecarboxylase" was discovered. "Codecarboxylase" was the original name for pyridoxal 5'-phosphate, the active coenzyme form of vitamin B-6. In 1953, vitamin B-6 deficiency was associated with clinical and biochemical lesions in infants and young children (Snyderman et al., 1953). Possibly, it was these intriguing findings in humans that fostered much of the additional research on this vitamin.

Structures and Chemistry

Vitamin B-6 exists in three primary forms: pyridoxine (PN), pyridoxamine (PM), and pyridoxal (PL) with each also existing as the respective 5'-phosphorylated compound (PNP, PMP, PLP). A fourth less well recognized form, 5-O-(B-D-glycopyranosyl) also exists, and for simplicity will be referred to as PNG. All three of the primary 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 moiety. If there is a carboxyl group at C-4, the derivative is termed four-pyridoxic acid (4-PA). Position five is the site of phosphorylation and glucoside linkage.

Collectively, all of the B-6 vitamers are considered to be labile. However, each vitamer varies in its degree of degradation. All forms of vitamin B-6 are light sensitive in solution with the degree of sensitivity being influenced by pH (Ang, 1979; Schaltenbrand et al., 1987). The salts of all three primary B-6 vitamers, along with the 5'-phosphate forms of these compounds are soluble in water, but only minimally soluble in organic solvents. The three non-phosphorylated forms of vitamin B-6 are all relatively heat stable in acid but heat labile within an alkaline medium.

Sauberlich (1985) has reported PLP to be the coenzyme for over 100 different enzymatic reactions. The major types of biochemical reactions facilitated by PLP's highly reactive nature include: alpha-carbon transaminations, racemizations, decarboxylations, oxidative deaminations, and loss of side chains; beta-carbon replacements and eliminations; and finally gamma-carbon replacements/exchanges, eliminations, and cleavages (Leklem, 1993). Of greatest importance to this paper are the alpha-carbon transaminations. Amino transferases/transaminases catalyze the reversible interconversion of amino acids with their respective alpha-keto analogues. Pyridoxal 5'-phosphate (PLP), the active coenzyme form of vitamin B-6, is covalently bound to enzymes via a Schiff base with an epsilon-amino group of lysine in the enzyme. Aminotransferases can be found in either a PLP or PMP form (i.e. the forms "oscillate"). Following Schiff base formation, the proton is split off the alpha-carbon of the amino acid and the C-4'of the PLP-form is protonated. Hydrolysis of the bond between the alpha-carbon and nitrogen gives an aldehyde. The PMP-form then condenses with the aldehyde, after which the reaction runs in the opposite direction: deprotonation at C-4', protonation at the alpha-carbon, and transaldimination lead to the ultimate formation of an alpha-amino acid or amine, and the cofactor is once again in the PLP form. Virtually all

reactions which are catalyzed by aminotranferases utilize glutamate as a reactant. In general, the transaminases are named for the two amino acids which take part in the reaction (White et al., 1973). White et al. (1973) suggest that the main task for aminotransferases are to ensure a supply of amino acids for protein synthesis because the composition of the diet does not completely fullfill this requirement. However, aminotransferases are also important in energy metabolism by supplying glucose to tissues by means of the Cori-Alanine cycle. Of the total twenty amino acids, there are only four, threonine, lysine, proline, and hydroxyproline which are not substrates of transaminases (Driskell, 1984).

Food Sources and Bioavailability

Vitamin B-6 is found in a wide variety of foods. See Table 1 for a list of data on the vitamin B-6 content of selected foods. In general, for animal foods the phosphorylated forms of vitamin B-6 are most predominant within our diet. Animal foods tend to contain larger proportions of PL and PM than plants, which are higher in PN and PM. Plants also contain relatively high levels of PNG. Evidence suggests that the bioavailability (i.e. the amount of a nutrient absorbed and utilizable by a cell) of this glucoside of pyridoxine is lower than the primary forms of vitamin B-6, but only marginally (Shultz and Leklem, 1987). The glycosylated form is absorbed (Kabir et al., 1983a) but not fully utilized (Trumbo and Gregory, 1988; Gregory et al., 1991). In comparison to PN, the uptake and cellular conversion of PNG to PLP within isolated rat hepatocytes is only 20% and 0.2%, respectively (Zhang et al., 1993). The decreased uptake is thought to be a result of the existence of permeability barriers sterically hindering PNG transporters and the decreased metabolism is likely a result of the rate limiting action of B-glucosidase. Bioavailability studies have found an inverse correlation between urinary 4-PA excretion and PNG content in several foods (Bills et al., 1987). Meats are a rich source of vitamin B-6 and contain no PNG. Kabir et al. (1983b) found the vitamin B-6 in tuna

Table 1. Vitamin B-6 content of selected foods and percentages of the three forms

Food	Vitamin B-6 (mg/100 gm)	PN (%)	PL (%)	PM (%)
Vegetables				
Beans lima, frozen	0.150	45	30	25
Cabbage, raw	0.160	61	31	8
Carrots, raw	0.150	75	19	6
Peas, green, raw	0.160	47	47	6
Potatoes, raw	0.250	68	18	14
Tomatoes, raw	0.100	38	29	33
Spinach, raw	0.280	36	49	15
Broccoli, raw	0.195	29	65	6
Cauliflower, raw	0.210	16	79	5
Corn, sweet	0.161	6	68	26
Fruits				
Apples, red delicious	0.030	61	31	8
Apricots, raw	0.070	58	20	22
Apricots, dried	0.169	81	11	8
Avocados, raw	0.420	56	29	15
Bananas, raw	0.510	61	10	29
Oranges, raw	0.060	59	26	15
Peaches, canned	0.019	61	30	9
Raisins, seedless	0.240	83	11	6
Grapefruit, raw	0.034	-	-	-
Legumes				
Beans, white, raw	0.560	62	20	18

Table 1, continued

	Vitamin B-6	PN	PL	PM
Beans, lima, canned	0.090	75	15	10
Lentils	0.600	69	13	18
Peanut butter	0.330	74	9	17
Peas, green, raw	0.160	69	17	14
Soybeans, dry, raw	0.810	44	44	12
Nuts				
Almonds, without skins, shelled	0.100	52	28	20
Pecans	0.183	71	12	17
Filberts	0.545	29	68	3
Walnuts	0.730	31	65	4
Cereals/Grains				
Barley, pearled	0.224	53	42	6
Rice, brown	0.550	78	12	10
Rice, white, regular	0.170	64	19	17
Rye flour, light	0.090	64	22	14
Wheat, cereal, flakes	0.292	79	11	10
Wheat flour, whole	0.340	71	16	13
Wheat flour, all purpose, white	0.060	55	24	21
Oatmeal, dry	0.140	12	49	39
Cornmeal, white and yellow	0.250	11	51	38
		-	-	-
Bread, white	0.040	-	-	-

Table 1, continued

	Vitamin B-6	PN	PL	PM
Bread, whole wheat	0.180	-	-	-
Meat/poultry/fish				
Beef, raw	0.330	16	53	31
Chicken breast	0.683	7	74	19
Pork, ham, canned	0.320	8	8	84
Flounder fillet	0.170	7	71	22
Salmon, canned	0.300	2	9	89
Sardine, Pacific canned, oil	0.280	13	58	29
Tuna, canned	0.425	19	69	12
Halibut	0.430	-	-	-
Milk/eggs/cheese				
Milk, cow, homog.	0.040	3	76	21
Milk, human	0.010	0	50	50
Cheddar	0.080	4	8	88
Egg, whole	0.110	0	85	15

All values taken from Orr, 1969

to be 25% and 37% more bioavailable than of whole wheat bread and peanut butter, respectively. Some fruits and many vegetables also contain significant amounts of vitamin B-6; however, this is primarily in the PNG form, making these foods lower in bioavailability compared to foods containing greater amounts of the primary forms of vitamin B-6. Orange juice contains approximately 50% of its vitamin B-6 in the PNG form (Kabir et al., 1983a). Consistent with these findings, it has been observed that vitamin B-6 from orange juice is only 50% as well absorbed as crystalline pyridoxine (Nelson et al., 1976). Milk is relatively low in vitamin B-6 content. In lesser developed nations, where food availability is reduced, grains/cereals and legumes can contribute large percentages to the total vitamin B-6 intake.

In the production of white flour from the whole wheat kernel, as much as 85% of the 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 grams of wheat bran in the diet reduces vitamin B-6 bioavalability by up to 17% (Lindberg et al., 1983). Therefore, with respect to bread consumption, the vitamin B-6 in white bread supplemented with pyridoxine hydrochloride (PN-HCl) is more bioavailable than the vitamin B-6 in non-supplemented whole wheat bread (Leklem et al., 1980). Foods representing the "average" American diet are estimated to be 71-79% bioavailable with respect to vitamin B-6 (Tarr et al., 1981). In addition to storage and processing, heating of food causes a loss of vitamin B-6. For example, in the toasting of bread 50-70% losses are incurred (Gregory and Kirk, 1981). Heat sterilization of milk results in the conversion of PL to PM (Bernhart et al., 1960). Heat-treated milk, which has been stored, shows a decrease in vitamin B-6 due to the formation of new compounds. Since milk is relatively low in vitamin B-6, and therefore is not a major source of vitamin B-6 intake from the diet, the health (and economic) benefits from heating (and storing) milk appear to exceed the losses in vitamin B-6 content of heat-treated milk.

Requirements

Several methods have been used for assessing vitamin B-6 requirements. The majority of the metabolic studies designed for assessing vitamin B-6 status have included experimental tests such as the tryptophan load test, urinary 4-PA excretion, aminotransferase activity, and plasma PLP concentrations. Table 2 lists normal values for the above mentioned tests (i.e. status indicators). In general, requirement studies have been depletion-repletion studies and often did not experiment with lesser bioavailable forms of vitamin B-6, such as PNG. In the words of James E. Leklem "our ability to set a requirement is only as good as the criteria we use for determining the status of vitamin B-6 (Leklem, 1988a)". In addition to dependable criteria, the experimental designs need to be well controlled and relatively consistent among research groups. If not, the data become very difficult to compare and evaluate, making a recommendation for the vitamin B-6 requirement difficult at best.

The current RDA for vitamin B-6 is approximately 1.6 and 2.0 mg/day for women and men, respectively (National Research Council 10th ed.). Based on relatively few studies (Snyderman et al., 1953; Bessey et al., 1957; McCoy, 1978), it is recommended that infants consume 0.3 mg/day and increase to 1-2 mg/day from childhood to adolescence. Pregnancy and lactation both add 0.5 mg/day to the RDA for women. The above figures should cover all aspects of potential vitamin loss from decreased bioavailability.

Further work is required to assess whether physical activity leads to an increased requirement for vitamin B-6. Limited data are 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; Dunton et al., 1992, 1993). In addition to physical activity several additional factors may affect vitamin B-6 requirements. See Table 3 for a list of these factors. Of the dietary factors listed, by far protein has received the most attention. As protein intake rises, so does the need for vitamin B-6 (Miller and Linkswiler, 1967; Miller

Table 2. Methods for evaluating vitamin B-6 status and suggested values for adequate status in adults (Data from Leklem, 1990)

Methods	Suggested values for adequate status[1]
Direct	
Blood	
Plasma PLP	> 30 nmol/L
Plasma PL	NV
Plasma total B-6	> 40 nmol/L
RBC PLP	NV[2]
Urine	
4-PA	> 3.0 umol/day
Total B-6	> 0.5 umol/day
Indirect	
Blood	
EGOT (transaminase)	< 1.25, activity coefficient
EAST (transaminase)	< 1.80, activity coefficient
Urine	
2 gram tryptophan load	< 65 umol/day
3 gram methionine load	< 350 umol/day
Oxalate excretion	NV
Diet intake	
Vitamin B-6, weekly average	> 1.2-1.5 mg/day
Vitamin B-6:protein ratio	> 0.020
Other	
EEG pattern	NV

[1]These values are dependent on sex, age, and for most, protein intake
[2]NV= no value established; limited data are available.

Table 3. Factors affecting an individual's vitamin B-6 requirement

1. Dietary
    a. Physical structure of a food
    b. Forms of vitamin B-6; those due to processing
    c. Binding of forms of vitamin B-6
2. Defect in delivery to tissues
    a. Impaired g.i. absorption
    b. Impaired transport-albumin, synthesis, and binding, phosphatase activity
3. Physiological/Biochemical
    a. Physical activity-increased loss, gluconeogenesis
    b. Protein-enzyme induction
    c. Increased catabolism/turnover-phosphatase activity, illness
    d. Impaired phosphorylation and/or interconversion, competing pathways, nutrient deficiencies, drugs
    e. Pregnancy-demand of fetus
    f. Growth-increased cell mass, repair
    g. Excretion rate-urinary, sweat, menstrual loss
    h. Lactation-adequate levels in milk
    i. Sex-differences in metabolism
    j. Age differences in metabolism
4. Genetic
    a. Apoenzyme defects-altered binding to apoenzyme
    b. Altered apoenzyme levels-biochemical individuality
5. Disease prevention/treatment
    a. Which? heart, cancer, diabetes, PMS, kidney, alcohol

Adapted from Leklem, 1993

et al., 1985). Therefore, a vitamin B-6 to protein ratio of 0.020 mg B-6/gm protein has been proposed (Dietary Std. Canada, 1975).

Absorption, Transport, Metabolism, and Excretion

All nutrients ingested in the diet must be absorbed within the small and large intestines, otherwise they simply pass through the body in feces, non-utilized. Once absorbed, transport via the circulatory system provides a means of nutrient distribution to tissues for subsequent metabolism and storage. Any excess of a particular nutrient must be dealt with, either by storage or by renal, fecal, and sweat excretion.

The absorption of vitamin B-6 has been most extensively studied using animal models, particularly the rat. Most rat studies suggest that all three non-phosphorylated forms of vitamin B-6 are absorbed within the proximal jejunum (Middleton, 1977; Middleton, 1982) by a non-saturable process (Henderson, 1985). However, some research indicates the possibility of a saturable component of uptake, especially within the duodenal section of the small intestine (Middleton, 1985). If such a mechanism exists in humans, this would have obvious implications for megadose supplements of PN. In the rat, absorption has been shown to decrease from the proximal to the distal small intestine (Middleton, 1985). Alkaline phosphatase is required for intestinal uptake, since phosphorylated compounds do not readily cross membranes (Middleton, 1977, 1978). At relatively high concentrations, however, there is limited absorption of phosphorylated B-6 vitamers (Mehansho et al., 1979; Hamm et al., 1979). Once the B-6 vitamers have crossed the intestinal mucosa, they must then be transported to the liver where hepatocytes are involved in interconversions and metabolism.

In the blood, albumin binds PL and PLP (Dempsey and Christensen, 1962). However, gel filtration experiments suggest that PLP's binding affinity to albumin is significantly greater than that of PL (Anderson et al., 1974). As previously mentioned, phosphorylated compounds generally do not cross membranes. Therefore, PL is the most important form of vitamin B-6

uptake into cells (Lumeng et al., 1974). Pyridoxal 5'-phosphate (PLP) bound to albumin must initially be released from this transporting protein prior to hydrolysis of the phosphate moiety by alkaline phosphatase. Then the PL released from albumin easily crosses cell membranes and within the cell PL is usually rephosphorylated. Erythrocytes rapidly take up PN and PL by simple diffusion (Mehansho and Henderson, 1980). In contrast to PN, the driving force behind PL uptake in the red blood cell is not coupled to rephosphorylation, but rather by an affinity to hemoglobin and conversion to PLP (Friedrich, 1988). Thus the uptake of PL and PN into erythrocytes depends upon different mechanisms.

The metabolism of vitamin B-6 occurs primarily within the liver. All three non-phosphorylated forms of vitamin B-6 are taken up by passive diffusion followed by metabolic trapping through phosphorylation (Mehansho et al., 1980; Kozick and McCormick 1984). In the liver phosphorylated forms of vitamin B-6 predominate, since pyridoxal kinase is at least 5-fold higher in activity than the phosphatase enzyme (Merrill and Henderson, 1990). First, all three forms are phosphorylated at the 5'-position by pyridoxal kinase (McCormick et al., 1961). The reaction is reversible, 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 excess of vitamin B-6 (i.e. PL) in the liver is irreversibly converted to 4-PA by an FAD-dependent aldehyde oxidase and/or an NAD-dependent dehydrogenase. Four-pyridoxic acid is excreted renally, and on a daily basis may account for 40-60% of an adequate vitamin B-6 intake (Leklem, 1988a). The most important forms of vitamin B-6 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. 70-90%) under conditions of

normal intake, other forms also exist (Shultz and Leklem, 1981; Leklem, 1990). Next in abundance is PL with even lower levels of PN and PM found in the blood. Pyridoxamine 5'-phosphate (PMP) and PNP are either found in extremely low concentrations or not detected at all (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 in the blood are the best indicators of vitamin B-6 status (Leklem, 1988b). The importance of PL arises from its ability to easily be supplied to tissues, while PLP must first be dephosphorylated. If the rate of dephosphorylation were sufficient, PLP could likely serve as a source of PL.

By using various amounts and mixtures of oral vitamin B-6 supplements, dose experiments provide a useful technique to study vitamin B-6 utilization. Studies have quantitatively observed the changes in blood B-6 vitamers over time (Lumeng et al., 1974, 1980; Wozenski et al., 1980; Ubbink et al., 1987). Within one-hour about 80% of an oral PN supplement reaches the liver (Snell and Haskell, 1971). Lumeng et al. (1974) noted a four to five-fold increase and plateau in plasma PLP with 25-50 mg/day PN administration two to four days after the beginning of supplementation. This corresponded to a value of about 500 nmol/L with the 50 mg/day supplement. Recall Table 2, where the suggested value for adequate status is only greater than 30 nmol/L. The suggested value is easily accomplished with intakes near the RDA, provided that there is normal absorption, transport, and metabolism of vitamin B-6.

The major form of vitamin B-6 excreted in urine in humans and animals is 4-PA. The three non-phosphorylated forms of vitamin B-6 are also found in urine, but 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. Urinary 4-PA is, however, considered to be a short term indicator of vitamin B-6 status since 4-PA does change rapidly in response to a change in dietary vitamin B-6 intake. Exercise can also increase 4-PA

excretion. Studies (Borisov, 1977; Hatcher, 1982; Manore et al., 1987; Dunton et al., 1992, 1993; Rokitzki et al., 1994) have shown that 4-PA is significantly increased in 24-hour urine samples of subjects on exercise test days, and to some extent may be even higher in trained versus untrained individuals (Manore et al., 1987). Based primarily upon urinary 4-PA excretion Rokitzki et al. (1994) suggest that a mean loss of one mg of vitamin B-6 occurs as a result of a single marathon race. 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 increase with age; however, sex differences are small (Lee and Leklem, 1985). 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 between 3.0-7.1 umol/day for men and 2.4-6.5 umol/day for women (Simon et al., 1982). More recently, Leklem (1990) suggests that 3.0 umol/day of 4-PA should be excreted in the urine of males and females alike to be considered adequate in vitamin B-6 status. Often in vitamin B-6 deficiency, 4-PA is not detected in urine. Therefore, its evaluation in nutritional status is important (Snell and Haskell, 1971).

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

Body Pools/Stores

As a general rule, water soluble vitamins are not stored to any large degree within the body. Thus large intakes are simply excreted in the urine. Vitamin B-6 appears to be a noteworthy exception to the rule. With large supplementary doses, the increased 4-PA

excretion in urine does not account for all the dietary intake of vitamin B-6. Therefore, vitamin B-6 must be retained somewhere within the body or excreted as an additional form or metabolic product other than 4-PA. 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 concentrations of PLP in human organs are found in the liver followed by the brain, kidney, and spleen, respectively (Shin et al., 1983). In mammals, most of the PLP in the tissues is bound to proteins. In the erythrocytes PLP is found bound to hemoglobin, and in plasma it is bound to albumin. In rat and human muscles, the coenzyme is found in association with glycogen phosphorylase. This binding to a 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 in vitamin B-6 concentration following supplementation are the blood and muscles. In healthy persons receiving 100 mg PN-HCl/day for one to three weeks, it was observed that baseline levels of total plasma vitamin B-6 increased from 114 nmol/L before supplementation to 655 nmol/L afterwards. Based on studies in rodents, 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). Additional studies in rats helped confirm the muscle's role in storage (Black et al., 1977,1978). When rats were given vitamin B-6 supplements, glycogen phosphorylase content increased by 300% 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. Recently, Coburn et al. (1991) examined if similar results occur in humans. By using the technique of muscle biopsy, they 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, they demonstrated that with six weeks of 0.98 mmol PN-HCl/day (i.e. 166 mg/day PN), vitamin B-6 increases in muscle in humans are slight relative to the increases observed in rats. The

increase was on the order of 25%. From this study there was no evidence supporting the notion that vitamin B-6 supplementation in humans might cause an abnormal increase in glycogen phosphorylase, as was noted in rats.

Functions

The functions of vitamin B-6 within the body are quite extensive. As previously mentioned, vitamin B-6 is involved in over 100 known enzymatic reactions within biological systems. This involvement of vitamin B-6 in these diverse enzymatic reactions makes it important in several cellular processes which include: immune system function, glycogenolysis, gluconeogenesis, erythroctye function, niacin formation, nervous system function, lipid metabolism, and hormone modulation. As seen in the above list, carbohydrate, fat, and protein metabolism are all affected by PLP. This suggests a potential role for PLP as a fuel modifier through its metabolic association with these energy producing macronutrients.

More recently vitamin B-6 has been associated with improved immune function in elderly humans as determined by lymphocyte response. While Talbott et al. (1987) noted that with supplemental vitamin B-6 improvements occur in immune function, van den Berg et al. (1988) have found that with marginal vitamin B-6 deficiency there are adverse effects on lymphocyte production and on antibody antigen responses. Meydani et al. (1991) studied the effect of vitamin B-6 deficiency on immune status in eight, healthy, elderly persons and they found that vitamin B-6 deficiency (i.e. 3ug/kg/day for 20 days) impaired *in vitro* indices of cell mediated immunity. Such adverse responses may be related to alterations in 1-carbon metabolism which can subsequently lead to changes in nucleic acid synthesis (Axelrod and Trakatellis, 1964; Chandra and Puri, 1985).

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 the energy demands of

working muscles by phosphorylating glucose (from muscle glycogen) to G-1-P (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. However, contrary to the work of Black et al. (1977) in rats, Coburn et al. (1991) found there to be a statistically significant 13% decrease in glycogen phosphorylase enzyme activity in men after a period of vitamin B-6 supplementation compared to baseline conditions. The supplement was administered orally in the form of PN-HCl at a dose of 0.98 mmol (i.e. 166 mg/day PN) for six weeks, after which muscle biopsies were performed on the gastrocnemius muscle for various tests which included phosphorylase activity. These findings in humans suggest that vitamin B-6 supplements theoretically should not enhance glycogenolysis at all, but however depress the rate of glycogenolysis. With this in mind, one might then speculate if vitamin B-6 would then have any effect on gluconeogenesis.

Gluconeogenesis, often confused with glycogenolysis, is an entirely different metabolic process. In gluconeogenesis, non-carbohydrate precursors (i.e. amino acids, lactate, and glycerol) are all ultimately 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-Alanine cycle, the keto analogues of specific amino acids require PLP for conversion to alanine. In addition, alanine aminotransferase utilizes PLP as a coenzyme for the conversion of alanine to pyruvate. This reaction is reversible in both liver and muscles, and both directions require either PLP or PMP as a coenzyme. In rats, a vitamin B-6 deficiency has been shown to decrease alanine aminotransferase activity (Angel, 1980). Females who were given 0.19 mg/day of vitamin B-6 for four weeks, did not show a significant change in fasting plasma glucose concentrations (Rose et al., 1975). This suggests that low vitamin B-6 intakes may not compromise the gluconeogenic process to any significant degree. However, an important

question is whether supplementation of vitamin B-6 enhances the process. Unfortunately, no direct evidence answers this question. In rats, inhibition of gluconeogenesis with mercaptopicolinic acid (MPA) resulted in a decrease in endurance capacity by 25-30% during exhaustive endurance exercise (John-Alder et al., 1986). Thus gluconeogenesis appears to be an important energy producing pathway during prolonged physical activity.

Pyridoxal 5'-phosphate not only functions in the formation of red blood cells (RBC), but PLP also functions in RBC metabolism through transamination reactions and oxygen (O_2) affinity. The synthesis of erythrocytes requires PLP to serve as a cofactor for delta-aminolevulinic acid synthetase (Kikuchi et al., 1958), which catalyzes the condensation between glycine and succinyl-CoA to delta-aminolevulinic acid. Since gamma-aminolevulinic acid is the initial precursor in heme synthesis (Bottomly, 1983), vitamin B-6 plays a key role in erythropoiesis. Therefore, a deficiency of vitamin B-6 can lead to hypochromic microcytic anemia. Both PL and PLP have been found to bind to hemoglobin (Mehansho and Henderson, 1980; Ink et al., 1982). Pyridoxal binds to the alpha-chain of hemoglobin resulting in increased O_2 affinity (Maeda et al., 1976), while PLP binds to the beta-chain of hemoglobin resulting in decreased O_2 affinity (Reynolds and Natta, 1985). These results clearly suggest that altered ratios of PL to PLP within the RBC can lead to changes in O_2 affinity for hemoglobin; and, thus possibly affect gas exchange within the pulmonary system at the level of the alveoli and affect gas exchange within muscle tissue at the level of the capillaries. Within the body adequate gas exchange is of paramount importance, particularly during strenuous exercise.

Within the tryptophan-niacin pathway, PLP functions in at least four enzymatic steps. However, there is only one direct PLP-requiring step in the conversion of tryptophan to niacin, which is the conversion of 3-hydroxykynurenine to 3-hydroxyanthranilic acid by kynurinase (Brown, 1985). Leklem et al. (1975) found that after four weeks of a low vitamin B-6 intake, urinary excretion of distinct metabolites increased following a two gram L-tryptophan load test.

They interpreted this to suggest that low vitamin B-6 intakes have a slight negative effect on the conversion of trytophan to niacin.

The formation of serotonin within the nervous system also occurs via tryptophan metabolism and similarly is vitamin B-6 dependent. The conversion of 5-hydroxytryptophan to 5-hydroxytryptamine (serotonin) is catalyzed by a PLP-dependent enzyme, 5-hydroxytryptophan decarboxylase. Recently, serotonin has been used to explain a concept known as mental/central fatigue, which is thought to be a potential factor in exhaustion during endurance exercise (Newsholme et al., 1992). This will be discussed in a later section of this literature review (see Exercise Physiology-Protein/Amino Acids). Vitamin B-6 (as PLP) also is involved in the synthesis of additional neurotransmitters which include: taurine, dopamine, norepinephrine, histamine, and gamma-aminobutyric acid (Dakshinamurti, 1982). Vitamin B-6 deficiency has been associated with neurological abnormalities (e.g. convulsions and abnormal EEG patterns) in human infants (Coursin, 1954; Maloney and Parmalee 1954) and adults (Canham et al., 1969). In infants, supplements of 30-100 mg/day are sufficient to prevent convulsions and to normalize EEG tracings. In animals, vitamin B-6 deficiency has also been associated with incomplete nervous system development (Kurtz et al., 1972; Thomas and Kirksey, 1976; ; Morre et al., 1978a, 1978b; Chang et al., 1981). These results obviously suggest that adequate PLP supplies are necessary for the embryo to undergo rapid and normal mitosis during periods of development.

Most of the research relating vitamin B-6 to lipid metabolism has been done using the rat model. Early studies, dating back to the 1930's, found that a vitamin B-6 deficiency in rats resulted in a decrease in body fats (McHenry and Gauvin, 1938). Future studies on vitamin deficiency in rats were equivocal as to whether a deficiency increased or decreased fat synthesis (Desikachar and McHenry, 1954; Sabo et al., 1971; Angel and Song, 1973; Angel, 1975). Vitamin B-6 does appear to be involved in the conversion of linoleic acid to arachidonic acid (Witten and Holman, 1952). In addition, vitamin B-6 appears to have an effect on

carnitine synthesis (Cunnane et al., 1985; Cho and Leklem, 1990). However, this may have no effect on plasma levels of fatty acids. Human studies suggest that only minor changes occur in the blood levels of fatty acids following a moderate vitamin B-6 deficient condition (Mueller and Iacono, 1963), and no significant change in plasma cholesterol occurs (Bayasal et al., 1966). In humans, supplementation of vitamin B-6 has been shown to have either no effect on the lipoprotein profile (Pike et al., 1990) or to significantly increase the HDL subfraction (Kleiner et al., 1980).

Pyridoxal 5'-phosphate not only binds to steroid receptors (Litwalk et al., 1985; Allgood and Cidlowski, 1991), but in addition binds to receptor DNA binding domains. Both sites are located on the receptor itself, and contain lysine residues with which PLP interacts. The net result of PLP's interaction at these two sites is a decreased expression and biological response of the steroid. Currently, no human studies have been conducted to suggest any physiological significance to these findings. However, with the known effects of steroids in a variety of biological systems, it is highly possible that vitamin B-6 status may be a contributing factor to the action of hormones in these systems.

Amino Acids

Several studies have investigated the effect of vitamin B-6 on plasma amino acid levels. However, the majority of this research has examined the effects of deficiency, not supplementation, on amino acids. With deficient intakes of vitamin B-6 in animals, researchers have shown plasma concentrations of glycine and cystathionine to be elevated, while plasma threonine, alanine, and serine levels are decreased (Beaton et al., 1953; Swendseid et al., 1964; Wolfson et al., 1986). In human studies, glycine, serine, and threonine have been reported to increase by as much as 15%, 17%, and 25%, respectively with vitamin B-6 deficiency (Donald et al., 1971; Park and Linksweller, 1971; Henderson et al., 1989). Kang-Yoon and Kirksey (1992) investigated the effect of 22 mg/day PN supplementation for 14 days

on plasma amino acid levels and found glutamic acid concentrations to decrease significantly by 47%; thus suggesting that the transamination reaction involving alanine and pyruvate is depressed, since glutamic acid is formed from alpha-ketoglutarate in the reaction. However, other reasons entirely unrelated to transamination could also account for the depressed glutamic acid levels (e.g. protein intake, hydration status, plasma volume changes). Under conditions of moderate to severe exercise, one would actually expect to observe glutamic acid concentrations to increase, as plasma volume decreases and alanine is actively converted to glucose. In addition, they found alanine, cysteine, arginine, phosphoserine, and urea concentrations to increase significantly by 16%, 33%, 31%, 29%, and 15%, respectively.

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). 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 can potentially phosphorylate 147 ADP's. Following transamination, selected amino acids are converted to specific Krebs cycle intermediates, and their location of entry into the cycle determines the amount of ATP formed. Oxidation of each mole of leucine, isoleucine, and valine (i.e. branched chain amino acids-BCAA) 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, or enough energy to perform maximal 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 five-fold that of ATP (McArdle et al., 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, by freeing energy in ATP, myosin and actin filaments can slide relative to each other. This is known as the sliding filament theory (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 individual's 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 et al., 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 latter 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/RER) 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 principle a numerical value is assigned to each macromolecule, ranging from 0.70-1.0. Thus when carbohydrate alone serves as the fuel for the body, an equimolar amount of carbon dioxide is produced per mole of oxygen consumed, giving a value of 1.0.

An example of glucose oxidation:

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

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

An example of palmitic acid oxidation:

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

When proteins are considered, the scenario becomes slightly 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.

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 be elevated to somewhere near 1.1. However, 0.70 is the bottom end of the scale and this value is commonly noted in persons in 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 their 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 proportional to the intensity of exercise (Van Beaumont et al., 1981). Gore et al. (1992) have found that the reduction in plasma volume, which occurs with cycle ergometry, is essentially complete within the first five minutes of activity. Thus they suggest that oral water ingestion during prolonged exercise may be more essential for homeostasis of extravascular water rather than plasma volume. At submaximal intensities of 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. With the use of specific equations, the concentration changes of the plasma constituents are used to interpret plasma fluid shifts. 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 the use of hemoglobin concentrations to be the most reliable. Since different indices may give different results in calculating plasma volume changes, 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. They have demonstrated that a loss of RBC mass occurs as a result of dehydration, which is corrected for by measuring hemoglobin concentrations. In contrast, a method by Van Beaumont (1972) assumes a constant RBC mass and only measures hematocrit changes in the blood with exercise to determine plasma volume shifts.

Fuel Sources

Carbohydrates

Carbohydrates are compounds which have a ratio of one carbon atom to water ($C:H_2O$). They are often categorized as either simple sugars ($C_6H_{12}O_6$) or complex sugars. The simple sugars consist of the monosaccharides glucose, galactose, and fructose, along with the disaccharides sucrose, lactose, and maltose. The disaccharides are formed by the condensation of glucose to either itself, galactose, or fructose to yield a disaccharide. Of the simple sugars, glucose is the most important for energy metabolism. The complex sugars represent thousands of glucose molecules linked in a polymeric form and are found within plants as starch and within animals as glycogen.

Blood glucose and muscle and liver glycogen are the immediate sources of carbohydrate available to the actively exercising muscles. It is estimated that the total body carbohydrate storage equals 375-475 grams, of which approximately 325 grams are muscle glycogen , 90-110 grams are liver glycogen and 15-20 grams are blood glucose (Felig and Wahren, 1975). At four kcal/gram the average person is therefore capable of 1500-2000 kcal of energy production at an R-value of 1.0. Glycogen is stored in tissues with 2.7 grams of water per

gram of glycogen (Olsson and Saltin, 1970), thus reducing the effective caloric value of glycogen on a per gram basis relative to fat. The glycogen concentration in skeletal muscle at rest depends upon an individual's past history relative to diet, exercise, and state of training (Gollnick, 1985). Bergstrom et al. (1967) clearly showed a correlation between diet, initial muscle glycogen levels, and endurance. In subjects consuming a high carbohydrate diet (i.e. 82% of kcal), muscle glycogen levels were six times greater compared to high fat diet, as determined by needle biopsy. Time to exhaustion on the high carbohydrate diet was nearly 200 minutes during submaximal exercise of 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. Karlsson and Saltin (1971) field tested this new "glycogen loading" theory and found that every subject ran his best time under carbohydrate loaded conditions. The subjects did not run faster initially, but rather maintained their normal pace longer during the 30 km run, thus suggesting the need for carbohydrate as a high intensity fuel source. A more recent study involving trained female cyclists further confirms the positive effect of carbohydrates on endurance (O'Keefe et al. 1989). Three separate isocaloric diets of 13%, 54%, and 72% carbohydrate resulted in times to exhaustion of 60, 98, and 113 minutes respectively, during cycle ergometry at 80% VO_2 max. It should be noted that these three studies (as well as the majority of other carbohydrate loading studies) used a relatively short adaption period, because investigators have shown endurance not to be impaired at workloads of 65% VO_2 max following adaptation to low carbohydrate diets (i.e. 20 gm/day) for one month (Phinney et al., 1983). This may be somewhat related to an increase in carnitine palmityl transferase activity in response to a chronic high fat, low carbohydrate diet (Fischer et al., 1983). 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. In fact, not all muscle fibers are equally depleted of

glycogen at exhaustion. Histological techniques, such as the periodic acid-Schiff (PAS) stain, have shown a selective depletion of slow twitch (ST) fibers during submaximal exercise ranging from 31-84% VO_2 max. At maximal and supramaximal intensities, glycogen depletion of fast twitch (FT) fibers predominates (Gollnick et al., 1974).

If 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 kcal) for three days following exhaustive depletion exercise, and thereafter for three days consume a high carbohydrate diet (i.e. 79% of kcal). This procedure, known as "supercomposition" or "glycogen loading", can increase muscle glycogen levels by 200-300% (Hultman et al., 1971). Due to the impracticality of this technique, 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 and amount of carbohydrate affects the synthesis of muscle glycogen. A diet consisting of simple sugars induces greater synthesis of glycogen than a diet of complex sugars during the first six hours after exhaustive exercise. However, there is no difference in glycogen synthesis between simple or complex sugars 20, 32, or 44 hours post-exhaustive exercise (Kiens et al., 1990). Current research suggests that the glycemic index of foods is a better determinant for glycogen re-synthesis (Burke et al., 1993). Burke et al. (1993) recommend foods with a high glycemic index following exhaustive exercise in order to optimally restore muscle glycogen. Interestingly, Zawadzki et al. (1992) found that a carbohydrate-protein complex restored muscle glycogen better than either protein or carbohydrate alone. They suggest this to be a result of an interaction between carbohydrate and protein on insulin secretion. Apparently, muscle glycogen synthesis predominates over liver glycogen synthesis during recovery from exercise (Ferrannini et al., 1985). As for the

liver, fructose may be better than glucose for replenishment of glycogen (Nilson and Hultman, 1974), possibly due to higher fructose kinase activity than glucose kinase activity in the liver (Heinz, 1972). There do appear to be differences, although minimal, in the total amount of carbohydrate ingested for optimal glycogen resynthesis during exercise recovery (Ivy et al., 1988b). More importantly, one should strive for early consumption of carbohydrate post-exercise, as it has been observed that glycogen re-synthesis in muscles is highest immediately after exercise and decreases in the subsequent hours (Ivy et al., 1988a). The single most important factor in supercomposition is exercise induced glycogen depletion (Evans and Hughes, 1985), which results in a marked elevation in glycogen synthase activity (Piehl, 1974).

Until now, it has not been determined why muscle glycogen depletion causes fatigue. What is it about glycogen that makes it essential for endurance, and why are free fatty acids, or amino acids not able to substitute for glycogen to prevent fatigue? Speculation about these questions has led to several theories. Most popular of these is the anaplerotic theory (Dohm et al., 1985; Gollnick, 1985). This theory suggests that glycogen provides glucose moieties that are subsequently metabolized to pyruvate. The pyruvate is then carboxylated to oxaloacetate, with the net effect being to continue the citric acid cycle. With glycogen depletion citric acid cycle intermediates decrease, which limits the processing of acetyl groups from beta oxidation, and the muscle cell ultimately fails to synthesize adequate amounts of ATP. From a mechanistic viewpoint, the theory appears plausible, however, it is yet to be proven. Based on rat studies (Aragon and Lowenstein, 1980), Conlee (1987) proposes that amino acids could likewise supply citric acid cycle intermediates just as readily as glycogen. If so, this would have obvious implications for amino acid supplements during endurance exercise.

Carbohydrate ingested in the immediate hours prior to exercise is readily oxidized by muscles (Rauvisson et al., 1979; Jandrain et al., 1984) and this is independent of pre-exercise muscle glycogen levels. Although carbohydrate feedings in the days prior to exercise

demonstrate positive effects, the intake of simple sugars within one-hour of activity may ultimately prove detrimental. Keller and Schwartzkopf (1984) found that 1.5 gm glucose/kg body weight given 60 minutes prior to exhaustive exercise at 85% VO_2 max decreased cycling performance by 25% in very highly trained runners. Possibly, this is due to glucose's action on insulin, which decreases plasma glucose and FFA, thereby placing a greater demand on muscle glycogen stores (Costill et al., 1977). Fructose ingestion appears to cause smaller perturbations on plasma glucose and insulin, but apparently it is no more effective at sparing muscle glycogen than glucose (Koivisto et al., 1985). At intensities ranging from 62-77% VO_2 max, however, there are numerous reports of positive effects on performance with simple sugar ingestion immediately prior to exercise (Devlin et al., 1986; Gleeson et al., 1986; Hargreaves et al., 1987; Okano et al., 1988; Wright and Sherman, 1989; Peden et al., 1989). Thomas et al. (1994) have observed that a low glycemic index (GI) food, such as lentils, eaten one hour prior to endurance exercise at 65% VO_2 max increases performance duration by 20 minutes relative to a high GI food such as potatoes. They suggest low GI foods provide a constant supply of glucose to muscles without compromising the release of FFA as would occur from a high GI food. It is currently recommended that pre-exercise feedings contain 1-5 grams of carbohydrate/kg body weight and should be consumed 1-4 hours before exercise for optimum performance (Sherman, 1991).

One of the most logical ways to spare glycogen during exercise is to provide an increased delivery of glucose to the muscle. As early as 1924, researchers found that when runners, who were susceptible to hypoglycemia, were supplemented with carbohydrate they performed better under race conditions from one year to the next, and were void of the previous year's symptoms of hypoglycemia (Levine et al., 1924). Christensen and Hansen (1939) fed men 200 grams of glucose at complete exhaustion and found that they could exercise for an additional 60 minutes. The glucose ingestion resulted in rapid increases in blood glucose and relief of neuroglucopenic symptoms. Carbohydrate ingestion during prolonged exercise has

repeatedly been demonstrated to enhance performance, even in persons not suffering from neuroglucopenia (Coyle et al., 1983, 1986; Coyle and Coggan, 1984; Coggan and Coyle, 1987, 1988, 1989). This may be because insulin responses to carbohydrate fed during exercise are supressed relative to the responses observed during very light activity or resting conditions. Generally, fluids are the preferred form of supplemental carbohydrate. Their osmolarity has a large impact on the rate of gastric emptying, regardless of whether the drinks contain glucose, fructose, or sucrose. With sugar concentrations as high as 150 mmol/L and exercise intensities between 70-75% VO_2 max, solutions will be delayed in the stomach (Costill and Saltin, 1974). Studies do suggest, however, that at least 50 grams of glucose can be emptied during one hour of activity (Costill, 1985). In conclusion, for carbohydrate feedings to be successful in delaying the time to exhaustion, the uptake and utilization of exogenous glucose by muscles must exceed the lost energy from a decrease in FFA oxidation.

Blood glucose provides an additional or alternate carbohydrate source to the working muscles, which can only be provided by the liver due to the lack of glucose-6-phosphatase in muscle. In the fasted condition at rest, there is limited uptake of plasma glucose by skeletal muscles. Most of the glucose produced by the liver is used for central nervous system function (Andres et al., 1956). During the early minutes of exercise, both blood glucose and glycogen serve as the primary energy sources. Early in exercise blood glucose uptake by muscles may increase by ten to twenty-fold above rest with a graphical representation showing a curvilinear relationship with exercise intensity (Wahren, 1971; Katz et al., 1986). During cycle ergometer exercise, glucose uptake by muscles increases from approximately 0.05 gm/min at rest to approximately 0.2, 0.4, and 0.7 gm/min after 40 minutes of exercise at intensities of approximately 25%, 50%, and 75% VO_2 max, respectively (Wahren, 1971). Most of this blood glucose is probably directly oxidized, thus accounting for roughly one-fourth to one-third of the total carbohydrate metabolized for energy during the early periods of exercise (Coggan, 1991). Rates of uptake as high as 1.4 gm/min have been observed at 100%

VO_2 max (Katz et al., 1986). Blood glucose utilization is not only related to intensity but to exercise duration as well. Ahlborg and Felig (1982) found that during cycle ergometry, glucose uptake by legs increased by 40% between 40 to 90 minutes of exercise at 60% VO_2 max. Rates of blood glucose utilization as high as 1.2 gm/min have been observed at the end of 67% VO_2 max exercise to fatigue (Broberg and Sahlin, 1989). The observed increases in blood glucose utilization with time are thought to be related to the steady decline in the rate of muscle glycogenolysis (Coggan, 1991; Hargreaves et al., 1992). Thus, late in exercise when glycogen is low, blood glucose may account for almost all the carbohydrate being oxidized (Coggan and Coyle, 1987, 1988; Broberg and Sahlin, 1989). Using the euglycemic clamp technique, Coggan and Coyle (1987) found that intravenously infused glucose accounted for 76% of the total carbohydrate oxidation at the end of prolonged exercise. These results further support the administration of carbohydrate supplements during endurance exercise events. Interestingly, glucose infusion rates of 2.0 gm/min (i.e. twice that of the normal rate of oxidation) appear not to have an effect on glycogen degradation at 73% VO_2 max (Coyle et al., 1991). It therefore is unlikely that adequate glycogen synthesis occurs from glucose infusions such that net glycogen breakdown is decreased. Liver glycogen depletion, as is seen during exercise, may lead to eventual hypoglycemia and this is often associated with fatigue. However, hypoglycemia should not be confused with muscle glycogen depletion. Non-invasive techniques (i.e. endurance performance times) of predicting true exhaustion can be a difficult and somewhat inaccurate process; therefore, muscle biopsies or plasma samples should be collected to obtain information on other possible causes of fatigue. Both carbohydrate sources do represent limited fuel stores, so it is logical to expect that an alternate fuel such as fat contributes significantly to the energy demands of working muscles during prolonged exercise.

Fats

Fats, like carbohydrates, are composed of carbon, hydrogen, and oxygen. However, fats differ in that about 90% of the molecule is carbon and hydrogen. Fats are predominantly triesters of fatty acids and glycerol, commonly referred to as triglycerides. More specifically, fats consist of a three carbon glycerol "backbone" esterified with long chain fatty acids. Following hydrolysis of the triglyceride by a lipase, the FFA are then readily utilizable by muscle fibers. Both the 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 separately. The fatty acids can vary in chain length as well as in their degree of saturation. Most fatty acids, which are important to fuel metabolism, contain between 12-18 total carbons. Palmitic acid (16:0) represents a good indicator of all the the FFA utilization in plasma (Havel et al., 1964).

The total amount of fat stored in the body varies widely. It may range from 3-5% in extremely lean male endurance athletes to in excess of 50% in severely obese individuals. On average, males are betwen 15-25% body fat, while females are between 25-35%. 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. The primary form of adipose fat is triglyceride, which accounts for more than 95% of the total fat storage in man. Triglycerides are also found within muscle cells at concentrations ranging from 7-25 umol/gm wet weight, with highly oxidative fibers possessing more stored lipid than low oxidative fibers (Saltin and Gollnick, 1983). Compared to muscle glycogen this represents a small amount of stored fuel.

The energy derived for muscle contraction during moderate intensity exercise shifts with time from mostly carbohydrate at the onset to predominantly fats near exhaustion. One of the primary reasons for this is that there is a considerable time lag between the onset of exercise and lipolysis of triglyceride in adipose tissue, and thus release of FFA into plasma. This slow increase in plasma FFA comes from the fact that lipolysis in adipose tissue is regulated by the

sympathetic nervous system and that diffusion barriers exist at several sites of the system. Normally, plasma FFA show a significant increase in the blood between 20-30 minutes into exercise (Havel et al., 1959; Pruett, 1970; Carlson et al., 1971; Pernow and Saltin, 1971). Ahlborg et al. (1974) found that from one to four hours of prolonged exercise, FFA uptake into the muscles increased about 70%, and fats supplied 80% of the total energy requirement during this period. The hormonal effects of decreased insulin and increased glucagon, which accompany lowered blood glucose during endurance exercise, probably stimulated this increase in fat mobilization and subsequent metabolism of fats (Saudek and Felig, 1976). It appears that mobilization from adipose tissue is a key factor to fat oxidation, because plasma FFA concentrations are related to utilization (Havel et al., 1966). High plasma FFA levels signify greater utilization, and conversely low plasma concentrations represent a decreased usage of FFA for fuel. Interestingly though, no more than 2-4% of the FFAs delivered to the muscle are actually taken up by the muscle, and only part of those are oxidized (Saltin and Astrand, 1993). Thus uptake along with mobilization are key factors in the metabolism of FFA.

Some 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 of FFA) 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 complement the fuel mixture so that adequate energy substrates are provided 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 individuals (Holloszy and Coyle, 1984). A major problem which arises with complete carbohydrate depletion is that citric acid cycle intermediates are needed to continue oxidative metabolism, and it is via carbohydrates 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 relative lack of oxaloacetate can have a retarding effect on fat metabolism (Gollnick, 1985).

Currently there is debate as to the role that intramuscular triglyceride stores play in the total fuel utilization during exercise. This in part is due to the regional variation of triglyceride deposits that exist both within and around muscle fibers. Taking this into account and thus sampling relatively large pieces of muscle tissue, it was observed that intramuscular triglyceride provided only about 5% of the total energy during exercise (Froberg and Mossfeldt, 1971). Hurley et al. (1986) have shown there to be lower plasma FFA and glycerol concentrations as a result of endurance training, suggesting decreased fat availability. However, their respiratory exchange data revealed that overall fat utilization increased from 35% to 57% of the total exercise energy expenditure. In addition, muscle triglyceride concentration after exercise decreased roughly two-fold with training, and this difference correlated with the total increase in fat lipolysis. These findings suggest that dietary manipulation to increase muscle triglyceride may prove beneficial to endurance performance. Interestingly, Muoio et al. (1994) found that subjects' endurance performance times were 91 and 76 minutes while consuming isocaloric diets of 38% and 15% total fat calories, respectively. In addition, they found that VO_2 max values were on the order of seven ml/kg/min higher on the high fat diet compared to the high carbohydrate diet. They attributed these findings to be a result of increased muscular triglyceride through dietary manipulation.

Certain food items, namely caffeinated beverages, have been demonstrated to have positive effects on fat utilization and endurance performance. When the equivalent of two to three cups of brewed coffee (i.e. 330 mg of caffeine) were consumed one hour prior to moderately strenuous 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, resulting in increased cAMP levels and epinephrine (Arogyasami et al., 1989). It should be noted that not all studies involving caffeine, endurance performance, and fuel utilization 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/Amino Acids

Proteins, like carbohydrates and fats, are composed of carbon, hydrogen and, oxygen. However, they are unique in that they also contain nitrogen as a primary element of their structure. 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. The metabolism of branched chain amino acids (BCAA) is somewhat unique compared to the other amino acids. Initially, the BCAA are reversibly transaminated in the muscle to form branched chain keto acids via a PLP-requiring transaminase enzyme. Next a complex dehydrogenase irreversibly decarboxylates the keto acids and finally after several steps CoA derivatives enter the TCA cycle to form energy.

Based primarily on urea nitrogen excretion in urine and sweat, amino acids (i.e. proteins) are not believed to supply more than 5-15% of the total energy output during exercise (Lemon and Mullin, 1980). However, factors such as high intensity exercise, long duration exercise, chronic training, and decreased carbohydrate availability promote increased amino acid

oxidation (Lemon, 1987). Proteins are involved in exercise in: (1) enzymatic pathways to produce ATP, (2) oxygen delivery, (3) muscle formation and contraction, and (4) tissue repair.

Although eating three times the RDA 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 current RDA of 0.8 gm/kg body weight. The basis for this statement comes from a study involving well trained runners (Friedman and Lemon, 1989). When subjects consumed the RDA for protein, nitrogen balance was negative (i.e. -5.29 $\pm$ 2.58 gm/day) during an exercise test day. However, following a diet 70% higher in protein, retention of nitrogen was observed (i.e. 2.41 $\pm$ 1.99 gm/day). 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).

Of the 20 alpha-amino acids in the body, only the BCAA (leucine, isoleucine, and valine), alanine, glutamate and aspartate are oxidized at a significant rate in skeletal muscle (Golberg and Odessey, 1972). Quantitatively alanine is oxidized at the highest rate. The gluconeogenic precursor, alanine, which is actively converted to pyruvate (PLP-dependent) and further to glucose, increases more than five-fold in the plasma after strenuous exercise compared to a resting condition. In total, this alanine glucose cycle can supply as much as 45 % of the hepatic glucose output and 10-15% of the total energy requirement during four hours of continuous exercise (Felig and Wahren, 1971). During exercise, protein synthesis is believed to be depressed (Dohm et al, 1980). This allows movement of amino acids, mostly in the form of alanine, from muscle to the liver where gluconeogenesis increases. Approximately 80% of the free amino acids in the body pool are found within skeletal muscle with 0.2-6.0% being contained in the plasma (Poortmans, 1984). Therefore, the increase in plasma concentrations of amino acids is most likely from muscle release of amino acids. Amino acids can be thought

of as "time released glucose". Exercise lasting longer than two hours is thought to partially deplete the amino acid pool within the body. Several studies (Harlambie and Berg, 1976; Decombaz et al., 1979; Refsum et al., 1979; Rennie et al., 1981), which examined plasma amino acids before and after extremely long duration exercise (i.e. 100 km run), showed amino acid levels to decrease by up to 33% at post-exercise. In such a case, alanine content in the blood no longer reflects the transamination of glucose-derived pyruvate (Poortmans, 1984).

In addition to alanine the BCAA are available for oxidation (White and Brooks, 1981; Lemon et al., 1982). In the muscle BCAA provide 18% of the amino acid residues of muscle. These particular amino acids can contribute significantly to energy production during exercise, as one mole of leucine, isoleucine, and valine yields between 32-43 moles of ATP. In contrast to alanine, which increased three-fold in arterial concentration during exercise compared to rest, Ahlborg et al. (1974) showed there to be a net uptake of the BCAA into the muscle during four hours of moderate intensity exercise in men. Odessey et al. (1974) have proposed a mechanism which would help explain these results. They have suggested that the BCAA act as nitrogen donors to form alanine from pyruvate and they have termed this the "branched-chain amino acid-alanine cycle". The BCAA most likely work in a similar fashion with glutamine, since approximately 60-80% of the total amino acid nitrogen is transported via muscle release of alanine and glutamine combined (Felig, 1981). Previously, it was believed that the liver was the primary site of amino acid degradation in mammals (Shinnick and Harper, 1976). However, subsequent research has demonstrated that non-hepatic tissues (i.e. muscle) can oxidize amino acids, especially the BCAA (Dohm et al., 1977).

In experiments where there was adequate exercise intensities and durations, Kasperek and Snider (1987) have documented an increased activity of branched-chain ketoacid dehydrogenase within skeletal muscle. This would clearly have an effect to enhance muscle oxidation of the BCAA during exercise. Of the BCAA, leucine has been most extensively researched because its oxidation rate is quantitatively the highest (White and Brooks, 1981;

Rennie et al., 1981; Hagg et al., 1982; Lemon et al., 1982; Wolfe et al., 1982) and because it is closely associated with the release of alanine from muscle (Odessey et al., 1974). As a result of leucine's high oxidation rate during exercise, the dietary recommendations for leucine are likely to be inadequate (Hood and Terjung, 1990). For adults, Young and Bier (1987) have suggested that the recommended dietary intake (RDI) for leucine be increased from the present 14 mg/kg/day to 30 mg/kg/day in order to optimize rates of whole body protein synthesis. Intakes of 1.61 gm/kg/day of protein for a 70 kg individual would easily satisfy the leucine requirement.

Gluconeogenesis is not restricted to alanine and the BCAA. Virtually all amino acids can potentially be converted to TCA cycle intermediates. Other amino acids, primarily glutamic acid and arginine, are glucogenic. In addition, glycine can be converted to acetyl CoA, which is the two carbon product that initially enters the TCA cycle. Similar to alanine, glutamine shows a linear increase in the plasma with increasing exercise intensities (Babij et al., 1983). However, Babij et al. (1983) believed that the rise in glutamine is used primarily as an "ammonia sink", not as a fuel source.

Although aspartate can be oxidized in muscle (Goldberg and Odessey, 1972), its primary role in energy metabolism is likely to be as an amide donor for the reamination of inosine monophospate in the purine nucleotide cycle (Terjung et al., 1986). This cycle functions to replenish cellular ATP levels. Aspartate can be formed via transaminations of the BCAA, which further emphasizes the importance of the BCAA in energy metabolism during exercise.

Plasma amino acids are believed to directly affect the physiology of the central nervous system, whereby they may have a strong influence on mental/central fatigue during exercise. In particular, the concentration of tryptophan in circulation relative to other amino acids can influence neurotransmitter levels within the brain (Fernstrom and Wurtman, 1971). Via the 5-hydroxyindole pathway, L-tryptophan is ultimately converted to 5-hydroxytryptamine

(serotonin). Since L-tryptophan and other large neutral amino acids (LNAA) (i.e. BCAA, tyrosine, phenylalanine) all utilize the L-system for uptake across the blood brain barrier (BBB), serotonin synthesis is highly dependent upon the ratios of tryptophan:LNAA with higher ratios resulting in higher serotonin synthesis. These ratios are primarily susceptible to change by diet (Fernstrom and Faller, 1978), and in addition they are manipulatable by exercise (Blomstrand et al., 1988; Blomstrand et al., 1989; Blomstrand et al., 1991). Serotonergic neurons are involved in many aspects of behavior (Wurtman et al., 1981). However, exercise physiologists have formed their hypotheses from the clear relation that serotonin induces sleep (Griffiths et al., 1972; Hartman and Spinwever, 1979; Leathwood and Pollet, 1984; Nedopil et al., 1984; Demisch et al., 1987). The rationale is that increased serotonin levels within the brain result in central fatigue (i.e. sleepiness/tiredness). Clearly, if one is sleepy or tired, exercise would be a difficult undertaking. Over the past six years, Blomstrand, Newsholme, and co-workers have done the majority of the research in the area of plasma amino acid concentrations and their possible role in central fatigue. Their work (Blomstrand et al., 1988; Blomstrand et al., 1989; Blomstrand et al., 1991) can be summarized as follows: (1) the ratio of tryptophan:BCAA increases during endurance exercise, most likely due to an uptake of BCAA from blood by muscles, (2) in rats tryptophan levels increase within the brain, which suggests that an increase in 5-HT results, and (3) when runners are given BCAA supplements during a marathon, (which competitively decreases the uptake of tryptophan across the BBB and thus depresses 5-HT levels in the brain), their performance is enhanced. Until now, the evidence for plasma amino acid changes resulting in central fatigue has been inconclusive. The research has, in part, been based on other indirect studies (i.e. sleep, pain, cognitive research). In addition, in a double blind design Segura and Ventura (1988) observed that subjects were in fact able to exercise 49% longer following L-tryptophan supplementation, which theoretically should have elevated brain serotonin concentrations. They suggested that elevated brain

serotonin had an analgesic effect, thus allowing an increased pain tolerance during exercise. More research is obviously necessary in this area to draw definitive conclusions.

Lactic Acid

Lactic acid (lactate), a byproduct of the Embden-Meyerhof pathway (glycolysis), is often mistakenly considered a waste product of exercise. However, it actually serves as an important energy source. Because lactic acid is a strong organic acid, at physiological pH values it will readily dissociate to a proton (H^+) and an anion ($C_3H_6O_3$-). Both the proton and anion exist separately during exercise and perhaps ultimately take different pathways of metabolism (Stainsby and Brooks, 1990).

Two relatively recent hypotheses, The Glucose Paradox (Newgard et al., 1983; Foster, 1984) and The Lactic Acid Shuttle (Brooks, 1985; 1987) were developed to help explain lactate's role in energy metabolism. 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). Thus a given plasma lactate concentration is simply the net balance between the formation and removal of lactate within tissues. Epinephrine has been shown to increase net lactate output transiently, and in addition to reduce lactate removal in particular tissues (Stainsby and Brooks, 1990). 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 mmol/L 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 person's maximal capacity and, depending on the level of training, higher maximal 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. By the usage of radioactive tracers, Stanley et al. (1986) have shown the same phenomenon to occur in trained humans. At the point when lactate 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. In addition to training, lactate levels can be influenced by diet. Compared to low carbohydrate diets, high carbohydrate diets result in increased 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; Donovan and Brooks, 1983). By vascular shuttling of lactic acid, muscles depleted of glycogen can be restored during recovery after strenuous 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. 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. Also, 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. In summary, evidence to date strongly suggests that lactate is an important fuel source during 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 further degraded to pyruvic acid. Pyruvate then proceeds through the TCA cycle, and in total 22 ATP can be formed aerobically 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 ten-fold 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. 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 separate 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

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 (min); Int- Intensity as a % of VO_2 max; DC-Dietary conditions; fast = fasting; fed = non-fasting; Conc-Concentrations of substrates in mmol/L; Pre-before exericise; During-60 minutes into exercise; Post-immediately after exercise; Post 30 and 60-30 and 60 minutes after exercise

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 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 increased throughout the duration of exercise in each study described, except in the study of 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 increased from pre-exercise to 60 minutes into exercise and from 60 minutes to the end of exercise. Plasma levels of glycerol decreased in recovery. Similar to the levels of glucose, there were variable results for FFA levels. 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.

Listed in Table 5 is a quantitative summary of normal plasma amino acids along with the results of a single study (Bazzare et al., 1992), which utilized an endurance-exhaustive exercise protocol to study changes in plasma amino acid levels. The table shows that large decreases, ranging from 13-38%, in plasma amino acids occured following exercise for alanine, glycine, valine, threonine, leucine, histidine, serine, lysine, and isoleucine. All other amino acids with

Table 5. Normal plasma amino acid concentrations and the effects of endurance exercise

Amino Acid	[1]Normal (umol/L)	[2]Baseline (umol/L)	[2]Exhaustion (umol/L)
Alanine	419	398	283
Arginine	99	121	109
Aspartate	13	10	12
Cystine	-	19	18
Glutamate	40	32	28
Glycine	303	278	213
Histidine	104	61	38
Isoleucine	67	64	53
Leucine	144	124	105
Lysine	212	159	139
Methionine	29	42	42
Phenylalanine	69	57	60
Serine	153	111	98
Threonine	172	197	122
Tyrosine	97	63	68
Valine	271	220	175

[1]Data from Smith and Rennie, (1990)
[2]Data from Bazzarre et al., (1992); 22 trained males; 90 min treadmill run plus 90 min cycle ergometer; 70% VO_2 max; Fasting condition

the exception of tyrosine, which increased slightly, showed either no change or decreased by less than 10% during the exercise trial.

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 creatine phosphate (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. Knowledge of carbohydrate's role in intense exercise dates back to the previous century. Measurements of R values 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 (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 values, which is indicative of greater reliance on fat. Edwards et al. (1934) showed that while running 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 initial value of 90% to roughly 20% of the total energy expenditure. 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. 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 sparing carbohydrate (which is presumably at much higher levels), a trained individual can sustain a constant workload longer than if he/she were sedentary. It has been shown that 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). This may be somewhat related to a trained muscle's ability to store higher amounts of triglyceride relative to untrained muscles (Reitman et al., 1973).

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 a greater length of time for gas exchange of O_2 and CO_2 to occur. 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. Thus with training, R values are lower, muscle glycogenolysis is less, lactate accumulation is less, and fatty acid oxidation is greater compared to pre-trained conditions.

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 RER 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 three-fold and muscle glycogen utilization decreased four-fold during exercise after a four week ketogenic diet compared to a diet higher in carbohydrate. In relation to the micronutrients, niacin given in pharmacologic doses (i.e. 1-3 gm/day) appears to alter fuel utilization by stimulating carbohydrate metabolism and suppressing fat metabolism (Heath et al., 1993).

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). In general, fasting promotes the utilization of fats and decreases the utilization of carbohydrates compared to a fed state (Maughan and Williams, 1981; Loy et al., 1986). With the aid of naturally occuring ^{13}C-glucose isotopes, an estimation of exogenous glucose metabolism can be made. Laughlin and Armstrong (1983) found that ingestion of 100 grams of glucose resulted in 88% of the total amount of glucose being oxidized during four hours of exercise at 45% VO_2 max. Others have 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) provided evidence that males and females possibly rely on a different proportion of 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

An extensive amount of research has 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; Weight et al., 1988); 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) relating 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 was 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), and does not appear to be related to exercise intensity or duration (Sampson et al., 1993). 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, 1988b) has proposed an interesting mechanism and rational for the release of PLP from muscle during exercise. 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 PLP concentrations in response to water 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 led to changes in plasma PLP, the next line of focus shifted to fuel utilization. Less 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/day) 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 minutes. 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., 1975; Leklem, 1985). In addition, with high carbohydrate diets lactate levels are higher compared to low carbohydrate diets (Bergstrom, 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) primarily studied plasma urea and ammonia as they related to vitamin B-6 and exercise. However, she 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.

The latest in a series of diet and exercise experiments at OSU (Virk et al., 1992), which directly examined the possibility of vitamin B-6 as a "fuel modifier", presented inconclusive evidence as to whether the vitamin does in fact stimulate carbohydrate metabolism. In contrast to the prestudy hypothesis, it was found that during the exhaustive exercise trial (on a cycle ergometer) following a vitamin B-6 supplementation phase, the mean plasma lactic acid concentrations were actually lower compared to a non-supplemented condition. In addition, post-exercise mean plasma glucose concentrations remained virtually at pre-exercise levels during the vitamin B-6 supplemented ride, whereas a decline in plasma glucose was observed in the non-supplemented test. The results led the researchers to speculate that early in exercise vitamin B-6 was exerting its influence on glycogenolysis, but later in exercise vitamin

B-6 was possibly playing a greater role in gluconeogenesis to maintain plasma glucose levels as body carbohydrate stores were becoming depleted. 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., 1975). 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 (i.e. 42%) lactic acid levels. Unfortunately, no explanation was provided for the elevated lactic acid levels in the vitamin B-6 group.

Years later, Marconi et al. (1982) tested the effects of a supplemental alpha-ketoglutarate-pyridoxine complex on human maximal aerobic and anaerobic performance. The supplement consisted of 30 mg/kg body weight with a stoichiometric ratio of 46.35 alpha-ketoglutarate:53.65 PN (i.e. approximately one gm PN/day). At the end of the 30 day treatment period, subjects were administered a VO_2 max test and a test for anaerobic glycolytic metabolism. Interestingly, there was a statistically significant 5.8% increase in VO_2 max following supplementation. In addition, plasma lactates were statistically lower during the treatment condition compared to the placebo trial. Neither alpha-ketoglutarate nor PN administered separately produced similar results. It was hypothesized that these findings might

be a consequence of alpha-ketoglutarate enhancing the flow of reducing equivalents across the mitochondrial membrane by means of the malate-oxaloacetate shuttle, thus increasing VO_2 max and decreasing anaerobic glycolysis. Secondly, increased alpha-ketoglutarate and glutamate had been shown by others to activate the transamination of pyruvate to alanine, thereby enhancing a "non-glycolytic anaerobic pathway". This might account for part of the observed decrease in lactate during the treatment test compared to the placebo test. The benefit of taking the complex versus each constituent separately is a more effective reabsorption of alpha-ketoglutarate across the mitochondrial membrane (i.e. PN enhances alpha-ketoglutarate uptake into the mitochondria). From a performance standpoint, these results obviously have beneficial endurance effects for short supramaximal work loads; and, theoretically if the above hypotheses are in fact correct, then supplemental alpha-ketoglutarate-pyridoxine should likewise have positive effects on submaximal endurance activities. However, this remains to be tested.

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 consuming the same diet as the active group (i.e. 4.2 mg B-6/day). 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 (i.e. 30-40% of the ingested load) compared to the untrained subjects (i.e. 41-58% of the ingested load) led 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 vitamin's role as a possible fuel modifier. After reviewing the literature, it appears that most of the research has 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, 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 male 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. The tests were separated by approximately two weeks. For six days before and three 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 and circulating hormones), blood and urine samples were collected and respiratory gases were analyzed. Plasma was analyzed for glucose, lactic acid, glycerol, FFA, and amino acids.

Subjects

Five, healthy, trained, male cyclists were recruited from the OSU campus and the local community by a flier and by word of mouth to participate in the study. The study was approved by the OSU Human Subjects Committee. Training was defined as 180 minutes of aerobic activity (i.e. running, biking, swimming) per week, in 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. A pre-study questionnaire and a brief interview were used to evaluate training status. 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 ECG at rest and at maximal heart rate

4. Normal blood pressure at rest (i.e. 120/80) and with exercise
5. No vitamin supplements used for at least four weeks prior to starting the study (evaluated from a pre-study questionnaire and a brief interview)
6. No nicotine for at least one year or use of drugs known to interfere with vitamin B-6 and hormone metabolism or methodology
7. Sign an informed consent
8. Undertake a max VO_2 test to assess fitness level
9. Undertake an orientation/practice ride to determine 75% of VO_2 max.

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).

Exercise Testing

All exercise testing was carried out in the Human Performance Laboratory located in the Womens Building on the OSU campus using the the same cycle ergometer (Monark, Quinton Instruments). A total of four exercise tests per subject were conducted. These consisted of a VO_2 max test, an orientation ride, and two endurance rides. The VO_2 max test protocol consisted of increasing the workload in 30 watt increments until each subject showed a plateau in oxygen consumption along with an R value of greater than 1.1 and a maximum heart rate of greater than 90% of the predicted maximum or until each subject requested to stop the test. An average of the three highest oxygen consumption values obtained during the max test was used to set subsequent workloads during the orientation ride. In this session, which lasted

about 20 minutes, the workload which corresponded to 75 $\pm$ 1% VO_2 max was determined. On the morning of the seventh day of each dietary period, the cyclists exercised to exhaustion at 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 the test, heart rate was monitored by an ECG using three limb leads (Quinton Instruments, Model 630 A). Respiratory gases were collected for three minutes at 10-minute intervals to calculate R values and measure oxygen consumption (Sensor Medics Metabolic Cart Model 2900). Only 100 ml of water was given to subjects while cycling (at 60 minutes into exercise). In addition, after the post-blood draw 100 ml of water was given to subjects. 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 asked of subjects every 10 minutes, 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. 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 composed of 60% carbohydrate, 23% fat, and 17% protein (approximately 3700 total kcal). See Table 6 for a list of the diet. As mentioned in Table 6, subjects were allowed certain foods ad libitum. These were used primarily to adjust for each individual's variation in caloric need, as determined by appetite and daily weight monitoring. 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 (Miller and Edwards, 1981).

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

Breakfast:	220 gm 1% milk
	200 gm orange juice, reconstituted in water
	60 gm whole wheat bread
	60 gm Life Cereal™
	50 gm raisins
Lunch: sandwich:	60 gm whole wheat bread
	60 gm tuna, water packed
	35 gm lo-cal mayonnaise
	20 gm egg white, cooked
	15 gm dill pickle
	10 gm iceberg lettuce
	240 gm apple juice
	200 gm pears, canned, light syrup
	70 gm raw carrot sticks
	12 gm gelatin mixed in 240 gm Koolade™ (reconstituted)
	10 ea vanilla wafers (50 gm)
Dinner: salad:	80 gm iceberg lettuce
	50 gm red kidney beans, canned
	50 gm cheddar cheese
	15 gm red cabbage
	10 gm raw carrot, grated
	220 gm 1% milk
	200 gm green beans, canned
	200 gm peaches, canned, light syrup
	120 gm low-fat frozen yogurt, vanilla
	110 gm turkey breast
	70 gm white rice, dry
	30 gm whole wheat bread
	12 gm gelatin mixed in 240 gm Koolade™ (reconstituted)
Snack:	200 gm grape juice
	12 ea graham crackers (2 packets)

Free foods: selected candies, soda pop, coffee, tea, French salad dressing, margarine, jelly

Phase one was supplemented with a placebo capsule, while phase two was supplemented with an additional 20 mg PN/day 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 RDA's. When possible, all foods were purchased from the same lot, and careful preparation by means of weighing foods to $\pm$ 0.1 gm assured consistency within and between the diets of all subjects. Alcoholic beverages were prohibited throughout the study, and caffeine was not allowed on the day before, the day of, or the day after 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, Salem, OR).

Blood and Urine Collections

Blood was drawn in the week prior to the start of the experiment, on the two exercise test days, and once during the vitamin B-6 supplementation phase midway between the two dietary phases by a registered medical technologist. The mid-study non-exercising blood sample provided blood data on vitamin B-6 status. 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 was taken 30-40 minutes prior to exercise after the subject had rested for 10-15 minutes. The second sample was taken 5-10 minutes prior to starting exercise. The third sample was taken 60 minutes into exercise. During this time the workload was decreased slightly to allow for a safe blood draw. The fourth and fifth samples occurred in the exercise recovery phase at immediately post-exercise and post-60 minutes of exercise, respectively. Heparinized blood tubes were used for collection, and all samples were kept on ice approximately 5-10 minutes until centrifuged. After extraction of the plasma portion, the separately aliquoted samples were frozen at either -40°C or -80°C. Plasma for amino acid analysis was treated with sulphosalicylic acid prior to freezing at -80°C.

Urine was collected daily for each of the two diet periods. 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 subject's daily urine sample.

Risks/Benefits

Prior to max testing all subjects were screened to aid in the 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 $ 50.00 from OSU. In addition, all data compiled on individual blood chemistry values, body composition, and exercise information were provided free of charge. Each participant also received 18 days of well balanced meals at no cost.

Analyses

Plasma glucose was measured by the glucose oxidase method (Trinder, 1969) using a Technicon Autoanalyzer System II (Alpkem). All samples were done in duplicate. The intraassay ($n=7$) coefficient of variation (CV) for the assay was 2.3% for control samples ($n=7$).

Plasma lactic acid was measured spectrophotometrically (Henry, 1968) by means of a Beckman DU 40 spectrophotometer. A kit from Sigma Chemical Co. was used (Procedure No. 726-UV/826-UV). All samples were done in duplicate. The intra ($n=2$) and interassay ($n=3$) CVs were $<1\%$ and 1.8%, respectively, for control samples.

Plasma glycerol was measured by a modified enzymatic method (Pinter et al., 1967) for triglycerides by omitting the saponification step. A kit from Sigma Chemical Co. was used (Procedure No. 320-UV). All samples were done in duplicate using a Beckman spectrophotometer. The intra (n=2) and interassay (n=4) CVs were 4.7% and 14.4%, respectively, for control samples.

Plasma FFA were measured by a colorimetric method (Falholt et al., 1973). Following extraction of FFA by chloroform-heptane-methanol and phosphate buffer, the copper soaps of FFA were determined colorimetrically with diphenylcarbazide and a concentration was determined directly from palmitic acid standards. Most important to this procedure is the vortexing of the initial extraction mixture with copper triethanolamine (CuTEA). This requires vortexing the mixture extremely vigorously until the polar CuTEA is dispersed within the non-polar extraction solvent. Visually, when vortexing is complete, the mixture resembles hundreds of tiny micelles floating within a gel-like medium. In general, mixing was complete within 20-30 seconds, however, upon occasion several minutes were required. All samples were done in triplicate using a Beckman spectrophotometer. The intra (n=6,6,3) and interassay (n=3) CVs were 4.4% and 14.5%, respectively, for control samples.

Amino acids were determined by HPLC (Rosenlund, 1990). Plasma was deproteinized by mixing an equal volume of 6% sulfosalycilic acid with plasma, vortexing for 10 seconds, allowing the mixture to stand in an ice bath for 10 minutes, and centrifuging at high speed for 15 minutes. The supernatant was stored at -80°C. Derivatization was carried out by mixing fresh (daily) ethanol-triethylamine-phenylisothiocyanate (PITC Pierce Chem., 80 uL) and one mmol/L norleucine (40 uL), an internal standard, with either deproteinized plasma (40 uL) or Standard H (40 uL). A Standard H amino acid mixture (Pierce Chemical Co.) contained the following L-amino acids at a concentration of 2.5 mmol/L: Ala, Arg, Asp, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Tyr, Val, and Cys (1.25 mmol/L). Derivatized samples were hard frozen in liquid N_2 and lyophilyzed overnight. Freeze dried samples were reconstituted in

H_2O and 20 uL of sample were eluted on a Spherex (Phenomenex Columns) 5u C8 column (250 x 4.6 mm) at a column temperature of 45°C. The mobile phase consisted of eluent A, aqueous sodium acetate (3.28 g anhydrous) with dilute phosphoric acid (0.4 ml of a 1:10 dilution of concentrated acid) in 500 ml H_2O, and eluent B, a mixture of eluent A-acetonitrile-methanol (200:150:50). The pH of buffer A was 6.5 and the pH of buffer B was 7.5. Both buffers were degassed prior to utilization. A linear gradient was used, which consisted of changing the concentration of eluent B from 10% to 100% and back to 10% over a period of 45 minutes. Gradient details follow:

Time	Buffer B Concentration
0.01	10
20.0	50
25.0	50
27.0	100
35.0	100
37.0	10
45.0	10

The flow rate was 1.0 ml/min and the detection wavelength was set at 254 nm (UV). All samples were run on a Shimadzu HPLC: LC-10AD solvent delivery module; SIL-10A autoinjector; SPD-10A uv-vis detector; SCL-10 system controller; CR5-1 chromatopac integrator. Calculations were done as follows:

$$\text{Concentration} = \frac{\text{pk ht of sample amino acid} \times 2 \times .25\ \text{mmol/L} \times 1000}{\text{pk ht of std H amino acid}}$$

$$= \text{umol/L}$$

Note: The number two is the dilution factor in the concentration calculation.

Norleucine was used to correct concentrations. Only one sample per person was done in duplicate. The intra (n=5,3) and interassay (n=2) CVs for each separate amino acid eluted are listed:

Amino acid	Intraassay CV (%)	Interassay CV (%)
Serine	4.9	1.8
Threonine	2.5	4.3
Alanine	4.6	2.9
Proline	1.9	1.1
Histidine	2.6	1.6
Arginine	3.2	1.7
Tyrosine	3.6	3.6
Valine	4.1	3.3
Methionine	5.7	1.4
Isoleucine	5.8	4.3
Leucine	4.8	5.0
Phenylalanine	6.5	7.7

Tryptophan was measured fluorometrically (Bloxam and Wharen, 1974). Thirty uL of plasma were deproteinized with three ml of 10% TCA. The supernatant was collected and to this was added 2% formaldehyde and 6 x 10^{-3} M $FeCl_3$. This was mixed and heated in a stoppered tube for 60 minutes in a boiling water bath. Samples were cooled and read at an excitation wavelength of 373 nm and an emission wavelength of 452 nm. Standards were prepared by a 1:50 dilution of a stock tryptophan standard (1 umol/ml in 0.1 N NH_4OH) in 0.1 N NH_4OH. The standard curve was obtained from samples containing 0-2.0 nmols tryptophan/tube. All samples were done in triplicate. The intra (n=6) and interassay (n=3) CVs were 2.9% and 5.8%, respectively, for control samples.

Computerized R analysis of expired air during exercise was used to assess 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 on an absolute and relative basis.

Hemoglobin and hematocrit were measured by microhematocrit and cyanomethemoglobin methods, respectively. Hemoglobin was done in triplicate and hematocrit was done in duplicate. Plasma volume changes were measured by using hemoglobin and hematocrit data from two separate blood samples and applying the equations of Dill and Costill (1974) and Van Beaumont (1972).

Creatinine was determined by an automated procedure (Pino et al., 1965) for assessing compliance of subjects urine collections. The intraassay CV for the assay was 2.8% for control samples ($n = 10$).

Urea nitrogen was determined by an automated procedure (Henry, 1968) for assessing compliance of subject's urine collections.

Statistics

The data were analyzed by standard statistical methods (Statgraphics Software): 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 for the paired t-test. A 95% confidence interval (CI) using the least significant difference (LSD) method was used for ANOVA. In the Results section, if a difference occurs between the tests, a p-value will be given. However, if changes occur between time points recall that a 95% CI was used.

RESULTS

To better help understand the following results, recall that blood samples were obtained at the following time points: Pre-1 (30-40 minutes prior to exercise), Pre-2 (5-10 minutes prior to exercise), during-exercise (60 minutes into exercise), post-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. test1 = non-supplemented control test; test2 = vitamin B-6 supplemented experimental test). In test1, the mean exercise time to exhaustion was 1:34:49: (hr:min:sec), and in test2 the mean times to exhaustion were 1:31:56. See Table 7 for a description of subject characteristics. The table shows that subjects were relatively well trained and lean with a mean relative maximal oxygen uptake of 54.7 ml/kg/min and a mean body fat percentage of 13%. See Table A.24. for individual plasma PLP concentrations.

Plasma Volume Changes

Listed in Table 8 are the mean plasma volume changes and standard deviations for the exercise tests. Table 9 lists the mean hematocrit and hemoglobin values which were used to calculate the plasma volume changes. There were no significant differences between the two tests at any of the specific time points. However, there were statistically significant changes in mean plasma volume over time for both tests. In general, there appeared to be a larger mean decrease in plasma volume during test2 compared to test1 at all time points with both the Dill and Costill and the Van Beaumont methods. A comparison of the two methods showed that the Dill and Costill method produced plasma volume changes between 2.2% to 4.1% more negative than the Van Beaumont method. However, both methods produced identical relative responses in plasma volume changes over time. In test1, the mean post-exercise values were significantly more negative than the baseline pre-exercise values, and in test2, both the during and post-exercise values were significantly more negative than the baseline pre-exercise values.

Table 7. Subject Characteristics

Age	29±7
Body Weight (kg)	83±12
Height (cm)	185±6
Body Fat (%)	13±7
VO_2 max (L/min)	4.5±0.3
(ml/kg/min)	54.7±6.2

Mean±SD; n=5
Body weights depict data from body composition measures (mid-study)
Body fat percentages were measured hydrostatically (mid-study)
VO_2 max was determined on a cycle ergometer (pre-study)

Table 8. Mean plasma volume changes: effects of vitamin B-6 supplementation and exercise to exhaustion

		Method	
		Dill and Costill	Van Beaumont
		% Change	
Test 1	During	-9.0±3.4	-6.9±4.4
	Post	-11.3±6.9	-7.7±8.8
	Post-60	-3.4±5.8	0.7±7.9
Test 2 (+B6)	During	-13.9±2.0	-10.3±2.6
	Post	-16.1±5.8	-13.9±6.5
	Post-60	-4.7±4.5	-2.2±4.4

Mean±SD relative to pre exercise; n=5 except During n=3
Test 1 refers to the non-supplemented exhaustive endurance ride
Test 2 refers to the vitamin B-6 supplemented exhaustive endurance ride
Dill and Costill (1974) and Van Beaumont (1972) refer to separate methods for determining % change
During: 60 minutes into exercise; Post: immediately after exercise; Post-60: 60 minutes after exercise

Table 9. Mean hematocrit (Hct) and hemoglobin (Hgb): effects of vitamin B-6 supplementation and exercise to exhaustion

	Pre1	Pre2	During	Post	Post-60
Hct (%)					
Test 1	44.5±3.1	44.9±3.2	47.6±3.1	46.8±3.1	44.6±2.0
Test 2 (+B6)	44.5±3.6	44.3±3.8	48.7±3.8	48.2±2.6	45.0±2.9
Hgb (gm/L)					
Test 1	148±8	149±6	160±11	161±7	154±6
Test 2 (+B6)	151±13	150±13	169±14	166±13	156±11

Mean±SD; n=5 except for During n=3
Test 1 refers to the non-supplemented exhaustive endurance ride
Test 2 refers to the vitamin B-6 supplemented exhaustive endurance ride

Glucose

Listed in Table 10 and Figure 1 are the mean plasma glucose concentrations and standard deviations for the two exercise tests. There were no statistically significant differences between the mean plasma glucose levels for the two tests at any of the time points nor was there a significant change in mean plasma glucose over time during either of the exercise tests. The changes over time for both tests were slight, showing maximum changes of 4% and 3% for test1 (non-supplemented) and test2 (vitamin B-6 supplemented), respectively. Although all three subjects making it to the during-exercise time point had higher plasma glucose concentrations in test-2 compared to test-1, the differences were not statistically significant. No other trends were observed when reviewing each individual's contribution to the overall means.

Lactic Acid

Listed in Table 11 and Figure 2 are the mean plasma lactate concentrations and standard deviations for the two exercise tests. There were no significant differences between the plasma lactate means for the non-supplemented and vitamin B-6 supplemented tests at any of the specific time points. However, there was a significant change in mean plasma lactic acid concentrations over time for both tests. In test1 (non-supplemented), mean plasma lactate increased 2.4-fold from pre-exercise concentrations (1.14 mmol/L) to 60 minutes into exercise compared to test2 (vitamin B-6 supplemented) in which the increase from pre-exercise concentrations (1.23 mmol/L) was more dramatic at 3.3-fold. For both tests the mean during-exercise plasma lactate concentration was statistically higher than either of the respective pre and post-60 minute samples, and in test2 the during-exercise sample was statistically higher than the post-exercise sample. In test1, mean plasma lactate concentration increased by approximately 12% from 60 minutes into exercise until exhaustion (note: $n=3$ for subjects exercising beyond one-hour). However, in test2 there was a decrease in mean plasma lactate

Table 10. Mean plasma glucose concentrations: effects of vitamin B-6 supplementation and exercise to exhaustion

		Pre-1	Pre-2	During	Post	Post-60
				mmol/L		
Test 1	mean	4.82	4.83	4.81	5.03	4.83
	SD	0.33	0.41	0.24	0.49	0.32
Test 2 (+B6)	mean	4.81	4.88	4.95	4.96	4.87
	SD	0.46	0.39	0.16	0.77	0.29

All values are means and SD; n=5 except for During n=3
Test 1 refers to the non-supplemented exhaustive endurance ride
Test 2 refers to the vitamin B-6 supplemented exhaustive endurance ride
Pre-1 and Pre-2: prior to exercise; During: 60 minutes into exercise;
Post: immediately after exercise; Post-60: 60 minutes after exercise

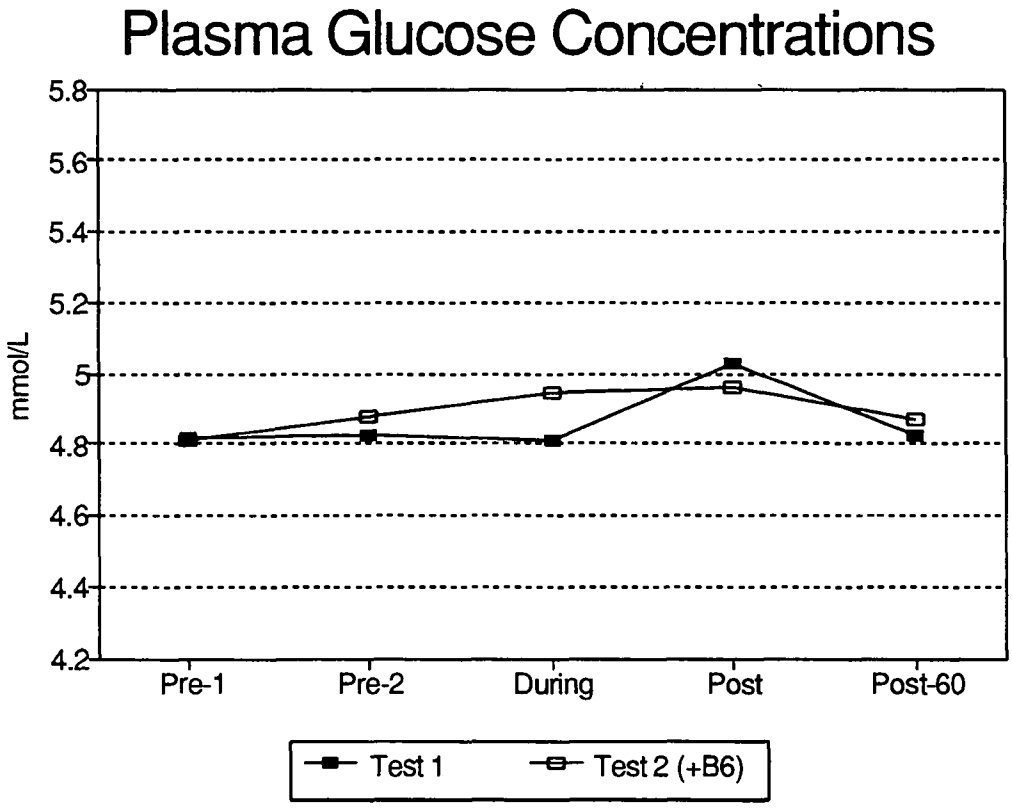


Figure 1. Mean plasma glucose concentrations: effects of vitamin B-6 supplementation and exercise to exhaustion

Table 11. Mean plasma lactate concentrations: effects of vitamin B-6 supplementation and exercise to exhaustion

		Pre-1	Pre-2	During	Post	Post-60
				mmol/L		
Test 1 a	mean	1.01	1.14	2.64	3.01	1.21
	SD	0.14	0.40	0.67	0.53	0.15
Test 2 (+B6) b	mean	1.12	1.23	3.92	2.83	1.21
	SD	0.25	0.42	0.97	0.49	0.38

All values are means and SD; n=5 except for During n=3
Test 1 refers to the non-supplemented exhaustive endurance ride
Test 2 refers to the vitamin B-6 supplemented exhaustive endurance ride
Pre-1 and Pre-2: prior to exercise; During: 60 minutes into exercise;
Post: immediately after exercise; Post-60: 60 minutes after exercise
a-the During and Post means are statistically higher than Pre-1,2 means
b-the During and Post means are statistically higher than Pre-1,2 means; the During mean is statistically higher than the Post mean

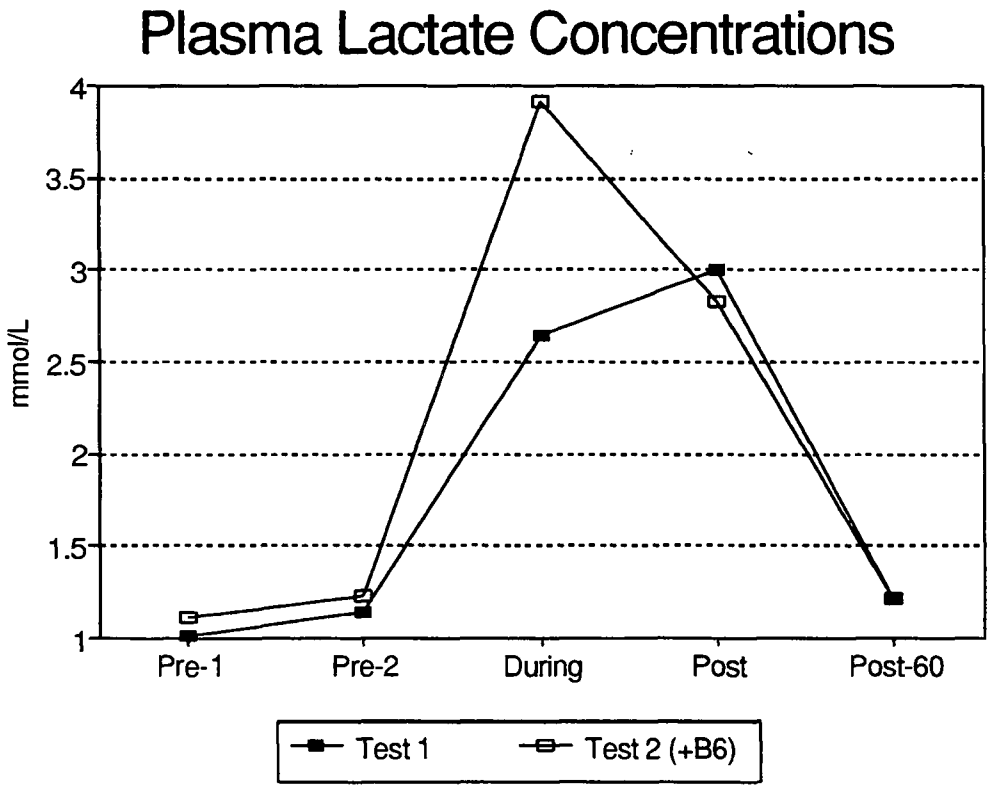


Figure 2. Mean lactate concentrations: effects of vitamin B-6 supplementation and exercise to exhaustion

concentrations of approximately 28% during this same time period. Both post-exercise sample means were statistically higher than either of their respective pre- and post-60 sample means. By the end of the recovery phase from exercise, the mean plasma lactate level for test1 was still 6% higher than the pre-exercise mean. However, in test2 (vitamin B-6 supplemented) the mean post-60 values were 2% lower than the mean pre-exercise mean. Similar to the glucose results, there was a consistent individual trend for subjects during-exercise samples to contain higher lactate levels in test2 relative to test1. However, these were not statistically higher. No other consistent trends in individual responses were observed for any of the specific blood sampling time points.

Glycerol

Listed in Table 12 and Figure 3 are the mean plasma glycerol concentrations and standard deviations for the two exercise tests. There were no significant differences between the plasma glycerol means for the two tests at any of the specific time points. However, there was a significant change in mean plasma glycerol concentrations over time for both tests. In the non-supplemented condition (test1), mean pre-exercise plasma glycerol levels were 0.20 mmol/L and in the vitamin B-6 supplemented condition (test2), mean pre-exercise levels were 0.22 mmmol/L. In test1, the mean plasma glycerol concentration increased by 130% by 60 minutes into exercise compared to only 77% in test2. For both tests the during-exercise mean plasma glycerol concentrations were statistically higher than either of the respective pre-exercise samples. By the end of exercise in test1, mean plasma glycerol was 3.4-fold that of pre-exercise mean values, and in test2 was 2.5-fold that of pre-exercise mean values. Both of the post glycerol values were statistically greater than either of their respective during-exercise mean values. By 60 minutes post-exercise, mean plasma glycerol concentrations decreased by an average of 52% for both tests relative to post-exercise levels. However, mean concentrations for both tests were still 75% and 30% greater than pre-exercise

Table 12. Mean plasma glycerol concentrations: effects of vitamin B-6 supplementation and exercise to exhaustion

		Pre-1	Pre-2	During	Post	Post-60
				mmol/L		
Test 1 a	mean	0.20	0.19	0.46	0.67	0.35
	SD	0.06	0.05	0.15	0.09	0.07
Test 2 (+B6) b	mean	0.22	0.21	0.39	0.54	0.29
	SD	0.03	0.02	0.03	0.17	0.09

All values are means and SD; n=5 except for During n=3
Test 1 refers to the non-supplemented exhaustive endurance ride
Test 2 refers to the vitamin B-6 supplemented exhaustive endurance ride
Pre-1 and Pre-2: prior to exercise; During: 60 minutes into exercise;
Post: immediately after exercise; Post-60: 60 minutes after exercise
a-the During and Post-60 means are statistically higher than Pre-1,2 means;
the Post mean is statistically higher than During and Post-60 means
b-the During and Post means are statistically higher than Pre-1,2 means;
the Post mean is statistically higher than the During mean

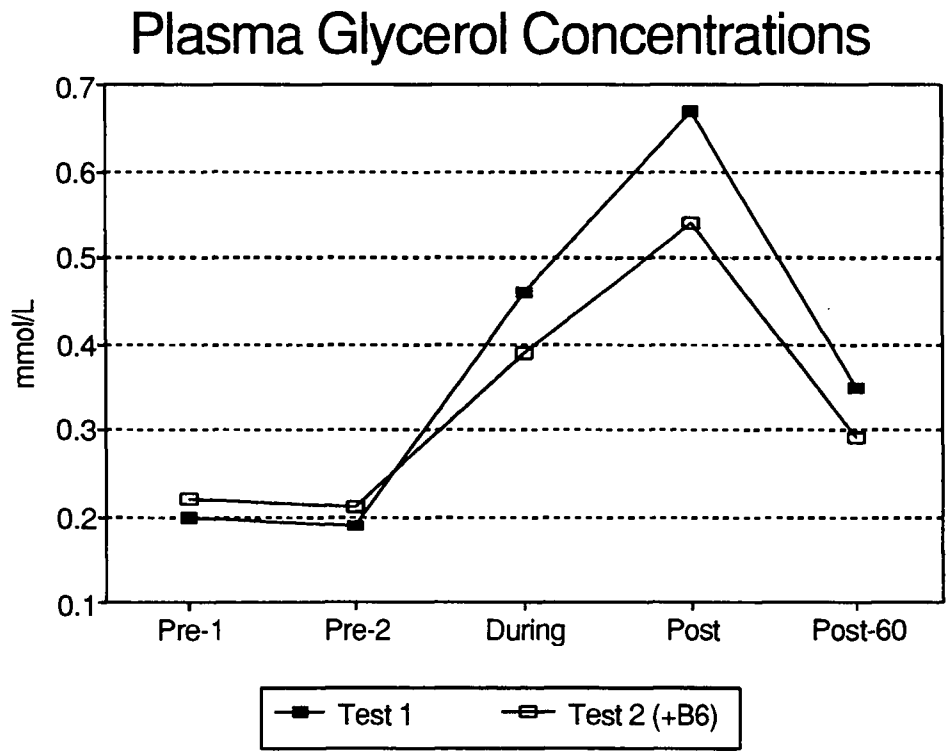


Figure 3. Mean plasma glycerol concentrations: effects of vitamin B-6 supplementation and exercise to exhaustion

concentrations for test1 and test2, respectively. Only in test1 was this continued elevation statistically significant compared to pre-exercise values. There were some observed trends among the subject's individual plasma glycerol concentrations. All but one of the subjects had lower plasma glycerol levels prior to exercise in test1 compared to test2. In addition, all but one individual had higher plasma glycerol levels at exhaustion in test1 relative to test2.

Free Fatty Acids

Listed in Table 13 and Figure 4 are the mean plasma FFA concentrations and standard deviations for the two exercise tests. There were no significant differences between the plasma FFA means for the two tests at any of the specific time points. However, there was a significant change in mean plasma FFA concentrations over time for both tests. The line graphs which represent the changes in plasma FFA pre-, during-, and post-exercise virtually mimic each other in both test1 and test2, with all of the test2 means being lower than the test1 means. In test1 (non-supplemented), mean pre-exercise FFA concentrations were 0.45 mmol/L and in test2 (vitamin B-6 supplemented), mean pre-exercise concentrations were 0.37 mmol/L. After the first 60 minutes of exercise (i.e. during-exercise), mean plasma FFA levels increased by 33% and 21% for test-1 and test-2, respectively. By the end of exercise in both tests, the mean level of plasma FFA had increased by approximately 2.8-fold compared to resting levels. However, the absolute change in mean values was 0.87 mmol/L in the non-supplemented test and 0.67 mmol/L in the vitamin B-6 supplemented test. In both tests the post-exercise sample resulted in a statistically significant increase in concentration relative to the pre-exercise concentrations. Following 60 minutes of recovery, mean plasma FFA levels were still more than double the mean pre-exercise resting levels, which represented a statistically significant elevation. Similar to the other fuels, there were some observed trends when reviewing each individual's data. For four out of the five subjects, their fasting pre-exercise plasma FFA concentrations were lower in test2 than in test1. In addition, all three

Table 13. Mean plasma FFA concentrations: effects of vitamin B-6 supplementation and exercise to exhaustion

		Pre-1	Pre-2	During	Post	Post-60
				mmol/L		
Test 1 a	mean	0.47	0.45	0.61	1.33	0.94
	SD	0.19	0.12	0.15	0.37	0.29
Test 2 (+B6) b	mean	0.38	0.37	0.46	1.05	0.82
	SD	0.11	0.05	0.09	0.49	0.33

All values are means and SD; n=5 except for During n=3
Test 1 refers to the non-supplemented exhaustive endurance ride
Test 2 refers to the vitamin B-6 supplemented exhaustive endurance ride
Pre-1 and Pre-2: prior to exercise; During: 60 minutes into exercise;
Post: immediately after exercise; Post-60: 60 minutes after exercise
a-the Post and Post-60 means are statistically higher than all other means
b-the Post and Post-60 means are statistically higher than all other means

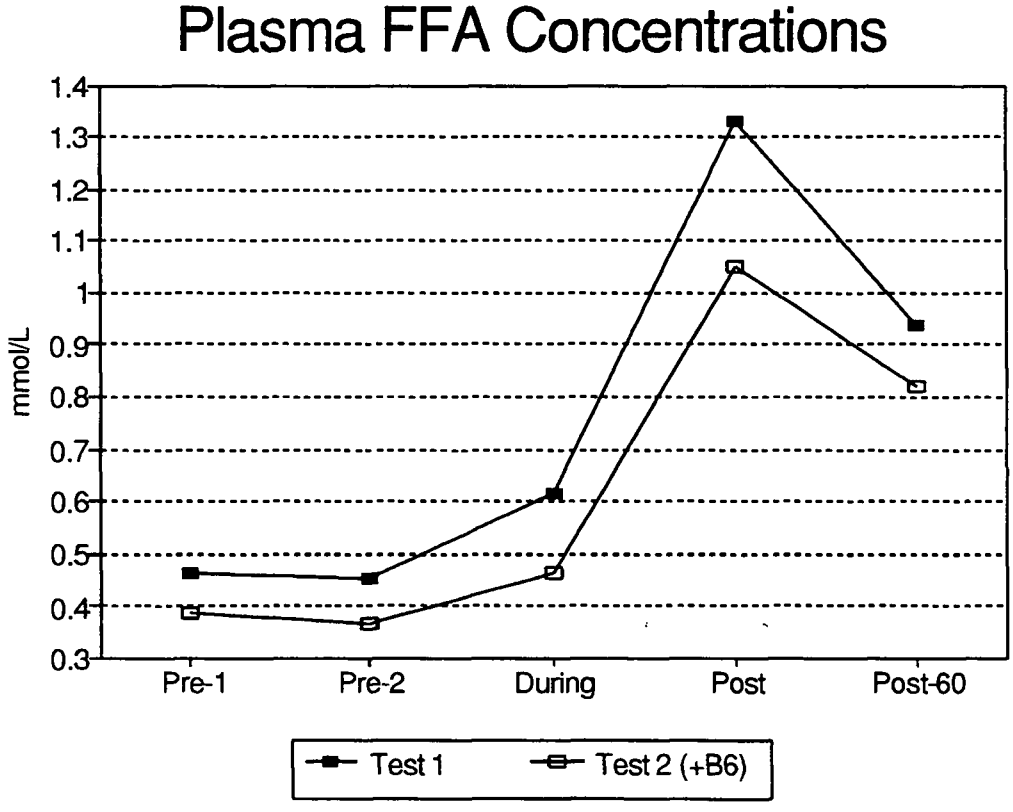


Figure 4. Mean plasma FFA concentrations: effects of vitamin B-6 supplementation and exercise to exhaustion

subjects making it to the one-hour blood sampling time point had lower plasma FFA levels in test2 compared to test1.

Amino Acids

Listed in Table 14 is a quantitative summary of the mean plasma values of the 13 methodologically obtainable amino acids. See Figures 5 through 17 for a visual representation of the relative changes in concentrations pre-, during-, and post-exercise. There were statistically significant differences between the plasma levels of tyrosine and methionine, and there were statistically significant changes over time for the mean plasma alanine and plasma histidine concentrations. The plasma concentration of no other amino acid differed between the two tests nor did any change significantly over time within a test. In general, the plasma concentration of the second pre-exercise sample in test2 (vitamin B-6 supplemented) tended to be higher than the first pre-exercise sample. This trend was not observed in test1 (non-supplemented).

Immediately prior to exercise, the mean plasma levels of tyrosine were 71 umol/L and 70 umol/L for test1 and test2, respectively. By 60 minutes into exercise, mean plasma tyrosine concentrations increased by roughly 8% over pre-exercise levels in test1, whereas test2 mean tyrosine levels showed vitrually no change at all. By the post-exercise time point in test1, mean plasma tyrosine concentrations had increased an additional 3% over the during-exercise time point. In contrast, no change in mean plasma tyrosine levels over this respective time period was observed in test2. After 60 minutes of recovery, mean plasma tyrosine was back to pre-exercise concentrations in test1. However, in test2 mean tyrosine values dropped to approximately 6% below pre-exercise values. At post-60 minutes of exercise these differences between the two tests were the only statistically significant differences ($p = 0.007$).

Methionine was the only other amino acid which changed significantly between test-1 and test2. Pre-exercise mean concentrations of methionine were 32 umol/L and 35 umol/L in test1

Table 14. Mean plasma amino acid concentrations: effects of vitamin B-6 supplementation and exercise to exhaustion

Amino Acid	Pre-1	Pre-2	During	Post	Post-60
			(umol/L)		
Serine					
T1	144±22	144±26	152±37	144±24	128±26
T2 (+B6)	138±35	148±37	143±56	138±35	120±28
Threonine					
T1	174±15	172±13	166±11	164±8	153±9
T2 (+B6)	159±20	170±21	163±39	153±20	138±22
Alanine					
T1	305±62	312±61	458±35#	415±34#	307±54
T2 (+B6)	319±73	326±61	535±180#	428±98	296±65
Proline					
T1	240±34	237±40	236±45	224±41	198±37
T2 (+B6)	239±59	248±58	255±84	212±61	202±38
Histidine					
T1	75±5	76±6	85±3*	85±3	77±6
T2 (+B6)	80±10	84±5	93±11	86±6	81±10
Arginine					
T1	160+32	161±27	145±32	156±30	157±37
T2 (+B6)	140+15	165±12	128±9	150±15	148±33
Tyrosine					
T1	73±10	71±12	77±10	80±6	71±7[a]
T2 (+B6)	69±6	70±3	71±13	71±4	66±4
Valine					
T1	237±38	235±44	217±54	220±44	204±36
T2 (+B6)	235±38	244±37	239±77	218±37	209±43
Methionine					
T1	33±4	32±7	36±4	36±4[b]	29±3
T2 (+B6)	30±5	35±2	32±11	32±3	29±2
Isoleucine					
T1	81±21	79±23	68±17	76±20	65±11
T2 (+B6)	82±14	78±14	76±27	70±12	67±10
Leucine					
T1	149±29	146±28	133±27	142±28	130±19
T2 (+B6)	143±16	149±23	147±49	143±18	133+18
Phenylalanine					
T1	69±11	67±9	76±7	82±12	68±2
T2 (+B6)	70±8	74±12	73±17	77±3	68±8
Tryptophan					
T1	60±8	60±9	61±3	57±5	52±3
T2 (+B6)	56±9	56±9	60±5	56±5	55±4

Mean±SD; n=5 except During n=3; Note: See Figures 5-17 (pgs 85-97)
[a]p=0.007 difference between the tests; [b]p=0.03 difference between the tests
# and * statistically different means within a given test relative to all other means
T1 refers to the non-supplemented exhaustive endurance ride; T2 refers to the vitamin B-6 supplemented exhaustive endurance ride; Pre-1 and Pre-2: prior to exercise; During: 60 minutes into exercise; Post: immediately after exercise; Post-60: 60 minutes after exercise

Figure 5. Mean plasma serine concentrations: effects of vitamin B-6 supplementation and exercise to exhaustion

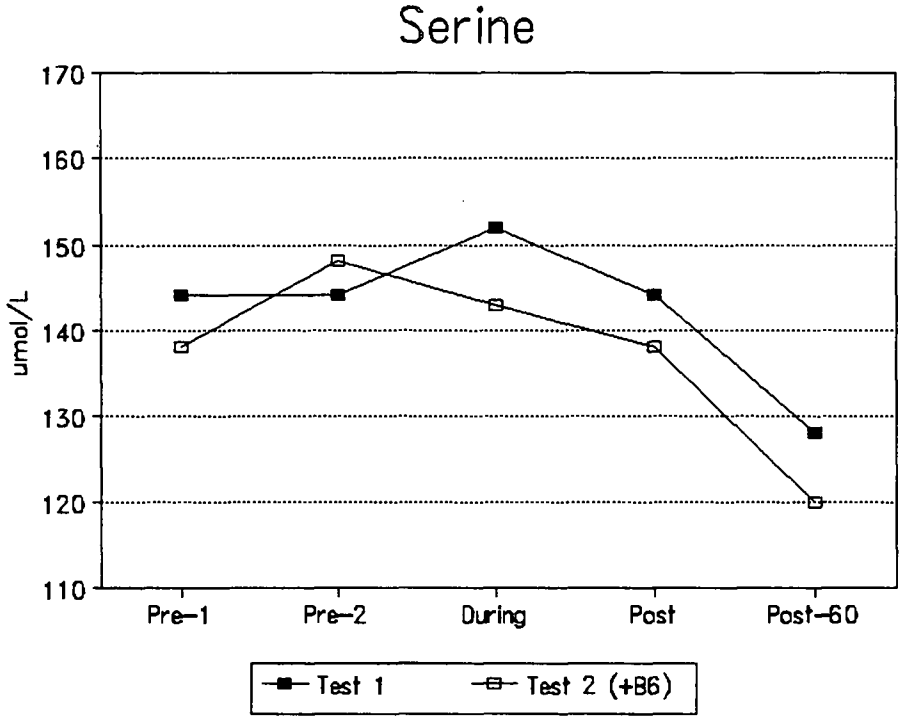


Figure 6. Mean plasma threonine concentrations: effects of vitamin B-6 supplementation and exercise to exhaustion

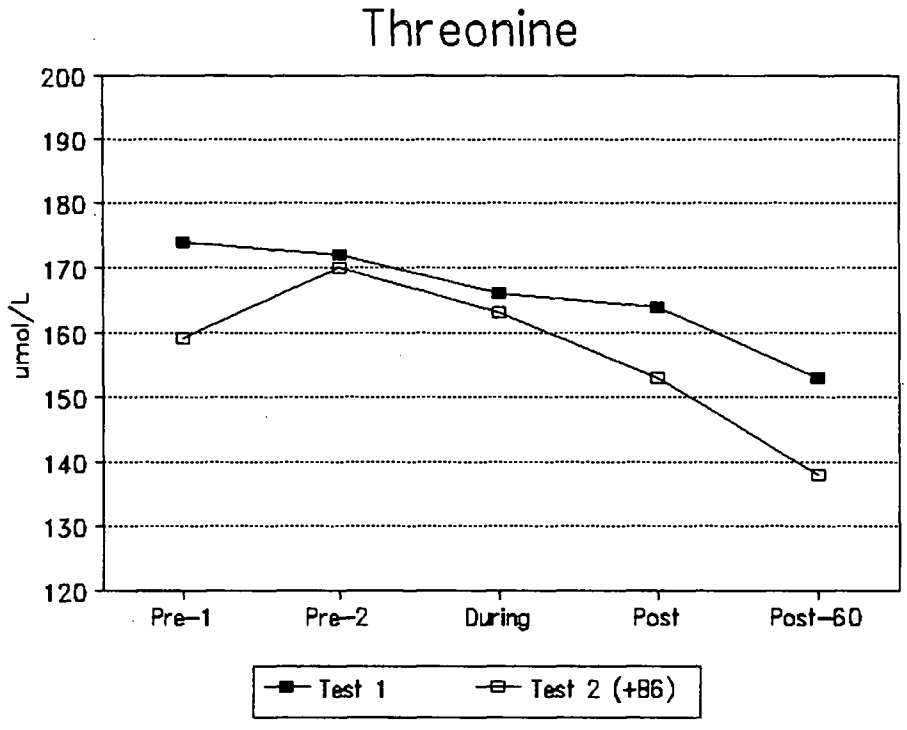


Figure 7. Mean plasma alanine concentrations: effects of vitamin B-6 supplementation and exercise to exhaustion

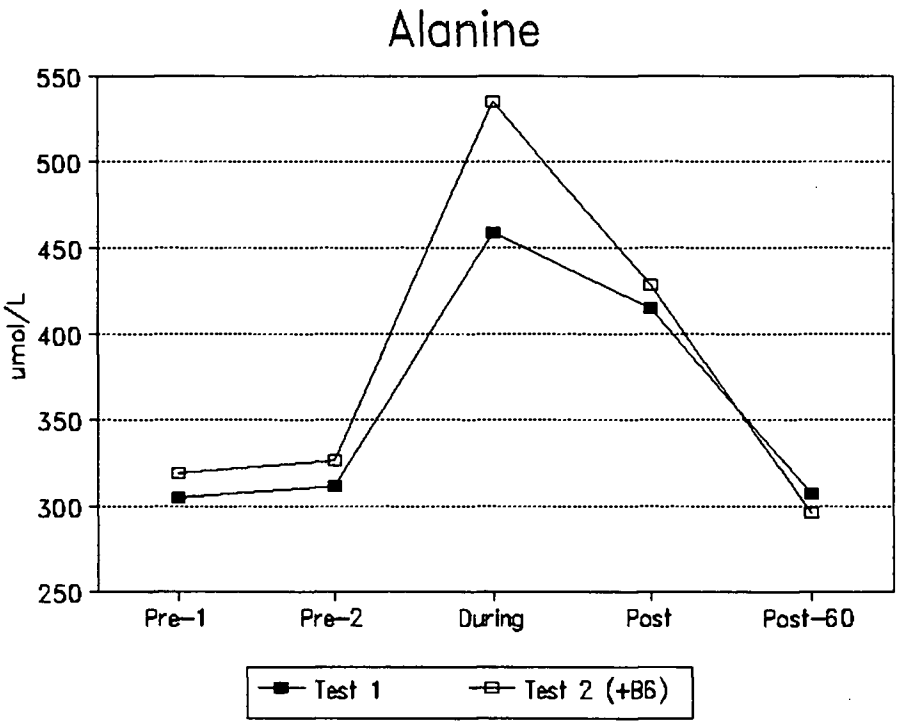


Figure 8. Mean plasma proline concentrations: effects of vitamin B-6 supplementation and exercise to exhaustion

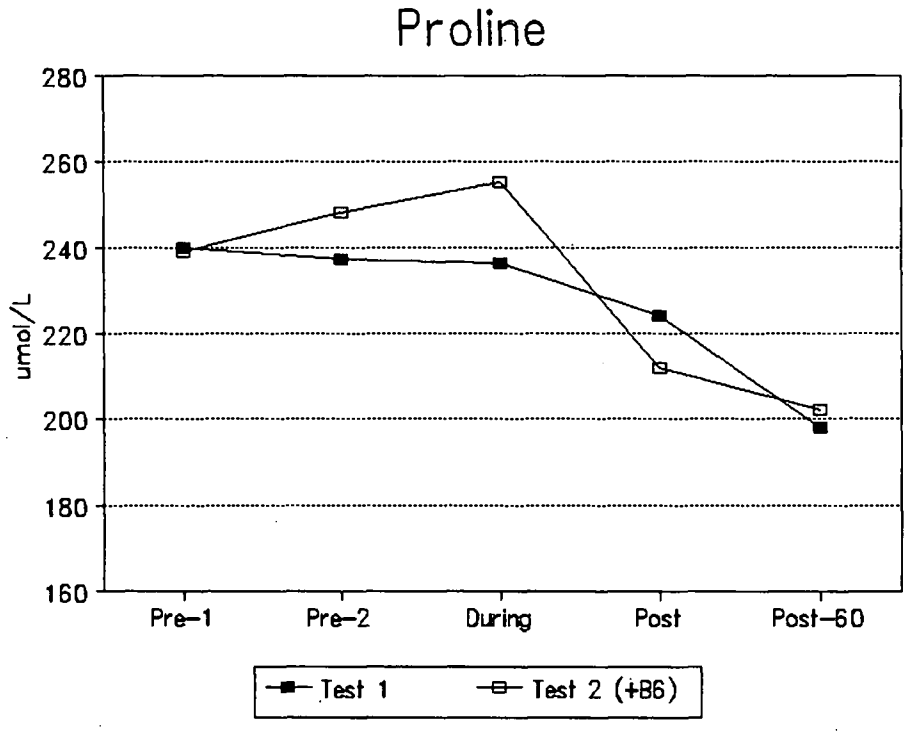


Figure 9. Mean plasma histidine concentrations: effects of vitamin B-6 supplementation and exercise to exhaustion

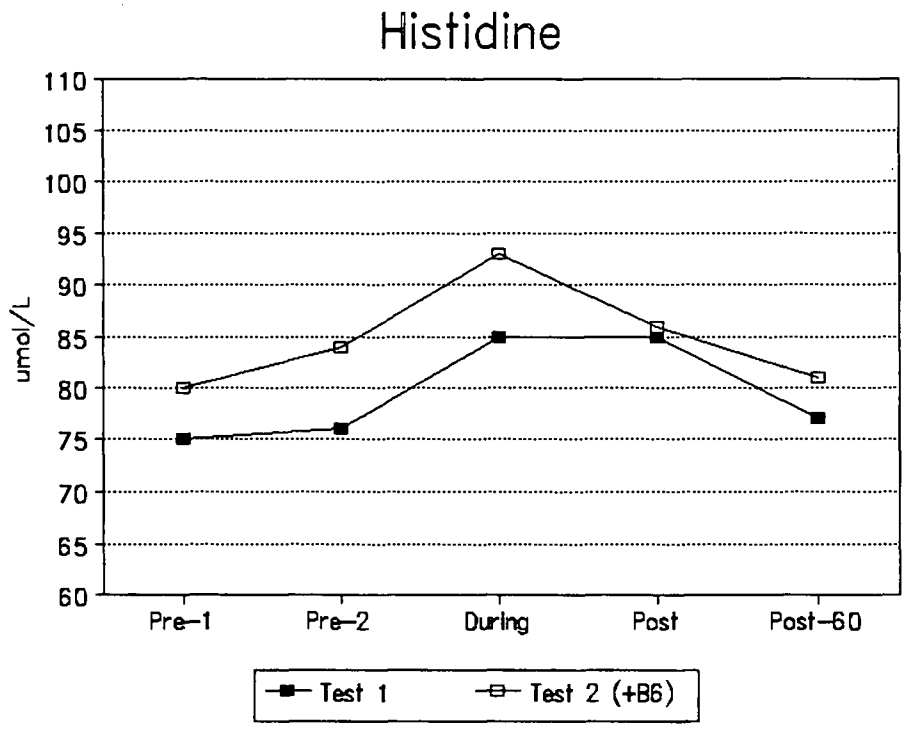


Figure 10. Mean plasma arginine concentrations: effects of vitamin B-6 supplementation and exercise to exhaustion

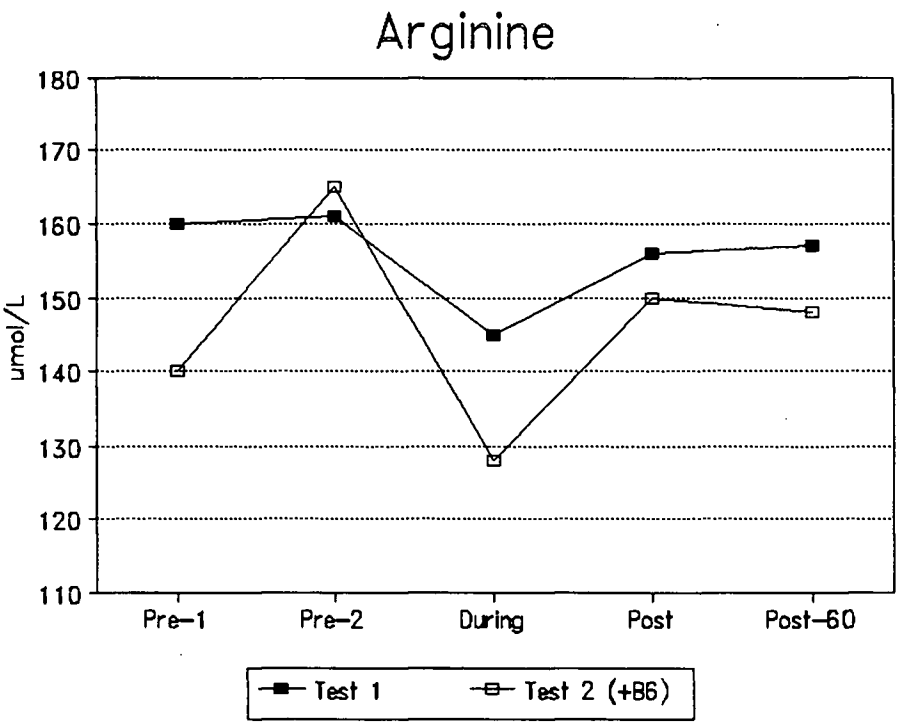


Figure 11. Mean plasma tyrosine concentrations: effects of vitamin B-6 supplementation and exercise to exhaustion

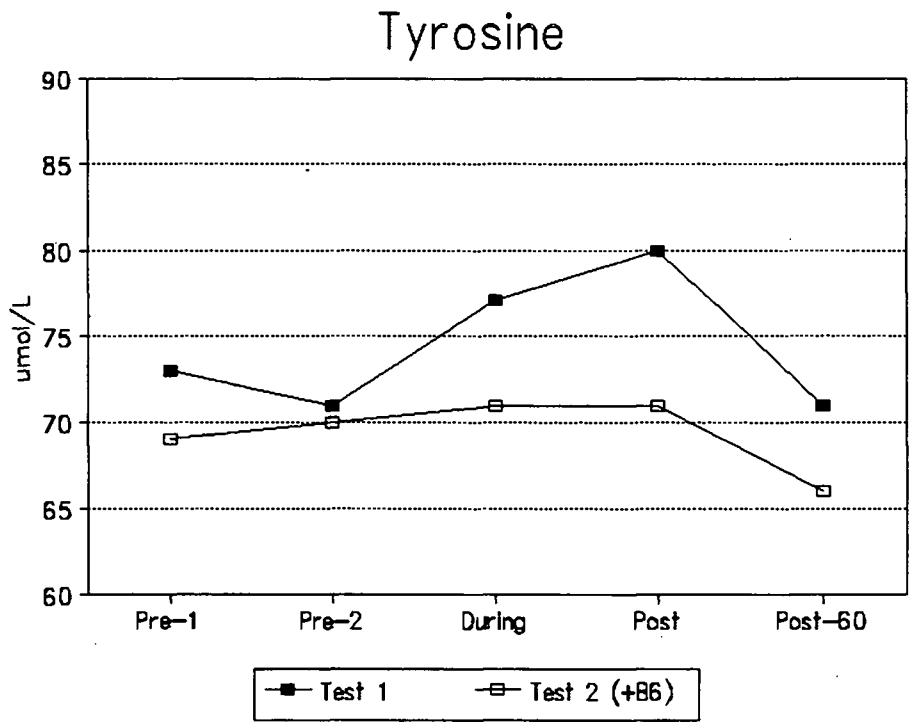


Figure 12. Mean plasma valine concentrations: effects of vitamin B-6 supplementation and exercise to exhaustion

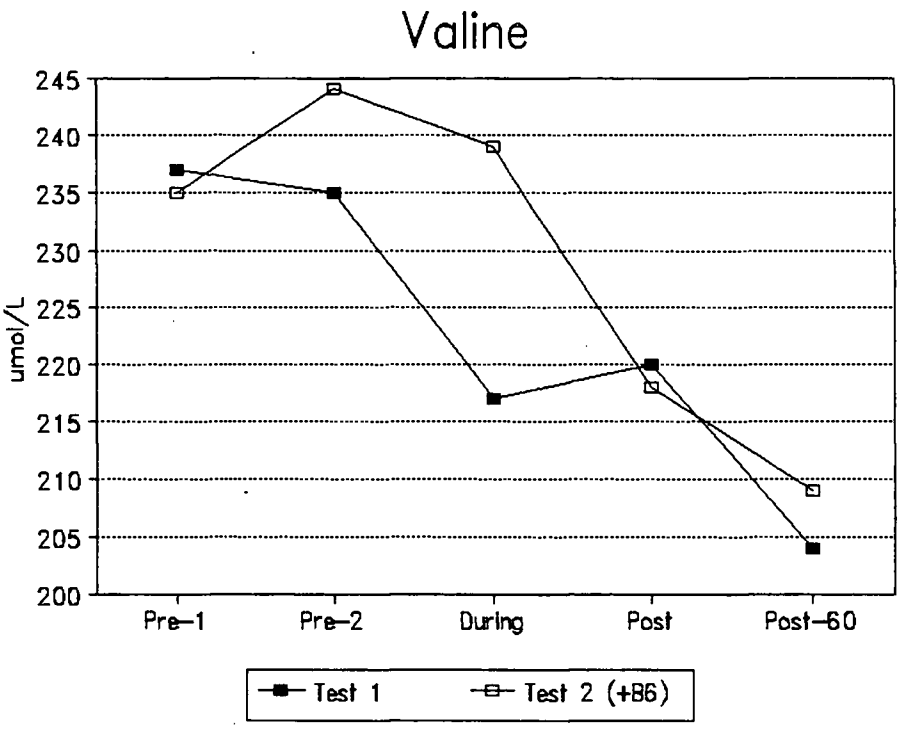


Figure 13. Mean plasma methionine concentrations: effects of vitamin B-supplementation and exercise to exhaustion

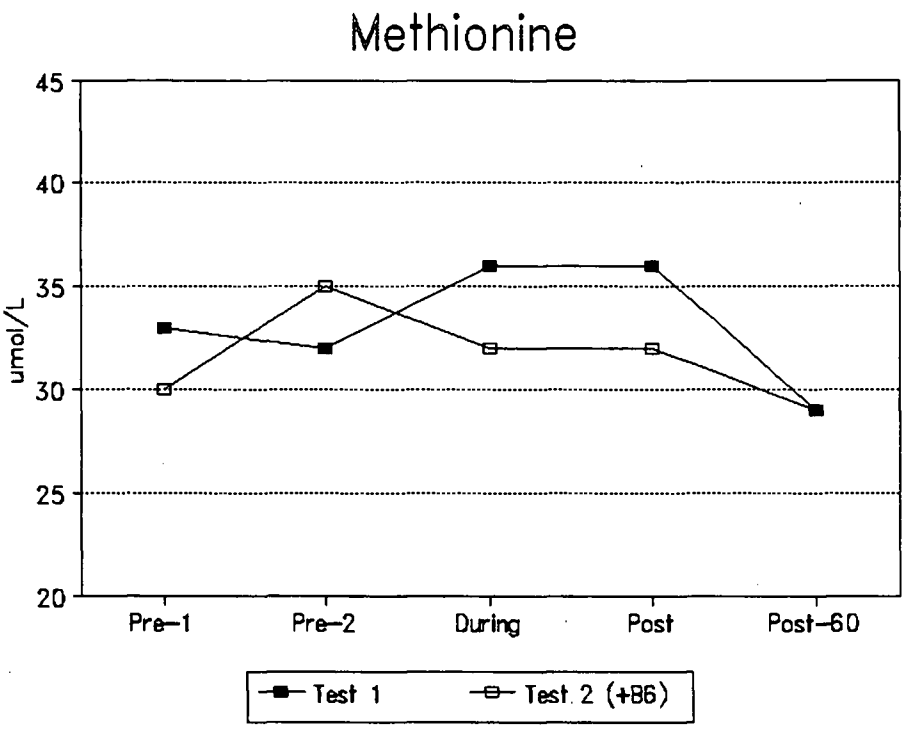


Figure 14. Mean plasma isoleucine concentrations: effects of vitamin B-6 supplementation and exercise to exhaustion

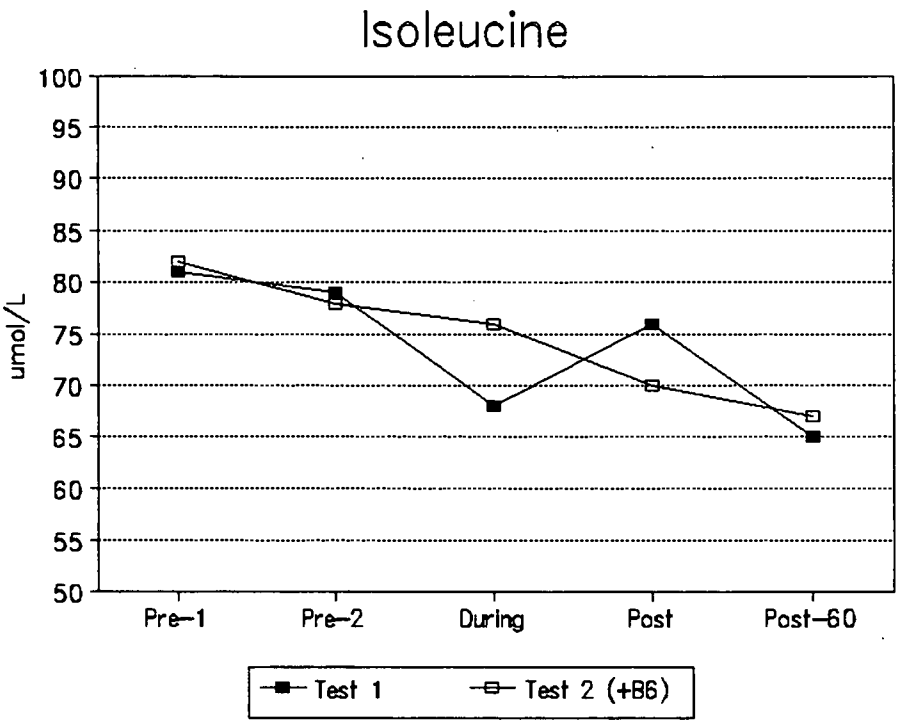


Figure 15. Mean plasma leucine concentrations: effects of vitamin B-6 supplementation and exercise to exhaustion

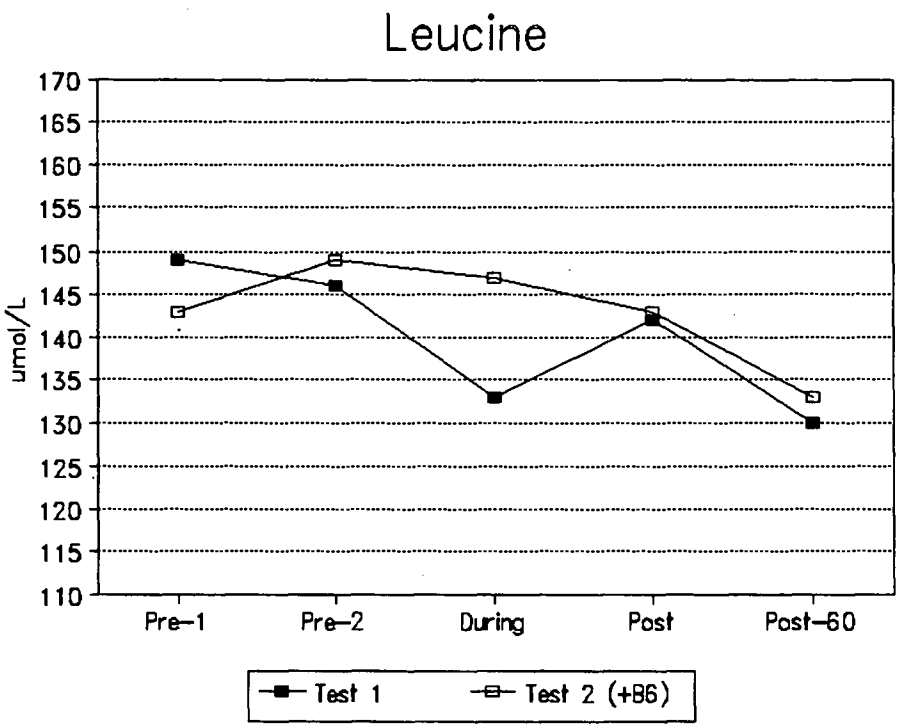


Figure 16. Mean plasma phenylalanine concentrations: effects of vitamin B-6 supplementation and exercise to exhaustion

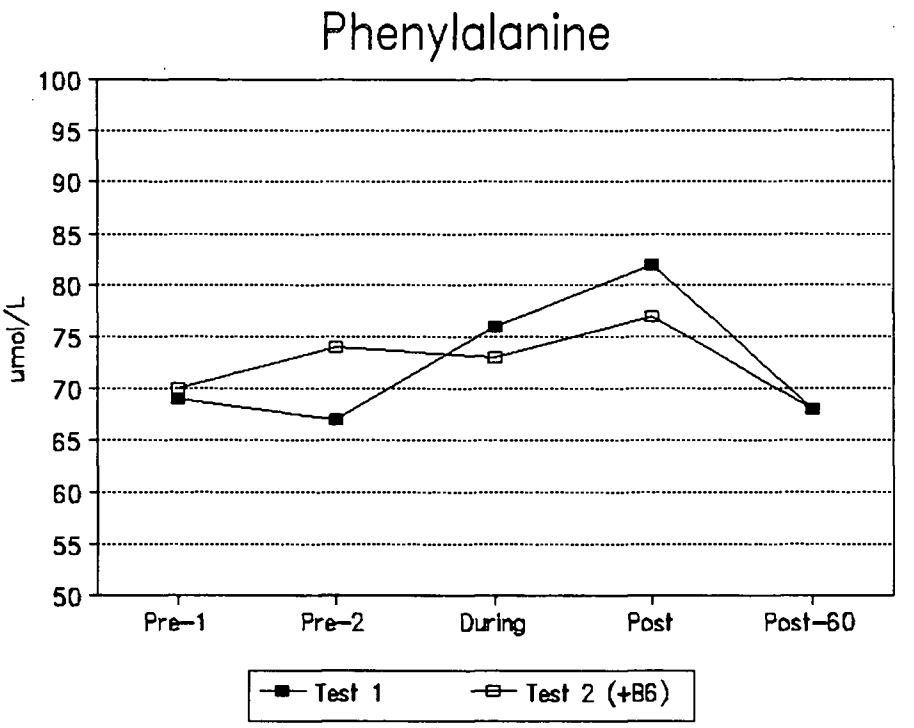
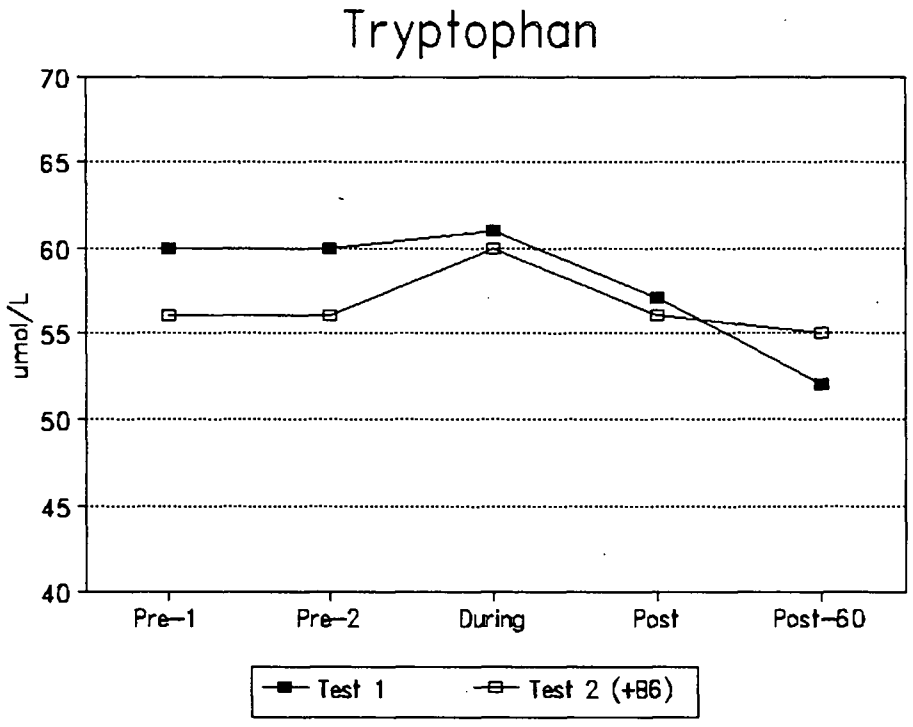


Figure 17. Mean plasma tryptophan concentrations: effects of vitamin B-6 supplementation and exercise to exhaustion



and test2, respectively. At the during and post-exercise time points, methionine increased in test1 but remained essentially unchanged in test2. The difference at these time points between the two tests was on the order of 11%, but was statistically higher only in test1 relative to test2 at the post-exercise time point ($p = 0.03$). By post-60 minutes of exercise in both tests, mean plasma concentrations of methionine were identical. These levels were just slightly below those observed pre-exercise.

Mean, pre-exercise, plasma alanine levels were 312 umol/L and 326 umol/L in test1 (non-supplemented) and test2 (vitamin B-6 supplemented), respectively. During exercise, plasma alanine concentrations increased by 33% in test1 and 40% in test2. These increases represented statistically significant changes compared to pre-exercise values. By the end of exercise, mean plasma alanine concentrations were very similar between the two exercise tests, and were still on average 25% higher than pre-exercise concentrations. In the non-supplemented test, this represented a statistically significant elevation over pre-exercise concentrations, but not in the vitamin B-6 supplemented test. By the end of the recovery phase, mean plasma alanine levels returned to just below pre-exercise levels for both tests.

At all time points, mean plasma histidine levels showed a trend of being 1% to 10% higher in test2 relative to test1. However, this was not a statistically significant difference between the tests. In both tests mean plasma histidine concentrations increased by approximately 10% after 60 minutes of exercise compared to pre-exercise. However, only in the non-supplemented situation was this increase statistically significant. Mean plasma histidine concentrations remained constant in test1 from the during-exercise time point to post-exercise, which represented a continual statistically significant elevation relative to mean pre-exercise concentrations. In the vitamin B-6 supplemented condition, at post and post-60 minutes of exercise, histidine levels had returned to essentially pre-exercise levels. Similarly, at all the post-60 time points in test-1, mean plasma histidine concentrations were virtually identical to pre-exercise concentrations.

Expired Gases

Listed in Table 15 and Figure 18 are the mean R values and standard deviations for the exercise tests. There were no significant differences in mean R values between the two tests at any of the specific collection time points. Nor was there a significant change in mean R values over time during either of the tests. Although it was not statistically significant, it did appear that within the first hour and one-half of exercise, mean R values tended to be higher by approximately 0.02 units in the vitamin B-6 supplemented test relative to non-supplemented test. On an individual basis, no trends were observed among the subjects. This suggests a limited possibility of statistical differences with a larger sample size.

Listed in Table 16 and Figure 19 are the mean relative oxygen consumption values for the exercise tests. There was a statistically significant difference between the two tests at a single collection time point. However, there was not a significant change over time during either of the tests. During the first 10 minutes of exercise, mean relative oxygen consumption values were significantly ($p=0.02$) higher in test2 compared to test1. In addition, for at least four additional time points (i.e. 47-50; 57-60; 97-100; 107-110 minutes) there was a trend for all individual subject's oxygen consumption values to be higher in test2 than in test1.

Diet

Listed in Table 17 are the nine day total kilocalorie (kcal) means and standard deviations for each subject during each of the controlled dietary periods. There was a statistical difference ($p=0.0008$) in energy intake between the two feeding periods for only subject number two. On average, he consumed 222 kcal less in period2 compared to period1. The actual vitamin B-6 content of the diet was slightly lower than anticipated. In period1, subjects consumed an average of 1.89 mg/day of vitamin B-6, while in period2 they consumed an average of 1.93 mg/day of vitamin B-6 through foodstuffs.

Table 15. Mean R values: effects of vitamin B-6 supplementation and exercise to exhaustion

	Test 1			Test 2 (+B6)		
Time (min.)	n	mean	SD	n	mean	SD
10	5	0.93	0.04	5	0.93	0.03
20	5	0.92	0.03	5	0.92	0.05
30	5	0.91	0.03	4	0.94	0.04
40	5	0.91	0.02	4	0.93	0.04
50	5	0.91	0.04	4	0.93	0.04
60	3	0.90	0.05	3	0.94	0.06
70	2	0.93	0.04	3	0.92	0.06
80	3	0.88	0.05	3	0.91	0.04
90	3	0.87	0.06	2	0.93	0.01
100	2	0.91	0.01	2	0.91	0.01
110	2	0.89	0.01	2	0.91	0.01
120	2	0.92	0.01	1	0.92	
130	2	0.89	0.01	1		
140	1	0.88		1	0.89	
150				1	0.88	
160				1	0.89	
170				1	0.88	
180				1	0.89	
190				1	0.89	

All values are means and SD; n refers to the number of subjects; Time refers to the duration of exercise; Test 1 refers to the non-supplemented exhaustive endurance ride; Test 2 refers to the vitamin B-6 supplemented exhaustive endurance ride

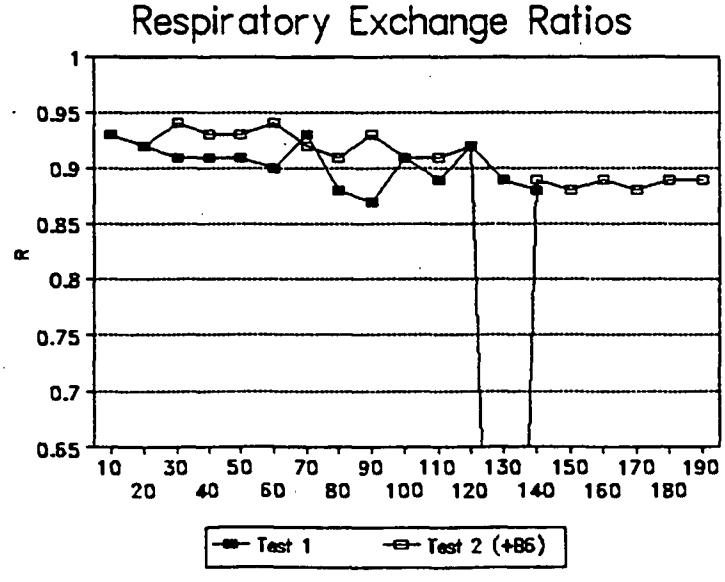


Figure 18. Mean R values: effects of vitamin B-6 supplementation and exercise to exhaustion

Table 16. Mean relative oxygen consumption: effects of vitamin B-6 supplementation and exercise to exhaustion

	Test 1			Test 2 (+B6)		
Time (min.)	n	mean	SD	n	mean	SD
a 10	5	39.2	4.6	5	41.5	5.7
20	5	39.8	5.1	5	41.4	5.5
30	5	39.4	5.1	4	39.1	3.7
40	5	39.7	4.6	4	39.4	2.7
50	5	39.3	5.1	4	40.5	3.8
60	3	38.5	1.6	3	42.9	3.0
70	2	40.7	0.8	3	42.1	4.5
80	3	39.5	3.1	3	42.7	5.9
90	3	39.9	2.6	2	44.3	5.3
100	2	41.9	1.9	2	44.5	4.6
110	2	41.0	0.2	2	44.2	4.0
120	2	43.2	0.8	1	41.7	
130	2	43.1	0.7	1	42.3	
140	1	42.3		1		
150				1	42.0	
160				1	43.8	
170				1	42.6	
180				1	43.1	
190				1	43.7	

All values are means and SD (ml/kg/min); (a) refers to $p = 0.02$ different means; Time refers to the duration of exercise; n refers to the number of subjects; Test 1 refers to the non-supplemented exhaustive endurance ride; Test 2 refers to the vitamin B-6 supplemented exhaustive endurance ride; Mean 75% of VO_2 max = 41.0 ml/kg/min

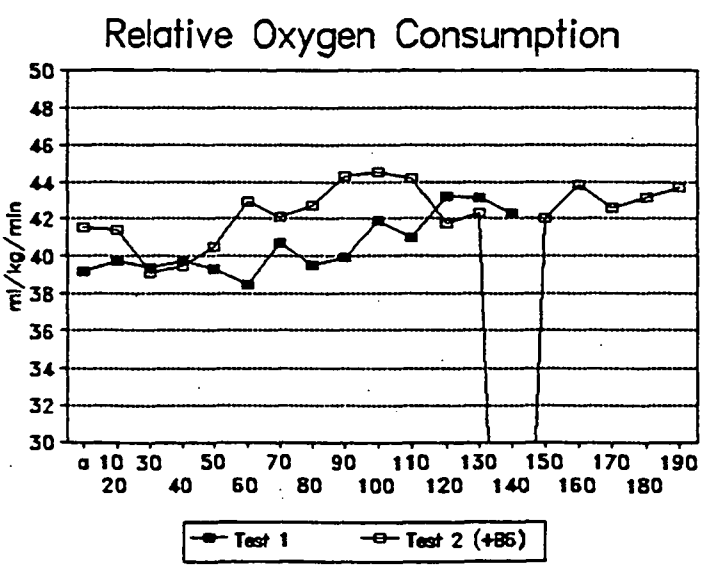


Figure 19. Mean relative oxygen consumption: effects of vitamin B-6 supplementation and exercise to exhaustion

Table 17. Mean nine day kilocalorie intakes during the controlled dietary periods.

Subject	Period-1	Period-2
1	3943±234	4171±490
[a]2	3766±131	3544±94
3	3816±198	3810±197
4	3973±184	3993±163
5	3724±211	3637±310

Mean±SD; [a]refers to statistically different means ($p = 0.0008$)
Period-1 refers to the non-supplemented dietary phase
Period-2 refers to the vitamin B-6 supplemented dietary phase

Times to Exhaustion

Listed in Table 18 are the mean exercise times to exhaustion and standard deviations for the two exercise tests. In addition, within the table are each subject's individual data. There were no statistically significant differences in mean exercise times to exhaustion between the tests. Three of the five subjects showed a decrease in time to exhaustion ranging from 12 to 31 minutes, while two subjects showed an increase in time to exhaustion ranging from 3 to 44 minutes, thus resulting in no net effect of vitamin B-6 on endurance exercise performance. There were no statistically significant differences in ratings of perceived exertion (RPE) between either of the two tests (See Appendix Table 18). However, RPE did increase significantly over time during each test as expected.

Table 18. Exercise times to exhaustion: effects of vitamin B-6 supplementation

Subject	Test 1	Test 2(+B6)
1	2:10:03	1:50:20
2	0:53:39	0:22:18
3	0:52:19	0:55:47
4	1:32:00	1:20:57
5	2:26:04	3:10:20
mean±SD	1:34:49±0:42:56	1:31:56±1:03:50

Time in hour:min:sec
Test 1 refers to the non-supplemented exhaustive endurance ride
Test 2 refers to the vitamin B-6 supplemented exhaustive endurance ride

DISCUSSION

Until now, there has been evidence both for and against a vitamin B-6 effect on fuel utilization during exercise. This study was designed to gain new information on whether or not vitamin B-6 supplementation in conjunction with exercise affected circulating plasma amino acids. Catecholamines and growth hormone were investigated by two other graduate students. If there were any effects on plasma amino acids, it would thus provide a possible mechanistic theory by which vitamin B-6 may be altering fuel metabolism. We also sought to "fine tune" and repeat a study (Virk et al., 1992) done earlier in order to confirm or negate whether vitamin B-6 does in fact alter energy metabolism during exhaustive endurance exercise in men. The previous study resulted in relatively few statistically significant differences in plasma substrate concentrations, expired gas data, and in exercise performance times to exhaustion between a control and vitamin B-6 supplemented condition. However, this was due in part to a small subject number (n) and not necessarily to an absent vitamin B-6 effect. This second study was done to determine if the consistent trends observed in the previous study might actually result in statistically significant differences by increasing the n and by applying our previous experience to more fully control the exercise tests as well as the diet and urine collections.

One of the inconsistencies found in the literature in this area of study is whether to measure plasma volume changes and further whether to correct plasma substrate concentrations based on the observed plasma volume changes. Often, authors claim that they have corrected their data with respect to plasma volume changes; however, more times than not, an equation or method for their data manipulation is not provided. In this portion of the study, plasma volume changes were measured; however, the plasma substrate concentrations were not normalized for the observed plasma volume changes. The primary reason for this arises from the lack of consistency (i.e. validity) and the degree of confidence with the methods used to measure plasma volume changes. In this study we utilized two separate

methods (Dill and Costill, 1974; Van Beaumont, 1972) for measuring changes in plasma volume and although these two methods are well acknowledged in the literature, there were differences between the methods in the calculated plasma volume changes. In general, in the present study the Dill and Costill method tended to generate values ranging form 2.2-4.1% more negative than the Van Beaumont method, thus raising the question of which method, if either, actually is providing a true measure of the change in intravascular volume, which is known to occur with exercise. Before values can be corrected or normalized, one should have complete confidence in the tool used for manipulating the data. Both the Dill and Costill and Van Beaumont methods require a hematocrit measurement. Although hematocrits were done in duplicate and in general there was consistency in duplication, it should be noted that there is a $\pm$0.5 mg% error associated with this measurement. The Dill and Costill method requires a hemoglobin measurement in addition to hematocrit; and, although this was done in triplicate, it should be noted that the method is associated with a measurement error of approximately $\pm$3% Harrison, 1985). These combined measurement errors can potentially alter plasma volume values by 1-5%. In a review, Harrison (1985) suggests that simply one's posture or mode of exercise can have significant effects on plasma volume changes. In specific, with exercise in a supine or seated position, larger decreases in plasma volume occur compared to exercise in a standing position. Although each blood draw was performed in a seated position, these variables were not completely controlled for in this study. Subjects sat for a minumum of 10-15 minutes (after standing) for each blood draw except for the post-exercise blood draw. At exhaustion (i.e. post-exercise) subjects stood temporarily for 10-30 seconds immediately prior to being seated and having their blood drawn. Harrison (1985) suggests that a minimum of 20 minutes in a given posture is required to minimize a posture effect on plasma volume. In reviewing each individual's plasma volume change, there are clear inconsistancies in the direction and magnitude of plasma volume changes. For example, subjects number one and six showed approximately a 3% smaller plasma volume change with the Dill and Costill method

at post-exercise than at during-exercise time point. Rationally, one would expect to observe a reversal of this finding, as dehydration becomes more prevalent with continued exercise, provided fluids are not replaced. Correction of the data with plasma volume changes may add a confounding factor in the interpretation of the results. In the discussion that follows, plasma volume changes will be used to provide a possible explanation for observed changes or lack of these between the control and vitamin B-6 supplemented tests for a given plasma substrate; however, recall that there are limitations involved in the measurement of plasma volume changes.

Based on the knowledge of vitamin B-6's role in glycogenolysis and gluconeogenesis, another objective of this study was to determine if vitamin B-6 supplementation would result in higher sustained plasma glucose levels during exercise, as compared to a non-supplemented condition. Virk (1992) observed that, under conditions of vitamin B-6 supplementation, plasma glucose concentrations showed a trend of being between 5-11% higher during, post, and post-60 minutes of exercise compared to a non-supplemented condition. No such finding was observed in this study. In this research project, mean plasma glucose levels changed very little as a result of vitamin B-6 supplementation and as a result of exercise. Generally, blood glucose concentrations slowly fall later in exercise as hepatic glucose output fails to keep pace with muscle utilization (Ahlborg and Felig, 1982). The absolute mean values obtained in this study are consistent with the 1992 study at all specific time points with the exception of post- and post-60 minutes of exercise. The absolute mean plasma glucose values are also consistent with those found by others (see Table 4, pg 48); however, the relative lack of a change in mean plasma glucose levels over time in this study is only consistent with the results of Hoffman et al. (1991). In the 1992 study mean pre-exercise plasma glucose levels averaged 4.73 mmol/L, and in this study mean pre-exercise plasma glucose values averaged 4.84 mmol/L for both the supplemented and non-supplemented conditions. A comparison of the during-exercise mean plasma glucose values for the two studies shows that values in test1 and

test2 are 1.6% lower and 6% higher, respectively, in the present study. By post exercise the absolute concentration differences in mean plasma glucose between the studies were 82 mmol/L (16%) and 33 mmol/L (6.4%) for the non-supplemented and vitamin B-6 supplemented tests, respectively; and, by post-60 minutes of exercise, differences were 75 mmol/L (11.4%) and 56 mmol/L (15.5%) for respective tests. As noted in Table 4, plasma glucose concentration can either increase, decrease, or show virtually no change at all as a result of exercise. Provided there was a change in plasma glucose between the two exercise tests or over time, one might speculate that this was a result of altered glucose uptake by the muscles or of altered hepatic output of glucose by the liver. Manore and Leklem (1987) have hypothesized that high vitamin B-6 intakes might lead to a more rapid utilization of muscle glycogen. This would most likely have the effect of decreasing the uptake of plasma glucose during exercise, provided adequate glycogen reserves were present, thus sustaining plasma glucose levels at adequate levels early in exercise. However, later in exercise when glycogen stores become severely depleted, hepatic output of glucose would have to increase to keep pace with muscle utilization. If not, plasma glucose would fall to abnormally low levels, a condition which has been associated with fatigue (Christensen and Hansen, 1939). Vitamin B-6, through its active involvement in gluconeoegenisis as a coenzyme for transaminases, should theoretically help in the maintenance of plasma glucose levels during the later stages of exercise. Unfortunately, the results of this study do not fully support these hypotheses because both the control and supplemented conditions resulted in less than a 4% change in plasma glucose over time. Based on an average of the Dill and Costill and Van Beaumont methods for calculating plasma volume changes, mean plasma volume changes were 4.2%, 5.5%, and 2.1% more negative in the supplemented test versus the non-supplemented test, at during-, post-, and post-60 minutes of exercise, respectively. Adjusting for these differences in mean plasma volume changes for test1 and test2 would make the during and post-60 mean plasma glucose values more similar, and would make the post-exercise means more different.

Although the post-exercise means increase in test1 and decrease in test2, when correcting for plasma volume changes, there were still no statistically significant differences between the mean plasma glucose concentrations for both tests.

Previous studies in the area of vitamin B-6 and exercise have observed either an increase (Lawrence et al., 1975), a decrease (Marconi et al., 1982; Virk, 1992) or no change at all (Manore and Leklem, 1987) in mean plasma lactate concentrations during exercise following a period of vitamin B-6 supplementation compared to a control situation. In this study it was hypothesized that plasma lactate levels would be lower during and post-exercise in a vitamin B-6 supplemented state relative to a non-supplemented state. This hypothesis was based primarily on the findings of Virk (1992), where it was observed that mean plasma lactate concentrations showed a trend of being between 6-23% lower at all plasma sample collection points (pre, during, and post-exercise) in the vitamin B-6 supplemented state compared to a non-supplemented state, possibly due to an increased rate of gluconeogenesis as a result of supplementation. Lactate can be either directly oxidized for energy (Brooks, 1988) or can be converted to glucose/glycogen by the process of gluconeogenesis (Stanley et al., 1986). In contrast to the pre-study hypothesis, in this study mean plasma lactate levels tended to be higher under conditions of vitamin B-6 supplementation versus a control situation. Both exercise intensity (Hurley et al., 1986) and diet (Bergstrom et al., 1967; Karlsson and Saltin, 1971; Bergstrom and Hultman, 1972) can potentially alter plasma substrate (i.e. lactate) levels. However, exercise intensity and diet were essentially identical in this study for both the first and second diet periods. A possible explanation for some of the observed difference may be in part due to the greater decrease in plasma volume in the vitamin B-6 supplemented exercise test compared to the non-supplemented test, which would result in a greater hemoconcentration in test2 relative to test1. However, the plasma volume changes do not account for the majority of the observed differences, thus suggesting a possible vitamin B-6 effect on circulating plasma lactate levels. For example, at the during-exercise time point, the

mean plasma lactate levels were 33% higher in the vitamin B-6 supplemented test compared to the non-supplemented test. Assuming plasma volume changes are correct, they can only account for approximately 6% of the total change in the during-exercise mean plasma lactate levels. The results of this study are consistent with those observed by Lawrence et al. (1975), where subjects who received oral PN supplements of 51 mg/day exhibited significantly higher (i.e. 42%) plasma lactate levels during swimming exercise compared to a control trial. Unfortunately, they offered no explanation for their findings. As mentioned in the discussion on plasma glucose, vitamin B-6 is thought to possibly enhance carbohydrate metabolism by enhancing a more rapid rate of glycogen metabolism. The lactate results of this study support this hypothesis, particularly at the during-exercise time point where mean plasma lactates (n=3) were higher (not statistically significant) during test2 compared to test1. An increased rate of carbohydrate metabolism would obviously result in higher tissue and plasma levels of lactate, since only carbohydrates can enter into the Embden-Meyerhof pathway (glycolysis) which ultimately forms lactate from pyruvate (as a reversible end product).

At all specific time points, the absolute mean plasma lactate concentrations observed in this study are higher than the values observed in the 1992 study, particularly in the non-supplemented test. In addition, the mean concentrations are higher than those listed in Table 4 (pg 48). The values in this study as well as those in the 1992 study most closely resemble a combination of the values obtained by Hoffman et al. (1991) and Coyle et al. (1983). In this study (as in 1992), it was observed that mean plasma lactates changed significantly over time during both the supplemented and non-supplemented tests. In both tests the during-exercise and post sample values were statistically elevated relative to each test's respective pre or post-60 values, and in test-2 the during-exercise sample value was statistically greater than the post-value. With the initiation of intense exercise, lactate removal can no longer match lactate production (Brooks, 1985). Thus the plasma lactate level increases during exercise.

The tendency for higher mean plasma lactates under conditions of vitamin B-6 supplementation compared to the non-supplemented condition in conjunction with the glycerol and FFA results does suggest a shift to enhanced carbohydrate metabolism in the supplemented state. Rationally, one would expect that if carbohydrate metabolism increased then lipid metabolism would decrease, since the two combined fuels contribute almost exclusively to energy production during exercise (Lemon and Mullin, 1980; Plante and Houston, 1984). In addition to plasma FFA, plasma glycerol concentration was measured to better estimate the mobilization of FFA from adipose tissue (Hetenyi et al., 1983). Glycerol itself is not considered to be a significant contributor to energy production (Gleeson et al., 1986). Consistent with the pre-study hypotheses/objectives, mean plasma glycerol concentrations tended to be lower during, post, and post-60 minutes of exercise in the vitamin B-6 supplemented test compared to the control test, however, the differences were not statistically significant. Recall that the plasma volume shifts in test2 were also greater than in test1 such that hemoconcentration would result to a larger extent in test2. Thus the mean glycerol values in test2 are potentially even lower than those in test-1 if they were corrected for plasma volume shifts. In general, the absolute values observed in this study are higher (30-100% greater than the plasma glycerol values of Tarnopolsky et al., 1990) in comparison to the glycerol data for the studies listed in Table 4; however, the relative mean plasma glycerol changes over time are not as great in this study compared to the studies within Table 4. An approximate doubling of mean plasma glycerol levels was observed after 60 minutes of exercise, which is consistent with the findings of Hurley et al. (1986). Both Hurley et al. (1986) and Tarnopolsky et al. (1990) found that mean plasma glycerol concentrations increased by 4.3-fold from the start to the end of exercise. Under control conditions in this study, it was found that mean plasma glycerol concentrations increased by 3.4-fold from the start to the finish of exercise compared to supplemental condition in which the rise was only 2.5-fold. Thus, particularly under conditions of vitamin B-6 supplementation, there appeared

to be a suppression of lipolysis which was reflected in lower plasma glycerol levels in test2 relative to test1.

Although not statistically significant, at all of the sample collection time points, the mean plasma FFA levels were lower under conditions of supplementation compared to the control condition despite a greater plasma volume change in test2 relative to test1. This is consistent with the pre-study objective and the hypothesis that vitamin B-6 stimulates glycogenolysis, thereby decreasing FFA mobilization due to decreased utilization. It was observed that four out of five subjects had lower fasting pre-exercise plasma FFA levels in the vitamin B-6 supplemented condition relative to the control (test1). Manore and Leklem (1987) and Virk et al. (1992) found there to be statistically significantly lower mean fasting pre-exercise plasma FFA concentrations following vitamin B-6 supplementation relative to a control situation. All three subjects making it to the during-exercise time point also had lower plasma FFA concentrations following the supplemental phase versus the placebo phase. This could either be reflective of a decrease in total fat oxidation, which is supported by the observed R values or it could be a result of increased intramuscular triglyceride utilization thus reducing the reliance on blood-borne fatty acids. Training (Hurley et al., 1986) and diet (Muoio et al., 1994) are both believed to enhance one's capacity to utilize intramuscular lipid stores. However, these were closely regulated/monitored for both the control and vitamin B-6 supplemented exercise sessions. The absolute values as well as the relative changes over time in mean plasma FFA levels observed in this study are consistent with those listed in Table 4 and with the 1992 study. Although mean plasma FFA increased slightly from pre-exercise to the during-exercise time point in both tests, a 60 minute interval was not sufficient time to result in a statistically significant rise in mean plasma FFA levels relative to pre-exercise levels. Only at exhaustion and after one-hour of recovery were mean plasma FFA levels significantly elevated above resting pre-exercise levels. This is no surprise since fat utilization increases with increasing duration of exercise (Edwards et al., 1934; Christensen and Hansen, 1939) due to

carbohydrate stores becoming depleted and as a result of circulating hormonal regulators of lipid oxidation rising to favorable levels.

The novelty of this study came from the analysis and quantification of 13 individual amino acids and the effect that vitamin B-6 supplementation in conjunction with exhaustive endurance exercise had upon their concentrations within the plasma. Until now, no similar studies that we are aware of have been conducted. Several aspects led to incorporating this aspect of the research into the project. Vitamin B-6 is not only involved in glycogenolysis but also in gluconeogenesis via its role as a coenzyme for a variety of transamination and deamination reactions which convert non-glucose precursors (i.e. selected amino acids) into glucose. Thus it was hypothesized that supplemental vitamin B-6 might stimulate these PLP-dependent reactions and therefore have an effect upon the levels of a variety of amino acids within the plasma. It has been observed both recently (Kang-Yoon and Kirksey, 1992) and previously (Merrow et al., 1966; Park and Linkswiler, 1971) that alterations in dietary vitamin B-6 intake result in plasma increases of several amino acids in humans. Specific amino acid values will be discussed below. In addition, measuring particular amino acids (i.e. alanine and the BCAA) provides a more complete perspective for explaining the levels of the other plasma substrates. For example, in the 1992 study, it was observed that mean plasma glucose levels tended to be higher during exercise following the supplemental phase versus the control phase. The explanation for this observation was that supplemental vitamin B-6 was enhancing the transamination of pyruvate to alanine within the muscle, which through the glucose-alanine cycle subsequently led to a maintenance of plasma glucose via hepatic output throughout exercise in a supplemented state. In the non-supplemented state, a decline in plasma glucose was noted throughout exercise. Recently, Carraro et al. (1994) suggested that there is little doubt that alanine flux from muscle results from an increased intracellular pyruvate. Enhanced glycolysis (i.e. glycogenolysis), which potentially occurs with B-6 supplementation, would result in higher intracellular concentrations of pyruvate. This in turn would favor the release

of alanine from the muscle and alanine would travel via the circulatory system to the liver where gluconeogenesis would progress.

Of all the amino acids quantitated, there was an observed vitamin B-6 effect only on tryrosine and methionine, and there was an exercise time effect only on alanine and histidine. Although mean plasma tyrosine levels tended to be lower at all of the sample time points in test2 relative to test1, only the post-60 minute sample resulted in a statistical difference between the tests ($p = 0.007$). The significance of this is difficult to explain at best and would appear to have no relevance to fuel utilization. However, the lower mean plasma tyrosine levels in the vitamin B-6 supplemented condition relative to the unsupplemented condition can be somewhat explained by the results of other studies. In a study by Donald et al. (1971), young adult women were fed a diet low in vitamin B-6, which resulted in significant increases in the plasma concentration of tyrosine as well as the concentration of numerous other amino acids (i.e. serine, threonine, glutamic acid, and methionine). The diet low in vitamin B-6 was suggested to result in a temporary shifting of free amino acids into the plasma followed by removal of some amino acids either by redistribution in tissues or by excretion. Possibly with supplemental vitamin B-6, as this study incorporated, plasma tyrosine levels would shift in the opposite direction. It has been observed in men between the ages of 20-30 years who were given 40 mg/day doses of PN-HCl that plasma concentrations of serine, tyrosine, and histidine decreased by the end of the 30 day study (Speitling et al., 1988 as cited by Kang-Yoon and Kirksey, 1992). One of the metabolic fates of tyrosine is its conversion to dopamine and further to the catecholamines, norepinephrine and epinephrine. One of the enzymes in this conversion is DOPA decarboxylase. Supplemental vitamin B-6 has been shown to result in elevated DOPA decarboxylase (PLP-dependent) activity in animals (Roberge, 1976) which might therefore enhance the flux of tryosine through the pathway, possibly resulting in lower levels of plasma tyrosine. In this study, following an overnight fast and 60 minutes of recovery from exhaustive endurance exercise, there would obviously be an increased need for elevated

plasma catecholamines as lipolysis and gluconeogenesis would be the primary pathways for energy in the body. Therefore tyrosine may have been utilized in the production of catecholamines. In fact, the catecholamine data generated by Jenny Young in another portion of this study showed that mean plasma catecholamine levels were 13% and 8% higher at post 60 minutes of exercise in test2 compared to test1 for epinephrine and norepinephrine, respectively.

By the end of exercise in test2, mean plasma methionine levels were statistically ($p = 0.03$) lower in comparison to test1 means despite a 4-5% greater plasma volume change in test-2 relative to test-1. The significance of this finding, as mentioned in the tyrosine discussion, has limited application to fuel utilization and furthermore to exercise performance. However, from purely a research perspective, the finding is significant. Currently, there is little information regarding the effect of large supplemental doses of vitamin B-6. Kang-Yoon and Kirksey (1992) suggest that in view of the current widespread therapeutic use of large doses of vitamin B-6 in the treatment of various disorders (e.g. premenstrual syndrome and psychosis), studies of the effects of large doses of vitamin B-6 on plasma amino acid concentrations appear warranted. It is difficult to explain why it required a mean exercise time of approximately 93 minutes for mean plasma methionine levels to show a statistically significant change in test2 relative to test1.

One of the pre-study objectives was to determine if selected glucogenic amino acids would be higher during the vitamin B-6 supplemented exercise test compared to the placebo test. In particular, it was expected that alanine would show an increase in the plasma during exercise following supplementation as a result of supplemental vitamin B-6 enhancing the muscle's transaminase capabilities and therefore the transamination process of pyruvate to alanine. Alanine would then be released into the plasma, where its concentration would increase, and would readily be taken up by the liver as a gluconeogenic substrate. It was observed that at all blood collection time points, with the exception of post-60 minutes of exercise, there was

a trend for higher mean plasma alanine levels in test2 (vitamin B-6 supplemented) compared to test1. Recently, Kang-Yoon and Kirksey (1992) have observed that after 14 days of vitamin B-6 supplementation of 27 mg/day in young women, plasma alanine concentrations significantly increased by approximately 16%. They suggest this response to supplementation to be a result of accelerated protein and/or amino acid metabolism. It was observed that mean pre-exercise plasma alanine concentrations were higher (not statistically significantly) in the vitamin B-6 supplemented condition versus the non-supplemented state. Although not statistically significant, in this study mean plasma alanine concentrations were 14% higher in the supplemented condition versus the control condition at the during-exercise time point. In addition, mean plasma levels of each of the BCAA tended to be higher in the supplemented condition at 60 minutes into exercise (i.e. remained near pre-exercise levels or decreased very little). Bazzare et al. (1992) noted that after 45 minutes of exercise at 75% VO_2 max in men, there was approximately a 7-8% decrease in plasma BCAA levels, which is similar to the results observed in this study in test1 but not in test2. What potentially is occurring to the plasma levels of BCAA in this study under conditions of vitamin B-6 supplementation can be explained by the interorgan relationship between the skeletal muscles and the liver as they both relate to amino acid metabolism. Within muscle tissue there is a relatively low activity of branched-chain keto acid dehydrogenase and a relatively high activity of branched-chain keto acid transaminase in comparison to the liver, which shows a reversal of these activities. As exercise progresses and thus the need for gluconeogenesis, within the muscle the BCAA would act as nitrogen donors to form alanine from pyruvate (PLP-dèpendent) as a result of high transaminase activity. Both the branched chain keto acids (BCKA) and alanine would exit the muscle tissue, and hence alanine would increase within the plasma. Within the liver the BCKA would be reaminated from various other amino acids and nitrogen containing compounds and released into the circulation and ultimately travel to the active muscles such that they could repeatedly be used to transaminate pyruvate to alanine. Hence the plasma levels of the BCAA

would increase with the increased need for gluconeogenesis. Consistent with this rationale, increased blood alanine and BCKA turnover have been reported during exercise (Evans et al., 1983; Dohm et al., 1985). Of all the amino acids quantitated, alanine showed the greatest statistically significant percentage changes over time with peak concentrations occurring after one-hour of exercise in both tests, followed by a slight decline from the during-exercise to the post-exercise time point. By the end of 60 minutes of recovery, mean plasma alanine levels had returned to essentially baseline levels. Whole body alanine release does increase in exercising humans (Wolfe et al., 1984), due in most part to muscle release (Felig and Wahren, 1971).

Similar to alanine, histidine plasma levels increased significantly over time in both exercise tests. However, there were no statistically significant differences between the plasma concentrations despite the fact that mean plasma histidine levels were between 1-10% higher at all blood sampling points in the supplemented exercise test compared to the unsupplemented test. Others have observed either no change (Kang-Yoon and Kirksey, 1992) or a decrease (Speitling et al., 1988 as cited by Kang-Yoon and Kirksey, 1992) in mean plasma histidine levels following two to four weeks of PN supplementation of greater than the 20 mg/day administered in this study. Vitamin B-6 (as PLP) is involved as a coenzyme in the metabolism of histidine in two distinct metabolic pathways. These include the primary pathway of the deamination of histidine with the ultimate formation of glutamate and the decarboxylation of histidine to histamine. During exercise, the deamination pathway would be expected to increase as the body works to maintain a steady output of hepatic glucose. Unfortunately, glutamic acid was not detectable with the amino acid methodology used in this study. If measurable, glutamate might provide a better explanation of the different trends between the tests as well as the changes over time in mean plasma histidine levels.

Originally, fatigue in the exercising muscle was linked to either a depletion of available fuel in the form of carbohydrate or to metabolic acidosis as a result of lactate accumulation within

the muscle, thus suggesting peripheral mechanisms of action. However recently, the concept of central/mental fatigue has received interest in the area of exercise physiology as a potential factor in an individual's capacity to perform physical activity for extended periods of time (Newsholme et al., 1992). Of particular importance is the ratio of tryptophan relative to the LNAA (i.e. BCAA, tyrosine, and phenylalanine), with higher ratios resulting in the formation of the sleep-inducing neurotransmitter serotonin. In test1 and test2, the ratios were similar and equalled 0.095 and 0.097, respectively. Although PLP is directly involved as a coenzyme in the metabolism of tryptophan to serotonin and as well is involved in the metabolism of each of the LNAA, there was no significant difference in the tryptophan:LNAA ratio between the supplemented and non-supplemented exercise tests, thus suggesting no vitamin B-6 effect on central fatigue (as related to amino acid levels). Although not statistically significant, at all specific time points except for post-60 minutes of exercise, mean plasma tryptophan concentrations were lower in the vitamin B-6 supplemented test compared to the non-supplemented test. On an individual basis, in four out of five subjects it was observed that plasma tryptophan levels were lower in test2 at both pre-exercise time points and at the post exercise time point. Only subject #2 consistently had higher plasma tryptophan concentrations in the vitamin B-6 supplemented test relative to the non-supplemented test. This particular subject did show approximately an 8% greater plasma volume change in test2 (supplemented) relative to test1, thus possibly explaining his higher plasma tryptophan levels in the vitamin B-6 supplemented test versus the non-supplemented test. These limited data suggest that vitamin B-6 may potentially have an effect to reduce plasma tryptophan levels.

The absolute values of each of the amino acids quantitatied are consistent with the values listed in Table 5 (pg 50) with the exception of alanine and arginine, which are respectively 24% lower and 32% higher than the values in the table. However, the mean plasma alanine concentrations in this study are very similar to those of Milsom (1979) and the mean concentrations of arginine are very similar to those of Ye et al. (1989). The use of

sulphosalicylic acid as a deproteinizing agent has recently been shown to result in an interfering peak at or near where arginine elutes (Aristoy and Toldra, 1991), thus providing a possible explanation for the relatively high arginine values observed in this study. The plasma proline values in this study are consistent with those obtained by Jeevanandam et al., (1989).

During the first one and one-half hours of exercise, the mean R values in this study tended to be 0.02 units higher in vitamin B-6 supplemented test relative to control test. Assuming all subjects were exercising below their respective anaerobic/lactate thresholds (AT/LT), then this translates out to approximately a 6-7% increase in carbohydrate utilization with a similar decrease in fat utilization in test2 compared to test1. Since the AT/LT was not actually measured and since there was the likelihood that some subjects might have been exercising at an intensity above their AT/LT, an accurate percentage breakdown of carbohydrate and fat cannot be provided. Exercising above one's AT/LT would tend to mistakingly add to the proportion of carbohydrate utilized for energy and falsely decrease the proportion of fat oxidized. In endurance trained individuals the AT/LT usually occurs at 70% to 80% of a persons VO_2 max (Farrell et al., 1979). Assuming that subjects were exercising at a level below their AT/LT, then the mean contributions of carbohydrate and fat over the first 90 minutes of exercise to the total energy expenditure are 77.4% and 22.6% for test2 and 70.8% and 29.2% for test1. These findings are consistent with the work of Campuzano (1988) and Virk (1992) as well as with the prestudy hypothesis of anticipating higher R values following a period of vitamin B-6 supplementation as compared to a control period. This supports the hypothesis of a more rapid rate of glycogenolysis under conditions of vitamin B-6 supplementation.

Interestingly, it was observed that mean VO_2 values were statistically higher ($p = 0.02$) during the first 10 minutes of exercise in the supplemented exercise test compared to the non-supplemented test. In addition, at four subsequent collection time points surrounding the one-hour mark there was a trend for all individual subject's oxygen consumption values to be higher

in test2 than in test1. These findings are in complete contrast to the 1992 study, in which it was found that mean oxygen consumption values were in fact lower, however, not statistically different, at all similar collection time points between the tests. Since submaximal oxygen consumption directly increases with increasing exercise intensity (Weneger and Bell, 1986), this raised the potential question of whether subjects were exercising at higher intensities in test2 compared to test1. There was no difference in mean heart rates between the two tests as would be expected from different intensities of exercise. Marconi et al. (1982) observed that, following a period of PN-alphaketoglutarate supplementation, individual's max VO_2 values increased by 5.8%. However, neither PN nor alphaketoglutarate alone produced similar results. Thus similar findings of a B-6 effect on oxygen consumption have not yet been documented elsewhere.

As in the 1992 study, other major questions of this study were to determine: 1) if vitamin B-6 supplementation would affect fuel utilization during exhaustive endurance exercise in trained males, and 2) how such possible alterations in fuel metabolism would affect performance. The second question provided a practical application to this somewhat technical research. Since fatigue or exhaustion has been highly correlated with muscle glycogen depletion (Bergstrom et al., 1967), and since supplemental vitamin B-6 is thought to enhance the rate of glycogenolysis within muscles (Manore and Leklem, 1987), it was hypothesized prior to this study that exercise following a period of vitamin B-6 supplementation would result in shorter submaximal, exhaustive, endurance times compared to a non-supplemented period. Virk's findings in 1992, in which supplemental vitamin B-6 had no effect upon endurance times to exhaustion at submaximal intensities were supported in this study. Vitamin B-6 had no effect upon exercise times to exhaustion as mean times differed by less than three minutes between the tests. Thus based on these studies, it cannot be recommended that endurance athletes not supplement their diets with relatively high intakes of vitamin B-6 due to the possibility of a detrimental effect on performance. Although there is some evidence to suggest

that vitamin B-6 may be affecting fuel utilization during exercise, the effect does not appear to be of such a magnitude that one's endurance capacity is compromised.

Other factors, which despite our knowledge of are uncontrollable, may have in fact overshadowed any vitamin B-6 effect. For example, boredom or lack of motivation is definitely a potential state of being as an individual endlessly cranks the pedals of a cycle ergometer for several hours. An individual can literally feel as if he/she is simply "spinning their wheels". Therefore, a person may choose to prematurely terminate an exercise session, not because of fatigue or glycogen depletion but as a result of boredom. Muscle tissue biopsies are currently the most direct technique available for measuring glycogen depletion, in contrast to using the indirect method of endurance times to exhaustion. However, the resources of this laboratory are not set up to perform this invasive biopsy sampling procedure. Another potential reason for not observing a vitamin B-6 effect upon endurance times to exhaustion is that an order/training effect may have occurred. Primarily as a result of the length of time required for the effects of vitamin B-6 supplementation to return to baseline levels within the body in combination with our limited resources (i.e. budget, time, personnel), it was highly impractical to randomize each subject's supplemental phase. Thus all subjects were tested for a vitamin B-6 effect following a previous placebo test; and, although a single-blind design was chosen, this may have contributed to an order/training effect as subjects were more familiar with the test the second time through the protocol.

Although one subject (#2) did consume significantly ($p = 0.0008$) fewer kilocalories (222 kcal) in the second controlled dietary period compared to the first period, it is highly unlikely that this contributed to any differences between the tests in either plasma substrate levels or endurance times to exhaustion. The dietary periods were tightly controlled in both macronutrient and micronutrient composition. Thus body weights were not different in subjects between the two exercise tests nor was there a difference in vitamin B-6 content of the diet between the two separate feeding periods.

In conclusion, the plasma substrate data, expired gas results, and exercise times to exhaustion in this study do not provide conclusive evidence for or against a vitamin B-6 effect on fuel utilization during exhaustive endurance exercise in young men. Although there were no statistically significant effects on plasma glucose, lactate, glycerol, and FFA levels, there were observed trends for mean plasma lactate levels to be higher and mean plasma glycerol and FFA levels to be lower under conditions of vitamin B-6 supplementation compared to a non-supplemented condition. Thus, along with a trend for higher R values following a period of supplementation, providing some evidence of an enhanced rate of carbohydrate metabolism or glycogenolysis in a vitamin B-6 supplemented state relative to a non-supplemented state. However, despite whether an effect is present or not, vitamin B-6 consistently appears not to have a strong enough effect such that one's endurance capacity is compromised.

Statistically significant differences were observed between the two tests for mean plasma tyrosine and methionine levels at selected blood collection time points. However, the relevance of these differences to energy production during exercise is most likely insignificant. Of importance, however, may be the observed trends for higher mean alanine levels during the vitamin B-6 supplemented exhaustive ride compared to the non-supplemented ride. This may have implications in sustaining plasma glucose at adequate levels during prolonged exercise via the process of gluconeogenesis.

The observed trends in this study may have been statistically significant differences had there been a greater number of subjects. Since both the 1992 study and this study were essentially identical in design, there is the possibility of combining the data which are common to both studies. This would obviously increase the statistical power and increase the likelihood of finding statistically significant differences between the non-supplemented and vitamin B-6 supplemented conditions, provided that the data showed consistent trends in both studies. In order to draw more definitive conclusions from additional research projects in this area, it

is recommended that a minimum of 8-10 subjects be used in future studies investigating the relationship between vitamin B-6, fuels, and amino acids.

As a result of this study, there are still several unanswered questions. The first which comes to mind is whether further vitamin B-6 and exercise studies should be conducted, and second is, if additional research is worthy of continuation, how can we better test the possibility of vitamin B-6 having an effect on energy metabolism during exercise? Being an individual who habitually exercises on a daily basis and one who has at times competed in athletic events, I am obviously biased towards continuing this line of research. Any performance conscious athlete is continually striving for a competitive edge, be it through mental and physical training, dietary habits, or any practice which may potentially enhance their ability to perform. From the data gathered in the 1992 study as well as in this study, there is some evidence to suggest that vitamin B-6 may be altering fuel metabolism in men during exercise to exhaustion at submaximal intensities. Although the research up to this point does not suggest that vitamin B-6 supplementation affects performance, simple study design flaws may be the reason for not observing a vitamin B-6 effect on performance. One of the limitations to this area of research is that it requires strict dietary control and thus a large demand on resources (i.e. time, budget, peronnel). This makes it difficult to test a large number of subjects and it necessitates an almost perfect study design. If future studies in the area of vitamin B-6 and exercise are to continue, I would recommend three relatively minor modifications of the study design, which might help uncover any potential vitamin B-6 effects on exercise performance and fuel utilization. First and foremost is that prior to initiation in the study all subjects have their anaerobic/lactate (AT/LT) threshold determined. The AT/LT should be used to set a given individual's intensity level, not an individual's VO_2 max per se. By setting an individual's workload safely below their AT/LT, it should allow individuals to exercise longer, which would not only increase the number of data points for an individual but it would also increase the likelihood of glycogen depletion and not metabolic acidosis causing the

fatigue. By quantifying the AT/LT, it would also allow a more accurate quantification of the amount of carbohydrate and fat utilized for energy. The second modification of this study design would be to regulate water intake while subjects are exercising. This should theoretically minimize plasma volume changes and thus better help compare plasma fuel levels between the control and experimental tests. In addition, by providing fluids during exercise, it would minimize the potential of dehydration causing fatigue. The third and final modification of the study design would be to insist that subjects abstain from any form of exercise for a minimum of 72 hours prior to their exercise tests. This requires subject compliance, but from a study design perspective it ensures that glycogen stores would be at high levels prior to exercise, and thus increase one's capacity to perform for long durations of time. These relatively minor changes would better help to test a vitamin B-6 effect by reducing the effect of additional variables on fuel utilization during exercise.

It is hoped that the findings of this study will not only stimulate additional vitamin B-6 and exercise studies, but that further research, be it exercise or not, will be conducted on the relationship between vitamin B-6 and plasma amino acid levels. Both vitamin B-6 depletion and supplementation can significantly alter an individual's plasma amino profile. Currently, there is widespread therapeutic use of pharmacological doses of vitamin B-6 in the treatment of various disorders; however, the clinical significance in relation to amino acids has not been established. With limited data available in this area (particularly in relation to vitamin B-6 supplementation), it is recommended that future studies investigate this relationship between vitamin B-6 and amino acids.

SUMMARY AND CONCLUSIONS

It was hypothesized that supplemental vitamin B-6 (i.e. 20 mg/day PN) would alter plasma fuel substrate and amino acid concentrations 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 concentrations would be higher during exercise with supplemental vitamin B-6, 2) to determine if plasma lactate, glycerol, and FFA levels would be lower during exercise with supplemental vitamin B-6, 3) to determine if selected amino acid concentrations would be higher during exercise with supplemental vitamin B-6, 4) 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 five, trained, male cyclists. They were exercised to exhaustion twice at $74.5 \pm 7.8\%$ 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 seven 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 (i.e. plasma) was analyzed for glucose, lactic acid, glycerol, FFA, and amino acids.

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.

There were no statistically significant effects of vitamin B-6 supplementation on plasma glucose, lactate, glycerol, and FFA. However, there were statistically significant changes over time for each of the aforementioned substrates with the exception of plasma glucose. Although not statistically significant, there were observed trends for R values to be higher in the vitamin B-6 supplemented state versus the unsupplemented state, thus providing some evidence for a vitamin B-6 effect upon fuel metabolism. Despite this possible vitamin B-6

effect, there was no observed effect on endurance performance, as determined by exercise time to exhaustion. Statistically significant differences were observed between the two tests for mean plasma tyrosine ($p=0.007$) and methionine ($p=0.03$) levels at distinct plasma sampling points. In addition, both plasma alanine and histidine concentrations changed statistically significantly over time during exercise in both tests. During the first 10 minutes of exercise, mean oxygen consumption values were statistically significantly higher ($p=0.02$) in the vitamin B-6 supplemented condition compared to the non-supplemented condition. The results of this study suggest that vitamin B-6 may have an effect upon energy metabolism during moderate intensity endurance exercise; however, the effect is highly variable among individuals and does not consistently compromise or enhance performance.

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APPENDIX

Table A.1. Individual plasma lactate concentrations (mmol/L)

Subject		Pre-1	Pre-2	During	Post	Post-60
1	Control	1.07	1.74	3.21	3.15	1.28
	B-6	1.28	1.28	3.43	2.34	1.47
2	Control	1.14	0.95		3.34	1.15
	B-6	0.94	0.87		2.51	1.07
3	Control	0.83	0.73		2.08	0.98
	B-6	0.91	0.87		2.90	0.88
4	Control	0.88	0.95	1.91	3.12	1.29
	B-6	0.98	1.25	3.30	2.79	0.89
5	Control	1.11	1.33	2.81	3.34	1.34
	B-6	1.49	1.88	5.04	3.62	1.75

Pre-1 and Pre-2: prior to exercise; During: 60 minutes into exercise;
Post: immediately after exercise; Post-60: 60 minutes after exercise
Control refers to the unsupplemented exhaustive endurance ride
B-6 refers to the supplemented exhaustive endurance ride

Table A.2. Individual plasma glucose concentrations (mmol/L)

Subject		Pre-1	Pre-2	During	Post	Post-60
1	Control	4.57	4.60	4.69	5.10	4.84
	B-6	4.38	4.40	4.87	4.68	4.68
2	Control	4.78	4.71		5.44	4.81
	B-6	4.61	4.67		4.15	4.83
3	Control	4.94	5.04		5.53	4.79
	B-6	4.49	4.98		6.19	4.75
4	Control	4.46	4.37	4.64	4.37	4.40
	B-6	5.05	4.87	4.83	4.61	4.68
5	Control	5.31	5.42	5.08	4.72	5.29
	B-6	5.50	5.46	5.13	5.14	5.37

Pre-1 and Pre-2: prior to exercise; During: 60 minutes into exercise;
Post: immediately after exercise; Post-60: 60 minutes after exercise
Control refers to the unsupplemented exhaustive endurance ride
B-6 refers to the supplemented exhaustive endurance ride

Table A.3. Individual plasma glycerol concentrations (mmol/L)

Subject		Pre-1	Pre-2	During	Post	Post-60
1	Control	0.118	0.120	0.319	0.583	0.387
	B-6	0.195	0.187	0.358	0.496	0.297
2	Control	0.189	0.193		0.704	0.300
	B-6	0.246	0.195		0.489	0.244
3	Control	0.161	0.169		0.568	0.233
	B-6	0.186	0.200		0.528	0.223
4	Control	0.283	0.268	0.616	0.712	0.390
	B-6	0.216	0.205	0.411	0.378	0.241
5	Control	0.236	0.215	0.451	0.771	0.390
	B-6	0.258	0.244	0.400	0.822	0.444

Pre-1 and Pre-2: prior to exercise; During: 60 minutes into exercise;
Post: immediately after exercise; Post-60: 60 minutes after exercise
Control refers to the unsupplemented exhaustive endurance ride
B-6 refers to the supplemented exhaustive endurance ride

Table A.4. Individual plasma FFA concentrations (mmol/L)

Subject		Pre-1	Pre-2	During	Post	Post-60
1	Control	0.255	0.390	0.633	1.817	1.078
	B-6	0.251	0.373	0.557	1.208	1.055
2	Control	0.461	0.573		1.631	0.711
	B-6	0.555	0.379		0.880	0.491
3	Control	0.723	0.567		0.928	0.551
	B-6	0.413	0.431		0.986	0.571
4	Control	0.571	0.439	0.756	1.159	1.182
	B-6	0.322	0.287	0.455	0.464	0.723
5	Control	0.313	0.287	0.453	1.182	1.177
	B-6	0.375	0.362	0.377	1.808	1.266

Pre-1 and Pre-2: prior to exercise; During: 60 minutes into exercise;
Post: immediately after exercise; Post-60: 60 minutes after exercise
Control refers to the unsupplemented exhaustive endurance ride
B-6 refers to the supplemented exhaustive endurance ride

Table A.5. Individual plasma tryptophan concentrations (umol/L)

Subject		Pre-1	Pre-2	During	Post	Post-60
1	Control	63.41	65.40	60.62	53.30	53.39
	B-6	59.34	56.71	57.83	49.90	56.18
2	Control	51.89	52.81		51.51	49.08
	B-6	57.87	61.61		58.10	58.03
3	Control	53.49	50.26		60.29	49.08
	B-6	44.61	44.61		56.07	53.30
4	Control	71.14	71.94	64.22	63.11	49.75
	B-6	67.71	65.87	65.43	62.78	48.90
5	Control	60.50	61.63	59.21	56.66	56.52
	B-6	52.19	50.46	57.46	53.50	56.38

Pre-1 and Pre-2: prior to exercise; During: 60 minutes into exercise;
Post: immediately after exercise; Post-60: 60 minutes after exercise
Control refers to the unsupplemented exhaustive endurance ride
B-6 refers to the supplemented exhaustive endurance ride

Table A.6. Individual plasma serine concentrations (umol/L)

Subject		Pre-1	Pre-2	During	Post	Post-60
1	Control	121	128	130	118	106
	B-6	109	110	108	107	107
2	Control	138	137		138	134
	B-6	150	177		156	148
3	Control	136	133		146	118
	B-6	117	129		120	116
4	Control	179	190	195	184	171
	B-6	194	197	207	192	147
5	Control	146	134	132	135	113
	B-6	121	127	113	116	81

Pre-1 and Pre-2: prior to exercise; During: 60 minutes into exercise;
Post: immediately after exercise; Post-60: 60 minutes after exercise
Control refers to the unsupplemented exhaustive endurance ride
B-6 refers to the supplemented exhaustive endurance ride

Table A.7. Individual plasma threonine concentrations (umol/L)

Subject		Pre-1	Pre-2	During	Post	Post-60
1	Control	182	187	178	164	157
	B-6	142	143	133	133	129
2	Control	173	164		175	160
	B-6	157	178		161	158
3	Control	166	162		165	141
	B-6	139	155		138	159
4	Control	155	162	157	154	146
	B-6	187	194	207	182	139
5	Control	195	186	164	175	161
	B-6	171	180	148	152	106

Pre-1 and Pre-2: prior to exercise; During: 60 minutes into exercise;
Post: immediately after exercise; Post-60: 60 minutes after exercise
Control refers to the unsupplemented exhaustive endurance ride
B-6 refers to the supplemented exhaustive endurance ride

Table A.8. Individual plasma alanine concentrations (umol/L)

Subject		Pre-1	Pre-2	During	Post	Post-60
1	Control	381	404	493	418	349
	B-6	304	308	423	373	264
2	Control	264	275		367	331
	B-6	242	296		403	308
3	Control	230	244		406	258
	B-6	271	307		377	239
4	Control	298	305	457	460	356
	B-6	427	433	742	602	403
5	Control	351	331	424	424	240
	B-6	350	285	439	387	265

Pre-1 and Pre-2: prior to exercise; During: 60 minutes into exercise;
Post: immediately after exercise; Post-60: 60 minutes after exercise
Control refers to the unsupplemented exhaustive endurance ride
B-6 refers to the supplemented exhaustive endurance ride

Table A.9. Individual plasma proline concentrations (umol/L)

Subject		Pre-1	Pre-2	During	Post	Post-60
1	Control	248	250	228	200	203
	B-6	187	184	180	159	163
2	Control	257	230		203	213
	B-6	235	256		235	231
3	Control	196	196		209	163
	B-6	177	192		168	206
4	Control	282	298	286	271	249
	B-6	314	303	346	307	247
5	Control	218	209	196	176	162
	B-6	283	305	239	189	165

Pre-1 and Pre-2: prior to exercise; During: 60 minutes into exercise;
Post: immediately after exercise; Post-60: 60 minutes after exercise
Control refers to the unsupplemented exhaustive endurance ride
B-6 refers to the supplemented exhaustive endurance ride

Table A.10. Individual plasma histidine concentrations (umol/L)

Subject		Pre-1	Pre-2	During	Post	Post-60
1	Control	72	79	83	85	81
	B-6	92	93	96	92	90
2	Control	69	67		82	74
	B-6	66	83		81	76
3	Control	76	76		84	68
	B-6	77	84		84	92
4	Control	81	84	88	88	83
	B-6	86	81	102	92	82
5	Control	79	76	85	88	80
	B-6	77	79	81	81	67

Pre-1 and Pre-2: prior to exercise; During: 60 minutes into exercise;
Post: immediately after exercise; Post-60: 60 minutes after exercise
Control refers to the unsupplemented exhaustive endurance ride
B-6 refers to the supplemented exhaustive endurance ride

Table A.11. Individual plasma tyrosine concentrations (umol/L)

Subject		Pre-1	Pre-2	During	Post	Post-60
1	Control	82	82	74	77	74
	B-6	71	69	71	71	67
2	Control	58	56		71	64
	B-6	63	72		66	75
3	Control	79	71		82	65
	B-6	62	68		69	71
4	Control	80	82	88	86	80
	B-6	77	75	84	73	59
5	Control	66	63	68	84	72
	B-6	70	68	59	77	58

Pre-1 and Pre-2: prior to exercise; During: 60 minutes into exercise;
Post: immediately after exercise; Post-60: 60 minutes after exercise
Control refers to the unsupplemented exhaustive endurance ride
B-6 refers to the supplemented exhaustive endurance ride

Table A.12. Individual plasma valine concentrations (umol/L)

Subject		Pre-1	Pre-2	During	Post	Post-60
1	Control	200	205	174	160	153
	B-6	207	207	194	185	177
2	Control	214	198		216	202
	B-6	205	238		202	191
3	Control	278	270		273	225
	B-6	232	257		237	271
4	Control	277	293	278	251	249
	B-6	298	301	328	273	235
5	Control	215	207	198	202	189
	B-6	231	219	195	193	170

Pre-1 and Pre-2: prior to exercise; During: 60 minutes into exercise;
Post: immediately after exercise; Post-60: 60 minutes after exercise
Control refers to the unsupplemented exhaustive endurance ride
B-6 refers to the supplemented exhaustive endurance ride

Table A.13. Individual plasma methionine concentrations (umol/L)

Subject		Pre-1	Pre-2	During	Post	Post-60
1	Control	39	42	39	42	33
	B-6	36	36	35	35	29
2	Control		23		31	28
	B-6	28	36		30	28
3	Control	31	32		38	26
	B-6	28	32		32	31
4	Control	34	35	38	36	29
	B-6	36	37	41	35	27
5	Control	29	27	31	34	
	B-6	24		20	29	

Pre-1 and Pre-2: prior to exercise; During: 60 minutes into exercise;
Post: immediately after exercise; Post-60: 60 minutes after exercise
Control refers to the unsupplemented exhaustive endurance ride
B-6 refers to the supplemented exhaustive endurance ride

Table A.14. Individual plasma isoleucine concentrations (umol/L)

Subject		Pre-1	Pre-2	During	Post	Post-60
1	Control	81	83	59	56	55
	B-6	75	73	66	61	60
2	Control	61	55		72	63
	B-6	72	82		67	58
3	Control	107	101		103	73
	B-6	68	73		73	79
4	Control	97	101	88	79	79
	B-6	98	99	107	89	78
5	Control	59	57	58	59	56
	B-6	69	62	55	60	60

Pre-1 and Pre-2: prior to exercise; During: 60 minutes into exercise;
Post: immediately after exercise; Post-60: 60 minutes after exercise
Control refers to the unsupplemented exhaustive endurance ride
B-6 refers to the supplemented exhaustive endurance ride

Table A.15. Individual plasma leucine concentrations (umol/L)

Subject		Pre-1	Pre-2	During	Post	Post-60
1	Control	137	144	112	105	106
	B-6	137	141	126	123	123
2	Control	125	125		142	140
	B-6	145	168		144	132
3	Control	107	101		103	73
	B-6	68	73		73	79
4	Control	97	101	163	155	152
	B-6	169	171	203	166	140
5	Control	120	112	123	128	115
	B-6	128	113	111	128	108

Pre-1 and Pre-2: prior to exercise; During: 60 minutes into exercise;
Post: immediately after exercise; Post-60: 60 minutes after exercise
Control refers to the unsupplemented exhaustive endurance ride
B-6 refers to the supplemented exhaustive endurance ride

Table A.16. Individual plasma phenylalanine concentrations (umol/L)

Subject		Pre-1	Pre-2	During	Post	Post-60
1	Control	68	73	76	67	65
	B-6	75	77	79	76	71
2	Control	54	53		79	70
	B-6	69	84		74	70
3	Control	83	71		99	68
	B-6	76	82		81	79
4	Control	77	75	83	78	70
	B-6	71	75	87	73	60
5	Control	63	61	70	86	67
	B-6	57	54	54	79	61

Pre-1 and Pre-2: prior to exercise; During: 60 minutes into exercise;
Post: immediately after exercise; Post-60: 60 minutes after exercise
Control refers to the unsupplemented exhaustive endurance ride
B-6 refers to the supplemented exhaustive endurance ride

Table A.17. Individual plasma arginine concentrations (umol/L)

Subject		Pre-1	Pre-2	During	Post	Post-60
1	Control	175	179	179	178	172
	B-6	141	153	136	153	157
2	Control	175	161		175	158
	B-6	128	170		163	146
3	Control	196	188		176	169
	B-6	165	181		164	196
4	Control	123	158	141	142	152
	B-6	135	166	131	144	130
5	Control	129	119	116	110	132
	B-6	115	154	118	127	109

Pre-1 and Pre-2: prior to exercise; During: 60 minutes into exercise;
Post: immediately after exercise; Post-60: 60 minutes after exercise
Control refers to the unsupplemented exhaustive endurance ride
B-6 refers to the supplemented exhaustive endurance ride

Table A.18. Individual ratings of perceived exertion

Time		Subject Number 1	2	3	4	5
7-10	Control	12	13	12	16	10
	B-6	12	16	13	16	12
17-20	Control	13	13	12	16	11
	B-6	12	16	13	16	12
27-30	Control	14	15	13	17	12
	B-6	13		13	16	12
37-40	Control	14	16	14	17	12
	B-6	13		14	17	12
47-50	Control	14	17	16	17	12
	B-6	14		15	17	11
57-60	Control	15			17	12
	B-6	14			17	11
67-70	Control	14			17	12
	B-6	15			18	11
77-80	Control	15			18	13
	B-6	15			19	11
87-90	Control	15			18	13
	B-6	15				11
97-100	Control	15				13
	B-6	17				11
107-110	Control	16				14
	B-6	17				11
117-120	Control	16				14
	B-6					11
127-130	Control	17				15
	B-6					11
137-140	Control					15
	B-6					12
147-150	Control					
	B-6					12
157-160	Control					
	B-6					12
167-170	Control					
	B-6					13
177-180	Control					
	B-6					13

Time refers to minutes of exercise
Control refers to the unsupplemented exhaustive endurance ride
B-6 refers to the vitamin B-6 supplemented exhaustive endurance ride

Table A.19. Individual R values

Time		Subject Number 1	2	3	4	5
7-10	Control	0.97	0.93	0.91	0.86	0.96
	B-6	0.95	0.89	0.92	0.90	0.97
17-20	Control	0.94	0.94	0.92	0.86	0.93
	B-6	0.96	0.89	0.91	0.86	0.98
27-30	Control	0.93	0.91	0.91	0.86	0.92
	B-6	0.96		0.91	0.89	0.98
37-40	Control	0.91	0.91	0.89	0.89	0.93
	B-6	0.94		0.90	0.88	0.98
47-50	Control	0.95	0.92	0.87	0.87	0.93
	B-6	0.95		0.91	0.88	0.97
57-60	Control	0.95			0.85	0.91
	B-6	0.94			0.87	0.99
67-70	Control	0.96				0.90
	B-6	0.95			0.85	0.95
77-80	Control	0.93			0.83	0.89
	B-6	0.94			0.86	0.93
87-90	Control	0.94			0.82	0.90
	B-6	0.92				0.93
97-100	Control	0.91				0.90
	B-6	0.91				0.90
107-110	Control	0.90				0.88
	B-6	0.90				0.92
117-120	Control	0.93				0.91
	B-6					0.92
127-130	Control	0.90				0.88
	B-6					
137-140	Control					0.88
	B-6					0.89
147-150	Control					
	B-6					0.88
157-160	Control					
	B-6					0.89
167-170	Control					
	B-6					0.88
177-180	Control					
	B-6					0.89

Time refers to minutes of exercise
Control refers to the unsupplemented exhaustive endurance ride
B-6 refers to the vitamin B-6 supplemented exhaustive endurance ride

Table A.20. Individual oxygen consumption values (ml/kg/min)

Time		Subject Number 1	2	3	4	5
7-10	Control	40.34	46.21	33.84	36.68	39.12
	B-6	43.86	50.05	35.60	37.87	40.30
17-20	Control	39.69	47.88	34.68	36.49	40.10
	B-6	43.44	49.93	36.03	38.44	39.13
27-30	Control	39.50	47.29	33.00	37.78	39.26
	B-6	43.62		35.45	37.02	40.59
37-40	Control	39.62	46.89	34.50	37.07	40.25
	B-6	42.84		36.63	37.85	40.15
47-50	Control	39.80	46.79	32.49	37.63	39.71
	B-6	45.63		37.27	37.99	41.00
57-60	Control	39.48			36.69	39.32
	B-6	46.25			40.57	41.86
67-70	Control	41.21				40.14
	B-6	47.21			38.58	40.42
77-80	Control	41.56			35.90	41.06
	B-6	49.43			38.43	40.14
87-90	Control	41.94			37.03	40.77
	B-6	47.98				40.53
97-100	Control	43.25				40.64
	B-6	47.74				41.28
107-110	Control	41.12				40.98
	B-6	47.09				41.38
117-120	Control	43.78				42.62
	B-6					41.70
127-130	Control	43.57				42.53
	B-6					
137-140	Control					42.31
	B-6					42.27
147-150	Control					
	B-6					41.98
157-160	Control					
	B-6					43.77
167-170	Control					
	B-6					42.58
177-180	Control					
	B-6					43.14

Time refers to minutes of exercise
Control refers to the unsupplemented exhaustive endurance ride
B-6 refers to the vitamin B-6 supplemented exhaustive endurance ride

Table A.21. Individual plasma volume changes (%)

Subject		DC During	DC Post	DC Post-60	VB During	VB Post	VB Post-60
1	Control	-5.1	-2.1	1.8	-1.9	3.1	6.2
	B-6	-13.2	-10.6	-5.5	-8.2	-9.1	-4.5
2	Control		-16.9	-0.8		-14.0	7.37
	B-6		-23.6	-4.8		-22.8	-1.6
3	Control		-19.3	-11.7		-18.4	-11.5
	B-6		-19.9	-8.9		-18.3	-5.9
4	Control	-11.1	-10.0	-0.5	-8.6	-7.7	4.1
	B-6	-12.3	-10.4	2.77	-9.53	-7.7	5.1
5	Control	-10.9	-8.3	-7.2	-10.2	-1.6	-2.6
	B-6	-16.2	-16.1	-7.3	-13.2	-11.5	-4.0

DC refers to the Dill and Costill method and VB refers to the Van Beaumont method
Control refers to the unsupplemented exhaustive endurance ride
B-6 refers to the supplemented exhaustive endurance ride
During: 60 minutes into exercise; Post: immediately after exercise; Post-60: 60 minutes after exercise

Table A.22. Individual hematocrit values

Subject		Pre-1	Pre-2	During	Post	Post-60
1	Control	46.25	47.75	47.50	46.25	45.50
	B-6	44.75	45.00	47.00	47.25	46.00
2	Control	45.00	44.50		48.50	43.00
	B-6	41.25	41.00		47.50	41.50
3	Control	41.00	41.50		46.25	44.25
	B-6	43.00	43.00		48.00	44.50
4	Control	48.50	48.50	50.75	50.50	47.50
	B-6	50.50	50.50	53.00	52.50	49.25
5	Control	41.75	42.00	44.50	42.25	42.50
	B-6	43.00	42.00	46.00	45.50	43.50

Pre-1 and Pre-2: prior to exercise; During: 60 minutes into exercise;
Post: immediately after exercise; Post-60: 60 minutes after exercise
Control refers to the unsupplemented exhaustive endurance ride
B-6 refers to the supplemented exhaustive endurance ride

Table A.23. Individual hemoglobin concentrations (gm/L)

Subject		Pre-1	Pre-2	During	Post	Post-60
1	Control	149	152	157	156	152
	B-6	148	147	163	158	153
2	Control	149	145		165	150
	B-6	138	137		160	143
3	Control	142	146		163	154
	B-6	155	153		176	165
4	Control	161	158	172	171	164
	B-6	171	171	185	183	170
5	Control	140	142	151	153	151
	B-6	143	143	160	161	151

Pre-1 and Pre-2: prior to exercise; During: 60 minutes into exercise;
Post: immediately after exercise; Post-60: 60 minutes after exercise
Control refers to the unsupplemented exhaustive endurance ride
B-6 refers to the supplemented exhaustive endurance ride

Table A.24. Individual plasma PLP concentrations (nmol/L)

Subject		Pre-study	Pre	During	Post	Post-60
1	Control	59.4	37.0	38.7	30.0	29.4
	B-6		177	193	174	156
2	Control	209	29.4		36.4	28.7
	B-6		198		227	173
3	Control	18.5	17.6		23.5	15.9
	B-6		133		157	126
4	Control	49.5	32.7	34.9	29.8	24.3
	B-6		170	169	179	144
5	Control	130	45.1	53.8	43.3	36.4
	B-6		183	204	159	132

Pre: prior to exercise; During: 60 minutes into exercise;
Post: immediately after exercise; Post-60: 60 minutes after exercise
Control refers to the unsupplemented exhaustive endurance ride
B-6 refers to the supplemented exhaustive endurance ride
Data taken from Nancy Dunton