AN ABSTRACT OF THE THESIS OF

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Title: The Influence of Exercise on Vitamin B-6

Metabolism

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James E. Leklem

The present study was designed to investigate the effects of exercise on vitamin B-6 metabolism. Four groups of subjects (intermittent, college, untrained, and high school) consisting of nineteen male and two female individuals, participated in the study. The subjects exercised either on a bicycle ergometer, by running three 1500 meter intervals, or both. Blood samples drawn prior to exercise (pre), after exercise (post), and 30 minutes after exercise (30 minute post), were analyzed for plasma pyridoxal 5'-phosphate (PLP), plasma B-6 (PB6), glucose, hemoglobin and hematocrit. A 24-hr urine collection the day before and the day of exercise was analyzed for urinary B-6 (UB6), 4-pyridoxic acid (4PA), creatinine, and urea nitrogen.

The dietary intake of the four groups was greater than the RDA in vitamin B-6, riboflavin, thiamin, niacin, vitamins A and C, calcium and iron. The B-6/protein ratios of the college and untrained groups were adequate while the high school group's ratios were considered inadequate.

The bicycle ergometer had a significant effect on the plasma PLP levels of the college and untrained groups ($P< 0.005$) and PB6 levels of the college group following exercise ($P< 0.005$). The 30 minute post plasma PLP levels were significantly lower for the college group ($P< 0.005$).

During the run, the college group had significantly higher post exercise levels of plasma PLP ($P< 0.005$) and PB6 ($P< 0.005$) as compared to the pre exercise sample. The high school group also had significantly higher levels of plasma PLP following exercise for all three runs ($P< 0.005$, $P< 0.025$, and $P< 0.01$, respectively) as well as higher PB6 levels ($P< 0.025$, $P< 0.01$, and $P< 0.025$, respectively).

The college athletes had a greater percent change in plasma PLP ($P< 0.01$) from the pre to post sample during the run as compared to the high school athletes.

Urinary B-6 and 4PA were not significantly altered during either exercise suggesting a shift in PLP and the unphosphorylated forms of vitamin B-6 from one compartment to another. The significantly higher levels of plasma PLP and PB6 following exercise were attributed to an increased utilization of glycogen phosphorylase in the skeletal muscle with a subsequent release of PLP.

The Influence of Exercise on Vitamin B-6 Metabolism

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"Happy are those who dream dreams and are ready to pay the price to make them come true."

-L. J. Cardinal Suenens

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LIST OF ABBREVIATIONS

PL	Pyridoxal
PN	Pyridoxamine
PN	Pyridoxine
PLP	Pyridoxal 5'-Phosphate
PMP	Pyridoxamine 5'-Phosphate
PNP	Pyridoxine 5'-Phosphate
4PA	4-Pyridoxic Acid
UB6	Total urinary vitamin B-6
NRC	National Research Council
mg	milligrams
gm	grams
µmole	micromole
nmole	nanomole
kp	kilopond
RDA	Recommended Dietary Allowance
$\bar{x}$	mean
S.D.	Standard Deviation

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THE INFLUENCE OF EXERCISE ON VITAMIN B-6 METABOLISM

I. INTRODUCTION

Our understanding of the role of vitamin B-6 in numerous metabolic reactions has been developed and broadened considerably since the vitamin was first discovered. The expansion of this knowledge is often due to the unexpected relationships uncovered from metabolic studies. Such is the case involving the effects of exercise on vitamin B-6 metabolism. During a metabolic study in this laboratory, involving varying doses of vitamin B-6, an unexplained rise in plasma B-6 was reported for one subject. It was discovered that the subject exercised following a test dose of vitamin B-6 and prior to the blood sampling. To further investigate these results, the study was expanded to include exercise for all the subjects involved. The data indicated a positive significant effect on plasma vitamin B-6 resulting from exercise, although the relationship was not clear.

The role of vitamin B-6 in the production of energy needed for exercise has been studied by numerous researchers in the past. However, these studies have not included blood levels of vitamin B-6, urinary excretion of the vitamin, or its major metabolites. It is known that pyridoxal 5'-phosphate is required both in the functioning of the enzyme glycogen phosphorylase, which initiates the conversion of glycogen to glucose-1-phosphate, as well as other vitamin B-6 dependent enzymes involved in the production of glucose through gluconeogenesis. Because of this relationship of pyridoxal 5'-phosphate to energy production, studies have been done to test whether an increase in endurance

could be achieved with an increased intake of vitamin B-6. However, the study did not measure levels of plasma B-6 or pyridoxal 5'phosphate, nor were any urinary measurements conducted (Lawrence et al., 1975). In a Russian investigation using laboratory animals, a major metabolite of vitamin B-6, 4-pyridoxic acid, was measured while the rats were being deprived of pyridoxine, a form of vitamin B-6, during exercise. Although one form of vitamin B-6 was analyzed in the urine, no blood analyses were done.

Thus, the degree to which exercise alters vitamin B-6 metabolism in the body is not yet known. The need for investigating the relationship between an increased need for energy and the alteration, if any, on the various forms of vitamin B-6 in the blood and urine is the purpose of this study. The objectives, therefore, are first, to observe any changes in vitamin B-6 metabolism following exercise. To accomplish this objective, vitamin B-6 in the form of plasma pyridoxal 5'phosphate and plasma B-6 will be measured as well as the urinary excretion of 4-pyridoxic acid and total urinary vitamin B-6. Other blood constituents, such as glucose, hemoglobin, and hematocrit will also be measured. In addition, the urinary output of creatinine, and urea nitrogen will be assessed to complete the laboratory analyses. Secondly, the effects of training on the alteration of vitamin B-6 metabolism will be investigated. It is known that athletes trained for aerobic events utilize more fat and spare glycogen when compared to an untrained athlete. Thus, the trained athletes need for vitamin B-6 may not be as great. Both untrained and trained athletes will be used as subjects in the study. Thirdly, the difference, if any, in an adult athlete's response as

compared to an adolescent athlete, will be studied. An adult depends on vitamin B-6 to repair and maintain the already mature tissues. In addition, an adolescent requires vitamin B-6 for protein metabolism during the peak stages of growth and maturity. To satisfy this objective, both college and high school-aged athletes will be exercised and compared as to their vitamin B-6 response. Finally, the difference between two exercises, namely the bicycle ergometer and running, will be compared.

II. REVIEW OF LITERATURE

Vitamin B-6

Importance

The existence of vitamin B-6 was first established by György in 1934, when the substance was identified as a factor preventing a pellagra-like dermatitis in laboratory rats (György, 1935). Within the next year, the basic chemical structure was identified and isolated. Due to the structure of the vitamin, it was initially given the name pyridoxine until subsequent work by Snell and associates revealed the existence of other forms, i.e., pyridoxal and pyridoxamine (Snell et al., 1942).

Since the essential role of vitamin B-6 in the rat was identified, numerous other functions have been recognized. The versatile vitamin is a coenzyme for over 60 different enzymatic reactions, most of which involve amino acid metabolism (Sauberlich, 1968). The vitamin B-6 dependent enzymes include transaminases, decarboxylases, racemizases, oxidoreductases, isomerases, desulfhydrases, and deaminases. Transaminases are involved in the transfer of the α-amino group of an amino acid such as alanine, arginine, asparagine, aspartic acid, cysteine, isoleucine, lysine, phenylalanine, tyrosine, valine and tryptophan, to the α-carbon atom of an α-keto acid. The formation of these substances are important constituents in the citric acid cycle. Another group of enzymes, decarboxylases, form amines from tyrosine, histidine, dehydroxyphenylalanine and tryptophan. The reaction involving the substrate tryptophan, leads to the formation of the neurotransmitter serotonin. Racemization enzymes convert L-forms of amino acids to

D-forms. Vitamin B-6 is also involved in the conversion of cysteine to pyruvic acid via desulfhydration, oxalate to glycine, and in the synthesis of α-amino levulinic acid, a porphyrin precursor (Sauberlich and Canham, 1973).

In addition to amino acid metabolism, vitamin B-6 is also essential in carbohydrate metabolism and may play a role in fat metabolism (Coursin, 1961). Glycogen phosphorylase, an enzyme that catalyzes the breakdown of glycogen to glucose-1-phosphate, depends on pyridoxal phosphate for activation. In lipid metabolism, the actual function of vitamin B-6 is still unclear, but the role is thought by some researchers to be a secondary one (Sebrell and Harris, 1968; and Sauberlich and Canham, 1973).

Biochemistry

Vitamin B-6 is found abundantly in nature. Six different forms of the vitamin exist: pyridoxal (PL), pyridoxamine (PM), pyridoxine (PN), and the phosphorylated forms pyridoxal 5'-phosphate (PLP) pyridoxamine 5'-phosphate (PMP), and pyridoxine 5'-phosphate (PNP). Figure 1 illustrates the chemical structures of each form and their interconversions. Generally, the vitamers exist as white crystals and are soluble in water. PM, PN, and PL, and probably their phosphorylated forms, are unstable in visible and ultraviolet light when in an alkaline or neutral solution and PL in particular is easily destroyed by heating (Snell, 1981). The biological activity of the forms of the vitamin differ. The conversion of pyridoxal to 4-pyridoxic acid (4PA) occurs via aldehyde oxidase and/or aldehyde dehydrogenase. This metabolic

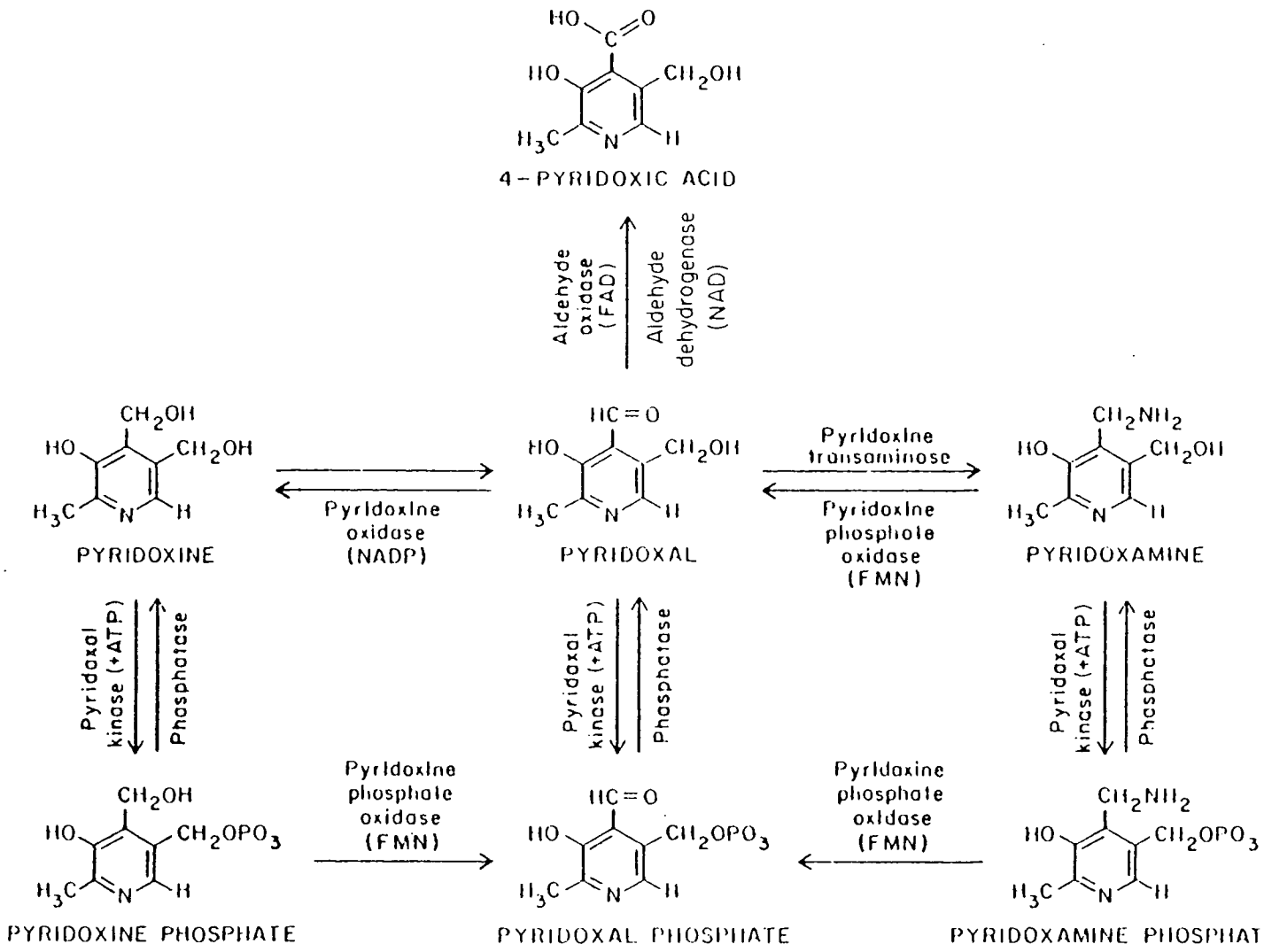

Figure 1. Interconversions and structures of vitamin B-6.

by-product is not biologically active, cannot be reconverted to active form, and is therefore excreted in the urine (Brin, 1978). PLP is the most active form of vitamin B-6, although pyridoxamine 5'-phosphate (PMP) can also activate a number of B6-dependent enzymes (Sauberlich and Canham, 1973; and Linkswiler, 1967). PN, PM, and PL are converted through enzymatic pathways to the PLP form.

Sources

Vitamin B-6 is widely distributed in a variety of foods. PL and PM, as well as their phosphorylated forms, predominate in animal products. PN is found primarily in foods of plant origin (Rabinowitz and Snell, 1948). Most of vitamin B-6 in nature is found in the bound form, probably as enzyme-coenzyme complexes (Brin, 1978).

Absorption

Absorption of vitamin B-6 has been shown to occur rapidly in the jejunum, with smaller amounts absorbed in the ileum (Booth and Brain, 1962). Serebro et al. (1966) suggested that absorption occurs by passive diffusion. This is supported by Middleton's research (1972), utilizing an everted sac technique. Phosphorylated forms of the vitamin are hydrolyzed to the free form before being absorbed. The hydrolysis occurs through the enzymatic action of alkaline phosphatase which is present in mammalian intestinal tissues (Yamada, 1980).

Determination of the extent of absorption was initially done by measuring urinary excretion of the vitamin. Rabinowitz and Snell (1948) measured the urinary excretion of various forms of vitamin B-6 in

humans following large doses of PN, PM, and PL. Results indicated that with saturation of the vitamin, the amount of urinary excretion was greater. Studies done in this laboratory (Wozenski et al., 1980) indicate a difference in the rate of absorption and/or metabolism of the three forms of vitamin B-6. With equimolar doses of PL, PN, and PM, the urinary excretion of 4PA was greater after the ingestion of PL than PN and PM. Total urinary excretion of vitamin B-6 was lower after PL than PN and PM.

Levels of vitamin B-6 in the blood have also been measured. The form of B-6 that predominates in the plasma has not been clearly determined. Sauberlich et al. (1972) stated that the major form is PLP, whereas Anderson et al. (1971) and Kelsay et al. (1968) reported it to be PL. Anderson measured changes in whole blood, plasma, and red cells after oral ingestion of 50 mg of PN. Both PL and PLP were found in the red cells, but only PL was found in plasma. Kelsay et al. found only PL in the blood when subjects were given daily 1.66 mg or less of vitamin B-6. When given 50 mg of PN for 2 days prior to a fasting blood sample, Kelsay et al. found small amounts of PN, however, no PM was observed. Sauberlich argues that the form present is PLP but that hydrolysis during analysis forms PL, which would explain Kelsay's and Anderson's observations. In support of Sauberlich, Lumeng et al. (1974) and Wozenski et al. (1980) have shown that plasma contains significant amounts of PLP.

Interconversions of Vitamin B-6

The interconversions of vitamin B-6 are shown in Figure 1. The conversion of PN to the active forms was first demonstrated in rabbit liver extracts (Wada et al., 1959). Lumeng et al., (1974), utilizing

dogs, support this research and state that the main site of production of plasma PLP from either PL or PN is the liver. Once phosphorylated, the PLP leaves the liver bound to albumin (Li et al., 1974). Interconversion also occurs in the red blood cell (Anderson et al., 1971). Lumeng and Li (1974) confirmed the pathway in the red cell by identifying the presence and activity of the enzymes needed for conversion. However, the importance of the role of the red cell in the conversion of vitamin B-6 has not been determined. Recent studies by Anderson (1980) demonstrated a very active uptake of both PN and PL by the red cell. PLP is not taken up by the red cell or other tissues due to its binding to albumin which prevents entry. Figure 2 illustrates the conversion of PN in the red cell. Anderson (1980) suggests that a significant portion of blood vitamin B-6 enters the red blood cell and that most PL in the blood is the result of this conversion. The red cell may also serve as a reservoir for PL and a transport mechanism to other tissues for the uptake of vitamin B-6. Therefore, the red cell appears to be active in the metabolism, transport, and storage of vitamin B-6 in the body.

Indirect measurements of the rate of interconversion in the liver have been conducted on human subjects. Small equimolar doses of PN, PL and PM were given to young men to examine the changes in the levels of vitamin B-6 compounds in timed blood collections (Wozenski et al., 1980). Results indicated doses of PN had a highly significant effect ($P < 0.01$) on the percent increase for total PB6 and plasma PLP. The time required for plasma PLP and PB6 to reach a peak appears to depend on the size of the dose given. The peak concentrations were reached later with a 10 mg PN dose then the 4 mg PN dose (Wozenski et al., 1980).

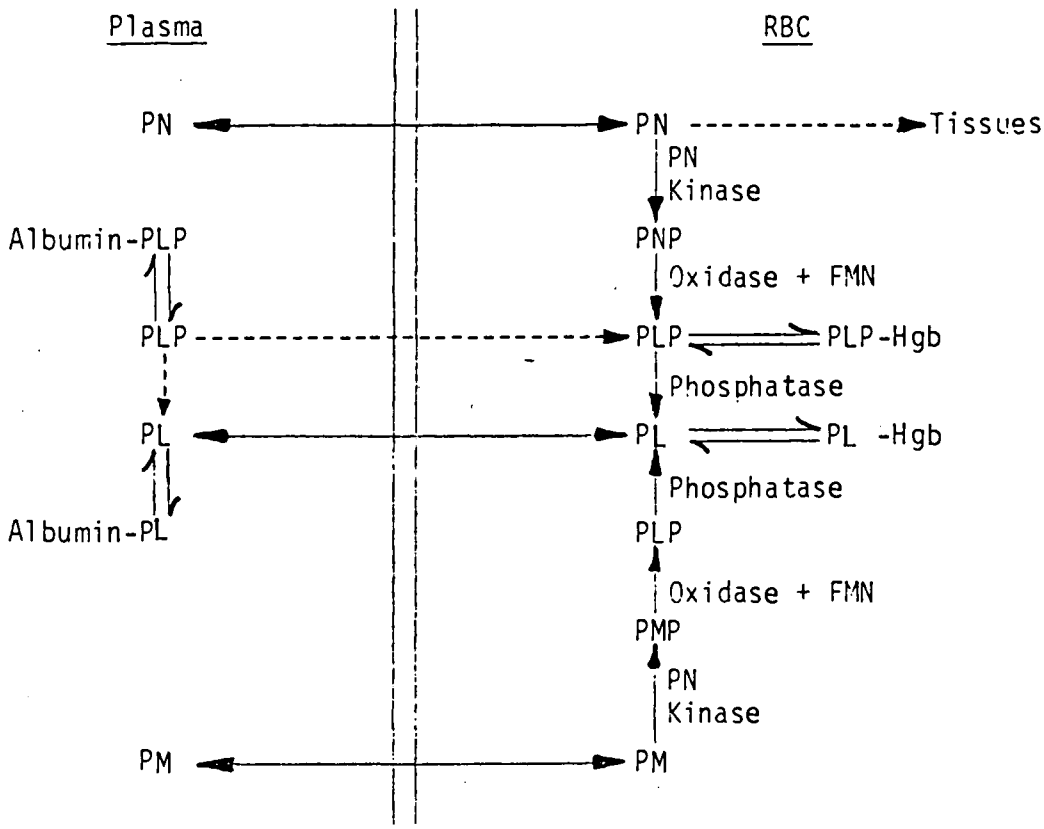

Figure 2. Interconversions of vitamin B-6 in the red blood cell.

Adapted from Shane, 1978 and Anderson, 1980.

The authors suggested the increased dose of PN resulted in either a retention of the vitamin in the body or a conversion to a product not measured. Anderson et al. (1971) reported PL and PLP in whole blood, plasma, and red cells reached a peak one hour after a 50 mg dose of pyridoxine hydrochloride was administered, whereas, a 100 mg oral dose of pyridoxine hydrochloride administered by Contractor and Shane (1968) took two hours for PB6 to reach a peak. However, not all the PN dose is converted to PLP. Wozenski et al. (1980) reported that plasma PLP concentrations following the PN doses were not as high as the PB6.

The major excretion product of vitamin B-6 is 4PA. The level of this vitamin B-6 metabolite excreted in the urine has been found to decrease rapidly with dietary depletion and increase with the repletion of the vitamin (Baysal et al., 1966; Linkswiler, 1967; Kelsay et al., 1968; Donald, 1978).

Free forms of the vitamin are also excreted in the urine. When the intake of vitamin B-6 is about 1.6 mg daily, the form ingested doesn't appear to affect the form excreted (Kelsay et al., 1968). Subjects were reported to excrete approximately twice as much PL as PM with a supplement of 1.5 mg PN. However, when large amounts of B-6 are consumed, the form fed does appear to affect the form excreted. Kelsay et al. (1968) report a urinary excretion of 4% PL and 10% PN when a 50 mg dose of PN is given. Rabinowitz and Snell (1948) obtained similar results in human subjects following doses of 82 mg PN.

Tissue Levels and Storage

Vitamin B-6 is found in most tissues of the body. As mentioned

previously, the red blood cell may serve as a reservoir for the vitamers (Anderson, 1980). Organs such as the liver, brain, kidney, skeletal muscle and heart also contain vitamin B-6 . In studies using rats, the highest total amount or amount per gm of tissue of vitamin B-6 was in the liver, then kidney, brain, skeletal muscle and heart (Thiele and Brin, 1966). However, the muscle contains the most vitamin B-6 in terms of total levels in the body. The concentration of vitamin B-6 in various tissues could reflect the metabolic need for the vitamin rather than serving as a storage depot. An exception to this is the skeletal muscle, where the major form is PLP. Krebs and Fischer (1964) estimated that nearly half of all vitamin B-6 in the body exists as PLP bound to muscle phosphorylase. This is not startling due to the fact that PLP plays such an essential role in the enzyme glycogen phosphorylase. Phosphorylase a, the more active form of the enzyme, catalyzes the initial step of glycogen breakdown to glucose-1-phosphate. Baranowski et al. (1957) first identified the presence of significant amounts of PLP in rabbit skeletal muscle phosphorylase. PLP was later identified as being a required coenzyme for catalytic activity (Cori and Illingworth, 1957). However, the mechanism of PLP in activating phosphorylase was not clear. Cori and Illingworth (1957) found that phosphorylase could be converted into an inactive apoenzyme and release PLP. Fischer et al. (1958) determined that a free aldehyde group on the PLP molecule was not necessary for phosphorylase activity. It was concluded that the mechanism of PLP involved in activating phosphorylase was not the same as that of other vitamin B-6 enzymes that require a free aldehyde group (Snell, 1958). Fischer et al. (1963) believe the function to be

more of a stabilizing effect on the structure of phosphorylase.

More recent evidence supports Cori and Illingworth's earlier work. Vitamin B-6 appears to be accessible from phosphorylase based on evidence that enzyme concentration fell to 35% in normal rat muscle when fed a vitamin B-6 deficient diet (Black et al., 1977). To test the hypothesis of muscle phosphorylase acting as a reservoir for vitamin B-6 , Black et al. (1977) fed rats a diet containing 10% of the level recommended by the National Research Council (NRC). Results showed a slow accumulation of phosphorylase at the same rate as muscle mass increased. Next, rats were fed ten times the NRC-recommended level of vitamin B-6 . This time the enzyme concentration of the gastrocnemius muscle increased dramatically. To further test the reservoir hypothesis, the accessibility of vitamin B-6 in phosphorylase was measured (Black et al., 1978). Results showed that phosphorylase concentration decreased over time when a vitamin B-6 deficient diet was fed, but the total content of phosphorylase in gastrocnemius muscle remained constant. Black et al. (1978) concluded that the enzyme acts as a reservoir for vitamin B-6 and that starvation, rather than vitamin B-6 deficiency, caused a muscle depletion of phosphorylase. Starvation initiates the change in phosphorylase concentration, and therefore, releases vitamin B-6 for use in other metabolic processes such as gluconeogenesis.

Lumeng et al. (1974) supported the theory that phosphorylase is a storage form for vitamin B-6. After investigating the levels of PLP in plasma and other tissues in the rat, these authors concluded that plasma and other muscle PLP are storage pools for vitamin B-6 that are

easily mobilized during time of metabolic need.

Recommended Dietary Allowances

Various methods are used to determine vitamin B-6 requirements, with the amount needed to restore levels to pre-depletion values being the usual criterion. Investigators, generally agree that 1.0 mg per day of vitamin B-6 is not sufficient and 2.2 mg per day is more than most people need. The requirement for vitamin B-6 appears to be directly related to protein intake (Cinnamon, 1970; Kelsay et al., 1968; Coursin, 1961; Sauberlich et al., 1972; Linkswiler, 1978). This relationship was noted when humans were fed a diet of 160 gm protein and 2.0 mg vitamin B-6 per day for one week followed by a two week vitamin B-6 depletion diet. A significant drop in the urinary excretion of B-6 was noted (Sauberlich et al., 1972). Baysal et al. (1966) describes the effects of a diet containing 0.16 mg of vitamin B-6 and 100 gm of protein on the blood levels of the vitamin and the excretion of 4PA. After five days of the diet,there was a significant decrease in the blood vitamin B-6 concentration, UB6 and 4PA. This decrease continued until the end of the depletion period, when there was little 4PA excreted and quite low blood vitamin B-6 levels.

In Canada, vitamin B-6 dietary requirements for women are based on studies by Donald (1978) and others in which a recommendation of 0.02 mg of vitamin B-6 per gm of protein was adapted (Bureau of Nutritional Sciences, 1975).

The vitamin B-6 nutritional status of the adolescent has not been adequately studied. Present recommendations are based on the pre-

adolescent child with allowances made for the adolescents unique nutritional requirements. These requirements are affected by a period of very rapid growth and onset of sexual maturation. Until research can be conducted to adequately evaluate the needs of the adolescent, the recommended dietary allowance has been set at 1.8 to 2.0 mg per day (National Research Council, 1980).

Effects of Exercise

Little research has been conducted on the effects of exercise on vitamin B-6 metabolism. Russian investigators exercised rats to exhaustion and deprived them of either PN, nicotinic acid or gave both in adequate amounts. The excretion of 4PA was measured to indicate B-6 metabolism. Those animals deprived of PN reached exhaustion sooner than either the low nicotinic acid diet or the vitamin supplied animals (Efremov and Zaburkin, 1972). The excretion of 4PA decreased in all swimming rats, but it decreased especially in the rats deprived of PN. The authors concluded that rats exercised to exhaustion require increased amounts of niacin and vitamin B-6.

Lawrence et al. (1975) reported using human subjects to test the effects of vitamin B-6 and vitamin E on the endurance of trained swimmers. Each participant was given a supplement of 17 mg pyridoxine hydrochloride and 300 I.U. of α-tocopherol acetate, or a placebo. The improvement of the well-conditioned swimmers appeared to reflect training rather than the supplementation.

Blood levels of vitamin B-6 following exercise have not been measured by investigators to date. However, in our laboratory, investigators

noticed a great rise in PB6 values for one subject two hours after 0.5 mg PN had been administered. Upon investigation, the subject reported a run of two miles between the one hour and two hour samples. Therefore, it appeared that strenuous exercise might have resulted in the high PB6 levels (Wozenski, 1977).

The importance of vitamin B-6 in metabolism during exercise is related to the function it serves in glycogenolysis and gluconeogenesis. These two pathways for supplying glucose are especially important in sustaining submaximal levels of exercise. The ability of the body to furnish the fuel sources necessary to meet energy demands may be related to the availability of vitamin B-6 as well as other physiological factors.

Fuel Utilization During Exercise

Various factors affect the contribution of protein, fat and carbohydrate used as a supply of energy for muscular work. Protein is not used as a fuel as long as the energy supply is adequate (Krogh and Lindhard, 1920). This can be demonstrated by the fact that nitrogen excretion is not significantly increased during muscular work in the fed individual (Hedman, 1957). The choice of fuel, therefore, is limited to carbohydrate and fat. The percentage of these two nutrients used in energy metabolism is greatly affected by the activity the subject is involved in as well as the individual's level of training. To aid in determining the type of major energy system or systems being used to perform any activity, general guidelines have been developed as Table 1 illustrates. As shown, three major areas can be outlined to

Table 1. Work effect areas with performance times, major energy system(s) involved and examples of the type of activities

Classification	Area	Performance Time	Major Energy System(s) Involved	Examples of Type of Activity
MES	1	Less than 30 seconds	ATP-PC	Shot-put, 100 yd. sprint, base stealing, golf and tennis swings
MEM	2	30 seconds to 1½ minutes	ATP-PC Lactic acid	220-440 yd. sprints, halfbacks, fullbacks. speed skating, 100 yd. sprint
MEL	3	Greater than 3 minutes	Oxygen	Soccer and lacrosse, cross-country skiing, marathon run, jogging

Adapted from Fox and Mathews, Interval Training, 1974.

include various activities. Area 1 includes the utilization of ATP-phosphocreatine and is termed a MES activity (muscular exertion of a high intensity, short duration). This area encompasses activities such as a 100 yard sprint or tennis racket swings. The second area is the MEM activity or muscular exertion of medium duration and utilizes the ATP-phosphocreatine system as well as the utilization of lactic acid through the Cori cycle. Examples of this system would be a 220 yard sprint, or the activities of a football player. The third area is called MEL or muscular exertion of a long duration. This area utilizes oxygen as the major energy system involved and would include activities such as marathon running or jogging. The efficient utilization of energy for each of these three areas is increased by training for a specific activity. Physical training affects many of the biochemical parameters involved in energy production including the availability of oxygen, free fatty acids, blood glucose, the utilization of muscle glycogen and enzyme activity.

Oxygen Uptake

Fuel utilization of carbohydrates and fat ultimately depends on the availability of oxygen to the working tissues. The oxidation of free fatty acids requires oxygen to accept hydrogen. Thus, without an adequate supply of oxygen, the fuel source is limited to carbohydrates. Anaerobic versus aerobic utilization of carbohydrates also is determined by the oxygen supply. With limited amounts of oxygen available, carbohydrate oxidation proceeds through the glycolytic pathway with the subsequent accumulation of lactic acid. The build-up of lactic acid in the muscle impairs the function of the muscle cells (Astrand

and Rodahl, 1977), as well as inhibiting the mobilization of free fatty acids suppressing fatty acid oxidation even further (Fredholm, 1969). Therefore, the greater the maximal oxygen uptake, the greater an individual's ability for fat utilization. Training has been shown to maximize the oxygen uptake during exercise (Issekutz et al., 1965; Ekblom et al., 1968; Bloom et al., 1976). Issekutz et al. (1965) demonstrated this effect of training on dogs. In the trained dog, the utilization of free fatty acids increased, whereas it declined in the untrained animal. Another indication of a greater maximal oxygen uptake is that the lactic acid was lower during exercise in the trained versus untrained dogs, suggesting an increase in aerobic capacity.

Human studies conducted with distance runners report a smaller increase in lactate and oxygen debt after training than before training began (Hagberg et al., 1980; Boyd et al., 1974; and Farrell et al., 1979). The authors suggest this could be the result of two factors. First, an increased delivery of oxygen to the muscle as illustrated by the rapid rise in heart rate in the trained individual compared to the untrained. A second factor could be due to an increased extraction of oxygen from the blood as a result of training. Farrell et al. (1979), suggested that the accumulation of plasma lactate in runners is related to the pace set for the long distance races. Runners set the pace to utilize the largest amount of oxygen and prevent a rise in plasma lactate. Boyd et al. (1974) interpreted the low plasma lactate levels as a necessary function for maintaining free fatty acids at an optimal level, thus allowing for the maximum utilization of carbohydrate.

Researchers have shown that oxygen uptake can be measured indirectly

on the basis of a recorded heart rate and work load during exercise. The individual relationship between the heart rate and varying work loads is linear. As shown in Figure 3, the linear relationship enables one to predict the maximal oxygen uptake at a given heart rate and work load. The estimation of oxygen uptake from the recorded heart rate has been found to be accurate at ±15% (Astrand and Rodahl, 1977).

Free Fatty Acids

Training has been shown to increase the utilization of free fatty acids as a fuel source. Holloszy and Booth (1976) stated that individuals who have adapted to endurance exercise obtain more energy from fat and less from carbohydrates than untrained individuals. Research reported thus far supports this theory. In human studies conducted by Costill et al. (1977), the increased availability of plasma free fatty acids slows the utilization of carbohydrate during exercise and increases the oxidation of fat for energy. Comparing the metabolic responses of trained cyclists and non-trained individuals, the plasma free fatty acids in both groups fell initially but the trained cyclists increased the levels after eight minutes of exercise and continued to increase throughout the exercise. The untrained athletes plasma FFA levels continued to decrease until 16 minutes of exercise was completed and then began to rise to reach pre-exercise values. Similar results have been reported by other researchers (Bloom et al., 1976; Rennie et al., 1974; and Sutton, 1978). All conclude that there was a greater degree of lipolysis and fat utilization in the trained athletes.

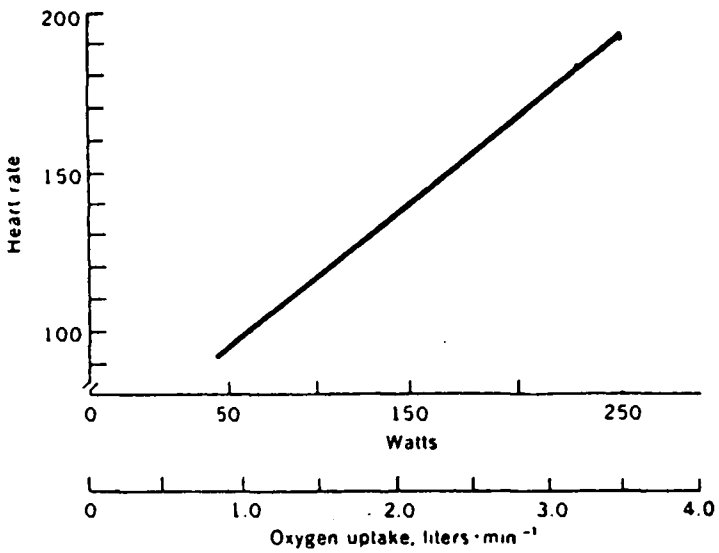

Figure 3. Prediction of maximal oxygen uptake from heart rate and work load on a bicycle ergometer.

Adapted from Astrand and Rodahl, Textbook of Work Physiology: Physiological Bases of Exercise, 1977.

Blood Glucose

Plasma glucose mobilization and oxidation appears to play a minor role in supplying energy to the working muscles in trained athletes compared to plasma free fatty acids. Available information on the influence of training on blood glucose levels is contradictory. Some studies have shown that a trained individual demonstrates a much greater rise in blood glucose during exercise than untrained individuals (Rennie et al., 1974; Bloom et al., 1976; Costill et al., 1977). Holloszy and Booth (1976) suggested this was due to a decrease in the utilization of glucose by trained muscle and increased fatty acid oxidation. However, Sutton (1978) reported a significant elevation of blood glucose in the unfit subjects as compared to fit subjects while exercising on a bicycle ergometer.

Muscle Glycogen

The degree of glycogen utilization differs in the fit and unfit individual. Most researchers agree that the use of stored glycogen depends upon the extent to which other fuels from blood, such as free fatty acids, can supply the energy needs. Therefore, the trained individual being able to mobilize and utilize fat more efficiently, would spare glycogen. Costill et al. (1977) demonstrated a glycogen sparing effect when plasma free fatty acids were elevated. Another investigation followed untrained subjects through an endurance training program and found a significantly lower rate of glycogen depletion after training (Saltin and Karlsson, 1971). Hermansen et al. (1967) compared trained versus untrained subjects worked to exhaustion on a

bicycle ergometer. The glycogen content determined by muscle biopsy indicated that the untrained subjects depleted their muscle glycogen content earlier in exercise and the trained subjects utilized less glycogen overall.

Enzyme Activity

Exercise increases the activity of many enzymes involved in energy production. In trained laboratory animals, both aspartate transaminase and malate dehydrogenase in the cytoplasm and mitochondria are increased (Sutton, 1978). Huston et al. (1975) reported that rats have a higher muscle hexokinase, glycogen phosphorylase, and phosphofructokinase activity after training. In contrast, Holloszy et al. (1971) were not able to show any alterations in glycogen phosphorylase or phosphofructokinase after training. Huston's study suggests that training was associated with an increased muscle glycolytic capacity. Training was also related to the ability of the liver to increase phosphoenolpyruvate carboxykinase activity during exercise which increased gluconeogenic capacity (Huston et al., 1975). Another enzyme important to endurance exercise is glycogen synthetase. Total glycogen synthetase activity was increased in skeletal muscles of humans and rats in response to exercise training, suggesting an increased ability to synthesize glycogen in trained muscles (Jeffress et al., 1968; Taylor et al., 1972).

III. METHODS

Nineteen men and two women volunteered for the study and were grouped according to level of training and age. The two females' values were not included in the overall means although their data are reported separately. The first group (intermittent) consisted of three males and one female and served as a pilot study. The next two groups consisted of cross country runners, one group of seven male college aged athletes (college) and the other of six male students from a local high school (high school). The fourth group was composed of three male and one female untrained college students (untrained). Each group exercised by either a bicycle ergometer regime, a predetermined run, or both. Vital statistics for each individual are listed in Table 2. Consent forms approved by the Human Subjects Committee of Oregon State University were signed by all subjects before beginning the study (see Appendix).

The terms trained versus untrained have been used to describe the subject's ability to perform under a given exercise regime. Training indicates the subject has been stressed through a particular type of exercise and has specifically adapted to the demands imposed by that exercise. The trained subjects utilized for this study have been "trained" to adapt to an endurance aerobic exercise such as cross country running. Endurance can be defined as the ability to persist in physical activity and to resist muscular fatigue. Untrained subjects have not undergone any particular exercise to alter physiological responses.

Table 2. Vital statistics of the subjects

Group	Subject No.	Sex	Age	Height (cm)	Weight (kg)
Intermittent	100[a]	F	23	165	55.4
	110	M	32	168	62.7
	120	M	25	178	77.2
	130	M	38	180	88.5
	$\bar{x}$		32	173	76.9
	S.D.		7	7	14.1
College	300	M	22	178	62.2
	310	M	28	168	60.2
	320	M	21	154	65.8
	330	M	23	173	63.6
	340	M	19	183	71.6
	350	M	18	179	64.9
	360	M	34	190	79.5
	$\bar{x}$		24	175	66.8
	S.D.		6	12	6.6
High School	200	M	17	172	78.1
	210	M	16	169	68.1
	220	M	16	187	68.6
	230	M	16	177	67.2
	240	M	16	184	68.6
	250	M	15	175	55.4
	$\bar{x}$		16	177	67.7
	S.D.		1	7	7.2
Untrained	400	M	28	175	74.9
	410	M	27	173	72.6
	420[a]	F	21	158	52.4
	430	M	29	170	82.6
	$\bar{x}$		28	169	76.7
	S.D.		1	8	5.2

a values not included in calculations

Test Descriptions and Procedures

Bicycle Ergometer

To determine the effects of exercise on vitamin B-6 metabolism, each subject in the intermittent, college and untrained groups exercised on a bicycle ergometer[1] while being monitored by an ECG.[2] The bicycle ergometer is a stationary bicycle whose front wheel is driven by the subjects' pedalling. A bicycle ergometer has several advantages. First it is easily accessible. Second, the subject's upper body is relatively stable and allows for accurate ECG monitoring. Third, the work load is expressed in standard units of work and thus enables work comparisons. Work load is independent of body weight because resistance is applied to the bicycle.

The exercise procedure consisted of three, seven minute intervals for a total of 21 minutes[3]. Female subjects began the exercise at a work load of 0.5 kp and for males at 1.0 kp[4]. At the end of the first seven minute interval, the work load was increased relative to the average heart rate for the sixth and seventh minute. Figure 4 is a flow chart illustrating the work load adjustments made during the exercise relative to the heart rate for females and males. Subjects continued to exercise for the second seven minutes at the adjusted work load. At the end of fourteen minutes, the same procedure was followed to determine the work load for the third seven minute interval.

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1 Monark, Quinton Instruments, Seattle, Washington.

2 Birtcher, Model 344, El Monte, Calif.

3 Procedure adapted from the Y's Way to Physical Fitness, 1973.

4 Kilopond is the force acting on the mass of one kilogram at normal acceleration of gravity (Astrand and Rodahl, 1977).

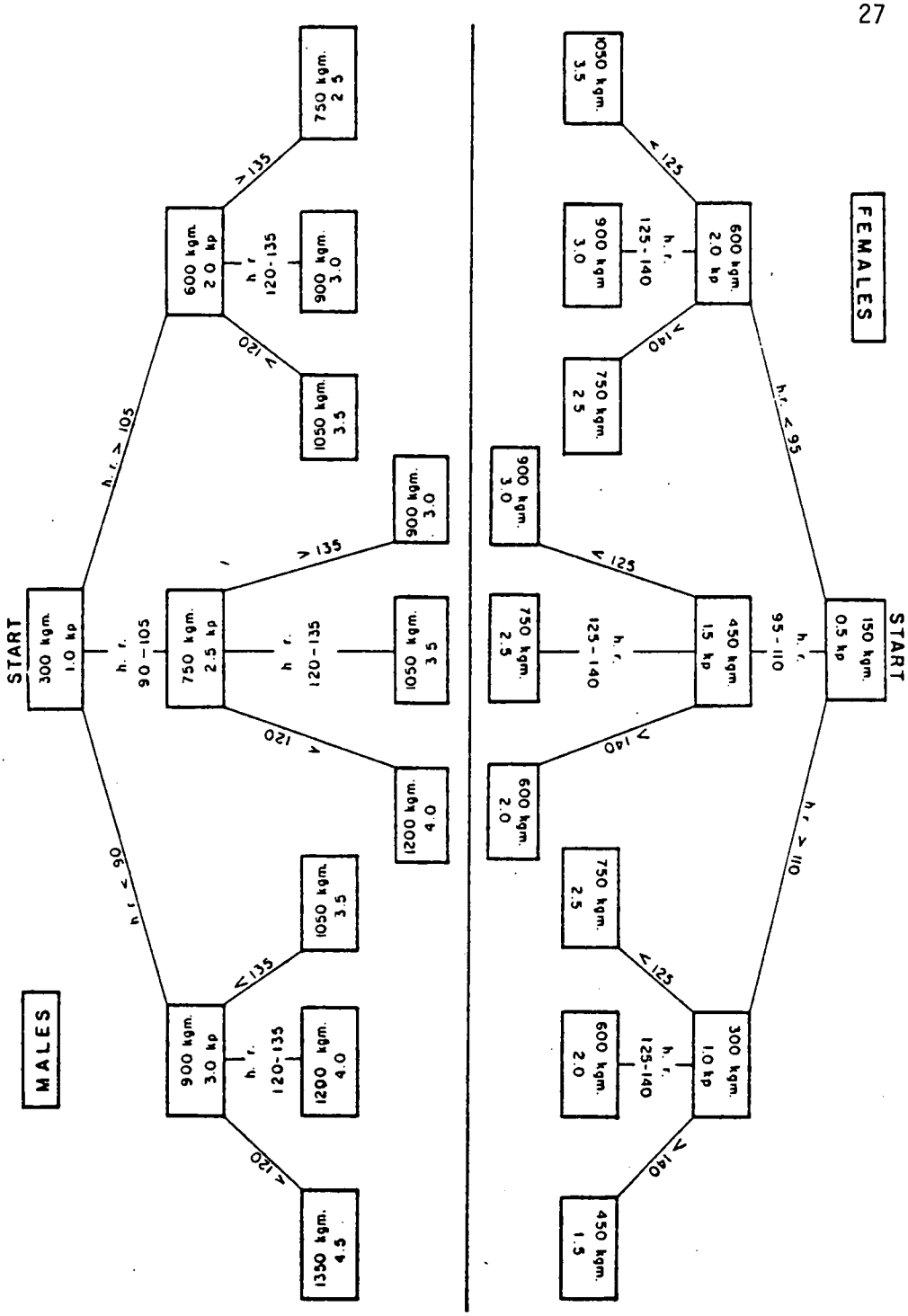

Figure 4. Flow chart for work load adjustments during the bicycle ergometer regime.

Throughout the exercise, subjects maintained a speed of twenty kilometers per hour to provide a constant pedal frequency. For the college and untrained groups, the exercise was continuous for twenty-one minutes without rest between intervals. A brief pause in pedalling was made between work load adjustments for the intermittent study to allow for blood drawing. In that study, each subject stopped the exercise for approximately two minutes.

Blood was sampled five times during the exercise period for the intermittent group and three times for the college and untrained groups. For the intermittent study, samples were taken prior to the beginning of exercise (pre), at the end of the first (1st) and second (2nd) seven minute intervals, at the end of the exercise (post), and thirty minutes following the end of the exercise (30 minute). The college and untrained blood samples included a pre, post, and 30 minute specimen. For each sampling period, the medical technologist drew 10 ml for the intermittent study and 20 ml for the college and untrained groups. Blood was drawn from the antecubital vein of the forearm into heparinized tubes.

Run

The second type of exercise consisted of a predetermined run involving the college and high school groups. The study was conducted during a scheduled team interval work-out for both groups. Each group ran for 21-26 minutes at submaximal levels. The work-out for the high school group consisted of six 1500 meter distances, each run in a nine minute interval. All the subjects began the work-out together and

maintained a designated pace through the 1500 meter distance. Runners were allowed nine minutes of run and rest per 1500 meter interval. The amount of time not used for the run was used as a rest period before beginning the next 1500 meter interval. After three 1500 meter intervals had been completed, post exercise blood samples were drawn. Blood was drawn within five minutes after finishing the run.

The college group also ran a similar interval work-out consisting of 1500 meter distances. The pace was set to be equivalent to a five minute mile with a three minute rest between each interval. The post exercise blood sample was drawn after the completion of three 1500 meter distances.

Prior to the pre blood draw, each group completed warm-up exercises. The high school group participated in team warm-ups consisting of approximately ten minutes of easy jogging, ten minutes of group calisthenics, followed by five minutes of individual stretches. The college group completed their warm-ups individually as needed.

Within five minutes following the pre exercise warm-ups, and one to two minutes immediately following the exercise, 20 ml of blood were drawn for the pre exercise sample. The same procedure for drawing blood as described for the bicycle ergometer, was followed on each occasion.

The heart rate of each subject prior to the pre exercise blood draw, but following warm-up exercises, was measured at the carotid artery. Immediately following exercise, a post exercise heart rate was also recorded for each individual.

Groups

Intermittent Group

Four apparently healthy individuals, three males (age 25, 32, and 38) and one female (age 23) served as subjects in the study. Prior to beginning the study, the subjects were determined to be in good health on the basis of a health questionnaire. None of the subjects reported taking vitamin supplements. Vital statistics for each subject are given in Table 1.

This group was exercised on the bicycle ergometer. The study was conducted in the morning to allow for the exercise to be performed in a fasting state.

Each subject was asked to record their self-selected food intake two days before the exercise day and the day of exercise for a total of three days. One male subject did not submit a diet record for evaluation.

Height and weight values were obtained the day of exercise. Unlike the other groups, no other anthropometric measurements were taken.

College Group

Seven male cross country runners, aged 18 through 34, comprised this group. Each subject exercised using the bicycle ergometer. One week later this group also participated in the predetermined run to compare the differences in the stress imposed on the body by the two exercises.

The exercise study was conducted five weeks into the cross country

season. All subjects completed the bicycle ergometer regime. However, one subject did not participate in the run due to a knee injury sustained five weeks prior to the study.

Height, weight, arm circumference, wrist circumference, and tricep and subscapular skinfolds were recorded the day of the bicycle ergometer exercise.

Total 24 hour urine collections under toluene were made the day before exercise and the day of exercise on the bicycle ergometer. Urine samples were also collected on the day of the run. Once received, the urine samples were frozen at -20°C until analyzed.

Diet records of self-selected food intake were recorded by each subject the day before and the day of the bicycle ergometer exercise, as well as the day of the run. Written as well as verbal instructions were given prior to the study, for recording dietary intake. In addition, breakfast was provided for each subject four hours prior to the bicycle ergometer exercise to control dietary intake. The same meal was provided for lunch four hours before the run. Table 4 describes the food composition of the meal served.

Four of the six subjects indicated the use of vitamin supplements ten days prior to the beginning of the study.

Untrained Group

Four untrained individuals, one female, age 21, and three males, ages 27, 28 and 29, volunteered for the study. None of the subjects engaged in any daily physical training other than walking to and from class.

The bicycle ergometer regime was used as the exercise for this group. The same anthropometric measurements were taken, as with the college group. In addition to these measurements, the supra-iliac skinfold was included. Twenty-four hour urine collections were made the day before and the day of exercise, and frozen utilizing the same procedure described for the other groups.

Diet records were recorded two days prior to exercise and the day of exercise. Verbal and written instructions for recording dietary intake were given to the subjects prior to the study. The same breakfast as used for the college study was provided four hours before exercise.

High School Group

Six male members of the high school cross country team volunteered for the study. Table 2 lists the vital statistics of the team members. Each subject completed the same health questionnaire given to all groups. In addition, anthropometric measurements were taken prior to exercise on the first and second days of the exercise study. Consent forms were signed by both the subjects and their parents.

The exercise regime was conducted on three different days over a training period of six weeks. The first day was approximately two weeks after the initial training program began. All subjects participated in the first study. Subjects were exercised by the run previously described. The second study was conducted three weeks later at the midpoint of training and all subjects participated in this study. The third exercise study was completed three weeks after the

second session at the closing of the training program, with only four subjects participating.

All subjects had been members of the cross country team for at least one year and most for two or three years.

The normal training schedule consisted of two days of interval work-outs, one at the beginning and one midway through the week. The season lasted for approximately three months. Training was geared to an individual's needs with daily records kept of the subjects' progress.

Twenty-four hour urine collections under toluene were made the day before exercise and the day of exercise. Once received, the samples were frozen at -20°C until analyzed. Diet records were maintained by each subject the day before and the day of exercise for each of the three exercise days. Prior to the study, verbal as well as written diet instructions were given to aid each subject in determining portion sizes and to provide accuracy in recording dietary intake. The exercise regime was conducted in the late afternoon 4 hours after the midday meal which was consumed at the high school cafeteria. No other food was consumed. Subjects 210, 220 and 250 indicated having taken vitamin supplements. However, those subjects reported the last supplement was taken two weeks before the study began. All subjects were instructed not to take any vitamins during the study.

Height, weight, arm circumference, and tricep and subscapular skinfolds were recorded on the first and second exercise periods prior to blood draws. From this information, the percentage body fat was calculated using the formula adapted from Frerichs et al. (1979):

Body fat% = 51.73 + (0.28 x weight) + (-0.35 x height) +
(0.78 x triceps skinfold)

A summary of the diet records, and the blood and urine sampling periods of all groups is given in Table 3.

Sample Analyses

Blood Determinations

The modified method of Chabner and Livingston (1970) was used for assaying PLP in human plasma. The original procedure was altered by using (1) 5.0 M potassium acetate as a buffering solution, (2) sidearm incubation flasks and (3) liquid scintillation counting fluor. The method is based on the amount of $^{14}CO_2$ released during the decarboxylation of L-tyrosine-1-^{14}C. The gas is trapped in a solution of 0.2 ml of NCS tissue solubilizer (Amersham, Corp.) and quantitated using a Beckman liquid scintillation counter (Model L5-3133P). Each sample was counted in 10 ml of a toluene based fluor. Samples were assayed in duplicate with a recovery for each subject included. For each 0.5 ml sample of plasma, 0.6 ml of 1.0 M perchloric acid was added to precipitate the plasma and release any protein-bound PLP. The inter-assay variability of the control sample was 4% (n=10) with the recoveries averaging 92 ±10%.

Plasma was assayed for total vitamin B-6 using the microbiological assay detailed by the AOAC (1980).[1] The organism Saccharomyces uvarum 4228; ATCC No. 9080, which is differentially

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1 Margaret Edwards assisted in the total plasma vitamin B-6 analysis.

Table 3. Summary of exercise performed, diet analyses and blood and urine samples

Group	Number of Subjects	Type of Exercise	Blood Samples	Urine Samples	Diet Analyses	Meal Provided
Intermittent	4	bicycle ergometer	pre, 1st, 2nd, post, and 30 minute[a]	none	3 days	none
College	7	bicycle ergometer	pre, post, and 30 minute	2 days	2 days	breakfast
		run	pre and post	1 day	1 day	lunch
High school	6	run	pre, post	2 days	2 days	none
	6	run	pre, post	2 days	2 days	none
	4	run	pre, post	2 days	2 days	none
Untrained	4	bicycle ergometer	pre, post, and 30 minute	2 days	3 days	breakfast

a (pre) prior to exercise, (1st) after 7 minutes of exercise, (2nd) after 14 minutes of exercise, (post) after 21 minutes of exercise, and (30 minutes) following a 30 minute post exercise rest.

responsive to the various non-phosphorylated forms of vitamin B-6, was used for the assay (Storvick et al., 1964; Storvick and Peters, 1964). Due to the shortage of sample, duplicates were not run. An inter-assay control was used, representing a mean of 18.4 ± 0.5 ng/ml (n=4) and a variability of 3%. Recoveries were also included reflecting a mean of 94 ± 4%. Initially, a 2 ml sample was precipitated with 10 ml of 10% trichloroacetic acid (TCA) in centrifuge tubes. After 30 minutes, the samples were centrifuged for ten minutes at 1510 x g and the supernatant collected. A second and third washing of the samples was done using 5 ml of 10% TCA and again centrifuged for ten minutes. The supernatant was collected from each wash and the precipitate discarded. The accumulated supernatant for each sample was covered with a watch glass and autoclaved for 30 minutes at 102 kPa This step hydrolyzes all the phosphorylated free forms. Therefore, the values represent the total concentration of both the non-phosphorylated and free forms of vitamin B-6.

Plasma glucose was determined on a Technicon Autoanalyzer (Technicon Corporation, Terrytown, New York) after being diluted 1:11 with 0.9% saline. The method is a modification of the procedure described by Hoffman (1937). Each sample was analyzed in duplicate.

Hemoglobin and hematocrit were determined immediately following the exercise. Hematocrit was determined in duplicate on the fresh blood using the microhematocrit method. Hemoglobin was measured in triplicate utilizing a standard method of analysis.

Urine Analysis

Total urinary vitamin B-6 was assayed by the method of the AOAC (Horwitz, 1980) using Saccharomyces uvarum[1]. Each sample was analyzed in duplicate. Prior to the determination, the samples were subjected to acid hydrolysis. Ten ml or 1% of the total urine volume was autoclaved with 50 ml of 0.1 N HCL at 102 kPa for 30 minutes. The pH was adjusted to 4.5 using either KOH or HCL. The volume was adjusted to 100 ml with redistilled water and the solution was then filtered through Whatman No. 1 filter paper. This filtrate was used in the microbiological assay for total urinary B-6.

The method used for determining 4-pyridoxic acid (4PA) was that of Reddy et al. (1958).[2] This method involved ion exchange chromatography to separate 4PA from other fluorescent compounds in the urine, followed by fluorometric determination. An Aminco-Bowman spectrophotofluorometer (American Instrument Co., Inc., Silver Spring, Maryland) was used to read the fluorescence. Interassay variability of a control sample was 2% (n=6).

Creatinine and urea nitrogen in the urine were determined on a Technicon Autoanalyzer (Technicon Corporation, Tarrytown, New York). A modified method of the Jaffee reaction was used for the creatinine determination (Pino et al., 1965). The urine samples for the urea nitrogen assay were diluted 1:51 in a 0.9% saline solution before being analyzed and run in duplicate. The method of Wybenga et al. (1971)

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1 Margaret Edwards analyzed urinary vitamin B-6.

2 Lynda Barstow helped in the determination of 4PA.

was followed.

Food Composites

Composites, excluding the milk, were made of the breakfast meal served to the college and untrained group. The milk was analyzed separately for vitamin B-6 content. The diet composite was prepared by blending together each item of known weight in a Waring blender, and pureed for two minutes. The total weight of the composite was noted and one to two grams of a well-homogenized sample was accurately weighed into a beaker. The aliquot was then acid hydrolyzed and analyzed for vitamin B-6 by the S. uvarum method (AOAC, 1975). Vitamin B-6 content of the composite is given in Table 4.

Dietary Record Analysis

Daily food intake recorded by each subject was carefully coded for computer nutrient analysis. The nutrient data bank used was developed by Ohio State University Hospital and School of Allied Medical Professions (Schaum, Mason, and Sharp, 1973), and modified for use by Oregon State University, Department of Foods and Nutrition.

The vitamin B-6, in micrograms (μg) was calculated for each subject, manually, on a per day basis. These figures were used to check the computer values for vitamin B-6 for each subject. The vitamin B-6/protein ratio was then calculated from the individual computer values.

Data Analysis

The data representing the subjects' responses to exercise were

Table 4. Food composition of controlled breakfast and lunch provided in metabolic unit[a]

	Amount (g)	Energy[a] (Kcal)	Vitamin B-6[ab] (mg)
Whole wheat bread	75	228	
Rice cereal (dry)	30	117	
Milk 2%	244	145	0.117[c]
Orange juice	249	92	
Grapefruit sections (canned)	100	41	
Peaches (canned)	100	85	
Margarine	21	204	
TOTAL	819	912	0.417

a From Nutrient Composition of Foods, Agriculture Handbook No. 8 (1975)

b Assayed by Margaret Edwards using S. uvarum method (AOAC, 1975).

c Assayed separately, but included in total B-6 content for the meal.

calculated in several ways. First, values for the bicycle ergometer and run were calculated separately. For the blood values, the percent change from pre to post, post to 30 minutes, and pre to 30 minutes was determined. The percent change in the metabolism of vitamin B-6 may reflect the stress imposed on the body. Next, the absolute change in nanomoles (nmoles) was calculated for the same categories, to determine any effects which may not have been reflected in the percent change. Third, the percent of the total PB6 value found to be PLP was also analyzed for the pre, post and 30 minute categories. Urine values were calculated as the percent change from the day before exercise to the day of exercise.

Paired t-tests were conducted to determine the significance of the changes in blood and urine values for PLP, PB6, 4PA, UB6, glucose, as well as B6/protein, B6 intake, and the percent of total PB6 found to be PLP. Student's t-test on the various group means was utilized to evaluate the effect of the level of training (McClave and Dietrick, 1979). In addition, Pearson's Coefficient of Correlation (McClave and Dietrich, 1979) was calculated to determine if linear relationships existed between any of the variables. The statistical analysis was performed utilizing the Hewlett Packard Model 10 calculator. The null hypothesis was rejected at the 0.05 level of significance.

IV. RESULTS

Dietary Intake

The dietary intake of each subject was analyzed for seven nutrients. Table 5 lists the mean intake as percent of the RDA by group for these nutrients. Appendix Table 1 lists the individual values. For the untrained group, only two of the three subjects submitted records of their dietary intake during the study. Subject 420 had recorded the diet but the forms were misplaced before they could be submitted. Also, subject 130 of the intermittent group did not record his dietary intake during the study. The daily intake of vitamin B-6 for all the athletes ranged from as low as 12% to as high as 326% of the RDA. Subject 110 of the intermittent group consumed the largest amount of vitamin B-6 for this group (262%, two days prior to exercise). The college athletes had satisfactory vitamin B-6 intakes with a mean of 111 ± 13%, while the two untrained subjects 400 and 410, averaged 97 ± 77% and 108 ± 23%, respectively for the three days. The overall mean vitamin B-6 intake for the high school athletes for all the diets calculated, was slightly below the RDA at 97 ± 11%. Two of the subjects from this group reported very low intakes for the six days recorded. Subject 220 ranged from an intake of 22% to a more satisfactory intake of 96% of the RDA for vitamin B-6 and subject 240 had values that ranged from 12 to 43% of the RDA.

The calculations of the vitamin B-6/protein ratio (mg/g) revealed an individual range of 0.003 for subject 240 to 0.450 for subject 360. This reflects the vitamin B-6 and high protein intake mentioned previously. The 1980 RDA for protein for males age 15 and greater is 56 gm (National Research Council, 1980). Computer analysis showed only two

Table 5. Nutrient intake of the subjects

Subject No.	Energy (Kcal)	Protein (gm)	Fat (gm)	Carb. (gm)	8-6/ Protein (mg/gm)	Vit. 8-6[1] %	Riboflavin %	Niacin %	Thiamin %	Vit. C %	Vit. A %	Calcium %	Iron %
Intermittent group													
110													
o	5992	220	92	1109	0.024	262	227	190	437	262	178	166	546
a	1813	73	83	185	0.018	67	71	84	124	96	76	62	186
b	1576	41	58	202	0.039	78	90	93	61	272	66	78	80
120													
o	1961	62	62	245	0.029	89	127	163	106	191	252	149	166
a	3387	115	133	415	0.017	95	133	210	174	379	207	177	256
b	2765	62	94	356	0.018	53	84	85	141	525	77	112	139
College group													
a_1 $\bar{x}$	3189	110	113	425	0.019	109	170	119	176	883	129	215	201
S.D.	±981	±45	±46	±122	±0.005	±64	±91	±41	±94	±567	±93	±101	±70
n=7													
b_1 $\bar{x}$	3179	114	114	448	0.021	125	163	139	167	1228	215	221	164
S.D.	±948	±27	±27	±178	±0.012	±101	±28	±57	±68	844	78	46	±44
n=6													
b_2 $\bar{x}$	3675	131	127	526	0.016	100	215	152	202	770	127	219	233
S.D.	±504	±19	±28	±167	±0.003	±11	±43	±38	±41	231	56	37	±43
n=5													
High school group													
a_1 $\bar{x}$	3196	148	115	406	0.013	92	188	166	150	319	200	197	134
S.D.	±546	±56	±35	±61	±0.006	±43	±121	±77	±45	±179	±205	±109	±66
n=6													
b_1 $\bar{x}$	3834	160	164	451	0.015	110	202	164	179	345	263	203	136
S.D.	±687	±38	±54	±98	±0.006	±32	±80	±35	±58	±164	±387	±62	±39
n=6													
a_2 $\bar{x}$	3118	154	125	379	0.014	108	221	141	155	337	165	195	100
S.D.	±606	±27	±42	±83	±0.012	±85	±68	±72	±35	±195	±89	±47	±23
n=6													
b_2 $\bar{x}$	3283	146	134	393	0.014	104	228	163	155	289	227	177	107
S.D.	±558	±34	±30	±67	±0.009	±70	±106	±91	±53	±182	±313	±82	±26
n=6													
a_3 $\bar{x}$	3056	127	127	370	0.013	84	176	131	128	180	193	148	90
S.D.	±494	±36	±33	±63	±0.013	±75	±73	±75	±26	±70	±178	±61	±18
n=5													

Table 5. Nutrient intake of the subjects cont'd.

Subject No.	Energy (Kcal)	Protein (gm)	Fat (gm)	Carb. (gm)	B-6/ Protein (mg/gm)	Vit. B-6[1] %	Riboflavin %	Niacin %	Thiamin %	Vit. C %	Vit. A %	Calcium %	Iron %
High school group cont'd.													
b_3 $\bar{x}$	3111	132	119	399	0.014	87	230	146	160	232	152	192	96
S.D.	±796	±42	±19	±137	±0.006	±31	±100	±30	±42	±73	±83	±94	±50
n=5													
Untrained group													
400													
o	1815	55	64	246	0.020	51	84	44	95	586	65	133	84
a	3272	130	157	365	0.028	185	191	182	165	367	110	247	269
b	2505	61	104	349	0.018	54	105	55	74	408	75	180	128
410													
o	2259	85	95	289	0.019	81	145	86	139	897	168	216	156
a	2197	92	84	293	0.026	122	166	98	316	409	48	183	163
b	2772	98	116	309	0.024	121	181	173	188	593	165	210	125

1 Percent of the 1980 RDA
o Two days prior to exercise
a One day prior to exercise
b Day of exercise

of the 64 daily diets recorded (3%) with a protein consumption of 56 gm or less, whereas 75% of the recorded diets reflected an intake of over 100 gm per day of protein. The overall group means for vitamin B-6/protein ration were quite different with a mean of 0.019 ± 0.008 mg/g for the college group and 0.014 ± 0.008 mg/g for the high school athletes.

As expected, the average caloric intake of the college and high school athletes was approximately 28% higher than the untrained subjects. Overall, the caloric intake did not appear to change dramatically from one day to the next except for the day of the run for the college group and the first exercise day for the high school group. Both groups consumed larger quantities of food that day, especially those foods higher in fat.

Of the other nutrients listed in Table 5, intakes of very few were below the RDA. The intake of two of these nutrients, thiamin and riboflavin, which are directly involved in energy metabolism, was higher than the RDA. Of the 64 diets calculated, only seven (11%) were below the RDA for thiamin and only six (9%) were less than the RDA for riboflavin. Interestingly, 55% of the diets exceeded the RDA for thiamin and 64% for riboflavin by at least one and a half times the RDA. For vitamin C, only 6% were below the RDA, whereas 84% were at least two times greater. The college subjects had a much higher mean intake of vitamin C than did the high school athletes, while the intake of the other nutrients was similar.

Bicycle Ergometer

Intermittent Group

The intermittent group will be discussed separately from the college and untrained groups for two reasons. First, this group was not homogeneous in the level of training. Secondly, the intermittent subjects had a two minute rest following each seven minute interval while blood was being drawn. The college and untrained groups exercised continuously with only pre and post exercise blood samples drawn.

The values for both plasma PLP and plasma B-6 will be discussed individually. Plasma PLP is the active coenzyme form of vitamin B-6, and therefore is discussed separately from plasma B-6 which includes both the unphosphorylated and phosphorylated forms of the vitamin.

Vital Statistics. Table 2 lists the vital statistics for all four groups. The intermittent group, composed of three male subjects ranging in age from 25-38 years old, had a mean height of 176 ± 8 cm with a mean weight of 76.9 ± 14.1 kg. Two of the subjects (110 and 130) were untrained while subject 120 was a trained marathon runner. As mentioned proviously, subject 100 was a female and, although she participated in the study, her values were not included in the calculations.

Plasma PLP. Prior to the beginning of exercise, the intermittent group displayed a mean plasma PLP level of 3.88 ± 2.14 nmoles/100 ml of PLP, as shown in Table 6. After completing seven minutes of exercise, one of the three subjects showed increased plasma PLP levels resulting in mean change of 0.6 ± 17.8% for the group. Little change in plasma

Table 6. Mean plasma pyridoxal 5'-phosphate levels during the bicycle ergometer regime

Group	n	Sample[a]	$\bar{x}$ ± S.D. nmoles/100 ml
Intermittent	3	Pre	3.88 ± 2.14
		1st	4.15 ± 3.00
		2nd	4.03 ± 2.60
		Post	4.32 ± 2.61
		30 minute post	3.61 ± 1.99
College	7	Pre	5.07 ± 1.33
		Post	6.12 ± 1.58
		30 minute post	4.97 ± 1.60
Untrained	3	Pre	3.75 ± 1.64
		Post	4.12 ± 1.61
		30 minute post	3.44 ± 1.32

a Blood samples were drawn prior to exercise (Pre), after 7 minutes (1st), 14 minutes (2nd), 21 minutes (Post) of exercise and following a 30 minute rest (30 minute post).

PLP levels was noted at the end of 14 minutes as compared to the seven minute level. During that interval, two of the three subjects increased their plasma PLP levels. This reflected a mean change of 1.0 ± 8.3%. The largest change between time intervals during the exercise occurred during the second to post interval. The plasma PLP levels increased 8.5 ± 7.5%, with a rise in plasma PLP for all three subjects. Overall, the effect of exercise resulted in a 9.1 ± 10.9% increase in plasma PLP in the pre to post category, as shown in Table 7. Following the 30 minute resting period, plasma PLP dropped to a level which, interestingly, was 7.0 ± 6.6% lower than the mean pre exercise value. As illustrated in Figure 5, the percent change was the greatest for the post to 30 minute interval (-14.4 ± 4.8%). In Table 8, the change in nmoles/100 ml of plasma PLP is shown as positive for the pre to post interval (0.44 ± 0.50 nmoles/100 ml) and negative for both the 30 minute post exercise (0.71 ± 0.62 nmoles/100 ml) and the pre to 30 minute post exercise intervals (0.28 ± 0.25 nmoles/100 ml).

Table 9 lists the percent of total plasma vitamin B-6 present as PLP for the pre, post, and 30 minute post exercise samples. Following the exercise, two of the three subjects showed a greater percentage of plasma vitamin B-6 as PLP as compared to the pre value. For all but one subject, the percent of vitamin B-6 as plasma PLP continued to drop slightly following a 30 minute rest.

Plasma B-6. As seen with the plasma PLP, a similar effect of exercise was also evident for PB6. Table 10 lists the mean values for PB6 with the percent change given in Table 11. Pre exercise levels averaged 7.44 ± 4.13 nmoles/100 ml. An increase in two of the three

Table 7. Mean percent change in plasma pyridoxal 5'-phosphate during the bicycle ergometer regime

Group	n	Time Interval[a]	$\bar{x}$ ± S.D. %
Intermittent	3	Pre to 1st	0.6 ± 17.8
		1st to 2nd	1.0 ± 8.3
		2nd to Post	8.5 ± 7.5
		Post to 30 minute post	-14.4 ± 4.8
		Pre to Post	9.1 ± 10.9
		Pre to 30 minute post	-7.0 ± 6.6
College	7	Pre to Post	22.0 ± 14.4 [b]
		Post to 30 minute post	-18.8 ± 12.7 [b]
		Pre to 30 minute post	-3.0 ± 8.2
Untrained	3	Pre to Post	12.6 ± 8.8 [b]
		Post to 30 minute post	-16.3 ± 2.6
		Pre to 30 minute post	-5.7 ± 8.9

a Time intervals were compared at 0 to 7 minutes (Pre to 1st), 7 to 14 minutes (1st to 2nd), 14 to 21 minutes (2nd to Post), following the end of exercise to a 30 minute rest (post to 30 minute post), 0 to 21 minutes (Pre to Post), and 0 to 30 minutes after the end of exercise (Pre to 30 minute Post).

b $P < 0.005$

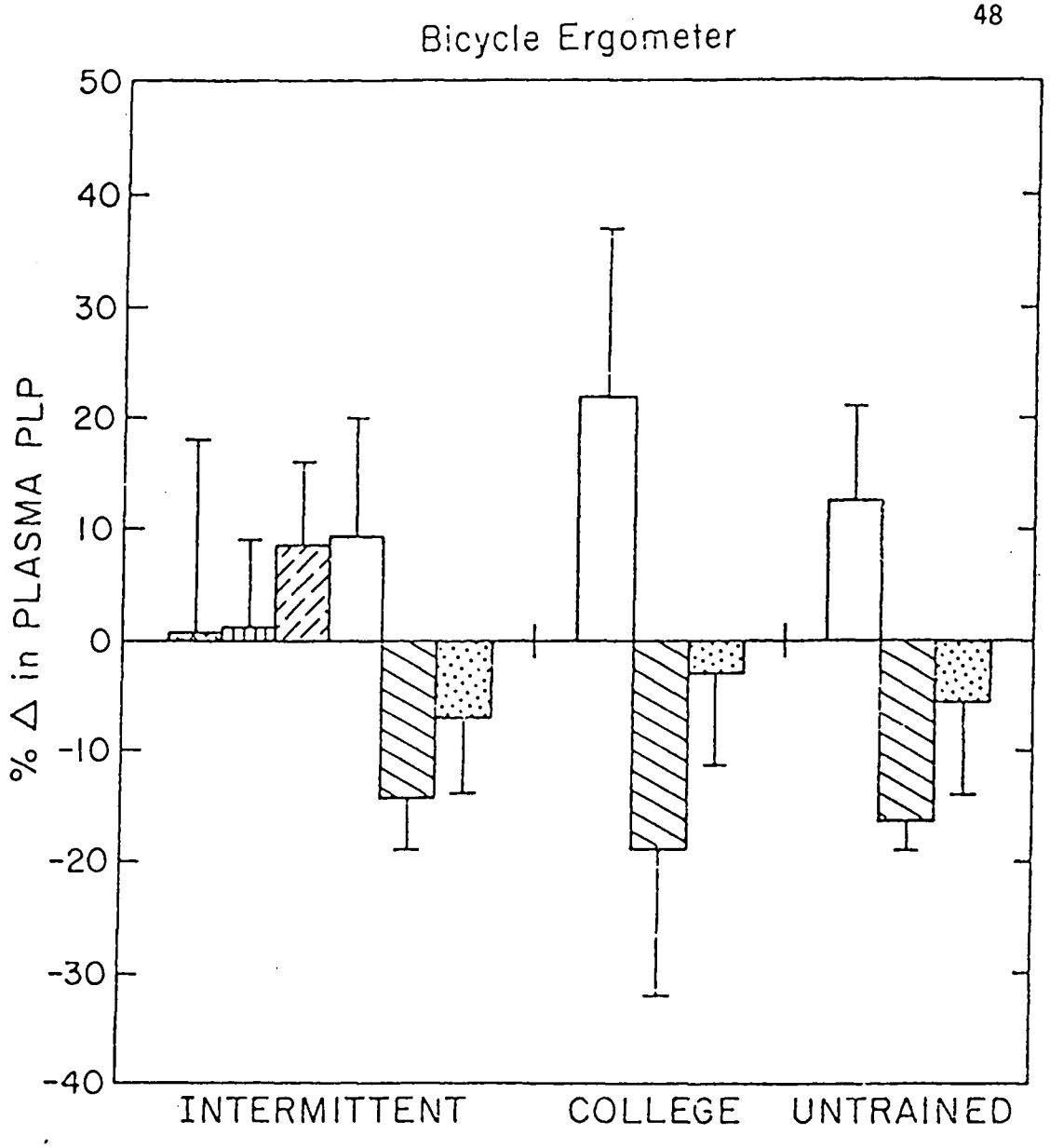

Figure 5. Percent change in plasma pyridoxal 5'-phosphate during the bicycle ergometer regime. Intervals represented are:

- Pre exercise to 7 minutes
- 7 to 14 minutes
- 14 to post exercise
- Pre to post
- Post to 30 minute post
- Pre to 30 minute post exercise

Table 8. Mean net change in plasma pyridoxal 5'-phosphate during the bicycle ergometer regime

Group	n	Time Interval[a]	$\bar{x}$ ± S.D. nmoles/100 ml
Intermittent	3	Pre to Post	0.44 ± 0.50
		Post to 30 minute post	-0.71 ± 0.62
		Pre to 30 minute post	-0.26 ± 0.25
College	7	Pre to Post	1.05 ± 0.60
		Post to 30 minute post	-1.15 ± 0.78
		Pre to 30 minute post	-0.10 ± 0.44
Untrained	3	Pre to Post	0.38 ± 0.05
		Post to 30 minute post	-0.68 ± 0.32
		Pre to 30 minute post	-0.31 ± 0.36

a Time intervals are described in Table 7.

Table 9. Percent of total plasma vitamin B-6 as pyridoxal 5'-phosphate during the bicycle ergometer regime

Group	n	Sample[a]	$\bar{x}$ ± S.D. %
Intermittent	3	Pre	50.7 ± 12.7
		Post	43.4 ± 6.1
		30 minute post	40.9 ± 3.3
College	7	Pre	75.2 ± 12.8
		Post	78.1 ± 11.5
		30 minute post	76.7 ± 13.5
Untrained	3	Pre	62.9 ± 18.1
		Post	60.6 ± 9.9
		30 minute post	59.3 ± 3.3

a Blood samples are described in Table 6.

Table 10. Mean plasma vitamin B-6 levels during the bicycle ergometer regime

Group	n	Sample[a]	$\bar{x}$ ± S.D. nmoles/100 ml
Intermittent	3	Pre	7.44 ± 4.13
		1st	8.74 ± 5.01
		2nd	8.87 ± 4.69
		Post	9.77 ± 5.46
		30 minute post	8.65 ± 4.09
College	7	Pre	6.69 ± 0.96
		Post	7.78 ± 1.45
		30 minute post	6.60 ± 1.33
Untrained	3	Pre	5.78 ± 1.57
		Post	6.62 ± 1.73
		30 minute post	5.66 ± 1.20

a Blood samples are described in Table 6.

Table 11. Mean percent change in plasma vitamin B-6 during the bicycle ergometer regime

Group	n	Time Interval [a]	$\bar{x}$ ± S.D. %
Intermittent	3	Pre to 1st	5.8 ± 26.7
		1st to 2nd	11.6 ± 14.0
		2nd to Post	9.2 ± 3.3
		Post to 30 minute post	-8.9 ± 15.5
		Pre to Post	28.9 ± 37.9
		Pre to 30 minute post	15.1 ± 26.1
College	7	Pre to Post	15.9 ± 6.5 [b]
		Post to 30 minute post	-14.6 ± 11.3
		Pre to 30 minute post	1.3 ± 13.1
Untrained	3	Pre to Post	15.0 ± 14.9
		Post to 30 minute post	-12.2 ± 9.7
		Pre to 30 minute post	-0.9 ± 6.2

a Time intervals are described in Table 7.
b $P < 0.005$

subjects was seen for the first interval of exercise representing a mean change of 5.8 ± 26.7%. This was greater than that observed for plasma PLP. After 14 minutes of exercise, the mean value increased in two out of three subjects (11.6 ± 14.0%). All three subjects had increased PB6 levels at the end of the exercise reflecting an increase of 9.2 ± 3.3% from the second interval. As seen with plasma PLP levels, PB6 dropped (8.9 ± 15.5%) after a 30 minute rest. The largest percent change between intervals occurred in the pre to post category (28.9 ± 37.9%) as illustrated in Figure 6. In contrast to the plasma PLP, an overall positive change of 15.1 ± 26.1% was found for PB6 from the pre exercise to the 30 minute post exercise sample as compared to a negative change for plasma PLP.

As compared to the change in plasma PLP (0.44 ± 0.50 nmoles/100 ml), a greater change in nmoles of PB6 (2.33 ± 2.08 nmoles/100 ml) was seen in the pre to post category (Table 12). Similarly, during the post to 30 minute post exercise interval, there was a larger decrease in PB6 (1.12 ± 1.82 nmoles/100 ml) as compared to plasma PLP (0.71 ± 0.62). Unlike the first two time intervals, the PB6 levels increased (0.21 ± 1.50 nmoles/100 ml) during the pre to 30 minute post exercise level, while a decrease was noted in plasma PLP (0.26 ± 0.25 nmoles/100 ml) for the same interval.

Plasma Glucose. The group mean values for plasma glucose, shown in Table 13, fell within the normal range of 80-110 mg/100 ml (Henry, 1974). As listed in Table 14, the mean percent change in plasma glucose for the pre to post category and post to 30 minute post exercise category was slightly negative (3.8 ± 5.8 and 2.7 ± 5.4%, respectively).

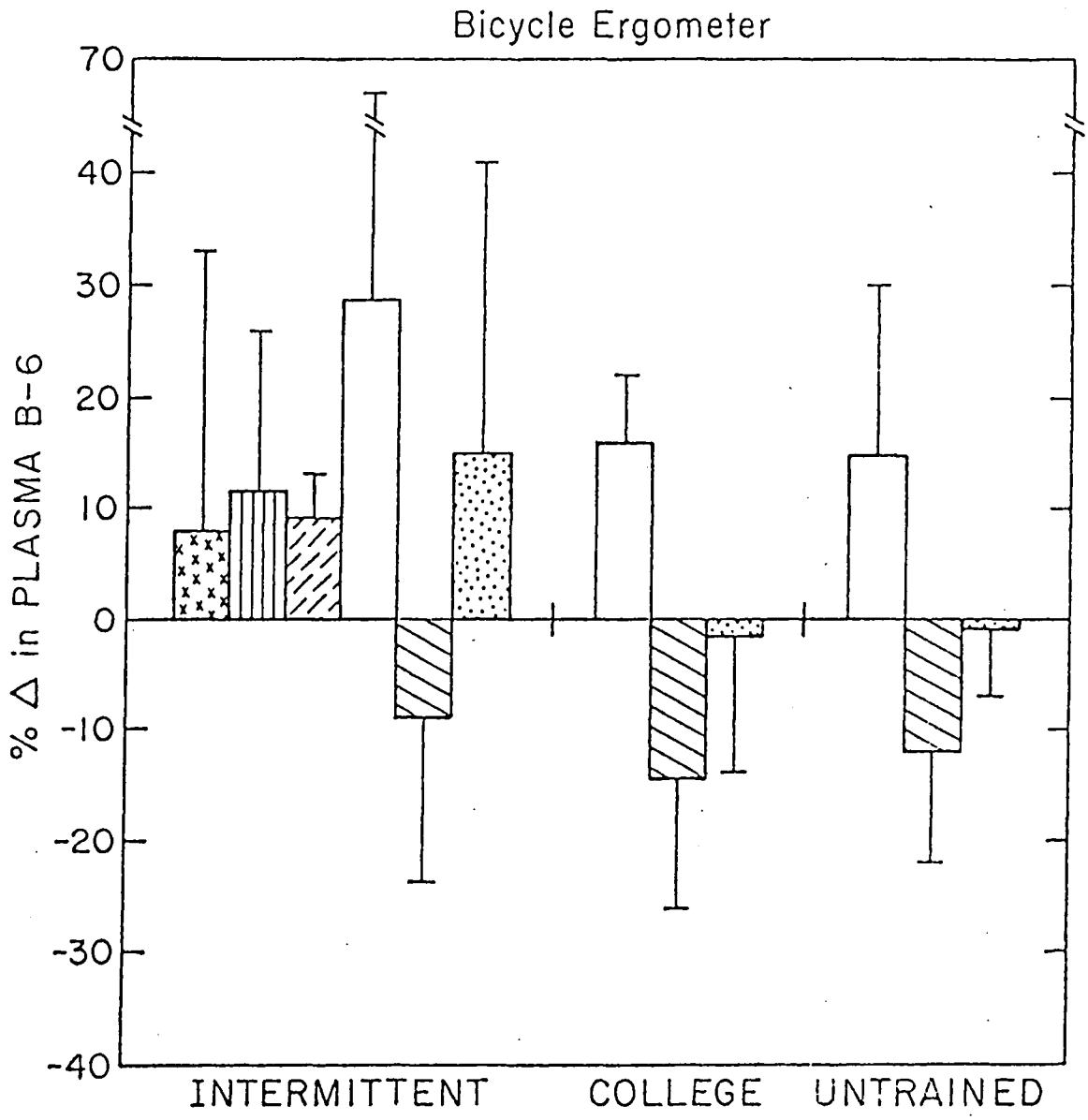

Figure 6. Percent change in plasma B-6 during the bicycle ergometer regime. Intervals are described in Figure 5.

Table 12. Net change in plasma vitamin B-6 during the bicycle ergometer regime

Group	n	Time Interval[a]	$\bar{x}$ ± S.D. nmoles/100ml
Intermittent	3	Pre to Post	2.33 ± 2.08
		Post to 30 minute post	-1.12 ± 1.82
		Pre to 30 minute post	0.21 ± 1.50
College	7	Pre to Post	1.10 ± 0.54
		Post to 30 minute post	1.19 ± 1.05
		Pre to 30 minute post	-0.09 ± 0.97
Untrained	3	Pre to Post	0.84 ± 0.88
		Post to 30 minute post	-0.95 ± 0.78
		Pre to 30 minute post	-0.12 ± 0.41

a Time intervals are described in Table 7.

Table 13. Mean plasma glucose levels during the bicycle ergometer regime

Group	n	Sample[a]	$\bar{x}$ ± S.D. mg/100 ml
Intermittent	3	Pre	88 ± 6
		1st	84 ± 11
		2nd	88 ± 8
		Post	84 ± 8
		30 minute post	82 ± 4
College	7	Pre	85 ± 5
		Post	88 ± 7
		30 minute post	87 ± 7
Untrained	3	Pre	79 ± 11
		Post	81 ± 4
		30 minute post	72 ± 11

a Blood samples are described in Table 6.

Table 14. Mean percent change in plasma glucose during bicycle ergometer regime

Group	n	Time Interval[a]	$\bar{x}$ ± S.D. %
Intermittent	3	Pre to Post	-3.8 ± 5.8
		Post to 30 minute post	-2.7 ± 5.4
		Pre to 30 minute post	-6.2 ± 10.1
College	7	Pre to Post	6.5 ± 14.5
		Post to 30 minute post	-1.9 ± 10.9
		Pre to 30 minute post	3.4 ± 8.3
Untrained	3	Pre to Post	3.6 ± 11.6
		Post to 30 minute post	-11.3 ± 11.8
		Pre to 30 minute post	-9.0 ± 2.3

a Time intervals are described in Table 7.

The pre to 30 minute post exercise interval showed the largest decrease in glucose (6.2 ± 10.1%). It must be noted,however, that there was a wide variation in the subjects' response, as evidenced by the large standard deviation.

Hemoglobin and Hematocrit. Hemoglobin and hematocrit mean values and percent change are listed in Table 15. All hemoglobin values were normal (13-18 gm/100 ml) as were the hematocrit values (40-50%). The largest decrease in hemoglobin was recorded for the post to 30 minute post exercise interval (10.5 ± 8.6%). An increase in hemoglobin was seen in the pre to post category (8.5 ± 2.1%) but overall the pre to 30 minute post exercise changed very little (1.0 ± 5.6%). There was a smaller effect of exercise on the hematocrit values compared to hemoglobin with an increase in hemoglobin for the pre to post category of 5.4 ± 6.7% and a decrease of 2.9 ± 8.9% for the post to 30 minute post exercise interval. In contrast to the hemoglobin, the hematocrit decreased overall for the pre to 30 minute post exercise period (2.9 ± 2.5%).

Female Subject's Response. The one female subject in the intermittent group had results similar to those found for the male subjects. The pre exercise plasma PLP value was slightly lower (2.18 nmoles/100 ml) but as was seen with the male subjects, the plasma PLP increased (20.6%) following 21 minutes of exercise and decreased (15.9%) during the post to 30 minute post exercise time interval. Plasma B6 also increased following exercise (27.9%) and decreased following 30 minutes of rest (10.0%). Also within normal range was the plasma glucose pre exercise value, which dramatically increased following exercise (42.1%). Hemoglobin

Table 15. Mean and percent change in hemoglobin and hematocrit levels during the bicycle ergometer regime

Group	n	Sample[a]	Hb $\bar{x}$ ± S.D. gm/100 ml	Hct $\bar{x}$ ± S.D. %	Time Interval[b]	Hb $\bar{x}$ ± S.D. % change	Hct $\bar{x}$ ± S.D. % change
Intermittent	3	Pre	16.4 ± 1.1	46 ± 2	Pre to Post	8.5 ± 2.1	5.4 ± 6.7
		1st	17.2 ± 1.5	46 ± 3	Post to 30 minute post	-10.5 ± 8.6	-2.9 ± 8.9
		2nd	18.0 ± 2.0	48 ± 3	Pre to 30 minute post	1.0 ± 5.6	-2.9 ± 2.5
		Post	18.2 ± 1.7	47 ± 6			
		30 minute post	16.5 ± 1.3	44 ± 3			
College	7	Pre	16.0 ± 1.2	46 ± 2	Pre to Post	6.7 ± 4.5	4.9 ± 4.5
		Post	17.0 ± 1.6	48 ± 4	Post to 30 minute post	-8.2 ± 2.8	-7.1 ± 1.5
		30 minute post	15.6 ± 1.6	45 ± 3	Pre to 30 minute post	-2.1 ± 3.7	-2.2 ± 3.8
Untrained	3	Pre	15.8 ± 1.3	45 ± 4	Pre to Post	5.4 ± 1.6	7.3 ± 1.5
		Post	16.6 ± 1.2	48 ± 4	Post to 30 minute post	-6.7 ± 2.5	-6.1 ± 1.7
		30 minute post	15.5 ± 1.0	45 ± 3	Pre to 30 minute post	-1.7 ± 2.2	0.7 ± 0.9

a Blood samples are described in Table 6.

b Time intervals are described in Table 7.

and hematocrit values were normal with only slight increases in both blood values for the pre to post category (6.3 and 4.7%, respectively).

College and Untrained Groups

The results of the study involving the college and untrained groups were compared to ascertain whether or not training had an effect on vitamin B-6 metabolism. Similarities existed between the two groups such as: (1) the same procedure was followed during the bicycle ergometer exercise; (2) each group was given the same meal four hours before the exercise; (3) the subjects were male, ranging in age from 18 to 34. However, they were not similar in the level of training, which could result in different fuels being utilized during exercise, such as glycogen, blood glucose, and free fatty acids. Therefore, the change in blood values of plasma PLP and glucose may be different between the two groups.

Vital Statistics. The college group was composed of males ages 18-34 with a mean height of 175 ± 12 cm and weight of 66.8 ± 6.6 kg. In comparison, the untrained group was similar in age (21 to 29 years of age), of approximately the same height (173 ± 3 cm) but somewhat heavier (76.7 ± 5.2 kg). Other anthropometric measurements for both groups are given in Appendix Table 2.

Plasma PLP. As shown in Table 6, the college group had a higher, although not significant, mean pre exercise plasma PLP value (5.07 ± 1.33 nmoles/100 ml) as compared to the untrained group (3.75 ± 1.64 nmoles/100 ml). Values for the subjects in the college group ranged from 3.61 to 6.76 nmoles/100 ml. In the untrained group, the lower mean was due to the pre value of subject 430 (1.85 nmoles/100 ml) while

subjects 400 and 410 had higher values of 4.65 and 4.74 nmoles/100 ml, respectively. Following the exercise, the college plasma PLP value increased to 6.12 ± 1.58 nmoles/100 ml, while the untrained group's value increased to 4.12 ± 1.61 nmoles/100 ml. In both groups, the plasma PLP levels decreased after the 30 minute post exercise interval with the college group having a mean value of 4.97 ± 1.60 nmoles/100 ml and the untrained group a slightly lower level (3.44 ± 1.32 nmoles/100 ml).

The change in plasma PLP for the pre to post interval represents an increase of 22.0 ± 14.4% for the college group as compared to a much lower increase of 12.6 ± 8.8% for the untrained subjects. Table 7 lists the mean percent changes for the three categories. In the college group, five of seven subjects showed an increase for the pre to post category ranging from 14.6 to 18.3%. However, one subject, 310, increased by only 5.5%, whereas subject 360 showed a dramatic increase of 51.4%. Two of the untrained subjects, 400 and 410, had a smaller percent increase in plasma PLP of 6.9 to 8.2%, respectively. Subject 430, whose pre plasma PLP values were low, increased 22.7%, for the pre to post category. The percent change for plasma PLP is illustrated in Figure 5. For both the college and untrained groups, paired t-tests of the plasma PLP values indicated a positive significant difference ($P < 0.005$) between the pre to post time interval. When the mean percent change of the two groups was compared, no significant difference was found.

The change in plasma PLP levels for the post to 30 minute post exercise interval reflected a decrease of 18.8 ± 12.7% for the college group. The untrained group showed a slightly lower decrease for the

same category (16.3 ± 2.6%). As was seen in the pre to post category, subject 360 from the college group decreased 40.9% from the post to 30 minute post exercise level, which was a dramatic negative percent change in comparison to subject 330 who decreased only slightly (3.7%). The three untrained subjects, 400, 410, and 430, were basically similar in their response following the exercise for the post to 30 minute post exercise interval (-14.3, -19.3, and -15.3%, respectively). A negative significant difference was found for the college group for the post to 30 minute post exercise interval ($P < 0.005$), but the change was not significant for the untrained group. No significant difference was observed between groups when the mean percent change for the post to 30 minute post exercise interval was compared.

The final category, pre to 30 minute post exercise, is important because it may reflect the body's homeostatic mechanism to return to the pre exercise level. Both groups had a negative percent change in plasma PLP, although the college group was slightly lower than the untrained group (3.0 ± 8.2 and 5.7 ± 8.9%, respectively). Paired t-tests showed no significance between the pre and 30 minute post exercise values for either group nor did the Student's t-test show any statistical difference between the mean percent change of the two groups for the pre to 30 minute post exercise time interval.

The change in nmoles of plasma PLP for the pre to post, post to 30 minute post exercise, and pre to 30 minute post exercise categories was greater for the college than for the untrained groups. The mean change in nmoles/100 ml of plasma PLP is listed in Table 8 for both groups, while the college subjects' response is illustrated in

Figure 7. Student's t-test showed no significant difference between the two groups.

Plasma B-6. As seen with the plasma PLP levels, the college and untrained groups responded to exercise with an increased level of PB6. The pre exercise levels of PB6 were 6.69 ± 0.96 and 5.78 ± 1.57 nmoles/100 ml for the college and untrained groups, respectively (see Table 10). Following exercise, the mean post exercise level increased to 7.78 ± 1.45 for the college and 6.62 ± 1.73 nmoles/100 ml for the untrained group. All ten of the subjects had increased levels of PB6 after 21 minutes of exercise. The 30 minute post exercise values were lower than the post levels with the college group having a slightly higher mean than the untrained group (6.60 ± 1.33 and 5.66 ± 1.20 nmoles/100 ml, respectively).

The percent change for PB6 is shown in Table 11 and illustrated in Figure 6. As can be seen, both groups showed a similar increase in PB6 (15.9 ± 6.5 and 15.0 ± 14.9% for the college and untrained groups, respectively). The decrease in levels from the post to 30 minute post exercise interval was slightly greater for the college group (14.6 ± 11.3%) than for the untrained group (12.2 ± 9.7%), while for the final category, pre to 30 minute post exercise, both groups decreased to the same degree (1.3 ± 13.1 and 0.9 ± 6.2% for the college and untrained groups, respectively). The change between the pre and post interval was significantly different for the college group ($P < 0.005$) but not for the untrained group. The other intervals were not significant for either group, nor did the Student's t-test indicate any difference between the mean values of the groups for any of the three time intervals.

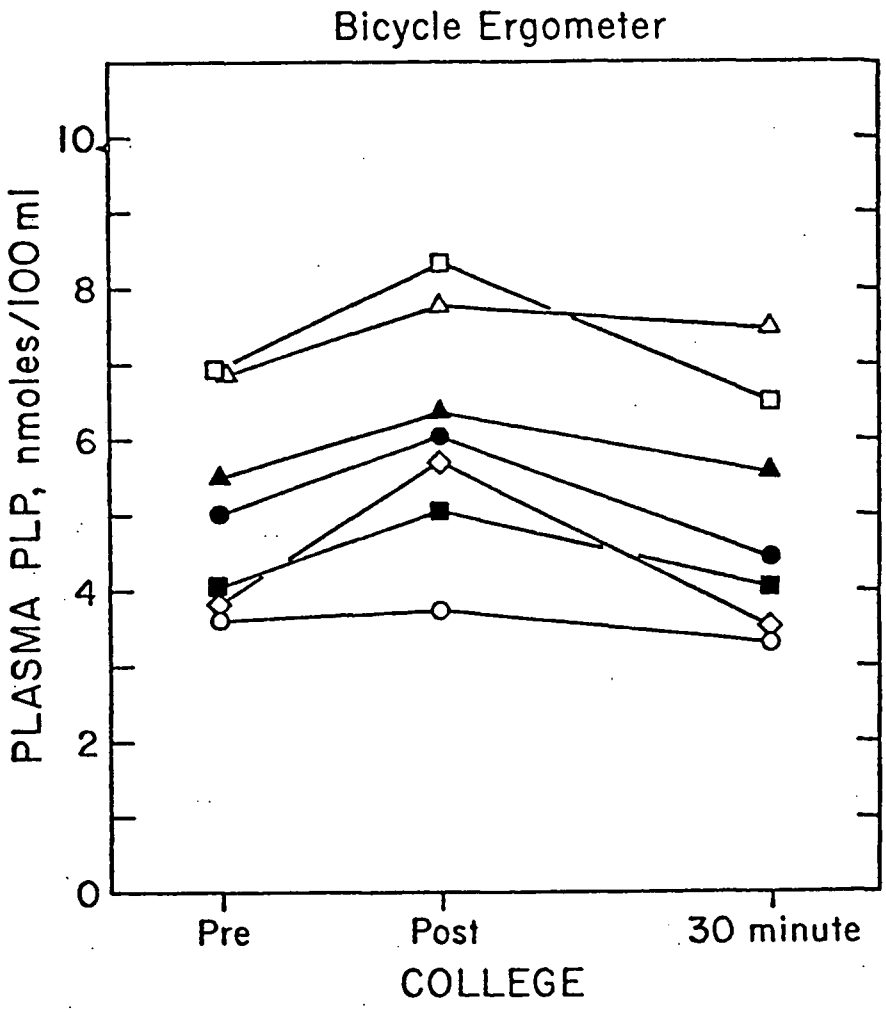

Figure 7. Plasma pyridoxal 5'-phosphate levels during the bicycle ergometer regime.

- ● Subject 300
- ■ Subject 340
- ○ Subject 310
- □ Subject 350
- ▲ Subject 320
- ◇ Subject 360
- △ Subject 330

The net change in nmoles for PB6 is listed in Table 12, with Figure 8 representing the college group's response. Student's t-test revealed no significant difference between the two groups in the mean change in nmoles/100 ml for any of the three time intervals.

In comparing the net change of both plasma PLP and PB6, the two groups responded to different degrees. For the pre to post category, the college group increased the same amount for plasma PLP and PB6 (1.05 ± 0.60 nmoles/100 ml and 1.10 ± 0.54 nmoles/100 ml, respectively) while the untrained group showed a much larger increase in PB6 than plasma PLP (0.84 ± 0.88 nmoles/100 ml and 0.38 ± 0.05 nmoles/100 ml, respectively). It can also be seen that the overall net change was greater for the plasma PLP and PB6 of the college than untrained group. For the post to 30 minute post exercise category, the college group decreased to a much larger degree for both plasma PLP and PB6 (1.15 ± 0.78 nmoles/100 ml and 1.19 ± 1.05 nmoles/100 ml, respectively) as compared to the untrained group (0.68 ± 0.32 nmoles/100 ml plasma PLP and 0.95 ± 0.78 nmoles/100 ml PB6) although the untrained group showed a larger decrease in PB6 than PLP. During the third category, pre to 30 minute post exercise, the college group had a similar decrease for both plasma PLP and PB6 (0.10 ± 0.44 nmoles/100 ml and 0.09 ± 0.97 nmoles/100 ml, respectively) while the untrained group showed a more dramatic drop in plasma PLP than PB6 (0.31 ± 0.36 nmoles/100 ml and 0.12 ± 0.41 nmoles/100 ml, respectively). The intermittent group responded similarly to the untrained group in all three time intervals except for the pre to post PB6 response. For this interval, the intermittent group's levels of PB6 increased much more dramatically than did

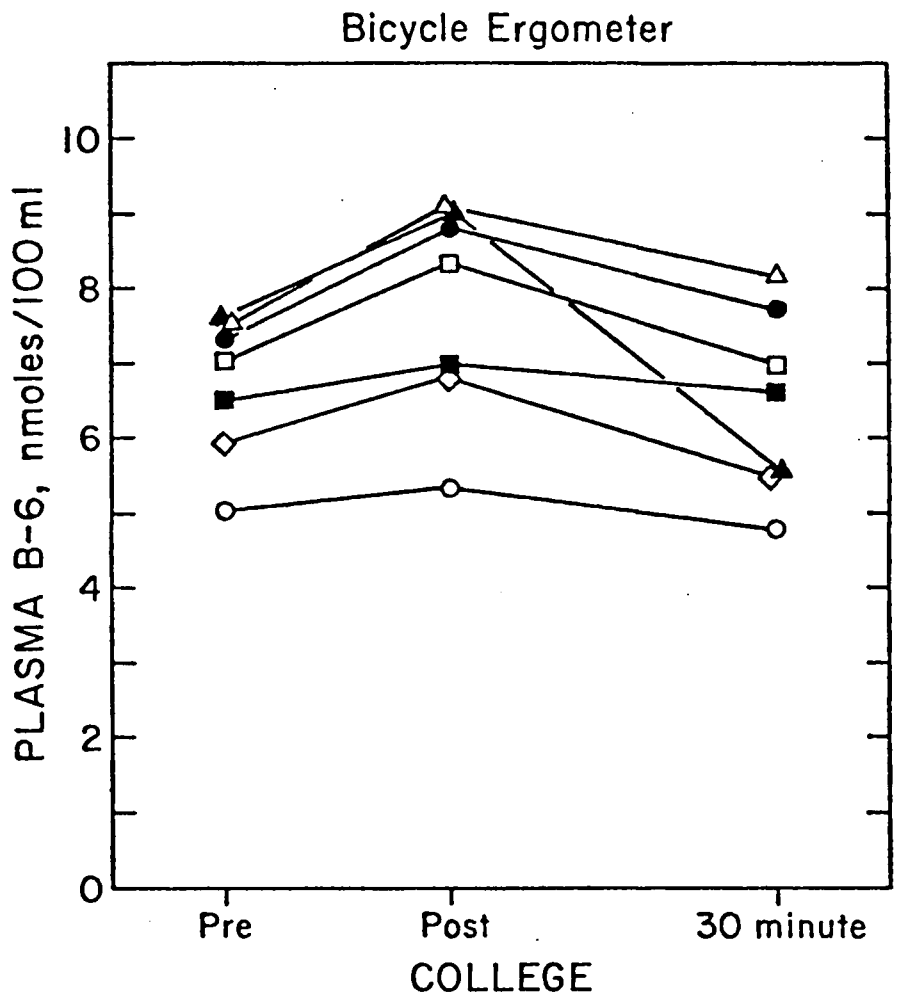

Figure 8. Plasma B-6 levels during the bicycle ergometer regime. Symbols representing each subject are described in Figure 7.

the untrained group (2.33 ± 2.08 nmoles/100 ml and 0.84 ± 0.88 nmoles/100 ml, respectively).

Regression analysis showed a positive correlation ($P < 0.01$) between the pre exercise plasma PLP and PB6 levels as well as the post ($P < 0.02$) and 30 minute post exercise ($P < 0.05$) levels.

Plasma Glucose. The pre exercise value of glucose for all subjects in the college and untrained groups (Table 13) fell within the normal range with the exception of subject 400 in the untrained group, whose pre exercise value was 67 mg/100 ml. Because of the lower value for subject 400, the mean level of glucose for the untrained group was slightly lower (79 ± 11 mg/100 ml) than the college group (85 ± 5 mg/100 ml). Following the 21 minutes of exercise, six of the seven college subjects had increased glucose levels although there was a wide variation in their response. The increase resulted in a positive percent change of 6.5 ± 14.5% (Table 14) with a mean of 88 ± 7 mg/100 ml for the post sample. The 30 minute post exercise glucose levels were lower than the post sample. The 30 minute post exercise glucose levels were lower than the post values for five of the seven college subjects. The group mean of 87 ± 7 mg/100 ml reflected a percent change of 1.9 ± 10.9% for the post to 30 minute post exercise time interval. The percent change for the pre to 30 minute post exercise category was 3.4 ± 9.3% for the college group.

Following the 21 minutes of exercise, the untrained group had a mean value of 81 ± 4 mg/100 ml of glucose for the post sample which reflects an increase of 3.6 ± 11.6%. Two of the three subjects in the untrained group increased from the pre to post sample. The 30 minute

post exercise mean value was 72 ± 10 mg/100 ml for the untrained subjects, or a decrease from the post level of 11.3 ± 11.8%. This reflected a negative change of 9.0 ± 2.3% for the untrained subjects. In comparing the two groups, the pre, post and 30 minute post exercise mean values of glucose were higher for the college group (85 ± 5, 88 ± 7 and 87 ± 7 mg/100 ml, respectively) than for the untrained group (79 ± 11, 81 ± 4, and 72 ± 10 mg/100 ml, respectively). The percent change for the pre to post interval was slightly greater for the college group (6.5 ± 14.5%) than the untrained subjects (3.6 ± 11.6%). The opposite was found for the post to 30 minute post exercise interval. The college group showed a slight decrease during that interval (1.9 ± 10.9%) as compared to a much greater decrease for the untrained group (11.3 ± 11.8%). As a result of exercise, there was an increase in glucose for the college group of 3.4 ± 9.3% whereas the untrained group decreased 9.0 ± 2.3%. Although there were definite differences in the values from one level to the next, paired t-tests showed the differences were not significant within each group, nor did the two groups differ significantly.

Hemoglobin and Hematocrit. The hemoglobin and hematocrit values for all subjects in the college and untrained groups were within the expected range for this age group (Henry, 1974). As seen in Table 15, following exercise the values for hemoglobin and hematocrit measured for both groups. However, the increases were not significant, and the two groups did not differ in their response. The largest change was seen for hemoglobin (-8.2 ± 2.8%) which occurred during the post to 30 minute post exercise interval for the college group. The other changes

were all lower than 7%. Interestingly, both groups increased in hemoglobin and hematocrit following exercise, and decreased in both values during the post to 30 minute post exercise interval.

Urinary Vitamin B-6. The amount of vitamin B-6 excreted in the urine of the college and untrained groups is listed in Table 16 for both groups. No significant difference was found between the two groups for either urine sample or for the percent change between samples. The college group showed relatively little change between the two days (-0.1 ± 14.2%) while the untrained group decreased slightly from the day before to the day of exercise (-8.4 ± 22.4%). Paired t-tests showed no significant difference between the samples for either the college or untrained group.

4-Pyridoxic Acid. The college group had a higher mean level of 4PA (9.56 ± 2.23 umoles/24 hr) than the untrained group (5.72 ± 2.01 umoles/24 hr) although there was not a significant difference (see Table 17). One subject in the college group (320) had an extremely high level of 4PA (51.93 umoles/24 hr) possibly due to the medication he was taking for a knee injury which may have interferred with the analysis, and therefore the value was not included in the calculations. The 4PA decreased slightly from the day before to the day of exercise in five out of six college subjects but increased in two of the three untrained subjects. The changes were not significant for either the college or the untrained group. A positive correlation was found between the UB6 and 4PA levels the day before exercise ($P < 0.001$) and the day of exercise ($P < 0.01$).

Creatinine and Urea Nitrogen. Creatinine was in part, analyzed to assess the completeness of urine samples as well as any effect of exercise. Table 18 lists the mean values for the college and untrained groups. Although the untrained group mean

Table 16. Total urinary vitamin B-6 excreted during the bicycle ergometer regime

Group	n	Sample	$\bar{x}$ ± S.D. µmoles/24 hr	% change
College	7	Day before exercise	1.01 ± 0.17	-0.1 ± 14.2
		Day of exercise	1.01 ± 0.18	
Untrained	3	Day before exercise	0.76 ± 0.21	-8.4 ± 22.4
		Day of exercise	0.67 ± 0.09	

Table 17. Mean 4-pyridoxic acid excreted during the bicycle ergometer regime

Group	n	Sample	$\bar{x}$ ± S.D. µmoles/24 hr	% change
College	6	Day before exercise	9.56 ± 2.23	-5.0 ± 22.5
		Day after exercise	9.41 ± 4.62	
Untrained	3	Day before exercise	5.72 ± 2.01	12.9 ± 24.6
		Day of exercise	6.21 ± 1.94	

Table 18. Mean creatinine excretion during the bicycle ergometer regime

Group	n	Sample	$\bar{x}$ ± S.D. gm/24 hr
College	7	Day before exercise	1.70 ± 0.22
		Day of exercise	1.82 ± 0.26
Untrained	3	Day before exercise	1.46 ± 0.50
		Day of exercise	2.10 ± 0.44

was much higher on the day of exercise, paired t-tests showed no significant difference between the day before and the day of exercise for either group.

The urine was also analyzed for urea nitrogen for the day prior to exercise as well as the day of exercise. Table 19 lists the group means for urea nitrogen. Paired t-tests indicated no significant difference between either the day before or the day of exercise for either group. Nor did the two groups differ in their response. Interestingly, regression analysis showed a positive correlation ($P < 0.001$) between the average urea nitrogen excretion and the average vitamin B-6 intake. Contrary to what was expected, no significant correlation was found between the excretion of urea nitrogen and the average protein intake calculated from the day before and the day of exercise.

Physical Working Capacity. Figures 9 and 10 illustrate the physical working capacity of the college and untrained groups, respectively. The level of training differs for both groups in that the rate of rise in heart rate with increasing exercise load was lower for the college group than the untrained group. The estimated mean amount of work to produce a heart rate of 170 beats per minute for the college group was approximately 1095 kilogram-meters per minute. In contrast, the untrained group required approximately 930 kilogram-meters per minute of work. The heart rate of 170 is usually used as the level above which no significant increase in work load occurs aerobically. The rise in heart rate usually increases linearly with increasing work loads. However, the college group mean appears to be less than linear. Two

Table 19. Mean urea nitrogen excretion during the bicycle ergometer regime

Group	n	Sample	$\bar{x}$ ± S.D. gm/24 hr
College	7	Day before exercise	12.6 ± 4.2
		Day of exercise	13.6 ± 3.6
Untrained	3	Day before exercise	10.6 ± 1.9
		Day of exercise	12.8 ± 2.2

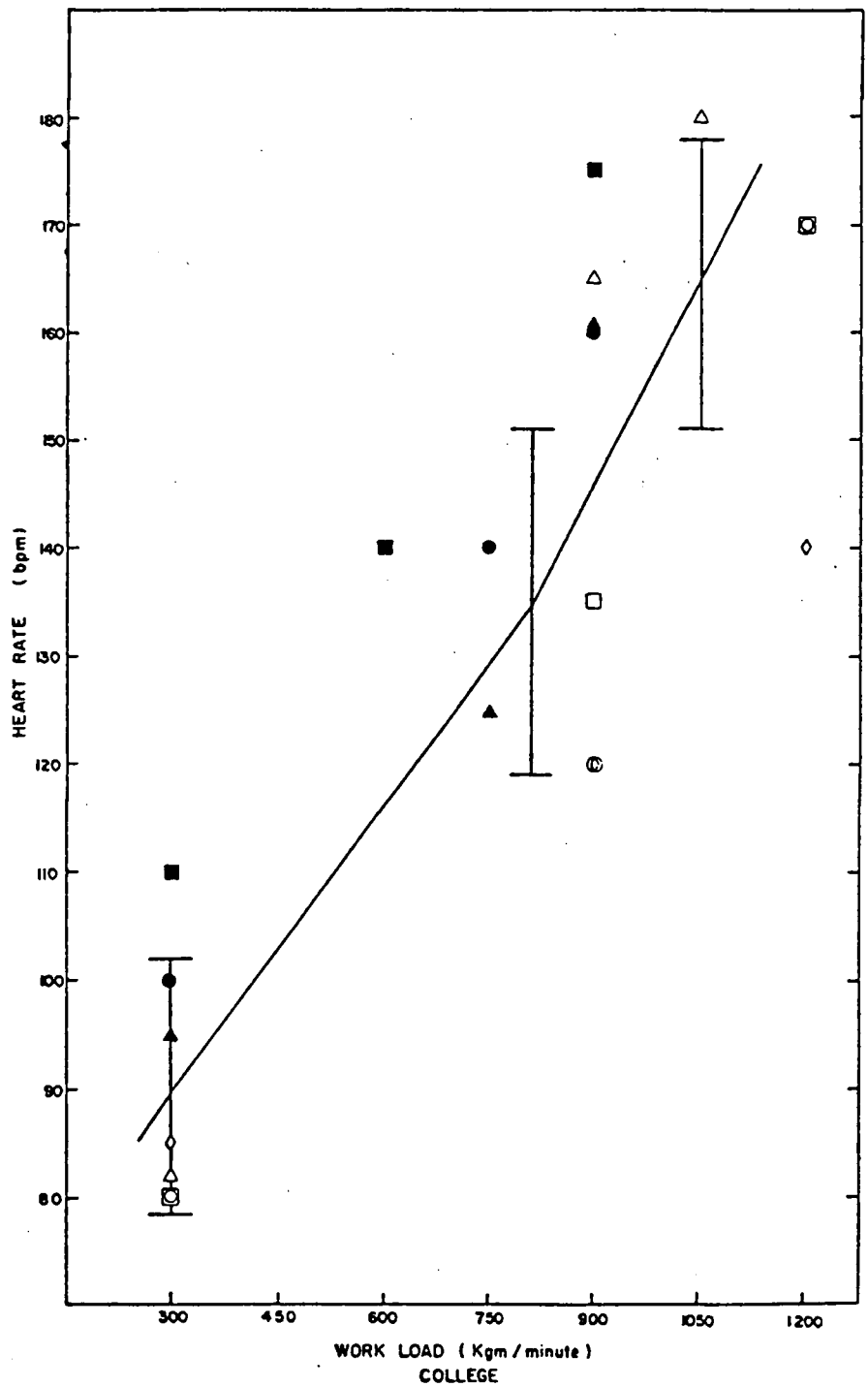

Figure 9. Physical working capacity for the college group during the bicycle ergometer regime. Symbols representing each subject are described in Figure 7.

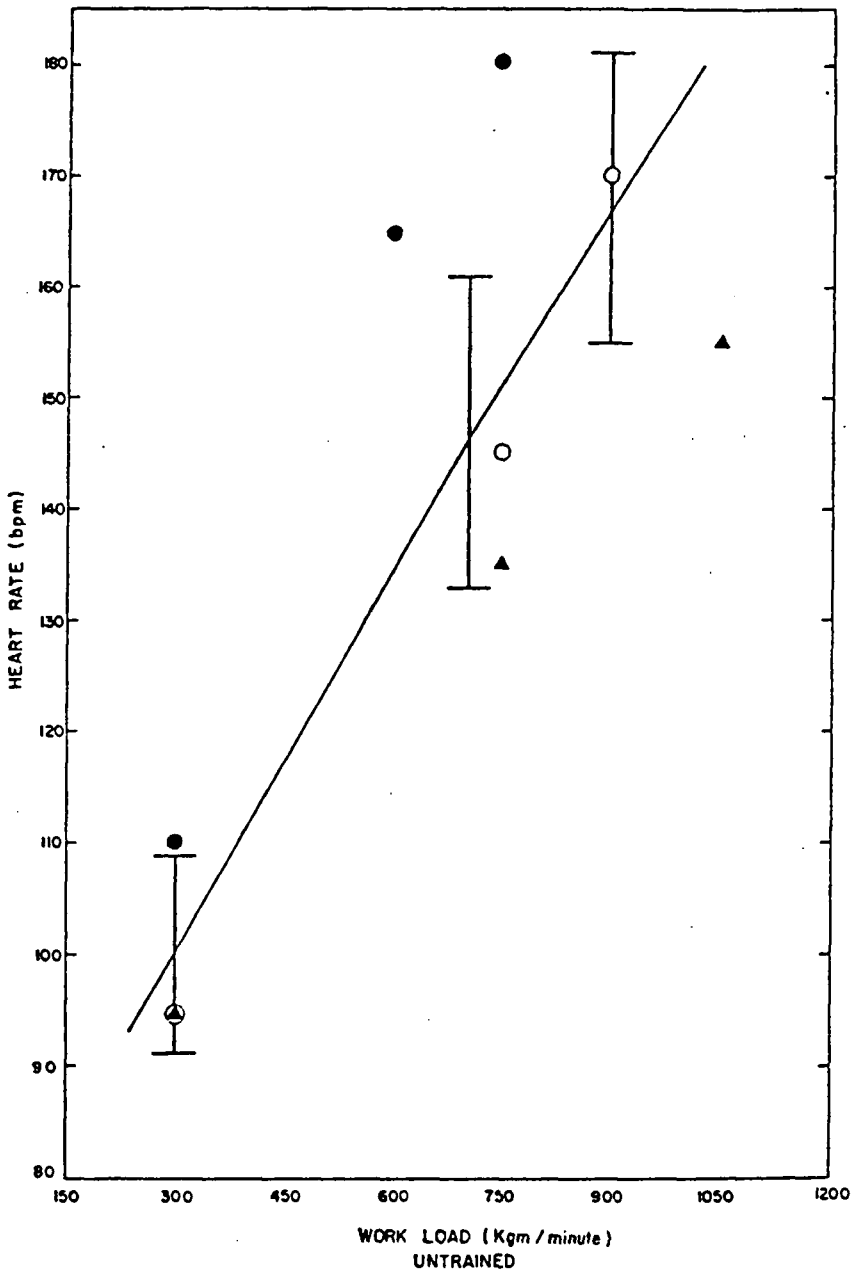

Figure 10. Physical working capacity for the untrained group during the bicycle ergometer regime.

● Subject 400

○ Subject 410

▲ Subject 430

subjects, 310 and 320, did not produce linear rises in heart rate whereas the other five subjects did.

Female Subject's Response. In contrast to the female subject in the intermittent group, the untrained female athlete did not show an increase in plasma PLP for the pre to post interval, but rather, the levels remained unchanged. After the 30 minute rest, the plasma PLP values dropped 9.2% or 0.76 nmoles/100 ml which was slightly less than the first female subject. Unlike plasma PLP, the PB6 values increased 9.0% for the pre to post time interval and decreased approximately 10.0% or 1.21 nmoles/100 ml after a 30 minute rest. Plasma glucose values increased slightly following exercise (4.2%) as did the hemoglobin and hematocrit values.

Run

College Group

Plasma PLP. Six of the seven college subjects (subject 320 could not participate due to a leg injury) also exercised by running. Table 20 lists the absolute values for plasma PLP. Values for the six subjects ranged from a low of 2.61 nmoles/100 ml for the pre exercise samples to a high of 7.46 nmoles/100 ml, with the mean pre exercise plasma PLP for the run (5.21 ± 2.10 nmoles/100 ml) being similar to the values for the bicycle ergometer study (5.07 ± 1.33 nmoles/100 ml). Following the run, the mean plasma PLP levels increased to 6.95 ± 2.66 nmoles/100 ml. This represented a 35.0 ± 8.9% change. Figure 11 graphically illustrates the change for each subject following exercise. In comparison, the bicycle ergometer exercise resulted in a smaller increase of 22.0 ± 14.4%.

Table 20. Mean plasma pyridoxal 5'-phosphate levels, net change and percent change during the run

Group	n	Sample[a]	$\bar{x}$ ± S.D. nmoles/100 ml
College	6	Pre	5.21 ± 2.10
		Post	6.95 ± 2.66
		Net change	1.73 ± 0.65 [b]
		% change	35.0 ± 8.9%
High School	6	Pre	4.96 ± 2.55
		Post	6.07 ± 2.81
		Net change	1.11 ± 0.34 [b]
		% change	24.3 ± 6.9%
	6	Pre	5.88 ± 4.74
		Post	6.96 ± 5.53
		Net change	1.42 ± 1.01 [c]
		% change	19.4 ± 9.0%
	4	Pre	4.38 ± 1.97
		Post	5.54 ± 2.33
		Net change	1.17 ± 0.49 [d]
		% change	27.5 ± 10.7%

a Blood samples are described in Table 6.
b $P < 0.005$
c $P < 0.025$
d $P < 0.01$

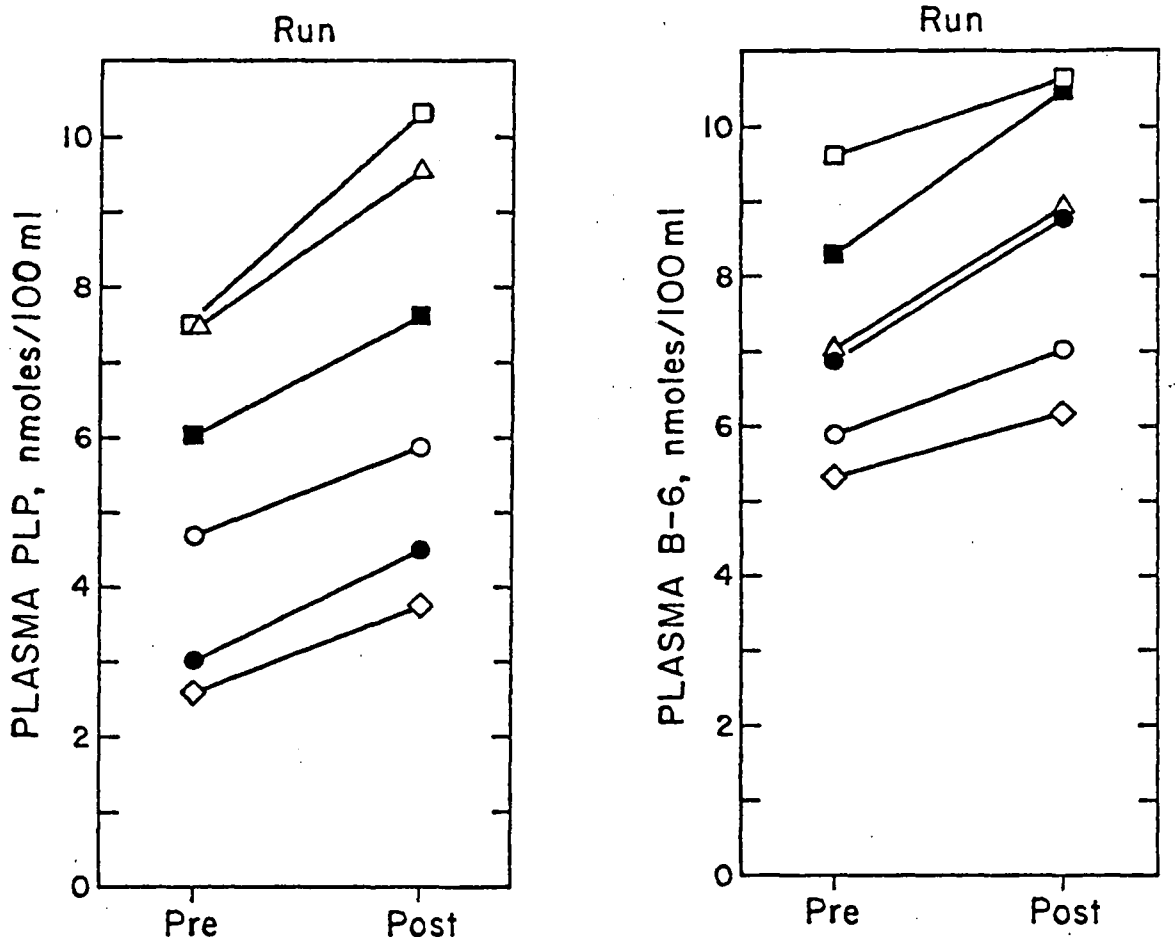

Figure 11. Plasma pyridoxal 5'-phosphate and plasma B-6 levels during the run for the college group. Symbols representing each subject are described in Figure 7.

All six subjects showed an increase and ranged from 25.8% to 45.9%. The positive increase in plasma PLP reflected a change of 1.73 ± 0.65 nmoles/100 ml for the run, which was slightly higher than the bicycle ergometer response (1.05 ± 0.60 nmoles/100 ml). With the paired t-test, there was a positive significant difference between the pre and post values for plasma PLP ($P < 0.005$).

Table 21 lists the percent of the total plasma vitamin B-6 found as plasma PLP during the run. Pre exercise levels were lower than those found prior to the bicycle ergometer regime (64.6 ± 16.1 and 75.2 ± 12.8%, respectively). Following exercise, the percent as plasma PLP increased to 72.7 ± 17.6%, which corresponded to the bicycle ergometer values (78.1 ± 11.5%). Paired t-tests revealed no significant difference between the percent of plasma PLP for the pre and post values.

Plasma PB6. The group means for PB6 are given in Table 22. The mean pre exercise PB6 value (7.18 ± 1.59 nmoles/100 ml) was slightly higher than that noted for the bicycle ergometer (6.69 ± 0.96 nmoles/100 ml). After the exercise, the post levels of PB6 rose to a mean of 8.73 ± 1.84 nmoles/100 ml. As seen in Figure 11, all six subjects had elevated PB6 levels following the exercise. The increase represented a change of 21.7 ± 6.4%, or 1.54 ± 0.54 nmoles/100 ml. In contrast, the bicycle ergometer elicited a smaller positive change of 15.9 ± 6.5% or 1.10 ± 0.54 nmoles/100 ml.

Plasma Glucose. The change in plasma glucose following the run is given in Table 23. Pre exercise values were within the normal range for all six subjects, however, the mean value was higher than that measured prior to the bicycle ergometer regime (91 ± 5 and 85 ± 5

Table 21. Percent of total plasma vitamin B-6 as pyridoxal 5'-phosphate during the run

Group	n	Sample[a]	$\bar{x}$ ± S.D. %
College	6	Pre	64.6 ± 16.1
		Post	72.7 ± 17.6
High School	6	Pre	61.0 ± 6.3
		Post	63.9 ± 4.1
	6	Pre	57.4 ± 2.8
		Post	57.9 ± 5.1
	4	Pre	54.9 ± 6.2
		Post	56.4 ± 6.9

a Blood samples are described in Table 6.

Table 22. Mean plasma vitamin B-6 levels, net change, and percent change during the run

Group	n	Sample[a]	$\bar{x}$ ± S.D. nmoles/100 ml
College	6	Pre	7.18 ± 1.59
		Post	8.73 ± 1.84
		Net change	1.54 ± 0.54
		% change	21.7 ± 6.4% [b]
High School	6	Pre	7.97 ± 3.48
		Post	9.55 ± 4.59
		Net change	1.58 ± 1.31
		% change	18.4 ± 8.7% [c]
	6	Pre	10.48 ± 9.03
		Post	12.25 ± 10.20
		Net change	1.77 ± 1.26
		% change	18.1 ± 7.1% [d]
	4	Pre	8.30 ± 4.75
		Post	10.23 ± 5.60
		Net change	1.93 ± 0.93 [c]
		% change	24.2 ± 7.0%

a Blood samples are described in Table 6.
b $P < 0.005$
c $P < 0.025$
d $P < 0.01$

Table 23. Mean plasma glucose levels and percent change during the run

Group	n	Sample[a]	$\bar{x}$ ± S.D. mg/100 ml
College	6	Pre	91 ± 5
		Post	112 ± 17
		% change	23.4 ± 21.8%
High School	6	Pre	88 ± 16
		Post	91 ± 11
		% change	4.9 ± 9.5%
	6	Pre	96 ± 8
		Post	101 ± 16
		% change	7.0 ± 18.9%
	4	Pre	91 ± 9
		Post	111 ± 3
		% change	23.0 ± 11.9%[b]

a Blood samples are described in Table 6.

b $P < 0.025$

mg/100 ml, respectively). Interestingly, the run resulted in an increase of glucose to 112 ± 17 mg/100 ml or 23.4 ± 21.8%, whereas the bicycle ergometer resulted in a much lower increase to 88 ± 7 mg/100 ml or 6.5 ± 14.5%. The changes in plasma glucose during the run were not statistically significant due to the wide range of responses among subjects.

Hemoglobin and Hematocrit. Hemoglobin values, which were considered normal, increased slightly from 15 ± 1 gm/100 ml to 16 ± 1 gm/100 ml or 2.8 ± 3.1% (see Table 24). The percent change was lower compared to the 6.7 ± 4.5% increase that occurred following the bicycle ergometer regime. Pre exercise hematocrit values were also well within normal range. After exercising, the hematocrit values increased to 46 ± 3%, or a positive change of 4.7 ± 2.0%. A similar percent change of 4.9 ± 4.5% was seen following the bicycle ergometer exercise. The pre to post exercise changes for both the hemoglobin and hematocrit were not statistically significant.

Urinary Vitamin B-6 and 4-Pyridoxic Acid. Urine samples for the run were collected only on the day of exercise. The UB6 values (Table 25) were similar to the values recorded for the bicycle ergometer (0.95 ± 0.20 and 1.01 ± 0.18 umoles/24 hr, respectively). In contrast to the UB6 levels, 4PA excretion was lower during the run (7.27 ± 2.96 μmoles/24 hr), as shown in Table 26. Regression analysis showed a positive correlation ($P < 0.001$) between the UB6 and 4PA values during the run.

Creatinine and Urea Nitrogen. Table 27 lists the creatinine values for the day of exercise. The mean of 1.64 ± 0.26 gm/24 hr was similar

Table 24. Mean hemoglobin and hematocrit levels during the run

Group	n	Sample[a]	Hb $\bar{x}$ ± S.D. gm/100 ml	Hct $\bar{x}$ ± S.D. %
College	6	Pre	15.3 ± 0.9	44 ± 2
		Post	15.7 ± 0.8	46 ± 3
		% change	2.8 ± 3.1%	4.7 ± 2.0%
High School	6	Pre	14.8 ± 0.9	42 ± 2
		Post	15.4 ± 1.4	44 ± 2
		% change	3.9 ± 5.9%	3.9 ± 1.5%
	6	Pre	14.9 ± 0.8	43 ± 1
		Post	15.1 ± 0.7	44 ± 2
		% change	1.2 ± 3.6%	2.0 ± 2.5%
	4	Pre	13.4 ± 0.5	42 ± 1
		Post	14.9 ± 0.8	43 ± 2
		% change	10.7 ± 3.6%	4.7 ± 1.2%

a Blood samples as described in Table 6.

Table 25. Total urinary vitamin B-6 excreted during the run

Group	n	Sample	$\bar{x}$ ± S.D. µmoles/24 hr
College	6	Day of exercise	0.95 ± 0.20
High School	4	Day before exercise	0.94 ± 0.26
		Day of exercise	0.99 ± 0.25
	6	Day before exercise	1.05 ± 0.43
		Day of exercise	0.98 ± 0.28
	5	Day before exercise	0.93 ± 0.25
		Day of exercise	0.89 ± 0.17

Table 26. Mean 4-pyridoxic acid excretion during the run

Group	n	Sample	$\bar{x} \pm$ S.D. µmoles/24 hr
College	6	Day of exercise	7.27 ± 2.96
High School	4	Day before exercise	9.82 ± 2.51
		Day of exercise	12.36 ± 4.65
	6	Day before exercise	10.65 ± 6.42
		Day of exercise	10.18 ± 4.43
	5	Day before exercise	11.21 ± 6.26
		Day of exercise	9.56 ± 2.95

Table 27. Mean creatinine excretion during the run

Group	n	Sample	$\bar{x}$ ± S.D. gm/24 hr
College	6	Day of exercise	1.64 ± 0.26
High School	4	Day before exercise	1.73 ± 0.35
		Day of exercise	1.80 ± 0.24
	6	Day before exercise	1.62 ± 0.34
		Day of exercise	1.66 ± 0.30
	5	Day before exercise	1.56 ± 0.21
		Day of exercise	1.24 ± 0.23

to the 1.82 ± 0.26 gm/24 hr found during the bicycle ergometer regime. The same was true for the urea nitrogen excretion (Table 28). The mean excretion was 12.08 ± 4.44 gm/24 hr as compared to 13.58 ± 3.56 gm/24 hr during the bicycle ergometer study.

High School Group

Vital Statistics. The high school group was studied on three separate occasions throughout the training sessions. As listed in Table 2, the mean age of the subjects was 16.0 ± 0.6 years, with a mean height of 177 ± 7 cm and weight of 67.7 ± 7.2 kg.

Plasma PLP. Table 20 lists the plasma PLP values for each of the three runs, as well as the percent change and the change in nmoles/100 ml. Pre exercise plasma PLP levels were similar for runs 1 and 3 (4.96 ± 2.55 and 4.38 ± 1.97 nmoles/100 ml, respectively) while the second run had a slightly higher value (5.88 ± 4.74 nmoles/100 ml) due to the high plasma PLP levels of subject 210 (15.40 nmoles/100 ml). All three exercise days resulted in positive increases in plasma PLP following the exercise. The first and third exercise days had similar increases (24.3 ± 6.9 and 27.5 ± 10.7%) compared to a smaller increase of 19.4 ± 9.0% for run 2. As shown in Figure 12, subject 210 had much higher pre and post exercise plasma PLP levels than the other athletes for each of the three runs. However, the change in nmoles/100 ml of plasma PLP for subject 210 from the pre to post interval was highest for run 2 resulting in a larger overall change for the group (1.42 ± 1.01 nmoles/100 ml) compared to runs 1 and 3 (1.11 ± 0.34 and 1.17 ± 0.49 nmoles/100 ml, respectively). Subject 210 had a history of vitamin

Table 28. Mean urea nitrogen excretion during the run

Group	n	Sample	$\bar{x}$ ± S.D. gm/24 hr
College	6	Day of exercise	12.08 ± 4.44
High School	4	Day before exercise	13.26 ± 5.22
		Day of exercise	13.17 ± 4.06
	6	Day before exercise	12.33 ± 2.74
		Day of exercise	11.69 ± 4.20
	5	Day before exercise	11.67 ± 5.25
		Day of exercise	14.29 ± 4.76

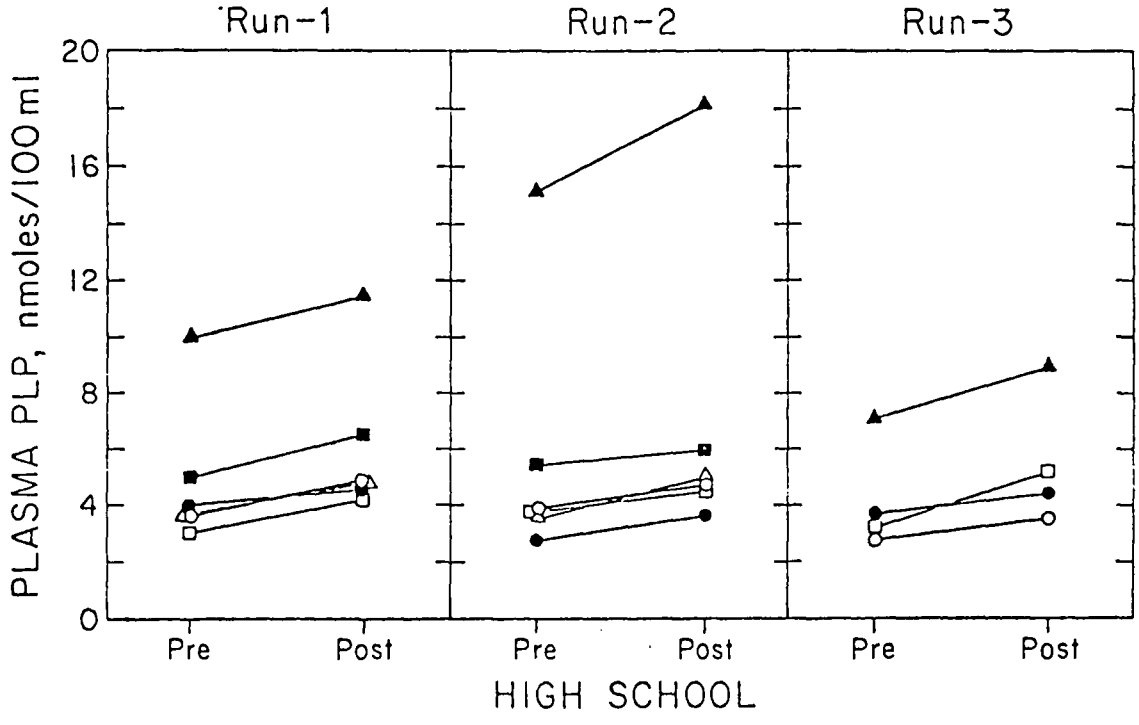

Figure 12. Plasma pyridoxal 5'-phosphate levels for the high school group during the run.

- ● subject 200
- ▲ subject 210
- ○ subject 220
- ■ subject 230
- △ subject 240
- □ subject 250.

intake although he claimed he had not taken vitamins two weeks prior to or during the study. From the paired t-tests, the pre and post plasma PLP values were found to be significantly different for run 1 ($P < 0.001$), run 2 ($P < 0.025$) and run 3 ($P < 0.01$).

The percent of total plasma vitamin B-6 found as plasma PLP, given in Table 21, shows that the pre exercise values decreased as the season progressed. The first exercise day was somewhat higher than the second or third run (61.0 ± 6.3, 57.4 ± 2.8, and 54.9 ± 6.2%, respectively). Following exercise, the percent of PB6 as plasma PLP was essentially the same as the pre values for all three runs.

Plasma B6. The changes in PB6 following exercise were similar to the plasma PLP results. Pre exercise values for run 1 and 3 (see Table 22) were similar (7.97 ± 3.38 and 8.3 ± 4.75 nmoles/100 ml, respectively) whereas the subjects in run 2 had higher pre PB6 values (10.48 ± 9.03 nmoles/100 ml). As Figure 13 illustrates, subject 210 had higher pre exercise levels of PB6 than the other subjects for all three runs (14.70, 28.73, and 15.39 nmoles/100 ml, respectively). The smallest percent increase for the three runs was seen during run 2 (12.1 ± 7.1%) with runs 1 and 3 having a greater increase following exercise (18.4 ± 8.6 and 24.2 ± 7.0%, respectively). Paired t-tests indicated a significant difference between the pre and post levels of PB6 for run 1 ($P < 0.025$), run 2 ($P < 0.01$), and run 3 ($P < 0.025$). There was a positive correlation of the pre exercise values of PB6 with the pre exercise plasma PLP values ($P < 0.001$) and the post exercise PB6 and plasma PLP values ($P < 0.001$). Also, the change in nmoles/100 ml of PB6 was positively correlated with the change in nmoles/100 ml of plasma PLP ($P < 0.005$).

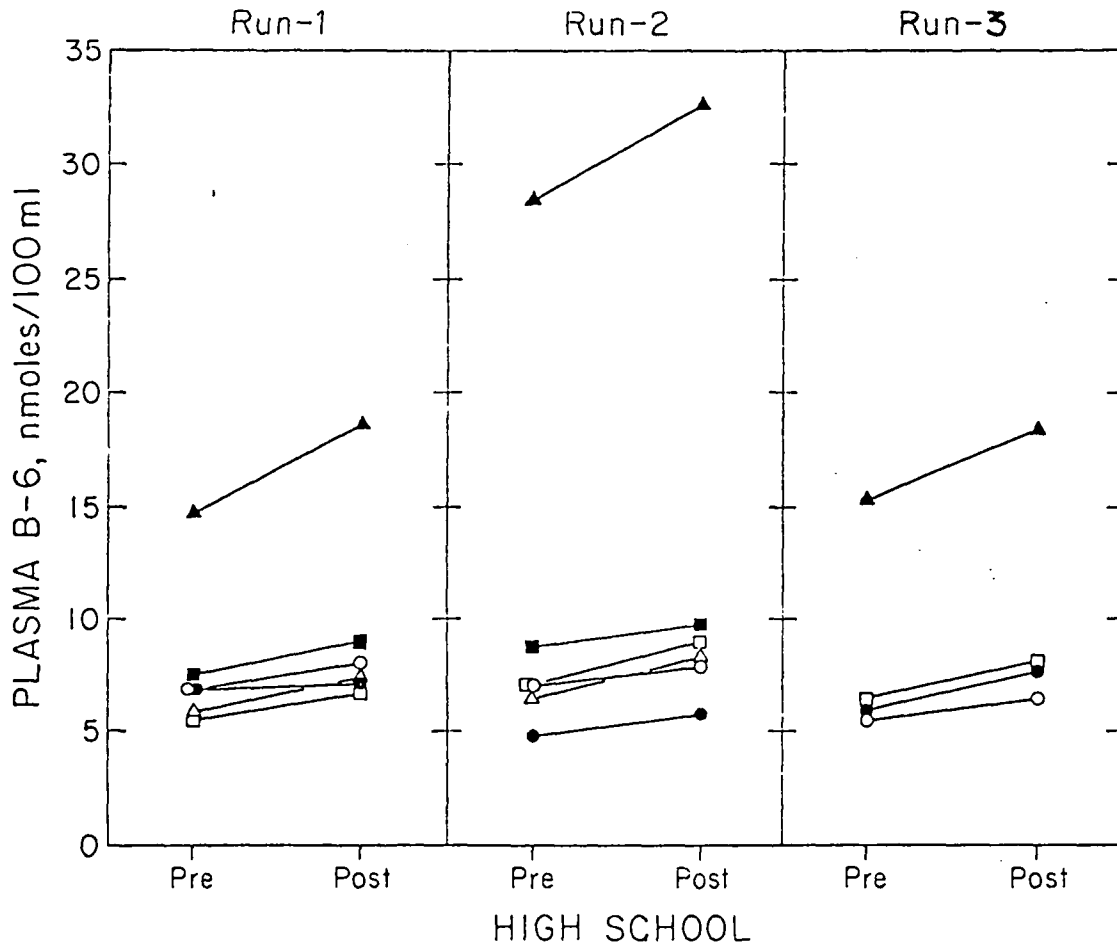

Figure 13. Plasma B-6 levels for the high school group during the run. Symbols representing each subject are described in Figure 12.

Plasma Glucose. Glucose values for the three runs fell within normal values for all subjects. The pre exercise glucose levels ranged from 67 mg/100 ml to 116 mg/100 ml. Group means for runs 1, 2, and 3 are given in Table 23 and illustrated in Figure 14. For each run, there was an increase in plasma glucose following exercise. Run 1 changed from 88 ± 16 mg/100 ml to 91 ± 11 mg/100 ml for an increase of 4.9 ± 9.5%. Although the overall group mean increased, the response was not the same for all subjects. Three of the five subjects had higher glucose levels after exercise, whereas one subject decreased (250) and one subject (240) maintained levels similar to the pre exercise value. The overall mean for run 2 was 96 ± 8 mg/100 ml for the pre exercise value. This increased to a mean of 91 ± 11 mg/100 ml (7.0 ± 18.9%) following exercise. As illustrated in Figure 14, three subjects decreased from the pre exercise levels, while three increased. Finally, run three showed three subjects increasing more dramatically than either run 1 or run 2 and one subject increased slightly. The group mean for run 3 was 91 ± 9 mg/100 ml before exercise which increased to 111 ± 3 mg/100 ml following exercise or 23.0 ± 11.9%. Therefore, the response to exercise throughout the study was not consistent for plasma glucose although the mean percent change in glucose from the pre to post level increased as the season progressed. Statistically, only run 3 had pre and post values that were significantly different ($P < 0.025$).

Hemoglobin and Hematocrit. Hemoglobin values were normal for all subjects during the three runs (Table 24). For runs 1 and 2, the mean pre exercise levels of hemoglobin were the same (15 ± 1 gm/100 ml) and did not significantly change following exercise. Run 3 had a lower mean

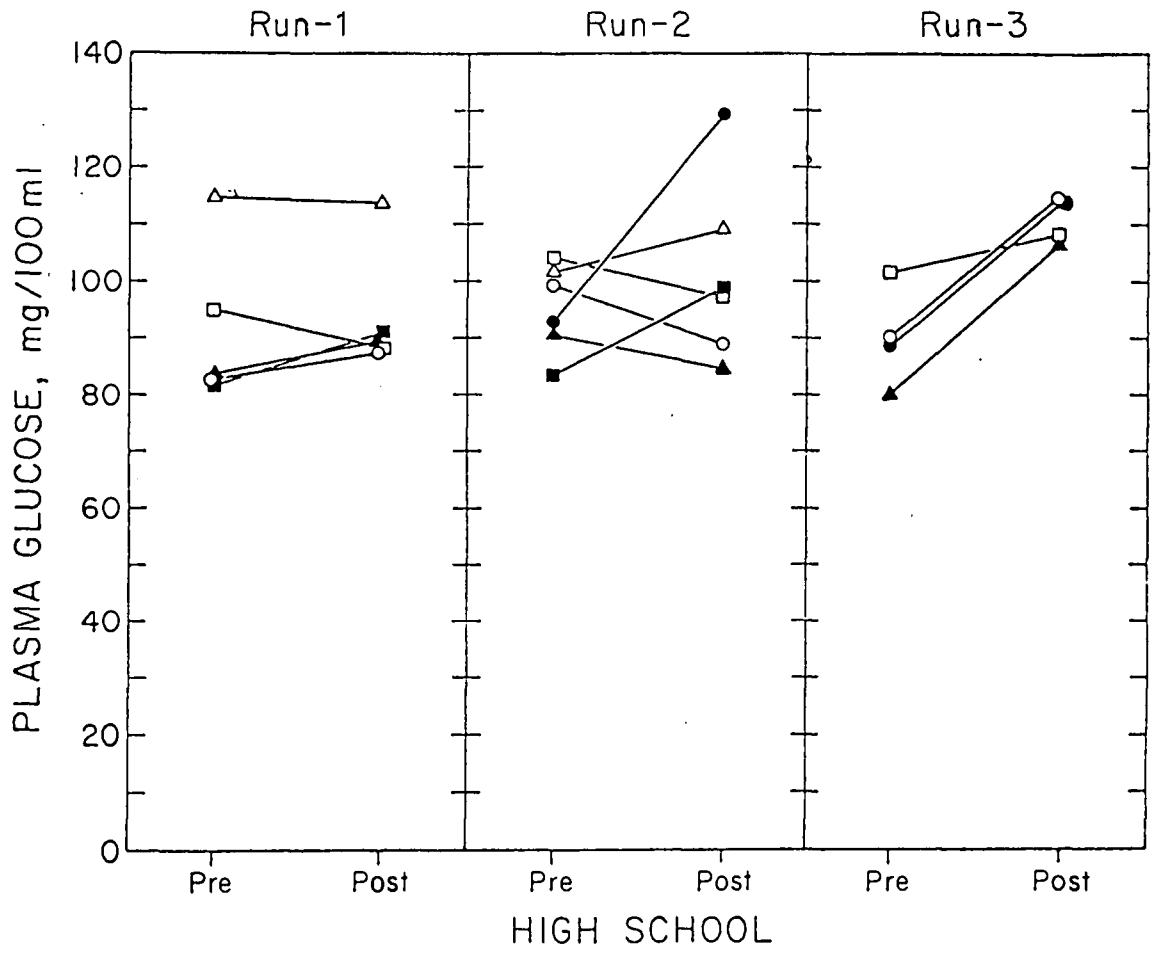

Figure 14. Plasma glucose levels for the high school group during the run. Symbols representing each subject are described in Figure 12.

pre exercise level (13 ± 1 gm/100 ml) that increased to 15 ± 1 gm/100 ml (10.7 ± 3.6%) but the change was not significant. The hematocrit values, also shown in Table 24, were similar for all three runs and considered normal. Pre exercise mean hematocrit values increased following exercise in all three runs (3.9 ± 1.5, 2.0 ± 2.5, and 4.7 ± 1.2%, respectively). However, paired t-tests did not indicate that the changes were significant.

Urinary Vitamin B-6 and 4-Pyridoxic Acid. The mean UB6 values (Table 25) the day before exercise were considered normal (0.94 ± 0.26, 1.05 ± 0.43, and 0.93 ± 0.25 μmoles/24 hr for runs 1, 2, and 3, respectively), as were UB6 values obtained from the day of the study for the three runs (0.99 ± 0.25, 0.98 ± 0.28, and 0.89 ± 0.17 μmoles/24 hr, respectively. The 4PA levels were somewhat similar on all occasions (see Table 26). Paired t-tests indicated that the values were not significantly different.

Creatinine and Urea Nitrogen. Creatinine mean values for each run are listed in Table 27. The values for the day before exercise and the day of exercise were not significantly different for either of the three runs.

Similarly, no significant difference in urea nitrogen was found between the day before or day of exercise for either run 1, 2 or 3 (Table 28). As expected, regression analysis showed a significant correlation ($P < 0.05$)between urea nitrogen excretion and dietary protein intake.

Effects of Age. The difference between the response of adolescent athletes versus adult athletes was compared using a Student's t-test.

Run 2 of the high school study was used for comparison as it represents the midpoint of training for both groups of athletes. Figure 15 illustrates the responses of both groups for the percent change of plasma PLP and PB6 from the pre to post exercise levels. As can be seen, the percent change for the college group was greater than the high school response for either run 1, 2, or 3 (see Table 20). During run 2, the college athletes had a mean percent plasma PLP change of 35.0 ± 8.9% as compared to the mean change of 19.4 ± 9.0% for the high school athletes. The percent change of plasma PLP was statistically different between the two groups ($P < 0.01$) while the percent change in PB6 was not significant. Interestingly, the percent of plasma vitamin B-6 as plasma PLP was 12% higher after the run for the college athletes as compared to only 0.9% during run 2 for the high school athletes. No other significant differences were found to suggest age affected the results. The calculation of percent body fat was made for each subject (Appendix Table 2), indicating a mean of 13.0 ± 4.4%. No correlation was found between the percent body fat and the changes in blood values.

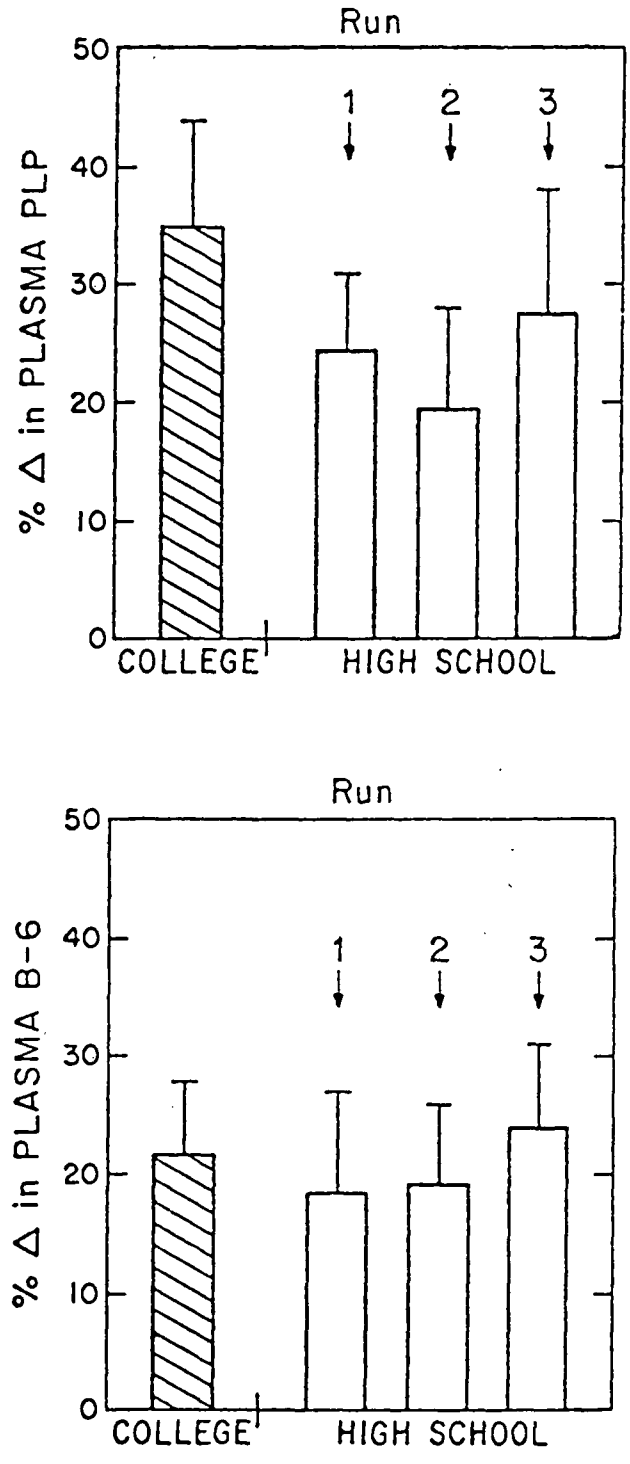

Figure 15. Comparison of the percent change in plasma pyridoxal 5'phosphate and plasma B-6 levels of the college and high school groups during the run.

V. DISCUSSION

Dietary Intake

The RDA was utilized to evaluate the diet of all the subjects involved. The point must be emphasized, however, that dietary guidelines for athletes have not yet been defined and, even though the RDA does not consider strenuous daily exercise, it is the only means available to effectively assess the adequacy of nutrient intake.

The caloric consumption for the college and high school groups was greater than the untrained subjects, as would be expected, due to the higher amount of energy expended during training. The protein intake was consistently very high in all subjects but especially so in the college and high school groups. As discussed previously, a higher protein intake would tend to raise the requirement for vitamin B-6 (Donald, 1978). However, the vitamin B-6 intake during this study was not high enough to meet the requirements of the high protein intake, and thus, inadequate B-6/protein ratios resulted. Donald (1978) has suggested that a B-6/protein ratio of 0.019 can be considered adequate, while a 0.017 ratio is not. Following these guidelines, the high school intake of B-6/protein was inadequate, while the ratios found for the college and untrained groups were adequate. In reviewing the dietary intake of the high school group, it was noted that the ingestion of large amounts of milk, which is high in protein while being relatively low in vitamin B-6, was responsible for the inadequate ratio. The protein intakes during this study were similar to those of a similar age group of non-athletes reported by Driskell and Chrisley (1981).

The dietary intake of vitamin B-6 for all four groups was, in most

cases, satisfactory in terms of the RDA. As previously shown, the high levels of other nutrients measured, such as riboflavin, thiamin, niacin, vitamins A and C, calcium and iron were more than adequate for a healthy, normal adult. The high levels of nutrients were not surprising, considering the large amount of calories consumed by those subjects in training. The consumption of a diet high in calories does not, however, insure its quality. Jette et al. (1978) found the high calorie/carbohydrate diet of some athletes to be markedly low in niacin and riboflavin, while being high in vitamin C. Interestingly, in the present study, the quality of the diet was still satisfactory in the lower caloric diets of the untrained subjects. Overall, the subjects in this study appear to have selected balanced diets reflecting well-nourished adults.

A final point must be made in regards to the use of self-selected dietary records. While the subjects were instructed in recording dietary intake, the assessment may be altered by many factors. First, there is a lack of information on the vitamin B-6 content of many foods and its bioavailability (NRC, 1980, and Wozenski et al., 1980). Second, vitamin B-6 can be subject to destruction before being consumed and may not be totally absorbed (Gregory and Kirk, 1976). Third, additional sources of error can arise due to the food composition tables used, inaccurate food records, erroneous interpretations of those records and the calculation of dietary intake (Chrisley and Driskell, 1979). However, research has shown that if the utmost care was taken in recording the dietary intake, the errors made in estimating the food eaten do not interfere the average nutrient intake on a group basis (Young et al., 1953).

Vitamin B-6 Metabolism During the Bicycle Ergometer

Pre exercise plasma PLP and PB6 levels, combined with the UB6 and 4PA values, indicated that all the subjects in the study are normal in terms of their vitamin B-6 status. Unlike the research reported by Shultz and Leklem (1981), the pre plasma PLP levels did not correlate with the dietary intake of vitamin B-6 or the vitamin B-6/protein ratios. However, the pre plasma PLP was not a fasting sample as in the study reported by Shultz and Leklem, and as shown in Wozenski's study (1980) diet would not cause an increase in plasma levels of PLP four hours after the ingestion of food. In Wozenski's study (1980), plasma PLP returned to fasting levels within one hour with a 0.5 mg PN dose. The lack of correlation may be due to either the small number of subjects and/or the inaccuracy of the dietary records; or perhaps the metabolism of the athlete differs from the population studied by Shultz and Leklem. It is suggested therefore, that in future studies, the diet of the athletes should be controlled to eliminate this source of error.

Based on the data reported here, exercise on a bicycle ergometer has a significant effect on plasma PLP and PB6 levels. The urinary forms of vitamin B-6 measured, UB6 and 4PA, were not significantly altered, suggesting a shift in plasma PLP and the unphosphorylated forms of vitamin B-6 from one compartment to another, rather than an increased conversion and subsequent increased excretion of the vitamers. Since plasma PLP is the predominant active form of the vitamin, and functions in energy metabolism, the rise would be expected to be related to the intensity of the exercise. Using the bicycle ergometer enabled us to determine the work intensities at the different time intervals. Although

the intermittent group did not show a significant increase in plasma PLP or PB6, the blood samples taken after each interval enables the rise in plasma PLP to be followed as the work load increased. The lack of significance for this group in the plasma PLP and PB6 response was probably due to the large variation in subjects' ages, training, and pre-exercise history. One subject (120) was a trained marathon runner, who had competed in a marathon race the day before the study. This subject appeared to be highly trained aerobically, as evidenced by the bicycle ergometer workout. He worked at the highest work load possible and still only achieved a heart rate of 120. Also, his levels of plasma PLP and PB6 only slightly increased during the exercise. Two possible explanations exist for the small change in the vitamers. First, the subject was probably oxidizing mostly free fatty acids for energy and the utilization of muscle glycogen was not necessary. Second, the glycogen may have been depleted during the marathon the day before and would not have been available for energy had the intensity of work been greater. It should be noted, however, that even though the results for the intermittent group were not statistically different from one interval to the next, there was a rise in both plasma PLP and PB6 in all subjects.

The significantly higher levels of post exercise plasma PLP and PB6, as compared to the pre exercise values, were found for the college and untrained groups. Although no data exist for the plasma PLP values at seven and fourteen minutes of exercise, based on the intermittent study, it is assumed that the largest increases occurred prior to the end of exercise. During the first seven minute interval of exercise, the subjects worked at what one could consider a light to moderate rate

of work which increased to very heavy and finally to an extremely heavy rate (Astrand and Rodahl, 1977). The increased rate of exercise could have resulted in a decrease in blood volume, which has been suggested as a possible explanation for the increased levels of plasma PLP and PB6 seen in this and other studies. To follow plasma volume changes, hemoglobin and hematocrit levels were analyzed. Researchers have reported significant correlations between hemoglobin concentration and changes in plasma volume during exercise (Beaumont et al., 1972; Harrison et al., 1975). In the present study, the change in hemoglobin concentrations from the pre to post interval for the college group (4.9 ± 4.5%) did not indicate a dramatic decrease in plasma volume. Whereas, the percent change in plasma PLP and PB6 for the same interval (22.0 ± 14.4 and 15.9 ± 6.5%, respectively) indicated an alteration in plasma PLP and PB6 levels. Other calculations were reported by Dill et al. (1974) utilizing both the hemoglobin and hematocrit levels to accurately assess changes in plasma volume. Utilizing the calculations presented, the change in plasma volume in this study could possibly account for 9 to 13% of the changes in the B-6 vitamers. However, the relationship between plasma volume changes and the increased levels of plasma PLP and PB6 is not clear. It is hypothesized, therefore, that the increase in plasma PLP and PB6 for the pre to post interval may be related to the fuels being utilized to meet the energy demands. With a moderate work intensity, the energy required to perform the activity is provided mainly by the oxidation of free fatty acids. But initially, other fuel sources, such as glycogen, will be utilized anaerobically. As an increased amount of oxygen is delivered to the skeletal muscle, at low intensity work loads,

the free fatty acids are sufficiently oxidized to meet the energy requirements. As the work load increased, glycogen then would be used at a faster rate. Hermansen et al. (1967) reported the decrease in glycogen would occur in the second and third time intervals, with less glycogen in the muscle at the end of the exercise. With this in mind, the plasma PLP and PB6 would be expected to rise with the increased work loads, and as seen with the intermittent group, this did occur. Thus, as mentioned previously, the college and untrained groups largest increase in plasma PLP and PB6 most likely occurred during the last interval of the exercise when the intensity of work was greatest. The question can then be asked as to what was the source of the vitamers. However, because this study was not designed to answer that question directly, only general assumptions can be made.

The rise in plasma PLP and the rise in PB6, which includes the phosphorylated as well as unphosphorylated forms of vitamin B-6, could be the result of an increased secretion from the liver, a release of vitamers from the red blood cell or from the skeletal muscle. Although Lumeng et al. (1974) reported that the appearance of PLP in the plasma of dogs was due to the liver's ability to phosphorylate the PN and PL forms of the vitamer, the liver can also release PL into the blood. This PL could then be subsequently taken up by the skeletal muscle, phosphorylated, and bound to glycogen phosphorylase. Alternatively, a portion of the PL can be taken up rapidly by the red blood cell for storage and transportation (Anderson, 1980). As reported by Li and Lumeng (1981), PLP, PL, and 4PA are the major vitamin B-6 compounds released into the plasma by the liver, and not PN, PMP, PNP, or PM, which means

the increase in PB6 would be due mainly to the release of PL or PLP. However, there doesn't appear to be a metabolic advantage to the liver releasing large amounts of the two vitamers into the blood stream. One function of the liver is to replenish blood glucose, which is also used as a fuel source during exercise, by either the breakdown of liver glycogen or through gluconeogenesis. Both processes involve enzymes requiring PLP. The liver's ability to produce PLP is dependent on PL, which is the preferred substrate for the kinase enzyme during the phosphorylation to PLP. Thus, the release of PL in large amounts would leave less to be converted to PLP. It is concluded, therefore, that the increased amounts of plasma PLP and PB6 seen during this study are not due to a release of the vitamers from the liver.

Another source of the increased levels of plasma PLP and PB6 in the form of PL, is the red blood cell. As discussed previously, the red blood cell is an easily accessible source of PL, which functions as a reservoir and transports PL to target tissues in the body (Anderson, 1980). However, the importance of the red blood cell in vitamin B-6 metabolism has not yet been determined. Because no data is available in the present study to clarify this role, the possible role the red cell might play in the observed increases must await further work.

If the source was not the liver or the red blood cell, then the increased plasma PLP and PB6 levels seen during exercise was probably due to a release of the vitamers from the skeletal muscle. Black et al. (1978) reported that in times of starvation, phosphorylase in the muscle of laboratory rats is significantly depleted. This would also make PLP available for release into the blood. The release of PLP would provide

an endogenous source of aid in the synthesis of gluconeogenic enzymes which are essential for ensuring a source of glucose precursors during the starvation period. Prolonged heavy exercise, in which the local stores of glycogen have been depleted, could possibly simulate starvation. If that is the case, then the theory that PLP is being released from the breakdown of glycogen phosphorylase would explain the rise in plasma PLP and PB6 following extremely heavy work as seen at the end of the 21 minutes of bicycle ergometer exercise. However, one question still remains unclear. If the source of the increased plasma PLP is the muscle, by what mechanism is it released into the blood? Lumeng et al. (1978) reported that the PLP freed from the breakdown of glycogen phosphorylase in the skeletal muscle is transferred directly or indirectly to other tissues, probably after first being hydrolyzed to PL. However, the data presented here suggest the possibility that PLP is freed from glycogen phosphorylase and released directly into the plasma without first being dephosphorylated, for transfer to the liver during times of extreme physical exertion when local stores of glycogen phosphorylase were decreasing. The uptake of plasma PLP by the liver for gluconeogenic processes is supported by the significantly lower levels of plasma PLP following a 30 minute rest as compared to the post exercise levels. Interestingly, only the trained college athletes had significantly lower levels while the untrained subjects did not, possibly due to the increased enzymatic activity following training. This is supported by the research of Huston et al. (1975) in which training was found to increase phosphoenolpyruvate carboxykinase activity in rats during exercise, therefore increasing the gluconeogenic processes.

Although the PB6 levels were not significantly different following a 30 minute rest due to the high standard deviation, the levels of PB6 did drop. Thus, it is concluded that the increased levels of plasma PLP and PB6 were the result of the release of the vitamers from the skeletal muscle.

Another aspect of this study was to investigate the difference, if any, between a trained and untrained subject in their vitamin B-6 metabolic response to exercise. Because an aerobically trained individual utilizes more fat and spares glycogen during submaximal exercise (Hermansen et al., 1967), it was hypothesized that the need for PLP would be greater for the untrained subject who would utilize more glycogen initially than the trained athlete. Because only pre and post samples were taken, no data exists to analyze when the rise actually occurred. In the present study, the response between the two groups was not statistically different. It is noted, however, that the percent increase in plasma PLP from the pre to post levels was somewhat lower for the untrained group as compared to the trained subjects. This suggests that the trained subjects have a larger capacity for storing the necessary PLP than the untrained. However, there were only three subjects in the untrained group. The percent of total vitamin B-6 as plasma PLP appears to be higher prior to exercise for the trained, as compared to the untrained group, although not significant. Earlier reports by Lumeng et al. (1978) have shown that plasma PLP concentration correlates with the PLP content of skeletal muscle. Since training increases muscle mass (Astrand and Rodahl, 1977), which subsequently increases phosphorylase content (Krebs and Fischer, 1955; Sevilla and Fischer, 1969) with concomittant increases in PLP storage capacity in

laboratory rats (Black et al., 1977), the amount of PLP present in the muscle of the trained subjects would probably be greater than that of the untrained subjects, and thus a higher plasma PLP content. Further study is needed to explore this theory, such as a study involving the training of an individual from the unfit stage to an aerobically trained state.

The nutritional implications must not be overlooked. It is apparent from the present study, that the plasma and muscle function as reservoirs for vitamin B-6 which are easily mobilized during strenuous physical exertion. Because the potential for extensive storage capacity exists (Black et al., 1977) the physiological significance of a dietary intake of at least normal amounts can be established. It has been reported that with an excess intake of PN, both muscle PLP content and glycogen phosphorylase activity increases proportionately (Black et al., 1977). Because glycogen phosphorylase is essential to the performance of an endurance trained athlete, it is evident that the need for sufficient levels of vitamin B-6 in the diet are necessary for the athlete to efficiently meet the energy requirements as well as normal functioning. The question then arises as to the amount necessary to meet these needs. A controlled study involving the administration of certain levels of dietary vitamin B-6 is therefore recommended.

Interrelationships with Vitamin B-6

Because PLP is an essential element in the production of glucose from either muscle or liver glycogen or from non-carbohydrate sources during gluconeogenesis, an interrelationship between blood glucose

levels and plasma PLP would be expected. However, no significant correlations were found. This was probably due to the intensity and duration of the exercise. In research reported by Wahren et al. (1971) in which a-v differences in glucose were studied, glucose uptake during leg exercise was found to increase gradually during 40 minutes of exercise in which the intensity of exercise was increased. Although the present study measured the absolute levels of glucose, no apparent increase or decrease during the bicycle ergometer for any of the groups was noted. It is hypothesized that if the exercise during the present study had continued for a longer period of time, perhaps the rise in plasma PLP would have correlated with a significant rise in blood glucose. Research demonstrated that rates of glucose utilization are not affected by vitamin B-6 deficiency (Krebs and Fischer, 1964). It would appear of interest to further study the effects of normal to high levels of dietary vitamin B-6 on the production of glucose during moderate to intense exercise of a long duration.

Vitamin B-6 Metabolism During the Run

The results of this study were similar to the bicycle ergometer study, in that exercise by running increased the plasma PLP and PB6 levels in the blood. The UB6 and 4PA levels did not change appreciably from one day to the next. However, because the athletes were in training, the day before the study could not be considered a non-exercise day. Thus, the effects of exercise on the urinary values could not be assessed. Any differences noted were probably related to variations in dietary intake of the vitamin, although direct comparisons were not made.

As discussed previously, the increased plasma PLP and PB6 levels are probably due to a release of the vitamers from the skeletal muscle. As the exercise was submaximal and lasted approximately 21 to 26 minutes, the utilization of glycogen should have increased towards the end of the exercise, resulting in a release of PLP and PL from the muscle to be utilized by the liver for gluconeogenesis.

This study also investigated the difference in the vitamin B-6 metabolic response between an adult versus an adolescent athlete. The response of both the college and high school groups to exercise was similar in that all subjects increased plasma PLP and PB6 levels during the exercise. However, one significant difference did emerge. The college athletes had a greater percent change in plasma PLP from the pre to post exercise interval ($P < 0.01$) than the high school athletes. As previously discussed, if the percentage of skeletal muscle was greater for the college than the high school athletes, the amount of stored PLP would be larger, which would enable the college group to mobilize a larger percentage of PLP. Although Table 2 lists the vital statistics such as height and weight as being similar for the two groups, no data for comparing accurately the skeletal muscle were obtained. Also, the fact that the college athletes may have been better trained could be responsible for the results. Therefore, further research is needed to compare the adult versus adolescent reponse of vitamin B-6 metabolism to exercise.

Another aspect of this study was to observe any changes in the vitamin B-6 response for the same subjects throughout a training period. The high school group was studied on three separate occasions during the

training season to satisfy this objective. The results, however, were not significant. The data presented suggest that the training achieved during the study did not affect the response of vitamin B-6 to exercise. The lack of significance may be due to the length of time involved and the level of training already achieved at the commencement of the study. Any further studies on the effects of training on vitamin B-6 metabolism should include the response of an untrained individual through the training period to accurately assess the body's adjustment to the exercise demand.

Finally, the effects of different types of work, namely the bicycle ergometer and the run, were compared. The bicycle ergometer and run were designed to approximate submaximal exercise to achieve a comparable test. Numerous studies in the past have shown a greater maximum oxygen consumption when exercise was performed on a treadmill as compared to a bicycle ergometer. The differences ranged from 5 to 10% (Astrand and Rodahl, 1977; McKay and Banister, 1977). Research by Pannier et al. (1980) found the difference between the two test procedures was due to the training conditions of the subjects. Trained runners had lower maximum oxygen uptake on the bicycle ergometer than on the treadmill. In the present study, statistical analysis showed no significant difference between the college athletes response to either exercise, in terms of plasma PLP and PB6 alterations. Because this study suggests that the type of exercise used to investigate the response of vitamin B-6 metabolism does not affect the results, the recommendation is made that the bicycle ergometer be the preferred exercise procedure used in future studies.

VI. SUMMARY AND CONCLUSIONS

The purpose of this thesis was to: (1) observe any changes in vitamin B-6 metabolism following exercise including plasma and urinary levels of the vitamin; (2) to compare the vitamin B-6 metabolism of a trained versus untrained adult subject following exercise; (3) to compare the response of vitamin B-6 metabolism of trained adult versus trained adolescent athletes following exercise; (4) to observe any changes in the vitamin B-6 metabolic response of an adolescent athlete during the training period; and (5) to compare the effects of a bicycle ergometer exercise versus running on vitamin B-6 metabolism.

Nineteen men and two women were recruited for the study and grouped according to level of training and age. The first group consisted of three males and one female of various ages and levels of training and served as a pilot study (intermittent). The next two groups were composed of seven college aged trained athletes (college) and six male students from a local high school (high school), while the fourth group was made up of three male and one female untrained college students (untrained). Each group exercised by either a bicycle ergometer, a predetermined run, or both.

Exercise on the bicycle ergometer was performed by the intermittent, college and untrained groups. It consisted of three, seven minute intervals for a total of 21 minutes. Each subject began the exercise at a work load of 0.5 kp for females and 1.0 kp for males. At the end of each seven minute interval, the work load was increased relative to the average heart rate, while the pedal frequency was maintained at 20 kilometers per hour. Blood was sampled three times, prior

to exercise (pre), following exercise (post) and following a 30 minute rest (30 minute). The intermittent study also included two additional blood draws, one after the first (1st) and second (2nd) seven minute interval.

The predetermined run was conducted during a scheduled team interval work-out for the college and high school groups. The work-out for the high school group consisted of six 1500 meter distances each run in a nine minute interval. All the subjects began the work-out together and paced himself through the 1500 meter distance. The amount of time remaining of the nine minutes alloted was used as a rest period before beginning the next 1500 meter interval. Prior to the beginning of exercise, blood was drawn (pre) as well as a post exercise sample (post) after three 1500 meter intervals were completed.

The college group also ran a similar interval work-out consisting of 1500 meter distances. The pace was set for a five minute mile with a three minute rest between each interval. Blood samples were drawn prior to exercise (pre) and following three 1500 meter distances (post).

Besides blood samples, a 24-hour urine collection, the day before and the day of exercise, and two day dietary intake records were obtained from each subject. Blood analysis included plasma PLP, PB6, plasma glucose, hemoglobin and hematocrit levels for all samples collected. Urine samples were analyzed for UB6, 4PA, creatinine, and urea nitrogen.

Prior to the bicycle ergometer exercise, a meal was provided for the college and untrained groups, which was analyzed for vitamin B-6 content. The daily food intake that was recorded by each subject was coded for computer nutrient analysis.

The data collected were statistically evaluated using paired t-tests to determine the significance of the changes in blood and urine values for plasma PLP, PB6, 4PA, UB6, and glucose, as well as B-6/protein, intake, and the percent of plasma total vitamin B-6 found to be plasma PLP. Student's t-test on the various group means was utilized to evaluate the effect of level of training and age. In addition, regression analysis was conducted to determine if linear relationships existed between any of the variables.

The dietary intake of the four groups was greater than the RDA in vitamin B-6, riboflavin, thiamin, niacin, vitamin A and C, calcium, and iron. The B-6/protein ratios of the college and untrained groups were adequate while the high school group had an inadequate vitamin B-6 intake for the amount of protein consumed.

Based on the data presented here, exercise on a bicycle ergometer or by running has a significant effect on plasma PLP and PB6 levels. The other B-6 vitamers measured, UB6 and 4PA, were not significantly altered, suggesting a shift in PLP and the unphosphorylated forms of vitamin B-6 from one compartment to another, rather than an increased conversion and subsequent excretion of the vitamers. It is concluded that the increased levels of plasma PLP and PB6 seen in this study may be due to an increased utilization of glycogen phosphorylase in the skeletal muscle, during the more intense exercise with a release of PLP from glycogen phosphorylase. The PLP and PL were then transferred to the liver for use in gluconeogenesis. This theory is supported by the significantly lower levels of plasma PLP found following a 30 minute rest for the college group.

The comparison of the vitamin B-6 metabolism of trained versus untrained subjects found no significant difference. However, the percent increase in plasma PLP from the pre to post interval was somewhat higher for the college trained subjects as compared to the untrained group. This suggests that the trained subjects have a larger capacity for storing the necessary PLP than the untrained subjects.

Although the college athletes had a greater percent change in plasma PLP ($P < 0.001$) than the high school athletes during the pre to post interval, the overall vitamin B-6 metabolic response was similar between the two groups. It is concluded that the greater percent change in plasma PLP for the college group may have been due to a greater percentage of skeletal muscle and therefore a larger amount of stored PLP than the high school group. The possibility also exists that the college group was better trained and therefore had a greater capacity for mobilizing the PLP due to the enhanced enzymatic activity achieved through training.

The response of vitamin B-6 to exercise in adolescent athletes during the training season did not significantly change. The data presented suggest that the training achieved during the study did not affect the response of vitamin B-6 to exercise possibly due to the length of time involved or the level of training already achieved at the commencement of the study.

The type of exercise used to study the response of vitamin B-6 metabolism does not appear to affect the results. Therefore, the conclusion is made that the ease and control obtained with the bicycle ergometer makes it the preferred exercise method.

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APPENDIX

Appendix Table 1. Individual nutrient intake by group

Subject No.	Energy (Kcal)	Protein (gm)	Fat (gm)	Carb. (gm)	8-6/ Protein (mg/gm)	Vit. 8-6[1] %	Riboflavin %	Niacin %	Thiamin %	Vit. C %	Vit. A %	Calcium %	Iron %
Intermittent Group													
110													
o	5992	220	92	1109	0.024	262	227	190	437	262	178	166	546
a	1813	73	83	185	0.018	67	71	84	124	96	76	62	186
b	1576	41	58	202	0.039	78	90	93	61	272	66	78	80
120													
o	1961	62	62	245	0.029	89	127	163	106	191	252	149	166
a	3387	115	133	415	0.017	95	133	210	174	379	207	178	256
b	2765	62	94	356	0.018	53	84	85	141	525	77	112	139
o $\bar{x}$	3978	141	77	677	0.019	175	177	177	272	227	215	158	356
S.O.	±2850	±112	±21	±611	±0.005	±122	±70	±19	±234	±51	±52	±12	±269
a $\bar{x}$	2600	94	108	300	0.021	81	102	147	149	238	142	120	221
S.O.	±1113	±30	±35	±163	±0.012	±20	±44	±89	±36	±200	±92	±82	±50
b $\bar{x}$	2171	52	76	279	0.016	65	87	89	101	398	71	95	110
S.O.	±841	±15	±26	±109	±0.003	±18	±4	±6	±56	±180	±8	±24	±41
College Group													
300													
a_1	2894	108	127	344	0.017	87	255	63	114	1416	312	307	151
b_1	4253	114	105	746	0.014	67	120	171	223	2847	273	151	163
b_2	4450	161	132	665	0.012	100	209	208	226	465	108	200	289
310													
a_1	2335	83	64	425	0.013	57	95	109	118	286	35	123	158
b_1	2824	103	84	443	0.023	119	185	97	134	775	145	266	144
b_2	3896	112	81	745	0.017	97	214	165	258	615	81	188	243
320													
a_1	2199	81	74	292	0.022	92	104	82	81	513	86	184	124
b_1	1612	71	51	195	0.017	62	142	83	92	822	296	190	111
330													
a_1	3678	79	131	569	0.013	50	84	118	128	228	50	179	172
340													
a_1	2456	77	100	339	0.026	101	151	127	179	823	130	138	210
b_1	3869	142	173	465	0.014	102	190	156	162	506	196	273	212
b_2	3495	127	147	425	0.017	102	182	155	192	983	130	197	251

Appendix Table 1. Individual nutrient intake by group cont'd.

Subject No.	Energy (Kcal)	Protein (gm)	Fat (gm)	Carb. (gm)	8-6/ Protein (mg/gm)	Vit. 8-6[1] %	Riboflavin %	Niacin %	Thiamin %	Vit. C %	Vit. A %	Calcium %	Iron %
350													
a_1	4805	147	198	615	0.018	133	171	158	320	1628	118	172	311
b_1	2892	108	103	377	0.014	74	161	98	118	1063	107	213	132
b_2	3262	132	124	407	0.013	85	183	112	150	993	93	229	184
360													
a_1	3959	196	197	390	0.025	240	326	179	294	1288	168	399	279
b_1	3623	145	143	462	0.045	326	183	230	271	1353	276	231	222
b_2	3273	121	150	387	0.019	115	288	120	185	795	222	278	196
a_1 $\bar{x}$	3189	110	113	425	0.019	109	170	119	176	883	129	215	201
S.O.	±981	±45	±46	±122	±0.005	±64	±91	±41	±94	±567	±93	±101	±70
b_1 $\bar{x}$	3179	114	114	448	0.021	125	163	139	167	1228	215	221	164
S.D.	±948	±27	±27	178	±0.012	±101	±28	±57	±68	±844	±78	±46	±44
b_2 $\bar{x}$	3675	131	127	526	0.016	100	215	152	202	770	127	219	233
S.D.	±504	±19	±28	±167	±0.003	±11	±43	±38	±41	±231	±56	±37	±43
Untrained Group													
400													
o	1815	55	64	246	0.020	51	84	44	95	586	65	133	84
a	3272	130	157	365	0.028	185	191	182	165	367	110	247	269
b	2505	61	104	349	0.018	54	105	55	74	408	75	180	128
410													
o	2259	85	95	289	0.019	81	145	86	139	897	168	216	156
a	2197	92	84	293	0.026	122	166	98	316	409	48	183	163
b	2772	98	116	309	0.024	121	181	173	188	593	165	210	125
o $\bar{x}$	2037	70	80	268	0.020	66	115	65	117	742	117	175	120
S.O.	±314	±21	±22	±30	±0.007	±22	±43	±30	±31	±221	±72	±59	±51
a $\bar{x}$	2735	111	121	329	0.027	154	179	140	241	388	79	215	216
S.D.	±760	±27	±52	±51	±0.001	±45	±17	±60	±107	±30	±43	±45	±75
b $\bar{x}$	2639	80	110	329	0.021	88	143	114	131	501	120	195	127
S.O.	±189	±26	±9	±28	±0.004	±47	±54	±83	±81	±130	±63	±22	±2
High School Group													
200													
a_1	2536	97	88	384	0.022	106	118	143	108	290	77	118	84
b_1	2764	105	113	371	0.024	126	142	177	115	324	92	137	79

Appendix Table 1. Individual nutrient intake by group cont'd.

Subject No.	Energy (Kcal)	Protein (gm)	Fat (gm)	Carb. (gm)	B-6/ Protein (mg/gm)	Vit. B-6[1] %	Riboflavin %	Niacin %	Thiamin %	Vit. C %	Vit. A %	Calcium %	Iron %
200													
a_2	3441	143	182	361	0.036	262	146	273	152	224	106	144	91
b_2	3610	144	178	414	0.033	236	138	339	176	532	60	101	115
a_3	2653	118	143	273	0.035	203	125	256	124	213	504	110	71
b_3	2334	109	114	246	0.020	108	137	181	127	133	258	129	68
210													
a_1	3761	201	172	354	0.009	88	302	176	163	105	51	253	131
b_1	3488	166	132	352	0.013	111	355	223	211	169	109	271	168
a_2	2345	203	94	285	0.008	78	159	68	102	131	101	157	89
b_2	3337	170	126	398	0.009	75	228	105	117	31	66	230	142
a_3	3613	124	165	420	0.009	59	190	108	144	73	99	146	111
b_3	3122	116	142	388	0.020	115	262	152	176	295	148	186	145
220													
a_1	3460	133	147	405	0.014	96	160	158	140	261	94	117	102
b_1	3673	121	154	468	0.015	91	335	141	162	607	1050	135	125
a_2	2747	144	72	385	0.004	28	215	124	166	629	78	184	88
b_2	3539	147	130	454	0.013	94	356	163	201	414	850	173	121
a_3	3088	103	120	424	0.003	22	140	97	126	148	106	116	106
b_3	3138	120	108	437	0.009	53	163	99	120	311	60	132	111
230													
a_1	3474	218	99	429	0.013	138	418	185	172	295	409	388	188
b_1	4733	195	198	550	0.014	134	343	174	268	178	179	278	126
a_2	3252	164	118	390	0.017	134	319	111	191	244	288	267	87
b_2	3967	196	160	445	0.011	109	355	163	226	282	246	305	109
a_3	3454	189	132	389	0.012	109	297	138	157	217	176	254	90
b_3	4391	206	135	607	0.011	108	387	157	224	207	209	355	24
240													
a_1	2478	79	89	348	0.003	12	128	70	98	312	57	112	63
b_1	4295	194	257	310	0.006	55	234	144	122	389	66	197	125

Appendix Table 1. Individual nutrient intake by group cont'd.

Subject No.	Energy (Kcal)	Protein (gm)	Fat (gm)	Carb. (gm)	B-6/ Protein (mg/gm)	Vit. B-6[1] %	Riboflavin %	Niacin %	Thiamin %	Vit. C %	Vit. A %	Calcium %	Iron %
a_2	2852	129	118	325	0.007	43	207	101	129	527	261	181	103
b_2	2480	114	108	268	0.006	36	173	91	106	335	63	170	74
250													
a_1	3469	160	97	513	0.014	112	229	137	219	651	513	192	235
250 b_1	4051	180	132	557	0.016	143	201	126	194	406	85	199	191
a_2	4070	140	164	527	0.014	102	280	167	188	270	158	233	145
b_2	2763	105	101	378	0.013	71	118	114	105	139	78	84	80
a_3	2470	103	77	346	0.005	26	126	59	89	248	80	111	74
b_3	2569	110	98	318	0.010	53	200	140	153	211	84	154	131
a_1 $\bar{x}$	3196	148	115	406	0.013	92	188	166	150	319	200	197	134
S.O.	±546	±56	±35	±61	±0.006	±43	±121	±77	±45	±179	±205	±109	±66
b_1 $\bar{x}$	3834	160	164	451	0.015	110	202	164	179	345	263	203	136
S.O.	±687	±38	±54	±98	±0.006	±33	±80	±35	±58	±164	±387	±62	±39
a_2 $\bar{x}$	3118	154	125	379	0.014	108	221	141	155	337	165	195	100
S.O.	±606	±27	±42	±83	±0.012	±85	±68	±72	±35	±195	±89	±47	±23
b_2 $\bar{x}$	3283	146	134	393	0.014	104	228	163	155	289	227	177	107
S.O.	±558	±34	±30	±67	±0.009	±70	±106	±91	±53	±182	±313	±82	±26
a_3 $\bar{x}$	3056	127	127	370	0.013	84	176	131	128	180	193	148	90
S.O.	±494	±36	±33	±63	±0.013	±75	±73	±75	±26	±70	±178	±61	±18
b_3 $\bar{x}$	3111	132	119	399	0.014	87	230	146	160	232	152	191	96
S.O.	±796	±42	±19	±137	±0.006	±31	±100	±30	±42	±73	±83	±94	±50

o two days before exercise.

a day before exercise.

b day of exercise.

1 nutrients listed as % ROA.

Appendix Table 2. Individual anthropometric measurements by group

	Subject No.	Arm Circumference cm	Triceps Skinfold mm	Subscapular Skinfold mm	Suprailiac Skinfold mm	Body Fat %
College Group	300	10.3	7.3	9.3		
	310	10.5	3.5	6.3		
	320	11.4	5.3	6.8		
	330	10.3	5.3	6.7		
	340	10.8	3.7	7.7		
	350	10.2	2.8	6.7		
	360	11.1	5.5	9.5		
	$\bar{x}$	10.7	4.8	7.6		
	S.D.	±0.5	±1.5	±1.3		
Untrained Group	400	11.0	9.9	18.0	16.8	
	410	11.3	5.1	8.3	6.5	
	420[a]	9.5	10.3	8.7	7.0	
	430	13.5	12.5	17.7	19.7	
	$\bar{x}$	11.9	9.2	14.7	14.3	
	S.D.	±1.4	±3.8	±5.5	±6.9	
High School Group	200	11.6	6.3			18.5
	210	11.2	10.0			19.5
	220	9.3	5.0			9.3
	230	11.3	6.2			13.3
	240	10.5	9.0			13.7
	250	9.3	4.3			9.2

Appendix Table 2. Individual anthropometric measurements by group cont'd.

	Subject No.	Arm Circumference cm	Triceps Skinfold mm	Subscapular Skinfold mm	Suprailiac Skinfold mm	Body Fat %
High School Cont;d.	$\bar{x}$	10.5	6.8			13.9
	S.D.	±1.0	±2.2			±4.4

a Subject 420 was a female participant and her values were not used in the calculations.

Appendix Table 3. Individual blood and urine values by group

Subject #	4-PA µmoles/ 24 hr	Urinary B-6 µmoles/ 24 hr	Creatinine gm/24 hr	Urea N gm/ 24 hr	Hct %	Hb gm/ 100 ml	Glucose mg/ 100 ml	Plasma B-6 nmoles/ 100 ml	PLP nmoles/ 100 ml
100 pre					43	14.2	87.1	8.6	2.18
1st					42	14.2	80.2	10.4	2.01
2nd					43	14.4	80.2	10.3	2.25
post					45	15.1	123.8	11.0	2.63
30 minute					43	14.2	94.1	9.9	2.27
110 pre					47	17.6	93.8	14.34	6.31
1st					49	18.3	95.6	11.72	7.56
2nd					51	18.6	96.9	14.23	6.98
post					52	19.3	85.0	16.03	7.23
30 minute					47	17.7	77.4	13.04	5.82
120 pre					44	15.8	87.1	7.14	3.05
1st					44	15.5	74.9	7.20	2.97
2nd					45	15.7	87.1	6.99	3.02
post					41	16.1	83.6	7.30	3.54
30 minute					42	15.1	84.3	7.95	3.05
130 pre					46	15.8	81.9	3.47	2.27
1st					46	17.8	80.8	4.67	1.92
2nd					47	19.6	80.2	5.50	2.09
post					48	18.8	83.6	5.98	2.19
30 minute					44	16.8	83.6	4.96	1.97
200 pre	12.51	0.93	1.80	17.43	42	14.6	67.1	6.99	4.00
post	-	-	-	-	43	15.0	79.2	7.14	4.63
pre	8.51	0.77	1.82	10.76	43	14.5	93.3	4.86	2.86
post	10.53	0.89	1.96	8.58	43	14.1	129.9	5.75	3.46

Appendix Table 3. Individual blood and urine values by group cont.

Subject #	4-PA µmoles/ 24 hr	Urinary B-6 µmoles/ 24 hr	Creatinine gm/24 hr	Urea N gm/ 24 hr	Hct %	Hb gm/ 100 ml	Glucose mg/ 100 ml	Plasma B-6 nmoles/ 100 ml	PLP nmoles/ 100 ml
200 pre	8.63	0.59	1.32	6.18	41	13.4	88.9	5.85	3.61
post	12.42	0.91	1.45	21.60	43	14.2	114.2	7.77	4.25
210 pre	12.76	1.20	1.87	14.45	47	16.7	83.8	14.70	10.01
post	19.03	1.30	1.65	14.23	48	17.4	89.8	18.74	11.56
pre	22.48	1.71	1.44	12.39	44	16.3	91.1	28.73	15.40
post	18.11	1.44	1.50	13.53	45	16.0	85.5	32.88	18.12
pre	22.19	1.18	1.37	13.87	42	14.1	80.3	15.39	7.28
post	12.55	1.10	1.30	15.12	45	16.0	107.2	18.55	8.89
220 pre	9.46	0.88	1.38	9.07	41	14.6	84.3	7.09	3.68
post	12.06	1.09	1.78	9.48	43	15.9	87.2	8.24	4.87
pre	13.17	1.39	1.89	13.77	41	14.3	99.9	6.98	3.96
post	10.94	1.03	1.46	9.51	41	14.5	89.8	7.86	4.73
pre	10.20	0.99	1.67	8.70	40	13.2	90.9	5.46	2.88
post	9.27	0.92	1.25	10.16	42	14.7	112.4	6.38	3.73
230 pre	10.39	1.07	2.16	20.15	42	14.7	82.0	7.55	5.14
post	9.27	0.85	2.15	18.43	44	16.0	91.4	9.20	6.54

Appendix Table 3. Individual blood and urine values by group cont.

Subject #	4-PA µmoles/ 24 hr	Urinary B-6 µmoles/ 24 hr	Creatinine gm/24 hr	Urea N gm/ 24 hr	Hct %	Hb gm/ 100 ml	Glucose mg/ 100 ml	Plasma B-6 nmoles/ 100 ml	PLP nmoles/ 100 ml
230 pre	8.53	0.95	1.73	16.84	45	15.3	83.8	8.86	5.45
post	9.14	1.02	2.11	19.49	45	15.8	98.9	9.81	5.91
pre	8.34	1.12	1.83	19.65	-	-	-	-	-
post	7.79	0.89	0.86	14.65	-	-	-	-	-
240 pre	-	-	-	-	40	13.9	115.8	6.03	3.63
post	8.84	1.15	2.17	14.98	42	14.8	112.3	7.29	4.65
pre	6.32	0.93	1.84	11.28	42	14.1	102.4	6.52	3.81
post	6.95	0.92	1.54	9.68	44	15.1	109.6	8.23	5.16
250 pre	6.68	0.60	1.51	9.37	42	14.3	94.7	5.47	3.31
post	9.09	0.73	1.62	10.53	44	13.3	87.2	6.67	4.19
pre	5.01	0.53	1.00	8.95	45	15.2	104.3	6.91	3.82
post	5.38	0.57	1.38	9.34	47	15.2	97.9	8.94	4.38
pre	6.68	0.75	1.59	9.97	43	12.9	101.8	6.48	3.73
post	5.75	0.62	1.36	9.90	44	14.5	108.1	8.21	5.29
300 pre	7.18	0.79	1.39	8.79	48	15.8	88.7	7.32	5.08
post	7.14	0.91	1.56	9.98	51	16.7	81.7	8.83	6.01
30 minute					46	14.8	86.7	7.68	4.39
pre	-	-	-	-	46	16.0	97.4	6.88	3.11
post	4.26	0.83	1.21	7.68	47	15.9	94.0	8.83	4.54

Appendix Table 3. Individual blood and urine values by group cont.

Subject #	4-PA µmoles/ 24 hr	Urinary B-6 µmoles/ 24 hr	Creatinine gm/24 hr	Urea N gm/ 24 hr	Hct %	Hb gm/ 100 ml	Glucose mg/ 100 ml	Plasma B-6 nmoles/ 100 ml	PLP nmoles/ 100 ml
310 pre	8.16	0.86	1.61	9.85	44	16.5	90.9	5.04	3.61
post	6.15	0.79	1.60	12.70	46	16.6	92.2	5.38	3.63
30 minute					43	15.7	83.4	4.87	3.36
pre	-	-	-	-	43	15.4	93.7	5.90	4.66
post	6.56	0.81	1.67	11.32	45	15.3	131.7	7.03	5.95
320 pre	51.93	1.85	1.82	13.75	46	16.1	80.8	7.61	5.49
post	11.87	1.26	1.75	13.48	48	16.4	104.4	8.94	6.39
30 minute					45	15.7	91.3	5.55	5.61
330 pre	10.63	1.13	1.53	8.44	49	17.9	86.0	7.53	6.76
post	10.05	1.21	2.03	13.14	55	20.0	101.7	9.31	7.75
30 minute					51	18.6	99.1	8.16	7.46
pre	-	-	-	-	48	16.7	95.6	7.09	7.46
post	5.74	0.81	1.61	9.51	51	17.0	90.9	8.94	9.57
340 pre	8.86	1.24	2.05	12.40	44	15.9	81.7	6.45	4.05
post	7.88	1.11	2.33	13.80	44	17.0	86.6	6.93	5.05
30 minute					41	15.2	81.8	6.56	4.07
pre	-	-	-	-	43	14.8	87.1	8.30	6.04
post	6.97	1.23	1.99	13.33	47	15.5	121.0	10.54	7.60

Appendix Table 3. Individual blood and urine values by group cont.

Subject #	4-PA µmoles/ 24 hr	Urinary B-6 µmoles/ 24 hr	Creatinine gm/24 hr	Urea N gm/ 24 hr	Hct %	Hb gm/ 100 ml	Glucose mg/ 100 ml	Plasma B-6 nmoles/ 100 ml	PLP nmoles/ 100 ml
350 pre	9.03	1.02	1.86	14.51	44	15.8	85.2	7.00	6.73
post	6.81	0.83	1.69	10.97	48	17.7	74.7	8.33	8.27
30 minute					45	16.1	88.7	7.89	6.51
pre	-	-	-	-	44	15.0	86.9	9.64	7.40
post	7.18	0.83	1.60	10.35	46	16.0	111.4	10.81	10.27
360 pre	13.50	1.03	1.65	20.50	43	13.7	78.2	5.86	3.77
post	18.42	1.18	1.78	21.01	44	14.8	86.9	6.80	5.71
30 minute					42	13.4	79.9	5.50	3.37
pre	-	-	-	-	43	14.1	87.8	5.28	2.61
post	12.92	1.19	1.77	20.29	43	14.7	123.6	6.20	3.75
400 pre	7.37	0.73	1.21	8.79	47	16.7	66.9	5.81	4.65
post	6.28	0.77	2.33	13.28	50	17.3	77.5	7.66	5.03
30 minute					48	16.5	60.1	5.87	4.31
410 pre	6.31	0.99	1.13	12.64	40	14.4	86.9	7.33	4.74
post	8.11	0.65	1.59	14.73	43	15.3	80.6	7.57	5.07
30 minute					41	14.4	81.4	6.75	4.09
420 pre	3.53	1.19	1.00	6.26	44	13.9	83.9	11.02	8.31
post	3.53	1.10	1.25	8.58	47	15.0	87.4	12.01	8.31
30 minute					43	13.6	80.7	10.80	7.55
430 pre	3.49	0.57	2.03	10.22	46	16.3	83.9	4.20	1.85
post	4.23	0.59	2.38	10.47	50	17.3	85.7	4.62	2.27
30 minute					46	15.7	75.1	4.37	1.92

REAGENT SOURCES

1. Technicon Corp., Tarrytown, New York: Technicon Autoanalyzer.
2. J. T. Baker Chemical Co., Phillipsburg, N.J.: trichloroacetic acid, PPO.
3. New England Nuclear, Boston, Mass.: L-tyrosine-1-^{14}C.
4. Sigma Chemical Co., St. Louis, Mass.: tyrosine decarboxylase, pyridoxine HCL, pyridoxal HCl.
5. Amersham Corp., Arlington Heights, Ill.: NCS tissue solubilizer.
6. Beckman Co., Fullerton, California: Beckman liquid scintillation counter (model I5-3133P).
7. ICN, Irvine, Calif.: POPOP.
8. Fisher Scientific Co., Fair Lawn, New Jersey: toluene.
9. Merck and Co., Inc., Rahway, N.J.: pyridoxal phosphate.

Informed Consent

Nutrition and Exercise

Our knowledge of the levels of vitamin B_6 in the blood during exercise is limited. Vitamin B_6 serves as a coenzyme for more than 60 enzymes, some of which are involved in exercise metabolism. Because of the metabolic relationship between vitamin B_6 and the production of energy necessary for performance, the levels of work and B_6 are important for an accurate understanding of the metabolic processes.

The purpose of this investigation is to determine the influence of various levels of work on the levels of vitamin B_6 in the blood.

A bicycle ergometer will be used to obtain the different work loads. The rate of work will be monitored by an ECG throughout the investigation. Blood samples will be collected and analyzed for vitamin B_6 levels at various intervals.

The levels of vitamin B_6 will be assessed in 5 subjects. Each subject will have a blood sample taken before exercise begins. The level of work for the 1st 7 minute interval of work will be the same for all subjects depending on sex and held at a constant rate. At the end of the 1st interval, blood will be drawn. The work load for the second interval will be determined for each subject depending on the heart rate recorded from the 1st interval. Blood will again be drawn at the end of the second interval. The procedure will be the same for the 3rd 7 minute interval of work with the work load being determined by the heart rate from the 2nd interval. Following the end of exercise, the subject will rest for 30 minutes and a final blood sample will be drawn.

Each subject will be asked to keep a detailed record of their diet for 3 days preceeding the investigation. The investigation will be performed in a fasting state.

The frequency and amount of blood drawn should not be a physical stress for a normally active healthy subject.

This study is a preliminary one in a series of experiments to determine the effects of vitamin B_6 and performance.

If you have further questions please contact Dr. James Leklem or Kathy Munoz at the Department of Foods and Nutrition, Oregon State University, phone #754-3561.

Consent Form - 3

Exercise Study

I give my consent to participate in a nutrition-exercise study. I further give my consent to allow collection of complete 24 hour urine samples and timed blood samples before and at intervals after exercising. The total amount of blood on any given day will not exceed 80ml. The blood will be drawn by a medical technologist. Sterile dipsosable needles and vacutainer tubes will be used to minimize the risk of infection.

It is also my understanding that on those occassions when meals are provided I will eat all the food provided. I also will provide information on previous diet intake, use of any vitamin or mineral supplements or use of any drugs and medications. I understand I am free to withdraw from this study at any time. The details of this study will be explained and all questions satisfactorily answered.

NAME _____

WITNESS _____

DATE _____

NUTRITION EXERCISE STUDY

Diet and Medical History

Medical History:

1. Do you have any medical problems requiring the care of a doctor? Yes____ No____. Describe if yes.

2. Are you on a special diet? Yes____ No____. Describe.

3. Age at which you had your first menstrual period ____. Do you have regular menstrual periods? Yes____ No____.

Dietary History:

1. Do you presently use vitamins? Yes____ No____. If yes, what type? (brand name)

2. Frequency of use of vitamins
   a.____ once per day
   b.____ 2-5 times per week
   c.____ once per week
   d.____ 2-4 times per month
   e.____ less than once per month

3. Past vitamin use? Yes____ No____

4. Last date vitamins taken _____.

5. Check meals eaten each day on a regular basis.
   Breakfast _____
   Lunch _____
   Dinner _____
   Snacks _____

6. Do you have any food allergies? Yes___ No___ Describe if yes.

COLLECTION OF URINE:

1. Collect all urine in containers provided.

2. Label all containsrs carefully and clearly with your initials and date.

3. Each day:

   Urine collections will be made on a 24-hr basis and run, for example, from 6:45 am one day until the same time the next day. Therefore, the collection made on rising in the morning belongs with the urine collected on the previous day and should be date accordingly. It is important that the collection made on rising is done at the same time each day.

4. Urine will be collected starting with breakfast on the day you start on the diet study. Return urine samples daily at any time convenient for you.

5. Store urine in a cool place and protected from light.

6. Please be careful not to spill or lose any urine. If this does happen, however, let us know.

7. Drink approximately the same amount of fluids each day.

OTHER:

1. Note any major changes in activity level from your usual pattern. (Example: 24 hour sleep-in, or a once in a lifetime 50 mile bike trip.)

INSTRUCTIONS FOR RECORDING FOOD

1. Please record each food and beverage you consume (except water) on a separate line. Be sure to indicate all snacks.

2. Record them in reasonably exact amounts: liquids in cups, fluid ounces or milliliters; vegetables and fruits in cups or inches using the ruler on the record sheets; beans, grains and pasta in cups dry or cups cooked; bread in slices, indicate what kind of bread; meats, fish and cheeses in ounces (an average meat portion is 3 oz., a slice of American cheese is about 1 oz.) or measure your servings with the ruler.

   If it is impractical to measure foods at certain meals, measure a comparable food at least once to establish in your mind the measure of certain quantities. Remember: the more accurate your record the more accurate the analysis will be.

3. Please specify if a food is consumed raw. Also indicate if it was prepared from fresh, canned or frozen products.

4. Indicate how the food was prepared, such as fried, boiled, baked etc.

5. If a food is a mixture (sandwich, soup, stew) list the major ingredients separately in their proportions or amounts as eaten.

6. Use brand names wherever possible, or mention comparable brand name products.

7. Specify if a food is fortified with vitamins and minerals, or if it is a diet product. Please include the brand names.

8. For fruits and vegetables indicate if skin was removed.

9. Provide any other information you feel might be helpful.

10. Indicate if milk is whole, skim, 2% or dry non-fat milk.

11. Be sure to include sauces, gravies, milk in coffee etc. Everything you eat or drink.

DIET RECORD SHEET

SAMPLE

CODE NO. _____

DATE CONSUMED: 5/30/79

LEAVE A BLANK SPACE BETWEEN EACH MEAL
USE A SEPARATE SHEET FOR EACH DAY

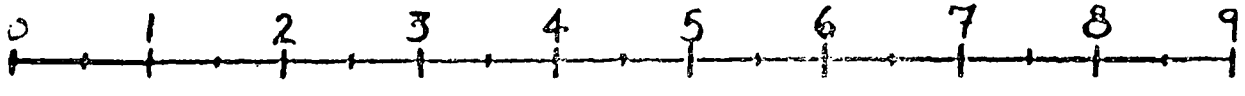
FOOD specify each food or beverage on a separate line	SOURCE canned, dried fresh etc.	BRAND be specific	PREPARATION fried, baked, raw etc.	AMOUNT measure in cups inches etc.	FOR OFFICE AMT. code	FOR OFFICE WT col
Egg	Chicken	—	scrambled	1 med.		
Orange juice	frozen	Flavorpac	diluted with water	6 oz.		
Bread, whole wheat	homemade	—	toasted	1 slice		
Butter	sweet cream	Marigold	on toast	1 tsp.		
Chicken noodle soup	canned	Campbell's	heated	3/4 cup		
Mashed potatoes	instant	Carnation	package directions	1/2 cup		
Butter	sweet cream	Marigold	on potatoes	2 tsp.		
Saltines	packaged	Sunshine	in soup	5 crackers 2" x 2"		
Milk, 2%	fresh, cow's	My Te Fine	—	10 oz.		
Apple	fresh	Winesap	raw, unpeeled	1 2" diam.		
Baked beans	canned	Valleys	heated	2 c.		
Cornbread	homemade	Jiffy	package directions	2 2"x2"		
Tea	tea bag	Lipton	boiled water	1 c.		
Sugar	white	—	in tea	1 tsp.		
Lettuce	fresh	Iceberg	salad	1 c.		
Ice cream	frozen	Olga's	cone	2 scoops		

DIET RECORD SHEET

CODE NO. _____ DATE CONSUMED: _____

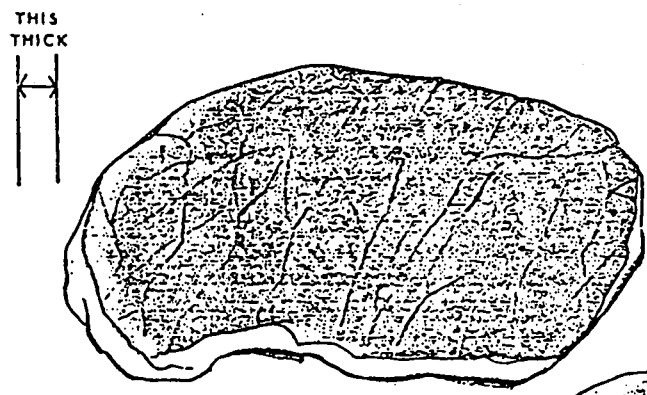
LEAVE A BLANK SPACE BETWEEN EACH MEAL
USE A SEPARATE SHEET FOR EACH DAY

FOOD specify each food or beverage on a separate line	SOURCE canned, dried fresh etc.	BRAND be specific	PREPARATION fried, baked, raw etc.	AMOUNT measure in cups inches etc.	FOR OFFICE USE AMT. code	FOR OFFICE USE WT. code

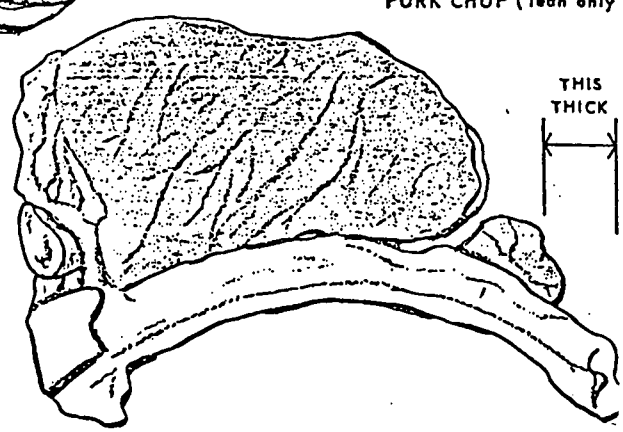


It takes 2 pieces of cooked meat without bone of the size pictured to equal 3 ounces.

ROAST BEEF ROUND (lean only)



PORK CHOP (lean only



ROAST TURKEY

DIET RECORD SHEET

SAMPLE

CODE NO. _____

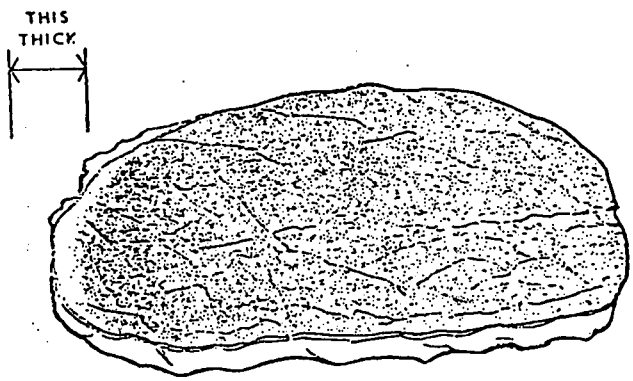
DATE CONSUMED: 5/30/79

LEAVE A BLANK SPACE BETWEIN EACH MEAL
USE A SEPARATE SHEET FOR EACH DAY

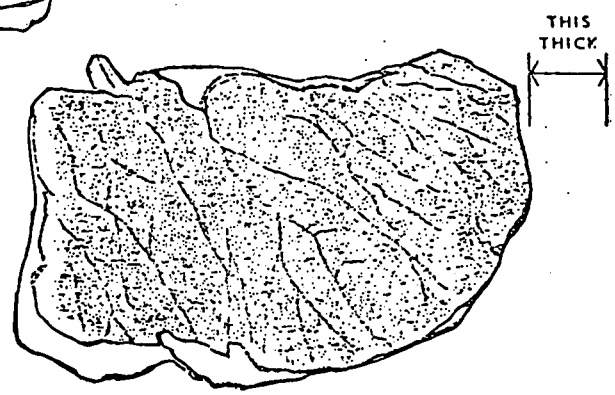
FOOD specify each food or beverage on a separate line	SOURCE canned, dried fresh etc.	BRAND be specific	PREPARATION fried, baked, raw etc.	AMOUNT measure in cups inches etc.	FOR OFFICE AMT. code	FOR OFFICE WT cod
Egg	Chicken	—	scrambled	1 med.		
Orange juice	frozen	Flavorpac	diluted with water	6 oz.		
Bread, whole wheat	homemade	—	toasted	1 slice		
Butter	sweet cream	Marigold	on toast	1 tsp.		
Chicken noodle soup	canned	Campbell's	heated	3/4 cup		
Mashed potatoes	instant	Carnation	package directions	1/2 cup		
Butter	sweet cream	Marigold	on potatoes	2 tsp.		
Saltines	packaged	Sunshine	in soup	5 crackers 2"x2"		
Milk, 2%	fresh, cow's	My Te Fine	—	10 oz.		
Apple	fresh	Winesap	raw, unpeeled	1 2" diam.		
Baked beans	canned	Valleys	heated	2 c.		
Cornbread	homemade	Jiffy	package directions	2 2"x2"		
Tea	tea bag	Lipton	boiled water	1 c.		
Sugar	white	—	in tea	1 tsp.		
Lettuce	fresh	Iceberg	salad	1 c.		
Ice cream	frozen	Olga's	cone	2 scoops		

The sketches below represent the actual size of a 3-ounce serving of cooked meat, without bone.

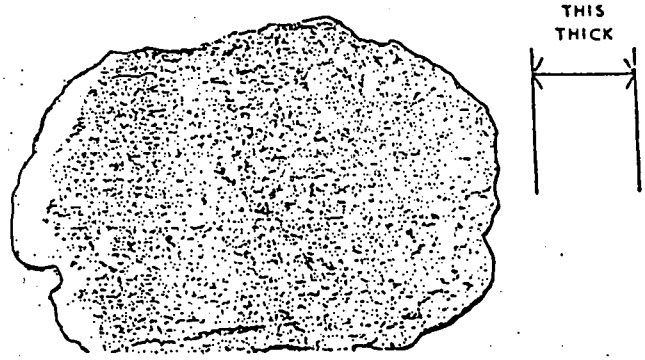
ROUND STEAK (lean only)



VEAL CUTLET (trimmed



HAMBURGER (lean)



COLLECTION OF URINE:

1. Collect all urine in containers provided.

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

3. Each day:

   Urine collections will be made on a 24-hr basis and run, for example, from 6:45 am one day until the same time the next day. Therefore, the collection made on rising in the morning belongs with the urine collected on the previous day and should be date accordingly. It is important that the collection made on rising is done at the same time each day.

4. Urine will be collected starting with breakfast on the day you start on the diet study. Return urine samples daily at any time convenient for you.

5. Store urine in a cool place and protected from light.

6. Please be careful not to spill or lose any urine. If this does happen, however, let us know.

7. Drink approximately the same amount of fluids each day.

OTHER:

1. Note any major changes in activity level from your usual pattern. (Example: 24 hour sleep-in, or a once in a lifetime 50 mile bike trip.)