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Title: Influence of Controlled Strenuous Exercise on Vitamin B-6 Metabolism in Man: Effects of Carbohydrate Depletion-Repletion Diets and Vitamin B-6 Supplements.

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Recent studies in men have shown plasma levels of vitamin B-6 and pyridoxal 5'-phosphate (PLP), the active form of vitamin B-6, to increase with exercise. It was hypothesized that muscle glycogen phosphorylase might be the source of these increases as this enzyme has been shown to increase with increasing vitamin B-6 (B6) intake in the rat, seemingly to store PLP. The investigation was designed to study the effects of diet-altered glycogen stores and B6 supplements on B6 metabolism during controlled strenuous exercise. The effect of exercise (EX) on the excretion of 4-pyridoxic acid (4PA), the major B6 urinary metabolite, was also studied.

The study consisted of three experimental weeks during which carbohydrate (CHO)-modified diets were fed and six EX tests were administered (one each Wednesday and Saturday). Four trained male cyclists (20 - 23 years) served as subjects. Week 1 a normal CHO diet was fed (NC diet, 40% of total kilocalories as CHO). During

week 2, which began 7 days after week 1, a low CHO diet was fed Sunday through Tuesday (LC diet, 11% CHO) to deplete muscle glycogen. In the same week, the LC diet was followed by a high CHO diet (HC diet, 71% CHO). The HC diet was fed Wednesday through Saturday to replete, or load, glycogen stores. The NC, LC, and HC diets contained 1.64, 1.55, and 1.82 mg of B6, respectively. Week 3, beginning 14 days after week 2, was identical to week 2, but with the daily addition of an 8 mg supplement of pyridoxine. Daily exercise was encouraged Sunday through Tuesday to facilitate glycogen depletion. The EX test consisted of 50 min of continuous bicycle ergometer exercise (30 min at 60% HRmax (maximal heart rate), 15 min at 80% HRmax, and 5 min at 90% HRmax).

Blood samples were drawn prior to the exercise test (pre), 2 min prior to the 90% HR max interval (during), immediately post EX (post), 30 min post, and 60 min post EX. Plasma samples were analyzed for PLP, PB6, creatine kinase (a muscle enzyme), and hematocrit and hemoglobin. Urine was collected in 24 hour aliquots and analyzed for 4PA and creatinine.

The HC diet was associated with significantly lower pre exercise PB6 and PLP levels than LC diet. This was attributed to the high CHO content of HC.

Increased plasma PLP and PB6 levels (pre versus post) were seen for all EX tests. This was significant for PB6 levels of all EX tests. Exercise following LC resulted in smaller pre to post increases in PB6 and PLP than other unsupplemented EX tests, but this was significant only for EX following LC versus NC(Wed). Supplementation resulted in greater pre to post increases in PLP

and PB6 than EX following unsupplemented diets, but this was significant only for LC versus LC+B6. Plasma PLP and PB6 levels dropped throughout the 60 min post EX period. The 60 min post PLP levels were significantly below pre for the EX tests following diets NC(Wed), LC, HC+B6. Neither plasma volume percent (%) changes (calculated from hematocrit) nor creatine kinase % changes correlated significantly with % changes in PB6 and PLP. Urinary 4PA was elevated on all EX test days as compared to non-test days, except for EX following LC.

Tissue redistribution of B6 appears to be occurring with exercise. With the LC diet, more B6 is needed for increased amino acid catabolism in the liver. In this situation tissue redistribution was not associated with increased conversion of B6 to 4PA. Greater increases in PLP with EX following supplementation suggests increased storage may have occurred. These findings are supportive of the hypothesis that increased PLP levels with exercise may originate from PLP bound to phosphorylase. The need for supplemental B6 for the athlete was not established, as status was adequate with normal intakes.

Influence of Controlled Strenuous Exercise on Vitamin B-6 Metabolism in Man: Effects of Carbohydrate Depletion-Repletion Diets and Vitamin B-6 Supplements

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LIST OF ABBREVIATIONS

PL	pyridoxal
PN	pyridoxine
PM	pyridoxamine
PLP	pyridoxal 5'-phosphate
PNP	pyridoxine 5'-phosphate
PMP	pyridoxamine 5'-phosphate
PB6	plasma total vitamin B-6
4PA	4-pyridoxic acid
%4PA	percent of vitamin B-6 intake excreted as 4PA
PV	plasma volume
CK	creatine kinase
Hgb	hemoglobin
Hct	hematocrit
CHO	carbohydrate
PRO	protein
NC	normal carbohydrate diet
LC	low carbohydrate diet
HC	high carbohydrate diet
LC+B6	low CHO diet with supplemental vitamin B-6
HC+B6	high CHO diet with supplemental vitamin B-6
RDA	Recommended Daily Allowance
HRmax	maximum heart rate
$\dot{V}O_2max$	maximal oxygen consumption in liters per minute
$Kpm \cdot min^{-1}$	a unit of work equal to 1 kilopond (the force acting on 1 kg at the normal acceleration of gravity) times meters per min
kcal	kilocalorie
g	gram
kg	kilogram
ml	milliliter
L	liter
min	minute
m	meter
nmol	nanomole, 10^{-9} moles
µmole	micromole, 10^{-6} moles
U/L	activity units per liter
SD	Standard deviation of the mean
r	correlation coefficient

Influence of Controlled Strenuous Exercise on Vitamin B-6 Metabolism in Man: Effects of Carbohydrate Depletion-Repletion Diets and Vitamin B-6 Supplements

I. INTRODUCTION

Physical exercise places extreme demands on the body's metabolic systems to provide energy for muscular work. Vitamin B-6, in its active form as pyridoxal 5'-phosphate (PLP), has two important roles in exercise energy metabolism, both relating to glucose homeostasis. First, PLP is the coenzyme for aminotransferases, which are involved in gluconeogenesis. Second, PLP is an integral part of the enzyme glycogen phosphorylase (EC 2.4.1.1) which cleaves glucose-1-phosphate from glycogen. Due to the role of PLP in phosphorylase, vitamin B-6 supplements have been tested for their ability to enhance athletic performance, with negative results. Most authorities on nutrition and athletics concur that exercise does not increase the need for vitamins and increased intake does not enhance performance. Conversely, recent studies have indicated that exercise may indeed modify vitamin B-6 metabolism. Dramatic increases in plasma vitamin B-6 and PLP following exercise have been documented by Wozenski (1), Leklem et al. (2), and Munoz (3) in male subjects of various ages and athletic abilities. The study of Leklem and coworkers suggested the urinary level of 4-pyridoxic acid (4PA), the major vitamin B-6 excretory metabolite, might also be increased by exercise. Data from the study of Munoz failed to show an effect on excretion. Neither study controlled the intake of vitamin B-6. Thus the question of whether excretion is increased

by exercise and therefore whether vitamin B-6 status is effected, remains to be determined.

Another question unanswered about the effect of exercise is the source of the increased plasma B-6 vitamers following exercise. Recent studies of the enzyme glycogen phosphorylase in rat muscle indicate that this enzyme may actually serve as a storage site for PLP, accumulating when vitamin B-6 is fed in excess (4, 5). Further studies have indicated this storage pool of PLP is not released in response to vitamin B-6 deficiency per se, but rather is rapidly depleted in response to short term starvation (5). These investigators suggest that release of PLP in response to short-term starvation would be adaptive, as PLP would be needed in other tissues such as the liver for gluconeogenic enzymes involved in sustaining glucose homeostasis. Exercise can be considered a form of short term starvation, in terms of the demand placed on glucose homeostasis. Therefore, it seems plausible to hypothesize that the source of increases in plasma PLP with exercise could be the muscle PLP-phosphorylase storage pool. The releases of PLP from this pool would be related to the energy metabolism of exercise, possibly relative to the depletion of glycogen.

The quantity of glycogen stored in the muscle and its utilization during exercise can be changed dramatically through manipulation of dietary carbohydrate and exercise. A technique popularly known as "Carbo-Loading" is currently used by long distance runners to greatly increase glycogen levels. Increasing the muscle glycogen has been shown to significantly increase endurance time for high intensity exercise such as long distance

running (6). To achieve high glycogen levels, glycogen is first depleted through a low carbohydrate (CHO) diet and heavy exercise for about three days. Depletion is then followed by a high CHO diet and decreased exercise for another three days. At the end of this regimen muscle glycogen levels can be more than doubled. In the glycogen depleted state energy for exercise is provided primarily by fat metabolism, while in the glycogen loaded state glycogen is the primary fuel.

This study of vitamin B-6 metabolism in exercise consists of three major objectives. The first two objectives are related to the hypothesis that the source of increased plasma PLP with exercise is glycogen phosphorylase. The first objective will be to study the effect of exercise on vitamin B-6 metabolism when glycogen stores have been manipulated (loaded and depleted states). The response of vitamin B-6 metabolism to controlled exercise tests in the glycogen depleted and loaded states will be compared to the response to exercise after a normal diet is consumed. Both total vitamin B-6 and PLP will be measured in plasma to evaluate vitamin B-6 metabolism. The second objective is to test the effect of increased PLP stores on the exercise response. To accomplish this the glycogen depletion and loading diet regimens will be repeated with an oral supplement of 8 mg of vitamin B-6. The same exercise test will be administered and vitamin B-6 and PLP will again be measured to evaluate vitamin B-6 metabolism. In addition, plasma levels of creatine kinase, a muscle enzyme, will be monitored during each exercise. This enzyme is known to be released from the muscle during exercise, signaling changes in

muscle tissue permeability that may also be related to exercise energy metabolism (7, 8). As current knowledge indicates that PLP must be dephosphorylated prior to membrane transport, the creatine kinase level may indicate permeability changes. Hematocrit and hemoglobin will also be followed throughout each exercise test. Trained male bicyclists will be recruited for subjects so that no training effect will occur as a result of repeated exercise tests. The bicycle ergometer will be used to administer exercise tests, as this mode of exercise is known to produce changes in vitamin B-6 metabolism. The third and final objective is to evaluate the effect of exercise on 4PA excretion under conditions of controlled vitamin B-6 intake. To accomplish this, the normal, glycogen depleting, and glycogen loading diets will be formulated to contain identical amounts of vitamin B-6 and protein. All urine will be collected and the quantity of 4PA analyzed. Excretion will be compared between non-exercise days and exercise test days.

II. REVIEW OF LITERATURE

Vitamin B-6

Metabolic Roles

Vitamin B-6 was named in 1934 by Paul György (9) when he determined a fraction of the B-complex was capable of preventing the pellagra-like dermatitis in the rat. The essentiality of vitamin B-6 for humans was confirmed in 1954, when infants developed convulsions after being fed an infant formula in which the vitamin B-6 had been accidentally destroyed (10, 11).

An amazing array of biological roles for vitamin B-6 have been elucidated. In addition to the well known role of vitamin B-6 in amino acid metabolism, vitamin B-6 is involved in carbohydrate metabolism, immune function, nucleic acid metabolism, lipid metabolism, and hormone function.

The active form, pyridoxal 5'-phosphate (PLP), is a coenzyme for reactions in the metabolism of amino acids and glycogen. There are over 60 PLP dependent amino acid enzymes, the majority are aminotransferases. Aminotransferases result in the removal of the α-amino groups from amino acids forming an α-keto acid. Other PLP dependent reactions included racemization, decarboxylation, cleavage, synthesis, dehydration and desulfhydration. Decarboxylation

reactions result in the formation of certain catecholamine and neurotransmitters. Several PLP enzymes are involved in the metabolism of tryptophan, giving rise to the formation of serotonin, a neurotransmitter and nicotinic acid, a vitamin. PLP is also required for the synthesis of aminolevulinic acid, an intermediate in the synthesis of the porphyrin ring of hemoglobin (12). Pyridoxamine 5'-phosphate is also active in some PLP dependent aminotransferases (13).

Vitamin B-6 is involved in carbohydrate metabolism through its role in glycogen phosphorylase (EC 2.4.1.1). This enzyme contains four moles of PLP per mole of phosphorlyase and catalyzes the breakdown of glycogen to glucose-1-phosphate (14). In addition to roles in amino acid and carbohydrate metabolism, it has long been known that vitamin B-6 deficiency in animals results in abnormal fat metabolism (15,16). Much research has failed to identify a vitamin B-6 dependent enzyme involved in fat metabolism; thus it is currently believed that vitamin B-6 plays only a secondary role in fat metabolism.

Chemistry

Vitamin B-6 is a collective term referring to all the biologically active forms of the vitamin which include: Pyridoxine (PN), the 4' alcohol form; pyridoxal (PL), the 4'aldehyde form; pyridoxamine (PM), the 4'amine form; and the three respective 5'-phosphate esters of these forms (PNP, PLP, PMP). The structures and their biological interconversions are shown in Figure 1. The excretory metabolite of vitamin B-6 is 4-pyridoxic acid (4PA), also

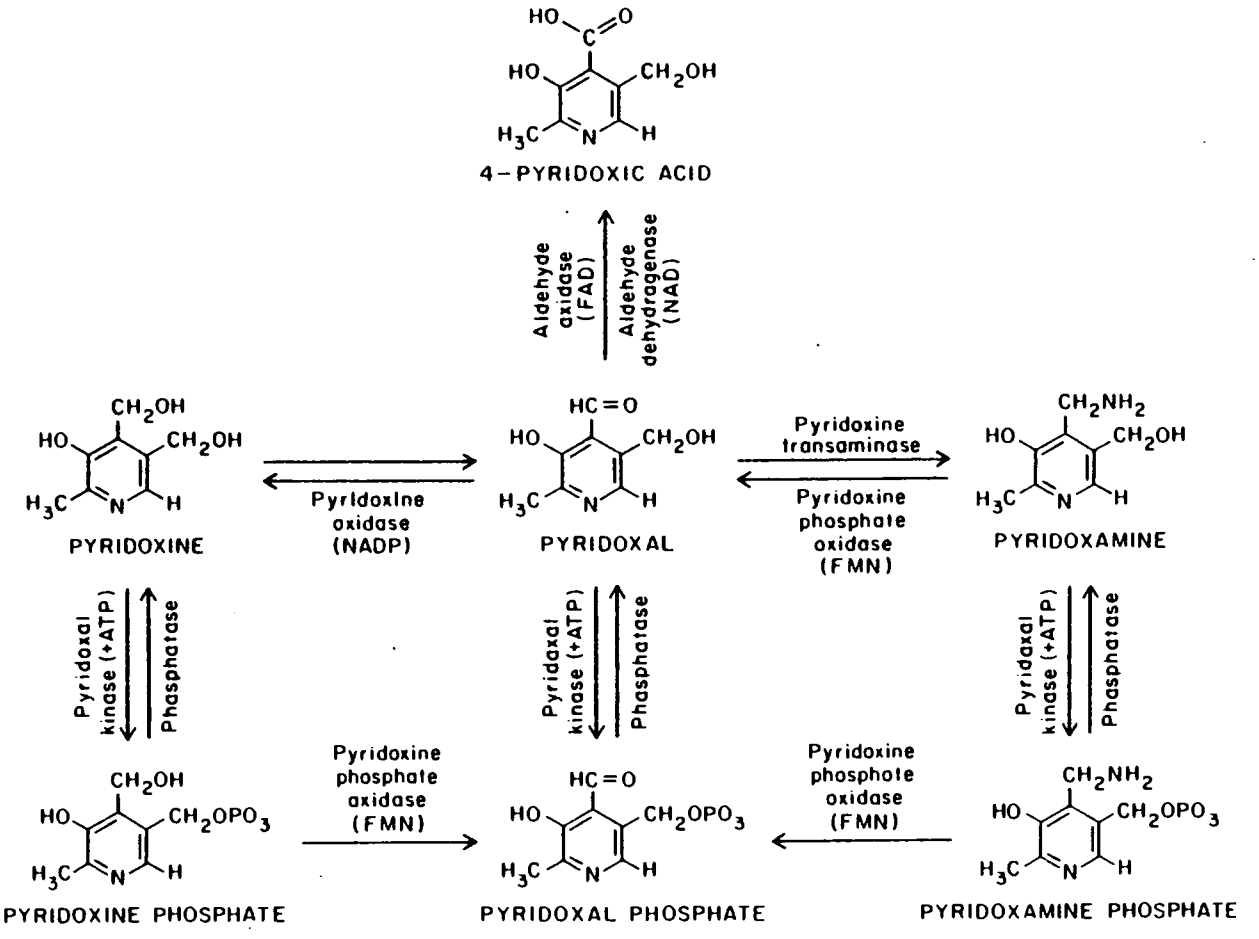

FIG. 1. Interconversions and structures of B-6 vitamers

shown in Figure 1. Aqueous solutions of the B-6 vitamers are unstable to white light (17). Pyridoxal hydrochloride is particularly unstable in alkaline solutions, while all three free-hydrochloride forms are stable in dilute mineral acids (17).

In general, PN is the major form in plant foods, whereas in animal foods PL and PM are predominating forms (17). Table 1 lists the vitamin B-6 content of a few foods which are good sources of the vitamin (19). The B-6 vitamers are commonly bound to the protein fraction in the food source (17, 20). The bound nature of vitamin B-6 in foods interferes with the biological utilization of the vitamin. The term bioavailability refers to this phenomonon, experimentally defined as the ratio of standardized biological activity (such as the growth response of an animal) to the total nutrient content determined chemically or microbiologically (21). Some fiber components in food appear to decrease bioavailability. For example, white bread vitamin B-6 is more available than whole wheat bread vitamin B-6 in man(22). Heat processing and storage may result in decreases in bioavailability in foods through the binding of vitamin B-6 in amino groups of lysyl residues in foods (21).

Absorption

Absorption of vitamin B-6 occurs mainly in the jejunum and to a lesser extent in ileum (23, 17, 24). Research to determine the absorption mechanism of vitamin B-6 was first carried out indirectly by evaluation of the urinary excretion of 4PA and other B-6 vitamers following test doses of vitamin B-6 (23, 25, 26). In man, urinary excretion was found to be linearly related to the size

TABLE 1
Vitamin B-6 content of selected foods.

Food	Total vitamin B-6, mg/100 grams
Liver, beef	0.840
Soybeans, raw	0.810
Beans, pinto	0.531
Banana, raw	0.510
Tuna, canned	0.425
Avocado, raw	0.420
Peanuts, roasted	0.400
Wheat flour, whole	0.340

of the test doses; this has been interpreted as indicating that absorption occurs by passive diffusion. Further research with everted rat intestinal sacs and isolated vascularly perfused intestinal segments confirm that the absorption mechanism is passive diffusion (27-30). Work of Hamm (29) and Menhansho (30) indicates that physiological levels of the phosphorylated forms of vitamin B-6 are hydrolyzed in the intestinal lumen prior to absorption. The phosphorylated forms are also transported into the mucosa, but at a much slower rate. Following dephosphorylation the free forms are transported into the portal circulation without further interconversion (27, 29, 30). Absorption of vitamin B-6 is rapid, even following very large test doses, with absorption being 95% complete in 2 hours (24).

Wozenski and coworkers studied the absorption and metabolism of small test doses of PN, PL, and PM in man (1). They reported that following a dose of PM, plasma and urinary responses were less than the responses to either a PN or PL dose suggesting a difference in absorption or metabolism or both. Work of Yamada and coworkers, reported in his recent review of absorption, has also indicated a difference in absorption of B-6 vitamers (24). Their research has suggested this order of absorption rates: PL>PN>PM.

The bioavailability of vitamin B-6 will affect the amount of vitamin B-6 remaining in the fecal matter, for example more vitamin B-6 is found in the feces when whole wheat versus white bread is fed (22).

Interconversions and transport

The interconversions of free and phosphorylated forms of vitamin B-6 have been reviewed by Snell and Haskell (13). These interconversions and the enzyme involved in each reaction are shown in Figure 1. PLP and PMP are the major forms of vitamin B-6 found in mammalian tissues, but since a large proportion of dietary vitamin B-6 is ingested as PN and the supplementary form of vitamin B-6 is usually pyridoxine-hydrochloride, it is important to understand the tissue pathways of conversion of PN to the active cofactors (31, 32). Studies in which tritiated or carbon-14 labelled PN have been fed to humans and rats indicate that the preferred pathway is first the phosphorylation of PN to PNP by PL Kinase (ATP: pyridoxal 5'-phosphotransferase; EC 2.7.1.35) followed by the oxidation of PNP to PLP by PNP (PMP) oxidase (EC 1.4.3.5) (33-38). Free PL is then formed by action of alkaline phosphatase on PLP (39). Formation of PMP from PLP occurs via the action of transaminases or PNP oxidase on PLP (13). Pyridoxal kinase, which will phosphorylate all of the free vitamers, has been found to be present in brain, liver, kidney, large and small intestine, spleen and muscle (40-41). Pyridoxine phosphate oxidase, in contrast, is present in large amounts in the liver, in small amounts in erythrocytes, brain and kidney, but is absent or in very small amounts in lung, heart, pancreas and muscle tissue (32, 36, 42). The prevalence of PL kinase indicates that most tissues are able to phosphorylate B-6 vitamers, but some such as the muscle are incapable of oxidizing the vitamers to PLP and therefore are dependent on other tissues to convert PN and PM to PL (36).

The liver and erythrocytes are the tissues regarded as important in the conversion of B-6 vitamers to PL and PLP for eventual uptake by other body tissues. Lumeng and coworkers (43) carried out organ ablation studies to ascertain the source of plasma PLP following a dose of PN or PL. Their study indicated that the liver was the sole source of PLP following the dose of PN or PL, since removal of the intestines, spleen, stomach or kidney did not reduce the response of plasma PLP to the dose as did liver removal. The red cell is known to avidly take up PN and convert it to PLP, but it is unable to release PLP back into the plasma (38, 43). The PLP formed in the red cell would first be hydrolyzed to PL and then released into the plasma.

Pyridoxal is considered to be the form transported across cell membranes, since phosphorylated forms, like most charged molecules, do not cross membranes readily (13). Membrane-bound alkaline phosphatase hydrolyzes PLP making PL available for uptake into tissues (39). In vitro studies suggest that the protein binding of PL and PLP appears to slow their transport (43, 44). Nearly all PLP is bound tightly to albumin, although a small pool of unbound PLP exists in equilibrium with the bound portion. This protein binding slows the action of alkaline phosphatase, but PLP can still be slowly hydrolyzed to PL (43). Pyridoxal is more loosely bound to albumin (44). Anderson has postulated that the red cell may transport vitamin B-6 directly to tissues without entering the plasma, in a manner analogous to the red cell transport of amino acids (45).

A diagrammatic presentation of the tissue relationships and

interconversions of PL and PLP is shown in Figure 2, adapted from Lumeng and co-workers (32) to additionally show the potential role of the red cell.

Tissue Distribution and Storage

In mammalian tissues, PLP and PMP are the predominate forms of vitamin B-6. In the rat nonphosphorlyated forms account for less than 10% of the total vitamin B-6 content (31, 46). The highest concentration of vitamin B-6 in the rat is found in the liver followed by the kidney, brain, skeletal muscle and heart in decreasing order of concentration (31, 46). The ratio of PLP to PMP varies with the tissue, Thiele and Brin (46) reported PMP as predominating in kidney, and brain, while PLP was greatest in skeletal muscle. Others also report a great predominance of PLP in muscle in contrast to all other tissues (31, 32, 47). Methodology studies by Vanderslice and co-workers (47) indicate that the variables of phosphatase inhibitors, various assay conditions, and sample storage have considerable influence on the concentrations found in tissues.

Studies in which rats have been fed diets with deficient or excess vitamin B-6 indicate that the tissues and blood fluids vary in their response to these challenges. In deficiency the plasma, muscle, spleen, thymus, adrenals, kidneys, and lung rapidly lose vitamin B-6 (46, 48-50). The liver concentration is also decreased by deficiency, but the rate of decrease appears to depend on the previous level of dietary vitamin B-6 (48). The plasma is most easily depleted followed by muscle and liver, with brain least

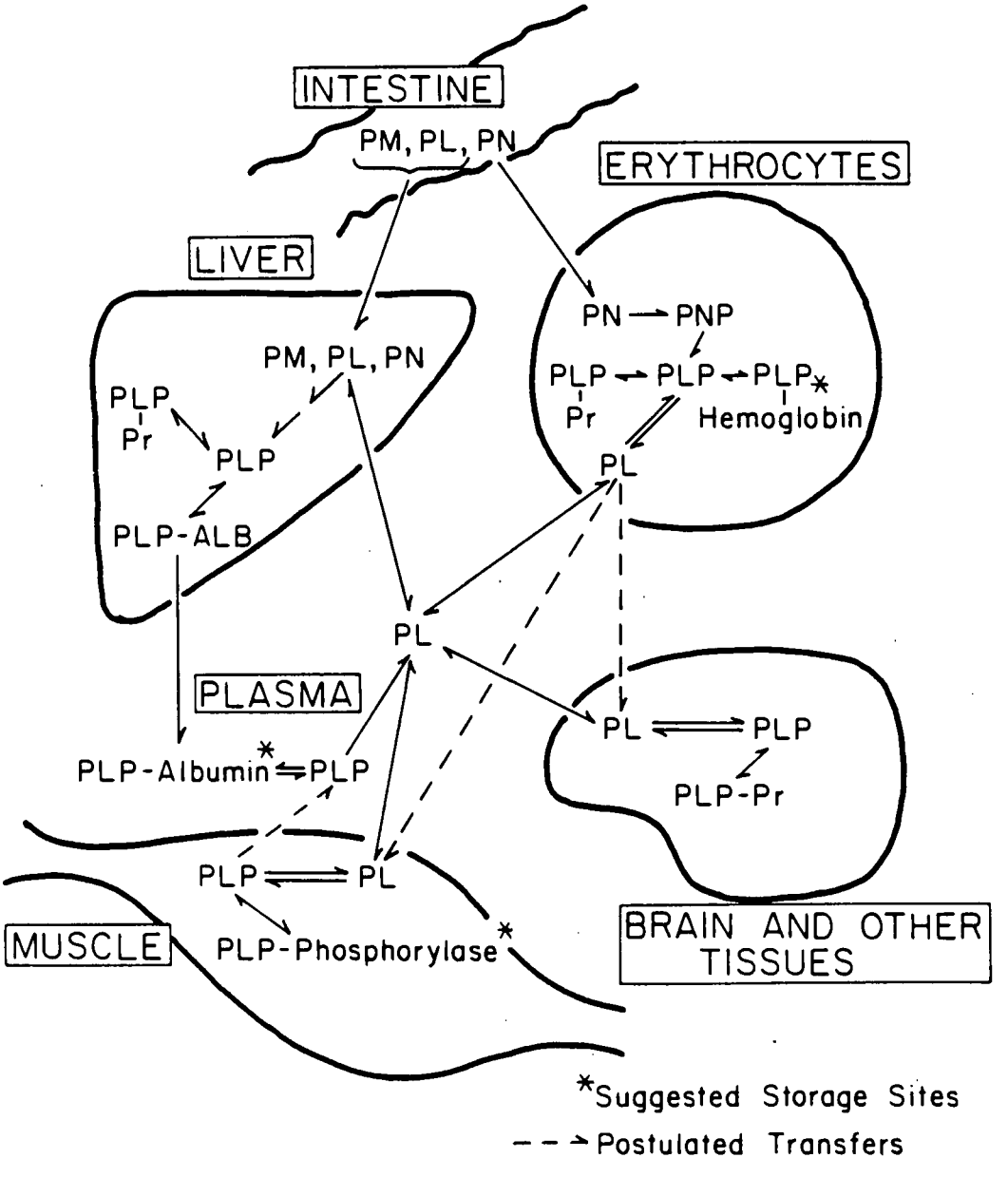

FIG. 2. Tissue interrelationships of pyridoxal and pyridoxal 5'-phosphate.

easily depleted (48, 50). In the rat the muscle has been shown to accumulate PLP, while the brain and liver do not (50). When a high protein diet is fed to rats, liver concentration of PLP is higher than in the liver of rats fed lower protein diet, suggesting more vitamin B-6 is being retained to metabolize the excess protein (49).

By nature of its total mass in relation to body size, the muscle is regarded to be the major pool of vitamin B-6 in the body, even though the actual concentration is low in comparison to other tissues. Krebs and Fischer (4) have estimated that half of the total body content of vitamin B-6 is contained in muscle. These investigators were the first to hypothesize that the muscle enzyme act as a repository for vitamin B-6, based on the decrease in phosphorylase with vitamin B-6 deficiency in rats. Several investigators have studied tissue phosphorylase content in vitamin B-6 deficiency and reported that the muscle, liver and adrenal phosphorylases declined in deficiency (51-54). Muscle phosphorylase a, the more active form, was shown by Eisenstein (52) to be reduced less than the total phosphorylase activity. The reduction of liver phosphorylase was much less marked than the muscle phosphorylase (52). Black et al. (55) studied the effect of increased B-6 intake on the rat muscle phosphorylase content, testing whether the proposed repository could expand when vitamin B-6 was in excess. In addition they evaluated the effect of increased intake on two PLP dependent aminotransferases in muscle. In response to a supplement ten times the recommended intake for rats, phosphorylase concentration increased throughout the six week study paralleling the increase in

muscle PLP. The aminotransferases increased for only two weeks. Rats fed on diets at 10% the recommended level did not show increases in muscle concentrations of phosphorylase. Thus phosphorylase responds to increased intake by expanding whereas other vitamin B-6 enzymes failed to do so. In a later study of Black and co-workers (5) the accessability of this PLP store was clarified. In agreement with earlier investigators, phosphorylase concentration was found to decrease after six to eight weeks of deficiency, but their analysis of whole muscle content indicated that the total quantity of phosphorylase was actually constant. The decrease in concentration was the result of dilution of phosphorylase by subnormal growth of muscle tissue, rather than an actual loss of the enzyme. A decrease in phosphorylase content of muscle was seen only when the deficient rats became anorectic and were losing weight. This led Black and coworkers to study the effect of short term starvation on the phosphorylase content of muscle. They confirmed that food deprivation and starvation were effective in decreasing phosphorylase but had no effect on muscle alanine and aspartate aminotransferases. Thus the reservoir of PLP in muscle appears to be mobilized in response to starvation rather than B-6 deficiency per se. In short term starvation, blood glucose is maintained by gluconeogenesis from amino acids and glycogenolysis. Liver PLP dependent transaminases have an important role in maintainance of glucose homeostasis by providing the precursors for gluconeogenesis. The release of PLP from muscle in periods of caloric deprivation would allow these processes to be prolonged. Whether PLP is released as such is not known, Black and co-workers (5)

have hypothesized PLP was hydrolyzed to PL prior to release.

Blood levels

The study of vitamin B-6 levels in blood and plasma is complicated by the multiplicity of vitamer forms and the lack of methodology to quantitatively discern all of these forms. In normal human subjects a controversy exists as to the major vitamer in plasma. This is largely due to the different methods employed by different investigators. Sauberlich et al. (56) and Lumeng et al. (57) report PLP as the major form in plasma. In contrast Anderson et al. (31) and Kelsay et al. (58) report PL as the predominant form. Vitamer content of plasma and whole blood are presented in Table 2. Note that Shane (59) reports PMP and PM as major forms in whole blood of adult males. Lumeng and co-workers (57) found very little PMP and PM in plasma, thus they suggest these vitamers exist primarily in red cells rather than plasma.

Normal plasma PLP values for healthy adults have been reported to range from 3.4 ± 1.5 nanomoles (nmol)/100 ml of plasma to 7.5 ± 2.0 nmol/100 ml by various investigators (47, 60-62). Plasma PLP concentrations are significantly lower in adult women (3.7 ± 1.5 nmol/100 ml) than adult men (5.2 ± 1.9 ml nmol/100 ml) (62). Plasma levels of PLP in men have been shown to be relatively constant over periods of eight hours, several weeks and several months provided a regular diet is consumed (1, 33, 57). The turnover of this vitamer is rapid in plasma and is responsive to changes in dietary intake (57, 63, 64). This sensitivity has lead some investigators to regard plasma PLP as a reliable indicator of the vitamin B-6 status in

TABLE 2
Human plasma and whole blood B-6 vitamer content.*

Source	PMP	PM	PNP	PN	PLP	PL	Total	4PA
Vanderslice, 1980 (47) n = 1	2.4	1.2	-‡	21.6	7.7	-	32.9	n.d.†
Lumeng et al., 1978 (57) n = 6	0.5	0.62	-	1.9	7.69	-	12.4	n.d.
Whole Blood Shane, 1978 n = 5 (59)	8.09	4.6	-	-	5.46	0.77	18.9	17.4

* concentration in nanomoles/100 ml plasma or whole blood.

† not determined.

‡ - undetected.

humans (12, 61). In the rat, plasma PLP was positively related to muscle PLP content when the rats were placed on deficient diets. Thus in the rat, plasma PLP is a reliable indicator of muscle tissue stores (50).

In man, when large doses of PN are fed for several weeks, plasma PLP, PL, and 4PA are the forms that increase in the plasma, while PMP and PN stay constant (57). Wozenski and co-workers (1) found that a dose of 0.5 mg of PN would produce measurable changes in plasma PLP and total vitamin B-6. With chronic ingestion of large doses the red cell accumulates PLP, bound to hemoglobin, with the whole blood to plasma ratio climbing from a normal of 1 to ratios of 5 to 50 (65).

Excretion

Vitamin B-6 is excreted in urine mainly as 4-pyridoxic acid (4PA), lesser amounts of the free forms, and very small amounts of the phosphorylated forms (26). The major metabolite, 4PA, is formed by the action of a NAD-dependent, aldehyde dehydrogenase and a FAD-dependent aldehyde oxidase.

The excretion of 4PA in man is a indicator of recent dietary B-6 intake as evidenced by decreased excretion in depletion and increased excretion in repletion of vitamin B-6 (58, 64, 66). Urinary total vitamin B-6 is also an indicator of immediate dietary intake. When large doses of vitamin B-6 are fed, the quantity of free vitamers and 4PA in the urine increase (1, 58, 64). Wozenski and co-workers (1) found that as the dose increased from 0.5 to 10.0

mg, the percentage of the dose recovered from the urine as 4PA and total urinary B-6 decreased. In comparing the rate of metabolism and excretion of equimolar doses of PN, PL and PM, they also found PL was more rapidly converted to plasma PLP and 4PA than other forms, while PM was most slowly converted.

Miller and Leklem (67) reported that men receiving a high level of dietary protein (2g/kg) and 1.5 mg of vitamin B-6 per day excreted less vitamin B-6 as 4PA. Urinary vitamin B-6 was also decreased compared to subjects on lower protein intakes. Sauberlich et al. (56) also reported this effect of dietary protein, while Linkswiler (66) reported no difference between those receiving 55 and 100 grams of protein per day.

Shultz and Leklem (62) correlated levels of dietary intake of vitamin B-6 to urinary 4PA and vitamin B-6. From their findings they have suggested minimum acceptable excretion levels below which dietary intake is considered inadequate. In adult males these recommendation are 5.00 to 5.67 μmoles 4PA/24 hours and 0.60 to 0.69 μmoles urinary vitamin B-6/24 hours. These recommendations are in agreement with those of Sauberlich (68) and Linkswiler (66) for minimum excretory levels.

The 4PA excretion was studied in five young men to which 1, 2, 4, and 10 mg doses of PN were given (1). The excretion of 4PA following the 10 mg dose was increased from 4.4 μmoles/24 hours to 19 μmoles/24 hours the day of the dose. The day following the 10 mg dose, 4PA was still increased. Doses of 4 mg and less did not result in elevated 4PA the day following the dose.

Recommended Intakes

The quantity of vitamin B-6 needed by man has been studied by numerous investigators in the last twenty years. An important fact gleaned from the numerous studies is the increased need for vitamin B-6 when dietary protein is increased (56, 66, 69-72). Recommended intakes by the National Research Council Food and Nutrition Board (73) in the United States and the Canadian Bureau of Nutritional Sciences (74) therefore consider protein intake. Canadian recommended allowances are 0.02 mg vitamin B-6 per gram of protein eaten, while the US recommendation is set at 2.2 mg for adult men (19 through 22 years) or 2.0 for adult women rather than being related to protein. In fact, protein intakes often exceed those recommended without commensurate increases in B-6, potentially leading to marginal vitamin B-6 status (75).

Exercise Metabolism

Exercise Standardization

The study of exercise metabolism requires that the exercise event be standardized such that it can be accurately described, compared to other studies, and replicated by other investigators. Three factors should be considered in the description of exercise: Intensity; duration and frequency; and mode of exercise. Intensity and duration of exercise are important determinants of which metabolic substrates are used to power exercise and whether the fuel metabolism is aerobic (through oxidative processes) or anaerobic

(through nonoxidative processes). Intensity is the factor most difficult to control and describe in research investigations.

Intensity of exercise is most accurately described as a percentage of an individual's maximal oxygen consumption ($\dot{V}O_2max$). The $\dot{V}O_2max$ represents the greatest oxygen uptake (liters of oxygen per minute) that an individual can attain when progressively greater work loads are applied (76). Two methods of work load application used are the bicycle ergometer and the motor driven treadmill. With the bicycle ergometer, work load is expressed in kilopond meters per minute ($kpm \cdot min^{-1}$) which is the force required to move one kilogram at the normal acceleration of gravity while maintaining a speed of one meter per minute. The force is adjusted while the pedaling speed is constant, usually 50 revolutions per minute. Treadmill work loads are expressed as speed and elevation. Application of the work load, such that a given $\%\dot{V}O_2max$ is attained, allows the investigator to apply the same relative exercise stress on individuals who vary greatly in maximal oxygen consumption. For example, a well trained person may need a work load of 1800 $kpm \cdot min^{-1}$ on a bicycle ergometer to reach 70% of his maximum oxygen consumption, while the poorly trained person would need only 750 $kpm \cdot min^{-1}$ to reach his maximum oxygen consumption. Adjusting workload to attain a given $\%\dot{V}O_2max$ is an example of a relative work load or intensity. Absolute work load is the application of work load without consideration of the individual's working capacity. For example in a study by Sutton et al., a work load of 750 $kpm \cdot min^{-1}$ was applied to all subjects of both high and low work capacities (77). Measurement of oxygen consumption indicated that this represented

an average of 85% $\dot{V}O_2$max for those of low capacities and 35% for those of high work capacities. Metabolic and hormonal responses are very different between subjects in this situation.

A linear relationship exists between heart rate and oxygen consumption (see Figure 3). Thus heart rate (HR) may also be used to standardize exercise intensity. The work load is then adjusted until a predetermined HR is attained. Maximal heart rate (HRmax) may be determined directly by an exercise test or estimated by subtracting age from 220 to adjust for the decline in HRmax with age (76). The %$\dot{V}O_2$max is somewhat less than the same %HRmax. For example, 65% HRmax would be approximately 55-60%$\dot{V}O_2$max. The accuracy of prediction of %$\dot{V}O_2$max from heart rate is ± 15% (76). Accuracy can be improved if each individual's heart rate-oxygen consumption relationship is predetermined.

The mode of exercise also influences metabolism during exercise. Different types of exercise utilize muscles of varying metabolic capacity and different amounts of the total body muscle mass. Maximal oxygen consumption determined on a bicycle ergometer is slightly less than that determined on a motor driven treadmill (76).

Energy metabolism in exercise

Research performed at the beginning of this century indicated that carbohydrate and fat were the primary sources of energy for muscular work (79). Protein is the third nutrient capable of suppling energy for muscular work. Protein has been considered an

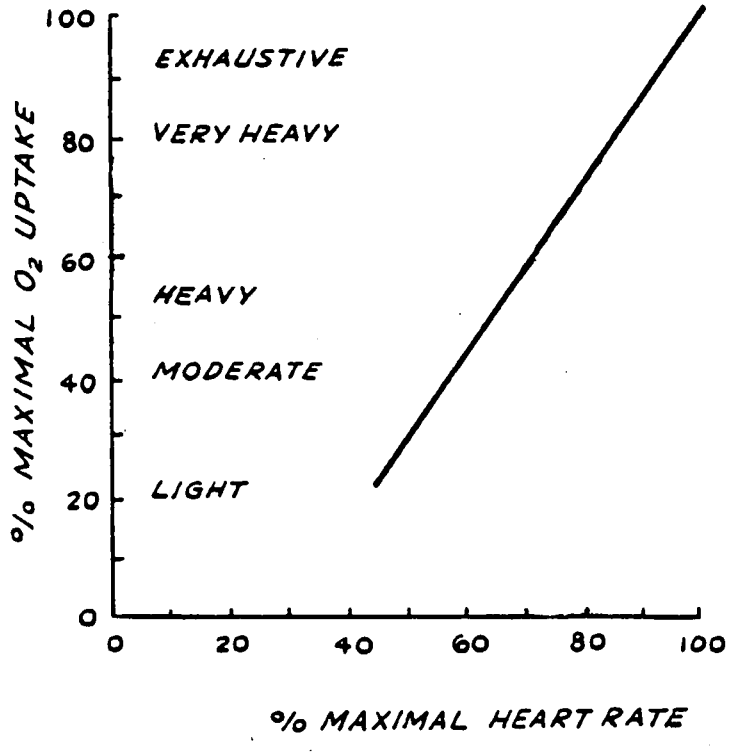

FIG. 3. Relationships between percent maximal oxygen uptake and percent maximal heart rate (78).

insignificant source of energy as nitrogen balance was shown to be unaffected by exercise (80). More recent studies have focused on factors regulating the utilization and availability of energy substrates, particularly muscle glycogen. Research into the role or protein and amino acids in exercise metabolism has also continued. This research has confirmed that nitrogen balance is only minimally affected while indicating amino acid catabolism occurs in prolonged exercise (81-85).

Sources. The human body has several energy stores that can be used by the muscle to fuel muscular work. These are adipose tissue, muscle glycogen, liver glycogen, and tissue protein. The high energy phosphate bond of adenosine triphosphate (ATP) is the ultimate currency of energy transfer used to power muscular contraction. ATP is not stored but must be regenerated constantly from catabolism of the previously mentioned fuel stores. A small amount of phosphate bond energy is stored as creatine phosphate (also called phosphocreatine). This is a very small store of energy that can regenerate ATP during the first few seconds of exercise. It is estimated that only 10 kilocalories (kcal) of creatine phosphate are stored in the muscle mass of man. The body store of carbohydrate is also limited. In muscle and liver, an estimated 2,000 kcal are stored as glycogen in normal man (76). Muscle glycogen can only be used in the cell in which it is stored, since muscle lacks the enzyme glucose-6-phosphatase which would allow release of glucose into blood. Liver glycogen is made available to muscle via blood glucose. The use of blood glucose for muscular work is limited somewhat due to the fact that tissues

such as the brain are dependent on this substrate. Glucose can be metabolized within the muscle cell either aerobically or anaerobically with the production of lactic acid.

Free fatty acids, hydrolyzed from triglycerides in adipose tissue and delivered via blood, represent the largest store of energy for exercising muscle. In normal weight man, an excess of 10^5 kcal are present (76). Fatty acids can be catabolized only in the presence of oxygen. The utilization of free fatty acids is thus limited by the availability of oxygen in the tissue and mobilization from adipose tissue rather than the size of the storage supply itself.

Protein and amino acids, as mentioned, are not usually considered sources of energy for muscular work. Teleologically it would seem counter-productive to catabolize proteins that function in enzymatic processes and muscular contraction as energy sources. Research in this area has shown indeed that prolonged exercise is similar to short-term starvation, in terms of substrate shifts and hormonal changes, where the liver gluconeogenesis is invoked to maintain blood glucose (81-88). The net output of alanine from muscle and net sphlanchnic output of branched chain amino acids in prolonged exercise have been observed (87). These observations combined with evidence of preferrential oxidation of branched chain amino acids in muscle has lead to the hypothesis of a branched chain amino acid-alanine-glucose cycle (87-89). In this cycle, glucose derived pyruvate would be transaminated by α-amino nitrogen group from branch chain amino acid catabolism in muscle. The alanine thus formed could be transaminated in the liver and

the pyruvate carbon skeleton used for gluconeogenesis. Branch chain amino acids could be derived from protein catabolism in the liver or the amino acid pool. Estimates indicate that gluconeogenesis accounts for between 3 and 10% of hepatic glucose output in prolonged exercise (85). Hypothetically this output of glucose could be crucial in maintainance of blood glucose when glycogen stores are depleted and hypoglycemia is impending.

The relative amounts of the two major energy substrates fat and carbohydrate (CHO), utilized to power exercise, are modified by the following factors:

1) Type of muscular work:
   a) intensity: light or heavy in relation to $\dot{V}O_2max$
   b) duration: brief or prolonged, intermittant or continuous.

2) State of physical training

3) Diet, particularly intake of carbohydrates

Each of these factors will be considered separately.

Intensity and duration. Skinner and McKellan (89) have prepared a chart (see Table 3) that indicates the relative usage of CHO and fat with increasing exercise intensity from rest to $\dot{V}O_2max$. Several authors have also reviewed the effect of intensity and duration on fuel metabolism (76, 82, 90, 91). In general, the rate of muscle glycogen utilization is directly proportional to the exercise intensity (92). Thus as intensity increases, glycogen usage is greatly increased. Uptake of blood glucose into the muscle has also been positively related to exercise intensity (82). As the intensity of exercise approaches the $\dot{V}O_2max$, lactate begins to accumulate in the blood. This point is termed the anaerobic

TABLE 3
Exercise intensity and predominant type of metabolism, energy substrates, heart rate, oxygen consumption and event examples (89).

	Phase I		Phase II		Phase III	
	Rest		Aerobic Threshold	Anaerobic Threshold		$\dot{V}O_2$ max
Predominant Metabolism	Aerobic				Anaerobic	
Predominant Substrate	Fat>Carbohydrate				Carbohydrate>Fat	
Relative Intensity (% $\dot{V}O_2$max)			40-65	65-90		
Heart rate (bpm)			130-150	160-180		
Blood Lactate mmol/L			~2	~4		
Event example	Slow walk			Marathon run		Sprint

threshold, as it indicates that CHO has become the major source of energy and is being used in an increasingly anaerobic manner (89). Lactate inhibits the mobilization of free fatty acids (FFA) from adipose tissue and therefore leads to even greater dependence on CHO (93).

As the duration of exercise is prolonged at low and moderate intensities (Phase I and Phase II in Table 3) fat mobilization is increased (90). The uptake and metabolism of FFA in muscle is proportional to the concentration of this substrate in the blood (91). Studies in which the plasma FFA have been artificially raised in man and rats indicate that increased availability of this substrate will markedly reduce utilization of muscle and liver glycogen (94-96).

Recovery. Following exercise the depleted glycogen stores must be repleted and any lactate produced must be disposed of in some manner. The fate of lactate during post exercise recovery is somewhat of a controversy. A study by Hermanssen and Vaage (97) in men suggests the fate of lactate in muscle is primarily one of conversion to muscle glycogen. Others feel that lactate is released into the blood and taken up by the liver to be oxidized or converted to glucose. Studies reported by Felig (87) indicate uptake of gluconeogenic precursors, such as lactate, alanine, and pyruvate, remains high in post exercise recovery. Liver glucose output drops sharply post-exercise, returning to basal about 40 minutes after exercise (87). Plasma levels of FFA increase sharply post exercise as the turnover rates decrease while mobilization is still elevated (90). The repletion of muscle glucogen is slow

post exercise. In the study of MacDougel et al. (98) muscle glycogen that had been reduced to 28% of the fasting level by exercise required 24 hours to return to the pre-exercise level. This slow repletion rate is in agreement with the findings of others (99, 100).

Training. The physiological adaptations that occur with aerobic or endurance training have been reviewed by several authors (101-104). The major change associated with endurance training is an increase in the maximal oxygen consumption. Increases of 16 to 22% (as a result of 2 to 3 months of training) have been reported in normal men (104, 105). Changes in muscle enzyme activities occur, particularly the activities of key oxidative enzymes such as succinate dehydrogenase(104). Activities of muscle glycogen phosphorylase, lactate dehydrogenase and other key glycolytic enzymes exhibit small or nonsignificant differences with training (101). This was also true when the enzyme activities of untrained persons are compared to trained (101, 106). Myoglobin, which facilitates oxygen transfer to muscle, also increases with training (101). The storage of glycogen has been found to be increased with chronic endurance training (107).

The net effect of training adaptations to endurance exercise results in a greater ability to utilize fat as a substrate for exercise, thereby sparing glycogen stores (101, 104, 105). Lower blood and muscle lactate values result from the same absolute exercise stress in trained versus untrained individuals (77, 97, 105).

Diet and exercise. The importance of dietary carbohydrate in exercise endurance was confirmed by studies of Christensen and

Hansen in 1939 (108). Men that were fed a diet high in carbohydrates for three days were able to work twice as long as those maintained on a high fat diet. Studies in the 1960's and subsequent research have sought to clarify the relationship between diet (particularly CHO) and exercise performance. The development of the needle biopsy technique has allowed quantitative study of intracellular muscle metabolites. Bergstrom and Hultman (109), utilizing this technique and themselves as subjects, were able to show that a high carbohydrate diet fed after exhausting exercise would double muscle glycogen stores. With the bicycle ergometer at intensities of 75 to 80%$\dot{V}O_2$max, exhaustion appears to coincide with glycogen depletion (6, 110). This finding in exercising man has lead to the theory that muscle glycogen store was the limiting factor in prolonged heavy (an hour or longer at 75 to 80%$\dot{V}O_2$max) exercise (6, 110). Karlson and Saltin (111) have further shown that increased muscle glycogen results in better running times.

The most effective method to increase muscle glycogen stores appears to be a low carbohydrate diet in conjunction with heavy prolonged exercise followed by a high carbohydrate diet and reduced activity. The glycogen loading regimens have varied considerably between investigators. The diets and exercise regimens utilized along with resulting muscle glycogen levels are summarized in Table 4. This can be compared to normal muscle glycogen levels in man averaging 1.6 g per 100 g muscle (6, 100, 111). While some investigators have increased the percentage of carbohydrate kcal in an isocaloric diet, others have simply added extra CHO to a normal diet (98). Instead of low CHO diets in combination with

TABLE 4
Glycogen supercompensation regimens and resulting muscle glycogen levels.

Investigators	Depletion Phase*	Repletion Phase	Muscle‡ Glycogen
Bergstrom, et al., 1967 (6)	PRO 53%, FAT 46%; 3 days 75%$\dot{V}O_2$max to exhaustion	PRO 18%, CHO 82%; 3 days	3.7 g/100 g muscle
Karlson, et al., 1971 (111)	2 hours heavy exercise "no CHO" 3 days	At least 2,500 kcal CHO 3 days	3.5 g/100 g muscle
Gollnick, et al., 1972 (112)	PRO 46%, FAT 51%, CHO 3%, 3 days 74%$\dot{V}O_2$max for 30 minutes	PRO+FAT 42%, CHO 57% 3 days	144 mmoles glucose units/kg muscle
Piehl, 1974 (113)	2 hours heavy exercise	CHO 60%, 2 days	150 mmoles glucose units/kg muscle
MacDougall, et al., (98)	140%$\dot{V}O_2$max to exhaustion	3,100 kcal mixed diet + 2,500 kcal CHO	88 mmoles glucose units/kg muscle
Kochan, et al., 1979 (100)	PRO 33%, FAT 57%, CHO 10% 3 days, 75%$\dot{V}O_2$max for 60 minutes	PRO 7.5%, FAT 2.5%, CHO 90% 3 days	4.0 g/100 g muscle
Forgac, 1979 † (114)	not less than 100 g CHO, exhaustive exercise	PRO 12%, FAT 23%, CHO 60%	not measured
Lemon and Mullin, 1980 (85)	70 to 75% $\dot{V}O_2$max for 60 minutes dinner "no CHO"	CHO at least 2,500 kcal 3 days	not measured
Sherman, et al., 1981 (115)	a) CHO 15%, 3 days 40 minutes at 73%$\dot{V}O_2$max	70% CHO, 3 days	207 mmoles glucose units/kg muscle
	b) CHO 50%, 3 days 40 minutes at 75%$\dot{V}O_2$max	70% CHO, 3 days	203 mmoles glucose units/kg muscle

* only exercise performed immediately prior to repletion phase is listed.

† Recommended dietary procedure.

‡ At the end of the repletion phase.

exercise to accomplish glycogen depletion some investigators have used heavy exercise alone (109, 113, 115). When followed by a high CHO diet, this depletion procedure is also effective in elevating muscle glycogen stores (113).

The repletion rate of muscle glycogen following depleting exercise has been studied by several investigators (98, 100, 113). At least 24 hours on a normal carbohydrate diet are required to restore glycogen levels to normal following depletion (98, 113). The study of Kochan et al., (100) showed that two days of high CHO diet were necessary to reach 193% of the normal glycogen. In this same study, four days on the high CHO diet produced a 234% elevation or supercompensation. Two days of high carbohydrate diet were necessary to double glycogen stores in the investigations of Bergstrom and Hultman (109). When 16.1 km runs (80%$\dot{V}O_2$max for approximately one hour) were performed on three successive days, a mixed diet (40 to 50% CHO) was insufficient to return muscle glycogen to normal between runs (99). Treadmill running at 80%$\dot{V}O_2$max results in less glycogen depletion in the m. quadraceps femoris than bicycle ergometer exercise at the same intensity and duration (116). This has been attributed to the percentage of total of muscle mass utilized to do work. A larger percentage of the total muscle mass is utilized to do work on a treadmill, thus less depletion may occur in any one muscle.

The substrates metabolized during exercise in the glycogen depleted and supercompensated states are different. In the glycogen depleted state more fat is utilized, as evidence by a lower respiratory quotient (6, 110, 117). When glycogen has been lowered

by diet and exercise blood lactate levels are much lower both after exhaustive exercise and after 30 minutes of exercise at approximately 75% $\dot{V}O_2$max (6, 112). Uptake of blood glucose by muscle was significantly less during 30 minutes of treadmill exercise after glycogen depletion (117). Lemon and Mullin (86) studied protein metabolism of exercise in the glycogen depleted state. Serum urea was significantly higher post exercise (one hour at 61%$\dot{V}O_2$max) in the glycogen depleted state versus the glycogen supercompensated state (86). Sweat urea was also increased. The authors intrepreted this as indicating protein catabolism was increased when the initial glycogen level was low.

Vitamin B-6 Metabolism and Exercise Interrelationships

Metabolic roles of Vitamin B-6 in exercise metabolism

Vitamin B-6 is involved in exercise metabolism through its coenzyme role in glycogenolysis and gluconeogenesis. Both of these roles relate to glucose homeostasis in exercise. The role of PLP in glycogenolysis is as a coenzyme of glycogen phosphorlyase (EC 2.4.1.1). This enzyme liberates glucose-1-phosphate from the glucose polymer glycogen. A recent review by Helmreich and Klein (118) concludes that the 5'-phosphate of PLP, in dianionic form, functions catalytically in glycogen breakdown. The total activity of glycogen phosphorylase in man is not increased by endurance training (106). The total activity of glycogen phosphorylase is increased by feeding excess vitamin B-6 in the rat, but as yet it is unknown if this occurs in man (55). The conversion of phosphorylase

b (dimeric form) to phosphorylase a (tetrameric) has long been believed to regulate the catalysis of glycogen. Recent studies in man in which muscle biopsies were examined indicate that this conversion does not occur with exercise that produces considerable lactate (119, 120). The control of glycogenolysis does occur at this point but the control must be other than the b→a conversion (120). Whether or not the total activity of muscle glycogen phosphorylase present could influence the rate of muscle glycogen usage is not known.

In a single blind study conducted by Lawrence and coworkers, the effect of a 17 mg supplement of pyridoxine hydrochloride on swimming performance and serum lactate levels was tested (121). Following six months of supplementation, the swimming performance of the young swimmers was not different than the control group. Post exercise serum lactate was elevated in the vitamin B-6 group. Thus it appears that glycogen had been utilized anaerobically to a greater extent than in the control group. The vitamin B-6 supplementation could have increased levels of glycogen phosphorylase, and this increase may have influenced glycogen utilization.

The second role vitamin B-6 plays in exercise metabolism is as a cofactor for aminotransferases. Liver and muscle amino-transferases have been postulated to function in maintaining blood glucose during prolonged exercise through the branched chain amino acid-alanine-glucose cycle (85, 87). A muscle enzyme, alanine aminotransferase, involved in this cycle is known to increase with endurance training (101, 103).

Carbohydrate metabolism, important in exercise of all intensities, appears to be modified by vitamin B-6 deficiency. Studies with vitamin B-6 deficient rats have shown decreases in fasting blood glucose levels, impaired glucose tolerance and impaired gluconeogenesis from alanine (122, 123). In women, vitamin B-6 deficiency coupled with oral contraceptive usage decreased fasting blood glucose levels and oral glucose tolerance (124, 125). These metabolic changes were not seen in vitamin B-6 deficient women who were not taking oral contraceptives .

Influence of exercise on vitamin B-6 metabolism

Investigations of the influence of exercise on vitamin B-6 metabolism are limited. Studies on vitamin B-6 metabolism with swimming exercise in rats have been conducted by Efremov and Zaburkin in Russia (126). Their findings indicate that animals deficient in vitamin B-6 reached exhaustion sooner, which may be related to the changes with deficiency of vitamin B-6 mentioned above. Excretion of 4PA was decreased following exercise in the rats, but this decrease was corrected by PN loading.

Metabolism of vitamin B-6 in man was found to be altered by exercise during a study in our laboratory. Wozenski (1) observed a sharp increase in plasma PLP in one of five subjects two hours after a 0.5 mg dose of PN and been given. The subject had exercised by running just before the blood sample was taken. This finding was pursued by Leklem and coworkers (2). They analyzed PLP in the plasma of seven male adolescent cross country runners, during a run at the beginning of the season and a run after six

weeks of training. Both exercises produced significant increases in PLP as compared to plasma PLP immediately prior to exercise. After training the PLP pre to post increase was greater (2).

Munoz (3), also working in this laboratory, compared vitamin B-6 responses to exercise in adolescent males versus adult males, trained versus untrained individuals, and the responses to bicycle ergometer exercise versus running exercise. Significant increases in plasma vitamin B-6 and plasma PLP were seen with all subjects in each exercise situation. Plasma PLP increases (pre to post exercise) averaged 1.05 nanomole per 100 ml for the college athletes after 21 minutes of bicycle ergometer exercise. This concentration change represented a 22% increase in plasma concentration of PLP. The plasma total vitamin B-6 for this group increased 1.10 nmole per 100 ml, approximately the same increase as PLP. The significance of these changes can be compared to the fact that a 1.0 mg vitamin B-6 ingestion would be required to produce this change. Significant differences in the plasma PLP or vitamin B-6 response to exercise were not seen between the trained and untrained subjects or between responses to bicycle versus running exercise. College athletes had significantly greater percent changes in plasma vitamers than did adolescent athletes. The author hypothesizes that this might be due to a larger muscle mass and therefore a larger PLP storage in college athletes as compared to adolescents. Another important finding of this study was a drop in plasma PLP and vitamin B-6 30 minutes post exercise compared to immediate post exercise levels in the college athletes. Decreased plasma PLP following exercise

indicates that the PLP may have been metabolized and excreted or taken up by other tissues.

Another potential interrelationship of vitamin B-6 with exercise is the binding of PLP to hemoglobin at the site 2,3 diphosphoglyceric acid (DPG) also binds (127). Binding of DPG and ATP to hemoglobin at this site causes the oxygen binding affinity of hemoglobin to decrease thereby favoring oxygen unloading at tissue sites. During sixty minutes of aerobic exercise blood levels of DPG were found to increase 10% above the fasting level. The elevation remained up to four hours post exercise (128). Pyridoxal 5'-phosphate will also decrease the oxygen binding affinity, but the concentration of DPG is usually several thousand times greater than PLP (45). With pharmacological doses of vitamin B-6 in man, PLP is known to accumulate in the red cell (65). Anderson reports that a facilitated oxygen transfer with vitamin B-6 doses could not be demonstrated in vivo, but she presents no data (45).

Creatine Phosphokinase and Exercise

Following exercise, several muscle tissue enzymes have been found to increase in the serum. This increase is thought to signal either tissue damage due to exercise or a change in tissue permeability. Creatine phosphokinase (ATP:creatine phosphotransferase, EC 2.7.3.2) (CK) is a cytosolic enzyme that has been studied extensively with exercise. Three isoenzymes of CK have been determined electrophorectically from human tissues (129). Skeletal muscle and heart tissue are characterized by the MM and

MB isoenzyme while brain, bladder, thyroid, and kidney have the fastest migrating isoenzyme BB. Exercise causes a selective increase in CK-MM and CK-MB, indicating the effect is specific to muscle tissues (13).

Heavy prolonged exercise such as a marathon, 90 km. of cross country skiing, 53 miles of race-walking or 80 minutes of rugby produces significant increases (50 to 400% increases over pre-exercise levels) in healthy men (131-135). Increases of 14 to 112% were documented in our laboratory in eleven adolescent cross country runners immediately following an average workout (Leklem, personal communication). Two subjects who had the lowest increases in this study had pre-exercise values already 60% above upper normal limits. Moderately severe exercise of medium duration such as one hour racketball, two hours bicycling at 50%$\dot{V}O_2$max or six to ten mile runs also produce dramatic increases in serum CK but the response is delayed, with peak increases from eight to 24 hours post exercise (7, 130, 136, 137). Walking one hour at three miles per hour, and 15 minutes strenous bicycle ergometer exercise were unable to produce changes in serum CK in normal women and men (138-140). The inability to see increased enzyme levels may be due to failure to measure enzyme activity later than one hour post exercise.

In women there is less release of muscle enzymes following exercise. Shumate and coworkers (137) found that although identical workloads per kg bodyweight were applied, men had significantly greater CK levels 18 hours post exercise than women (664 ± 546 versus 152 ± 111 Units/liter, respectively).

Training has been shown to decrease the release of CK. After periods of physical training of three to ten weeks the CK increase following exercise was eliminated or lessened (141-143).

Theories to explain the cause of enzyme efflux have centered around availability of ATP to maintain the integrity of the cell membrane (8, 134, 142, 144, 145). Prolonged exercise may cause decreases in energy substrate availability, such as depleted glycogen stores. The decreased energy to maintain cellular membranes could lead to progressive changes from reversibly increased permeability to irreversible cell necrosis. An increased concentration of ATP in vitro was shown to protect rat lymphocytes from enzyme efflux caused by phospholipases (145). Studies by Thompson and co-workers (144) with cat hind limb indicated that CK efflux did not occur until there were indications of marked ATP reduction. In contrast to these studies, Franseconi et al. (146) did not find a relationship between high energy phosphates and CK efflux. In this latter study a 30% efflux of intracellular CK in hyperthermic rats was not accompanied by any decrease in high energy phosphate availability. The cause of changes in muscle cells with exercise that would allow the escape of proteins with molecular weights as great as 89,000 is thus unclear at this time.

III. METHODS AND MATERIALS

Subjects

College-age men who were enrolled in classes in advanced cycling were invited to participate in this study. From this group, five subjects were selected and four subjects completed all aspects of the study. Consent forms approved by the Human Subjects Committee of Oregon State University were signed by all subjects before beginning the study (sample form is shown in Appendix). The subjects were deemed healthy and acceptable as subjects for this study on the basis of the following criteria:

1) Normal routine physical examination

2) Normal resting, backlying and post-hyperventilation ECG (electrocardiograph) as evaluated by staff physician at Oregon State University Student Health Center

3) Normal hemoglobin and hematocrit

4) Normal blood chemistry as determined by automated analysis at Good Samaritan Hospital (Corvallis OR) (see Appendix)

5) Avoidance of drugs, alcohol and caffeine during the experimental weeks

6) Normal body weight and regular participation in physical activities of an endurance (aerobic) nature (such as long distance bicycling)

Subject descriptions are presented in Table 5. Percent body fat was calculated by method of Sloan (147) utilizing mean skinfold measurements collected at the beginning of the study, following

TABLE 5
Subject descriptions

Subject	Age	Height	Weight*		% Body Fat†
			Initial	Final	
	yrs	m	kg	kg	
1	20	1.83	84.5	82.7	11.2
2	20	1.79	70.0	70.9	7.5
3	22	1.89	89.0	89.2	5.9
4	23	1.83	75.5	77.3	11.8
Mean	21.3	1.84	79.8	80.8	9.1
±SD	±1.5	0.41	±8.6	±7.8	±2.9

* Initial weight taken Sunday, week 1, Final weight Saturday, week 3.

† Determined by method of Sloan (147) from mean values of skinfolds of three separate determinations.

the second study week and following the third study week. The equation of Sloan uses thigh and subscapular skinfolds to predict body density. Body weights were recorded daily before breakfast. In addition body weights were recorded immediately before and after exercise tests.

Experimental Protocol

A summary of the experimental protocol (diet and exercise) is presented in Figure 4. The experimental protocol (exercise and diets) was approved by the Oregon State University Human Subjects Committee. The study consisted of three experimental weeks. Week 1 served as a control dietary situation. During week 2, dietary carbohydrate and exercise were manipulated to accomplish glycogen loading and depletion. Week 3 of the study repeated week 2 under the circumstance of supplemental dietary vitamin B-6.

Diet Treatments

During the three experimental weeks the subjects were instructed to eat only the food provided. Consumption of alcohol and caffeinated beverages was prohibited. Any drug used was recorded on daily activity records (see Appendix). The subjects consumed self-selected diets prior to study week 1, during the seven day nonexperimental period between study weeks 1 and 2, and during the fourteen day nonexperimental period between weeks 2 and 3. These self-selected diets were recorded by each subject on forms provided (see Appendix). Data from these self-selected diets will not be considered in this

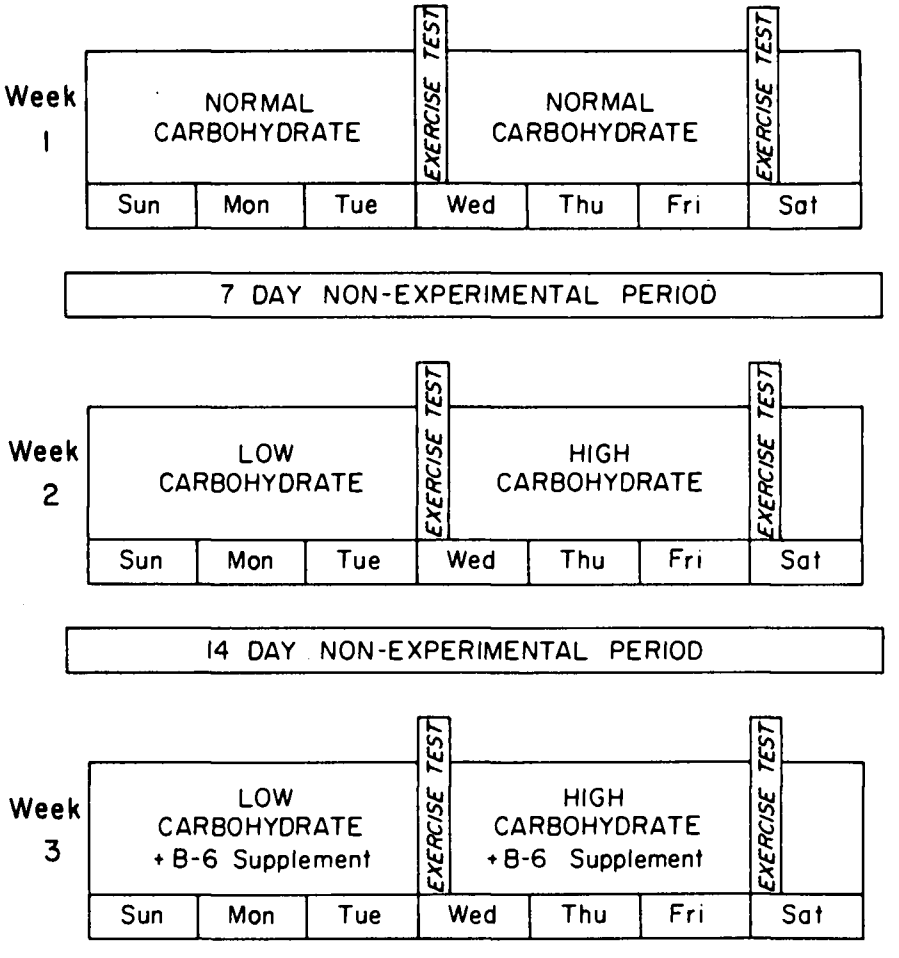

FIG. 4. Experimental protocol.

thesis. Three meals a day were provided during the experimental (study) weeks which ran from Sunday morning through Sunday morning. The subjects were allowed to return to self-selected diets at 8 a.m. on the final Sunday of each week.

Diets provided were calculated to provide 3500 kilocalories (kcal) and 2.0 mg vitamin B-6 (19, 148). The composition of the diets are listed in Table 6. During experimental week 1 a diet with 40% of total kcal as carbohydrate (CHO) was fed. This diet was patterned after diets normally consumed by the subjects in terms of carbohydrate composition. This diet will be referred to as the normal CHO or NC diet. Week 2 began with a diet low in carbohydrate (LC) providing only 11% of total kcal as CHO on days Sunday through Tuesday. The LC diet was followed by a high CHO diet (HC) (71% CHO) on days Wednesday through Saturday. These diets (LC and HC) were patterned after the exchange lists of Forgac (114) for glycogen depletion and loading. Diets fed during week 3 were identical in composition and sequence to week 2 diets with the addition of a daily oral supplement of 8 mg pyridoxine. Diets fed week 3 were abbreviated LC+B6 (Sun through Tue) and HC+B6 (Wed through Fri).

All diets were initially intended to provide a constant proportion of total kcal as protein. The protein content was increased on the LC diet after the first day as the high fat content caused subjects to complain of digestive upsets. The LC diet therefore contained approximately 4% more of total kcal as protein than the NC diet (22% and 18% of total kcal, respectively). This change increased acceptability of the diet.

TABLE 6
Experimental Diet Compositions

Component	Diet		
	Normal Carbohydrate (NC)	Low Carbohydrate (LC)	High Carbohydrate (HC)
Total Energy (kcal)	3505	3480	3480
Carbohydrate (Total g)	349	98	615
(% kcal)	40%	11%	71%
Protein (Total g)	165	194	145
(% kcal)	18%	22%	17%
Fat (Total g)	153	257	49
(% kcal)	42%	67%	12%
Vitamin B6, (mg pyridoxine)*	1.64	1.55	1.82

* Vitamin B6 content listed was determined by analysis of food composites, see Methods and Materials.

Although the diets were calculated to contain 2.0 mg vitamin B-6 (19), actual analysis of diet composites for each diet (see Sample Analysis) indicated that the actual content averaged 84% of the calculated value. The actual value from analysis is given in Table 6. In addition, analysis indicated that the diets did not contain equivalent amounts of vitamin B-6 as intended. The HC diet contained approximately 17% more vitamin B-6 than the LC diet.

The exact amounts of each food served are listed in the Appendix. All meals were prepared in the Department of Foods and Nutrition Metabolic Kitchen in Milam Hall at Oregon State University.

Supplemental vitamin B-6 given during study week 3 was prepared from pyridoxine hydrochloride (Calbiochem, San Diego CA) in a 0.5% acetic acid solution. The solution was prepared in one batch to contain 8.0 mg of PN per 10 ml. Aliquots were frozen at 0°C. Each morning an aliquot was thawed, protected from light, and 5.0 ml aliquots were pipetted into sample cups. Subjects were instructed to drink the aliquot then rinse the cup with water and drink the rinse. A 5.0 ml aliquot was given at breakfast and another was given at dinner. The PN solution was stored covered in the refrigerator between breakfast and dinner.

Daily exercise

Daily exercise activities were recorded during the study weeks and the non-experimental days (see Appendix for form). The normally pursued exercise regimens of the subjects were encouraged during Sunday through Tuesday. This exercise was encouraged to aid in glycogen depletion during the LC diets. Wednesday through Saturday

subjects were requested to restrict their activity level to less than 30 minutes of low intensity bicycling per day. This request was made to allow muscle glycogen stores to supercompensate (load) while consuming the HC diets.

Exercise Test Protocol

The same exercise test was administered on Wednesday and Saturday of each study week. Graduate students trained in graded exercise test application[1] and cardiopulmonary resuscitation administered the tests. For each test subjects reported to the Department of Physical Education Exercise Physiology Laboratory after an overnight fast. The starting time of each exercise test varied from approximately 5:30 a.m. for subject 1 to 7:00 a.m. for subject 4. Standardized Monark bicycle ergometers were used as the exercise modality (Quinton Instruments Seattle, WA). The exercise test protocol and timing of the blood sampling is presented in Figure 5. This test was designed to accomplish significant glycogen depletion through stepwise increases in exercise intensity during 50 minutes of continuous exercise. Heart rate was chosen to standardize the exercise intensity rather than oxygen consumption. Resistance (kp) was adjusted appropriately to maintain HR (heart rate) at the predetermined % HRmax, while subjects peddled at a constant rate of 20 km/hr. Maximal HR was estimated from the equation of Karvonen (76). Heart rate was monitored with

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[1] Art Seimann, Frank Goulard and Lauren Hatcher administered exercise tests.

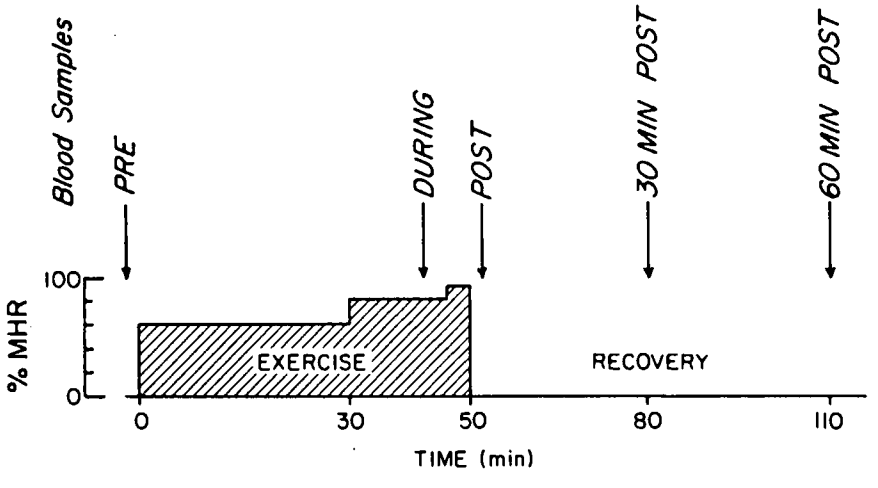

FIG. 5. Exercise test protocol and blood sampling times.

either a Bircher (Bircher, Model 344, El Monte CA) or a Parke-Davis ECG (Electrocardiograph) using a Blackburn CM-5 lead pattern. Heart rate was determined from a ECG tracing every two minutes throughout the 60% and 80% HRmax exercise intervals. Monitoring rate was increased to every minute during the five minutes of 90% HRmax and during the first six minutes following recovery. Work load was dropped to zero immediately at the end of the 90% HRmax period. Peddling rate was decreased stepwise over the next six minutes (active recovery). Heart rate was monitored every two minutes for another six minutes (seated recovery) while the subject rested while seated on the bicycle. Following seated recovery, ECG leads were removed and the subjects rested (seated) in a room adjoining the laboratory for the remainder of the one hour recovery period.

Work load, heart rate and ECG tracing were evaluated and are presented in the Master's thesis of Frank Goulard (1982) and Art Siemann (1982) both of Oregon State University Department of Health and Physical Education.

The six exercise tests and the responses to each test will be identified by the abbreviation of the diet fed the three days prior to the test. For example, the exercise test administered Wednesday morning of week 2 was preceded by 3 days during which the subjects consumed a low CHO diet, thus the exercise test will be referred to as exercise LC.

Sample Collection

Two certified medical technologists (Lynda Barstow and Karin

Hardin) were responsible for blood drawing. Figure 5 illustrates the timing of blood drawing with respect to exercise and recovery. Blood samples will be referred to by the labels indicated in Figure 5 (pre, during, post, 30 min post and 60 min post). Samples (20 ml) were drawn from the antecubital vein of the forearm into heparinized tubes with stasis. In addition to the 5 blood samples indicated in Table 5, a five ml sample was drawn on the morning after exercise prior to breakfast (24 hr post). The during exercise sample was drawn during the 80% HRmax period two to three minutes prior to the 90% HRmax interval. The subjects did not stop exercising for the sample to be drawn. Timing of sampling varied approximately ±2 minutes. Plasma was separated and stored at -70°C until analyzed. In addition to the analysis of this study, all blood samples were also analyzed for the fuel metabolites: glucose, lactate and free fatty acids.[2]

Urine was collected in 24 hours aliquots under toluene. On exercise days urine was collected into two aliquots: a 10 hour (including exercise) and a 14 hour aliquot. This division was made for urea analysis, which is not a part of this thesis, thus all urine data is expressed per 24 hours. Each 24 hr period started after the first morning voiding of the bladder. Sunday samples were collected but were considered practice samples and were discarded. Once received, the urine samples were measured and frozen at -20°C until analyzed.

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[2] Thesis project of Ann deVos (1983)

Sample Analyses

Diet Composites

Composites were made of each diet fed during the study. Foods were prepared exactly as if they were to be served then divided into animal and vegetable origin prior to composite preparation. The composite was prepared by blending together the weighed items with enough redistilled water (also weighed) to facilitate blending. The total weight was recorded and well blended aliquots were stored at -40°C until analyzed. An aliquot of one or two grams was weighed and then acid hydrolyzed. Analysis for total vitamin B-6 was performed by the S. uvarum method (149) (described more fully under PB6 analysis).[3] Vitamin B-6 contents of the diets are listed in Table 6.

Blood Analyses

Hemoglobin (Hgb) and hematocrit (Hct) were determined immediately following the completion of all exercise tests of a given day.[4] Hematocrit analysis was performed in duplicate using the microhematocrit method. Hemoglobin was determined in triplicate by the cyanomethemoglobin method.

Plasma was analyzed for total vitamin B-6 using the

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[3] Hossein Kabir performed the vitamin B-6 analysis of the diet composites.

[4] Karin Hardin and Lynda Barstow aided in Hct and Hgb analyses.

microbiological method detailed by Miller and Edwards (150). The organism Saccharomyces uvarum 4228; ATCC No. 9080, which is differentially responsive to all nonphosphorylated forms of vitamin B-6, was used for the assay. Due to the shortage of sample and the amount of sample necessary, duplicates were not run. All samples of a subject were assayed in the same assay. Inter-assay variability calculated from a control sample was 1.7% (n=4), with the control averaging 5.85 ± 0.10 nmol/100 ml.[5] A two ml plasma sample was precipitated with 10 ml of 10% trichloroacetic acid (TCA) in centrifuge tubes. This solution was allowed to set 30 min after which it was centrifuged and the supernatent collected. Second and third washings of the precipitate were performed using 5 ml of 10% TCA. Combined supernatents for each sample was covered with a watch glass and autoclaved at 102 kPa to hydrolyze all phosphorylated forms of PB6 present. The resulting values of PB6 represent the total concentration of B-6 vitamers present, both nonphosphorylated and phosphorylated.

Plasma PLP was determined by a modification of the method of Chabner and Livingston (157). The method of Chabner and Livingston was modified by using: 1) 5 M potassium acetate as a buffering solution; 2) sidearm incubation flasks; and 3) liquid scintillation fluor. The method is based on the amount of $^{14}CO_2$ produced through the decarboxylation of L-tyrosine-1-^{14}C by the action of tyrosine decarboxylase, a PLP dependent enzyme. The $^{14}CO_2$ released is trapped in NCS tissue solubilizer (Amershan Corp,

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[5] Karin Hardin performed all vitamin B-6 analyses.

Arlington Heights, IL). Ten ml of toluene based fluor was added and samples were quantitated using a Beckman liquid scintillation counter (Model L5-3133P). Samples were not extracted in duplicate as sample quantity was limited, but were assayed in duplicate. A recovery was analyzed for each individual. Samples were assayed by individual subjects with weeks 1 and 2 assayed together. Week 3 for each subject was assayed separately with any necessary samples repeated Plasma samples (0.5 ml) were precipitated with 0.6 ml of 1.0 M perchloric acid to release protein bound PLP. Inter-assay variability of the control sample was 5.7% (n=8). Recovery of added PLP averaged 90 ± 8%.

A kit for the determination of plasma creatine kinase (CK) was purchased from Sigma Chemical Co (Stock no. 46-10, Kit A Sigma, St Louis, MO). The method utilized is based on the coupling of ATP production by CK to the production of NADPH by the enzymes hexokinase and glucose-6-phosphate dehydrogenase. The rate of NADPH formed is then measured at 340 nm and this is directly proportional to the activity of CK present. All isoenzymes of CK are measured by this method. A Beckman DB Spectrophotometer with a water-jacketed cell was utilized to perform the assay. All samples were assayed in duplicate. All samples were assayed within one week after they were collected and were assayed in subdued light due to the sensitivity of CK. Enzyme samples from Hyland Diagnostics (Deerfield, IL) (Q-pak Chemistry Multi-enzyme control) were used as an enzyme control. In addition, a stored human serum was assayed with each analysis. Inter-assay variability on the control enzyme was 5.0% (n=15). Mean value

obtained for the enzyme was 449 ± 22.7 U/L. The range reported by Hyland Diagnostics was 315 to 514 U/L. Inter-assay variability on the stored human serum was 8.7%, with a mean of 62.8 ± 5.5 U/L (n=9).

Urine Analyses

Urinary creatinine was determined on a Technicon Autoanalyzer (Technicon Corporation, Tarrytaun, NY). The method of Pino et al. (152) was modified for creatinine analysis.

The method used for analysis of urinary 4-pyridoxic acid was that of Reddy et al. (153). After separation of 4PA from interfering urinary compounds by ion exchange chromatography, the 4PA is determined by fluorescence.[6] The fluorescence was read in an Aminco Bowman spectrophoto fluorometer (American Instrument Co., Inc., Silver Springs, MD). Samples were not assayed in duplicate, but at least 2 recoveries were analyzed for each subject. Control samples averaged 3.45 ± 0.44 μmole/24 hr with a variability of 12.6% (n=4). Recoveries averaged 94 ± 7.6% (n=11).

Data Reduction

The method of van Beaumont (154) was selected to calculate plasma volume change with exercise. This method involves multiplication by a proportionality factor since the percent change in hematocrit with exercise is known to underestimate the actual

[6] Linda Barstow helped in the determination of 4PA.

plasma volume (PV) change. The equation is:

$$\% \text{ change PV} = \frac{100}{100\text{-Hct}_1} \times 100 \frac{(\text{Hct}_1 - \text{Hct}_2)}{\text{Hct}_2}$$

A second method, that of Dill and Castill (155) was also used to calculate % change in PV. This method uses both hemoglobin and hematocrit to calculate change in PV. As PV changes calculated by the Dill and Castill method were equivalent in magnitude and direction to those generated by the van Beaumont method, only those of the van Beaumont were included. Calculations of percent change in plasma volume were made for each blood sample relative to pre exercise (pre to: during, post, 30 min post, and 60 min post).

Data collected from blood analyses (PB6, PLP, CPK) were analyzed in several ways. Percent changes were calculated for each blood sample as compared to the pre exercise blood value (pre to during, pre to post, pre to 30 min post, pre to 60 min post). The absolute difference from pre exercise (in nmol/100 ml) was also calculated for each blood sample. The percent of total plasma vitamin B-6 present as PLP was also calculated.

Urine excretion of 4PA was calculated as percent of the total daily intake of vitamin B-6.

Statistical Analyses

Paired t-tests were applied to blood data (PB6, PLP, CK) and calculations in a number of ways to test for three major effects, (1) the effect of diet independent of exercise on plasma levels, (2) the effect of exercise on plasma levels, and (3) the effect of

diet on the exercise responses. The effect of diet (1) was tested by comparing raw pre exercise values between the six exercise tests. The effect of exercise (2) was considered by testing each raw value against the pre exercise value and the 30 and 60 min post exercise values against the post exercise value within each exercise test session. The modification of exercise responses by diet (3) was considered by testing absolute difference from pre exercise and % changes from pre exercise between the six exercise test sessions.

Paired t-test were also applied to all combinations of daily % 4PA excretion within each week. Excretion of 4PA on exercise test days was compared between weeks.

Pearson's Correlation Coefficients were calculated for nmol/ 100 ml differences from pre of PB6 with PLP, for % change in plasma volume with each % change PLP and PB6, and for % change in CK with each % change PLP and PB6.

A Hewlett Packard Model 10 calculator was used for all calculations. The null hypothesis was rejected at the 0.05 level of significance.

IV. RESULTS

Blood Analyses

The abbreviation of the diet treatment fed three days prior to an exercise test will be used to identify a specific exercise test. This is to allow the reader to recall the diet treatment which may be expected to influence the response to the controlled exercise. The abbreviations of the exercise tests are as follows:

NC(Wed) for exercise test Wed Week 1
NC(Sat) for exercise test Sat Week 1
LC for exercise test Wed Week 2
HC for exercise test Sat Week 2
LC+B6 for exercise test Wed Week 3
HC+B6 for exercise test Sat Week 3

The abbreviation may also be used to refer directly to diet treatment. The diet treatment may also be refered to directly.

The blood analyses performed in this study will first be considered individually in the following order: plasma volume, plasma vitamin B-6, plasma PLP, and plasma creatine phosphokinase. After the individual considerations, correlations of these parameters will be considered.

Plasma Volume Changes

As plasma volume is known to change with exercise, it has been

suggested that changes in plasma constituents such as those measured in the study could be due to change in plasma volume. It was therefore deemed important to evaluate changes in plasma volume. Mean percent plasma volume changes as calculated from hematocrit by method of van Beaumont (154) are presented in Table 7; note that negative values indicate a decrease in plasma volume from the pre exercise volume (hemoconcentration) and positive values indicate expansion of the plasma volume from pre exercise (hemodilution).

As was expected, hemoconcentration occurred between the pre and during blood samples and between the pre and post samples. Considering mean values of the pre to during plasma volume change, a wide variation in the magnitude of hemoconcentration was observed from -2.7% for exercise NC(Sat) to -9.6% for exercise HC+B6. The low hemoconcentration observed for NC(Sat) from pre to during appears to be unique as all other days resulted in mean hemoconcentration in a narrower range from -6.5 to -9.6%. Mean hemoconcentration from pre to post exercise was more consistent among the exercise tests than was the pre to during change. The pre to post change ranged from -9.4% to -11.3%.

Values for plasma volume changes from pre to 30 min post exercise indicated that all subjects were returning to pre exercise plasma volume but some exceeded pre exercise thus exhibiting net hemodilution. Mean values for exercises NC(Wed and Sat) and LC indicated hemodilution from pre to 30 min post. Mean values for exercises HC, LC+B6 and HC+B6 indicated that hemoconcentration remained at 30 min post exercise relative to pre exercise. The mean values obscure the fact that for most exercise tests from pre

TABLE 7
Mean percent change in plasma volume for all exercise tests by the method of van Beaumont (154).

Week	Day	Diet	Percent Change from Pre exercise to: During	Post	30 min post	60 min post
1	Wed	NC	-6.5 ±4.3	-9.4 ±6.1	3.6 ±3.6	3.3 ±7.4
1	Sat	NC	-2.7 ±1.2	-9.3 ±2.1	5.8 ±5.5	7.6 ±3.1
2	Wed	LC	-9.3 ±3.2	-10.9 ±1.7	1.4 ±5.2	3.1 ±3.3
2	Sat	HC	-7.2 ±4.4	-10.0 ±3.0	-0.4 ±6.0	9.1 ±9.6
3	Wed	LC+B6	-8.7 ±6.3	-10.3 ±5.9	-2.6 ±6.4	-3.5 ±10.4
3	Sat	HC+B6	-9.6 ±2.9	-11.3 ±3.5	-0.2 ±4.2	3.9 ±2.5

All values are reported as mean ± 1SD.

to 30 min post at least two subjects (not always the same subjects) were hemodiluted.

Plasma volume changes from pre to 60 min post exercise exhibited more consistent results among the exerxise tests, with all but LC+B6 exhibiting mean hemodilution. With exercise LC+B6, three of the four subjects actually exhibited an increased hemoconcentration as compared to 30 min post exercise. In contrast, for all other exercises at 60 min post, hemodilution had occurred relative to pre, during, post, and 30 min post with only a singular individual exception for exercises NC(Wed) and LC.

Plasma Vitamin B-6 (PB6)

The mean plasma concentration of each time point of each exercise day and the difference of each time point from the pre exercise value are presented in Table 8. The effect of diet on pre exercise (fasting) plasma vitamin values will be considered first followed by the effect of exercise.

Pre exercise PB6 levels were compared statistically to assess effects of dietary treatments on fasting PB6 levels independent of exercise. The HC diet resulted in the lowest mean plasma concentration of all the diets (5.64 nmol/100 ml). This plasma level was significantly different than the pre exercise value for exercise LC, even though dietary intake of vitamin B-6 was actually 17% greater (1.82 mg pyridoxine) than the LC diet (1.55 mg PN). Supplementation resulted in PB6 concentrations that were significantly different (greater) than all other pre exercise concentrations. For exercise LC+B6, the pre exercise plasma level

TABLE 8
Mean plasma vitamin B-6 values for all exercise tests and differences from pre exercise for all values

Exercise			Plasma Vitamin B-6				
Week	Day	Diet	Pre	During	Post	30 min post	60 min post
			nmoles/100 ml				
1	Wed	NC	6.96[c,d] ±2.49	7.66* ±2.38	7.88* ±2.50	7.52 ±2.43	7.20 ±2.45
		-pre		0.66 ±0.25	0.92 ±0.48	0.56 ±0.53	0.24 ±0.51
1	Sat	NC	6.39[e,f] ±2.32	6.85 ±2.15	7.72* ±1.94	6.81† ±1.85	6.41† ±1.71
		-pre		0.46[a] ±0.56	1.32 ±0.60	0.42 ±0.58	0.02 ±0.70
2	Wed	LC	7.20[b,g,h] ±1.45	7.54* ±1.48	7.85* ±1.46	6.86† ±1.55	6.67† ±1.42
		-pre		0.34 ±0.06	0.65[k] ±0.20	-0.34 ±0.29	-0.53 ±0.37
2	Sat	HC	5.29[b,i,j] ±1.17	6.09 ±1.51	6.02* ±1.18	5.65 ±1.29	5.15† ±1.51
		-pre		0.80[a] ±0.60	0.73 ±0.44	0.36 ±0.30	-0.14 ±0.65
3	Wed	LC+86	17.69[c,e,g,i] ±5.83	19.99 ±5.15	20.34* ±6.20	17.69† ±5.07	18.23 ±5.98
		-pre		2.30 ±2.83	2.66[k] ±1.45	0.00 ±1.92	0.54 ±2.15
3	Sat	HC+B6	18.45[d,f,h,j] ±5.92	20.38* ±5.85	21.12* ±6.05	18.43† ±5.20	17.44† ±5.22
		-pre		1.93 ±0.52	2.66 ±1.07	0.02 ±0.79	-0.96 ±0.69

All values are mean ± 1SD
* Significantly different ($p<0.05$) from pre exercise value of the same day
† Significantly different ($p<0.05$) from post exercise value of the same day
k For those sharing this superscript, are significantly different with natural log conversion prior to paired t-test, ($p<0.05$)
a-j Those sharing the same superscripts are significantly different ($p<0.05$).

was 17.67 nmol/100 ml,representing a tripling of plasma levels over the HC diet. Pre exercise plasma levels for exercises LC+B6 and HC+B6 were not significantly different.

In general, the effect of exercise, regardless of dietary treatment, was to increase PB6 levels at the during and post exercise time points. With the post exercise recovery period plasma levels generally decreased, approaching the pre exercise value, but in many cases fell below the pre value. The effect of exercise on BP6 at the during and post time points will first be considered, followed by the 30 and 60 min post exercise responses.

The changes in absolute concentration of PB6 with exercise are shown graphically for each exercise session in Figure 6. The post exercise PB6 value was significantly different (greater) ($p<0.05$, by paired t-test) than the pre exercise PB6 value for each exercise session. Plasma vitamin B-6 levels measured at the during exercise time point were greater than pre for most of the exercise tests, but this difference was significant for only NC(Wed), LC, and HC+B6. The difference from the pre exercise value was calculated for each time point (Table 8) and these differences are presented graphically for comparison in Figure 7. As discussed above, the initial plasma vitamin B-6 value was affected by diet. Therefore, comparison of the changes with exercise should also be considered independent of the initial plasma value. The exercise sessions in order of increasing magnitude of the pre to post difference were: LC, HC, NC(Wed), NC(Sat), LC+B6, HC+B6. The smallest pre to post increase was 0.62 nmol/100 ml (a 9.4% change) for LC while the largest was more than four times larger when both exercises LC+B6

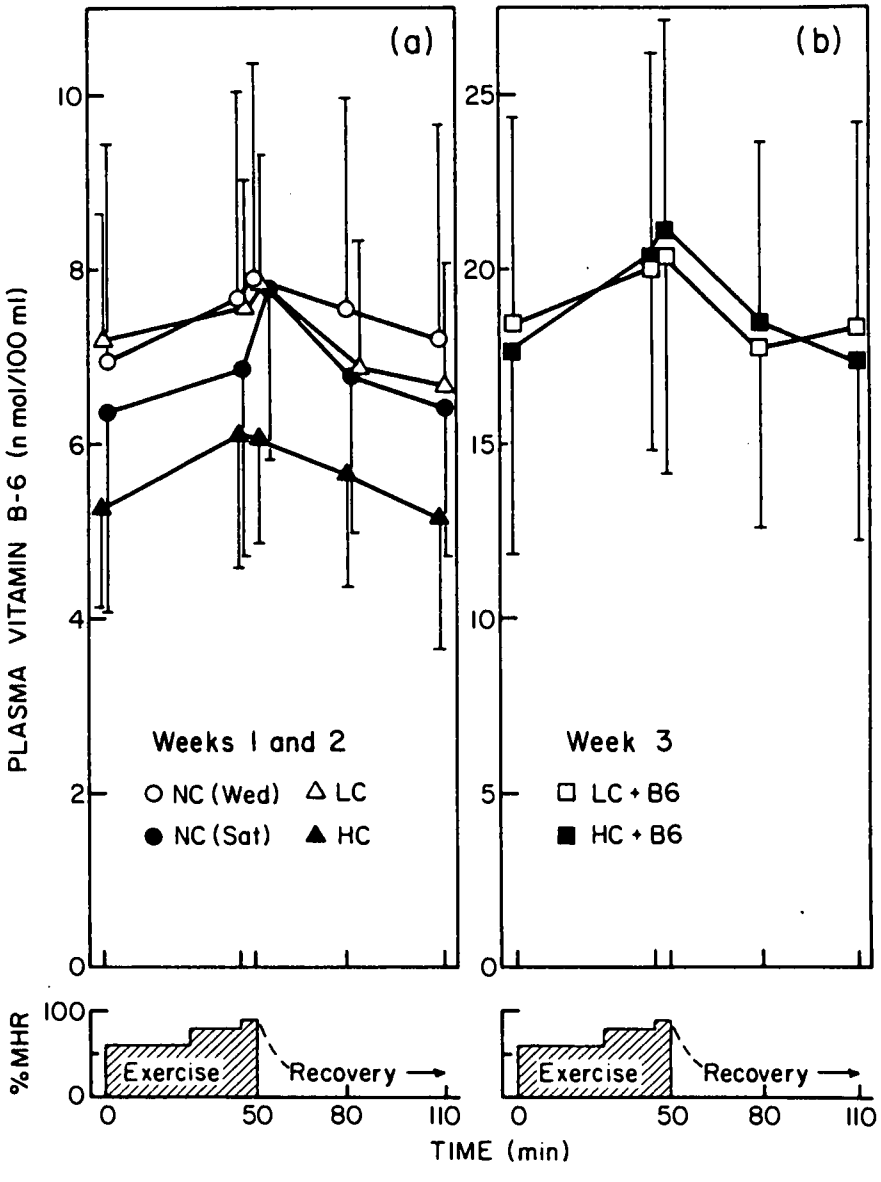

FIG. 6. Plasma vitamin B-6 response to all exercise tests. Each point represents the mean response (n=4). Vertical lines indicate ± 1SD.

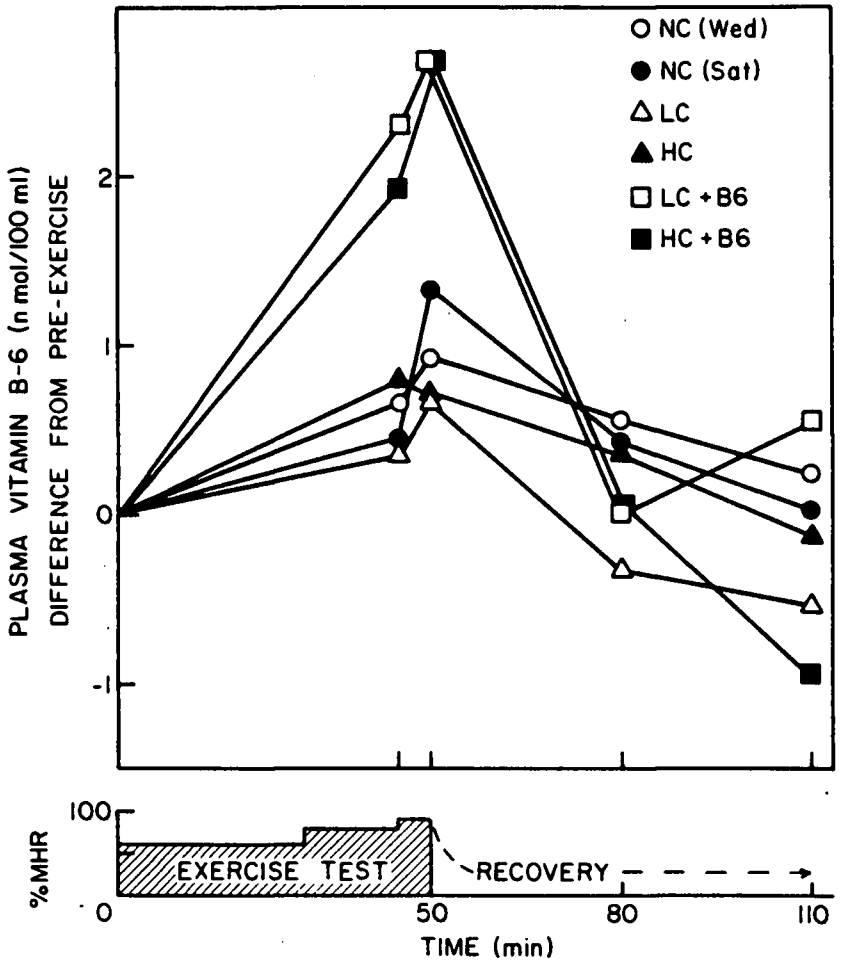

FIG. 7. Plasma vitamin B-6 response to all exercise tests: Differences from pre exercise values. Each point represents the mean response (n=4). Standard deviations were omitted for clarity.

and HC+B6 resulted in pre to post increases of 2.66 nmol/100 ml (both 15.7% changes). The LC and the LC+B6 pre to post PB6 differences could be shown to be significantly different when natural log conversion was applied to the differences prior to paired t-test. Statistical comparison of other differences between the exercise days of each week and between weeks revealed only one other significant difference: the NC(Sat) pre to during difference was less than the pre to during increase for exercise HC+B6. The pre to post difference of exercise LC+B6 was not significantly different than the NC(Wed) response although three of the four subjects had responses for BC+B6 that were two times greater than that seen for exercise NC. The pre to post difference for exercise HC+B6 was not significantly different than HC, although all four subjects had greater responses for HC+B6 (at least two times greater). The wide individual variation in the magnitude of the responses appeared to contribute to these differences being nonsignificant.

The short time period of intense exercise between the during and post exercise samples produced variable responses in PB6. Comparisons of mean during and post PB6 values indicate that for all exercises, with the exception of exercise HC, PB6 increases from during to post. The sharp change in the slope of the line (see Figure 8) indicates that the rate of change in PB6 increased for all but HC. For exercise HC only one subject(#3) actually exhibited a decrease in PB6. The exercise sessions in order of magnitude of the mean during to post differences were: HC, NC(Wed), LC, LC+B6, HC+B6, and NC(Sat). The order of the during to post difference varies from the order of the exercise sessions for the total pre to

post difference indicating that the during to post difference of each exercise session contributes a different amount to the total pre to post change of that session. For example, exercise NC(Sat), which had the largest during to post response, had a total pre to post response less than both LC+B6 and HC+B6, indicating that the during to post increase contributed a larger proportion of the total pre to post change for NC(Sat) than for LC+B6 or HC+B6.

Changes in PB6 levels with exercise were also considered as a percent of the pre exercise value. Mean percent change with exercise and recovery is reported in Table 9. In increasing order of magnitude of the percent change from per to post, the exercise tests were: LC, HC, NC(Wed), LC+B6 and HC+B6 and NC(Sat). The largest concentration change (2.66 nmol/100 ml for exercises LC+B6 and HC+B6) was less than NC(Sat) (1.32 nmol/100 ml) when considered as percent change: 15.7% for LC+B6 and HC+B6 versus 24.1% for NC(Sat). The smallest percent change (increase) with exercise from pre to post was for exercise LC, which also gave the smallest absolute change. These differences in percent change did not reach statistical significance. The pre to during percent changes were ordered somewhat differently with respect to magnitude (LC, NC(Sat), HC+B6, NC(Wed), HC, LC+B6) although LC again exhibited the smallest pre to during percent change.

All subject's PB6 values decreased with respect to post exercise at the 30 and 60 min post sampling times, but in relation to pre exercise, some had returned to pre while others dropped below pre values. At 30 min post, the mean PB6 remained above fasting (pre) for exercises NC(Wed and Sat) and HC. Following the LC exercise,

TABLE 9
Mean percent change in plasma vitamin B-6 for all exercise tests.

Week	Day	Diet	% Change Plasma Vitamin B-6, pre to: During	Post	30 min post	60 min post
				%		
1	Wed	NC	11.7 ±6.7	14.7 ±9.9	9.7[b] ±11.8	4.5 ±10.0
1	Sat	NC	8.4[a] ±10.0	24.1 ±14.1	9.2 ±11.6	2.6 ±8.9
2	Wed	LC	4.8 ±1.1	9.4 ±3.4	-4.9 ±4.0	-7.3 ±5.2
2	Sat	HC	15.0[a] ±10.3	14.5 ±9.5	6.6 ±6.0	-3.6 ±15.1
3	Wed	LC+B6	15.5 ±18.4	15.7 ±9.8	1.0[b] ±10.6	2.9 ±13.8
3	Sat	HC+B6	11.6 ±5.5	15.7 ±7.7	1.1 ±5.5	-4.9 ±2.4

All values are reported mean ± 1SD.

[a,b] Those sharing the same superscript are significantly different, ($p<0.05$).

PB6 values were below pre exercise, while plasma PB6 levels for exercises HC+B6 and LC+B6 were equal to pre exercise at 30 min post. The mean 30 min post PB6 value of exercises LC+B6 and HC+B6 actually represent extremely variable individual response. For example, for exercise LC+B6 two subjects dropped below pre PB6 values while the other two subjects still exhibited values greater than the pre PB6 values. At 60 min post exercise for exercises NC(Wed) and NC(Sat), the PB6 level was still slightly above the pre exercise value but had decreased relative to the 30 min post exercise level and thus appears to be approaching the pre exercise value. Plasma vitamin B-6 for exercises LC, HC, and HC+B6 was also decreased at 60 min post for all subjects versus pre and 30 min post. For exercise LC+B6, three of four subjects exhibited net increases in PB6 over pre and 30 min post values. This was a unique finding among the exercise tests. The largest mean decrease from pre to 60 min post exercise occurred for exercise HC+B6 (-0.96 nmol/100 ml). No exercise session had PB6 values at 30 and 60 min post that were significantly different from the pre exercise value.

Comparison of 30 and 60 min post PB6 values to post exercise PB6 values revealed several significant differences. The post and 30 min post exercise PB6 values were significantly different for exercises NC(Sat), LC, LC+B6, and HC+B6. At 60 min post for exercises NC(Sat), LC, and HC+B6, the mean PB6 value was still significantly different from the post PB6 value. In contrast to all other exercise, plasma vitamin B-6 values for LC+B6 at 60 min post exercise actually increased relative to pre and 30 min post exercise values. By 60 min post, the PB6 value for exercise HC had

also decreased enough to be significantly different from the post exercise value.

The percent change from pre to 30 and 60 min post exercise indicated that the greatest percent decrease was following exercise LC, although this was not significant. For the pre to 60 min post percent change, two subjects actually had greater percent decreases with HC+B6 than LC. Although exercise HC exhibited a mean negative percent change indicating a decrease relative to pre exercise at 60 min post, not all subjects (two of four) showed a negative percent change as observed for exercises LC and HC+B6. Mean values pre to 60 min post for exercises NC(Wed and Sat) and LC+B6 indicated a positive percent change, but in each case at least one subject exhibited a negative change.

Plasma Pyridoxal 5'-phosphate (PLP)

The mean values of PLP obtained with each exercise session and the difference from the pre exercise value for each time are given in Table 10. The pre exercise levels will first be considered for the effect of diet independent of exercise. The effect of exercise will then be covered beginning with the changes during and post exercise considered first followed by the 30 and 60 min post exercise responses.

At the beginning of the study (Week 1, Wed), all subjects exhibited fasting (pre) plasma levels of PLP were above the level considered marginal by Shultz and Leklem (62). During week 2 for exercise LC, the plasma PLP level of subject 3 dropped to 2.53 nmol/100 ml. As was shown with PB6, the pre exercise PLP value was

TABLE 10
Mean plasma pyridoxal 5'-phosphate for all exercise tests and difference from pre exercise for all values.

Exercise				Plasma Pyridoxal 5'-phosphate			
Week	Day	Diet	Pre	During	Post	30 min post	60 min post
				nmoles/100 ml			
1	Wed	NC	5.87[e,f] ±2.57	6.67* ±2.73	6.84* ±2.81	6.18† ±2.91	5.64† ±2.66
		-pre		0.79[a] ±0.19	0.97[b,m] ±0.42	-0.31[c] ±0.35	-0.23 ±0.52
1	Sat	NC	5.25[g,h] ±2.23	5.68* ±2.47	5.94* ±2.25	5.11† ±2.30	4.78*† ±2.06
		-pre		0.43 ±0.26	0.69 ±0.09	-0.01 ±0.27	-0.22 ±0.51
2	Wed	LC	5.49[d,i,j] ±1.66	5.73 ±1.51	5.81 ±1.56	5.17† ±1.70	4.79*† ±1.49
		-pre		0.23[a] ±0.21	0.32[b,n] ±0.25	0.35[c] ±0.30	-0.71 ±0.42
2	Sat	HC	3.64[d,k,l] ±0.83	4.14 ±1.07	4.53 ±1.42	3.93† ±1.26	3.47† ±1.29
		-pre		0.50 ±0.33	0.88 ±0.72	0.34 ±0.53	-0.17 ±0.56
3	Wed	LC+B6	14.15[e,g,i,k] ±6.21	15.42* ±5.98	16.44* ±7.47	14.02 ±5.50	13.05 ±5.31
		-pre		1.26 ±0.48	2.28[m,n] ±1.27	-0.15 ±1.45	-1.12 ±1.17
3	Sat	HC+B6	14.57[f,h,j,l] ±6.10	15.25 ±6.54	16.39* ±6.04	13.52 ±4.87	12.98*† ±5.57
		-pre		0.67 ±0.54	1.31 ±0.63	-1.06 ±1.74	-1.59 ±0.76

All values are expressed as mean ± 1SO.
* Significantly different ($p<0.05$) (paired t-test) from pre value of same day.
† Significantly different ($p<0.05$) from post value of same day.
a-l
Those sharing the same superscript are significantly different, ($p<0.05$).
m,n
Those sharing the same superscript are significantly different with natural log conversion prior to paried t-test, ($p<0.05$).

significantly lower ($p<0.05$) for exercise HC than for pre exercise LC. As anticipated, supplementation of 8 mg PN resulted in significantly different (greater) PLP when compared to pre exercise values for weeks 1 and 2. Mean pre exercise PLP levels with HC+B6 were approximately 3.9 times greater than the pre exercise value for the HC diet. It is interesting to note that with supplementation the subject with a marginal level of PLP increased (2.6 times) the least of all subjects.

Figure 8 illustrates the effect of exercise on the values of PLP. As with PB6, the mean values of PLP increased over pre at both the during and post time points for all exercises. The during plasma PLP concentration was significantly different (greater) than pre exercise only for the exercises NC(Wed and Sat) and LC+B6. Significant differences bewteen pre and post PLP were seen for NC(Wed), NC(Sat), LC+B6, and HC+B6, but not for LC or HC. The post value for LC was not significantly different than pre exercise LC since the plasma PLP of subject 1 failed to change. All subjects exhibited increases from pre to post exercise HC, but the response of subject 4 (+0.23 nmol/100 ml) was one-tenth the response of subject 2 (1.91 nmol/100 ml) resulting in the lack of significance. Comparison of the differences from pre is illustrated in Figure 9. The order of the exercise sessions in relation to the magnitude of the pre to post differences were (in order of increasing magnitude): LC, NC(Sat), HC, NC(Wed), NC+B6, LC+B6. Exercise following LC, as with PB6, resulted in the lowest pre to post change in PLP (0.32 nmol/100 ml) while LC+B6 resulted in the greatest change (2.28 nmol/100 ml). The pre to post difference for exercise LC was significantly

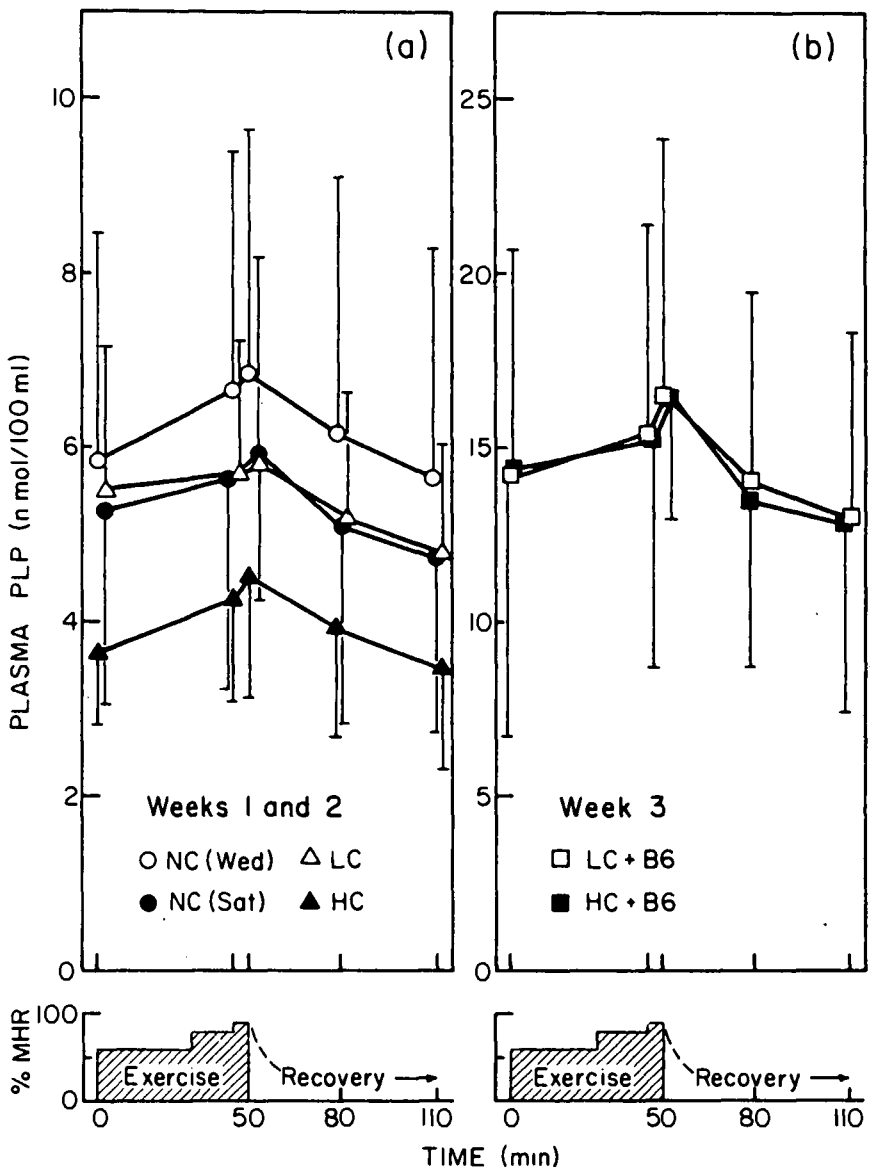

FIG. 8. Plasma pyridoxal 5'-phosphate response to all exercise tests. Each point represents the mean response (n=4). Vertical lines indicate ± 1SD.

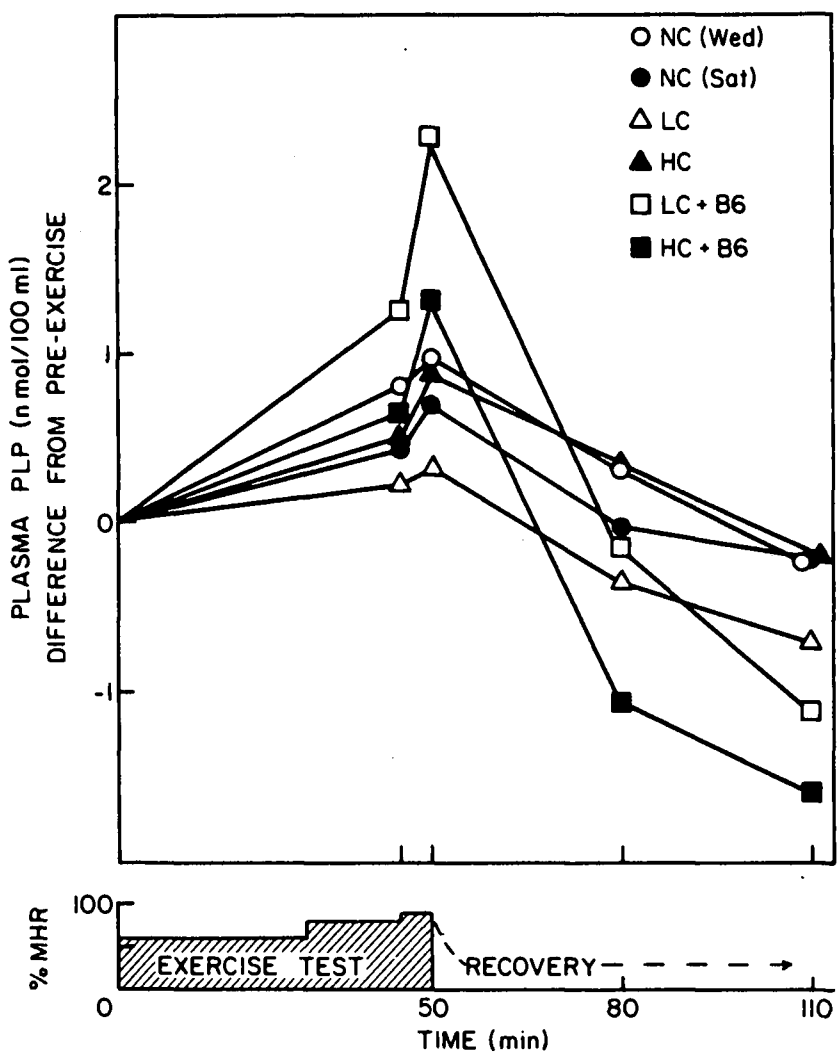

FIG. 9. Plasma pyridoxal 5'-phosphate response to all exercise tests. Differences from pre exercise values. Each point represents the mean response (n=4). Standard deviations were omitted for clarity.

different (less) than NC(Wed) and LC+B6 (with natural log conversion). Exercise LC+B6 was significantly different (greater) than NC(Wed) with natural log conversion. Although the mean difference pre to post for exercise HC was more than twice the LC pre to post difference, this failed to be significant as subject 3 had a greater increase with LC than HC in contrast to all other subjects. Interestingly the pre to post change for exercise HC+B6 was less than the pre to post for LC+B6 for all but subject 3, the subject who had a previously marginal plasma level of PLP. The pre to post difference for exercise HC+B6 failed to reach significance over NC(Sat) due to the response of subject 1 who did not show any greater response for HC+B6 than NC(Sat).

The short time period from during to post represents the response of PLP to a short period of intense exercise. Plasma PLP increased over this time period for all exercises, but as can be seen in Figure 9, the magnitude of this difference varied widely between exercise tests. The exercise sessions ordered with respect to the magnitude of the during to post increase were LC, NC(Wed), NC(Sat), HC, HC+B6, and LC+B6. The order was similar to the order seen for the total pre to post difference, with the only difference being exercise NC(Sat). However, for all exercise sessions at least one of the four subjects (usually a different person) showed no appreciable change in plasma PLP levels from during to post.

Table 11 lists the mean percent change (% change) from pre to PLP with exercise for each exercise session. Again, LC resulted in the smallest response (7.1%) of all dietary treatments. The order of the exercise sessions with respect to the magnitude of the % increase

TABLE 11
Mean percent change in plasma pyridoxal 5'-phosphate for all exercise tests

Week	Day	Diet	% Change Pyridoxal 5'-phosphate pre to: During	Post	30 min post	60 min post
					%	
1	Wed	NC	13.8[a] ±4.0	18.2[b] ±7.9	4.1[c] ±3.8	-4.1 ±7.3
1	Sat	NC	7.9[a] ±1.9	14.6 ±5.6	-3.0 ±5.4	-9.1 ±6.7
2	Wed	LC	5.5 ±6.8	7.1[b] ±7.7	-6.7[c] ±5.5	-12.6 ±7.2
2	Sat	HC	13.1 ±7.0	22.5 ±15.6	7.5 ±13.1	-7.0 ±16.6
3	Wed	LC+B6	11.0 ±6.6	15.3 ±2.6	0.2 ±7.7	-7.5 ±8.5
3	Sat	HC+B6	4.3 ±2.5	10.7 ±7.8	-5.2 ±8.8	-11.1 ±3.8

All values are mean ±1SD.

[a,b,c]Those sharing the same superscript are significantly different ($p<0.05$).

(in order of increasing magnitude) were: LC, HC+B6, NC(Sat), LC+B6, NC(Wed), HC. Thus, although LC+B6 resulted in the largest absolute change, as a % change this difference was less than NC(Wed) and HC (nonsignificant). LC pre to post % change was significantly different (less) than NC(Wed). Another significant difference appeared between the pre-during % change of NC(Sat) (7.9%) versus NC(Wed) (13.8%).

The 30 and 60 min post exercise responses in PLP represent decreases from the immediate post PLP level for all subjects at all times (see Table 10). For all subjects, with one exception, the 60 min value of PLP represents a continued decrease from the 30 min value. The plasma PLP of subject 2 for exercise HC+B6 increased from 18.53 at 30 min post to 19.42 nmol/100 ml at 60 min post (see Appendix). This was in direct contrast to the PB6 findings for LC+B6 where three of four subjects increased from 30 to 60 min post and HC+B6 where the same subject (#2) exhibited a net decrease from 30 to 60 min post. Mean pre difference values indicate that PLP remained above fasting at 30 min post for NC(Wed) and HC, while for all others (NC(Sat), LC, LC+B6, HC+B6) plasma PLP dropped below fasting. For all exercises, mean 60 min post PLP values were less than pre exercise values. These differences reached statistical significance for exercises NC(Sat), LC and HC+B6 when absolute concentration was compared for pre versus 60 min post PLP concentration. The magnitude of the pre to 60 min post differences (decrease) was greatest for HC+B6 followed by LC+B6, LC, NC(Wed), NC(Sat), and HC. In comparison with the immediate post exercise PLP concentration for exerciese NC(Wed and

Sat), LC and HC showed significant differences at 30 and 60 min post. Exercise LC+B6 was the only session that did not exhibit a significant difference (decrease) in PLP levels from post to 60 min post, because although all subjects decreased, the magnitude of the decrease varied considerably.

The % change from pre to 30 min post and 60 min post (See Table 11) revealed only one significant difference: The pre to 30 min post % change was less for LC (6.7%) than NC (4.1%). The mean values pre to 60 min post appear to indicate that LC resulted in the largest % change (decrease), but comparison of individual value (see Appendix) showed this was not a real difference.

Percent of Total Plasma Vitamin B-6 Present as Pyridoxal 5'-phosphate

Plasma levels of PLP were expressed as a percentage of the corresponding total plasma vitamin B-6 value to ascertain if other B-6 vitamers were changing with exercise independent of PLP. If the percent present as PLP remained constant with exercise and the magnitude of the changes in PLP and PB6 with exercise were of similar magnitudes, it could be concluded that changes in PB6 were due largely to changes in PLP.

Mean values of percent of total plasma vitamin B-6 present as PLP for each exercise session are presented in Table 12. There was a trend toward a decrease in the percentage of PB6 as PLP during the exercise recovery interval. This trend was significant only for exercise test LC where the percentage as PLP at 60 min post exercise was different than the percentage for pre exercise (74.9% for pre exercise and 70.5% for 60 min post).

TABLE 12
Percent of plasma total vitamin B-6 as pyridoxal 5'-phosphate for all exercise tests

Week	Day	Diet	%PLP/Total B6 Pre	During	Post	30 min post	60 min post
1	Wed	NC	82.5[a,b] ±7.5	85.0 ±9.5	85.4 ±9.4	79.4 ±13.5	76.2 ±9.6
1	Sat	NC	80.9[c,d] ±6.0	81.0 ±10.1	75.1 ±9.6	72.6 ±12.2	72.4 ±11.8
2	Wed	LC	74.9[a,c,e] ±9.0	75.1 ±6.1	73.2 ±8.4	73.7 ±10.2	70.5[e] ±8.1
2	Sat	HC	68.9[b,d] ±6.7	67.9 ±7.6	74.1 ±11.7	69.4 ±8.4	66.6 ±8.8
3	Wed	LC+B6	77.7 ±13.7	75.0 ±12.4	78.5 ±14.4	76.8 ±11.3	69.7 ±8.3
3	Sat	HC+B6	77.1 ±9.0	72.5 ±12.2	73.8 ±9.7	72.1 ±7.7	72.4 ±10.4

All values are mean ± 1SD.

[a-e] Those sharing the same superscript are significantly different ($p<0.05$).

The percentage of PB6 as PLP decreased from week 1 to week 2, with plasma % PLP for both LC and HC significantly less than either NC(Wed) or NC(Sat). The percentage of PB6 present as PLP pre exercise HC appears less than LC, but this was not significant.

The difference from pre exercise for PB6 was correlated with the corresponding difference from pre exercise for PLP by individual exercise test. The results of these correlations are presented in Table 13. For all exercise sessions the differences from pre for PB6 were significantly correlated ($p<0.05$) with the difference from pre for PLP.

Correlations of Percent Change in Plasma Volume to Percent Change in Plasma Vitamin B-6 and Pyridoxal 5'-phosphate

When plasma volume changes with exercise, the concentration of plasma constituents that may not leave the vascular compartment may change. It has been suggested that plasma volume changes with exercise could be responsible for all changes in plasma B-6 vitamers with exercise. Some of the change with exercise may well be due to hemoconcentration and hemodilution, but is all the change due to plasma volume change? The percent changes of these parameters (PV, PLP, PB6) were correlated to evaluate whether a linear relationship existed between the magnitude of changes. It can be seen by simply comparing the % change data that the concentrations of both PV and B-6 vitamers increase with exercise (a negative change for PV and a positive change for B-6 vitamers) and decrease during recovery, but it is more important to know if the magnitudes of the % change over a given time period are related. To do this the percent changes for all exercise tests over a given

TABLE 13
Correlation coefficients of the difference from pre exercise for PLP with the PB6 differences from pre exercise by exercise test.

Exercise Test	r
NC(Wed)	0.661*
NC(Sat)	0.569*
LC	0.831*
HC	0.554*
LC+B6	0.669*
HC+B6	0.819*

* Significant at $p<0.05$, df=14.

time the % change of the same time period for PV. These correlations are presented in Table 14.

For PB6 the percent changes from pre to during and pre to 60 min post were significantly correlated with the PV changes, with the highest correlation coefficient of 0.560. No other significant correlations were found for PB6 or PLP with PV. It should be noted that the lowest correlations obtained were for the % change from pre to post of both PB6 and PLP with PV change.

Plasma Creatine Phosphokinase (CK)

Plasma CK was measured to evaluate the effect of the exercise test employed in this study on muscle tissue permeability. Mean values of plasma CK with and following exercise are listed in Table 15. All fasting (pre exercise) values were within the range of normal reported by Sigma Biochemical Co. with two exceptions, subject 1 pre exercise LC and subject 3 for pre exercise LC+B6. Subjects were allowed to follow their normal exercise regimens Sunday through Tuesday to encourage glycogen depletion, therefore, the plasma CK values pre-exercise on Wednesdays (NC(Wed), LC, and LC+B6) could reflect the exercise performed on the previous day. Since exercise was not allowed Wednesday through Friday, pre exercise plasma CK levels should not reflect the effect of exercise. With the exception of subject 4, all subjects exhibited greater pre exercise plasma CK levels on Wed than on the Sat of the same week. This was reflected in mean pre exercise CK values with the exception of week 1 where the high value of subject 3 on Sat increased this mean value. In many cases, plasma CK on Wed was

TABLE 14
Correlation coefficients of percent change in plasma volume correlated with percent change in plasma vitamin B-6 and pyridoxal 5'-phosphate by time interval

Percent Change Time Interval	Correlation Coefficients of % Change Plasma Volume to:	
	PB6	PLP
	r	
pre to during	-0.453*	-0.223
pre to post	-0.073	-0.183
pre to 30 min post	-0.260	-0.293
pre to 60 min post	-0.560*	-0.373

* Significant at $p<0.05$, df=22.

TABLE 15
Mean plasma creatine kinase values for all exercise tests

Exercise			Creatine Kinase					
Week	Day	Diet	pre	during	post	30 min post	60 min post	24 hr post
					U/L*			
1	Wed	NC	71 ±16	77 ±23	89[a] ±27	76 ±19	67[b] ±19	-[†]
	Sat	NC	68 ±24	75 ±27	82[a] ±27	78[a] ±30	75 ±28	-[†]
2	Wed	LC	120 ±67	125 ±66	127 ±74	119 ±62	119 ±65	87 ±26
	Sat	HC	67 ±8	78[a] ±11	84[a] ±5	79[a,b] ±6	69[b] ±9	57[b] ±11
3	Wed	LC+B6	115 ±95	135 ±115	137 ±115	126 ±106	127 ±109	87 ±54
	Sat	HC+B6	59 ±18	67[a] ±21	66 ±22	60 ±16	57 ±16	81[‡] ±60

All values are reported mean ± 1SD, n = 4

* Units/liter plasma

† CK was not measured 24 hours post exercise for either NC(Wed) or NC(Sat)

‡ Excluding subject 3 (who increased 139% between 60 min post and 24 hr post) this value would be 52 ± 19.

[a] significantly different than pre value of same day

[b] significantly different than post value of same day

more than twice that of the Sat value.

The overall effect of exercise was to increase plasma CK at both the during and post time points relative to pre exercise. Plasma CK dropped following exercise at both the 30 and 60 min time points. Post exercise results will be considered separately. Percent change for each value from pre exercise CK was calculated and is presented in Table 16. Mean percent change in plasma CK as compared to pre exercise increases from pre to during ranged from 6.0% for LC (+5.1 U/L) to 17.4% for HC(+1.6 U/L). Mean plasma CK was significantly different (increased) from pre to post NC(Wed), NC(Sat) and HC. Mean percent increases from pre to post ranged from 4.4% for LC (+7.1 U/L) to 26.2% for HC (+16.9 U/L). Mean plasma CK increased consistently from during to post for all exercises, but many individuals actually exhibited small decreases in this short time period. For exercise HC+B6, three of the four subjects exhibited small decreases from during to post, while for exercises LC, NC(Wed), HC and LC+B6 at least one subject exhibited a net decrease from during to post. The exercise session NC(Sat) was the only session when all subjects increased from during to post.

At 30 min post exercise mean plasma CK decreased with respect to the post exercise value, but remained above or approximately equal to the pre exercise value for all exercise sessions. The 30 min post CK value was significantly different than pre only for NC(Sat) and HC. Mean percent change from pre to 30 min post ranged from 0.2% for LC to 14 % for NC(Sat). In comparison to post exercise plasma CK, 30 min post was significantly different only for HC.

TABLE 16
Mean percent change in plasma creatine kinase for all exercise tests

Exercise			Percent Change in Creatine Kinase, pre to:				
Week	Day	Diet	during	post	30 min post	60 min post	24 hr post
					%Δ		
1	Wed	NC	7.5 ±15.8	25.2 ±10.5	7.6 ±13.8	-5.7 ±13.0	-*
	Sat	NC	8.6 ±6.1	17.5 ±8.0	14.0 ±4.6	9.2 ±7.2	-*
2	Wed	LC	6.0 ±7.3	4.4 ±3.9	1.2 ±4.8	0.2 ±3.2	-21.6 ±19.4
	Sat	HC	17.4 ±4.2	26.2 ±8.7	16.2 ±12.3	1.6 ±12.9	-14.0 ±11.4
3	Wed	LC+B6	16.8 ±8.8	22.2 ±15.0	11.9 ±12.4	12.8 ±13.7	-7.6 ±34.9
	Sat	HC+B6	12.1 ±4.1	11.2 ±9.7	2.8 ±7.6	-2.6 ±3.4	-7.6† ±6.3

All values reported are mean ± 1SD.

* 24 hour post samples were not drawn during week 1

† Subject 3 value was excluded for this mean as he exercised nearly 2 hours resulting in a 139% increase between the 60 min post and 24 hr samples.

Mean plasma CK continued to decrease at 60 min post exercise except for exercises LC and LC+B6 when mean plasma CK did not change from the 30 min post value. No exercise session displayed a significant difference from pre to 60 min post. Plasma CK at 60 min post was significantly different (decreased) for exercises NC(Wed) and HC as compared to post exercise.

Plasma CK was measured at 24 hours post exercise beginning with exercise LC (Wed week 2). This was begun when it was concluded from preliminary data that the exercise test was of insufficient intensity and/or duration for the trained subject group to cause elevation of CK within 1 hr post exercise. In contrast to expected elevations at 24 hr post exercise, mean values indicated that plasma CK decreased with respect to pre exercise and 60 min post exercise. There were some individual exceptions, plasma CK of subject 4 increased between 60 min and 24 hr post for exercises LC (12.6%) and LC+B6 (11.2%). Another exception was the 139% increase (+97.6 U/L) of subject 3 between 60 min and 24 hr post exercise for session HC+B6, the largest single increase seen. This subject reported playing 2 hours of basketball between these blood samples.

The percent change in CK was correlated to the percent change in PB6 and PLP for the pre to during and pre to post time intervals for all exercise sessions. This calculation was made to detect any relationship in the magnitude of these changes. Percent change in CK was not significantly correlated with PB6 for either the pre to during ($r=0.310$) or the pre to post ($r=0.106$) time periods. Percent change in PLP was significantly correlated with

CK for the pre to during ($r=0.493$, $p<0.05$) time period but not for the pre to post interval ($r=0.330$).

Urine Analysis

Urinary Creatinine

Urinary creatinine was measured to assess completeness of the 24 hour urine collections. Individual values of daily creatinine excretion are given in the Appendix. Table 17 lists weekly means and three week means by individual. Subject 4 had the highest coefficient of variation for all weeks combined, due to the high standard deviation for week 1.

Urinary 4-pyridoxic acid (4PA)

Urinary 4PA was measured to test the effect of exercise on excretion of 4PA when vitamin B-6 intake was controlled. Excretion of 4PA was followed throughout the study to further test any modification of the effect of exercise by the diet treatments employed in this study. The excretion expressed as a percentage of dietary vitamin B-6 intake was tested statistically for the effects of diet and exercise rather than the absolute excretion level as dietary vitamin B-6 varied approximately 17% among the unsupplemented diets. Excretion in absolute amounts (μmol/24 hr) was considered to evalute vitamin B-6 status.

Group mean and individual daily urinary 4PA results are presented in Table 18. Shultz and Leklem (62) have suggested the

TABLE 17
Subject week means and three week mean of daily creatinine excretion

Week	Creatinine Excretion Subject 1	2	3	4
1	1.94 ±0.13	1.81 ±0.11	2.21 ±0.14	1.77 ±0.33
2	1.87 ±0.12	1.86 ±0.10	2.30 ±0.11	1.76 ±0.14
3	1.92 ±0.16	1.93 ±0.17	2.29 ±0.13	1.88 ±0.15
All weeks	1.91 ±0.13	1.87 ±0.13	2.27 ±0.13	1.81 ±0.22
Coefficient of variation (n=18)	6.8%	7.0%	5.7%	12.2%

All values reported are means ± 1SD

TABLE 18
Mean and individual daily urinary 4-pyridoxic acid excretion for all study weeks

		Urinary 4-pyridoxic acid					
Week	Subject	Mon	Tue	Wed	Thu	Fri	Sat
		µmol/24 hr					
1	1	5.79	5.55	6.44	5.74	4.79*	6.45
	2	6.02	5.86	6.54	5.05	4.73*	6.19
	3	7.38	5.91	7.37	5.91	6.11	6.14
	4	5.90	6.34	7.41	5.94	4.75*	7.92
	Mean	6.27	5.92	6.94	5.66	5.14	6.68
	±1SD	±0.74	±0.33	±0.51	±0.42	±0.66	±0.84
2	1	5.15	4.27*	5.52	5.08	4.51*	7.10
	2	5.90	4.65*	5.73	5.33	5.01	6.46
	3	6.67	5.32	5.92	5.71	5.91	7.05
	4	4.19*	4.95*	5.82	6.61	4.87*	5.42
	Mean	5.48	4.80*	5.75	5.68	5.08	6.51
	1SD	±1.06	±0.45	±0.17	±0.67	±0.60	±0.78
3	1	30.93	30.27	39.78	35.40	35.15	43.76
	2	28.30	29.14	37.91	34.76	33.66	41.64
	3	36.68	32.42	48.96	36.53	38.66	40.49
	4	31.80	28.51	36.54	34.79	36.21	36.19
	Mean	31.93	30.09	40.80	35.37	36.00	40.52
	1SD	±3.50	±1.72	±5.60	±0.83	±2.00	±3.19

* Values below the level (5.0 µmoles/24 hr) suggested as indicating marginal dietary vitamin B-6 intake (62).

range of 5.0 to 5.7 μmol/24 hr as excretory levels of 4PA associated with marginal vitamin B-6 intake. During weeks 1 and 2 (non-supplemental weeks), several subjects excreted quantities of 4PA below the level suggested by Shultz and Leklem as marginal. Although the diets were calculated to contain 2.0 mg of pyridoxine the U.S. RDA (73), diet composite analysis revealed actual diet levels at 80% of the calculated content. In view of the high protein intake associated with the diets (see Table 6) the dietary vitamin B-6 was indeed less than adequate (0.02 mg/g protein). Interestingly the urinary excretion of 4PA during weeks 1 and 2 increased above marginal levels on exercise test days even though dietary intake did not change. The LC diet, which provided the least vitamin B-6 (1.55 mg PN) and the most protein (194 g), produced the lowest levels of urinary 4PA (4.80 μmol/24 hr). The diet that produced the lowest plasma PLP was not LC, but HC which contained the greatest amount of vitamin B-6 (1.82 mg PN) of the unsupplemented diets and the least protein (145 g) of the diets. The 4PA excretion with the HC diet was only slightly greater after three days on the diet (5.08 μmol/24 hr) than after three days on the diet (4.80 μmol/24 hr). The lowest excretion seen while consuming the NC diet (week 1) was on Friday when excretion was 5.14 μmol 4PA/24 hr, just slightly greater than with the HC diet which provided more PN (1.64 mg PN for NC versus 1.82 mg PN for HC) With supplementation mean urinary 4PA ranged from 30.09 to 40.80 μmol/24 hr, levels that were from six to eight times greater than with the corresponding unsupplemented diets (4.80 to 6.51 μmol/24 hr).

Mean 4PA excreted as a percent of intake (% 4PA) is presented

in Table 19 and Figure 10. Mean % 4PA increased on all days of exercise tests except for exercise test LC as compared to non-test days. Excretion on non-exercise test days ranged from 47.1 to 64.9% versus 60.4 to 71.7% for exercise test days. (It should be remembered that non-exercise test days Mon and Tues were not necessarily non-exercise days, as normal exercise activities were allowed Sun, Mon and Tues.) As compared to the day prior to the exercise test, % 4PA excretion was significantly different (increased) on the following exercise test days: NC(Wed), HC, and LC+B6. For exercises NC(Sat) and HC+B6, only three or four subjects exhibited increased % 4PA excretion greater than the previous day.

Mean % 4PA excretion tended to be greater on Mon and Tue than Thu and Fri for both weeks 1 and 2. In support of this trend, % 4PA excretion on Mon (64.9%) during week 1 was found to be statistically different (greater) than Fri (53.2%), and during week 2, % 4PA excretion was statistically different (greater) Tue (52.3%) than Fri (47.1%). In contrast, during week 3 (supplemented) excretion was less Mon and Tue than Thu and Fri. This was statistically significant for Mon versus both Thu and Fri.

Between week comparisons of % 4PA excretion were made to assess the effect of diet on the exercise test day excretion. Excretion of 4PA as a percent of intake was found to be significantly different (less) on Wed during week 2 (LC) (53.4%) than Wed (NC) during week 1 (71.7%) or week 2 (LC+B6) (70.3%). Percent 4PA excretion on Sat week 2 (HC) was significantly different (less) than Sat week 3 (HC+B6).

TABLE 19
Mean daily 4PA excretion as a percentage of dietary vitamin B-6 intake.

	Urinary 4-pyridoxic acid					
Week	Mon*	Tue*	Wed†‡	Thu†	Fri†	Sat†‡
	% excreted/intake					
1	64.9[a,b] ±7.7	61.2[c] ±3.4	71.7[c,d,e,m] ±5.5	58.5[d] ±4.3	53.2[a,e] ±6.8	69.0[b] ±8.7
2	59.8 ±11.6	52.3[f] ±4.9	53.4[m,n] ±1.6	52.8 ±6.2	47.1[f,g] ±5.5	60.4[g,o] ±7.3
3	56.6[h] ±6.2	53.3[i,j,k,l] ±3.0	70.3[h,i,n] ±9.6	61.0[j,q] ±1.4	61.9[k] ±3.3	69.7[l,o,q] ±5.5

All values are reported as mean ±1SD

* Subjects were allowed to follow normal exercise regimens on these days

† Exercise was restricted on these days.

‡ Exercise test administered during early a.m. on these days.

a-q Those sharing the same superscript are significantly different ($p<0.05$).

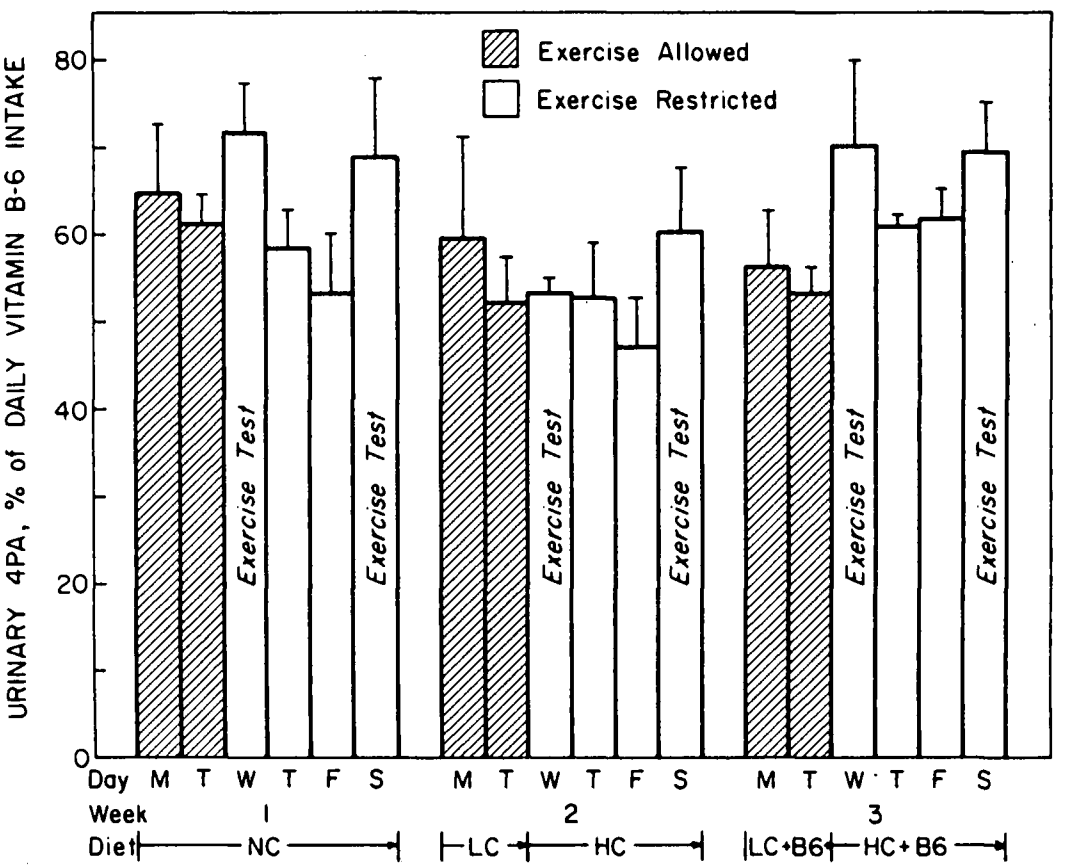

FIG. 10. Daily urinary 4-pyridoxic acid excretion: As a percent of daily vitamin B-6 intake for all study weeks. Each bar represents the mean response (n=4). Vertical lines at the top of each bar are ± 1SD. Day abbreviations M through S mean Monday through Saturday.

V. DISCUSSION

This study demonstrated several new findings relative to the effects of exercise and diet on vitamin B-6 metabolism. Plasma PLP and PB6 were observed to increase during and immediately following exercise under strictly controlled dietary conditions. It was observed for the first time that during the 60 minutes following exercise, plasma levels of PB6 and PLP decreased, in several cases significantly below the pre exercise fasting value. Dietary carbohydrate manipulation and vitamin B-6 supplements were found to modify the effect of exercise on plasma B-6 vitamers. The low carbohydrate diet, combined with daily exercise to deplete muscle glycogen, was found to decrease the response of PB6 and PLP levels (pre to post increase) to the exercise test as compared to the response following the NC and HC diets, although this was not significant. Supplementation resulted in much larger pre to post increases in PB6 and PLP. An important finding was that urinary 4PA excretion was elevated on the day of exercise tests, with the exception of exercise LC. An unexpected finding, unrelated to the effect of exercise, was that the HC diet resulted in significantly lower fasting (pre-exercise) levels of PB6 and PLP as compared to the diet low in carbohydrates (LC) which actually contained slightly less vitamin B-6.

The effects of diet on plasma B-6 vitamers seen in the present

study are considered separately, since diet may change blood vitamer levels independent of exercise. The three unsupplemented diets utilized in the present study were planned to be equal in vitamin B-6 content and protein content. However, laboratory analyses of composites of each diet revealed that the actual vitamin B-6 content was less than that calculated using Home Economics Research Report No. 36 (19). The differences between calculated and assayed values may have been due to cooking losses, particularly in those foods for which only uncooked vitamin B-6 content were available. The protein content of the LC diet was also greater than the other diets, as more protein foods were provided to increase the acceptability of this very high fat diet. All diets were originally planned to contain 18% of total kcal as protein (158 g), a level actually greater than average U.S. consumption of 107g for this age group (75). The increased protein for LC and the decreased vitamin B-6 for all unsupplemented diets, makes the adequacy of the dietary vitamin B-6 questionable. Although the Canadian Bureau of Nutritional Sciences (74) recommends intakes of 0.02 mg vitamin B-6 per gram of dietary protein, research studies have shown ratios as low as 0.011 mg per g protein were adequate for adult men as assessed by a 2 g tryptophan load test (69). In view of the latter research finding, the diets HC (0.013 mg per g protein) and NC (0.010 mg per g protein) were marginally adequate while the LC diet was inadequate at 0.008 mg per g protein.

The fasting plasma values were above the level designated as marginal by Shultz and Leklem (62), with the exception of subject

3 prior to exercise HC. Overall, the lowest mean plasma value occurred with the HC diet, which actually provided the highest quantity of vitamin B-6 per gram of protein of the unsupplemented diets. Despite the inadequate ratio of vitamin B-6 to protein for diet LC, the LC plasma values for PLP and PB6 averaged nearly 2.0 nmol/100 ml greater than the fasting value for the HC diet. The plasma values for LC were not significantly different from those seen with the NC diet. These results are in contrast to the research of Miller and Leklem (67) who found that high protein diets (2 g protein/kg body weight) led to decreased plasma PLP and PB6 compared to medium (1.0 g protein/kg body weight) and low (0.5 g protein/kg body weight) protein diets at the same vitamin B-6 intake. In their study less vitamin B-6 was metabolized and excreted as 4PA on the high protein diet. These results suggest that the lower plasma level was due to a change in tissue distribution through modified uptake or release rather than loss of the vitamin from the body through conversion to 4PA. Indeed, Itoh and Okada (49) have shown more PLP was found in the liver of rats fed a high protein diet. While the lowest protein intake provided in the present study was 1.8 g protein/kg body weight with the HC diet; the LC diet provided 2.4 g protein/kg body weight. The difference in protein contents was large enough that some difference should have been expected. The carbohydrate content of the protein-varied diets fed by Miller and Leklem (67) were not as extreme as those accompanying the LC and HC diets of the present investigation. It may be that dietary carbohydrate also has some effect on the tissue distribution of B-6 vitamers, possibly through an effect on

uptake or release of PLP. An acute glucose load has been found to decrease plasma PLP levels an average of 17.6% within 2 hours after the glucose load (156). This decrease in plasma PLP suggests carbohydrate also might change tissue vitamin B-6 content through modified uptake or release. It is difficult to compare this situation to the present study in which the plasma levels were measured after a 12 hour fast. When the LC and the HC diets were supplemented at five times the RDA (73), the difference in plasma levels disappeared. Thus the difference between plasma levels on the unsupplemented diets was also related to the relatively low vitamin B-6 levels of the diets.

The controlled exercise test administered in this study produced increases in PB6 and PLP over fasting levels. The increases were approximately the same magnitude as observed by Munoz (3) in trained college-aged men, who were administered a progressive 21 minute bicycle ergometer exercise test. The hypothesis from which the objectives of this investigation were drawn was that the increase in B-6 vitamers in the plasma was the result of release of PLP from muscle glycogen phosphorylase, as was also suggested by Munoz (3). The plasma level of B-6 vitamers measured at a single point in time represents the dynamic state of tissue uptake, release, and metabolism of the vitamers. A change in the plasma B-6 vitamer levels, in the absence of ingested vitamin B-6, could be explained by a modification of metabolism, tissue uptake, and/or release, leading to tissue redistribution of B-6 vitamers. The simplest explanation of the changes in B-6 vitamers would be that these changes are simply

reflections of plasma volume changes with exercise.

Evaluation of plasma volume change as calculated from hematocrit values indicated that part of the change in plasma vitamin B-6 and PLP might be due to plasma volume change. With the low correlation of the magnitude of PB6 and PLP with the magnitude of plasma volume changes over any of the time periods, it is concluded that the change in these vitamers represents real changes in the quantity of vitamin B-6 in the blood beyond the plasma volume change. However, the research literature on hemoconcentration indicates that changes in the plasma with exercise are much more complex than a simple loss of isotonic water leading to a concentration of other plasma constituents. Studies on plasma albumin and total protein suggest that changes in plasma proteins are not necessarily equal to plasma volume changes calculated by other methods, such as the method used in this investigation (155). Both net loss (155, 157) and net gain (158) of plasma protein and albumin have been demonstrated. It has been postulated that the loss of protein might occur through "leaky" capillaries, and gain of protein might occur by increased return of albumin-rich lymph into the vascular space. Pyridoxal 5'-phosphate exists in the plasma almost exclusively bound to albumin, thus changes in albumin might be expected to cause changes in plasma PLP levels. The modality, intensity, and duration of exercise appear to determine whether the net result will be a loss or gain of albumin with exercise. In a study comparing progressive exercise on the treadmill to similar exercise on a bicycle ergometer, there was a net loss of albumin during ergometer

work but not treadmill work (157). Dill and Costill (155) also found a net loss (6%) of total protein when subjects ran on a treadmill for two hours. If a net loss of plasma albumin occurred with exercise in this study, the increase in B-6 vitamers levels with exercise would be more, rather than less, significant. Conversely, another study of subjects exercising on an ergometer, at 55% $\dot{V}O_2$max for 50 minutes in a warm environment, exhibited net increases in total plasma proteins, which remained throughout 50 minutes of recovery (158). As albumin was not measured in this investigation, a conclusion cannot be drawn as to the relative contribution that changes in albumin may have made to the changes observed in plasma B-6 vitamers. Measurements of albumin in future studies would alleviate this uncertainty.

Discounting the premise that plasma volume change was responsible for changes in B-6 vitamers with exercise, it seems likely that the increase in PB6 and PLP with exercise represents an increased tissue release of the vitamers. Alternatively, the increase in plasma levels could be caused by decreased degradation or tissue uptake. If it is assumed the increase was the result of increased release from some tissue, at least two possible sources other than the muscle need to be considered. These tissues are the liver and the red cell.

The red cell could be a possible source of increased PLP with exercise since it has been shown to accumulate PLP when excess vitamin B-6 was fed (65). Studies on red cell have shown these cells to be incapable of releasing PLP, instead this vitamer must be dephosphorylated to PL prior to transport (45). This does not

discount a possible role of the red cell in the change of vitamer levels with exercise, since hemolysis and/or increased cellular permeability may occur with exercise. In addition the red cell could be releasing PL which might be phosphorylated elsewhere. At present, adequate methodology is not available for assaying PLP levels of whole blood.

Lumeng and coworkers (43) have concluded from their studies of organ ablation in dogs that the liver was the sole source of plasma PLP. As the data presented here indicates that PLP was quantitatively the major B-6 vitamer changing with exercise, the liver must seriously be considered as a possible source of the plasma PLP change with exercise. As Munoz has suggested, there appears to be no metabolic advantage for the liver to release PLP into the blood during exercise (3). A major function of the liver is maintainance of blood glucose through the processes of glycogenolysis and gluconeogenesis, both of which require PLP as a cofactor. Thus it would seem unlikely that the liver would release PLP, as PLP would be in demand as a coenyzme for active gluconeogenesis following exercise (86). In contrast to the muscle, it was shown by Li, Lumeng and Veitch (39) that when vitamin B-6 was fed in excess to rats, the liver content of PLP remained remarkably constant. Thus it is concluded that the liver does not appear to retain a large pool of PLP that would be readily available for release during exercise.

If muscle glycogen phosphorylase in man also functions to store PLP as it does in the rat, muscle could be considered a likely source of the plasma changes in B-6 vitamers with exercise.

Black and co-workers (5) demonstrated that depletion of muscle glycogen phosphorylase occurred quickly in the rat after one to four days of starvation. These investigators hypothesized that the depletion of phosphorylase was intimately related to glucose homeostasis, due to the key roles of PLP in phosphorylase and aminotransferases. The release of PLP would provide coenzyme for increased levels of amino acid catabolizing enzymes necessary to maintain glucose homeostasis during starvation. Prolonged exercise is similar to starvation as it disturbs glucose homeostasis through depleting local muscle glycogen stores and liver glycogen (82, 85). A study by Lemon and Mullin (86) has suggested that exercise following glycogen depletion may be even more similar to short-term starvation. They were able to demonstrate increased sweat and blood urea nitrogen levels indicating significant amino acid catabolism. In a relative sense, exercise in the glycogen depleted state could be considered more similar to starvation than exercise following glycogen loading. A relationship between the glycogen level and the release of B-6 vitamers into the plasma with exercise would support the possibility that the increase in B-6 vitamers originates from muscle glycogen phosphorylase. Since muscle glycogen levels were not directly measured, it has been assumed that the exercise-diet regimen administered in the present study brought about the intended glycogen modifications.

The data presented in this investigation suggest that PLP was quantitatively the major B-6 vitamer changing with exercise. Since PLP has been thought to be unable to cross muscle cell membranes without dephosphorylation, how is the PLP transported across the

muscle fiber membrane, if indeed it originates from muscle phosphorylase? One possibility would be that the changes that occur in muscle tissue permeability with exercise may allow PLP to cross the membrane without dephosphorylation. Increases in plasma creatine kinase, one muscle enzyme released with exercise, may signal the change in tissue permeability. Analysis of plasma CK identified only small increases immediately post exercise. No further increases in plasma CK occurred at 60 min post or 24 hours post. A further discussion of CK appears after the discussion of vitamin B-6 metabolism, but it can be concluded here that changes in plasma PLP and PB6 were not significantly correlated to changes in CK and therefore large CK changes (indicating large changes in tissue permeability) do not appear to necessarily accompany changes in PLP.

The first objective of this study was to test the effect of a change in glycogen stores on the metabolism of vitamin B-6 during exercise. To compare the responses of PB6 and PLP between the six exercise sessions, calculations of percent change and concentration differences from pre exercise were made over each time period. The comparison of concentration changes between the various exercise sessions yielded many more statistically significant findings than when the percent changes were compared. It is proposed that the concentration change may be more physiologically significant than the percent change for the following reason. Although Lumeng et al. (50) have shown that plasma PLP was significantly correlated with muscle tissue PLP content in the rat, Black and coworkers (5) have shown that muscle PLP (phosphorylase) does not decrease with

dietary vitamin B-6 insufficiency. Plasma PLP, conversely, has been shown to be very responsive to dietary changes (50). In some situations plasma PLP may not be directly related to muscle content of PLP, as plasma PLP may be more indicative of recent dietary intake, while muscle PLP might indicate more long term dietary vitamin B-6 intake. For example, in the situation where recent dietary intake had been deficient, plasma levels would be low even though muscle storage levels of PLP would be high. Thus if PLP in man is coming from muscle storage with exercise, the magnitude of the change would be related more to the concentration of the PLP in muscle than the plasma PLP concentration. The change in plasma B-6 vitamers expressed as a percent of plasma PLP would then be misleading.

Comparisons of the pre to post PLP and PB6 responses for NC(Wed) and NC(Sat) indicated that no significant difference exists between these exercise test responses, even though the mean values appeared different. Only a slight difference might be expected between these exercises, with regard to the metabolic energy substrates for exercise. As exercise was encouraged Sunday through Tuesday of each week, glycogen may have been slightly lower NC(Wed) than NC(Sat), as muscle glycogen may take 24 hours to return to normal levels after glycogen depleting exercise (113). Data from the analyses of the same blood samples for fuel metabolites during exercise suggest that no difference existed between these two exercise days with respect to fuel metabolism (159). Therefore it may be that these difference could represent individual variation in response to the standardized exercise test.

Comparisons of the responses of LC and HC to each other and to the NC exercise responses of PB6 and PLP revealed that the LC exercise resulted in different responses. Pre to post exercise increases in PB6 and PLP for LC, both as concentration differences and percent changes, were less than HC, NC(Wed) and NC(Sat). The mean exercise HC pre to post response did not appear different from NC(Sat), but was greater than the response of LC. Although these differences were not significantly different by paired t-test, they were supported by strong trends in the individual data. (Note that the percent change in plasma volume for exercise LC was not less than HC, NC(Wed), or NC(Sat).) The small pre to post response in PLP for exercise LC, as compared to NC and HC responses, was more distinctly different than the response of PB6 (pre to post) levels for LC as compared to HC and NC responses. During the short period of intense exercise between the during and post samples, a rapid utilization of glycogen could be expected. Over this time period, the change in PLP was least for exercise LC. For plasma vitamin B-6, exercise LC did not display the least change over this time period, but rather exercise HC exhibited the least change, since the PB6 level of subject 2 actually decreased. Thus it appears that in the state of depleted muscle glycogen stores (LC), the response of PLP to the controlled exercise test and to the short period of intense exercise was diminished. The metabolic situation with exercise after glycogen depletion most closely parallels the situation with short-term starvation (96). Therefore, the greatest glycogen phosphorylase depletion (5) (resulting in the greatest PLP release) was expected to occur, if indeed the PLP was coming

from muscle phosphorylase. Since the LC diet had been fed for three days (in combination with depleting exercise) prior to the exercise test, significant glycogen depletion and the lack of dietary CHO would have led to increased amino acid catabolism and gluconeogenesis. These events would have occurred prior to the time of the test on Wednesday. Therefore the reason for the diminished pre to post increase with LC may be that the transfer of PLP from muscle to the liver had already occurred prior to exercise in response to the increased gluconeogenesis processes brought on by the LC diet. Alternatively, the lower pre to post difference could be explained by a more rapid uptake of the PLP, since less would be measured in plasma if it were taken up by a tissue.

During the exercise recovery period (30 and 60 min post) plasma PLP and vitamin B-6 levels progressively decreased, in many cases dropping below the pre exercise level. Munoz (3) also observed decreases in plasma B-6 vitamers post exercise, but she did not measure B-6 vitamer levels later than 30 min post exercise. In only one exercise session (exercise LC+B6) and then only for PB6, did levels begin to increase during the recovery period. For all exercise sessions plasma PLP values continued to decrease at 60 minutes post exercise. These changes suggest that following the rise in plasma B-6 vitamer levels with exercise some tissue, such as the liver, was extracting B-6 vitamers from the blood. Since both PLP and PB6 values reflected this decrease during recovery (with the exception of LC+B6), the decrease in PLP was not merely a conversion of PLP to another vitameric form. A small non-significant decrease in the percent of PB6 present as PLP suggested

a small amount of conversion of PLP to other forms might have occurred late in the recovery period. Although exercise LC exhibited the smallest increase, the recovery period decrease in B-6 vitamers was not diminished. Indeed, during exercise recovery, plasma PLP and PB6 levels for exercise LC exhibited the largest decreases from pre exercise seen among the unsupplemented exercise sessions. This would support the proposal that the smaller pre to post difference with exercise LC was due to a more rapid uptake of the vitamers from the plasma.

The second objective of this investigation was to test the effect of vitamin B-6 supplements on the metabolism of vitamin B-6 with exercise. Supplements were given to increase the total vitamin B-6 intake to five times the US-RDA (73). By Wednesday of the supplemented week, the plasma levels of total vitamin B-6 and PLP were dramatically increased, but it is not known whether three days would be sufficient to increase the muscle tissue PLP-phosphorylase stores, (if man indeed stores PLP in this manner). Relatively higher muscle storage levels would be expected by Saturday (the 7th day of supplementation), if the muscle phosphorylase of man responds similarly to that of the rat (5).

The PN supplementation of the LC and HC diets increased the pre to post exercise concentration differences of plasma B-6 vitamer levels. Percent pre to post increases with exercises LC+B6 and HC+B6 were not significantly different as compared to other exercise sessions. The pre to post concentration differences were statistically significant for both PB6 and PLP of exercise LC+B6 versus LC, but not for exercise HC+B6 versus HC. The pre to

post differences in PB6 for HC+B6 were consistently greater (nearly two times) than HC for all subjects, although no such trend was apparent for the PLP values of these exercises. The mean increase in PB6 values with exercise was identical for exercises LC+B6 and HC+B6. While the mean pre to post increase in plasma PLP was slightly greater for LC+B6 than HC+B6, there were no consistent changes in the individual data that would substantiate this trend. Thus supplementation of the LC and HC diets appeared to eliminate the difference seen during week 2 between the exercise responses following the unsupplemented diets. This would suggest that the greater need for vitamin B-6 imposed by the LC diet and exercise, while vitamin B-6 intake was low, was the cause of the smaller increase with exercise LC. It also appears from this data that when vitamin B-6 was given in excess more was released, suggesting that supplementation may have increased storage.

During recovery the plasma PLP levels of the supplemented diets (LC+B6 and HC+B6) exhibited the greatest decreases in concentration below the pre exercise value of all exercise sessions. Although the supplementation lead to much higher pre exercise PLP values and greater increases with exercise, it did not prevent the plasma PLP levels from dropping far below the fasting levels during the post exercise recovery period.

The plasma vitamin B-6 recovery response was similar to PLP for HC+B6, but not for LC+B6. At 60 min post, 3 of the 4 subject's (subject 3 was the exception) plasma level of vitamin B-6 exhibited a sudden increase relative to pre exercise and 30 min post samples. This did not occur in PLP levels at the same time, thus release of

some vitamer form other than PLP conversion of PLP to PL must account for this change.

The third objective of this study was to assess the effect of exercise on 4PA excretion when vitamin B-6 intake was controlled. The exercise sessions, with the exception of LC, were associated with increased daily excretion of 4PA (as a percent of intake) in comparison to the day prior to exercise. The recovery period disappearance of B-6 vitamers that had been released into the plasma with exercise may represent conversion of these active vitamers to 4PA in the liver with subsequent increased excretion. Conversely, since the decrease in plasma vitamers after exercise LC were not accompanied by an increase in excretion of 4PA, this suggests that the vitamin B-6 was taken into a tissue and retained due to the greater need for vitamin B-6 in this circumstance. In the cases of exercises NC, HC, and HC+B6, the need for vitamin B-6 would not be as great since adequate glycogen stores for exercise would preclude a great need for amino acid catabolism. In the situation of LC+B6, the supplemental vitamin B-6 would have more than met the increased demand for coenzyme for increased amino acid catabolism, therefore excess could be metabolized to 4PA and excreted. Interestingly, the excretion of 4PA was decreased on the Thursday and Friday following the Wednesday exercises of weeks 1 and 2, as compared to Wednesday percent excretion and the Monday and Tuesday (daily exercise was allowed) excretions. Thus the non-exercise days appear to result in less excretion of 4PA. This also supports the finding that exercise led to increased 4PA excretion. Such an increased loss of vitamin B-6 may be a stimulus for increased

retention of vitamin B-6 after the depletion of stores by several days of exercise in combination with low vitamin B-6 intake. Further evidence of this comes from the finding that when the vitamin B-6 supplement was given, excretion was not decreased on the non-exercise days of Thursday and Friday.

The findings of this study concur with the findings of Leklem and co-workers that suggested that 4PA excretion might be increased in exercising male adolescents. However, studies with rats have shown the opposite was true. The study of Efremov and Bukin (126) and the Master's thesis of Stevens (160) indicate that the 4PA excretion may decrease with exercise, suggesting increased retention of vitamin B-6 with exercise in rats.

One problem with the results of the present study and the study mentioned above on the effect of exercise, was the failure to measure urinary total vitamin B-6. Since 4PA is the major urinary metabolite, it is probably indicative of the influence of exercise, but to be complete, urinary vitamin B-6 should be measured.

Some conclusions can be drawn from the evidence presented in this investigation as to the modification of the effect of exercise on vitamin B-6 metabolism by glycogen-altering diets. A direct relationship was not immediately obvious between the modified glycogen stores (assumed to be attained through diet and exercise) and the release of PLP, but there appeared to be a relationship nonetheless. When the glucose homeostasis system was stressed through glycogen depletion by the LC diet and daily exercise, the exercise test resulted in a samller pre to post change in B-6 vitamers, but was accompanied by a large decrease with exercise

recovery without an increased urinary 4PA excretion. Under the same circumstances when extra vitamin B-6 was provided, a large increase occurred in plasma PLP and PB6 with the exercise test which was accompanied by a large increase in 4PA excretion. Therefore in situations of adequate glycogen stores (NC, HC) or increased vitamin B-6 availability (LC+B6, HC+B6), greater increases occurred in plasma vitamers with exercise, and these increases were accompanied with increased excretion of 4PA as vitamin B-6 needs were either not increased or fulfilled by supplementation. The smaller response to exercise LC is proposed to represent the greater uptake and retention of vitamin B-6 by the liver rather than a decreased release of PLP. The modification of exercise-vitamin B-6 metabolism by the LC diet suggests a relationship did exist between glucose homeostasis and the exercise induced changes in vitamin B-6 metabolism which would be supportive of the hypothesis that glycogen phosphorylase was the source of exercise induced PLP release.

These results have several implications for both endurance athletes who might use the "Carbo Loading" technique for competitive edge and those who exercise regularly for the benefits of weight maintainance or cardio-vascular health. First, the low carbohydrate diet for glycogen depletion has been shown to be unnecessary to accomplish glycogen loading, depleting exercise alone followed by a high carbohydrate diet will allow equally great increases in muscle glycogen (115). In view of the stress this depletion diet places on glucose homeostasis and vitamin B-6 needs, athletes should no longer utilize dietary depletion as part of the "Carbo Loading" procedure. All subjects in the present study reported

adverse physiological (mostly digestive) and psychological responses to the LC diet. These reactions alone would suggest that the procedure was disadvantageous. Second, the increase in vitamin B-6 excretion with exercise, even though it appeared to occur only when vitamin B-6 was not needed, could affect vitamin B-6 status. If vitamin B-6 intake was only marginally adequate, increased excretion with regular exercise might predispose the exercising person to marginal vitamin B-6 status. The long term effects of marginal vitamin B-6 status have not been determined. In view of the fact persons who exercise can usually consume more kcal that non-exercising persons, it may be advantageous for those exercising to consume these extra kcal from vitamin B-6 rich foods to make up for any exercise-induced loss. The logical foods to recommend are foods such as whole grains, legumes, and beans, since they are high in vitamin B-6 in addition to carbohydrate which will aid in refilling exercise-depleted muscle glycogen stores. It should also be pointed out that these results do not suggest that the athlete needs to take vitamin B-6 supplements, since diets providing close to the RDA, other than the LC diet, were shown to be adequate.

The enzyme creatine kinase was measured throughout exercise to assess the effect of exercise on tissue permeability. Small increases were evident in plasma CK by the post exercise sample, ranging from 4 to 25%. Plasma levels decreased progressively following exercise and were not further elevated at 24 hours post exercise. Doubling and tripling of plasma CK levels within 30 min post exercise have been demonstrated following high intensity

exercise of long duration (e.g. marathons) (131-135). With less strenuous exercise or with high intensity exercise of shorter durations, peak elevations of 116 to 1130% have been reported from 8 to 24 hours post exercise (7, 130,137, 143). The small increases observed with exercise in this study suggest that the exercise was not strenuous enough to cause a dramatic change in CK. Galteau and coworkers (138) exercised aerobically fit men for 15 minutes on an ergometer at an intensity equivalent to that of the 5 minute 90% HRmax interval of this study, and could demonstrate no increases greater than 10%. Thus the exercise administered here was probably not demanding enough to produce any dramatic increase in CK. This conclusion is supported by two other observations. The first is that the pre exercise plasma levels varied more from Wed to Sat than within any exercise session. With the exception of Week 1, mean plasma levels of CK on Wed were twice the Sat values. For week 1 all subjects, except #4, had Wed values averaging 21 U/L greater than Sat values. Subject 4 reversed this trend by exhibiting a much greater Sat value. This suggests that the subjects regular exercise regimens influenced the plasma CK to a greater extent than did the exercise test. The difference between Wed and Sat pre exercise levels might also be considered to be the result of an influence of the dietary changes, since a smaller difference was seen between Week 1 Wed and Sat when the diet did not change. A second conclusion may be that the subjects did not exercise as much between Sunday and Wednesday of Week 1 as during weeks 2 and 3. This appeared to be the case from daily exercise records. The second observation was that when

subject 3 played two hours of basketball between the 60 min post and 24 hour post blood samples, a 139% increase was seen in his plasma CK levels. These results suggest that plasma CK levels may be useful in assessing whether there was compliance to desired exercise levels.

Increased plasma CK values were expected to accompany increases in B-6 vitamer levels. Although small increases occurred, there were very low correlations between the percent changes of CK with PB6 and PLP suggesting that the release of CK and B-6 vitamers occurred in response to different stimuli.

The experiences and results of the present investigations have also indicated directions for future research in this area. With respect to the hypothesis, conclusive evidence could obviously be obtained from muscle biopsy studies in exercising man. The effect of exercise on vitamin B-6 excretion, both as 4PA and urinary vitamin B-6, needs to be confirmed under conditions of more carefully controlled daily exercise. Quantitation of all exercise performed would be necessary. It is recommended that exercise test protocols use only a single intensity of work load. Changing the work load made the test application more difficult and less like the usual exercise circumstance.

VI. SUMMARY AND CONCLUSIONS

The effect of exercise on vitamin B-6 metabolism was studied in four men ages 20 to 23 years who were trained bicyclists. It was hypothesized that muscle glycogen phosphorylase might be the source of increased plasma vitamin B-6 (PB6) and its active form, pyridoxal 5'-phosphate (PLP) with exercise in man. This enzyme has been shown to act as a storage site for PLP in the rat. The most effective stimulus for depletion of the stored phosphorylase-PLP was shown to be short-term starvation which might be simulated by muscle glycogen depletion and exercise in man. There were three objectives to this investigation: 1) to determine the effects of muscle glycogen alterations prior to exercise on the subsequent vitamin B-6 metabolism during a controlled exercise test; 2) to determine the effects of vitamin B-6 supplementation on the response of vitamin B-6 metabolism to exercise; and 3) to study the effect of exercise on urinary 4-pyridoxic acid (4PA) excretion, the primary urinary vitamin B-6 metabolite, under conditions of carefully controlled vitamin B-6 intake.

The study consisted of 3 experimental weeks. Two exercise tests were administered each week (Wednesday and Saturday) during which blood was sampled to study vitamin B-6 metabolism. All food was provided during the experimental weeks. During week 1 a diet was fed that provided a "normal" proportion of calories (kcal) as

carbohydrate (CHO). This was designated the NC diet. Weeks 1 and 2 were separated by a 7 day nonexperimental period during which the subjects consumed self-selected diets. During week 2, Sunday through Tuesday, a diet low in CHO (11% of total kcal as CHO) (the LC diet) was fed to deplete muscle glycogen. This was followed by a high CHO diet (71% kcal as CHO) (the HC diet) to replete, or load, glycogen stores. Week 3 was separated from week 2 by 14-nonexperimental days. During week 3 the diets fed were identical to week 2, but in addition a daily supplement of 8 mg pyridoxine (PN) was given orally. These diets were designated LC+B6 and HC+B6, respectively. By diet composite analysis the actual vitamin B-6 content of the diets were as follows: NC, 1.64 mg PN; LC, 1.55 mg PN; HC, 1.82 mg PN. Daily exercise was encouraged Sunday through Tuesday to facillate glycogen depletion, but was limited Wednesday through Saturday to allow glycogen repletion. The exercise tests were administered after an overnight fast. The tests, on a bicycle ergometer, consisted of three progressive stages for a total of 50 min of continuous exercise: 30 min at 60% maximum heart rate (HRmax); 15 min at 80% HRmax; and 5 min at 90% HRmax.

Blood samples were drawn prior to each exercise test (pre), 2 min before the 90% HRmax stage (during), immediately post exercise (post), 30 min following exercise (30 min post) and 60 min post exercise (60 min post). Plasma samples were analyzed for hemoglobin (Hgb), hematocrit (Hct), total vitamin B-6 (PB6), pyridoxal 5'-phosphate (PLP) and creatine kinase (CK). All urine was collected throughout the experimental weeks in 24 hour aliquots and was analyzed for 4-pyridoxic acid (4PA) and creatinine.

The results of each exercise test were identified by the diet fed immediately prior to the test, since this diet would have influenced the muscle glycogen available for exercise. The exercise tests were: NC(Wed), NC(Sat), LC, HC, LC+B6, and HC+B6.

The raw and calculated data were statistically evaluated by paired t-test to determine significant differences. Regression analyses were also performed to assess relationships between certain variables.

The data obtained again demonstrated that exercise significantly altered vitamin B-6 metabolism. In addition, diet-glycogen alterations and vitamin B-6 supplementation were shown to modify the metabolism of vitamin B-6 during exercise. Significant differences (increases) in PB6 occurred pre to post exercise with all exercise tests. Significant increases occurred in PLP for all but exercises LC and HC. It was demonstrated that following the increase in PLP and PB6 with exercise, plasma levels of these vitamers drop continuously throughout 60 min post exercise. At 60 min post exercise some PLP values were significantly less than pre exercise. In only one case for PB6, and in no circumstance for PLP, did the drop in values post exercise appear to be reversing. Thus it was not known when plasma values returned to pre exercise values. The LC exercise test resulted in the smallest mean difference between pre to post in PB6 and PLP, but was among the largest mean decreases from pre to 60 min post. The need for vitamin B-6 would have been greatest in the situation of LC, due to increased amino acid catabolism for gluconeogenesis, and the additional stress of exercise. Supplementation (LC+B6 and HC+B6) was associated with

greater pre to post increases in PB6 and PLP (two times greater in most cases) than the corresponding unsupplemented exercises (LC, HC). This was significant for LC versus LC+B6 for both PB6 and PLP values. Greater increases following supplementation indicated more vitamin B-6 was made available with exercise, which would suggest that possibly more vitamin B-6 had been stored. Urinary 4PA excretion was increased on the day of all exercise tests as compared to non-test days, except for exercise test day LC. As the need for vitamin B-6 was increased with LC it was proposed that the smaller increase with LC and the lack of increased 4PA excretion represent redistribution of vitamin B-6 within the body without increased conversion to 4PA and excretion. In contrast, when need for vitamin B-6 was less or when the need was fulfilled by supplementation, the increase in B-6 vitamers was accompanied by increased conversion to 4PA. The modification of vitamin B-6 metabolism during exercise by the LC diet was taken to indicate that the release of B-6 vitamers was related to glucose homeostasis. In addition the relative need for vitamin B-6 appears to affect the levels measured in the blood. These findings were concluded to be supportive of the hypothesis that PLP was coming from muscle glycogen phosphorylase.

The percent changes in plasma volume (as calculated from hematocrit) were not found to be highly correlated with PB6 and PLP changes and it was concluded that the changes in PB6 and PLP were not caused solely by changes in plasma volume.

Based on the significant correlations of PLP with PB6 difference values and the similar magnitude of change in these parameters, it

was concluded that PLP is the major B-6 vitamer changing with exercise.

In addition to the effect of exercise on vitamin B-6 metabolism the HC diet was found to significantly lower fasting pre exercise PB6 and PLP levels (nearly 2 nmol/100 ml less) as compared to the LC diet. The high CHO content of the HC diet was suggested to be a possible cause of this difference.

Plasma CK was measured to ascertain changes in muscle tissue permeability with exercise, which might accompany the release of PLP. It was concluded that the exercise test administered was not strenuous enough to induce release of CK, but daily exercise practiced by the subjects did elevate pre exercise fasting levels. The small changes in CK were not highly correlated with percent changes in PLP and PB6. Therefore it was concluded that the release of B-6 vitamers occurs by a different mechanism or was the result of different stimulus than that for CK release.

The evidence obtained in this investigation has several implications for athletes. The LC diet increased vitamin B-6 needs and had adverse physiological (mostly digestive) effects on the subjects. As this diet was previously shown to be unnecessary to accomplish glycogen loading, it was recommended that this diet procedure (LC) no longer be followed by athletes. The increased excretion of 4PA with exercise suggests that adequate (US-RDA) vitamin B-6 intake should be consumed by athletes. Foods high in vitamin B-6 (whole grains, beans, legumes) and carbohydrate were suggested. A need for supplemental vitamin B-6 in those exercising was not established as adequate status was maintained without supplements.

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APPENDIX

Appendix Table 1
Individual blood chemistry screen results

Analysis	Subject 1		2		3		4		Normal Range
	before*	after*	before	after	before	after	before	after	
Glucose	90	82	92	87	88	93	89	85	65-110 mg/100 ml
BUN	18	20	14	18	19	20	25	20	10-20 mg/100 ml
Creatinine	2.3	1.2	1.1	1.1	1.4	1.4	1.1	1.2	0.7-1.4 mg/100 ml
Na+	144	148	144	149	141	147	142	148	135-145 mEq/100 ml
K+	4.9	5.1	6.5	5.0	4.6	5.6	4.3	5.4	3.5-5.0 mg/100 ml
Cl-	102	114	107	111	104	113	104	112	95-109 mEq/100 ml
Uric Acid	5.2	5.6	7.6	5.3	8.6	6.3	6.2	5.0	2.5-8.0 mg/100 ml
Ca	9.4	9.2	11.1	9.6	10.3	8.9	10.2	8.8	8.5-10.5 mg/100 ml
P	3.7	4.1	4.1	4.0	3.0	3.6	4.6	3.8	2.5-4.5 mg/100 ml
Total Protein	8.1	8.2	7.7	8.4	7.7	7.4	7.0	7.5	6.0-8.0 g/100 ml
Albumin	4.9	4.8	4.8	4.9	4.9	4.5	4.9	4.8	3.5-5.0 g/100 ml
Cholesterol	256	213	180	184	156	143	188	183	150-250 mg/100 ml
Triglycerides	-	78	91	110	57	48	63	174	30-135 mg/100 ml
Total bilirubin	0.5	0.5	0.8	0.3	1.8	0.9	1.1	0.4	0.15-1.0 mg/100 ml
Alkaline Phos.†	25	44	49	(30)	62	(31)	49	(28)	30-85 IU/100 ml
LDH	224	165	163	134	200	193	189	137	100-225 IU/100 ml
SGOT	37	37	27	41	39	51	25	37	7-40 IU/100 ml

* The before sample was taken about three days before the first week, the after sample was taken the last day of week 3.

† Methods were different for values in parentheses.

Appendix Table 2
Experimental diet compositions:
Normal Carbohydrate Diet (NC)

Food	Serving	Protein	Fat	Carbohydrate	Vitamin B6
Breakfast	g	g	g	g	mg
Eggs, scrambled	120	15.5	13.8	-	0.132
Toast, whole wheat	60	6.3	11.8	28.6	0.108
Orange Juice	250	1.8	0.3	26.8	0.07
Milk, whole fat	250	8.8	88.8	12.3	0.100
Margarine	55	-	44.0	-	-
Lunch					
Tuna, water pack	60	16.8	0.5	-	0.255
Cheese, cheddar	60	15.0	19.4	1.2	0.048
Salad dressing	40	0.4	17.0	5.0	-
Bread, whole wheat	100	10.5	3.0	47.7	0.216
Carrot sticks	100	1.1	0.2	9.7	0.150
Pears	200	0.4	0.4	31.2	0.028
Dinner					
Milk, whole fat	250	8.8	8.8	12.3	0.100
Hamburger, 21% fat	180	37.3	18.0	-	0.594
Cheese, ceddar	60	15.0	19.4	1.2	0.048
Rice, uncooked	60	4.0	0.3	48.2	0.102
Green beans, canned	100	1.4	0.2	5.2	0.070
Bread, whole wheat	330	5.3	1.5	23.9	0.054
Ginger snaps	90	5.0	8.0	71.8	0.030
Dried mixed vegetables	3.7	-	-	-	-
Candies, mixed	25	-	-	25.0	-
TOTAL GRAMS		153.2	165.2	349	2.105 mg
TOTAL CALORIES		613	1486	1398	
PERCENT OF CALORIES		18%	42%	40%	

Appendix Table 3
Experimental diet compositions;
Low Carbohydrate Diet (LC)

Food	Serving	Protein	Fat	Carbohydrate	Vitamin B6
Breakfast	g	g	g	g	mg
Cheddar Cheese	73	18.5	23.7	1.56	0.057
Eggs, uncooked	192	21.6	24.9	4.5	0.211
Bread, whole wheat	24	2.2	0.6	11.8	0.037
Orange juice	124	0.8	0.3	12.9	0.035
Canadian bacon	42	11.4	7.4	0.2	0.130
Margarine	38	-	30.7	-	-
Cream, heavy	30	0.6	11.2	1.0	-
Lunch					
Tuna, oil pack	160	46.1	13.1	-	0.680
Bread, whole wheat	48	4.4	1.2	23.6	0.074
Celery, fresh	60	0.6	0.05	2.3	0.036
Mayonnaise	56	0.8	44.8	1.2	-
Milk, whole fat	244	8.5	8.5	12.2	0.098
Dinner					
Cheese, cheddar	73	18.5	23.7	1.6	0.057
Hamburger	91	16.3	19.3	-	0.300
Cheese, cheddar	73	18.5	23.7	1.6	0.057
Milk, whole fat	244	8.5	8.5	12.0	0.098
Banana	25	0.18	0.02	3.8	0.125
Pineapple, with juice	44	0.01	0.01	4.5	-
Cottage Cheese	123	16.7	15.2	3.6	0.049
TOTAL GRAMS		194.1	256.8	98.2	2.044 mg
TOTAL CALORIES		776	2311	393	
PRECENT CALORIES		22.3%	66.4%	11.3%	

Appendix Table 4
Experimental diet compositions:
High Carbohydrate Diet (HC)

Food	Serving	Protein	Fat	Carbohydrate	Vitamin B6
Breakfast	g	g	g	g	mg
Bread, white	100	8.7	3.2	50.4	0.040
Cereal, puffed rice	70	0.4	0.3	62.3	0.075
Egg, scrambled	60	7.7	6.9	-	0.070
Orange juice	250	1.8	0.3	26.8	0.070
Milk, skim	1000	36.0	0.8	51.2	0.420
Sugar	15	-	-	15	-
Jam	46	0.3	-	32.2	-
Lunch					
Tuna, water pack	80	22.4	0.6	-	0.340
Salad dressing	20	0.2	8.5	2.9	-
Bread, white	120	10.4	3.8	60.5	0.050
Carrots, fresh	100	1.1	0.2	9.7	0.150
Pears canned, heavy sirup	200	0.4	0.4	31.2	0.028
Dinner					
Tuna, water pack	100	28.0	0.8	-	0.425
Rice, uncooked	90	6.0	0.5	72.3	0.153
Cheese, cheddar	30	7.5	9.7	0.6	0.024
Bread, whole wheat	50	5.1	1.5	23.9	0.090
Green beans, canned	100	1.4	0.2	5.2	0.070
Ginger snaps	125	6.9	11.0	99.8	0.040
Carbonated drink (7up)	400	-	-	48.0	-
Raisins	30	0.8	-	23.2	-
TOTAL GRAMS		145	43.7	615	2.041 mg
TOTAL CALORIES		580	438	2461	
PERCENT CALORIES		17%	13%	71%	

Appendix Table 5
Individual hematocrit values for all exercise tests

Exercise		Subject	Hematocrit				
Week	Day		pre	during	post	30 min post	60 min post
1	NC (Wed)	1	44.0	47.3	48.3	44.5	46.0
		2	47.3	47.8	47.5	46.3	45.5
		3	45.3	46.5	48.0	43.3	43.5
		4	47.3	49.0	50.0	47.3	45.8
1	NC (Sat)	1	47.0	47.8	49.0	44.0	44.8
		2	46.3	46.5	48.3	44.5	44.3
		3	45.5	46.3	48.0	45.0	43.3
		4	46.5	47.5	49.8	46.3	45.8
2	LC (Wed)	1	48.0	50.0	50.5	46.0	46.5
		2	45.8	47.5	48.3	46.3	44.5
		3	44.8	48.5	48.3	44.3	45.0
		4	46.0	48.3	49.0	46.8	45.5
2	HC (Sat)	1	43.8	47.3	47.5	46.0	43.5
		2	44.0	45.0	46.5	43.5	42.0
		3	44.8	45.8	46.5	43.5	39.8
		4	44.0	46.0	46.5	44.0	43.0
3	LC+B6 (Wed)	1	44.0	48.0	48.5	45.8	46.0
		2	47.5	47.5	48.3	46.3	45.3
		3	45.8	48.5	48.0	45.8	46.0
		4	45.0	47.5	48.5	47.3	49.0
3	HC+B6 (Sat)	1	43.5	46.8	46.8	44.8	43.0
		2	44.3	46.5	46.3	44.5	43.8
		3	44.5	47.5	48.8	44.5	43.5
		4	45.5	47.0	48.0	44.3	43.8

Appendix Table 6
Individual hemoglobin values for all exercise tests

Exercise		Subject	Hemoglobin				
Week	Day		pre	during	post	30 min post	60 min post
					g/100 ml		
1	NC (Wed)	1	16.6	17.8	17.6	15.8	17.0
		2	18.2	17.4	16.8	16.1	16.0
		3	16.8	17.3	17.8	16.0	15.9
		4	17.1	18.2	18.3	16.9	17.0
1	NC (Sat)	1	16.7	17.5	17.7	16.1	15.9
		2	16.4	16.7	16.7	15.5	16.0
		3	16.2	17.4	17.3	15.5	16.0
		4	17.0	17.9	18.0	16.8	17.0
2	LC (Wed)	1	17.7	18.2	18.2	16.7	16.7
		2	16.2	16.9	16.9	16.1	16.3
		3	16.6	17.3	17.4	16.0	16.3
		4	16.4	18.3	18.3	16.9	17.3
2	HC (Sat)	1	15.6	17.0	17.1	16.4	16.4
		2	15.4	16.8	18.4	15.5	14.9
		3	16.4	17.3	16.6	15.6	14.3
		4	16.4	17.0	17.3	16.3	15.5
3	LC+B6 (Wed)	1	15.4	16.8	17.2	16.4	16.8
		2	16.3	16.6	17.0	16.0	15.7
		3	16.3	17.6	16.9	16.4	16.2
		4	16.4	17.4	18.0	16.8	17.4
3	HC+B6 (Sat)	1	15.1	16.3	16.6	15.7	14.7
		2	14.5	16.1	16.0	15.1	14.9
		3	15.0	16.7	17.4	15.9	15.6
		4	16.2	17.1	17.3	16.1	15.6

Appendix Table 7
Individual and mean plasma vitamin B-6 values for all exercise tests

Week	Day	Diet	Subject	Plasma Vitamin B-6 pre	during	post	30 min post	60 min post
				(nmoles/100 ml)				
1	Wed	NC	1	4.62	5.49	5.26	5.87	5.48
			2	8.03	8.42	8.45	8.15	7.81
			3	5.23	6.05	6.72	5.39	5.11
			4	9.97	10.69	11.08	10.68	10.41
			Mean	6.96	7.66	7.88	7.52	7.20
			SD	±2.49	±2.38	±2.50	±2.43	±2.45
1	Sat	NC	1	4.92	5.89	6.06	5.23	5.29
			2	6.58	7.50	8.39	7.14	6.97
			3	4.47	4.51	6.25	5.58	4.81
			4	9.59	9.51	10.15	9.30	8.56
			Mean	6.39	6.85	7.71	6.81	6.41
			SD	±2.32	±2.15	±1.94	±1.85	±1.71
2	Wed	LC	1	7.08	7.40	7.93	6.40	6.02
			2	8.51	8.94	9.26	8.53	8.13
			3	5.21	5.53	5.83	4.94	5.01
			4	7.99	8.27	8.38	7.58	7.53
			Mean	7.20	7.54	7.85	6.86	6.67
			SD	±1.45	±1.48	±1.46	±1.55	±1.42
2	Sat	HC	1	4.72	5.96	5.96	5.42	5.35
			2	6.71	8.09	7.54	7.21	6.74
			3	4.02	4.42	4.67	4.08	3.10
			4	5.71	5.90	5.90	5.87	5.40
			Mean	5.29	6.09	6.02	5.65	5.15
			SD	±1.17	±1.51	±1.18	±1.29	±1.51
3	Wed	LC+B6	1	15.26	21.64	19.89	17.55	18.08
			2	24.97	24.81	27.75	22.69	23.74
			3	11.31	12.71	12.61	10.76	9.96
			4	19.20	20.79	21.12	19.74	21.12
			Mean	17.69	19.99	20.34	17.69	18.23
			SD	±5.83	±5.15	±6.20	±5.07	±5.98
3	Sat	HC+B6	1	17.29	18.92	19.55	18.86	16.55
			2	24.57	26.00	26.32	24.02	22.73
			3	10.78	12.81	13.22	11.61	10.58
			4	21.17	23.78	25.37	20.21	19.90
			Mean	18.45	20.38	21.12	18.43	17.44
			SD	±5.92	±5.85	±6.05	±5.20	±5.22

Appendix Table 8
Individual and mean plasma pyridoxal 5'-phosphate values for all exercise tests

Week	Day	Diet	Subject	Plasma Pyridoxal 5'-phosphate pre	during	post	30 min post	60 min post
				(nmoles/100 ml)				
1	Wed	NC	1	3.68	4.40	4.42	3.79	3.68
			2	6.96	7.70	7.53	7.33	6.01
			3	3.84	4.48	4.89	3.82	3.62
			4	8.99	10.05	10.52	9.76	9.26
			Mean	5.87	6.67	6.84	6.18	5.64
			SD	2.57	2.73	2.81	2.91	2.66
1	Sat	NC	1	3.84	4.20	4.40	3.58	3.49
			2	5.63	5.97	6.38	5.16	5.12
			3	3.30	3.51	4.03	3.37	2.97
			4	8.24	9.04	8.94	8.34	7.55
			Mean	5.25	5.68	5.94	5.11	4.78
			SD	2.23	2.47	2.25	2.30	2.06
2	Wed	LC	1	5.26	5.35	5.30	4.55	4.06
			2	6.95	7.00	7.13	6.58	6.10
			3	3.25	3.75	3.83	3.04	3.03
			4	6.51	6.78	6.97	6.50	5.95
			Mean	5.49	5.73	5.81	5.17	4.79
			SD	1.66	1.51	1.56	1.70	1.49
2	Sat	HC	1	3.50	3.99	4.19	3.66	3.02
			2	4.25	5.21	6.16	5.33	4.89
			3	2.53	2.72	2.76	2.38	1.92
			4	4.29	4.64	4.99	4.54	4.06
			Mean	3.64	4.14	4.53	3.93	3.47
			SD	0.83	1.07	1.42	1.26	1.29
3	Wed	LC+B6	1	13.28	15.12	15.49	13.91	12.89
			2	21.64	22.30	25.54	19.42	18.06
			3	6.55	7.75	7.34	6.49	5.74
			4	15.19	16.51	17.39	16.24	15.50
			Mean	14.17	15.42	16.44	14.02	13.05
			SD	6.21	5.98	7.47	5.50	5.31
3	Sat	HC+B6	1	13.08	13.18	13.58	11.88	11.27
			2	21.99	23.19	23.21	18.52	19.42
			3	7.31	7.63	8.84	7.49	6.28
			4	15.92	16.98	17.91	16.18	14.96
			Mean	14.57	15.25	16.39	13.52	12.98
			SD	6.10	6.54	6.04	4.87	5.57

Appendix Table 9
Individual and mean plasma creatine kinase values for all exercise tests

Week	Day	Diet	Subject	Creatine Kinase pre	during	post	30 min post	60 min post	24 hr post*
				Units/Liter					
1	Wed	NC	1	73	92	86	86	68	-
			2	86	88	119	79	79	-
			3	76	84	98	90	85	-
			4	48	43	55	48	41	-
			Mean	71	77	89	76	67	-
			SD	16	23	27	19	19	-
1	Sat	NC	1	45	48	55	50	47	-
			2	63	65	70	69	75	-
			3	64	75	82	76	66	-
			4	102	111	117	120	113	-
			Mean	68	75	82	78	75	-
			SD	24	27	27	30	28	-
2	Wed	LC	1	200	199	219	190	195	113
			2	114	114	118	114	111	90
			3	127	145	132	133	132	94
			4	38	42	38	40	38	50
			Mean	120	125	127	119	119	87
			SD	67	66	74	62	65	26
2	Sat	HC	1	67	79	83	74	61	52
			2	77	94	91	87	83	73
			3	65	73	81	78	65	50
			4	58	68	80	77	67	55
			Mean	67	78	84	79	69	57
			SD	8	11	5	6	9	11
3	Wed	LC+B6	1	110	125	123	116	114	75
			2	77	81	85	76	76	72
			3	248	298	302	277	283	165
			4	27	34	39	35	34	38
			Mean	115	135	137	126	127	87
			SD	95	115	115	106	109	54
3	Sat	HC+B6	1	56	60	59	55	53	48
			2	76	85	80	73	73	73
			3	70	82	88	73	68	168
			4	36	40	39	40	36	34
			Mean	59	67	66	60	57	81
			SD	18	21	22	16	16	60

* CK was not measured 24 hr post the first week.

Appendix Table 10
Individual urinary creatinine excretion per 24 hours

Week	Diet	Day	Subject 1	2	3	4
			g/24 hr			
1	NC	Mon	2.04	1.75	2.24	1.57
		Tues	1.83	1.78	2.10	1.72
		Wed	2.02	1.95	2.44	2.41
		Thur	1.92	1.72	2.12	1.73
		Fri	1.74	1.70	2.08	1.46
		Sat	2.07	1.95	2.25	1.73
2	LC	Mon	1.99	1.75	2.31	1.58
		Tue	1.76	1.82	2.14	1.65
	HC	Wed	1.97	1.91	2.24	1.95
		Thur	1.83	1.86	2.36	1.89
		Fri	1.69	1.81	2.34	1.72
		Sat	1.95	2.05	2.46	1.79
3	LC+B6	Mon	2.02	1.80	2.31	1.92
		Tue	1.80	1.80	2.18	1.64
	HC+B6	Wed	2.05	2.05	2.32	2.05
		Thur	1.79	1.79	2.23	1.81
		Fri	1.75	1.91	2.18	1.87
		Sat	2.12	2.21	2.52	2.01

Appendix Table 11
Individual urinary 4PA excretion as a percentage of total daily vitamin B-6 intake.

Week	Diet	Day	Urinary 4PA, % of Vitamin B-6 Intake					
			Subject				Group	
			1	2	3	4	Mean	SD
1	NC	Mon	59.9	62.3	76.3	61.0	64.9	± 7.7
		Tue	57.4	60.6	61.1	65.6	61.2	± 3.4
	NC	Wed	66.6	67.3	76.2	76.6	71.7	± 5.5
		Thur	59.4	52.2	61.1	61.4	58.5	± 4.3
		Fri	51.4	48.9	63.2	49.1	53.2	± 6.8
		Sat	66.7	64.0	63.5	81.9	69.0	± 8.7
2	LC	Mon	56.2	64.4	72.8	45.7	59.8	± 11.6
		Tue	46.6	50.7	58.0	54.0	52.3	± 4.9
	HC	Wed	51.3	53.2	55.0	54.0	53.4	± 1.6
		Thur	47.2	49.5	53.0	61.4	52.8	± 6.2
		Fri	41.9	46.5	54.9	45.2	47.1	± 5.5
		Sat	65.9	60.0	65.5	50.3	60.4	± 7.3
3	LC+B6	Mon	54.8	50.1	65.0	56.3	56.6	± 6.2
		Tue	53.6	51.6	57.4	50.5	53.3	± 3.0
	HC+B6	Wed	68.6	65.3	84.3	62.9	70.3	± 9.6
		Thu	61.0	60.0	62.9	59.9	61.0	± 1.4
		Fri	60.6	58.0	65.3	63.7	61.9	± 3.3
		Sat	75.4	71.7	69.3	62.3	69.7	± 5.5

Consent Form

I, _____, give my consent to participate in this study. The study has been explained to me and all my questions have been answered. I agreed to consume all food and vitamin supplements provided and to provide a record of food consumed the week prior to each experimental week. I agree to participate in the bicycle ergometer test which will be conducted on Wednesday and Saturday of each experimental week. I understand that there is a risk of a heart attack during a test such as this and that the type of exercise procedure to be conducted has been explained and the equipment used to in these procedures monitor heart (cardiac) function has been demonstrated. I further agree to participate only after clearance of a physician is given. I agree to allow 20 ml blood samples to be collected at five times during each exercise test and to collect 24 hour urine samples through each of the three experimental periods.

I understand that I will be paid $50.00 upon completion of the study. I understand that I am free to withdraw from the study at any time and that I can terminate the exercise part of the study at any time. I also understand that the investigators reserve the right to withdraw me from this study at any time.

All information concerning me will be kept confidential.

Name _____ Date _____

Witness _____ Date _____

Form I

VITAMIN B_6

NAME _____ TELEPHONE NO. _____

LOCAL ADDRESS _____

DATE OF BIRTH _____ HEIGHT _____ WEIGHT _____

Do you have any physical or metabolic defects? _____ If yes, please describe.

Are you taking any drugs, medications or vitamins or other dietary supplements? _____ If yes, please list (include brand names). Indicate length of use.

Do you have any food allergies? _____ If yes, please describe.

Describe briefly your daily physical activities.

Do you smoke? _____ How long? _____

What is your class and work schedule?

Thank You.

Name _____

Date _____

DAILY ACTIVITY SHEET

1. Record all activity for the day and length spent at each.

Activity	Length	Time of Day
Sleep		
Sitting		
Walking		
Running		
Other (describe)		

2. Record all "free" foods

Decaffinated Coffee (cups) _____

Herbal Tea (cups) _____

Sugar free beverages (cups) _____

3. How do you feel today? Excellent _____
Good _____
Fair _____
Poor _____

4. Any medication? (i.e., aspirin, etc.)

5. Unusual events - exams! and other.

6. Other comments.

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.

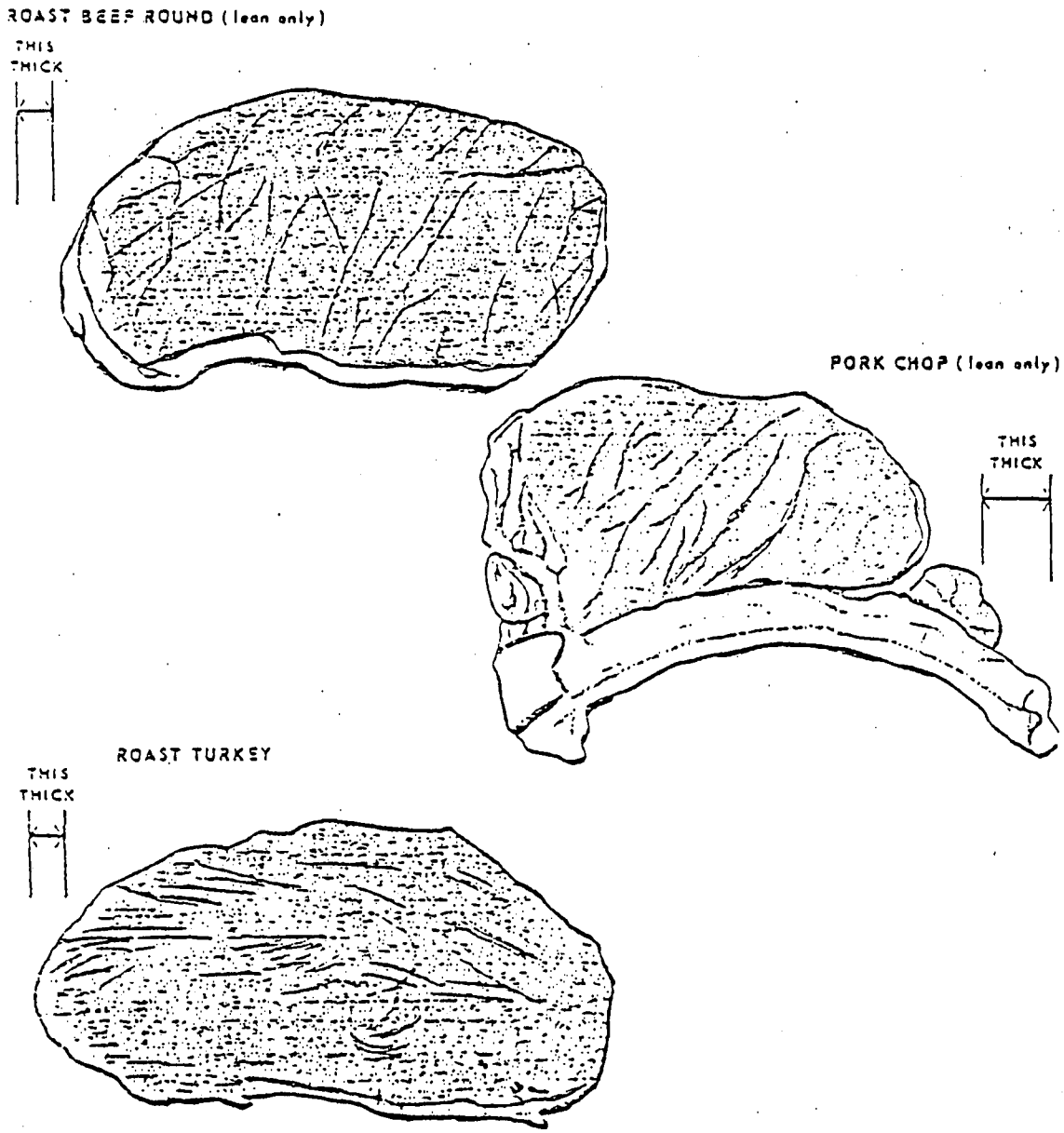
11. Be sure to include sauces, gravies, milk in coffee etc. Everything you eat or drink.

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 USE AMT. code	FOR OFFICE USE WT. code
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	Campbells	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	Ally Te Frie	—	10 oz.		
Apple	fresh	Winesap	raw, unpeeled	1 2" diam.		
Baked beans	canned	Nalleys	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		

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



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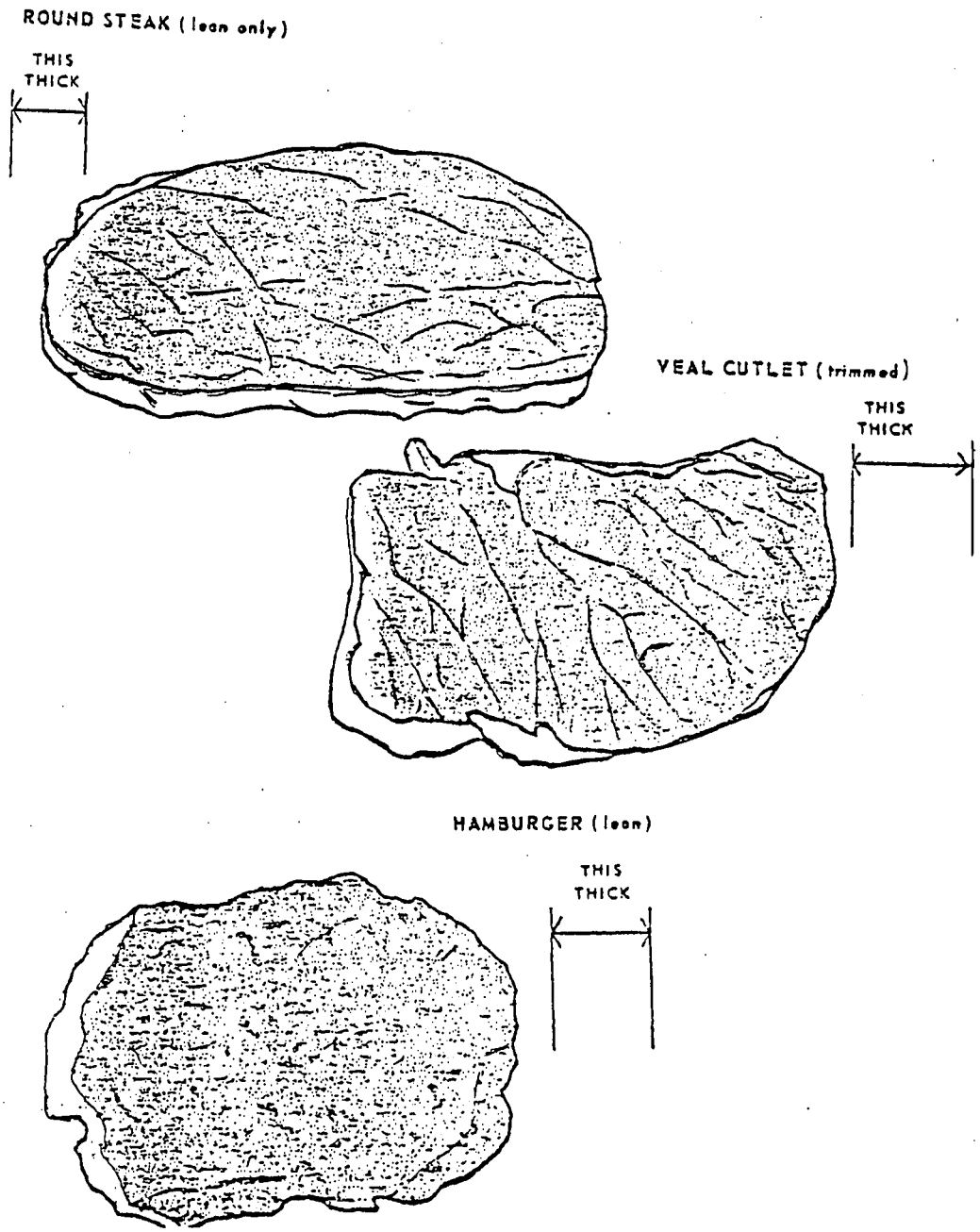
The sketches below represent the actual size of a 3-ounce serving of cooked meat, without bone.



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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	 WT. code