AN ABSTRACT OF THE THESIS OF

Ingrid A. Skoog for the degree of Masters of Science in Exercise and Sports Science presented on July 22, 1993.

Title: Effects of Vitamin B-6 Supplementation and Exercise to Exhaustion on Nitrogen Balance, Total Urinary Nitrogen & Urinary Urea in Trained Male Cyclists.

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Abstract approved: _____
Anthony Wilcox, Ph.D.

The role of protein (amino acids) as a fuel substrate during prolonged activity has been actively debated for the last 100 years. More recently, the study of gluconeogenesis during endurance exercise has shed more light on alternative glucose or energy production pathways.

The athlete who is better able to postpone glycogen depletion may have a competitive edge over his/her competitors. Conversely the individual who quickly becomes glycogen depleted may fatigue, and terminate activity more rapidly.

Our present investigation sought to explore three questions: 1) Will supplementation with vitamin B-6, in the form of pyridoxal 5'-phosphate (PLP), lead to a greater breakdown of muscle glycogen due to its role as a part of the glycogen phosphorlyase enzyme; 2) will a more rapid time to exercise induced exhaustion be observed due to supplementation and exhaustive cycle ergometer testing; and 3) if the above two hypotheses are met, will we observe a corresponding increase in urea production a by-

product of gluconeogenesis as evaluated through nitrogen balance assessments?

To test these hypotheses, six moderately trained adult male subjects were first tested maximally, using cycle ergometry. Two exhaustive exercise tests were performed separated by 3 weeks; workloads were set to elicit heart rates of 64-75% of each subject's maximal heart rate. The first (Test-1) exhaustive test was performed without supplementation with vitamin B-6 (Phase-1, US), the second (Test-2) following a 27 day supplementation with 20 mg/day oral vitamin B-6 capsules (Phase-3, S).

All subjects followed a strictly controlled isocaloric metabolic diet that provided 62% carbohydrate, 17% protein and 21% fat. Journals included records of dietary compliance, activity level and general feelings of wellness were kept by the subjects and reviewed daily by the researchers.

Subjects collected all urine in 24-hour time periods. Food composites and urine were analyzed in duplicate for urea nitrogen content. Total urinary and urinary urea nitrogen were obtained for each 8-day collection period surrounding each exhaustive exercise test, Days 1-6, prior to exercise testing and Day 7 and 8, post-exhaustive exercise testing. Based upon food composite analysis and nitrogen excretion, nitrogen balance was determined with estimates made for the lost plasma, sweat urea data, and other miscellaneous losses.

No statistically significant differences were observed in time to exercise-induced exhaustion between Test-1 (US) and Test-2 (S). Mean time to exhaustion for Test-1 was 121. 25 min. and Test-2 was 124.80 min.

Dietary intake of protein was 1.88 gm/kg body weight/day, which is 235% of the current RDA. Therefore it was of no surprise that all subjects

were in positive nitrogen balance (NBAL) during all days of both Diet Phase 1 (US) and 3 (S). There were no statistically significant differences observed between Dietary Phases 1 and 3 for days 3-8 pre-exercise collections for Urinary Urea Nitrogen (UUN) or Total Urinary Nitrogen (TUN). Additionally, no statistically significant changes in UUN excretion during the 24 or 48 hours post-exercise (Days 7 and 8) were observed between each treatment, however, a tendency towards an increased UUN excretion was observed for both days.

Nitrogen balance did shift towards a less positive NBAL state during the 24 and 48 hour periods of recovery. We observed a statistically significant decrease in positive NBAL between Day 7 (US) ($x=+2.27$ gm/24hrs) and Day 7 (S) ($x=+1.28$ gmN/24hrs) ($p<0.05$). Additionally, a statistically significant decrease in NBAL was observed between Day 8 (US); (+2.08 gmN/24hrs) and Day 8 (S); (+0.72 gmN/24hrs) ($p<0.05$), equaling a 188% decrease in positive nitrogen balance status (see Table 7a).

Based upon these findings, there appears to be an increased UUN elimination post-exercise while supplemented with vitamin B-6. Additionally, as observed through changes in NBAL status, the most significant changes were those observed in the 48 hours post-exhaustive exercise. It is suggested that vitamin B-6, despite no significant changes in subject's exercise duration, did perhaps increase the rate of muscle glycogen breakdown, therefore leading to a greater reliance on gluconeogenic production of glucose form amino acids. Furthermore, the observed NBAL changes were more pronounced during recovery due possibly to a depression in kidney function during Day 7, as glucose production from

amino acids continued during initial recovery, and, an increased rate of glycogen re-synthesis post-exercise, Day 7 and 8. These findings concur with those researchers who have observed an increase in UUN following long duration exercise. Additionally, they are in agreement with data that indicate a high protein intake will result in positive nitrogen balance despite long duration exercise.

Future research in this area may need to reduce total dietary protein intake and successfully analyze sweat and plasma as well as urinary urea concentration prior to, during, and after exercise. Employing more aggressive research methods, such as muscle biopsy for muscle glycogen content, utilizing amino acid tracers, monitoring plasma amino acid changes, and identifying tissue level shifts in amino acid concentration will help add vital information for elucidating the role of amino acids as an energy source for long duration activity.

Effects of Vitamin B-6 Supplementation and Exercise to Exhaustion on Nitrogen Balance, Total Urinary Nitrogen & Urinary Urea in Trained Male Cyclists

by

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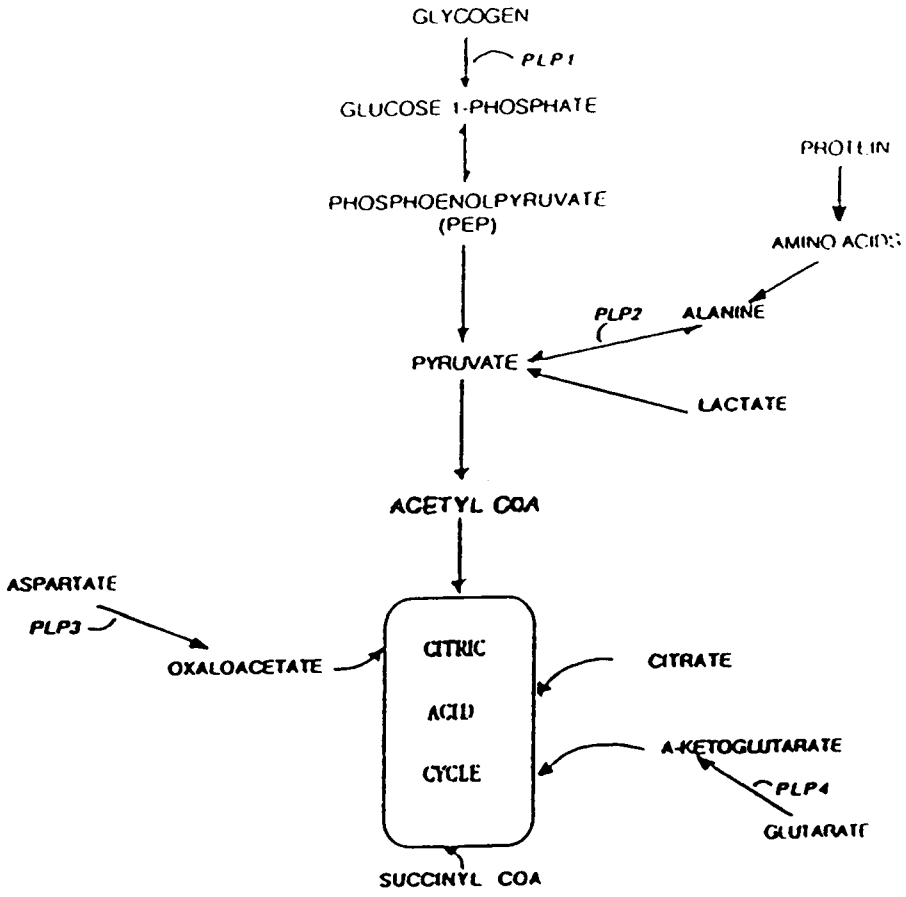
INTRODUCTION

At the initiation of moderate intensity exercise, blood glucose is the primary fuel substrate, but as exercise duration increases, blood glucose concentrations drop as a result of dwindling muscle glycogen stores (Manore & Leklem 1988; Costill, Coyle, Dalsky, Evans, Fink and Hoopes 1985,1980,1977; Astrand 1986).

Glycogen breakdown, glycogenolysis, involves the cleavage of glucose1-phosphate from the glycogen molecule (Krebs & Fisher, 1964). This reaction is dependent upon the enzyme glycogen phosphorylase (Figure 1) (Krebs et al. 1964). The glycogen phosphorylase enzyme requires the most active form of vitamin B-6, pyridoxal 5'-phosphate (PLP), to perform its functions (Low-Cam, Thadikonda & Kendall 1991; Schinzel 1991; Leklem 1990, 1983; Costill et al. 1977; Krebs et al. 1964). It has been estimated that one-half of all vitamin B-6 in the body exists as PLP bound to muscle glycogen phosphorylase (Devlin 1986; Black, Guirard & Snell 1978,1977; Hatcher, Leklem & Campbell 1982; Palm, Klein, Schinzel, Buehrer, Helmreich 1990).

As exercise continues an increase in free fatty acid utilization is observed as fat plays a greater and greater role as a fuel provider. Studies have shown that a trained individual has the ability to more quickly recruit fat as a fuel source compared to the untrained individual therefore sparing limited muscle glycogen stores (Astrand 1986;Costill & Miller 1980; Costill,

Figure 1. Pyridoxal 5'-phosphate (PLP) dependent reactions of energy production via the Citric Acid cycle (Adapted from Textbook of Biochemistry, Devlin, 1986).



PLP 1: glycogen phosphorylase
PLP 2: glutamate pyruvate transaminase
PLP 3: glutamate oxaloacetate transaminase
PLP 4: glutamate oxaloacetate transaminase

Coyle, Dalsky, Evans, Fink & Hoopes 1977). By delaying muscle glycogen depletion, endurance exercise performance may be enhanced (Rennie et al. 1976). However, despite an increased reliance on free fatty acids, glucose remains a vital fuel substrate for continued muscle function (Costill et al.,1977). Without additional carbohydrate from the diet, liver and muscle glycogen are depleted rapidly and plasma glucose concentrations drop. Under these circumstances, hepatic gluconeogenesis, a metabolic process which produces glucose from non-carbohydrate sources (amino acids, lactate and glycerol), plays an important, however, limited role in glucose production.

PLP is also an essential part of some of the enzymes required for gluconeogenesis (Devlin 1986, Leklem & Schultz 1983; Hatcher et al. 1982). Inadequate caloric intake and/or excessive energy demands which deplete blood glucose, liver and muscle glycogen concentrations stimulate gluconeogenesis. The liver and muscle are the primary sites for gluconeogenesis, and the glucose-alanine cycle (also called the Cori-Alanine cycle) facilitates gluconeogenesis by supplying necessary intermediates for energy production (Devlin 1986).

Within skeletal muscle, amino acids (primarily the branched chain amino acids; leucine, iso-leucine and valine) are transaminated by PLP-dependent aminotransaminase enzymes which cleave and transfer the amino group to α-keto glutarate then to pyruvate or oxaloacetate to form alanine or aspartic acid. Through this transamination reaction, alanine is produced from pyruvate (Russell, Bechtel, Easter 1985; Black 1978,1977).

Once alanine is formed, it is transported via the blood to the liver where it is transaminated back to pyruvate, depending upon the metabolic

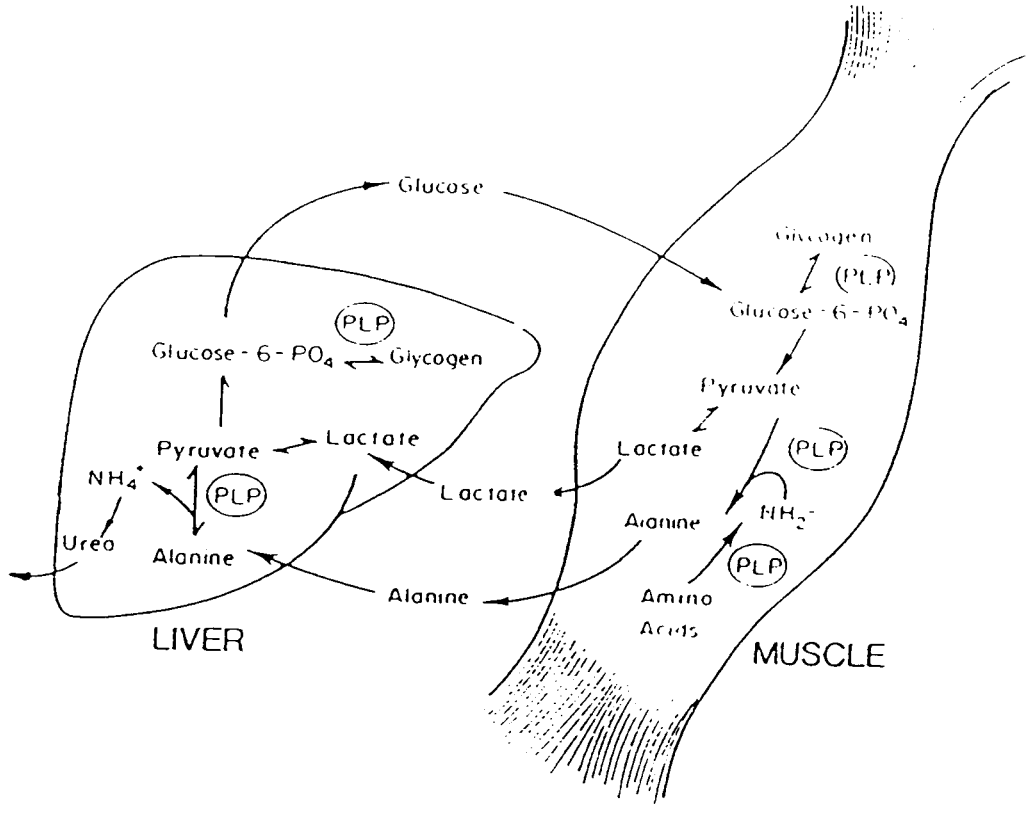
demands of the body. Pyruvate is then available to form glucose, to supply energy, glycogen, to replete depleted stores, or lactate (Figure 2) (Devlin, 1986).

Liver gluconeogenesis results in the release of excess ammonia from the transamination of alanine to pyruvate. Ammonia is converted to urea, which enters the blood stream as part of plasma. Urea can be excreted through sweat, urine, and, to a lesser extent, feces and sloughed skin (Lemon 1987; Rennie 1981a; Brooks & Fahey 1984; Williams 1985; Haramblie & Berg 1976; Cerny 1975; Wolfe, Goodenough, Wolfe, Royle, Nadel 1982; Calles-Escandon Cunninghamn, Snyder, Jacob, Huszar, Loke, Felig 1984; Konoplin & Haynes 1983; Lemon & Mullin 1980).

Though muscle stores of PLP are large, and its role in glycogen kinetics important, vitamin B-6's most significant role is as a coenzyme for enzymatic reactions necessary for protein metabolism (Sauberlich, 1985). Vitamin B-6 dependent enzymes include transaminases, decarboxylases, racemizases, oxidoreductases, isomerases, desulfhydrases and deaminases. Vitamin B-6 is essential for amino acid degradation, synthesis of non-essential amino acids and the exchange of amino groups during transamination (Devlin, 1986). Transaminases are involved in the transfer of the a-amino group of an amino acid, such as alanine, arginine, asparagine, aspartic acid, cysteine, isoleucine, lysine, phenylalanine, tyrosine, and valine to the a-carbon atom of an a-keto acid. The resultant compounds are important constituents in the citric acid cycle.

It was long believed that vitamin B-6 was not stored to any great extent in the body due to its properties as a water-soluble vitamin. Levels of the vitamin above body requirements were believed to be excreted

Figure 2. Pyridoxal 5'-phosphate (PLP) involvement in glucose and alanine metabolism in the Cori-Alanine cycle (Leklem, 1988).



in urine. However, research performed by Black and co-workers (1977) reported an increase in glycogen phosphorylase in rat muscle with vitamin B-6 supplementation (70 mg vitamin B-6/kg), thereby demonstrating the body's ability to store the vitamin in tissue. Inadequate vitamin B-6 intake, however, did not result in an increased availability of the stored form of the vitamin for body needs unless a caloric deficit also existed (Black, 1978).

Long duration activity has been likened to acute starvation and results in a hypoglycemic response due to the elevated energy demands of the body (Lemon and Nagle, 1981). In endurance trained men, Leklem and Shultz (1983) observed an increased plasma pyridoxal 5'-phosphate concentration during long duration exercise. It has been suggested that as exercise continues, PLP is released into plasma from muscle glycogen phosphorylase stores (Manore et al., 1988, Leklem, et al., 1983). This PLP shift from muscle to plasma is believed to facilitate gluconeogenesis by increasing the available PLP necessary for the transamination of specific amino acids and the gluconeogenic conversion in liver and muscle of the transamination by-products. Gluconeogenesis is believed to be enhanced, in part, due to limited muscle and liver glycogen stores as well as the seemingly unlimited availability of amino acids from body pools and skeletal muscle.

Based upon PLP's role in glycogenolysis as well as gluconeogenesis, the availability of dietary vitamin B-6 may impact substrate metabolism and exercise performance (Manore et al., 1988). Currently, there is little research on the effect of vitamin B-6 supplementation and substrate utilization during exercise, especially as it relates to amino acid metabolism.

The purpose of this research was to assess the contribution of amino acid degradation via gluconeogenesis by assessing time to exercise-induced exhaustion and measuring the changes in nitrogen balance via urinary, sweat and plasma urea concentrations during two bouts of exercise which occurred with and without prior vitamin B-6 supplementation. The time to exercise induced exhaustion provides an indication of the rate of glycogen depletion while the changes in nitrogen balance reflect the metabolic shifts in fuel substrate metabolism (ie. gluconeogenesis) during and following exhaustive exercise. See Appendix A for a more in depth literature review.

RESEARCH HYPOTHESES

A review of the literature leads to the hypothesis that with vitamin B-6 supplementation, muscle and liver glycogen will be more rapidly depleted, causing a decrease in time to exercise-induced exhaustion in trained college-aged male cyclists. In addition, the by-products of gluconeogenesis via amino acid metabolism (urinary, sweat and plasma urea) will be increased following exhaustive exercise in subjects supplemented with vitamin B-6. Consequently, a decreased nitrogen elimination as compared to dietary nitrogen intake (negative nitrogen balance) would be observed and reflect a greater use of amino acids as a fuel provider.

STATISTICAL HYPOTHESES

To assess the research hypotheses two statistical hypotheses, were applied.

1. Urinary Urea Nitrogen, a by-product of amino acid catabolism, will be increased during exhaustive exercise above dietary nitrogen intake following vitamin B-6 supplementation treatment.

   Ho: $Ms - Mus < 0$

   Ha: $Ms - Mus > 0$

   Where *Ms* is the mean measure of the by-products of protein catabolism for the vitamin B-6 supplemented (Phase 3), and *Mus* is the mean measure of the by-products of protein catabolism for the vitamin B-6 unsupplemented (Phase 1).

2. Time to exercise-induced exhaustion will decrease with vitamin B-6 supplementation treatment.

   Ho: $Ms - Mus > 0$

   Ha: $Ms - Mus < 0$

   Where *Ms* is the mean measure of time to exercise induced-exhaustion in the vitamin B-6 supplemented (Phase 3), and *Mus* is the mean measure of the time to exercise-induced exhaustion for the vitamin B-6 unsupplemented (Phase 1).

METHODS

Subjects

Six healthy, moderately trained male cyclists were recruited from the Oregon State University (O.S.U) campus and the local Corvallis community. Physical characteristics for each subject are given in Table 15, Appendix B. The term "moderately trained" means that the subjects were capable of performing the cycling activity at a higher workload and for a longer duration than untrained individuals, but they were not considered highly trained or elite within the sport. Subjects were accepted into the study if they maintained a regular cycling schedule of 120 minutes of aerobic activity in a minimum of 3 days per week for the previous year.

The subjects met the qualifications of the American College of Sports Medicine (ACSM) for placement in the "Apparently Healthy Individuals" classification of people undergoing exercise testing (ACSM, 1991). The apparently healthy individual is defined in Appendix B.

Prior to beginning the study, each subject's aerobic capacity and maximal heart rate were determined during progressive cycle ergometry testing. The ACSM criteria for exercise test termination were followed and are presented in Appendix B.

A three-day diet record, as well as health and exercise histories, were obtained during a pre-study interview, conducted by researchers, to establish health status (see Appendix B). Subjects were required to meet certain study criteria as outlined in Appendix B. If all criteria were met and the subject had no predisposing health risks, further baseline information was obtained: A blood draw at to assess plasma pyridoxal 5'-phosphate concentrations, a xylose absorption test to demonstrate normal carbohydrate

absorption, as well as normal cardiac rhythm and blood pressure response before, during, and after maximal exercise was required.

No smoking or drug use was allowed. Subjects could not have been taking any vitamin or mineral supplements for 6 weeks prior to being in the study, nor could they be taking any medications known to influence vitamin B-6 metabolism. Caffeine was not allowed during the two eight-day controlled diet phases.

Two subjects at a time started the study on consecutive days and proceeded through the study according to the time line in Table 1. Subjects were instructed to maintain their current level of fitness and pre-study weight throughout the experimental period. To monitor compliance, the subjects were required to keep daily journals during the controlled-diet phases. Journal entries included subject weight, exercise duration and perceived intensity, as well as any foods and beverages consumed that were not part of the controlled diet. Researchers reviewed journals daily and noted any dietary changes necessary to maintain pre-study weight.

An informed consent document containing the risks and benefits of the study was presented and explained, both verbally and in writing, to each subject prior to study participation (see appendix B). All exercise testing took place in OSU's Human Performance Lab under the supervision of a trained lab technician. This study was approved by OSU's Institutional Review Board.

Experimental Design

Prior to beginning the study, a test of maximal oxygen consumption ($\dot{V}O_2$ max) was performed by each subject. The purpose of this exercise test

Table 1. Study Calendar.

	sun.	mon.	tues.	wed.	thur.	fri.	sat.
OCTOBER:	14	14	16	17	18	19	20
	21	22	23	24	25	26	27
	28	29	30	31			
NOVEMBER:					1	2	3
	4	5	6	7	8	9	10
	11	12	13	14	15	16	17

Day 1: (October 16) first metabolic diet begins (8 day duration)*

Day 7: (October 22) exercise to exhaustion test, blood draw.

Day 9: (October 24) end of first metabolic diet period; vitamin B-6-supplementation begins.

Day 20-22: (November 4-6) 3 day 24-hour dietary records kept.

Day 21: (November 5) fasting blood draw.

Day 24: (November 8) second metabolic diet period begins (8 day-duration).

Day 30: (November 14) exercise to exhaustion test, blood draw.

Day 32: (November 16) end of second metabolic diet period, end of study.

Adapted from Duton, Thesis proposal, 1990.
*(prior to beginning of first metabolic diet a cycle ergometer max test was performed)

was to determine each subject's aerobic capacity and to obtain data necessary for establishing the subject's heart rate and workload for the endurance cycling trials. The three phases to this study (totalling 32 days) were as follows:

1) Days 1-8 (Phase 1): Controlled metabolic diet, no vitamin B-6 supplementation.

2) Days 9-23 (Phase 2): Self-selected diet, vitamin B-6 supplemented.

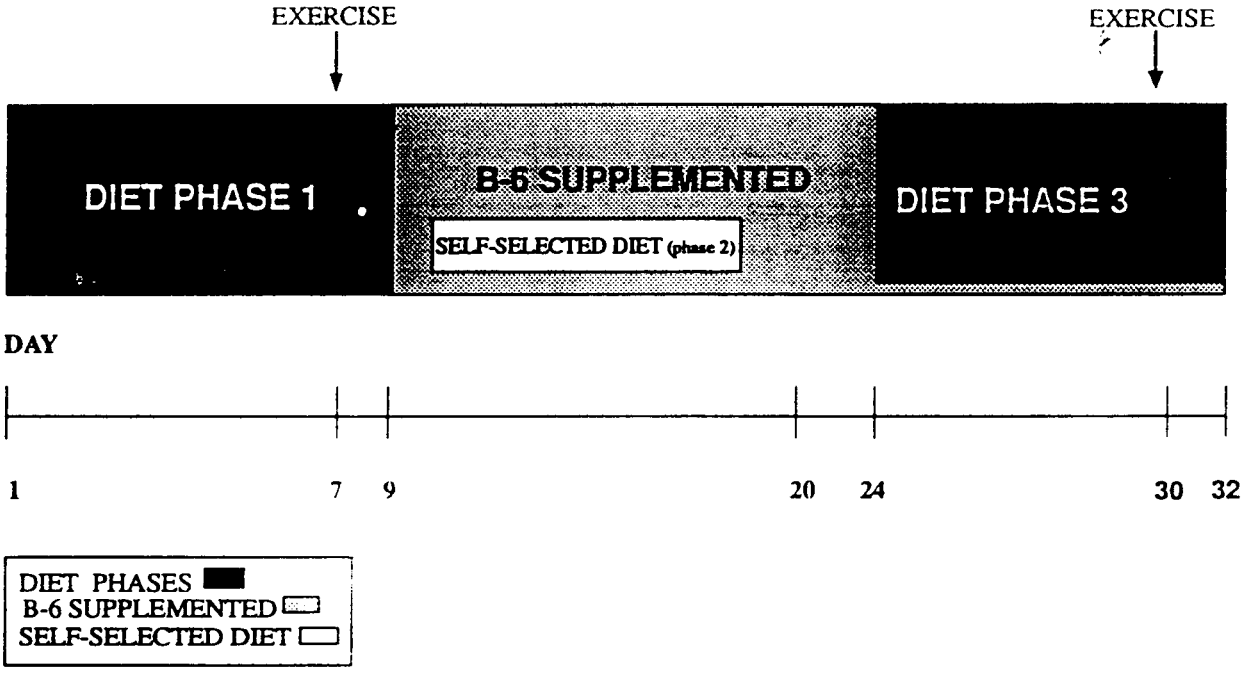
3) Days 24-32 (Phase 3): Controlled metabolic diet, vitamin B-6 supplemented (see Figure 3).

An exhaustive cycling trial was performed in the early morning hours of Day 7 and 31, the seventh days of Phases 1 and 3, respectively. The purpose of the 15 day self-selected diet (Phase 2) was to allow time between the first and third phases to normalize subjects to baseline levels, while supplemented with vitamin B-6, and to reduce the probability of confounding factors from the initial treatment (ie. prolonged, strictly controlled metabolic diet adherence).

Three to four weeks are required to return plasma B-6 vitamer concentrations to normal levels after supplementation has occurred. Therefore, in an effort to limit study time and cost as well as to improve subject compliance, the vitamin B-6 supplementation phase (Phase 3) followed the unsupplemented phase (Phase 1). As a result, it was not feasible to randomly assign subjects to treatment groups.

Subjects were provided with all daily meals as well as personalized results from blood chemistry profiles, maximal exercise testing and body composition assessments. A $50 stipend was given upon completion of the study.

Figure 3. Dietary Phases
(Adapted from Duton, N., Thesis Proposal, 1991).



Dietary Treatments:

This study included two controlled dietary phases, one supplemented with vitamin B-6 and one unsupplemented. The unsupplemented phase (Phase 1, days 1-8) preceded the supplemented phase (Phase 3, days 24-32). Each diet phase was identical in macro-nutrient quality and consisted of 62% carbohydrate, 17% protein, and 21% fat. The Appendix contains the menu and a detailed nutritional analysis. The mean caloric value of the diets was 3,456 calories/day. The controlled metabolic diet met 199% of the Recommended Daily Allowances (RDA) (1989), of all required micro-nutrients for men 18-35 years of age. Caloric intake was adjusted as necessary to maintain pre-study body weight. Necessary caloric adjustments were made by adding or subtracting foods that contained essentially no vitamin B-6. All such changes were recorded in daily journal entries.

The unsupplemented diet contained 2.3-2.4 mg/day of vitamin B-6. A placebo capsule was given with breakfast each day. Following the unsupplemented diet phase, a 20 mg vitamin B-6 supplement (as the hydochloride of pyridoxine) was given each day for the remainder of the study (days 9-32, a total of 24 days).

The supplemented controlled diet was given during days 24-32 and was identical in nutritional quality to the unsupplemented controlled diet with the exception of the vitamin B-6 capsule given at breakfast each day. Total vitamin B-6 ingestion during the supplemented phase was 22.3-22.4 mg/day. A 15-day self-selected diet separated the two controlled diet phases. During this two-week period, three 24-hour diet records were collected on days 20-22.

All foods served were purchased in bulk and from the same lot when possible in an effort to minimize variations in processing and nutritional quality. Food items were weighed prior to serving using a gram scale to ensure accurate portion sizes. Subjects were instructed to consume all foods served.

Food composites were collected in duplicate during each diet phase. Milk, plant and animal food composites were pureed in a blender, weighed, and a well-mixed duplicate sample was frozen at -18 degrees Celsius for future total nitrogen and vitamin B-6 analyses (see Appendix B for food composite lists, laboratory analysis procedures, and results).

All meals (breakfast, lunch and dinner) and snacks were prepared and served in the Metabolic Kitchen of OSU's Nutrition & Food Management Department. No alcoholic beverages were allowed. The study menus were analyzed for vitamin B-6, carbohydrates, protein, fat and caloric content using the Food Processor II nutrition analysis program (IBM version, ESHA Research, Salem, OR.).

Maximal Exercise Testing:

Subjects performed a graded Maximal Oxygen Consumption ($\dot{V}O_2$ max.) exercise test on a racing-modified cycle ergometer (Monark, Quinton Instruments, Seattle, WA.) to establish maximal heart rate and aerobic capacity. The $\dot{V}O_2$ max protocol consisted of a 35 watt increase every 2 minutes until volitional exhaustion occurred (see Appendix B). The criteria for determining $\dot{V}O_2$ max were:

1) an observed plateau in oxygen consumption .

2) a respiratory exchange ratio (RER) in excess of 1.10, and

3) a rating of perceived exertion (RPE) above 16 on a 6-20 Borg scale (Borg,1967).

4) achievement of age-predicted maximal heart rate (+/- 10 beats) (ACSM, 1991).

Oxygen consumption and carbon dioxide production were determined through gas analysis of expired air samples (Applied electro-chemistry SA-1 oxygen analyzer, Sensormedics LB-2 CO2 analyzer, Parkinson-Cowan CD-4 dry gas meter, and Rayfield REP-2000 software using an Apple II+ computer). Analyzers were calibrated immediately prior to all exercise testing. On-line determinations of gas concentration changes were recorded every 20 seconds.

Each subject's heart rate and cardiac cycles were continuously monitored electrocardiographically during maximal exercise testing using 12-lead electrocardiography (Quinton Instruments, Seattle, WA. Model CM 5). Blood pressure was assessed before, every 2 minutes during, and three to five minutes after exercise testing. See Table 16, Appendix B for the endurance exercise protocol and raw data collection forms.

Endurance Exercise Testing:

Two endurance exercise tests were performed using the same racing-modified cycle ergometer used in maximal testing. The first test was on Day 7 of Phase 1, the second on Day 7 of Phase 2. All exercise tests were conducted at similar times in the early morning hours. Subjects arrived for testing after an overnight fast and prior to consuming any foods or liquids.

Each test began with a 2 minute warm-up period at 0.5 kp (kilopounds) at 80 revolutions per minute (RPM) (240 kpm/min), followed by maintenance of a workload that elicited 65-75% of their pre-determined

maximal $\dot{V}O_2$, controlled by monitoring heart rate and workload, until exhaustion occurred. Subjects' individualized workloads are listed in Table 17, Appendix B. Exhaustion was defined as an inability to maintain the prescribed workload at 80 RPM's for greater than 30 seconds, and repeated more than two times within the final 10 minute collection phase.

Blood pressure and cardiac cycles were measured before, at 10 minute intervals during, and immediately following exhaustive exercise testing. Heart rate was calculated directly from the electrocardiographic tracing which was recorded every 10 minutes until exhaustion occurred.

The subjects' expired gases were collected and analyzed using the same methods during maximal exercise testing. Analyzers were calibrated every ten minutes during testing. Data obtained were processed by the Rayfield REP-2000 program on an Apple II+ computer system for on-line determination of oxygen and carbon dioxide concentrations. Data were collected before testing to establish a baseline, and for the last 3 minutes of every 10-minute interval during testing until exhaustion occurred. Subjects were not allowed any fluids during exhaustive exercise testing except for an occasional mouth rinse with water to minimize the effects of breathing through a mouth piece. No food or beverages were allowed until after the last blood draw, 30 minutes following test termination.

Body weight was measured without clothing before and immediately following exercise. Environmental conditions were maintained at 20-24 degrees Celsius, and at a relative humidity of 60%.

Body Composition Assessment:

Body composition was assessed at the beginning of the study using hydrodensitomitry. The subjects' weight, height, and age were obtained prior to weighing. Subjects were seated in the hydrostatic weighing tank on a plastic tubing chair hanging from an electronic digital scale. Water temperature was maintained at 36-37 C^{o} and recorded prior to each test. Ten underwater weighing trials were performed and recorded at the subject's calculated residual volume. Consistently repeated underwater weighing values obtained during the final 5 trials were used to calculate body density and percent body fat by use of the Siri equation. Residual volume was predicted as a percentage (28%) of each subject's measured vital capacity (Wilmore, 1969).

Subjects were asked to maintain their pre-study fitness and training programs for the duration of the study. Results of all exercise testing and hydrostatic weighing were withheld from the subjects until the completion of the study.

Daily Procedures:

Each subject was asked to follow specific daily procedures to assure compliance with the metabolic study. Subjects were given journal logs and daily activity forms to record the following information:

1) Exercise mode, duration, frequency and perceived intensity.

2) All non-caloric beverages and freely allowed foods (pure sucrose hard candies and non-caffenated sugar-free colas) consumed.

3) All medications taken; type and amount.

4) Overall well-being.

5) Any foods, and the amounts, eaten that were not a part of the

controlled diet.

6) Any unfinished foods or beverages, and the amounts.

7) Accuracy of urine collections.

8) Body weight.

9) Foods eaten to adjust for weight fluctuations.

During the self-selected diet phase, the subjects continued to keep the journals and completed a three-day record of dietary intake for analysis. Journals were reviewed frequently by researchers to monitor and improve study compliance.

Urine Collection:

Urine collections began with Day 1 of the first metabolic phase. Twenty-four hour collections were maintained through Diet Phases 1 and 3, as well as for 3 days during the self-selected diet phase (days 20-22) in order to re-establish a baseline prior to beginning the third phase. Urine was collected for two days, Day 7 and 8, following exhaustive exercise testing. All urine was collected in sterilized, distilled water rinsed containers. Five ml of toluene, a preservative, was added to each container before use.

Subjects delivered urine to the laboratory each morning. All containers were refrigerated by the subjects until transported to the lab. After volume determinations, samples were well mixed and aliquots collected. Urine samples were immediately frozen at -18 degrees Celsius for future analysis. Analysis performed were urinary urea nitrogen, total urinary nitrogen and creatinine concentration. Creatinine was used as an index of urine collection accuracy (Jackson, 1966).

Analytical Methods:

Analyses performed included total urinary and food composite nitrogen, urinary urea nitrogen, and urinary creatinine concentrations. Samples to be analyzed for plasma urea and sweat urea were lost due to freezer failure. A total of 19 days of samples were analyzed for each subject.

Total urinary nitrogen (TUN) excretion for each 24 hour urine collection was determined, in duplicate, using the Kjeldahl macro-method (Association of Official Analytical Chemists, 1965) (Oser, 1965). A similar method was applied to the diet composites analyzed for total nitrogen (see Appendix B).

Twenty-four hour urinary urea concentration was determined using a Technicon Auto Analyzer (Technicon Corporation, Tarrytown, New York). Urine samples were diluted 1:50 with a 0.9% saline solution before being analyzed in duplicate (Pino, Benoth & Gardyna,1965).

Twenty-four hour urinary creatinine excretion was measured via a modified method of the Jaffee reaction utilizing the Technicon Auto Analyzer (Pino et al. 1965, Jackson, 1966).

All analyses were performed in OSU's Foods & Nutrition Laboratory under supervision of Dr. James Leklem, Professor in the Nutrition & Food Management Department. Any duplicates with a greater than 4% difference between samples were reanalyzed.

The energy content and nutritional quality of the metabolic diet and self-selected diet recalls were analyzed using the Food Processor II nutritional analysis program on an IBM-compatible computer.

See the Appendix B for a detailed outline of the analytical methods.

Nitrogen Balance:

Nitrogen balance was determined by analysis of nitrogen intake from dietary sources minus nitrogen excretion via urinary urea nitrogen. Data from currently available research allowed estimation of miscellaneous nitrogen losses through sweat, feces and sloughed skin and endogenous amino nitrogen (Calloway, Odell, Margen, 1971). Nitrogen balance was determined using the following calculations:

Nitrogen intake (g N/24hrs): (protein intake in g/24hrs)
calculated from food composite analysis (6.25 g protein/g of nitrogen)

Nitrogen output (g N/24 hrs): urinary urea nitrogen + 4 g
(miscellaneous nitrogen loss)

Nitrogen balance: nitrogen intake = nitrogen output (Manan and Arlin, 1992)

Positive Nitrogen Balance: dietary nitrogen intake > nitrogen output.

Negative Nitrogen Balance: dietary nitrogen intake < nitrogen out put.

Statistical Analysis:

A paired *t* test (two-tailed) and 2-way Analysis of Variance (ANOVA) was used to determine if there were significant differences between the appropriate unsupplemented and supplemented with vitamin B-6 values after exhaustive exercise testing as well as to determine if there were significant differences between the respective means of time to exercise-induced exhaustion. All hypotheses tests were conducted at $p = <0.05$ level of significance.

RESULTS

Subject characteristics are shown in Table 2. Mean caloric and protein intake for each individual as well as by dietary phase are listed in Table 3. Unless otherwise noted, all statistical comparisons are made between data from study Phase 1 (days 3-8), without vitamin B-6 supplementation, and Phase 3 (days 3-8), with vitamin B-6 supplementation. Days 1 and 2 were not considered as they represent dietary adaptaion days.

Dietary Intake

During the controlled dietary phases, total caloric intake per phase averaged 3,352 calories/day during Phase 1 and 3,462 calories/day during Phase 3. Individual means for each subject are shown in Table 3. The daily dietary vitamin B-6 intake remained consistent between phases (2.30-2.35 mg vitamin B-6/day) based on laboratory analysis of food composites. See Table 17, Appendix B for nitrogen content of food composits for Phase 1 and Phase 3.

As a group, no statistically significant differences were observed between phases for caloric intake/day. On individual analysis subjects 1 and 2 did significantly increase their total calorie intake during Phase 3 as compared to Phase 1 ($p<0.05$). Subject 1 increased by 582 calories and Subject 2 by 101 calories, on average. Also Subject 5 showed a statistically significant decrease in calorie intake during Phase 3 as compared to Phase 1, the average change was -146 calories/day ($p<0.05$).

Table 2. Subject Characteristics (n=6).

	Mean	SD	Range
Age (yrs)	26.3	7.4	19 - 37
Body Weight (kg)	73.6	3.5	69.3 - 79.1
Height (cm)	179	2.6	175 -183
Body Fat (%)	12.6	3.0	9.5 -16.4
Max $\dot{V}O_2$ (L/min.)	4.4	0.5	3.6 - 4.9
Max $\dot{V}O_2$ (ml/kg/min.)	59.6	7.9	45.3 - 69.5
Mean Kcal intake			
Phase 1	3352	88.3	3227-3462
Phase 3	3462	168.5	3241-3809

(Adapted, in-part, from Virk, R. unpublished Thesis. 1992)
Body weights obtained at time of body composition assessments mid-study.
Body composition determined by hydrostatic weighing mid-study.
Max VO2 data obtained pre-study.
Kcal intake determined through computer nutritional analysis.

Table 3. Mean daily calorie intake per subject for unsupplemented and supplemented with vitamin B-6 phases

	DIET PHASE 1		DIET PHASE 2	
Subject	mean	SD	mean	SD
1	3227*	33	3809	318
2	3309*	61	3410	87
3	3462	116	3406	169
4	3300	85	3366	70
5	3387*	118	3241	72
6	3426	112	3538	249
x=	**3352**	**88.3**	**3462**	**168.5**

All values are means and SD calculated from 7 days of food logs.
Diet phase 1 refers to unsupplemented first diet period (days 1-8)
Diet phase 2 refers to supplemented with vitamin B-6 second diet period (days 24-32)
Statistically significant ($p<0.05$) Kcal intakes between diet phases are noted with an (*)

For subjects 1 and 2, the increased caloric intake came from carbohydrate sources: Subject 1 increased by 144 g (576 calories), Subject 2 by 17 g (68 calories), on average, each day of diet Phase 3. However, Subject 5 showed no change in total carbohydrate intake, therefore the decreased calorie intake was as a result of reduced fat intake in that there were no significant differences observed in Urinary Urea Nitrogen elimination.

Analysis of the food composites for each phase showed a 138.55 gram daily protein intake for Phase 1 and 139.84 g protein/24 hrs for Phase 3. Mean protein intake per kilogram body weight per day was 1.88 g/kg/day (+/- 0.895 g/kg/day) (235% of the RDA). Total nitrogen intake was 22.37 gN/24hrs intake Phase 1 and 22.17 gN/24 hrs Phase 3. As a percent, protein contributed 16.61% of the calories during Phase 1 and 16.08% of the calories during diet Phase 3.

Time To Exercise-Induced Exhaustion:

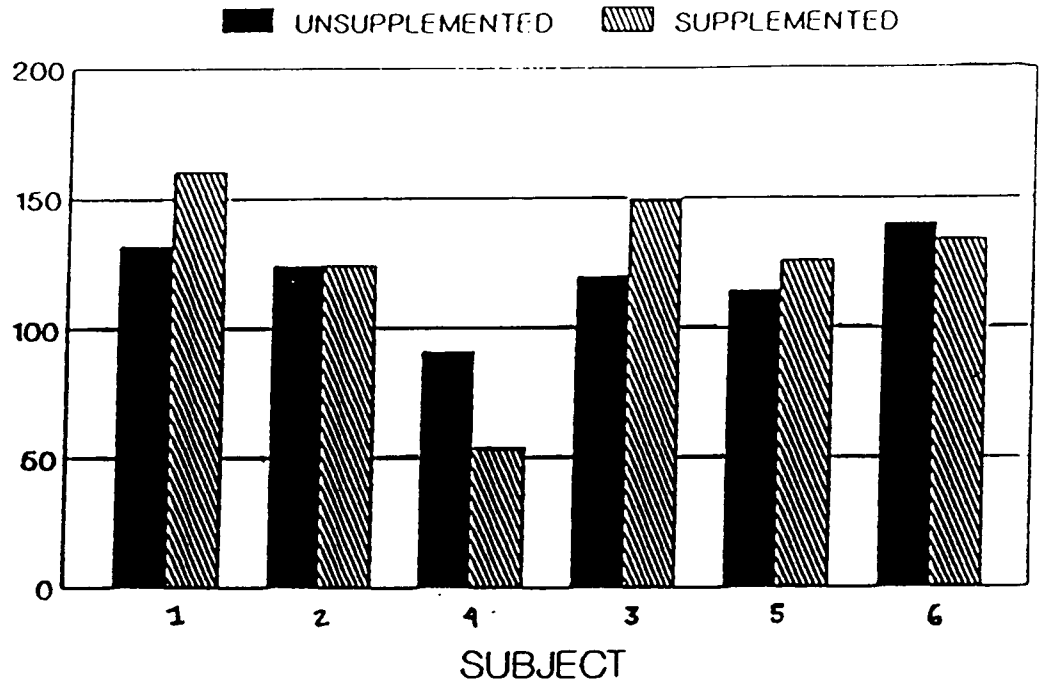
Table 4 lists the individual times to exhaustion for Test-1 (without vitamin B-6 supplementation) and Test-2 (with vitamin B-6 supplementation). There were no statistically significant differences in time to exhaustion between Test-1 and Test-2 ($p<0.05$). Three of the subjects did exercise longer during Test-2. Of these, two subjects extended their exercise time by 29 minutes and the third by 12 minutes. Of the three remaining subjects, two exercised for the same time (0 to 5 min) while the third subject terminated exercise 37 minutes earlier during Test-2 as compared to Test (Figure 4).

Table 4. Time to exercise-induced exhaustion while on diets without vitamin B-6 supplementation (Test 1) and while on diets supplemented with vitamin B-6 (Test 2).

	TEST 1	TEST 2		
Subject	**Time***	**Time***	**T1 - T2**	**$T\dot{V}0_2$**
1	131:50	160:32	+ 29:28	59.74
2	124:47	124:31	- 00:16	63.22
3	120:24	149:23	+ 28:59	69.47
4	91:44	54:00	- 37:44	61.38
5	114:54	126:28	+ 11:32	58.60
6	140:06	134:27	- 05:39	45.31
mean	121:25	124:51		59.62
S D	16:13	37:32		

(*) Time refers to minutes: seconds of exercise
$TV0_2$ data as ml/kg/min at maximal exertion
Test 1 subjects were tested while on a diet unsupplemented with vitamin B-6
Test 2 subjects were tested while on a diet supplemented with vitamin B-6
T1 - T2 is difference between first and second exhaustive test

Figure 4. Time to exercise-induced exhaustion (min.) by subject for Test-1, unsupplemented and Test-2 supplemented with vitamin B-6.



Total Urinary Nitrogen:

Table 5 and Figure 5 list and graphically illustrates the mean total urinary nitrogen (TUN) excretion in grams of nitrogen per 24 hour period (gN/24 hrs) for Diet Phase 1 and Phase 3.

As Figure 5 illustrates for days 3-6 of Phases 1 and 3, there were no statistically significant differences in TUN excretion ($p<0.05$). Additionally, the 48 hour post-exhaustive exercise collection (Day 7 & 8) showed no statistically significant reductions in TUN between dietary Phases 1 and 3.

Urinary Urea:

Table 6 and Figure 6 list the mean data and graphically illustrates urinary urea nitrogen excretion (UUN) in gN/24hr period for unsupplemented (Phase 1) and supplemented (Phase 3) with vitamin B-6 treatments. There were no statistically significant differences observed in UUN excretion between dietary Phases 1 & 3 for Days 3-6, which were pre-exercise, and Day 7 and 8 which were post exercise. Table 6a depicts the mean UUN excretion as a percent of TUN excretion for days 3-6, and 7 & 8. No statistically significant differences were observed between Dietary Phases 1 and 3.

Nitrogen Balance:

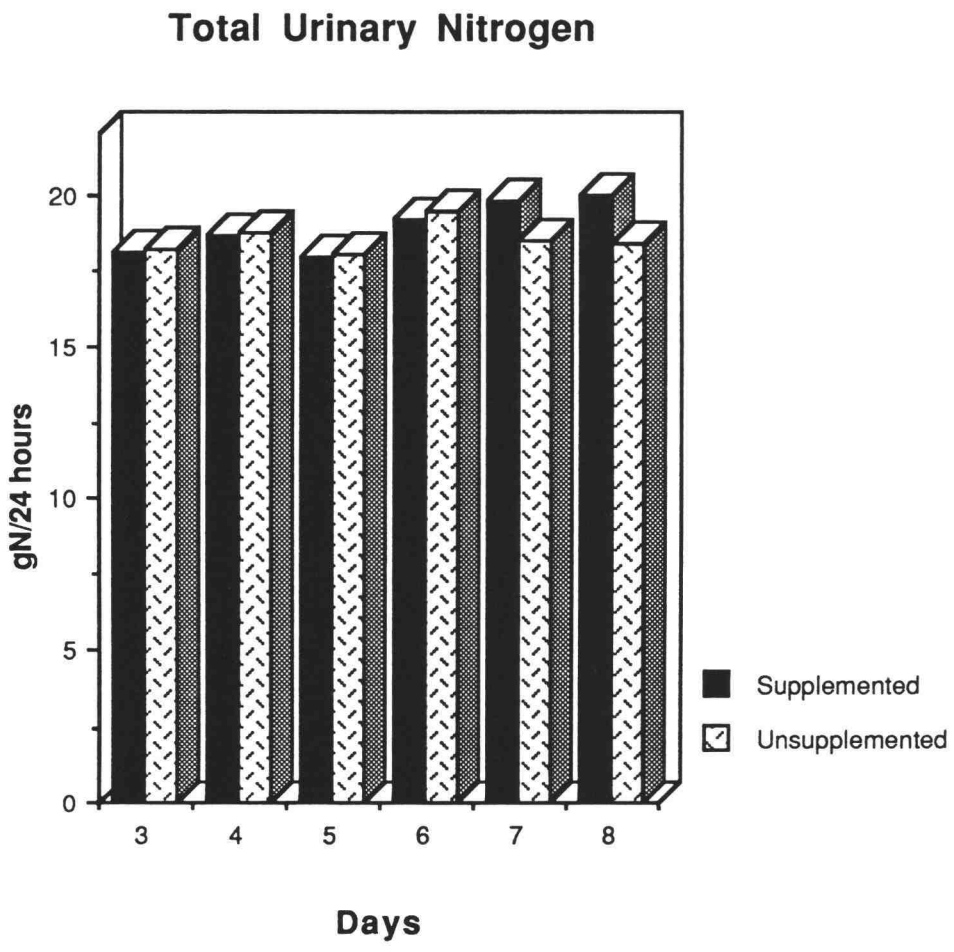
Table 7 shows the nitrogen balance results for each day of Dietary Phases 1 and 3. All subjects were in positive nitrogen balance during days 3-8 of Dietary Phases 1 and 3. The nitrogen intake calculated from analysis of food composites was 22.37 and 22.17 gN/24 hrs for Dietary Phases 1 & 3, respectively. Nitrogen balance results for Phases 1 & 3 are depicted in

Table 5. Total Urinary Nitrogen (TUN) excretion (gN/24hrs) while unsupplemented with vitamin B-6 (Phase 1) and supplemented with vitamin B-6 (Phase 3), days 3-8.

	Dietary Phase 1			**Dietary Phase 3**		
Day	mean	n	SD	mean	n	SD
3	18.22	6	1.93	18.11	6	1.80
4	18.71	5	0.40	18.67	6	1.02
5	18.03	6	1.20	17.95	6	1.80
6	19.40	6	1.40	19.20	6	1.27
7*	18.44	5	1.18	19.83	6	1.85
8*	18.38	5	0.93	19.96	4	1.10
mean	18.53		1.17	18.95		1.45

* post-exercise collections
All data in gN/24hrs
Dietary Phase 1: without vitamin B-6 supplementation
Dietary Phase 3: with vitamin B-6 supplementation

Figure 5. Total Urinary Nitrogen (TUN) excretion (gN/24hrs) while unsupplemented and supplemented with vitamin B-6 (days 3-8).



* Day 7&8 Post-Ex.

Table 6. Urinary Urea Nitrogen (UUN) excretion (gN/24hrs), days 3-8, without (Dietary Phase 1) and with (Dietary Phase 3) vitamin B-6 supplementation.

	Dietary Phase 1			**Dietary Phase 3**		
Day	mean	n	SD	mean	n	SD
3	15.78	6	1.90	16.02	6	1.90
4	16.23	5	0.70	16.44	6	0.92
5	15.88	6	1.00	15.61	6	1.52
6	17.48	6	1.50	16.35	6	1.17
7*	16.16	6	0.85	16.89	5	1.02
8*	16.27	5	0.93	17.45	4	1.19
mean	16.30		1.15	16.46		1.29

* post-exercise collections
All data in gN/24hrs
Dietary Phase 1: without vitamin B-6 supplementation
Dietary Phase 3: with vitamin B-6 supplementation (20 mg/day)

Figure 6. Urinary Urea Nitrogen (UUN) excretion (gN/24hrs) while unsupplemented and supplemented with vitamin B-6 (days 3-8).

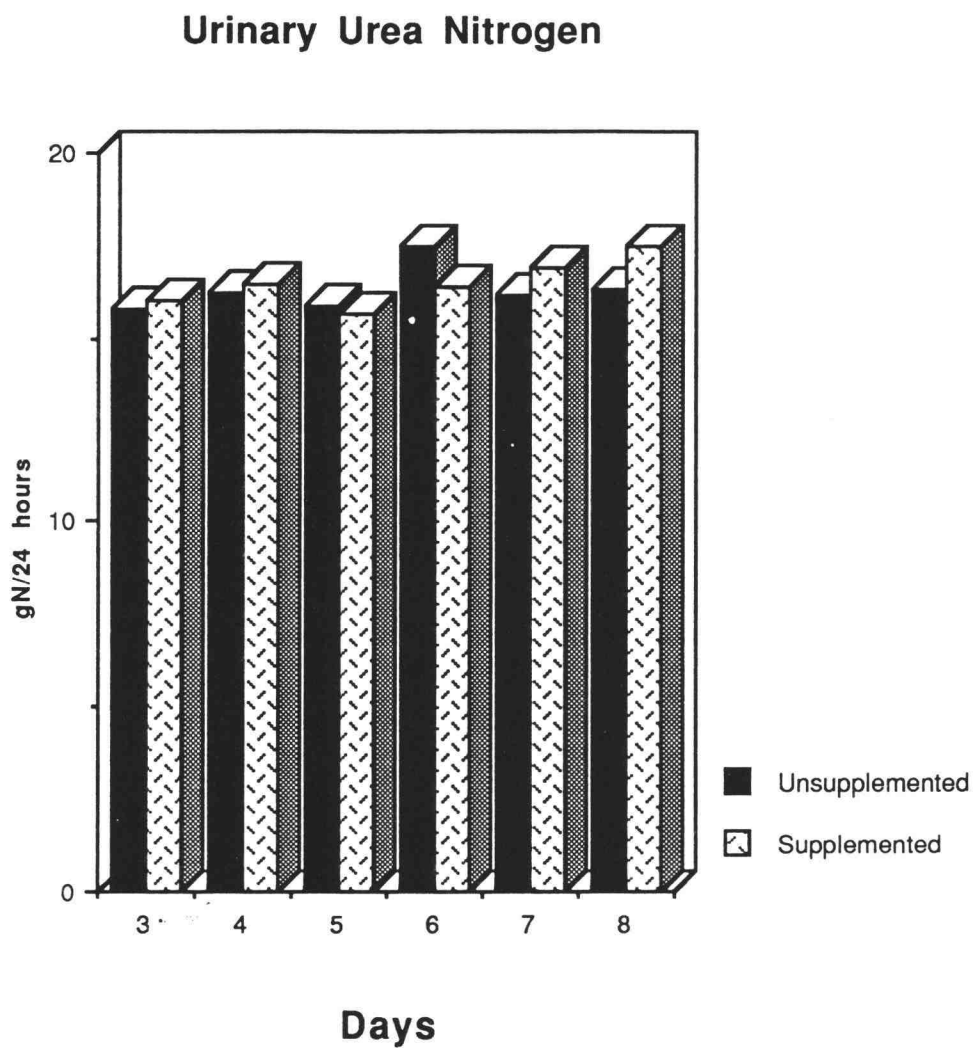


Table 7. Nitrogen balance data for all subjects, days 3-8, with and without vitamin B-6 supplementation.

Day	Dietary Intake*	Output*	Intake - Output*
Diet Phase 1**			
3	22.37	19.80	+2.57
4	22.37	20.23	+2.14
5	22.37	19.88	+2.49
6	22.37	21.47	+0.90
mean (3-6)		**20.34**	**+2.03**
7****	22.37	20.10	+2.27
8****	22.37	20.29	+2.08
Diet Phase 3***			
3	22.17	20.02	+2.15
4	22.17	20.45	+1.72
5	22.17	19.61	+2.56
6	22.17	20.35	+1.82
mean (3-6)		**20.10**	**+2.06**
7****	22.17	20.89	+1.28
8****	22.17	21.45	+0.72

* Values are gN /24 hrs
Output (urinary urea) adjusted for misc. Nitrogen losses (+4gms)
** Unsupplemented with vitamin B-6 dietary phase
*** Supplemented with vitamin B-6 dietary phase
**** post-exercise collections

Table 7a: Urinary Urea as a percent of Total Urinary Nitrogen.

	Diet Phase 1		Diet Phase 3	
Day	UUN/TUN*	%	UUN/TUN*	%
3	15.78/18.22	87	16.02/18.11	88
4	16.23/18.71	87	16.44/18.67	88
5	15.88/18.03	88	15.61/17.95	87
6	17.48/19.40	90	16.35/19.20	85
7**	16.16/18.44	87	16.89/19.83	85
8**	16.27/18.38	88	17.45/19.96	87
mean		88		87

* All data gN/24hrs excretion
** post-exercise collections
Diet Phase 1: without vitamin B-6 supplementation
Diet Phase 3: with vitamin B-6 supplementation

Figure 7. A statistically significant difference was observed between dietary Phases 1 and 3 for the 24 and 48 hour post-exercise collection periods ($p<0.05$). Table 7a illustrates the percent changes in TUN, UUN and nitrogen balance observed between Diet Phase 1 and 3 for pre exercise Days 3-6, 7 and 8, the 24 and 48 hours post exercise collections.

Figure 7. Mean Nitrogen Balance data for Diet Phase 1, unsupplemented and Diet Phase 3 supplemented with vitamin B-6 (dietary N intake - N output (urine + misc).

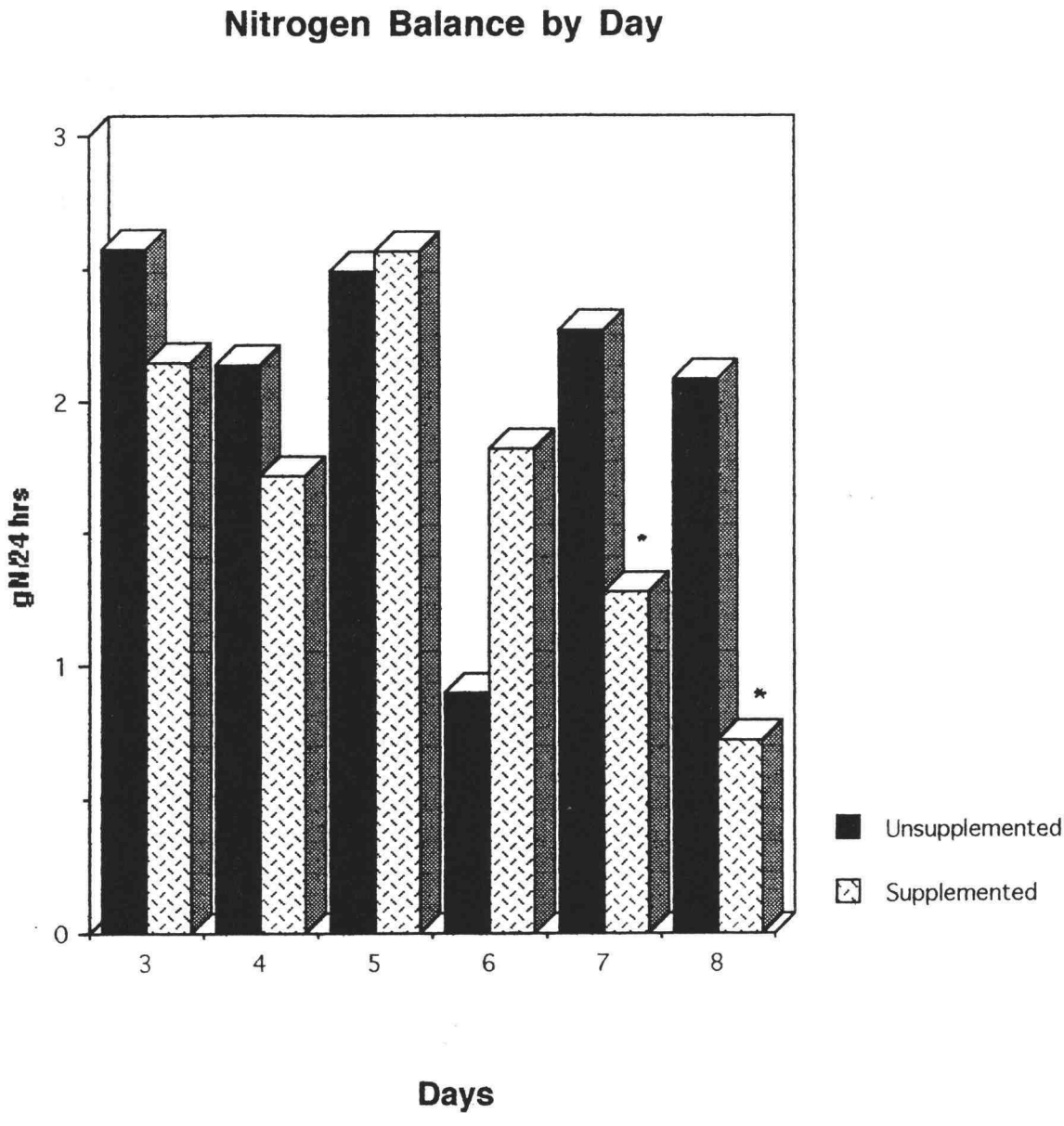


Table 7b Mean change in Total Urinary Nitrogen (TUN), Urinary Urea Nitrogen (UUN) and Nitrogen balance (NBAL) (gN/24hrs) for pre-exercise and post-exercise collections, Phase 1 & 3.

Days 3-6

	TUN	UUN	NBAL**
Phase 1	18.58	16.34	+2.03
Phase 3	18.48	16.11	+2.06
% change	**nc**	**nc**	**nc**

Day 7*

Phase 1	18.44	16.16	+2.27
Phase 3	19.83	16.89	+1.28
% change	**↑ 7%**	**↑ 4%**	**↓ 77%*** **

Day 8*

Phase 1	18.38	16.27	+2.08
Phase 3	19.96	17.45	+0.72
% change	**↑ 8%**	**↑ 7%**	**↓188%*** **

* post-exercise collections, ***Statistically significant ($p<0.05$)
**Nitrogen balance (NBAL) calculated via:
(dietary N intake - N output) + 4gm misc. losses.
All data gN/24hrs
↑ increase, ↓ decrease, nc no change
Diet Phase 1: without vitamin B-6 supplementation
Diet Phase 3: with vitamin B-6 supplementation

DISCUSSION

Whether or not endurance athletes need additional protein, above Recommended Dietary Allowances (RDA), has, and continues to be actively debated. Based upon currently available literature, recommended protein intakes for athletes range from slightly above, to 2 times the current RDA of 0.8g/kg/day (Zackin wt al. 1975; Gontzea et al. 1975; Wolfe et al. 1986; Lemon, 1984; Friedman and Lemon 1988; Tarnopolsky et al. 1988).

Despite the extensive amount of data available, a consensus has not been reached as to the role of protein as a fuel substrate during long-duration exercise. Several factors, surrounding the complexities of protein metabolism and the research methodologies applied, help explain the conflicting data. Namely, 1) the individuals intensity, frequency, duration and state of training, 2) dietary adaptation, composition (primarily carbohydrate), timing and overall energy balance, and 3) the difficulties of accurately assessing nitrogen elimination and balance, as well as the role of individual amino acids in fuel metabolism.

Our initial hypotheses were that through vitamin B-6 supplementation (20 mg/day) and exhaustive cycle ergometry testing, glycogen depletion would be accelerated and the hepatic (gluconeogenic) production of glucose increased. This due to pyridoxal 5'-phosphates (PLP) role in glycogenolysis, as a part of the glycogen phosphorylase enzyme. Though PLP is a water soluble compound, significant stores are located in muscle tissue as a part of this enzyme necessary for cleaving glucose 1-phosphate from the glycogen chain (glycogenolysis), to provide

glucose for energy production (Krebs and Fisher 1964; Manore and Leklem 1988).

Additionally, an expected decrease in exercise time would be observed, as muscle and liver glycogen were more rapidly depleted. The increased gluconeogenic production of glucose was expected to result in increased plasma urea nitrogen concentration and urea elimination in urine and sweat, possibly above that which was ingested via dietary sources. This is due to PLP's role as a part of the aminotransferase compound necessary to degrade amino acids to alanine and, subsequently, glucose (Manore and Leklem, 1988),

Increased gluconeogenesis during exercise has been well substantiated in the literature (Dohm 1985; Ahlborg et al. 1974, 1982) and is known to be regulated by both substrate availability and the prevalence of specific gluconeogenic enzymes. As exercise duration increases, glycogen becomes depleted and gluconeogenesis is elevated as amino acids donate their carbon skeletons to form alanine and subsequently glucose for energy. In a recent study by Anderson et al. (1990), subjects who exercised for 45 or 90 minutes (cycle ergometry, 70% $\dot{V}0_2$ max) showed a significant reduction in muscle glycogen content and an elevation in 24 hr urinary urea nitrogen elimination. Their results illustrate the effect of long duration exercise on urinary urea excretion. Ahlborg and associates (1974) observed an increased lactate, glycerol and alanine plasma clearance in exercising subjects and a corresponding increase in alanine by the working muscles. Thus, glucose precursors are delivered to and taken up by the liver more rapidly during exercise and their by-products are released for energy production. Metabolic regulation of gluconeogenic and glycolytic enzymes

also will affect substrate availability. In 1985, Dohm et al. showed an increase in the concentration of gluconeogenic enzymes pyruvate decarboxylase and fructose 1-6 biphosphatase with long duration exercise, while the concentration of glycolytic enzymes were decreased thereby supporting the increased role of exercise-induced gluconeogenesis. As alanine production is increased, a greater elimination of urea, via sweat, plasma and urinary losses is expected (Lemon et al. 1981; Rennie et al. 1981). Our urinary urea results did, in part, support these findings in that subjects supplemented with vitamin B-6 did show an increased urinary urea elimination (UUN) post-exercise as compared to the unsupplemented group. Mean UUN excretion prior to exhaustive testing (Day 3-6) were not significantly different between the unsupplemented (Phase 1) and supplemented (Phase 3) phases. During the 24 and 48 hours post-exercise UUN elimination did increase, though not statistically significant, by 4% and 7% respectively (Day 7 and 8). This data is in support of that observed by other researchers (Anderson et al. 1990, Ahlborg et al. 1974). Both Lemon et al. (1984) and Rennie et al. (1981) observed elevated urinary urea clearance during recovery after long duration exercise at above 50% of the subjects' $V0_2$ max. Lower intensity exercise (<50% $V0_2$ max) has consistently failed to show elevated UUN elimination (Wolfe et al. 1982; Cerny 1984; Lemon et al. 1982). If, following exercise, renal function returned to normal and hepatic gluconeogenesis continued to be elevated to supply substrate for glycogen re-synthesis, an increase in urinary urea was expected. However, the magnitude of our observed changes may have been greater had sweat data been successfully analyzed.

Total Urinary Urea (TUN) was assessed as an index of urinary urea Nitrogen elimination. The majority (85%) of nitrogen is excreted as urinary urea (Cerny, 1975). Prior to exercise testing, UUN as a percent of TUN remained close to the expected 85% (+/- 3%) between Phases 1 and 3 (Cerny, 1975). During recovery there were no observed differences between phases for the 24 or 48 hour recovery period (Day 7 and 8) 87% Phase 1 and 86% Phase 3.

Dietary intake of protein was 235% of the RDA, as determined from UUN and estimates for miscellaneous nitrogen losses (+4 g/day) (Manan and Arlin, 1992) As calculated, 15-16% of the subjects daily energy requirements were supplied via protein catabolism. This supports the observations and conclusions previously reported by Tarnopolsky et al. 1988 who reported 10-15% of the energy needs of athletes are supplied by protein.

Of the studies reviewed (Friedman and Lemon, 1989; Tarnopolsky et al. 1988; Zackin et al. 1986; Gontzea et al. 1975, 1974; Yoshimura et al. 1980; Meredith et al. 1989; Brouns et al. 1989a, b) only one reported a protein intake to maintain nitrogen balance above this studies intake, Yoshimura et al. 1980. In this study, 2 g/kg/day were required to maintain nitrogen balance in formerly sedentary subjects beginning a daily exercise program (first 1-2 weeks). Their results were substantiate by Butterfield and associates (1984) who agreed there is an increased need for protein, (negative nitrogen balance) but primarily for those who are beginning a regular endurance exercise program of moderate intensity. However, in contrast, as training continues, the body adapts to a lower protein intake and nitrogen balance returns to levels only slightly below pre-training

balance (Butterfield et al. 1984; Wolfe et al. 1984). All other studies reviewed found regularly training subjects to maintain nitrogen balance on a protein intake of 1.14-1.5 g/kg/day (95-188% of current RDA). In comparison with our own data, it is of no surprise that nitrogen balance remained positive throughout the entire study for both the unsupplemented as well as supplemented with vitamin B-6 phases. Excessive protein intake, if not used to build, repair or supply energy, will be readily converted to fat.

The significant changes in nitrogen balance observed during recovery appear to reflect a greater rate of amino acid catabolism, however several factors not directly assessed may have influenced these changes:

1) As a result of long duration exhaustive exercise the gluconeogenic production of glucose from amino acids may have lead to an increased UUN elimination thereby decreasing the positive NBAL status of the subjects following exercise. Our results are consistent with those repeatably observed in the literature and believed to represent an increase in exercise-induced gluconeogenesis (Anderson et al.1990; Brouns et al. 1989a,b; Friedman and Lemon 1989' Tarnopolsky et al. 1986). However, there were no significant differences in time to exercise-induced exhaustion observed between the two dietary phases. On one hand this may reflect incomplete glycogen depletion or more than likely, an inability of vitamin B-6 supplementation to significantly effect fuel substrate metabolism.

2) Depressed kidney function following exercise may have delayed the clearance of UUN until later in recovery. Research by Lemon et al. (1991a,b and 1986) have shown that kidney fuction is depressed for several (4-5) hours following long duration exercise and once normalized, result in an increased elimination of the excretable by-products of exercise, namely

urea. This change in clearance rate may in part explain the more significant changes in NBAL observed during the 48 hour collection period, Day 8, as compared to Day 7. 3) Following exercise there is an increased glucose and glycogen re-synthesis in a continued effort to maintain blood glucose concentrations and re-synthesize glycogen for muscle and liver stores. It has been reported that 24-36 hours are needed to fully replenish depleted muscle glycogen stores after intensive long-duration exercise (Astrand et al. 1986).

Though change in UUN elimination and NBAL was observed post-exercise, without sweat data it is difficult to draw cause and effect conclusions as to the source and use of amino acids catabolized during and/or following exhaustive exercise. However, these differences, between the unsupplemented and supplemented with vitamin B-6 groups, despite no observed changes in time to exercise-induced exhaustion, may reflect more of vitamin B-6's (as PLP) role in the rate of gluconeogenesis and not glycogenolysis.

Estimates of the additional losses of nitrogen via sweat, sloughed skin cells, endogenous nitrogen loss and feces may have been underestimated. Nitrogen balance techniques tends to overestimate nitrogen intake, if using nutrient analysis tables alone, and underestimate excretion (Lemon, 1991). Intake, as determined by total urea nitrogen analysis of food composites may be variable as accuracy depends upon precise composite sampling, mixing and analyzing procedures. Excretion estimates may be underestimated as there are several routes for nitrogen loss via urine, feces, sweat, sloughed skin cells and endogenous gastrointestinal cell loss, but few studies seek to measure all and settle instead for estimations establish through previous

research. Obviously this can be a substantial error in that no two human bodies have identical nutrient requirements, metabolic regulation, or rates of waste elimination.

Dietary composition and energy intake, though well organized and tightly controlled, may have had impact on the urinary urea and exercise performance data (Lemon, 1991). As discussed, our subjects protein intake was 230% of the current RDA. Two subjects consumed additional calories during Phase 3 as compared to Phase 1, primarily carbohydrate. Subject 1, an additional 582 and subject 2, 101 calories on average. Subject 1 did increase time to exercise-induced exhaustion (>29 min), however subject 2 did not (no change) . The increased exercise time for subject 1 may have been as a result of increased glycogen stores, and/or increased available blood glucose at test time. With supplementation (Phase 3) elevated plasma PLP may have increased the breakdown of glycogen but not to the degree that performance was impaired. In addition, if the increased caloric intake occurred in the latter part of a dietary phase (day 4-6) urinary urea excretion and nitrogen balance may have varied due to caloric intake alone and not the effects exercise induced gluconeogenesis. However, a corresponding increase in urinary nitrogen was not observed for either subject.

As mentioned in the review (Appendix A), 8-18 days may be necessary to adapt to dietary change depending on the severity of change (Oddoye and Margen 1979, Scrimshaw, Hussein, Murray, Rand, Young 1972). Dietary adaptation for our study was 6 days prior to exhaustive testing for Phase 1 and 3. However, on review of the data (Table 20, Appendix B), little variation in UUN excretion was observed beyond the first day of

controlled feeding. Urinary urea nitrogen as a percent of TUN remained at 84% +/- 2% for days 1 and 2. This may reflect a higher protein intake pre-study which, would not be surprising if the subjects caloric intake were high enough to maintain weight during training. Conversely, it may also demonstrate again the inconclusiveness of using urinary urea measures alone.

Exercise intensity and duration have been mentioned as impacting amino acid degradation and therefore nitrogen elimination. On examination of the individual data, those who significantly increased their exercise duration during Test-2 did not show a corresponding increase in urinary urea elimination. Had they been adequately glycogen depleted, and terminated exercise due to fatigue, UUN should have increased over pre-exercise levels. According to previous research, an intensity of 64-75% of $V0_2$ max set for the exhaustive exercise testing was high enough that urinary urea should have increased (Lemon 1984; Rennie et al. 1981). However, intensity, duration and the lack of significant urinary urea changes may point to mental fatigue or boredom as reason for test termination as opposed to exhaustion. Subjects were asked to not exercise on the day prior to exhaustive exercise testing which should have diminished the residual effects of a training bout on the recovery data obtained.

Additionally, our subjects were not allowed to drink fluids during exhaustive exercise testing which, apart form being different from their normal routines, may have increased sweat urea losses and decreased urinary urea losses beyond normal levels due to dehydration. Dehydration may have also affected exercise duration for both tests. A 10% reduction in

exercise performance has been reported in subjects, deprived of fluids, during long duration exercise (Astrand, 1986).

As previously mentioned, the nature of protein metabolism and the methodologies used to assess substrate shifts, complicate the study of protein as a fuel provider during exercise. Based upon these factors, and the powerful tools of retrospect and hindsight, several considerations for future research are possible.

Research design itself encompasses a major limitation of this project, namely, the order effect of treatments applied. As mentioned, beginning the study with the unsupplemented dietary phase (Phase 1) decreased overall project cost and duration, but, may have influenced the results and can not be ruled out as explanation for certain subjects' increase or decrease in exhaustive exercise times. A learning effect compounds this issue in that the supplemented with vitamin B-6 test (Test-1) followed the unsupplemented testing (Test-2). Familiarity with exhaustive exercise testing may have increased exercise duration during Test-2 for some individuals, and decreased exercise duration for others, depending on their level of interest and motivation.

Glycogen depletion was implied via, an overnight fast before early morning testing, and a exhaustive-exercise protocol that promoted more than 90 minutes of continuous exercise. However, true muscle glycogen depletion can only be assessed to any degree of accuracy by muscle biopsy techniques which were not available due to limited laboratory equipment necessary for analysis.

Though, ideally, research results should be applicable to a wide human population, continuity of the subjects physical and performance

characteristics are necessary in order to prove a cause and effect relationship. Training status and exercise routine are two such variables needing tight control. Training status has been shown to influence exercise capacity, duration and fuel substrate utilization (Astrand, 1986). Our subjects had somewhat variable fitness levels and functional capacities as measured by V02 max testing. And though training regimes were to be continued at the same level during as before study participation, there were variations between subjects. Some trained more consistently and aggressively while others continued a maintenance program only. Training, as is well known, results in an increased mitochondria concentration in muscle tissue, an increased glycogen storage capacity, as well as an increased concentration of metabolic enzymes (branched chain oxoacid dehydrogenase) necessary for specific branched chain amino acid oxidation. Therefore, the better trained individual, or one who simply increased training intensity through out the project, may have increased reliance of fatty acid metabolism and increased glycogen storage capacity during the two weeks between exhaustive tests.

A total of six subjects not only severely limits the application of results, it makes drawing conclusions about cause and effect relationships nearly impossible. A limited subject pool will always limit the power of any statistically significant findings, therefore larger studies, which include women as well as men, are needed.

Future research in protein utilization during endurance activities should incorporate, as standard procedure, post exercise urine collection with shorter collection time intervals, ie. urine collections post exercise testing at 2-3 hour intervals (for several days) adjusted after analysis to 24

hour values, would enable measurement of time coarse changes in nitrogen excretion. A minimum of 48 hours post exercise collection is necessary to allow kidney function to return to baseline unless hydration is maintained during exercise testing (Dolan, Hackney & Lemon, 1987)

Also, researchers should avoid nitrogen balance measures based solely on urinary urea excretion as the index of change in protein metabolism. Nitrogen balance techniques may not be sensitive enough to detect subtle changes in protein status. Additionally, nitrogen balance methods require strict subject compliance and cooperation through out the study period, substantial adaptation periods following dietary changes in protein and energy intake may be necessary in order to secure a reliable baseline (Scrimshaw et al. 1972; Goranzon & Forsum 1985; Munro 1951). plasma, RBC and muscle tissue needs closer evaluation during exercise testing and recovery. There is growing evidence that certain amino acids (leucine, isoleucine, valine, glutamate and aspartate) appear to be degraded during exercise while others during recovery (Hood and Terjung, 1990). More sophisticated research techniques able to estimate or directly measure endogenous amino acid turnover and the metabolic pathways are necessary. Plasma changes in urea and amino acid concentration, specifically the branched chain amino acids and alanine, should be measured while on strictly controlled diets with known amino acid supplementation during controlled exercise testing.

Overall, the NRC/RDA is still reluctant to increase protein or specific amino acid requirements for individuals who regularly engage in long duration exercise. Based upon the research reviewed, an intake above what can be supplied through a balanced, adequate calorie diet may not yet

be warranted. However, as research continues to investigate individual amino acid metabolism, the future may hold and RDA which has specific requirements for each essential amino acid, applicable to varied populations; athletic, growing and aging. Such research will need tight controls over diet composition, total caloric intake, meal timing, exercise intensity, duration and training status and utilize advanced techniques that trace amino acid degradation and synthesis.

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APPENDIX A

Literature Review

Vitamin B-6:

The metabolic roles of carbohydrate, fat and protein during exercise are well documented in the exercise science literature (Astrand, 1986). Research has shown that the predominant fuel substrates for long duration activity are fatty acids and to a lesser, but vital extent, glycogen and glucose. Long duration activity, though primarily utilizing fatty acids as fuel, requires a continuing supply of carbohydrate and glycogen to form blood glucose necessary to maintain muscle as well as central nervous system functions. The source of carbohydrate, during exercise, is predominantly stored muscle and liver glycogen and, to a lesser extent, the products of gluconeogenesis. As activity continues, the body's stores of glycogen are eventually depleted and activity becomes limited. Gluconeogenesis refers to the process of converting non-carbohydrate substrates (amino acids, lactate and glycerol) to glucose which can be used to maintain activity.

The athlete who is able to enhance and/or spare glycogen stores may be able to exercise for a longer duration and improve exercise performance. Glycogen storage can be affected by diet. Research shows that a high fat diet limits glycogen repletion and increases plasma free fatty acid concentration (Maughan, Williams, Campbell and Hepburn, 1978). In contrast, a high carbohydrate diet has been shown to decrease free fatty acid concentration during exercise and optimize glycogen storing capacity (Manore and Leklem,1988).

The ability to enhance or limit glycogen breakdown is metabolically regulated within the muscle by a series of enzymatic reactions, but predominantly by the enzyme glycogen phosphorylase (Krause's, 1992). This enzyme, when activated, acts to break down glycogen to form glucose 1-phosphate, a primary reaction of glycolysis. Once glucose-1-phosphate is

formed, it is then made available to the Kreb's Cycle within the mitochondria to form energy utilized by working muscle. Glycogen phosphorylase is dependent upon pyridoxal 5'-phosphate (PLP) for activation. PLP is the most active form of vitamin B-6 (Leklem, 1988a,b).

History:

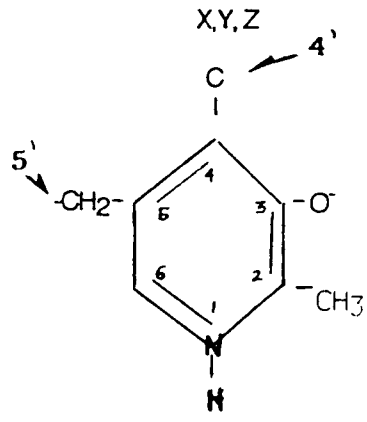
Vitamin B-6 was first discovered in 1934 by Gyorgy as a cure for a dermatitis in rats not attributed to deficiencies of other known B-vitamins. In 1938 Lepkovsky isolated a similar form of the vitamin from rice bran. Also during this year Kohn, Wendt, and Westpal synthesized pyridoxine (PN) and gave it its name (Merrill, 1988).

In 1939 the structure of pyridoxine was established and named 3-hydroxy-4,5-his(hydorxymethyl)-2-methyl pyridine. Pyridoxine was later accepted as the abbreviated name. In 1945 Snell discovered two additional forms of the vitamin; pyridoxamine (PM) and pyridoxal (PL). Soon after the chemical structure of pyridoxal 5'-phosphates (PLP) was identified and recognized as the active coenzyme form of vitamin B-6. Snyderman et al. (1953) first recognized and established B-6 requirements in humans (Merrill, 1988)

Structure and Classification:

All three free forms of vitamin B-6 (PN, PM, PL) are derivatives of 3-hydroxy-2-methyl-pyridine. The side chains of the pyridium ring at position 4 determine the primary forms (Figure 8). The three free forms also exist in the phosphorylated forms; pyridoxal 5'-phosphate (PLP), pyridoxine 5'-phosphate (PNP) and pyridoxamine 5'-phosphate (PMP). Position 5 on the

Figure 8. Chemical structure of Vitamin B-6 (Adapted from Merrill & Burnham, 1988).



GROUPS AT POSITION 5'	GROUPS AT POSITION 4'
HO	HC - OH (pyridoxine) | | H
$^{=}O_3PO$ (5'-phosphate)	
B-(D)- glucose (glucoside)	HC - NH_3^+ (pyridoxamine) | | H
	HC - C (pyridoxal) |
	C = O (4-PA) | | O

pyridium ring is the site of phosphorylation and glucoside linkage. A conjugated form of pyridoxine, 5-0-(B-D-glycopyranosyl) pyridoxine also has been identified and abbreviated as pyridoxine B-glucoside. The phosphorylated forms predominate in tissue and organ sites. PLP and PMP both function as coenzymes in tissue, however PLP predominates in this classification. Pyridoxic acid (4-PA) is the form of vitamin B-6 excreted in urine when excess B-6 is taken in or unabsorbed.

Sources:

In foods, the three free forms of vitamin B-6 are found in both the phosphorylated and non-phosphorylated forms. The best sources of vitamin B-6 are yeast, wheat germ, pork, liver, whole grain cereals, legumes, potato's, bananas and oatmeal (Leklem 1988,1980; Manan and Arlin 1992). Pyridoxine (PN) and PM predominate in plant foods. In addition, the conjugated form, pyridoxine B-glucoside, has been identified in several plant foods and, though well absorbed, appears not to be well utilized. The lower bioavailability of vitamin B-6 from plant foods has been attributed, in the past, to the presence of the conjugated form of the vitamin. Animal foods contain well absorbed and utilizable vitamin B-6. Milk, eggs, other vegetables and fruit contain small amounts of the vitamin (Krause's, 1992). Table 8 lists the vitamin B-6 and pyridoxine B-glucoside content of several foods (Leklem, 1988).

Chemical Properties:

Pyridoxine, a white, crystalline, odorless compound is soluble in water and alcohol. It is stable to heat in an acid medium, unstable in

Table 8. Vitamin B-6 and Pyridoxine B-Glucoside content of selected foods (Leklem, 1988).

Food	Vitamin B_6 (mg/100 g)*	Pyridoxine β-Glucoside (%)†
VEGETABLES		
Potatoes, cooked	0.394	42
Spinach, frozen	0.208	50
Carrots, raw	0.170	51
Cabbage, raw	0.140	46
Cauliflower, frozen	0.084	82
BEANS/LEGUMES		
Soybeans, cooked	0.627	57
Beans, navy, cooked	0.381	42
Peanut butter	0.302	18
Peas, green, frozen	0.122	15
NUTS/SEEDS		
Sunflower seeds, raw	0.997	36
Hazelnuts, raw	0.587	4
Walnuts, English	0.535	7
Almonds, roasted	0.086	0
FRUITS		
Avocados	0.443	3
Bananas	0.313	3
Orange juice, frozen	0.165	47
ANIMAL PRODUCTS		
Chicken, breast, raw	0.700	0
Beef, ground, cooked	0.263	0
Tuna, canned	0.316	0
Milk, skim	0.005	0

* All vitamin B_6 values are reported as pyridoxine equivalents.

† The percentage of pyridoxine β-glucoside was calculated by dividing the amount of pyridoxine β-glucoside by the total amount of pyridoxine (vitamin B_6).

alkaline and neutral pH environments. Exposure to light and high temperatures, food processing, and storage destroy the vitamin. For example, the processing of grains to flour may result in a 85% loss of available vitamin B-6 (Kies et al. 1984). Also, losses due to freezing of foods, may range from 30-55% (Sauberlich, 1985).

Recommended Dietary Allowances (RDA):

The RDA for vitamin B-6 is currently set at 1.6-2.2 mg/day for the healthy, disease free adult (NRC, 1989). The requirement for vitamin B-6 appears to increase as intake of protein increases (Miller, Leklem and Shultz, 1985). Adequate vitamin B-6 status can be maintained while consuming 0.016 mg B-6/g of dietary protein eaten. The RDA reflects a protein intake two times as large as the recommended intake of protein/B-6 as a safety margin, to account for individual differences. Extra protein needs associated with pregnancy and lactation should be paralleled by increases in vitamin B-6 intake. The RDA of vitamin B-6 for children and adolescents are not yet well defined and are currently based on average protein intakes. A special RDA for the elderly has not yet been identified, however, several metabolic studies have shown a substantial number of vitamin B-6 deficiencies present in this population, therefore further study is warranted (Manan and Arlin, 1992). Metabolic changes, as well as decreased health status in the elderly population may impact requirements (Manan and Arlin, 1992).

Physical activity and its effects on vitamin B-6 requirements and metabolism remains an active area of study. Some research reports suggest that exercise may increase vitamin B-6 requirements (Manroe et al.

1987, Dreon and Butterfield 1986). Table 9 lists other factors that may influence vitamin B-6 status.

Absorption and Excretion:

Absorption of the three free forms of vitamin B-6 occur by passive diffusion in the small intestines, predominantly the jejunum, and to a lesser extent the duodenum and ilium (Henderson, 1985). The phosphorylated compounds require alkaline phosphate to remove the phosphorylase from the pyridine ring, therefore allowing the compound to cross the cell membrane before absorption (Middleton, 1977, 1978). Once absorbed, the vitamin enters the blood and is transported to the liver. Metabolism of vitamin B-6 occurs primarily in the liver, where pyridoxamine 5'-phosphate and PNP are both converted to PLP and PL, the predominate vitamers. These innerconversions are possible due to the presence of a kinase catalyst, a riboflavin dependent oxidase, which acts to facilitate phosphorylation, or a phosphatase enzyme, which acts to remove the phosphorylase group (Merrill 1979; Merrill, Henderson & Wang, 1984; Lumen, Li, Lui 1985) (Figure 9). Figure 10 provides an overview of vitamin B-6 absorption, metabolism, transport and storage. The PLP formed but not directly used within the liver is released into circulation and found bound with albumin. Consequently PLP and PL are available for uptake by other tissues and cells. Erythrocytes (RBCs) are able to uptake PL and form PLP and are believed to act as a storage site and transporter for the vitamin. In addition, vitamin B-6 is required as an intermediate in the production of heme (Anderson, Perry, Clements, Greany 1989; Leklem 1983; Krause's 1992).

Table 9. Factors affecting vitamin B-6 requirements in humans (Leklem, 1988).

A. DIETARY- BIOAVAILABILITY

1. Physical structure of foods consumed.
2. Forms of vitamin B-6 in foods.
3. Binding of the various forms to macromolecules in foods.

B. DEFECTS IN TRANSPORT TO TISSUES AND CELLS

1. Impaired gastrointestinal absorption.
2. Impaired transport.

C. PHYSIOLOGICAL/BIOCHEMICAL

1. Protein intake
2. Increased catabolism
3. Growth
4. Sex differences
5. Aging process
6. Physical activity
7. Nutrient interaction-riboflavin, carbohydrate

D. GENETIC

1. Defects in apoenzymes requiring pyridoxal-5'-phosphate
2. Altered levels of apoenzymes requiring pyridoxal-5'-phosphate

Figure 9. Innerconversions of vitamin B-6 (Leklem, 1988).

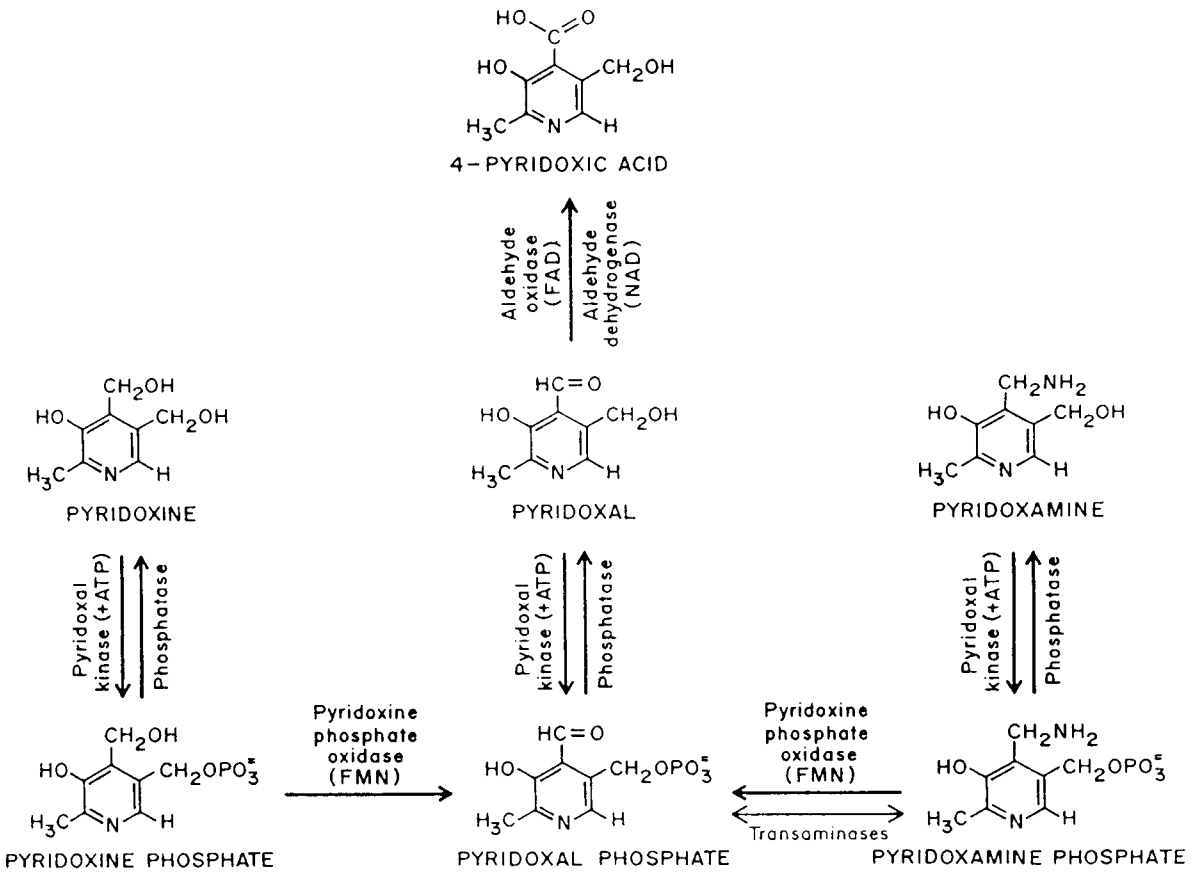
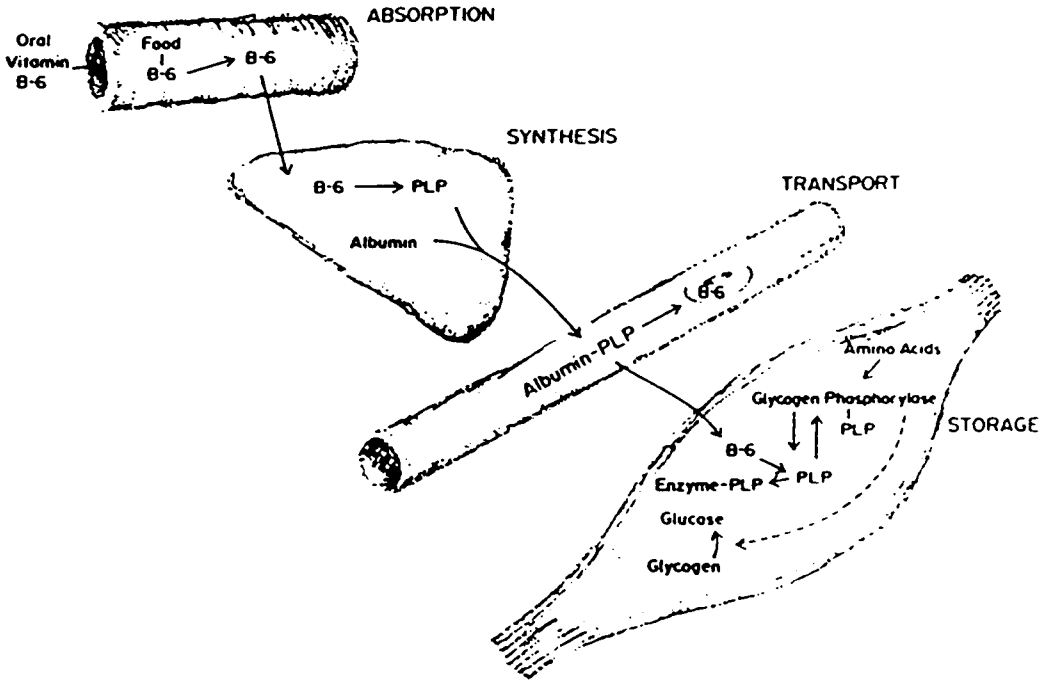


Figure 10. Vitamin B-6 (PLP) path of absorption, metabolism, transport and storage (Leklem, 1988).



The phosphorylated forms of vitamin B-6, primarily PLP, do not appear to readily cross cell membranes due to the content of phosphatase enzyme within tissues. However, the non-phosphorylated compound, PL, is the predominant form able to cross cell membranes. Once absorbed into the tissue cells, PL may be re-phosphorylated by action of the pyridoxal kinase catalyst to PLP (Leklem, 1988b). Because PL and PLP are the predominant forms, they are most often used to assess vitamin B-6 status.

Any excess or unabsorbed PLP is converted, in an irreversible reaction, to 4-PA and excreted in urine. Feces and sweat have also been found to contain measurable amounts of the vitamin's metabolites. 4-PA excretion accounts for 40-60% of the ingested, unabsorbed vitamin B-6 when taken in adequate amounts. However total excretion does not appear to completely account for intake, thus suggesting some retention of this water soluble vitamin. Oral supplementation with pyridoxine provides useful data concerning absorption, utilization, metabolism and excretion. Urinary excretion of PLP, PL and 4-PA are the most widely used methods for vitamin B-6 status research (Rabinowitz and Snell, 1948).

Body Stores:

Vitamin B-6 is found within most body compartments. The liver, brain, kidney, heart and skeletal muscle all contain vitamin B-6. The highest concentrations of the vitamin, per gram of tissue, are in the liver, followed by the kidneys, brain, skeletal muscle and heart (Thiele and Brin, 1966). However, it is the skeletal muscle which contains the most vitamin B-6 in terms of total body levels.

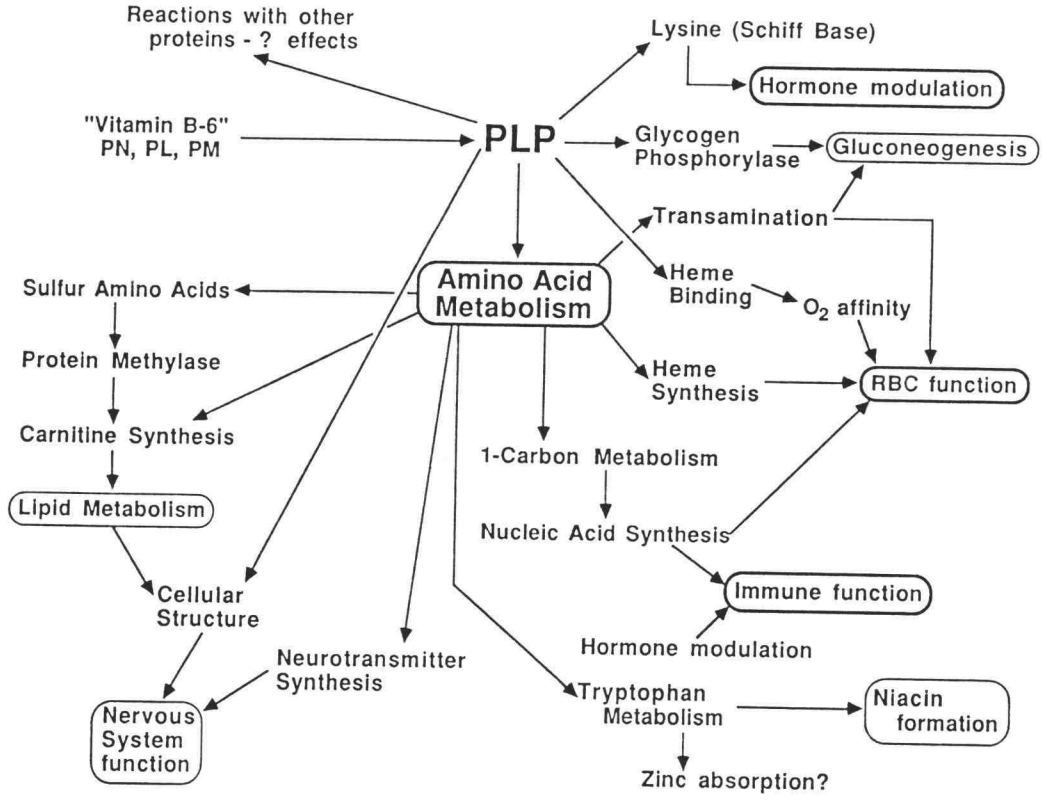
As a water soluble vitamin, it is accepted that excess B-6 intake would be eliminated in the urine and feces. However, with large doses, 4-PA excretion does not rise to the degree expected from the dose. Therefore, vitamin B-6 must be either stored in body compartments, or excreted as a B-6 vitamer different than the dose given. Krebs and Fisher (1964) have estimated that nearly one-half of all vitamin B-6 in the body exists as stored PLP bound to muscle glycogen phosphorylase, while Coburn and associates (1988) have more recently suggested that closer to 70% of PLP is found as a part of this enzyme. It is believed that PLP's bond to this coenzyme protects it from hydrolysis and thus serves as a reservoir for the vitamin (Fonda and Hawks, 1982). Black et al. (1977) research supports the reservoir theory. Rats given vitamin B-6 supplements showed an increase in muscle glycogen phosphorylase on muscle biopsy vs the unsupplemented control group.

Functions:

Vitamin B-6 is involved in many diverse metabolic processes (Figure 11). PLP itself acts as a co-enzyme in over 100 enzymatic reactions (Sauberlich, 1985). Some PLP dependent enzymes include: transaminases, racemizases, oxioreductases, isomerases, desulfhydrases and deaminases (Merrill and Burnham 1988; Devlin 1986).

Pyridoxal 5'-phosphate and PMP are the most active forms of the vitamin and are primarily involved in transamination and other reactions related to protein metabolism. Amino acid, lipid and carbohydrate metabolism are all affected by PLP enzymes. Two reactions of importance are, formation of a Schiff base with amino groups of amino acids/proteins,

Figure 11. Functions of pyridoxal 5'-phosphate (PLP) (Leklem, 1988).



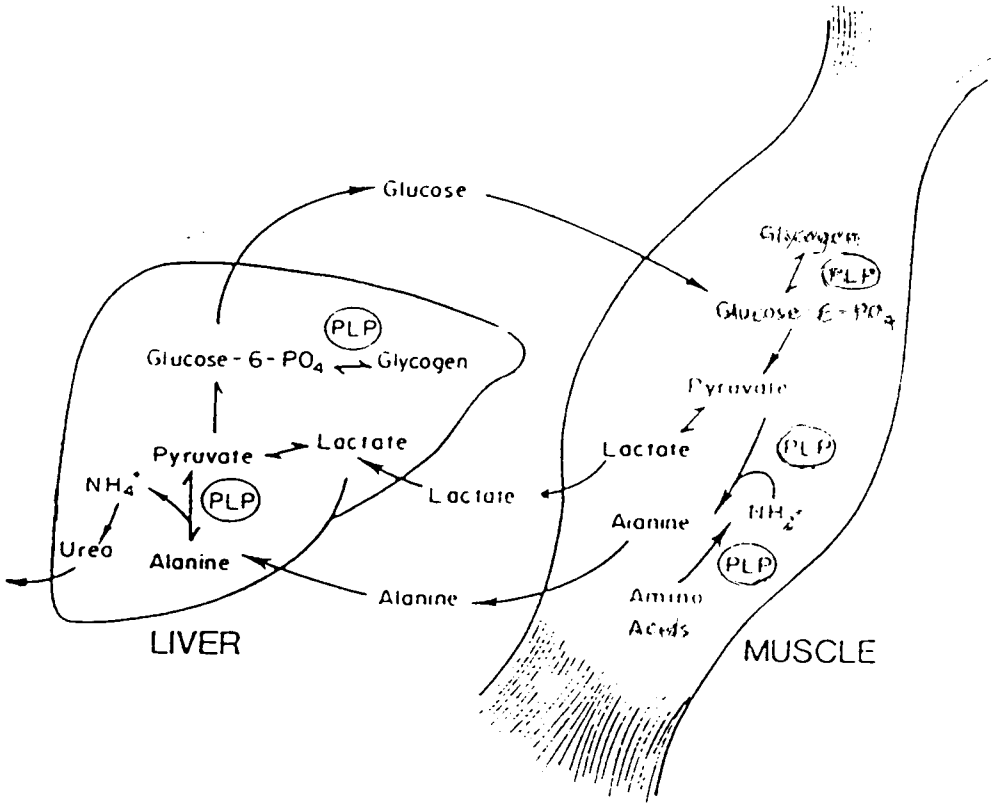
and secondly, PLP plays a major role as a part of the enzyme glycogen phosphorylase necessary to break down glycogen in muscle stores. Other cellular processes requiring PLP are gluconeogenesis, niacin formation, immune function, lipid metabolism, nervous system function, hormone modulation, erythrocyte function and amino acid catabolism (Leklem, 1988).

Perhaps PLP's most prolific roles are those associated with protein metabolism and, consequently, it is often referred to as "the" amino acid coenzyme (Bohinski, 1987). Transamination, the removal and transport of the a-amino group from one amino acid to the a-carbon of an a-keto acid, requires PLP. PLP is bound to the amino acid during transamination, therefore enabling its actions. The by-products of transamination form citric acid cycle intermediates (acetyl CoA) necessary to generate energy for cellular metabolism. Transamination is an essential step in not only the exchange of amino groups, but also the synthesis of non-essential amino acids and amino acid degradation.

Muscle, the largest storage site for PLP, also contains glycogen phosphorylase, an enzyme required to convert stored glycogen to glucose 1-phosphate during glycolysis (Cori & Illingsworth, 1957). This process is called glycogenolysis. PLP, as mentioned, found bound to the glycogen phosphorylase, is necessary for this enzyme's activity.

PLP also plays an important role in gluconeogenesis. Gluconeogenesis (via the livers glucose-alanine cycle) (Figure 12). PLP's roles are those of transamination and deamination of the various amino acids recruited to form alanine in skeletal muscle. The required PLP dependent enzyme for this reversible reaction is alanine-aminotransferase. Once formed, alanine travels to the liver, via the blood, and is deaminated,

Figure 12. Pyridoxal 5'-phosphate (PLP) involvement in glucose and alanine metabolism in the Cori-Alanine cycle (Leklem, 1988).

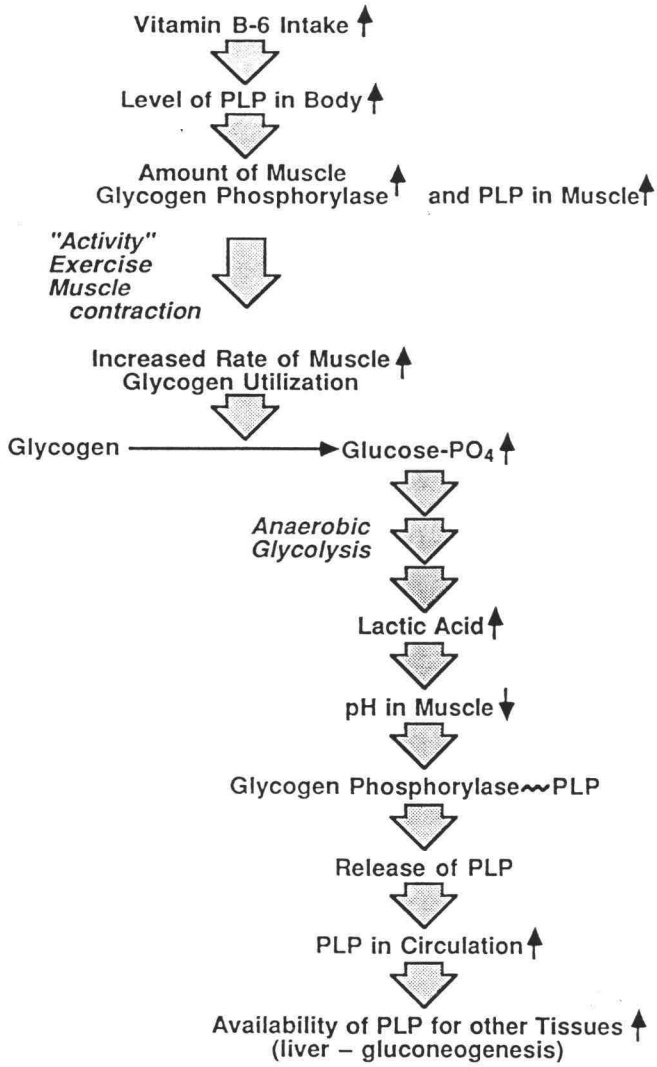


forming pyruvate and ammonia. The pyruvate is then available to produce glucose, glycogen or lactate, as dictated by the metabolic state of the body. The ammonia is eliminated via the kidneys, sweat, or feces as urea.

Leklem and Shultz (1983) observed an increased plasma PLP concentration in men following strenuous activity and proposed that with long duration exercise, a metabolic state similar to acute starvation would be achieved due to the excess calorie demands imposed through exercise. The caloric deficit was hypothesized to be a possible explanation for an increased plasma PLP level. Further, Leklem and Shultz believed that the PLP in plasma was released from muscle glycogen phosphorylase to be used to facilitate primarily the liver, in effort to generate glucose required to continue activity. Figure 13 illustrates the proposed mechanisms by which PLP facilitates hepatic gluconeogenesis.

More recently, Coburn and associates (1991) utilized muscle biopsy techniques to show that vitamin B-6 pools in muscle were unaffected by 6 weeks of vitamin B-6 deficient diets. Conversely, 6 weeks of supplementation with the vitamin resulted in slight (approx. 25%) increases in muscle PLP. Inadequate intake of vitamin B-6, or a dietary caloric deficit does not appear to effect muscle stores of the vitamin (as bound to glycogen phosphorylase). However, a exercise induced energy deficit increases PLP release. Perhaps, supplementation coupled with prolonged exercise may affect fuel metabolism, and performance, during exercise by increasing the loss of PLP bound to glycogen phosphorylase leading to rapid glycogen depletion and subsequently increasing hepatic gluconeogenesis to maintain glucose requirements.

Figure 13. Proposed mechanism for exercise-induced release of pyridoxal 5'-phosphate (PLP) from muscle glycogen phosphorylase (Leklem, 1988).



Protein:

The role of nutrition in performance is fast becoming a popular adjunct to successful training programs for professional athletes as well as the regular exerciser. Selection of a diet promoting optimum health and performance is a primary goal of the nutrition professional dealing with athletics.

Fuel supply for muscular activity and normal body functioning is maintained through dietary intake and body stores. The body obtains all necessary fuel from the by-products of carbohydrate, fat and protein metabolism. The two primary metabolic processes for energy production are:

1) Anaerobic: glycolytic & ATP-CP systems, and

2) Aerobic: tricarboxcylic acid cycle (TCA) & electron transport systems ETS (Astrand and Rodahl, 1986) (see Table 10).

It is well documented that carbohydrates and fats are the primary fuels for activity (Astrand, 1986). However, the role of protein as a fuel source during long duration activity has been repeatedly debated during the past 100 years (Butterfield, 1991; Lemon, 1987, 1991 ; von Liebig, 1842). Despite these researchers effort, there is still no consensus of proteins role as a fuel provider, due in part to the difficulties of quantifying protein absorption, synthesis and degradation.

Throughout history protein has gained attention as an aid believed to improve exercise performance. Since the times of Greek and Roman battles, competitors have believed that eating high protein foods, primarily from animal sources, would transfer to the athlete the strength and aggression the animals themselves possessed (Harris, 1966).

Table 10. Energy production pathways of the human body (A.C.S.M., 1991).

	Fuel substrate	O2 required	Speed of O_2 mobil	Total ATP production
Anaerobic Metabolism:				
A) ATP-CP systems	stored phos-phogens	NO	VERY FAST	VERY LIMITED
B) Glycolysis	glycogen glucose	NO	FAST	LIMITED
Aerobic Metabolism:				
A) TCA cycle, ETS	glycogen glucose fats protein	YES	SLOW	ESSEN. UNLIMITED

More recently, coaches, trainers and athletes still feel extra protein, above recommended dietary intake levels, will enhance strength, promote gains in muscle mass, and result in improved performance. A 1981 survey of university athletes and coaches revealed 98% believed performance would be improved by consuming a high protein diet (Grandjean et al., 1981). This belief has been perpetuated in professional athletics as well (Bentivegna, 1979).

Protein is an essential nutrient for life and accounts for 15% of human body weight. Table 11 lists body stores and the caloric potential of fat, protein and carbohydrate reservoirs. Protein, as can be seen, is second to fats in total caloric potential and 22 times that of carbohydrate reserves.

Structure and Classification:

Proteins, like fats and carbohydrates, contain carbon, oxygen and hydrogen. What makes these compounds unique is the presence of nitrogen, which makes up 16% of the protein molecule.

Proteins are formed by the linkage of amino acids to form chains or "building blocks" for cell constituents. Each protein molecule is a series of amino acids linked by peptide bonds formed between amino and carboxyl terminal ends of the individual amino acids (Robinson et al. 1986). Different arrangements of amino acids form different proteins as primarily dictated by the body's need to build, repair new tissues and, reluctantly, to provide energy (McArdle, 1990). 20 different amino acids are required by the body to assemble an infinite number of protein chains. Eight amino acids (9 in children and the elderly) are considered essential in that they cannot be synthesized in the body and must therefore be consumed as a part of the

Table 11. Human body reserves of fat, protein, carbohydrate (Adapted from Human Body Composition, Pass,ore & Brozek, 1965).

	Total body content (gm)	Caloric value (Kcal)
FAT	9,000	81,000
PROTEIN	11,000	44,000
CARBOHYDRATE	500	2,000

Calorie value based upon; fat = 9 kcal/gm, protein & carbohydrate = 4kcal/gm (FNC/NRC 1990)

diet. These essential amino acids are noted in Table 12. Two non-essential amino acids, cystine and tyrosine, are synthesized from the two essential amino acids, methionine and phenylalanine. The nine remaining amino acids are considered non-essential and are produced in the body from readily available compounds at a rate that meets the body's needs during the changing life cycles. Table 12 lists the non-essential amino acids.

Sources:

There are few foods which do not contain at least a limited supply of amino acids; however, plant and animal sources differ in their contributions to total amino acid requirements. Foods which contain all of the known essential amino acids are called complete proteins and are considered of higher biological value. Animal-origin foods are always complete. Sources of complete proteins include eggs, milk, meat, fish, and poultry. The egg is considered to be the highest quality protein and that to which all other foods are compared due to its wide array and concentration of amino acids. See Table 13 for a rating of protein quality in a variety of foods.

Plant foods generally have at least one missing or "limiting" essential amino acid and are considered to be of lower protein quality. Plant sources of proteins include lentils, dried beans, peas, nuts, cereals, grains, and, to a lesser extent, fruits and vegetables. It is possible however, to consume all of the essential amino acids on a high variety plant food diet (USRDA, 1989) that supplies necessary quantities of all essential amino acids. The body's requirement is not for complete protein chains, but for all essential amino acids. Table 14 lists the protein content of a variety of foods.

Table 12. Glucogenic and Ketogenic amino acids (Adapted from the Textbook of Biochemistry, Devlin, 1986).

Glucogenic	Ketogenic	Gluco- & Keto-genic
alanine	leucine*	isoleucine*
arginine		lysine*
asparagine		phenylalanine*
aspartate		tryptophan*
cysteine		tyrosine
glutamate		
glutamine		
glycine		
histidine*		
methionine*		
proline		
serine		
threonine*		
valine*		

(*) refers to essential amino acids

Table 13. Rating of common dietary protein sources (McArdle, 1990).

FOOD	PROTEIN RATING
Eggs	100
Fish	70
Lean beef	69
Cow's milk	60
Brown rice	57
White rice	56
Soybeans	47
Whole-grain wheat	44
Peanuts	43
Dry beans	34
White potato	34

Table 14. Protein content of foods (Adapted from Krause's Food, Nutrition and Diet therapy, 1992).

0-1gm	butter, margarine pear sugar
2-3 gm	cereal (1 oz) bread (a slice) corn (1/2 cup) french fries (1 serv)
4-6 gm	baked potato peas (1/2 cup)
7-8 gm	navy beans (1/2 cup) egg cheese (1 oz) tuna (1 oz) tofu (3.5 oz) milk (1 cup)
9-10 gm	peanuts (1oz) macaroni & cheese (3/4 cup) pizza, cheese (1/8 12" pizza)
12-15 gm	taco hamburger chili w/meat (1 cup)
22-26 gm	meat, lean (3 oz) Big Mac

Recommended Dietary Allowances (RDA):

Despite the long held theories of many coaches and athletes, muscle mass is not increased simply by eating high-protein foods. Consequently the Foods and Nutrition Board of the National Research Council (FNB/NRC) continue to recommend an intake of 0.8 g/kg body weight/ day (g/kg/d) for the healthy, disease-free adult (FNB/NRC:USRDA 1989). This recommendation has been based on nitrogen balance data assessing loss through urine, feces, sweat and skin, at rest in non-exercising adults. Due to the goals of the RDAs this intake is viewed as liberal and contains a safety margin of 0.35 g/kg/d to account for daily variations in dietary protein quality. Requirements vary for infants, adolescents, diseased, as well as pregnant and lactating women, and elderly adults. (For a list of these requirements refer to the FNB/NRC USRDA, 1989.)

Despite endorsements, for the current RDA of dietary protein, from the FNB/NRC, American Dietetic Association and other nutrition professionals, questions remain concerning the special needs of chronically training strength and endurance athletes. A growing body of research suggests that these individuals may have an increased requirement for protein or, more specifically, certain amino acids (Lemon,1991). Some researchers have proposed an intake of 1.2-1.4 g /kg/d (100-175% of the United States recommended intake) especially during early or intense training periods (Lemon et al.1991). The Dutch Nutrition Board (1988) is the only group which has actually proposed a 1.5 g/kg/d intake for active individuals. Despite the proposed increases in the protein RDA for American athletes, the increased needs would be adequately met through increased caloric intake without reliance on protein/amino acid supplements.

Americans typically consume excess protein due to a high intake of animal origin foods. For example, a 180 pound individual requires approximately 66 grams of protein per day (180 lbs/2.2 lbs/kg x 0.8 g/kg/day). This need can easily be met from a 6 oz can of tuna and 16 ounces of milk. Athletes on a high protein diet may consume 2-10 times the RDA from dietary sources as well as amino acid supplements and protein-fortified drinks.

Digestion & Absorption:

Protein digestion begins in the stomach, where proteins are split into proteoses, peptones,and large polypeptides by the digestive enzyme pepsin. However, most protein digestion takes place in the duodenum of the small intestines. Pancreatic proteolytic enzymes breakdown the intact protein and continue the digestive process, started in the stomach, until small polypeptides and amino acids are formed. Peptidases located in the brush border of the intestinal mucosa act on the polypeptides, cleaving them into amino acids, dipeptides and tripeptides. The final phase of protein digestion takes place when the dipeptides and tripeptides are hydrolyzed to their constituent amino acids by peptide dehydrogenases.

Amino acids are absorbed by four systems: one each for neutral, basic and acidic amino acids and one for proline and hydroxyproline. Absorbed amino acids and peptides are transported to the liver via the portal vein for release into general circulation (Kinney, 1988). The passage through the liver results in the removal of most amino acids except for the branched chain amino acids (leucine, isoleucine and valine). Branched chain amino acids make up approximately 8% of the dietary intake of amino acids but constitute 60% of the amino acids released into peripheral

circulation. Approximately 1% of the ingested protein is not absorbed by the time it reaches the jejunum and is then either utilized by bacteria in the gastrointestinal tract or eliminated in feces. Some amino acids remain in the epithelia cell and are used in synthesis of intestinal enzymes and new cells. Endogenous proteins from intestinal secretions and desquamated epithelia cells can be digested and absorbed from the small intestines.

There are no large reservoirs of amino acids in the body. Excess amino acids, above requirements, are not stored, but are used as energy or converted to fat. If used as immediate fuel the alpha-amino group is removed and the carbon skeleton is transformed into intermediates for the Krebs Cycle: acetyl-CoA, acetoacetyl CoA, pyruvate, -ketoglutarate, succinate, fumarate, or oxaloacetate. However, there does exist in the cellular proteins themselves a metabolic pool of amino acids in a state of dynamic equilibrium that may be used at any time to meet an appropriate need. Protein turnover in the body is probably necessary to maintain the amino acid pool so that the necessary amino acids are available when the body requires them for protein/tissue synthesis or catabolism for energy production. Figure 14 illustrates the fate of the the amino acid pools of the body (Lemon, 1991; Rennie, Edwards, Krywawych, Davies and Halliday 1981a; Dohm 1982). It has been estimated that 80% of the FAA pool is located within skeletal muscle, with the liver and kidney containing significant quantities as well (Poortmans 1988; Lemon 1991b;Rennie et al.1981a). The most active tissues for protein turnover (degradation to individual amino acids) are the plasma proteins, intestinal mucosa, pancreas, liver and kidney, whereas muscle, skin and brain are much less active sites. Once a part of the FAA pool, amino acids are available for

anabolism, the synthesis of new protein, or catabolism, the oxidation of amino acids for energy production in the liver and muscle (see Figure 14).

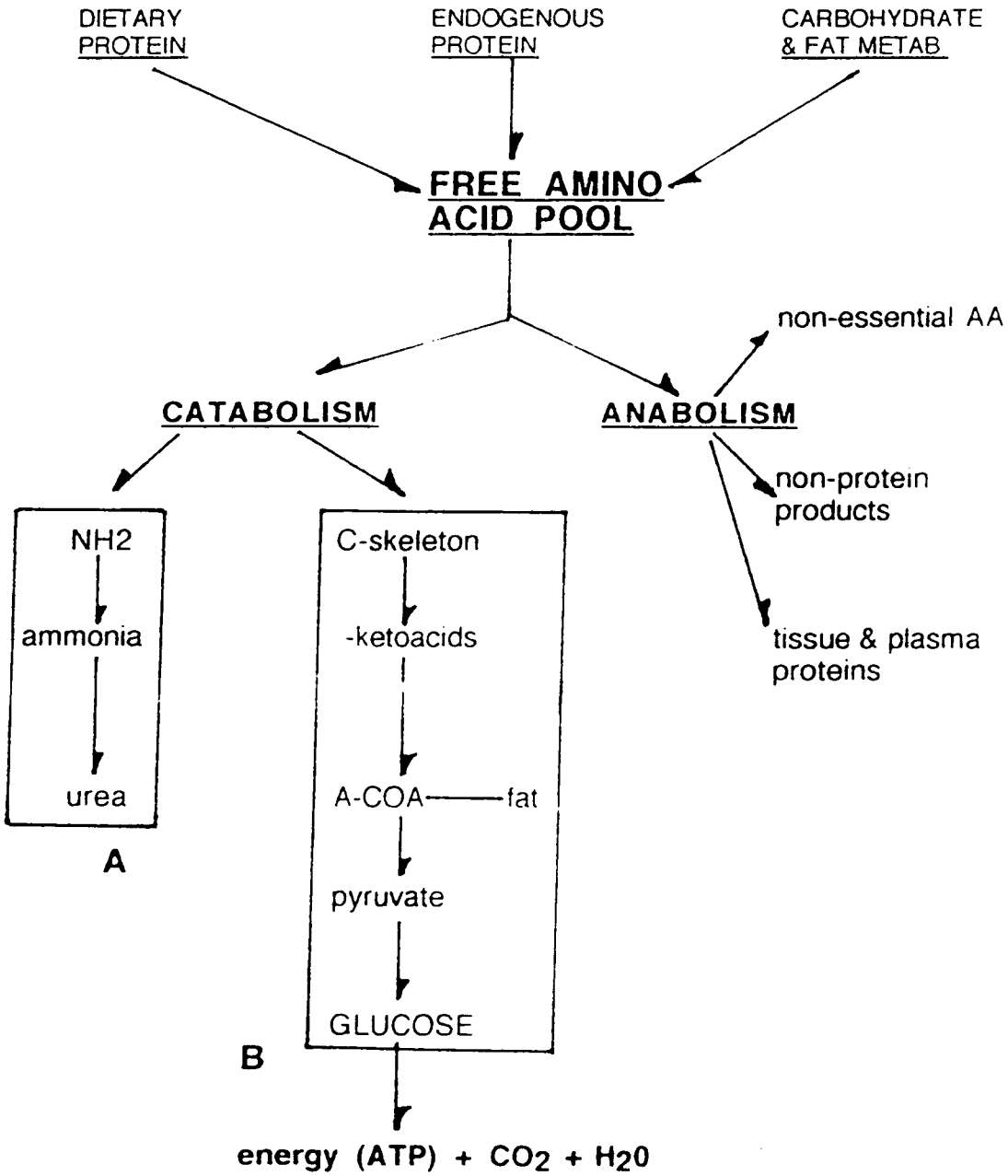
Functions:

Protein makes up approximately 12-25% of body mass; however, considerable individual variations exists at the cellular level. Skeletal muscle represents about 65% of the body's total protein and can be increased through resistance training and, to a lesser extent, with endurance training (Astrand and Rodahl, 1986).

Metabolic anabolism is the synthesis of cellular components, ie. muscle tissue. Growth demands a considerable proportion of amino acids for synthesis of new tissues. As the rate of growth declines, so does the demand for additional amino acids. However, the need for amino acids continues beyond growth cessation , as there is a constant turnover of tissue proteins. The appropriate amino acid must be available at the time of protein synthesis for a specific protein to be produced. When an inadequate intake of essential amino acids are consumed protein synthesis will be impaired and protein degradation increases. As a result whole body protein content is decreased. If continued, this process may result in decreased muscle mass, strength, endurance and perhaps impaired health.

Specific amino acids serve specific functions. Globular proteins make up nearly 2000 different enzymes that act as catalysts to speed up cellular reactions. Enzymes are critical requirements for energy production from fats, carbohydrates and proteins. In addition, enzymes are necessary for proper blood clotting reactions, oxygen transport, acid-base regulation, muscle contraction, hormone production and vitamin activation for metabolic

Figure 14. Amino acid pool of the body; Catabolism and Anabolism (Adapted from Williams, 1985).



A refers to the liver's urea cycle
B refers to gluconeogenisis

regulation. At rest, a normal adult may use amino acids to supply 2-5% of the body's energy needs, exercise may see this level rise to 10-15% (Lemon, 1991).

Nitrogen Balance:

When dietary nitrogen intake equals nitrogen excretion, the body is said to be in nitrogen balance. Positive nitrogen balance results from a greater intake than excretion and is typical during times of tissue growth and synthesis when more protein is being required. Growth, pregnancy, lactation, recovery from illness and intensive resistance training (to build mass) with adequate caloric intake, result in positive nitrogen balance (Manan and Arlin, 1992).

Negative nitrogen balance, where excretion exceeds intake intake, indicates the use of protein, primarily skeletal muscle, for an energy source. Negative nitrogen balance may be the result of protein catabolism, or an excess protein intake above recommended levels coupled with a low level intake of carbohydrate and fat (total caloric intake is low). If caloric intake continues to be inadequate, muscle wasting, or the loss of lean body mass, results and may lead to a decrease in athletic performance (Manan and Arlin 1992; Lemon 1991).

Exercise Effects on Protein Metabolism:

During prolonged exercise, lasting more than 2 hours, amino acids are made available for degradation by a reduction in protein synthesis and/or an increase in protein catabolism, if a calorie deficit exists, before or as a result of exercise (Rennie et al. 1981a). Regulation of protein metabolism may be under hormonal control during exercise in that exercise

results in a decreased plasma insulin level and an elevation of the plasma catecholamines, glucagon, and glucocorticoids. Lowered insulin levels lead to decreased protein synthesis and increased protein degradation. Elevated glucocorticoids and glucagon stimulate gluconeogenesis in the absence of available muscle or liver glycogen. The liver, and to a lesser extent muscle, are the principal suppliers of glucose during long duration activity via gluconeogenic production of glucose.

The body's metabolic response to prolonged exercise is not unlike that observed during acute starvation (Lemon, 1981), both result in decreased liver and muscle glycogen stores and an increased reliance on fatty acids to supply energy requirements. It would be ideal if all of the body's energy needs during activity could be met by stored glycogen and fat; however, research has shown that this is not entirely true. As exercise continues, liver and muscle glycogen are depleted but the body continues to require glucose for vital body, brain and central nervous system functions (Astrand and Rodahl, 1986). As a result, free amino acids from body pools as well as tissue amino acids donate their carbon chains to form glucose through a process called gluconeogenesis, the conversion of non-carbohydrate substrate (amino acids, lactate and glycerol) to glucose. Figure 16 depicts the conversion of amino acids to glucose. Specific amino acids are more readily utilized as energy via gluconeogenesis and are termed glucogenic amino acids. See Table 13 for a list of the glucogenic and ketogenic amino acids.

Gluconeogenesis:

In the liver, the primary substrate for gluconeogenesis is alanine, produced via the Glucose-Alanine cycle (see Figure 14). Quantitatively the branched chain amino acids (BCAA); leucine, isoleucine and valine are the most important amino acids available for the production of alanine (Felig and Wahren, 1971). Muscle has the capacity to degrade BCAA as well as the amino acids aspartate, asparagine and glutamate (Goldberg and Chang, 1978). Studies have shown that prolonged exercise results in an increase in BCAA uptake into skeletal muscle and an increased rate of BCAA release by the liver (Lemon & Mullin, 1980). Upon uptake into skeletal muscle, the amino acids are deaminated and transfer their amino groups to pyruvate to form alanine. The remaining carbon skeleton left after transamination is converted to one of several Krebs cycle intermediates and utilized directly in energy production (Lemon & Mullin, 1980).

The alanine produced circulates to the liver, where it is deaminated producing ammonia and pyruvate. The ammonia, combined with carbon dioxide, is eliminated as urea via the livers urea cycle in urine, sweat and feces. Depending upon the metabolic needs of the body, pyruvate may give rise to or be utilized for synthesis of lactate, glycogen or glucose. During prolonged activity the glucose produced is made available to skeletal muscle as useable fuel for cell metabolism. Ahlborg and associates (1976) have reported that as much as 45% of the glucose output needed to continue light activity for 4 hours is derived via the Glucose-Alanine cycle and gluconeogenesis. Greater exercise intensity increased the percent to 60. Felig and Wahren (1971) observed a 3-fold increase over resting values in alanine release from exercising muscle as intensity was increased.

Despite increases in amino acid oxidation, the total contribution of protein to the calorie demands of the body is small. It has been calculated that roughly 10-15% of the energy requirements necessary to perform prolonged exercise are supplied through gluconeogenic production of glucose from AA. Adequate caloric intake can easily supply the necessary AA (protein) requirements (Tarnopolosky et al. 1988).

Whether or not the RDA for athletes protein requirements needs to be increased remains to be decided. A growing body of research suggests that intake needs to be elevated for individuals beginning and continuing acute training programs but not for those maintaining a moderate intensity/ frequency program (Butterfield and Calloway 1984; Gontzea et al. 1975). However, research techniques thus far utilized have often been inconsistent and incomplete thereby limiting the ability to compare and contrast the available data.

DIETARY INFLUENCES ON PROTEIN REQUIREMENTS

Several diet related factors are known to affect nitrogen balance and possibly proteins use as a fuel source, these include energy balance, diet composition, meal timing and adaptation to dietary modifications.

Energy Balance & Diet Composition:

If caloric intake is inadequate, but protein intake adequate to meet the needs of the individual, they will still be found to be in negative nitrogen balance (Butterfield & Calloway 1984, Goranzon & Forsum 1985, Munro,

1951). An adequate caloric intake with excessive protein intake will result in positive nitrogen balance (Friedman & Lemon, 1989).

The combination of dietary modifications and long duration exercise have yielded interesting results that challenge our countries existing RDA's for athletes. For example, inadequate carbohydrate, but adequate protein intake will result in a more rapid depletion of stored muscle and liver glycogen during long duration exercise and an increased reliance on gluconeogenic pathways. This process, as mentioned previously, favors amino acids as its precursors in the absence of glycogen, and may lead to negative nitrogen balance (Anderson & Sharp 1990, Lemon & Mullin, 1980). The enzyme responsible for the gluconeogenic oxidation of the branched chain amino acids is branched chain oxoacid dehydrogenase. Activity of this enzyme is increased when glucose/glycogen stores are depleted, and exercise duration and/or intensity increased (Wagenmakers et al. 1990).

In a study performed by Friedman and Lemon, 1989, elite male runners were fed isocaloric diets with two different protein levels, supplied from a meat free diet, 1) the RDA of 0.86 g/kg/day and 2) a high protein diet (1.28 g/kg/day) 1.7 times the RDA. Subjects were exercise on a treadmill (75 minutes at 72%V02 max). Serum and urinary urea were measured for the day prior to, of and after exercise. Sweat data was collected only on the day of exercise. Their results showed a greater retention of dietary nitrogen for the high protein treatment group illustrating an increased protein requirement for the endurance trained athletes.

Meal timing:

Meal timing has also been found to affect proteins use as a fuel source.In a landmark study by Lemon & Mullin 1980, male cyclists were exercised (60 minutes at 61%$V0_2$ max) in a carbohydrate loaded (fed) and carbohydrate depleted (fasted) state. Analysis performed included urinary, sweat and serum urea measures pre, during and for 240 minutes post exercise. Their results were among the first to show that long duration exercise in a (assumed) more glycogen depleted state, increases serum, and sweat measures of urea. Urinary data showed an insignificant change as compared to the carbohydrate loaded individuals. Their conclusions suggested that depleted glycogen stores would result in a greater reliance on gluconeogenic production of glucose from amino acids.

Dietary Adaptation:

To accurately assess protein status, regardless of the type of dietary modification, an adaptation phase is necessary. This point is often overlooked and/or underestimated in the current body of research and ranges from 8 to 18 days, necessary before accurate analysis are possible, depending on the level of dietary manipulation . In a review article on protein requirements for athletes (Gatorade Sports Science Exchange, 1992) Butterfield & assoc. note that may studies fail to include adequate adaptation periods.

EXERCISE AND PROTEIN DEGRADATION

Training Status:

Training status and manipulations of exercise duration, frequency and intensity may affect the use of proteins as a fuel source. Limited data demonstrate that protein requirements are increased at the initiation of a regular training program. Gontzea et al. (1974 & 1975) found that subjects beginning a regular, moderate intensity endurance program have protein requirements up up to 1.5 g/kg/day for the first 20 days of training. (125-188% of the US RDA). Yoshimura et al. (1980) similarly found with protein intakes of 2.0 g/kg/day, total loss of blood proteins is minimized in endurance trained men during first 2 weeks of endurance training.

In a study performed by Einsphar & Tharp (1989) trained subjects exhibited a greater plasma alanine level (28% increase) during exercise than did the equally tested untrained group. Because of alanine's central role in gluconeogenesis it was believed that these results showed an increase requirement for protein in trained individuals. This data supports earlier work by Mole et al., 1973, which showed an increased level of cytoplasmic alanine aminotransferase activity in endurance trained men.

Exercise Duration & Frequency:

As previously explained as exercise duration continues to increase, blood glucose and muscle and liver stores of glycogen are depleted. The continued requirements for glucose result in an increased reliance on gluconeogenesis and its primary precursor: alanine. In this way exercise duration is believed to increase amino acid degradation and use as a fuel

provider (Paul, 1989). Haralambie & Berg (1976) measured changes in serum amino acid nitrogen and urea nitrogen in six groups of male athletes performing various types of activity (marching, running and cross-country skiing). Their results showed a significant increase in serum urea nitrogen, and a corresponding decrease in serum amino acid nitrogen, as exercise duration increased from 70 minutes to 765 minutes activity. As duration increased, so did the magnitude of change (Haralambie & Berg, 1976). Similar observations have been made by Dohm (1986) Lemon (1991) and Poortmans (1984).

In addition, an individual who is chronically endurance training (5-7 times per week) may be consistently depleting, or performing with limited muscle glycogen stores. At this training level, caloric intake is often inadequate to meet the athletes needs, therefore they are more likely to promote gluconeogenic use of amino acids to supply necessary glucose to continue activity.

In contrast, data from several other studies promote the possibility of an adaptation response to continued training. As a result, protein requirements are decreased as training is continued Butterfield & Calloway 1983, Gontzea et al. 1975). However, these studies typically utilized lower training intensities which in itself would facilitate greater reliance on fatty acid use and slowed glycogen depletion.

Overall increased protein utilization appears to be more related to availability of alternative substrate (ie. glycogen) than the exercise duration itself. This is supported by the fact that starvation, inadequate fuel availability, activates or increases exercise activation of branched chain oxo acid dehydrogenase (Wagenmakers et al., 1990).

Exercise Intensity:

Urea production has been shown to increase as exercise intensity is increased (Lemon et al., 1984a). Research utilizing varied intensities, 41%, 55% and 67% VO_2 max (treadmill tested) showed a greater sweat urea production at 55% and higher. Urinary urea concentrations were increased for 3 days following the 55% and greater intensities as well (Lemon et al., 1984). These results suggest a protein utilization threshold, above which, increases proteins use as an energy provider. Indirectly, Wolfe and assoc. (1982), supported these findings through a research project utilizing cycling (105 min) at 30% $V0_2$ max which did not result in an increase in urea production. A follow up study by Calles-Escandon et al. (1984) found that total urea excretion (urinary and sweat losses) increased by 100% during 90 minutes of cycling at 45% $V0_2$ max. These results lead researchers to agree that higher intensity exercise (above 45% $V0_2$ max) increases urea production as measured via sweat, urinary and plasma urea measures (Brouns et al. 1989a,b; Friedman & Lemon 1989; Lemon 1991b; Meredith et al. 1989; Tarnopolsky 1988, 1990). Additionally, an increased action of branched chain oxoacid dehydrogenase on amino acid degradation is believed to be enhanced at higher exercise intensities. Work by Tarnopolsky et al. (1988), Meredith et al. (1988) Friedman & Lemon (1989) Brouns et al. (1989a,b,) have provided supporting evidence for increased protein requirements with higher intensity, longer duration exercise.

Despite the observed effects of exercise intensity, frequency, and duration, these results can only be viewed from with in the context of the analytical methods employed. Confounding factors inherent in the study methodology can dramatically effect the interpretation of the data. Therefore

some discussion of assessment methods is important.

METHODS OF ASSESSMENT

Nitrogen Balance:

Methods used to assess protein requirements have become more sophisticated over the last few decades. Initially nitrogen balance techniques were the primary method for determining protein requirements, and those still used to determined the RDA's (Lemon, 1991b). The nitrogen balance measures employed simply utilized urinary urea nitrogen excretion versus dietary nitrogen intake to determine requirements. Urinary urea output is measured, adjusted for other extraneous nitrogen losses and compared to dietary protein intake. However, the current RDA, though containing a significant safety margin, is based on data from sedentary populations and does not account for possible variations as a result of regular, chronic endurance exercise. The growing body of research suggest that the effects of exercise, namely increased sweat and plasma urea concentrations, changes in kidney function, and continued data collection during recovery, reflect that the current RDA for protein may not be adequate for all athletes (Lemon and Proctor, 1991).

Exercise & Nitrogen Balance:

Early phases of exercise training may result in a transient negative nitrogen balance as the body develops necessary tissue and the metabolic energy pathways are trained to recruit greater amounts of the body's energy needs from free fatty acids (Astrand, 1986). Research has shown that an

untrained individual predominantly relies on carbohydrate as a fuel source during low to moderate intensity activity as compared to a trained individual who more quickly recruits fat as a fuel provider (Astrand, 1986). This being true the untrained individual will more rapidly deplete glycogen stores and increase the catabolism of amino acids. As training is continued, muscle and liver glycogen stores are increased and the individual shifts to an enhanced ability to utilize fatty acids. in this way, a transient, short term, negative nitrogen balance may surface as a result of substrate availability more so than the dynamics of exercise.

However, long duration exercise, similar to short-term starvation, results in negative nitrogen balance due to the lack of available energy from glycogen, and blood glucose in the trained as well as untrained individual. Therefore, endurance activities may result in a short and/or long term increases in amino acid requirements through increased reliance on gluconeogenesis.

Urinary Urea excretion:

Urinary urea has been a measure often to assess nitrogen balance Lemon and Proctor, 1991). The majority of nitrogen (85%) is excreted in urine as urea, therefore, monitoring changes in urinary urea elimination as a percent of total urinary nitrogen can identify shifts in nitrogen retention or elimination. Although less complete as a single measure, its ease of use for subjects and researchers cannot be overlooked. Typically, urinary urea measures alone underestimate protein utilization due to the effects of dehydration, blood flow shifts, depressed kidney function, and increased urea excretion via sweat (Lemon and Proctor 1991, Tarnopolsky 1988).

Exercise testing has yielded conflicting results as to whether urinary urea concentrations increase as exercise duration or intensity increases. Table 14a outlines several key studies and their results.

Based on more comprehensive techniques now utilized, it is widely accepted that urinary urea measures alone are a poor indicator and assessment tool for nitrogen loss during and after exercise but coupled with sweat and plasma urea excretion data under strictly controlled settings will provide a accurate assessment of nitrogen balance.

Sweat & Plasma Urea:

As an extension of nitrogen balance techniques, data reflecting plasma, sweat, and tissue changes in urea nitrogen concentrations are now more widely employed. Several studies have shown a significant elevation of plasma and sweat urea concentrations during and following endurance exercise testing (Dohm et al., 1982, Haralambie & Berg 1976, Lemon & Dolny 1986, Lemon & Mullin 1980, Refsum & Stromme 1974, Van Zant & Lemon, 1990).

In a study by Lemon and Nagle, 1981, a 14-fold increase over resting sweat urea nitrogen (mg/hr-1) concentrations was observed during exercise while on a low carbohydrate diet. Unfortunately, studies often overlook or inaccurately assess sweat urea concentrations. Currently the best method for sweat collection is the whole-body wash down procedure (Lemon, P.W.r., Yarasheski, K.E., Dolny, D.G. ,1986.

Plasma urea levels have been widely shown to increase with long duration exercise. Many of the studies concerning exercise intensity have utilized plasma urea measures as indexes of change.

Table 4a. Urea production and exercise: effects of duration & intensity (Adapted from Paul, 1989)

REFERENCE	MODE	DURATION	INTENSITY (%VO2 max)	RESULTS
Calles-Escandon et al. (1984)	Cycling	1.5 hr	45 %	↑SW, NC U
Cerny (1975)	Cycling	2 hr	60%	↑SW, ↑S. ↓U
Decombaz et al. (1979)	Running	100 km	-	↑SW, NC U
Haralambie & Berg (1976)	Running Nordic Skiing Walking	1.17-12.75 hr	-	↑S
Lemon et al. (1984a)	Running	1 hr	41, 55 & 67%	↑SW(55,67>41) ↑U (55,67), NC U (41)
Lemon & Mullin (1980)	Cycling	1 hr	60 %	↑SW, ↑S, ↓U
Rennie et al. (1981a)	Running	3.75 hr	50 %	↑P, ↓U (during) ↑U (after)
Wolfe et al. (1982)	Cycling	1.75 hr	30%	NC P

↑ increase, ↓ decrease
(NC) no change, (SW) sweat urea, (U) urinary urea, (P) plasma urea.

Individual Amino Acid Oxidation:

Recently, methods are being utilized that identify tissue level changes in specific amino acids, primarily the branched chain amino acids and alanine. Results indicate that concentration of these amino acids are increased at the tissue level following long duration exercise(Einsphar & Tharp, 1989).

Assessments of whole body leucine oxidation, a branched chain amino acid, have shown an increase in amino acid degradation during regular exercisers (Henderson et al. 1985, Hagg 1982, Lemon et al. 1987 & 1982, Rennie 1981, White & Brooks 1981). Branched chain amino acids are quantitatively more important because they are actively taken up by exercising muscle whereas other amino acids are released into the blood stream during endurance exercise (Ahlborg et al. 1974). Lemon & Nagle (1981) have proposed that the increase in leucine oxidation may be in part due to the increased activity of carnitine palmitoyltranferase following exercise training. Also mentioned were elevated resting levels of leucine, isoleucine and tyrosine in the trained vs. untrained groups. These results may point to an affinity of muscle for specific amino acids other than just the branched chain amino acids.

Wolfe et al. (1987), showed that the oxidation of lysine, an essential amino acid, is increased, but to a lesser extent than leucine, during long duration exercise. Individual amino acid oxidation continues to be the more active area of research in the question of the effects of exercise on protein metabolism.

Despite the variety of research and the methods used to assess nitrogen balance changes, no direct benefit to exercise performance has

been attributed to increased protein intakes in endurance athletes. The primary fuel substrate for activity continues to be carbohydrate and fatty acids. Additionally, adequate carbohydrate and caloric intake are the best methods for optimizing available energy substrate during activity for all individuals.

APPENDIX B

Supplemental Materials

Table 15. Subjects' physical characteristics.

SUBJECT	AGE	HT(cm)	WT(kg)	%FAT	VO_2(ml/kg)
1	34	177.8	75.3	9.5	59.74
2	21	175.3	69.3	12.1	69.47
3	37	182.9	73.3	12	61.38
4	22	179.1	70.8	9.5	58.6
5	25	174.0	79.1	5.8	45.31
6	19	177.8	73.9	16.4	63.22
x	26.3	179.0	73.6	12.6	59.60
SD	7.4	2.6	3.5	3.0	7.9

Table 16. Cycle Ergometer Workloads

AT 80 RPM's: MIN	WATTS	KG	KPM
1	80	1.00	480
2	110	1.375	660
3	140	1.75	840
4	170	2.125	1020
5	200	2.50	1200
6	230	2.875	1380
7	260	3.25	1560
8	290	3.625	1740
9	320	4.00	1920
10	350	4.375	2100
11	380	4.75	2280
12	410	5.125	2280
13	440	5.50	2640
14	470	5.875	2820
15	500	6.25	3000
16	530	6.625	3180
17	560	7.00	3360
18	590	7.375	3540
19	620	7.75	3720
20	650	8.125	3900

Table 17. Individual workload settings for exhaustive exercise.

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

Workloads kept consistent for both Test 1 (unsupplemented) and Test 2 (supplemented) with vitamin B-6
Watts = Kg x RPM

Table 18. Dietary protein intake(gN/24hrs) per Phase (based on food composit analysis).

Food Group	gm protein/ 24 hr intake
Phase 1	
dairy	21.18
animal	73.75
plant A	23.50
plant B	8.53
plant C	12.88
total =	139.84
Phase 3	
dairy	22.59
animal	72.40
plant A	22.46
plant B	8.68
plant C	12.42
total =	138.55

Grams of Nitrogen per 24 hours:

Phase 1: $\frac{139.84 \text{ gm protein/24 hrs}}{6.35 \text{ gm protein/gm Nitrogen}}$ = 22.37 gmN/24 hrs

Phase 3: $\frac{138.55 \text{ gm protein/24hrs}}{6.25 \text{ gm protein/gm Nitrogen}}$ = 22.17 gm N/24hrs

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Dietary Phase 1: unsupplemented with vitamin B-6
Dietary Phase 3: supplemented with vitamin B-6

Appendices

Table 19. Total Urinary Nitrogen data per subject.

subject	test	Day 1	2	3	4	5	6	7	8
1	US	-	14.46	13.09	16.1	17.47	19.7	15.89	-
	S	16.89	17.89	16.64	16.37	16.25	16.66	16.9	
2	US	15.85	17.85	16.08	17.14	15.17	18.26	14.91	15.04
	S	14.21	14.82	19.23	16.94	16.47	15.27	17.54	17.62
3	US	-	12.92	16.73	16.11	15.6	15.95	15.66	15.74
	S	-	15.22	15.22	15.01	14.97	14.97		-
4	US	13.11	16.86	16.81	15.19	14.92	17.69	16.41	16.33
	S	13.84	15.61	16.51	17.76	12.77	18.26	15.46	17.62
5	US	9.11	14.06	13.81	-	16.75	15.65	17.27	17.47
	S	12.55	16.89	13.94	16.02	16.62	16.55	18.1	15.83
6	US	-	14.75	18.18	16.61	15.38	17.6	16.82	16.77
	S	12.22	15.87	14.56	16.57	16.57	16.38	16.44	18.71
mean									
US		12.7	15.15	15.78	16.23	15.88	17.48	16.16	16.27
SD		3.4	1.8	1.9	0.7	1	1.5	0.85	0.93
S		13.94	16.05	16.02	16.44	15.61	16.35	16.89	17.95
SD		1.9	1.1	1.9	0.9	1.5	1.2	1.02	1.19

US: without vitamin B-6 supplementation, gN/24hrs
S: with vitamin B-6 supplementation, gN/24hrs
Day 7 exhaustive exercise
Day 7&8 post-exercise collections

Table 20. Urinary Urea Nitrogen data per subject.

subject	test	Day 1	2	3	4	5	6	7	8
1	US	-	16.52	14.68	18.9	20.13	21.53	18.19	-
	S	19.39	21.91	19.96	19.42	19.34	20	19.5	
2	US	18.65	18.89	17.83	18.61	16.89	19.96	16.3	17.03
	S	15.6	16.67	20.69	18.58	18	18.4	19.33	19.19
3	US	-	15.39	19.2	18.44	17.35	18.46	18.98	17.68
	S	-	17.75	17.75	17.49	17.24	17.24		-
4	US	16.09	18.72	19.42	18.29	17.25	18.58	19.26	18.8
	S	16.68	18.29	17.07	20.23	14.81	20.73	18.26	19.64
5	US	11.75	20.54	18.1	-	18.64	17.69	18.96	19.41
	S	14.74	20.23	16.25	17.79	19.79	19.91	23.02	19.43
6	US	-	18.93	20.07	19.3	17.9	20.05	19.5	18.98
	S	16.32	16.93	16.91	18.52	18.52	18.9	19.02	21.6
mean									
US		15.5	18.17	18.22	18.71	18.02	19.38	18.44	18.38
SD		3.5	1.9	1.9	0.4	1.2	1.4	1.8	0.93
S		16.55	18.63	18.1	18.67	17.95	19.2	19.83	19.96
SD		1.7	2	1.8	1.02	1.8	1.3	1.85	1.1

US: without vitamin B-6 supplementation gN/24hrs

S: with vitamin B-6 supplementation gN/24hrs

Day 7 exhaustive exercise

Day 7 & 8 post-exercise collections

Table 21. Urinary Creatinine excretion.

Subject	Test	Day 1	2	3	4	5	6	7	8
1	US	0	1.59	1.32	1.71	1.92	2.12	1.54	0
	S	1.9	1.93	1.99	1.7	2.16	2.22	2.12	0
2	US	1.76	1.87	1.86	1.95	1.81	2.5	1.81	1.81
	S	1.9	1.6	1.9	1.93	1.5	1.98	1.91	1.9
3	US	1.97	1.74	1.74		1.83	1.81	1.96	1.83
	S	1.78	1.97	1.71	1.87	1.8	1.89	1.94	1.71
4	US	0	2.43	2.24	2.11	1.17	1.4	2.25	2.13
	S	2.14	2.14	2.09	2.18	2.18	2.1	2.08	2.16
5	US	2.01	2.04	1.95	2.05	2.01	2.09	2.07	2.04
	S	1.62	2.01	1.94	2.14	2.04	1.93	2.15	2.31
6	US		1.7	1.9	1.83	1.74	1.95	2.25	1.9
	S	2.06	1.61	2.06	1.89	2.12	1.68	0	0
Mean	US	1.92	1.89	1.83	1.93	1.92	2.14	1.98	1.61
SD		0.14	0.31	0.3	0.16	0.17	0.22	0.3	0.14
Mean	S	1.87	1.87	1.95	1.95	1.97	1.97	2.04	2.02
SD		0.21	0.25	0.15	0.15	0.28	0.15	0.11	0.27

STUDY CRITERIA:
VITAMIN B-6 & EXHAUSTIVE EXERCISE

Subjects were required to meet the following criteria prior to the start of the study in order to minimize any adverse consequences to exercise testing;

1) Complete a health history questionnaire (with no abnormal health problems).

2) Normal blood chemistry profile.

3) Normal cardiac function as determined by electrocardiogram tracings (Quinton Instruments, Seattle, WA. Model CM-5) at rest, during and at maximal exercise.

4) Normal carbohydrate absorption as measured by xylose absorption.

5) Doctor's approval to participate.

6) Blood sample (10 ml) to determine plasma vitamin B-6 and pyridoxal 5'-phosphate concentrations.

7) Normal resting and exercising blood pressure response.

8) No vitamin supplements for at least 4 weeks prior to beginning the study.

9) No nicotine for at least one year.

10) No use of drugs known to interfere with vitamin B-6 metabolism.

Apparently Healthy Individual

Apparently Healthy Individuals are defined as "those who are apparently healthy and have no major coronary risk factors". The major risk factors are:

1) history of high blood pressure (>145/95)

2) elevated total cholesterol/high density lipoprotein cholesterol ratio (>5)

3) cigarette smoking

4) abnormal resting ECG

5) family history of coronary or other atherosclerotic disease prior to age 50

6) diabetes mellitus

Criteria for Exercise Test Termination

1) subject requests to stop
2) failure of monitoring system
3) progressive angina
4) two millimeter horizontal or downsloping ST-depression or elevation
5) sustained supraventricular tachycardia
6) ventricular tachycardia
7) exercise induced left or right bundle branch block
8) any significant drop (10 mm Hg) or systolic blood pressure, or failure of systolic blood pressure to rise with an increase in exercise load
9) lightheadedness, confusion, pallor, nausea
10) excessive blood pressure rise: systolic greater than 250 mm Hg; diastolic greater than 120 mm Hg
11) R on T premature ventricular contractions
12) unexplained inappropriate bradycardia
13) onset of second or third degree heart block
14) multifocal PVC's
15) increasing ventricular ectopy

INFORMATION SHEET
ENDURANCE EXERCISE TEST

1) Drink 2 10oz. glasses of water the morning of your endurance test before you report. DO NOT EAT ANYTHING AFTER 8 PM THE PREVIOUS EVENING.

2) Report to the Human Performance Lab (Women's Building, Rm. 19) for your endurance ride at your assigned time.

3) Bring: your urine bottles from the previous day, the same clothes you did your max test in, your own pedals (if you performed your max test with them), some good reading material, clothes to change into after the test. We will provide a shower area and towels.

4) Day of test procedure:
   a) report to the Human performance lab at _____.
   b) you will be weighed without clothes (don't worry, Ricky will do).
   c) rest for 10 minutes
   d) have first blood sample drawn (resting sample).
   e) have ECG hooked up (as was done in Max test)
   f) resting blood pressure will be read (blood pressures during this test will be less frequent than the max test, so we will not need to hook up the automatic blood pressure cuff).
   g) set the seat height, put on pedals (if using your own)
   h) sweat collector will be taped to upper corner of your shoulder
   i) you will be allowed to warm up for 2-5 minutes
   j) we will then set the workload on the bike to be 75% of the VO2 max level obtained from your max test.
   k) you will ride at 80 rpm, as you did in the max test, as long as you can.

5) Criteria for stopping the endurance ride
   a) maintaining less than 80 rpm for more than 15 sec
   b) you request to stop
   c) if the data we are monitoring dictates we stop the test

NOTE We ask that you wear no watches. We will inform you of your time at the completion of the entire study (after your second exercise test).

6) Blood drawing:
   a) the second blood sample will be drawn at 1 hour into the ride. While the blood is being drawn, your work load will be decreased.
   b) the third sample will be drawn 1-2 minutes after you finish the endurance ride.
   c) the fourth sample will be drawn 30 minutes after you finish.
   d) the last sample will be drawn 1 hour after you finish.

****NOTE*** Please do not lay down during this time. Breakfast will be available immediately after the last blood sample is drawn (1 hour after you finish the ride).
*** A controlled amount of water will be given at intervals during the ride.

MAXIMAL CYCLE ERGOMETER PROTOCOL

1) Adjust height of the saddle and handle bar to fit the patient. The knees should be flexed at approximately 5^{0} when the foot is at its lowest point. The subject should be instructed not to grip the handlebars tightly.

2) Pedal speed (RMP) will be set by the subject at a comfortable pace. Pedal speed will remain constant throughout.

3) The test begins by having the subject pedal with the lowest resistance possible for a 2 minute warm-up period.

4) External work will increase by 25-50 watts (150-300 kg) every .5 - 1 Kp.m 2 minutes (stage).

5) Heart rate will be taken every 1-2 minutes and blood pressure during the last minute of each stage with the arm free from griping the handlebar.

6) The ECG is recorded at the end of each stage or more frequently if indicated.

7) Termination of the test will follow the ACSM guidelines (1986).

8) For recovery, decrease the intensity and continue pedalling for up to 10 minutes. Blood pressure, heart rate and ECG will be measured at 3, 6, and 9 minutes during recovery.

(Adapted from the ACSM guidelines for max cycle test,1986)

DIETARY INTAKE

Breakfast:		240 gm orange juice
		30 gm raisins
		15 gm white sugar
		50 gm shredded wheat cereal
		220 gm 1% milk
		50 gm whole wheat bread
		3 ea jelly packets
Lunch:	sandwich	20 gm egg white, cooked
		60 gm whole wheat toast
		60 gm tuna water packed
		30 gm lo-cal mayo
		10 gm iceberg lettuce
		15 gm dill pickle
		70 gm raw carrot
		240 gm apple juice
		40 gm vanilla wafers
		160 gm pears
		17 gm gelatin mixed with 240 gm koolade
Dinner:		120 gm turkey
	salad:	50 gm iceberg lettuce
		15 gm red cabbage]
		40 gm red kidney beans
		40 gm cheddar cheese
		10 gm raw carrot, grated
		20 gm french dressing
		70 gm white rice, dry
		20 gm margarine
		200 gm green beans
		25 gm whole wheat bread
		240 gm 1% milk
		100 gm frozen desert
		150 gm peaches
		17 gm gelatin mixed with 240 gm koolade
Snack:		50 gm graham crackers
		240 gm grape juice

FOOD COMPOSIT 1 10/19/90

DAIRY:	FOOD	WEIGHT
	milk (dinner)	239.3
	milk (Bfk)	220.0
	cheese	39.7
	frozen yogurt	99.3
	water	13.6
		TW = 611.9 g
ANIMAL:	turkey	119.6
	tuna	59.1
	egg white	17.8
	gelatin (lunch)	16.9
	gelatin (dinner)	17.0
	water	200.1
		TW = 430.5 g
PLANT A	orange juice	238.0
	raisins	60.0
	white sugar	15.6
	shredded wheat	50.1
	WW bread (Bft)	49.2
	peaches	146.9
	WW bread (L)	25.2
	lettuce	9.7
	dill pickle	12.9
	white rice (ckd)	268.4
	carrots	69.9
	apple Juice	241.9
	water	26.1
		TW =1213.9 g

PLANT B

	vanilla wafers	25.2
	pears	159.1
	lettuce	49.5
	red cabbage	1 4.8
	kidney beans	37.5
	carrot (grated)	9.8
	green beans	199.1
	WW bread	57.7
	graham crackers	43.4
	grape juice	238.8
	water	25.7
	TW =	875.4 g
OTHER	jelly	42.0
	mayo-type dressing (lo-cal)	30.0
	french dressing	20.0
	margarine	20.0
	TW =	112.0 g

Blender and lid weight: 860.1 g.

Total protein intake in grams: 154.11

FOOD COMPOSIT 2 (11/18/90)

	FOOD	WEIGHT
DAIRY:		
	milk (dinner)	219.1
	milk (Bfk)	239.1
	cheese	39.9
	frozen yogurt	99.5
	water	44.1
	TW =	641.7 g.
ANIMAL:		
	turkey	118.8
	tuna	59.3
	egg white	19.4
	gelatin prepared (lunch)	260.7
	gelatin prepared(dinner)	253.8
	water	37.4
	TW =	749.4 g.
PLANT A		
	orange juice	238.8
	raisins	39.9
	white sugar	15.1
	shredded wheat	50.5
	WW bread (Bft)	47.0
	peaches	148.0
	WW bread (L)	59.7
	lettuce	9.6
	water	20.9
	TW =	629.5

PLANT B

dill pickle	14.7
white rice (ckd)	278.5
carrots	69.9
apple Juice	239.0
vanilla wafers	40.1
pears	159.1
water	33.9
TW =	834.9 g.

PLANT C

lettuce	49.8
red cabbage	14.9
kidney beans	36.9
carrot (grated)	9.8
green beans	198.5
WW bread	24.7
graham crackers	44.0
grape juice	239.0
water	25.3
TW =	642.9 g.

OTHER

jelly	42.0
mayo-type dressing (lo-cal)	30.0
french dressing	20.0
margarine	20.0
TW =	112.0 g

Blender and lid weight: 557.2 g

Nutritional Analysis of Controlled Diet.

Weight	3256 g
Calories	3476
Protein	155 g
Carbohydrate	552 g
Dietary Fiber	41.5 g
Fat Total	81.1 g

Calories from protein:	17%
Calories from carbohydrate:	62%
Calories from fats:	21%

APPENDIX FORM HEALTH/DIET/HISTORY
CONFIDENTIAL

Dr. Leklem
Foods and Nutrition Dept.
Oregon State University

Project Name _____
Date of Project _____

Code #: _____ Date: _____
Age: __ Birth Date: _____ State of County of Birth: ____
Predominant State of Residence: ____ City: _____
No of Yrs. _____ Present Employment: _____

Race (circle one): a. American Indian e. Chinese
b. Black f. Japanese
c. Caucasian
g. Other Oriental (specify) _____
d. Latin American
h. Other (specify) _____

Marital Status (circle one): a. Single b. Married
c. Divorced/Separated
d. Widowed

HEIGHT/WEIGHT; Ht. (ft. & in.) _____Present Wt. _____
Most weighed _____ What Year _____
Length of time you have maintained current wt. _____

MEDICAL HISTORY (Check any conditions for which you have been diagnosed and give the AGE at diagnosis):

___ a. diabetes
___ b. hypothyroidism
___ c. hyperthyroidism
___ d. goiter
___ e. hypoadrenalism (Addison's dis.)
___ f. osteoporosis
___ g. hepatitis
___ h. cirrhosis
___ i. kidney stones
___ j. nephritis
___ k. cystitis
___ l. high bl.pressure
___ m. angina
___ n. mental depression req. medication
___ o. insomnia requiring freq. medication
___ p. ulcer
___ q. pancreatitis
___ r. ulcer, colitis
___ s. spastic colon/ diverticulitis
___ t. recurring gastritis
___ u. allergies
___ v. heart problems (specify)_____
___ w. cancer (specify type)_____

Have you ever had a glucose tolerance test? ___yes____no
If yes, please explain the reason and the results:

_____

APPENDIX FORM . CONTINUATION

Do any of your close relatives have (had) diabetes?
_____yes _____no If yes, please check who of the relatives listed below have(had) diabetes

___a. mother ___d. brother ___g. uncle
___b. father ___e. cousin ___h. grandmother
___c. sister ___f. aunt ___i. grandfather

MEDICATION HISTORY (Check any which you take on a regular basis):

___a. sleeping tablets ___g. oral contraceptives
___b. barbiturates ___h. estrogens (female hormones)
___c. tranquilizers ___i. thyroxin
___d. bl. pressure tablets ___j. insulin
___e. diuretics ___k. cortisone
___f. antibiotics ___l. isoniazid
___m. other steroids (specify) _____

SURGICAL HISTORY (Please specify any type of surgery you have had and the date and age when it occurred):

Surgery	Date	Age
_____	_____	___
_____	_____	___
_____	_____	___

DIETARY HISTORY:

Are you a vegetarian? _____ yes _____ no
If yes, circle the type of vegetarian diet you follow:
a. ovo-lacto b. ovo c. lacto d. vegan

Do you take vitamins? (circle one):
a. yes, daily b. yes, frequently c. never
If yes, what type, amount, and how long have you taken them?

Type _____ Amount _____ How long? _____
_____ _____ _____
_____ _____ _____

Do you take other nutritional supplements?
Type _____ Amount _____

Please list all foods which you refuse to eat, cannot eat, of prefer not to eat:

Do you drink alcohol? (beer, wine, liquor): ___Yes ___No
If yes, give frequency and amount consumed:
Frequency (how often?) _____
How much (ounces per time) _____

APPENDIX FORM CONTINUATION

Do you drink coffee or tea? _____ Yes _____ No
If yes give number of cups per day _____
(Circle one): coffee tea
Do you drink decaffeinated coffee or tea? _____ Yes
_____ No
If yes give number of cups per day _____
(Circle one): coffee tea

Do you smoke? _____ Yes _____ No
If yes circle what you smoke: Cigarettes Cigars
How many per day _____ Packs per day _____

EXERCISE LEVEL: Do you have a daily fitness program?
_____ Yes _____ No
If yes, describe:

If no, what types of exercise would you get in a typical week:

B-6 & EXERCISE
SCREENING DATA

Name_____Date_____

Address_____

Phone (H)_____(W)_____Hrs:_____

Age_____ Ht._____Present Wt._____ Usual Wt_____

VITAMINS:

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

willing to stop? yes no

ACTIVITY:

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

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

DIET:

Food preferences Foods will not eat (or allergies) intolerances

MEDICATIONS:

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

OTHER:

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

Other helpful info?_____

(would this person be a good subject?)

Appointment schedule: date_____time_____with_____

INFORMED CONSENT

TITLE: The influence of vitamin B-6 supplementation and exercise to exhaustion on protein metabolism in college aged male trained cyclists.

PRINCIPLE INVESTIGATOR: James Leklem, Ph.D.
GRADUATE STUDENT INVESTIGATOR: Ingrid A. Skoog, B.S.

PURPOSE: The purpose of this research is to determine the influence supplemental vitamin B-6 and exercise to exhaustion has on protein metabolism by quantifing the by-products: urinary and sweat urea and 3-methylhistidine.

This investigation will involve 8 male, trained cyclists. The study will span 32 days (October 16 to the morning of November 16).

PRELIMINARY TESTS: I understand that each subject is required to complete a health history questionnaire prior to beginning the study. Each subject is to provide a complete list of all medications, including mineral, vitamin and other nutritional supplements taken in the last month. I understand that 17 ml. of blood will be drawn prior to the start of the study. A blood chemistry screen will be performed on part of the sample by Good Samaritan Hospital in Corvallis to determine normal health status. Part of the sample will be used to determine plasma vitamin B-6 and PLP levels.

I understand that each subject will have a xylose absorption test prior to beginning the study to ensure normal carbohydrate absorption. I understand that I will have my body composition analyzed prior to the start of the experiment by underwater weighing. I understand that I will keep a detailed 3 day 24-hour dietary history prior to the start of the study to assess typical caloric and vitamin B-6 intake.

$\dot{V}O_2$ MAX TEST: I understand that a $\dot{V}O_2$ max test will be performed prior to the start of the study to assess my level of aerobic conditioning. This test involves exercising on a cycle ergometer for about 20 minutes during which time the workload (intensity of exercise) is increased until maximal uptake of oxygen is achieved. The results of this test will provide the basis for determining the workload used for each of 2 exercise to exhaustion tests (75% of $\dot{V}O_2$ max). I understand that I may terminate the test on request at any time without penalty. I understand that the test of $\dot{V}O_2$ max has a chance of precipitating a cardiac event (such as abnormal heart rhythms) or even death. However, the possibility of such an occurrence is very slight (less than 1 in 10,000), since I am in good physical condition with no known symptoms of heart disease, and since the test will be administered by trained personnel who will be monitoring electrocardiographic and other physiological responses to the test. During the $\dot{V}O_2$ max test, an electrocardiogram will be monitored 5 minutes prior to, at 2

minute intervals during and at 3, 6 and 9 minutes following the exercise test. I understand the physical stress the exercise test will place on me.

EXERCISE TO EXHAUSTION TESTS: Each subject will exercise to exhaustion on the cycle ergometer on 2 separate occasions (day 7 and day 30) at 75% of their $\dot{V}O_2$ max, as determined by the VO_2 max test.
Exhaustion will be defined as the inability to maintain a workload at 75% of $\dot{V}O_2$ max. At this point, the investigator will stop the study. The test may be stopped at any time if requested by the subject. The exercise test will be stopped prior to exhaustion if indicated by the American College of Sports Medicine guidelines for stopping an exercise test (see criteria for discontinuing an exercise test). I understand that I will not be allowed to keep track of time during the exercise test, but will try to the best of my ability to cycle as long as possible during each exercise test. I understand that I will be monitored by an electrocardiogram at 5 minute intervals throughout the test and at 5 minutes following the test. I understand that each of the 2 exercise to exhaustion tests will be done in the fasted state (nothing but water after 7pm the preceeding day), and that I will not be allowed water until one hour after the test is completed. I understand that I will keep my daily exercise training level constant throughout the study.

DIET: On days 1-8 and days 24-32 of the study, I understand that each subject will be required to eat only the metabolic diet provided by the Department of Nutrition and Food Management at Oregon State University. I understand that I will eat my own food on days 9-23. I understand that on days 20-22, I will again keep a detailed 3 day 24-hour dietary intake record. I understand that each subject will take no vitamin, mineral or nutritional supplement other than the supplement provided. I understand that a capsule containing either citric acid, sugar or pyridoxine (vitamin B-6) will be given throughout the 31 days. I understand I will not know when the vitamin B-6 is being administered until after the completion of the study (day 32). I understand that I am to take one capsule per day on days 1-31. I understand that a person can not consume alcoholic beverages during the study, and must avoid caffeine containing items the day prior to, the day of and the day after each exercise test. I understand that a person is to inform the principle investigators if any prescription or non-prescription drugs are taken.

URINE: I understand that each subject will collect 24-hour urine specimens during days 1-8 and days 24-32. My understanding of 24-hour urine collections is as follows: After rising in the morning of the first day of urine collection, a person will completely empty their bladder. They will discard this urine. After this, each person collects all of their urine in the containers provided. Each morning the person completely empties their bladder at the same time as the day before and labels this urine as belonging to the collection period ending the preceding day (24- hour period). The

person will continue collecting their urine in this manner on the designated days. I understand that each person will bring their urine to the laboratory each morning. I understand each person is to report any accidental loss of urine to the investigators.

DAILY LOG: I understand that each subject will keep a daily log during the entire study. Recorded in the log will be: completeness of intake, any alteration of diet to maintain weight, non-caloric beverage intake, appetite, medications taken, overall health, accuracy of urine collections, body weight, and amount, type and perceived intensity of daily exercise.

BLOOD: I understand that 17 ml of blood will be drawn at each of 5 times during each of 2 exercise tests (a total of 10 exercise blood draws). These will be 5 minutes prior to exercise, 60 minutes into the exercise, 1-2 minutes after exercise, 30 minutes into the recovery phase and 60 minutes into the recovery period. 17 ml of blood will also be drawn before breakfast on day 21. Blood will be drawn from the antecubital vein by a registered medical technologist or Registered Nurse. I understand that there is a minimal risk of infection when blood is drawn and that sterile procedures will be used to minimize this risk. Persons at increased risk for Hepatitis B or HTLV III (AIDS) infections should not participate in this study.

EXPIRATORY GASES: I understand that prior to each exercise test and the $\dot{V}O_2$ max test, I will be given a 2-5 minute warm-up period of moderate intensity (heart rate of 130-140 bpm) at which time the gas collection devices will be attached to familiarize the subjects with the collection procedures. During the $\dot{V}O_2$ max test and subsequent exercise sessions (day 7 and day 30) gas collections will be made for 2 minutes at 10 minute intervals . This is done by wearing a mouthpiece and nose clamp that allows for inspired gas to be measured.

SWEAT COLLECTION: I understand that prior to each exercise session, I will have a sweat collection devise taped to my back. The devise will remain in place for the duration of each exercise test. The sweat collector is a 15X25 cm. strip of saran wrap glued to surgical tape using mender cement. The skin will be coated with vaseline under the collector. Samples will be collected every 30 minutes from a plastic tubing drain from the sweat collector, using a syringe. I understand that their may be minimal skin abrasion from the placement and removal of the sweat collector.

BENEFITS: I recognize that upon completion of the study, I will receive payment of $50 from Oregon State University. I will receive the chemistry blood screening values and body composition and fitness values obtained during this study. I will also receive 18 days of meals at no cost. I understand that each participant is free to leave this experiment at any time and that participation is voluntary. The Department of Nutrition and Food Management reserves the right to remove a subject from any study if he or she is uncooperative in following the protocol of the investigation.

CONFIDENTIALITY: The anonymity of each subject's data will be maintained by assigning each subject code numbers/letters upon entry into the study and recording all data by using this code rather than the subject's name. Only the principle investigators shall have access to the identity in any way in the presentation or publication of the results of this investigation.

This investigation has been explained to each participant and all questions have been answered. I give my consent to participate in this study. You may contact James E. Leklem (737-0969) concerning any questions about this research or any problems you develop which you feel may be associated with participating in this study.

_____ _____

Subject Date

_____

Address Phone Number

_____

Witness

B-6 & EXERCISE STUDY-FALL 90
NUTRITION & FOOD MGMT.
OREGON STATE UNIV.

NAME_____

DATE_____

DAILY ACTIVITY SHEET

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

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

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

RECORD ALL "FREE" FOODS IN EXACT AMOUNTS USED. INDICATE TYPE ~~ALSO~~ USED (:cDECAF, ETC.)

Coffee/Tea (cups)_____

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

candy/sugar_____

GENERAL HEALTH:

How do you feel today? Excellent___ Good___ Fair___ Poor___

MEDICATIONS:

Any medications?(aspirin,etc.)_____

UNUSUAL EVENTS:(injuries, exams)_____

TURNED IN URINE: yes no WEIGHT TODAY:_____

BODY COMPOSITION
LAB FORM

Name_____

Date_____

Age_____ Sex: Male Female

Height_____in (÷ 39.37 in/m) = _____meters

Weight_____lb (÷ 2.2 lb/kg) = _____kg

UNDERWATER WEIGHING

UWW trials: 1._____
2._____
3._____
4._____
5._____
6._____

Average of 3 highest values = _____

Subtract tare wt. _____(if appropriate)

UWW = _____kg

Wt in air_____kg

Residual volume_____liters

Body density_____g/cc

% Body fat_____%

Fat wt._____

Lean wt._____

Target %BF_____%

Target wt._____

Water temperature_____°C

Water density_____g/cc

RESIDUAL VOLUME DETERMINATION

	Impurity	Vol. O_2	N_2 initial	N_2 equil	N_2 final	Temp	BTPS
Trial 1							
Trial 2							
Trial 3							

$$\left\{\left[\frac{(\text{Vol } O_2 + 1.124)(N_2 \text{ equil} - \text{impurity})}{(N_2 \text{ initial} - N_2 \text{ final})}\right] - .082\right\} \times \text{BTPS} = \_\_\_\_\_\text{liters}$$

Max Vo_2 Test:

NAME_____ PB _____

DATE_____ TEMP_____

AGE_____ RH % _____

BICYCLE_____ RPM _____

SEAT HEIGHT_____ WT (#, KG)_____

CLOCK TIME	KG	HR (bpm)	BP	VO2 (ml/kg)	RPE (1-20)
REST	0				
W/U- 0-5 MIN	1.5-2.0				
1	2				
1.5	2				
2	2.5				
2.5					
3	3				
3.5					
4	3.5				
4.5					
5	4				
5.5					
6	4.5				
6.5					
7	5				
7.5					
8	5.5				
8.5					
9	6				
9.5					
10	6.5				
10.5					
11	7				
11.5					
12	7.5				
12.5					
13	8				
RECOVERY					
RECOVERY					
RECOVERY					

Data Collection

Date: Pb:

Initials/Code: Temp:

Test# %RH:

Hydrostatic Weight:

Density_____ % BF_____ RV_____

Time of Arrival:

Body Weight:

Pre-Test_____ Post Test:_____

Weight of clothes

Pre-Test:_____ Post-Test:_____

Last Meal/Food/ Drink (date/time:

Time EKG preped:

Seat Height:

Bike Ergometer#:

Blood Pressure:Pre-Test_____ During_____ Post-Test_____

How do you feel today?

Notes/ Comments: