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Title: The Effect of Glucose and Fructose Ingestions on Vitamin B-6 and Fuel Metabolism During Prolonged, Continuous Exercise in Trained Males

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The study was designed to indirectly understand muscle glycogen utilization during prolonged exercise when either glucose, fructose, or water is ingested. Eight trained adult males exercised on a cycle ergometer at 58±7% of VO2 max for 2 h on 2-4 occasions. At 0 minutes of exercise and at 30-minute intervals throughout the exercise, the subjects ingested 200mL of fluid containing either glucose, fructose, or plain water in a double-blind, randomized fashion. The carbohydrate (CHO) fluid concentration was based on each subject's body weight (BW): 1g CHO X kg^{-1} BW X L^{-1} water and ranged from 5.8-9.2% (average=7.5%) of BW. Blood samples were collected from subjects at rest and immediately prior to fluid ingestion during exercise and analyzed for hematocrit, hemoglobin, and plasma levels of glucose, lactate, and pyridoxal 5'-phosphate (PLP). ANOVA showed no significant difference among treatments at any time of exercise for mean plasma lactate and PLP levels

($p > 0.05$). Although not significant, mean plasma lactate and PLP concentrations tended to be lower when glucose was consumed as compared to fructose and water. The mean plasma glucose level, however, was significantly different among treatments at specific time points of exercise ($p < 0.05$). During exercise, mean plasma glucose decreased, and there was a higher plasma glucose level when glucose and fructose fluids were ingested as compared to water. At 60 minutes of exercise, this difference was evident for both glucose and fructose ingestion ($p < 0.05$). At 90 and 120 minutes of exercise, fructose ingestion produced a significantly higher mean plasma glucose level than either water or glucose ingestion ($p < 0.05$). It is hypothesized that the higher plasma glucose levels provided a greater blood glucose supply to working muscles, thereby sparing muscle glycogen stores. The findings indicate that for the long-term exerciser, consumption of a 5.8-9.2% fructose solution may promote less muscle glycogen utilization than either glucose or water, thereby possibly increasing endurance.

The Effect of Glucose and Fructose Ingestions On Vitamin B-6 and Fuel Metabolism During Prolonged, Continuous Exercise in Trained Males

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LIST OF ABBREVIATIONS

n	Sample Size
VO2 max	Maximal Oxygen Uptake
Hct	Hematocrit
Hgb	Hemoglobin
PV	Plasma Volume
PLP	Pyridoxal 5'-Phosphate
kcal	Kilocalorie
kpm	Kilopond times Meter
mmol	Millimole
mg	Milligram
g	Gram
kg	Kilogram
nm	Nanometer
cm	Centimeter
mL	Milliliter
L	Liter
nM	Nanomolar
mM	Millimolar
M	Molar
°C	Degrees Celsius
min	Minute
h	Hour
yr	Year
SD	Standard Deviation
ANOVA	Analysis of Variance
p	P-Value
CHO	Carbohydrate
C	Control fluid
G	Glucose fluid
F	Fructose fluid

The Effect of Glucose and Fructose Ingestions On Vitamin B-6 and Fuel Metabolism During Prolonged, Continuous Exercise in Trained Males

I. Introduction

Long distance athletes such as cyclists and runners are examining diet as a means of improving their performance. Typically, the long-term exerciser considers fluid drinks primarily as a replacement for body water lost in sweating. Researchers have shown that besides fluid replacement, drinks containing carbohydrate have an additional benefit: they can provide an increased fuel supply to the working muscle. Increasing the availability of carbohydrate to the exercising muscle via ingestion or infusion has been shown to slow muscle glycogen depletion, delay fatigue and improve endurance performance. These findings have made their way to the popular literature and the impact is obvious; the market for "sports drinks" now grosses an estimated $90 million per year (Costill 1985).

There are important considerations, however, in the carbohydrate drink used to improve performance: what <u>type</u> and <u>how much</u> dietary carbohydrate consumed during exercise contributes to the least amount of muscle glycogen utilized, thereby maximizing endurance performance? One carbohydrate <u>type</u> is sucrose (ordinary table sugar) composed of the two monosaccharides, glucose and fructose. Recent studies have investigated glucose (and its polymer)

and fructose to determine how they effect fuel metabolism and muscular endurance. Blood glucose homeostasis has been a major parameter studied since muscle glycogen is spared when blood glucose is maintained at a constant level. Both glucose and fructose in the diet are metabolized to yield blood glucose, but an important difference is that fructose is retained in the liver as glycogen in more than twice the amount than that for the same amount of glucose (Cori 1926). This reservoir of glycogen then allows for a more constant replenishment of blood glucose.

Two studies have shown, however, that glucose ingestions during exercise gave rise to similar blood glucose levels throughout exercise as did fructose ingestions (Fruth and Gisolfi 1983, Bjorkman et al. 1984). Although blood glucose levels were constant when fructose was ingested, endurance performance was not enhanced (Fruth ad Gisolfi 1983, Bjorkman et al. 1984); but endurance performance was enhanced with glucose ingestions (Bjorkman et al. 1984). Furthermore, the fructose fluids caused gastro-intestinal distress in all subjects (Fruth and Gisolfi 1983).

The amounts of carbohydrate used in the majority of studies investigating carbohydrate fluid intake during exercise are higher than an athlete might consume during an event. Most experimental fluids are 7 percent and greater in carbohydrate content. Costill and Saltin (1974) have shown that gastric emptying is delayed when the carbohydrate

concentration was 278 mM (5.0 percent solution) and above. The type and amount of carbohydrate in fluids that most enhance endurance of long-term exercisers are, as yet, unclear.

Fuel metabolism can be related to vitamin B-6 (B-6) in the body. The active form of B-6, pyridoxal 5'-phosphate (PLP) is the cofactor for the enzyme glycogen phosphorylase (EC No. 2.4.1.1). This enzyme is present in the liver and breaks down glycogen to glucose for blood glucose homeostasis. Glycogen phosphorylase is also present in the muscle and catalyzes the release of glucose from glycogen when fuel is needed. Additionally, PLP is the cofactor for aminotransferases involved in gluconeogenesis, a process which converts nonglucose substrates such as amino acids, lactate, pyruvate, and glycerol to glucose when blood glucose is at a lower level than optimal for tissues in demand of glucose (e.g. brain and working muscles). The process of gluconeogenesis occurs when liver glycogen stores become depleted. Investigations in this laboratory have shown that the metabolism of vitamin B-6 is altered with exercise. Specifically, it has been suggested that tissue redistribution of B-6 occurs with exercise (Hatcher 1983). It has not been shown what changes occur in vitamin B-6 metabolism when the exerciser, for instance the long-distance cyclist, consumes a carbohydrate drink during the exercise bout.

The present study was designed to obtain an indirect

understanding of muscle glycogen utilization during prolonged, continuous exercise in adult males trained in cycling and/or running when one type of carbohydrate is ingested as compared to another. Circulating levels of glucose, lactate, and PLP, as well as hematocrits and hemoglobins, were determined to gain information on vitamin B-6 and fuel metabolism in eight subjects cycling for 2 hours at 60 percent of maximal aerobic capacity. The carbohydrate forms selected were glucose and fructose. The amount ingested by each subject was based on body weight. Consequently, heavier subjects with greater fuel needs than those lighter in body weight, received more carbohydrate and calories than the lighter subjects. The administration of a carbohydrate dose similar to what an athlete might consume during an event was an important part of the experimental design.

The experimental hypothesis was that fructose, as compared to glucose or water, would promote less muscle glycogen utilization when ingested during prolonged, continuous exercise. The objectives of this investigation were threefold:

(1) to provide more knowledge of the effect of type and concentration of a carbohydrate drink on fuel metabolism when the carbohydrates are consumed during exercise;

(2) to demonstrate changes, if any, in vitamin B-6 metabolism during exercise when carbohydrates are consumed as compared to water;

(3) to determine the possible effects of glucose and fructose ingestions during exercise on blood volume changes and, consequently, on tissue hydration.

II. Review of Literature

Exercise: Biochemical and Nutritional Parameters

Since the movements of body parts are effected by muscle contraction, maximum athletic performance can be related to those factors which affect muscle structure and function. Foremost among these factors is the supply of energy. According to Holloszy (1982), the transition from rest to heavy exercise can involve as much as a 200-fold increase in the rate of energy utilization by skeletal muscle. Energy for contraction is achieved through the hydrolysis of adenosine triphosphate (ATP) to adenosine diphosphate (ADP) and inorganic phosphate (Pi). The amount of ATP present in muscle, however, is only sufficient to maintain full contraction for less than 1 second (Guyton 1981). For the continuation of muscular work, rephosphorylation of the resultant ADP is critical. The sources of energy or "fuels" used to reconstitute the ATP are determined by several factors.

Determinants of Available Fuels

The intensity and duration of work primarily determine the relative contributions of fuel sources to exercising muscles (Havel 1971). Work intensity is the force exerted over a distance per unit of time. A linear increase in work intensity will result in linear increases in heart rate and oxygen uptake until maximal levels are reached.

Maximum oxygen uptake is defined as the highest oxygen uptake an individual can attain during physical work while breathing air at sea level (Astrand and Rodahl 1977). "VO2 max" is the standard abbreviation for maximum oxygen uptake. Generally, high intensity exercise corresponds to 90-100 percent of VO2 max; moderate, 60-85 percent of VO2 max; low, less than 50 percent of VO2 max. The duration of work is the time to complete the exercise.

Muscular activity has been categorized according to work intensity and duration (Fox and Matthews 1974):

1) MES: muscular exertion of high intensity, short duration (e.g. 100 m dash);

2) MEM: muscular exertion of moderate intensity, medium duration (e.g. 220 m sprint);

3) MEL: muscular exertion of low intensity, long duration (e.g. marathon run).

The system of energy production (ATP reconstitution) for each category of muscular activity is dependent upon the characteristics of the activity. In MES activities, energy demand is great and urgent. Therefore, only those substrates within the muscle cell that are immediately available for energy are utilized. These are ATP and the high energy phosphate, creatine phosphate, which can reconstitute ATP. The process of energy-release from this system does not require oxygen, and thus is termed "anaerobic (Astrand and Rodahl 1977)."

When the intensity of work is decreased and the duration is increased as for MEM activites, an additional anaerobic system of energy production becomes operable: glycogen present in muscle is broken down (glycogenolysis) to re-form glucose which is metabolized (anaerobic glycolysis) to yield ATP. All anaerobic supplies, however, are limited and rapidly depleted. To meet the energy needs of the working muscle, oxygen is utilized (oxidative phosphorylation) for combustion of the substrates or "fuels," glucose and free fatty acids. "Aerobic" metabolism in MEM activities can only proceed for a short time due to the limited amount of oxygen available. Oxygen is limited because the existing oxygen stores in muscle cannot keep up with the rephosphorylation of high energy intermediates essential to muscle contraction (Astrand and Rodahl 1977).

In MEL activities, sufficient oxygen is available for aerobic metabolism. Oxygen is available because the increased metabolism does not exhaust oxygen stores before more oxygen is made available through increased respiration. The primary substrate used at rest and during prolonged, light exercise are free fatty acids. This changes during prolonged, heavy exercise, when blood-borne glucose and intramuscular glycogen contribute increasingly to ATP production (Astrand and Rodahl 1977).

Other factors can influence the contribution of fuel sources to exercising muscles. Besides exercise intensity

and duration, important factors that interrelate with carbohydrate utilization are training state, initial muscle glycogen levels, circulating substrates and hormone levels, participation of specific muscle groups, and skeletal muscle fiber type recruitment (Hickson 1983).

Fuel Metabolism During Exercise

Muscle Glycogen

Glycogen is a large polymer of glucose stored in muscle as well as liver cells. An average man in the resting, postabsorptive state stores 120 g of glygcogen in muscle (Newsholme 1977). This is equivalent to an energy store of 400 kilocalories. In the absence of oxygen, the metabolism of intramuscular glycogen can yield 3 moles of ATP per mole of glucose (Lehninger 1982):

$$(n+1)\text{glucose} + \text{ATP} \rightarrow (n)\text{glucose} + 4\ \text{ATP} + 2\ \text{lactate}$$

This process of glycogenolysis is shown in Figure 1 as part of a metabolic scheme inside a typical muscle cell. The control point is the irreversible phosphofructokinase step. When ATP is abundant, the rate of this reaction is inhibited, whereas high concentrations of ADP stimulates the reaction. Intramuscular glycogen can only be used by the exercising muscle. This is due to the one-way reaction of glucose to glucose-6-phosphate (Figure 1). In some tissues other than the muscle (e.g. liver), an enzyme, glucose-6-phosphatase, is present which cleaves the

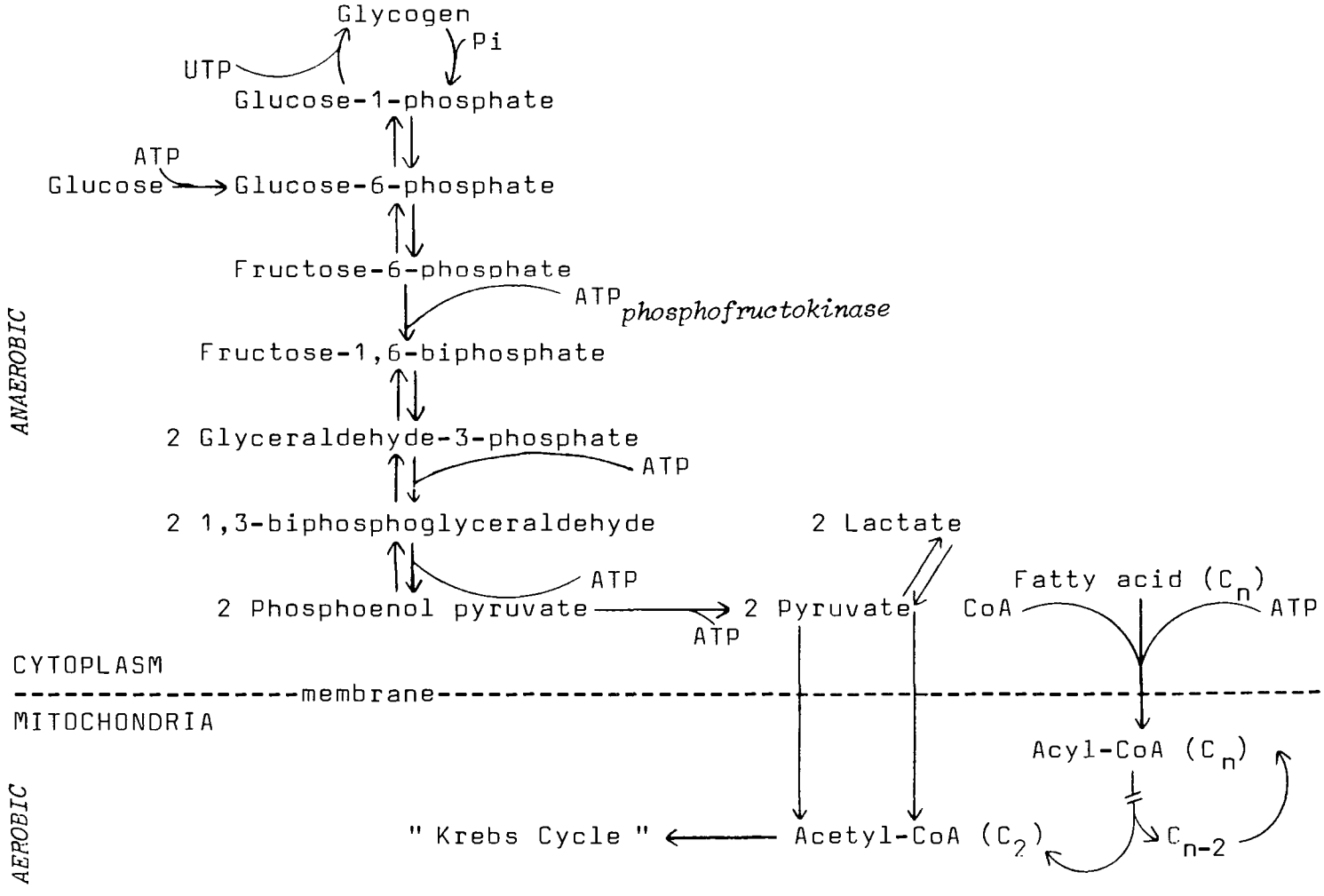

FIG 1. Metabolic scheme in a typical muscle cell. Abbreviations: ATP, adenosine triphosphate; UTP, uridine triphosphate; Pi, orthophosphate (PO_3^{-4}); CoA, coenzyme A. (Adapted from Astrand and Rodahl 1977.)

phosphate group from glucose; the glucose can then be released into the circulating blood and used by additional tissues (Lehninger 1982).

The greatest utilization of muscle glycogen occurs in the first few minutes of moderate-to-heavy work, then decreases thereafter (Bergstrom and Hultman 1967, Hermansen et al. 1967, Ahlborg et al. 1967, Baldwin et al. 1973). Bergstrom and Hultman (1967) observed a triphasic or exponential pattern of glycogen depletion in human muscle during exercise. Muscle biopsies showed an initial rapid rate of glycogenolysis most likely related to oxygen uptake in equilibrium with aerobic ATP production. The second phase was slower, possibly due to a steady combustion of muscle glycogen until low levels are reached. The third phase, when most muscle fibers had completely depleted glycogen supplies, was the slowest at utilizing intramuscular glycogen.

At low work intensities sustained over long durations (e.g. MEL activities), muscle glycogen is used slowly and levels may not be low at exhaustion (Saltin and Karlsson 1971, Essen 1977). Moderate intensity work causes marked muscle glycogen depletion with very low levels at exhaustion (Bergstrom and Hultman 1967, Hermansen et al. 1967, Ahlborg et al. 1967). Work at high intensities cause rapid depletion of muscle glycogen, but considerable levels can still be present at exhaustion (Saltin and Karlsson 1971, Thomson et al. 1979).

The types of muscle fiber recruited for each of the activities above have different glycogenolytic capacities which can explain the differing rates of glycogen depletion. In mammalian skeletal muscle, a mixture of three fiber types exist (Holloszy 1982):

Type I or "slow-twitch red fibers"
Low glycogenolytic capacity
High respiratory capacity
Low actomyosin ATP-ase activity;

Type II-A or "fast-twitch red fibers"
High glycogenolytic capacity
High respiratory capacity
High actomyosin ATP-ase activity;

Type II-B or "fast-twitch white fibers"
High glycogenolytic capacity
Low respiratory capacity
High actomyosin ATP-ase activity.

Glycogenolytic and respiratory capacities refer to the maximum capabilities of the fiber to either break down glycogen or proceed through aerobic metabolism, respectively, for ATP production. Actomyosin ATP-ase activity is the extent to which myosin, a muscle protein in association with actin (an additonal protein), yields ATP for muscle contraction.

As determined by glycogen depletion measurements during exercise at 30 to 85 percent of VO2 max, both human (Essen 1977, Costill et al. 1973, Gollnick et al. 1973) and animal (Baldwin et al. 1973) skeletal muscle preferentially recruit the red fiber types. In humans, the slow-twitch red fibers are the first to be depleted of muscle glycogen

during exhaustive exercise up to 85 percent of VO2 max; the fast-twitch red fibers follow. During higher intensity work, all fiber types are recruited. The fast-twitch fibers (especially the white), however, use the most muscle glycogen as compared to the slow-twitch red (Thomson et al. 1979, Baldwin et al. 1977).

Endurance training promotes slower rates of muscle glycogen depletion (Karlsson et al. 1974). This is a response to an increased ability of the trained individual to reconstitute ATP through aerobic metabolism. Training increases the maximal oxygen uptake of the athlete. This increase in respiratory capacity is induced by 1) increased muscle capillaries (Brodal et al. 1977), and 2) biochemical adaptations in skeletal muscle, i.e., increased mitochondrial enzymes (Holloszy and Winder 1979) and increased myoglobin content (Pattengale and Holloszy 1967).

Differences in muscle glycogen utilization have been shown to exist among various types of endurance exercise. Prolonged high intensity cycling (Ahlborg et al. 1967, Gollnick et al. 1973) uses more quadricep muscle glycogen than treadmill running (Costill et al. 1971) or cross-country skiing (Bergstrom et al. 1973) at similar exercise intensities. This is probably due to the differences in muscle fiber recruitment between cycling and running or skiing. The latter two types of exercise engage a greater muscle mass for performance. This means that although less glycogen is used by the quadricep muscles

during running and skiing as compared to cycling, glycogen stores in other muscles (e.g. deltoid) are being tapped for energy production (Bergstrom et al. 1973).

The ability to sustain prolonged, moderate-to-heavy work is dependent upon initial muscle glycogen levels. In human muscle, glycogen levels have been found to increase to above normal levels by dietary changes (Bergstrom et al. 1967). The regimen involved three days of a fat and protein diet after exhaustive exercise followed by three days of a carbohydrate diet after the same work. The amount of intramuscular glycogen has been found to be a major determinant of muscular endurance (Bergstrom et al. 1967, Gollnick et al. 1972).

Blood Glucose and Hepatic Glycogen

Blood glucose is derived from liver or "hepatic" glycogen through glycogenolysis, and constitutes an important source of energy for working muscles. Wahren et al. (1971) demonstrated in humans that glucose uptake by exercising muscles of the forearm and leg was increased over resting levels, and the magnitude was dependent upon the intensity and duration of the work performed. In heavy forearm exercise on a hand ergometer, total glucose uptake by the forearm muscle could potentially account for 100 percent of carbohydrate oxidation and 50 percent of total oxidative metabolism--both determined by the respiratory quotient (carbon dioxide production : oxygen consumption).

In leg muscle (Wahren et al. 1971) by the end of 40 minutes of cycling at 400, 800, or 1200 kilopond times meter (kpm) per minute, total glucose uptake could sustain 80, 70, or 60 percent, respectively, of the estimated carbohydrate oxidation; and 25, 25, and 40 percent, respectively, of the total oxidative metabolism. The increased glucose uptake by leg muscles during exercise at every workload was associated with a rapid, sharp increase in liver glucose production. At the highest workload (1200 kpm), the rise was most pronounced. The increase in liver glucose production was presumed to result mainly from hepatic glycogenolysis. Although hepatic gluconeogenesis increased during exercise at all workloads, by the end of exercise only 19, 12, and 9 percent of glucose production (respective to workloads of 400, 800, and 1200 kpm per minute) were found to be attributable to hepatic gluconeogenesis when estimated from liver uptake of lactate, pyruvate, glycerol, and α-nitrogen. The investigators did not speculate as to why the contribution of carbon skeletons to liver glucose production by gluconeogenic precursors decreases with increasing exercise intensity. Perhaps at the higher work intensities, hepatic glycogenolysis can produce glucose more efficiently, i.e. faster and with less ATP needed, than hepatic gluconeogenesis. Other studies have demonstrated that as exercise is continued beyond 40 to 60 minutes, blood glucose falls progressively, depending on work intensity

(Ahlborg et al. 1974, Ahlborg and Felig 1982).

Glucose uptake by resting muscle is stimulated by the hormone insulin--the main regulator of glucose uptake in insulin-sensitive tissues. During exercise, blood insulin levels decrease and glucagon increases in efforts to maintain blood glucose levels (Vranic et al. 1976a). An increase in the insulin level is not necessary for increased glucose uptake by muscle. The suppression of insulin secretion is important in that it increases the sensitivity of liver cells to the action of gluconeogenic and glyconeogenic hormones and other factors (Vranic et al. 1976b).

In humans, hepatic release of glucose increases progressively, then decreases with exercise duration (Ahlborg et al. 1974, Wahren 1977). Glucose output from liver during prolonged exercise is estimated at 16.7 mmol per minute (Rowell 1965). With prolonged exercise, glucose uptake by exercising muscles increases at a greater rate than liver output resulting in lower circulatory glucose levels. Exercise-induced "hypoglycemia," or abnormally-low blood glucose (less than 2.5 mmol per liter), may (Felig et al. 1982) or may not (Hermansen et al. 1967) be present at exhaustion.

In the resting, postabsorptive state, the average man stores 70 g of glycogen in the liver (Newsholme 1977). This is equivalent to an energy store of 200 kilocalories. When liver glycogen stores are depleted, hepatic

"gluconeogenesis" attempts to maintain blood glucose. The process produces glucose from noncarbohydrate sources formed by working muscle (e.g. lactate and pyruvate), by fat oxidation (e.g. glycerol), and by stored amino acids. Wahren et al. (1971) have shown that the uptake of gluconeogenic precursors by working muscle over 40 minutes was 19 percent that of liver glucose output. Over a longer duration of exercise (4 hours) at a lower exercise intensity, the uptake of gluconeogenic precursors accounted for 45 percent of liver glucose output (Ahlborg et al. 1974). Other means of postponing hypoglycemia during prolonged exercise are decreased glucose uptake by working muscles and increased free fatty acid oxidation by the muscle.

A simple comparison can be drawn between the roles of liver and muscle glycogen. Liver glycogen serves as the reservoir of glucose which readily yields glucose for release into the bloodstream, whereas muscle glycogen is an important source of ATP for muscular contraction through its degradation by glycolysis (Lehninger 1982).

Lactate

Lactate is derived from glycogenolysis in exercising muscles as previously stated and depicted in Figure 1. It is released into the blood from muscle cells when there is a high demand for energy in relation tc muscle oxygen supply. The accumulation of lactate causes metabolic pH to

decrease and oxygen consumption by respiration to increase (Keul et al. 1967). At the onset of exercise, when oxygen is limited, anaerobic lactate production increases and then decreases as exercise proceeds (Karlsson and Saltin 1971). This decrease in lactate reflects an increase in aerobic metabolism for ATP reconstitution.

High circulating levels of lactate inhibit FFA oxidation by working muscle by inhibiting the release of FFA from adipose tissue (Issekutz et al. 1965). When aerobic processes predominant, a large part of the lactate can be reoxidized to pyruvate in the liver (gluconeogenesis) that can proceed through glycolysis thereby sparing glycogen as fuel (Figure 2). Lactate may also be reoxidized by the heart, kidney, and resting and working skeletal muscles, and eliminated in urine or sweat. Working skeletal muscle produces, removes, and oxidizes lactate simultaneously. Although there is controversy over the amount of lactate reoxidized by skeletal muscle (di Prampero 1971, Issekutz et al. 1976), the majority proceeds by this pathway.

Free Fatty Acids

Free fatty acids (FFA) originate from three major sources (Williams 1976): adipose depot triglycerides, lipoprotein and chylomicron triglycerides, and intramuscular triglycerides. Of these three, adipose tissue triglycerides are primarily used for FFA oxidation

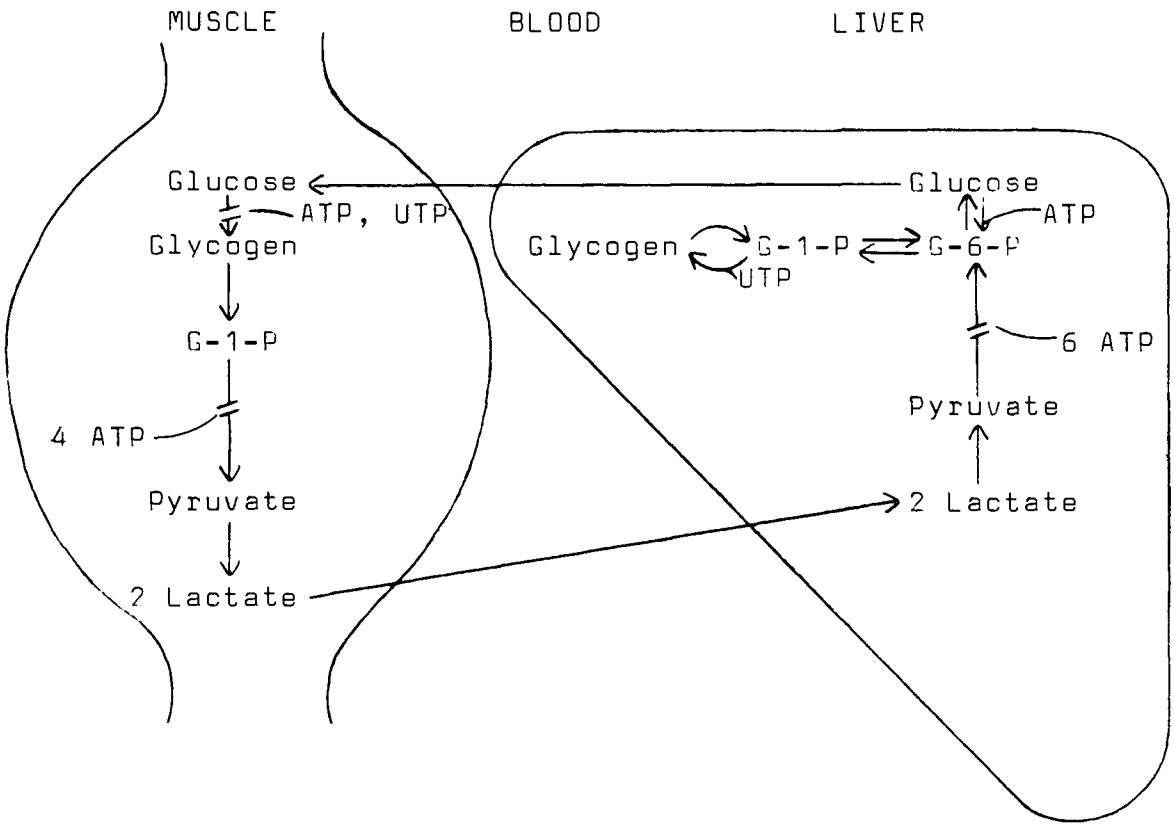

FIG 2. Lactate reoxidation by the liver. Abbreviations: ATP, adenosine triphosphate; UTP, uridine triphosphate; G-1-P, glucose-1-phosphate; G-6-P, glucose-6-phosphate.

during exercise. In the resting, postabsorptive state, the average man stores 15,000 g of triglyceride (Newsholme 1977) or 100,000 kilocalories in adipose tissue. The hydrolysis of triglycerides yields FFA and glycerol. Because muscles remove FFA in proportion to their concentrations in blood, the mobilization and delivery of FFA to working muscles determine the total amount oxidized (Hagenfeldt 1979). Figure 1 shows the entrance of FFA into the metabolic scheme of a typical muscle cell.

The production of FFA is stimulated by the increased activity of the sympathetic nervous system during exercise (Havel 1974). The catecholamine, norepinephrine, activates the adenyl cyclase system which catalyzes the conversion of ATP to cyclic adenosine monophosphate (cAMP). The latter stimulates the hormone-sensitive lipase in adipose tissue which catalyzes the hydrolysis of triglycerides.

Endurance training increases the activity or sensitivity to hormonal and nervous stimuli of the enzyme systems involved in FFA uptake (Nikklia et al. 1978) and mobilization (Askew et al. 1975). Adipose tissue adapts to training through the increase in metabolic activity of the cell while the cell size decreases (Askew et al. 1975). As a result, training increases the capacity of working muscles to oxidize FFA for fuel (Mole et al. 1971). The knowledge that trained individuals have an increased capacity to oxidize fat (Christensen and Hansen 1939) was first attained nearly 50 years ago.

The availability of oxygen to the working muscles, determined by the intensity and duration of work, governs the extent of FFA utilization. When work intensity is low-to-moderate, FFA oxidation occurs after a short lag time during which oxygen supply is increased to working muscles. At greater work intensities, the supply of oxygen is rate-limiting for FFA oxidation (Astrand and Rodahl 1977). An indirect benefit of FFA oxidation is the sparing of glycogen stores which may be needed to promote endurance in the last few minutes of prolonged exercise when anaerobic processes take effect. In turn, adequate muscle and liver glycogen stores contribute to optimal fat use during exercise by providing glycolytic intermediates to prevent severe ketosis, or the incomplete metabolism of fat.

Vitamin B-6 in Exercise Metabolism

Vitamin B-6 Function and Metabolism

Dietary vitamin B-6 (B-6) occurs in at least seven forms: pyridoxal (PL), pyridoxine (PN), pyridoxamine (PM), pyridoxal phosphate (PLP), pyridoxine phosphate (PNP), pyridoxamine phosphate (PMP), and glycosylated B-6. PN is the B-6 form found in plant sources (Rabinowitz and Snell 1948) such as pinto beans, peanuts, spinach, and bananas. PLP and PMP are the primary B-6 forms found in animal products (Lyon et al. 1962); rich sources of PLP and PMP are beef liver, chicken legs and breast, and tuna. Prior

to intestinal absorption, large amounts of PLP and PMP are hydrolyzed to yield pyridoxal (PN) and pyridoxamine (PM), although absorption of the phosphate esters can occur. The absorption of B-6 forms is nonsaturable (Buss et al. 1980, Mehansho et al. 1979, Middleton 1979, Hamm et al. 1979).

The three forms PN, PL, and PM and their 5'-phosphate esters can be interconverted (Figure 3) in various body tissues (Figure 4). The key enzyme involved in vitamin B-6 interconversions in tissues is pyridoxal kinase (EC No. 2.7.1.35). ATP is required in this reaction to phosphorylate PN, PL, and PM. Pyridoxal kinase is found in all mammalian tissues studied, but is present in the highest amounts in brain, liver and kidney; and in the lowest amounts in leg muscle, diaphragm, and whole blood (McCormick et al. 1961). Depicted also in Figure 3 is the conversions of PNP and PMP to PLP which is catalyzed by an oxidase. Regulation of vitamin B-6 occurs by product inhibition of PMP/PLP oxidase by PLP, and by hydrolytic destruction of PLP to PL; the PL is then oxidized to 4-pyridoxic acid (4-PA) and exits the body in urine (Lumeng and Li 1980).

PLP is the major form of vitamin B-6 transported in the blood. Of the total vitamin B-6 in the plasma, 60 percent is accounted for by PLP, 15 percent by PN, and 14 percent by PL (Lumeng and Li 1980). Albumin is the protein that binds PLP for transport in the bloodstream. Normal blood levels of PLP are > 39 nM for men and > 20 nM for women

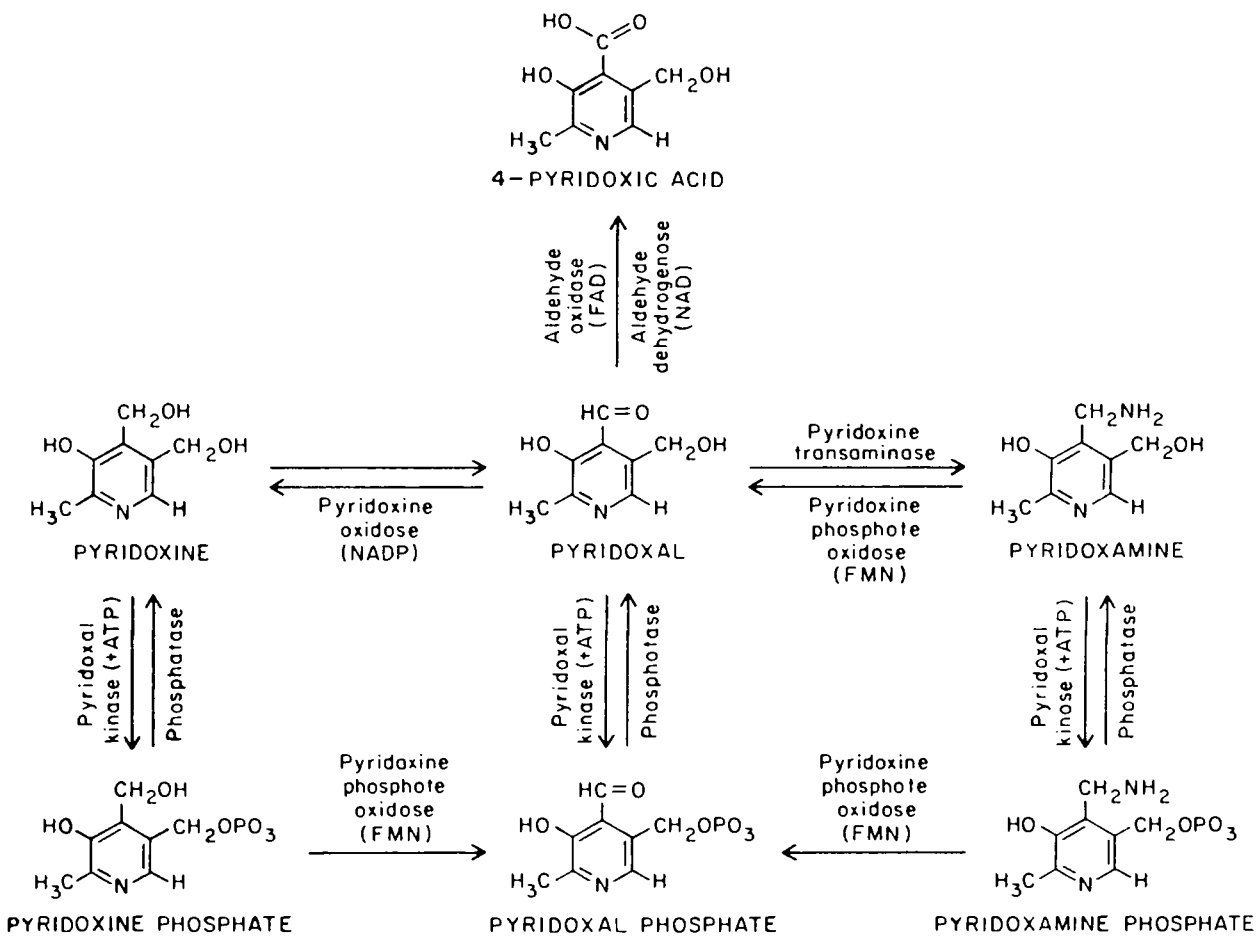

FIG 3. Interconversions of the B-6 vitamers.

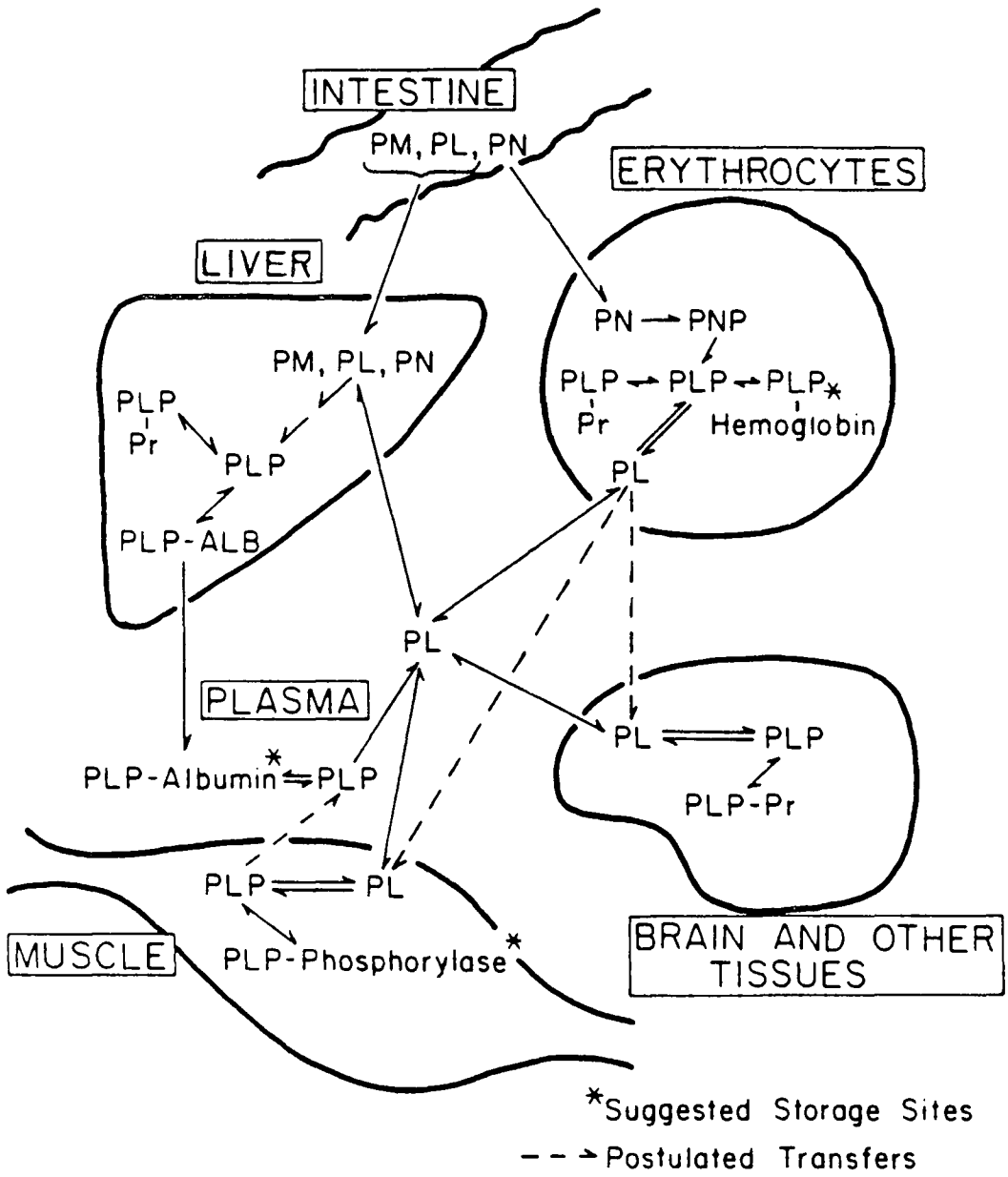

FIG 4. Tissue interrelationships of the B-6 vitamers. (Adapted from Hatcher 1983.)

(Shultz and Leklem 1981).

PLP is the active form of vitamin B-6. It is the cofactor for well over 60 enzymes involved in transamination, racemization, decarboxylation, cleavage, synthesis, dehydration, and desulfhydraton (Sauberlich and Canham 1980). Synthesis of PLP occurs primarily in the liver, but storage occurs in another tissue. The PLP content of the enzyme, glycogen phosphorylase, in mouse (Krebs and Fischer 1964) and rat (Black et al. 1977) muscle has led Krebs, Fischer, and Black to suggest that this enzyme is the major reservoir of stored vitamin B-6. Since glycogen phosphorylase constitutes 5 percent of muscle protein and muscle is 40 percent of body mass, the quantity of PLP in the phosphorylase reservoir is potentially large (Black et al. 1977).

PLP acts as the cofactor for glycogen phosphorylase. The function of glycogen phosphorylase is to cleave glycogen into glucose-1-phosphate units (Lehninger 1982). Glycogen phosphorylase is widely distributed in animal cells. This enzyme present in liver responds acutely to a decrease in blood glucose concentration and thus increases blood glucose concentration. This enzyme responds additionally to local energy needs through muscle glycogenolysis. Glycogen phosphorylase appears to be rate-limiting for glycogenolysis (Toews et al. 1979).

PLP is also the cofactor for muscle and liver aminotransferases. These enzymes in liver are in part

responsible for maintaining blood glucose levels during starvation and during prolonged exercise. The energy-yielding pathway is the branched chain amino acid-alanine-glucose cycle (Lehninger 1982). Ahlborg et al. (1974) demonstrated that after 40 minutes of low intensity exercise, the contribution of hepatic gluconeogenesis to glucose production increased progressively over 4 hours of exercise.

Effect of Exercise on Vitamin B-6 Metabolism

The first studies of vitamin B-6 and exercise revealed an increased excretion of the principal metabolite of vitamin B-6, 4-PA (see Figure 3), by rats after swimming (Efremov and Zaburkin 1972) and by humans after physical activity (Borisov 1977). Further investigations of the effect of exercise on vitamin B-6 metabolism have been performed in this laboratory.

Wozenski (1977) was the first from this laboratory to observe an increased plasma PLP in a male who had run between the administration of 0.5 g dose of PN and a 2-hour post-PN blood draw. Later studies using trained adolescent males (Leklem and Shultz 1983), in addition to adult trained males and both untrained adolescent and adult males (Munoz 1982), revealed significant increases in plasma PLP and total plasma B-6 (PB6) after submaximal running of short duration. Leklem and Shultz (1983) hypothesized that exercise, viewed as acute starvation, causes PLP to be

released from muscle glycogen phosphorylase into circulation.

Munoz (1982) additionally demonstrated that interval cycling produces the same results as interval running. Plasma PLP and PB6 significantly increased during cycling and the magnitude of this change was not significantly different from the increases seen in running. Additionally, significant decreases in both plasma PLP and PB6 were observed at 30 minutes post-exercise compared to the immediate increases after the exercise began. It was speculated that the return to pre-exercise levels may indicate that mobilized PLP was absorbed by tissues other than muscle, or metabolized and excreted.

In the study by Munoz (1982) of both running and cycling, the trained adult males had significantly greater changes in plasma PLP and PB6 than the adolescent males. A larger muscle mass of the trained adult male compared to the adolescent male, and thus a larger PLP store was suggested as an explanation. Leklem et al. (1983) observed sex differences in the magnitude of change in plasma PLP in trained athletes. Running or cycling resulted in significant plasma PLP levels in both trained males and females, but the magnitude of change was less for females. The smaller increase was probably due to the smaller female muscle mass.

Smaller increases in plasma PLP and PB6 during exercise have been observed after low, as compared to

normal and high, carbohydrate dieting in trained males (Hatcher 1983). The exercise was 50 minutes of cycling at progressive work intensities. As in the previous studies, significant increases in plasma PLP and PB6 occurred after the onset of exercise. Hatcher (1983) first observed that plasma PLP and PB6 levels 60 minutes post exercise were significantly lower as compared to immediate post-exercise. The smaller increases in plasma PLP and PB6 during exercise with the low carbohydrate intake was thought to result from lower levels of PLP stored in muscle due to the low carbohydrate intake and the three days of strenuous physical activity prior to the exercise test. Additionally, a decreased 4-pyridoxic acid excretion was observed after exercise. This could be due to the high protein and low B-6 content of the low carbohydrate diet, and the increased demand for gluconeogenesis to maintain blood glucose levels during exercise when muscle glycogen stores are low. Supplementation with 8 mg of vitamin B-6 for three days prior to exercise resulted in greater increases in plasma PLP and PB6 in the trained males than when nonsupplemented. This was suggested to reflect an increased storage of PLP in muscle.

DeVos (1983) studied changes in fuel metabolism in the study above (Hatcher 1983). After consumptions of the high carbohydrate diet supplemented with vitamin B-6, and exercise, increases (although nonsignificant) in plasma lactate levels were observed when compared to the normal or

low carbohydrate diets. DeVos (1983) speculated that vitamin B-6 supplementation may increase muscle glycogen depletion by increasing phosphorylase activity due to the large amounts of PLP present. Phosphorylase activity may have increased because of alterations in carbohydrate metabolism (simultaneously lower blood glucose and higher lactate levels) when B-6 supplementation was added to the glycogen depletion-repletion regimen.

Most recently, an investigation by Walter (1985) supports the evidence for increased PB6 and increased 4-PA excretion in humans during and after exercise, respectively. Trained adult male and females cycled for 20 minutes at high exercise intensity while on four different diets. After the consumption of either a moderate or a high carbohydrate diet supplemented with or without vitamin B-6, exercise resulted in significant increases in PB6 from pre to post and a significant decrease from post to post-60 minutes of exercise, the magnitude of the change being greater with supplementation. With all diets, 4-PA excretion increased significantly after exercise. Walter (1985) noted that the larger increase in PB6 with exercise after vitamin B-6 supplementation suggests an increased storage of the vitamin, most probably in muscle glycogen phosphorylase.

In summary, plasma PLP, the major circulating form of vitamin B-6, and total plasma vitamin B-6 (PB6) increase significantly immediately at the end of exercise and

decrease significantly 30 to 60 minutes after exercise. Exercise affects a significant increase in the excretion of the principal metabolite of vitamin B-6, 4-pyridoxic acid.

Blood Volume Changes During Exercise

Blood volume is about 75 mL per kg body weight for a normal male. This corresponds to 5 - 6 liters of blood in total and is known to be higher in the physically-trained man. About 55 percent of the total blood volume is plasma, which is roughly 9 percent solids and 91 percent water. The proteins in plasma play an important role in plasma volume (PV) and tissue fluid balance. They can cross the capillary wall when transporting ionic and nonionic substances to various sites (Astrand and Rodahl 1977).

During exercise, PV decreases in proportion to work intensity (Convertino et al. 1981; Wilkerson et al. 1977). The decrease primarily results from transcapillary filtration of intravascular fluid into working skeletal muscle. This is driven by an increased capillary hydrostatic pressure, and an increased tissue hyperosmolality due primarily to the release of lactate, bicarbonate, phosphates, and potassium ions from working muscles (Lundvall 1972). Part of the potential PV decrease is counteracted by osmotic withdrawal of fluid from inactive tissues. A slow restoration in PV is produced by increased lymph flow during exercise, although resultant fluid accumulation in interstitial spaces of working

muscles opposes further filtration of fluid (Jacobsson and Kjellmer 1964).

Diet: Carbohydrate Ingestions During Exercise

The Monosaccharides, Glucose and Fructose

Monosaccharides are commonly referred to as "simple sugars" and consist of a single polyhydroxy aldehyde or ketone unit. An unbranched single-bonded carbon chain is the backbone of a monosaccharide. When either 4, 5, 6, or 7 carbons are linked, the monosaccharide is respectively called a tetrose, pentose, hexose, or heptose. In each backbone, one of the carbon atoms is double-bonded to a oxygen atom. This is called a carbonyl group. The other carbons possess an hydroxyl group of oxygen and hydrogen. When the carbonyl group is at the end of the chain, the monosaccharide is an aldehyde and is formally called an aldose. D-glucose is an aldose, yet is often referred to as an aldohexose since it is a hexose as well as an aldose. If the carbonyl group is at any other position on the chain, then the monosaccharide is a ketone and called a ketose. D-fructose is a ketose and a hexose; therefore, it is termed a ketohexose (Lehninger 1982).

Three types of prefixes may be attached to the names of the monosaccharides. The first type is the designation of "(+)" or "(-)" immediately preceding the name. These prefixes are used when the monosaccharide contains at least one asymmetric or chiral carbon atom, and thus can occur in

two optically active forms. The term optically active refers to a substance that can rotate plane-polarized light in one direction or the other. The form or isomer of the monosaccharide that rotates plane-polarized light to the right is called dextrorotatory and is designated "(+)." The other isomer is termed levorotatory because it rotates plane-polarized light to the left; it is designated "(-)."

A second type of prefix is "D" or "L" placed immediately before the name of the monosaccharide or before the designation of the optically active isomer. The prefixes "D (introduced earlier)" and "L" refer to the configuration of the chiral carbon farthest from the carbonyl carbon atom. A D-sugar is one which projects the hydroxyl group on the chiral carbon to the right. When the projection is to the left, it is an L-sugar.

The third prefix type, "α" or "β," is used when referring to the anomeric form of a D-sugar. When an aldose or a ketose with 5 or more carbons reacts with alcohol, the straight-chain monosaccharide becomes cyclic and acts as though it had one more asymmetric center i.e. the carbonyl carbon becomes chiral. This carbon atom is called the anomeric carbon. For example. when the aldose D-glucose is crystallized from water, the α-D-glucose anomer is formed. In the presence of pyridine, β-D-glucose results. Both are 6 carbon rings called glucopyranoses and are identical in composition but different in structure at the anomeric carbon positon. The ketohexose, D-fructose,

also occurs in α and β anomeric forms. In the presence of an alcohol, however, this compound type can form a 5 carbon furanose ring or a 6 carbon pyranose ring. The appropriate terminology is fructofuranose and fructopyranose, respectively. "α" and "β" prefixes precede the "D" designation (Lehninger 1982).

The monosaccharides are colorless, crystalline solids. They are soluble only in polar solvents such as water. At 20 °C, glucose is soluble to the extent of 47 percent while fructose is very soluble at 79 percent (Kimura and Carr 1976). Monosaccharides, characteristically. are capable of donating electrons for the reduction of oxidizing agents (Lehninger 1982).

α-D-glucose and α-D-fructose are present in foods such as fruits, honey, and corn syrup. Together as sucrose, α-D-glucose and β-D-fructose are constituents of beet and cane sugars, molasses, and maple syrup. Dietary glucose and fructose can be absorbed by humans and animals, but the individual monosaccharides in sucrose must be made available for absorption by the enzyme sucrase. When dietary sucrose is consumed, it is enzymatically hydrolyzed by sucrase into glucose and fructose in the brush border cells of the intestinal epithelium. The mechanisms for absorption of glucose and fructose by the epithelial cell are different. Glucose is actively transported into and across the intestinal epithelial cell whereas fructose is not (Crane 1960), and glucose is absorbed faster than

fructose (Dehmel et al. 1969).

The presence of glucose or another monosaccharide, galactose, in the gut facilitates fructose absorption (Solomons 1985). Ten to 20 percent of ingested fructose, however, can be converted to glucose in the intestinal tract prior to absorption (Cook 1969). Due to the absence of intestinal glucose-6-phosphatase in human or rats (Ginsberg and Hers 1960), conversion of fructose to glucose inside the intestinal epithelial cell is theoretically unable to occur yet has been demonstrated in humans (Ockerman and Lundborg 1965, White and Landau 1965).

After intestinal absorption, both monosaccharides travel unchanged in the portal circulation to the liver. At this point, glucose metabolism differs from that of fructose. The entry of fructose into liver cells and the initial steps of metabolism are independent of insulin. Fructose uptake from the bloodstream occurs rapidly by various compartments of the body, however, the liver has been shown to be the primary site of uptake (Mendeloff and Weichselbaum 1953).

Inside the cell, each monosaccharide is prepared by enzymes to enter glycolysis (Figure 5). The purpose of this preparatory phase is to collect the carbon chains of the metabolized hexoses in the form of one common product, glyceraldehyde-3-phosphate. Glucose enters the preparatory phase of glycolysis and is enzymatically phosphorylated by ATP at C-1 and then at C-6 to yield

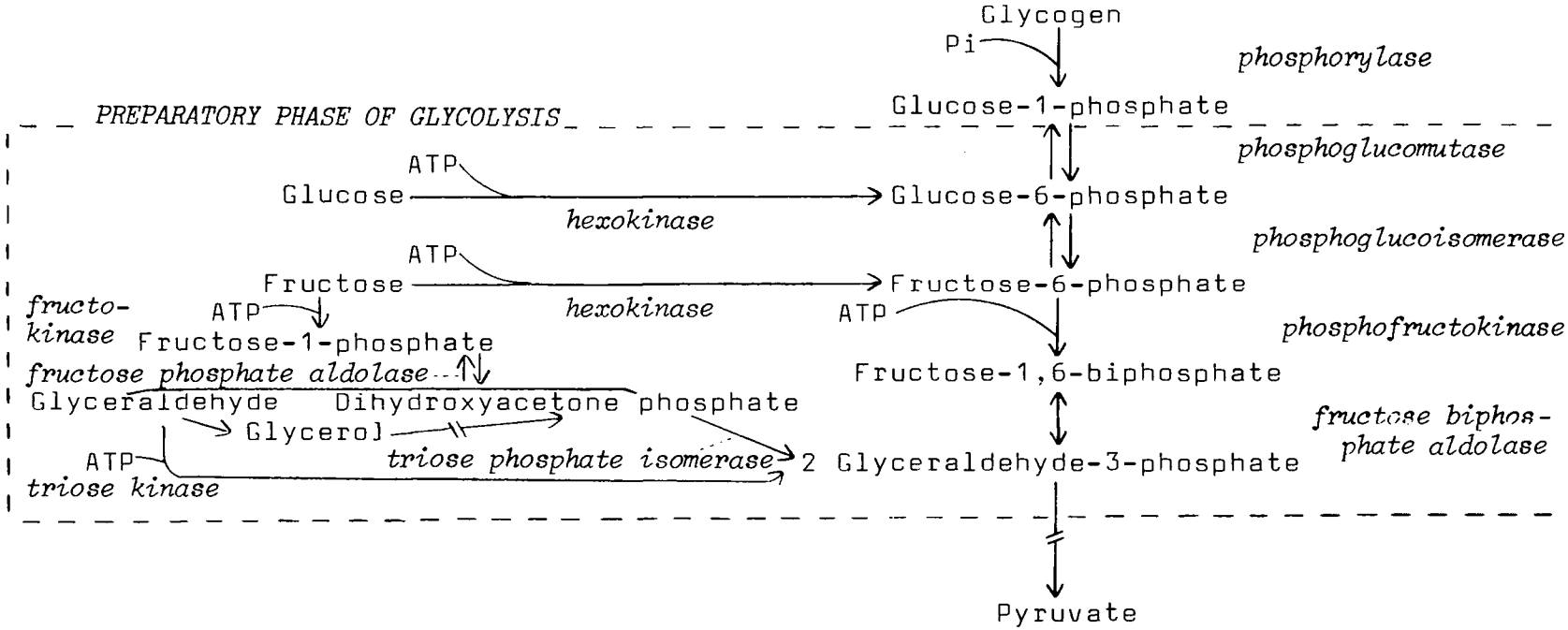

FIG 5. Metabolism of glucose and fructose in the preparatory phase of glycolysis. Abbreviations: ATP, adenosine triphosphate; Pi, orthophosphate (PO_3^{-4}). (Adapted from Lehninger 1982.)

fructose-1,6-biphosphate. This latter compound is cleaved to form two molecules of glyceraldehyde-3-phosohate. Fructose has two options: it may be enzymatically phosphorylated by ATP at the 1-C or 6-C position. Due to high concentrations of fructokinase in the liver, the formation of fructose-1-phosphate is the preferred pathway.

Ingested fructose can form glycerol, pyruvate (and consequently lactate), and glucose in the liver. Most of the glucose that is formed is stored as glycogen, thereby resulting in only a modest rise in blood glucose levels (Nilsson and Hultman 1974). The percent of ingested sugar that is absorbed and retained as liver glycogen is more than twice as great for fructose (38 percent) than for glucose (17 percent) (Cori 1926). The ingestion of fructose effects a decrease in the blood level of glycerol, a minimal increase in the blood level of insulin. and an increase in the blood levels of fructose, pyruvate and lactate. Ingestion of glucose, on the otherhand, increases the blood level cf insulin, decreases the blood level of glycerol, and has no effect on the blood levels of fructose, pyruvate, and lactate (MacDonald 1978).

Contribution to Endogenous Fuel Supply

Metabolic tracing using naturally labeled C-13 glucose has been used to analyze the fate of glucose ingestions during exercise. In a study by Pirnay et al. (1982), adult males consumed 100 g of C-13 glucose in 400 mL of water 15

minutes into a 105-minute treadmill run. The run by each subject was at a specified exercise intensity: 22, 39, 51, or 64 percent of VO2 max. Between 22 and 51 percent of VO2 max, total carbohydrate and lipid oxidation (determined by indirect calorimetry) and dietary glucose oxidation (using expiratory CO2 measurements) were linearly correlated with the relative workload. Between 51 and 64 percent of VO2 max, dietary glucose and lipid oxidation leveled off, whereas endogenous carbohydrate oxidation was markedly increased. The results support the evidence of previous studies (Pirnay et al. 1977, Ravussin 1979) for the use of glucose ingestions as a rapidly available energy source for working muscle.

Other investigators (using carbon-14 glucose) have found, however, that during 90 minutes of cycling or running at 60 to 72 percent of VO2 max (Costill et al. 1973), and during 3 hours of cycling at 50 percent of VO2 max (van Handel et al. 1980), ingested glucose was poorly oxidized. In the study by Costill et al. (1973), male subjects consumed 31.8 g of C-14 glucose in 300 mL of water 30 minutes into the 90 minute exercise bout. As determined by expired air measurements, it took 5 to 7 minutes for the C-14 glucose to appear in blood. This time was not different from the control situation (resting). During the last 20 minutes of exercise it was found that the ingested glucose comprised 5 percent of total carbohydrate oxidation. In the study by van Handel et al. (1980).

trained male subjects consumed 25 or 105 g of C-14 glucose in 400 mL of water at 120 minutes of the 3-hour exercise. By the end of exercise, the recovered C-14 in air, and in sweat and urine could only account for 10 and 2 percent, respectively, of the dose. These investigators suggested that most of the ingested glucose remained in compartments within an unoxidized glucose pool or was taken up by liver.

The discrepancy in findings by these two investigative teams could be due to the time or mode of glucose administration. Krzentowski et al. (1984a) investigated whether the time of glucose ingestion during exercise could explain the difference in results. Male subjects ran on a treadmill for 4 hours at 45 percent of VO2 max. After 15 or 120 minutes of exercise, 100 g of C-13 glucose in 400 mL of water was consumed by each subject. As determined by indirect calorimetry, total carbohydrate oxidation and lipid oxidation were similar under both conditions. Dietary glucose oxidation was also similar and approximated 55 percent of the glucose load. The investigators concluded that glucose ingested during prolonged exercise is effectively and similarly oxidized when ingestions take place at 15 and at 120 minutes after the start of exercise.

Although no studies of the mode of glucose administration have been reported, Krzentowski et al. (1984b) investigated the effect of physical training on the utilization of a glucose load during exercise. Before and after six weeks of aerobic training, male subjects cycled

for 105 minutes at 40 percent of pre-trained VO2 max during which 100 g of C-13 glucose in 400 mL of water was ingested at minute-15. There was a 17 percent increase in dietary glucose oxidation in the trained versus the untrained state, which the investigators concluded could be an indication of significant sparing of endogenous carbohydrates.

In all of the above studies, glucose was the carbohydrate administered, and the focus was the fate of glucose in the body. Others have investigated metabolic responses to glucose as well as additional types of carbohydrate. These were glucose polymers, sucrose, and fructose.

Ahlborg and Felig (1976) studied the influence of glucose ingestion on fuel-hormone response during prolonged exercise. Male subjects consumed 200 g of glucose in approximately 600 mL of water at the 90th minute of a 4-hour cycle at 30 percent of VO2 max. After glucose ingestion, arterial levels of glucose rose 35 percent by 140 minutes and remained 25 to 30 percent above resting levels and 60 to 90 percent above the controls (water ingested) at 180 and 240 minutes of exercise. Arterial FFA and glycerol levels decreased 60 to 70 percent by 180 and 240 minutes when glucose was ingested as compared to water. After glucose ingestion, arterial insulin levels increased fourfold compared to the 40-minute level and decreased thereafter yet were two to three times greater than when

water was ingested. Plasma glucagon remained unchanged after glucose ingestion but increased fourfold in controls by 240 minutes of exercise. Glucose uptake by exercising legs was two-fold higher for subjects who ingested glucose. This accounted for 60 percent of leg oxygen consumption. Liver output of glucose increased rapidly after glucose ingestion to twice the amount in controls although liver uptake of gluconeogenic precursors (lactate, pyruvate, and glycerol) decreased 70 to 100 percent after glucose ingestions. The total liver release of glucose represented 42 percent of the oral glucose load.

In a later study, Felig et al. (1982) studied glucose ingestions in the prevention of hypoglycemia and whether the prevention of blood glucose decreases by glucose ingestions delays exhaustion or alters the responses of plasma catecholamines to prolonged exercise. Ten g or 20 g of glucose in 200 mL of water was ingested by male subjects every 15 minutes of a cycle ride to exhaustion at 60 percent of VO2 max. Glucose ingestions prevented hypoglycemia and resulted in a smaller increase in epinephrine, but did not change perceived exertion or consistently delay fatigue. In the study introduced earlier, Krzentowski et al. (1984b) studied plasma levels of several hormones and other metabolites. From 0 to 15 minutes of exercise (prior to glucose feeding), the rate of total carbohydrate oxidation was slightly lower and lipid oxidation slightly higher after training. Physical

training did not affect the response of blood glucose, plasma insulin, or plasma FFA to the glucose ingested during exercise; however circulating levels of epinephrine, glycerol, and lactate were significantly decreased after training.

The effect of glucose polymers were studied by Ivy et al. (1979). The ingestion of 90 g of glucose polymer (12.8 g / 15 min) during 2 hours of isokinetic cycling (80 rpm) by trained males had no effect on total work production or VO2 max during the first 90 minutes. Glucose polymer ingestions were, however, effective in decreasing fatigue over the last 30 minutes of cycling. Total carbohydrate oxidation did not differ from controls although plasma glucose and insulin levels were maintained at higher levels with glucose polymer ingestions. Coyle et al. (1983) further demonstrated the effects of glucose polymer ingestions on fatigue rate. One g of glucose polymer per kg of body weight in a 50 percent solution of water and 0.25 g of glucose polymer per kg of body weight in a 6 percent solution of water were ingested, respectively, at the 20th minute and at 20-minute intervals thereafter of cycling at 70 to 79 percent of VO2 max by trained males. Blood glucose concentrations were 20 to 40 percent higher during exercise after glucose polymer ingestion than without. The exercise-induced insulin response was prevented with glucose polymer ingestions. Respiratory exchange ratio (RER) remained unchanged. Fatigue was postponed by glucose

polymer ingestions in 7 of 10 subjects, which was mediated in two subjects by the prevention of hypoglycemia.

The influence of sucrose ingestions was studied by Hargreaves et al. (1984). Forty-three g of sucrose in 400 mL of water was ingested by subjects at 0, 1, 2, and 3 hours of a 4-hour exercise. The subjects cycled for 20 minutes at 50 percent of VO2 max followed by 10 minutes of intermittent exercise (30 seconds at 100 percent of VO2 max followed by a 2 minute rest) for each 30 minutes. No differences in VO2, heart rate, or total energy expenditure were observed between trials, although the RER was significantly higher in the group ingesting sucrose. Blood glucose was significantly higher 20 minutes post-feeding. but by 50 minutes no difference was observed between trials until 230 minutes. Muscle glycogen utilization was significantly lower in the group ingesting sucrose. During the sprint rides to exhaustion at the end of each trial, subjects who had ingested sucrose performed 45 percent longer.

Two groups have reported the effects of fructose ingestions during exercise. Fruth and Gisolf (1983) exercised trained males at 70 percent of VO2 max on a treadmill until exhaustion. At 0-minute and at 20-minute intervals thereafter, 20 g of glucose or fructose in 200 mL of water or water only (control) was consumed. VO2 and RER were the same for glucose, fructose, and control. Serum glucose concentration during exercise were nearly identical

when subjects ingested glucose or fructose, and were not significantly higher than control. Serum insulin concentration was the same for glucose, fructose and control, and insulin concentration did not change with time. Serum glycerol concentration was elevated during the first 60 minutes of exercise for glucose, fructose, and control, but at 90 and 120 minutes, glycerol levels were significantly higher for fructose as compared to glucose and control. The concentration of blood lactate was significantly higher at 30 minutes of exercise when fructose was ingested as compared to glucose and control; at 60 minutes. the lactate level was significantly higher with fructose than with glucose ingestions. There was no significant difference between the treatments with respect to run time to exhaustion. Fructose ingestions were associated with an average run time of 16 and 13 percent less than when glucose and control were ingested, respectively. Furthermore, fructose caused gastro-intestinal distress in all subjects.

In a later study, Bjorkman et al. (1984) examined the influence of glucose and fructose ingestion on the capacity to perform prolonged heavy exercise. Trained males exercised on a cycle ergometer at 70 percent of VO2 max until exhaustion on three occasions. During the cycling, subjects consumed 17.5 g of glucose or fructose in 250 mL of water, or just water every 20 minutes. Glucose ingestions during exercise as compared to fructose and

water resulted in a significantly prolonged exercise duration. Plasma arterial glucose levels decreased slightly by 20 minutes of exercise and then was maintained at the basal level throughout the exercise when either glucose and fructose was ingested. When water was ingested, glucose levels fell significantly, resulting in lower values during exercise than with glucose or fructose ingestions. No subject had glucose levels fall below 3 mM.

In the study by Bjorkman et al. (1984), arterial FFA levels rose with all treatments after 40 minutes of exercise. Glucose ingestions were associated with FFA levels that were significantly lower than those of controls. FFA levels after fructose ingestions were not significantly different than when glucose or water was ingested. Arterial glycerol levels increased significantly less during exercise when glucose was ingested as compared to fructose or water. Arterial lactate levels rose two- to threefold after all treatments but reached higher values with fructose than with glucose. Arterial insulin levels decreased to about half of the restino level with all treatments but was significantly higher when glucose was ingested as compared to water. Exercise affected a similar increase in the arterial level of norepineohrine levels with the consumption of each drink. Arterial epinephrine levels increased five- to eightfold with all treatments during exercise but the increase was more pronounced with water than with glucose consumption.

The measurement of muscle glycogen levels was unique to the Bjorkman et al. (1984) glucose/fructose/exercise study. At exhaustion, muscle glycogen levels were half that at rest for all treatments. The mean rate of muscle glycogen degradation, however, was significantly lower with the ingestion of glucose as compared to fructose or water. The investigators concluded that intermittent glucose ingestons during prolonged heavy cycling postpones exhaustion and exerts a glycogen-conserving effect in working muscles. The investigators reported that fructose ingestions during exercise maintains the glucose levels during exercise but fails to influence either muscle glycogen degradaton or endurance performance.

To summarize, depending on the type of sugar, carbohydrate ingestions during prolonged exercise can decrease muscle glycogen utilization, delay fatigue, and increase endurance performance. All sugars in varying concentrations except fructose have elicited these beneficial effects. The ingestion of 7 or 10 percent fructose fluids during exercise have been associated with blood glucose levels that were not significantly different than when glucose fluids were ingested. Moreover, Fruth and Gisolf (1983) found that 10 percent fructose fluid ingestion caused G-I distress in every subject. Gastric emptying is known to slow when the carbohydrate fluid is 278 mM (5.0 percent solution) and above (Costill and Saltin

1974). Although, investigators have administered other sugars in concentrations greater than 278 mM, fructose ingestions may not be appropriate at higher levels. Further investigation is warranted to determine if fructose ingestions in varying concentrations are detrimental to athletic performance.

III. Materials and Methods

Preface

A study was designed and conducted during the Summer of 1984 to determine the effect of carbohydrate ingestions on vitamin B-6 and fuel metabolism during prolonged, continuous exercise in four trained males. The investigation was approved by the Human Subjects Committee of Oregon State University and served as the initial study for this research work. The study was expanded to include four additional subjects. Two of the initial subjects returned for continued testing. No changes in the pre-exercise meal, carbohydrate dose prescription, or exercise testing were made in the expanded research.

Subject Selection

Eight adult males, aged 21 to 39 years, were recruited to participate in this study. Health and physical activity history questionnaires were completed (see appendix), and each subject met four criteria:

1) aerobically-trained in cycling and/or running;
2) no history of diabetes and/or glucose intolerance;
3) no use of drugs that affect glucose or insulin metabolism;
4) apparently healthy.

The subjects were given detailed information on the expectations of the investigators at the times of the initial screenings, and each subject signed a subject consent form (see appendix). It was particularly critical to this research that each subject consent to maintain body weight and training level, in addition to refraining from taking vitamin or mineral supplements during the course of the study. At the times of the initial screenings, all subjects were asked to collect one urine sample. This was tested for glycosuria using Clinistix reagent strips (Miles Laboratories, Elkhart, IN). The result from each test was negative. No subject had glycosuria.

Subject Baseline Data Collection

Four days before the initial study commenced, the four subjects reported to the Oregon State University Human Performance Laboratory for the collection of baseline data. Height had been prerecorded on health questionnaires therefore, only weight and maximum oxygen uptake (VO2 max) measurements were performed at this time. In the later study, body weights of the returning two initial subjects were recorded outside the laboratory four or eight days before exercise testing and reported to the principal investigator. (This was done because of the long time lapse that occurred between the initial study and the expanded research). Weight losses of 5.0 kg and 2.8 kg by Subjects #1 and #2, respectively, were observed. VO2 max

was not, however, re-established in these two returning subjects because training levels were unchanged. The four new subjects reported to the Human Performance Laboratory one week prior to commencement of the later study for baseline data collection which included weight and VO2 max measurements. The physical statistics of all subjects are presented in Table 1.

VO2 max was determined using a Monark cycle ergometer (Quinton Instruments, Seattle, WA). The cycle seat height was adjusted for the comfortability of each subject and recorded. The pedal rate was held constant at 50 rpm using a metronome. Heart rate was monitored with an electrocardiograph (Quinton Instruments, Seattle, WA) using a torso mounted limb lead system. The negative electrode was placed at the C5 position. Each subject pedalled initially for 3 minutes with no resistance. The exercise load was then increased by 0.5 kp every 3 minutes thereafter until the subject could no longer maintain the designated cadence. Heart rate and oxygen consumption were measured during the last 30 seconds of each work load. The "maximum oxygen uptake" value occurred at the point where an increase in workload did not increase oxygen uptake by the subject (Wilmore and Costill 1974).

Experimental Design

Timeline

The experimental design is summarized in Table 2. The

Subject	Age	Height	Weight	VO2 max*
#	y	cm	kg	mL/kg/min
1	39	177.8	62.0†	49.1
2	33	170.2	58.3†	55.4
3	26	185.4	80.0	44.9
4	21	188.0	80.8	47.9
5	21	174.0	84.3	54.1
6	27	180.3	74.1	58.1
7	37	180.3	71.8	64.8
8	34	182.9	91.8	57.8
Mean ± SD	29.8 ± 7.0	179.9 ± 5.8	75.4 ± 11.2	54.0‡ ± 6.5

* VO2 max determined by graded exercise testing (Wilmore and Costill 1974).
† The body weight in June of 1984 was higher: 67.0 kg (Subject #1, four days pre-study) or 61.1 kg (Subject #2, eight days pre-study).
‡ This value is equivalent to 4.13 L/min.

TABLE 1
Physical statistics of the subjects.

DAY	June 21 1984	June 25 1984	June 29 1984	Nov 20 1984	Dec 4 1984	Dec 10, 14 1984	Dec 11 1984	Dec 18 1984	Jan 7 1985
PROTOCOL	Base-line data collec	Ex test	Ex test	Base-line data collec	Ex test	Base-line data collec	Ex test	Ex test	Ex test
SPECIFICS OF PROTOCOL	Body weight + VO2 max	Pre-ex meal 2 h ex @ 60% Trtmt C G F	Pre-ex meal 2 h ex @ 60% Trtmt C G F	Body weight + VO2 max	Pre-ex meal 2 h ex @ 60% Trtmt C G F	Body weight	Pre-ex meal 2 h ex @ 60% Trtmt C G F	Pre-ex meal 2 h ex @ 60% Trtmt C G F	Pre-ex meal 2 h ex @ 60% Trtmt C G F
SUBJECT(S) INVOLVEMENT(S) (BY NUMBER)	1 2 3 4	1 4 2 3	2 1 3 4	5 6 7 8	5 6 7 8	2 1	7 5 2 8	2 1 7 5 8 6	6 1
SUMMARY OF INVESTIGATIONS	During initial study four subjects experienced two of three treatments.			Continued research for later study involved four additional subjects who experienced all three treatments. Two returning subjects experienced the treatment missed plus one more.					

TABLE 2
Experimental Design. Abbreviations: Nov, November; Dec, December; collec, collection; ex, exercise; Pre-ex, Pre-exercise; Trtmt, Treatment; C, Control; G, Glucose; F, Fructose; h, hour; 60%, 60% of VO2 max.

initial study was conducted in June of 1984 and the later study completed in November/December of 1984, and January of 1985. Baseline data collection preceded exercise testing for all subjects. Four hours before all exercise tests, a standard breakfast was consumed by each subject. This meal is described in the next section. To determine the effects of glucose and fructose ingestions during exercise on selected metabolic parameters, each subject consumed either control, glucose, or fructose fluids on a given test day. Over a period of three test days, all treatments were to have been experienced. This was done for the four new subjects in the expanded research. Since two of the initial subjects could not return for continued testing, they experienced only two treatments. The returning two initial subjects experienced four treatments. The additional measure of one treatment was used to evaluate reproducibility of results. The minimum time lapse between exercise tests was four days. This allowed for a recuperation period, and for three-day dietary records (see appendix) to be completed by each subject for review of food intake prior to each exercise test day.

Pre-exercise Meal

Four hours before each exercise test and after an overnight fast, each subject reported to the metabolic kitchen for a standard breakfast. The hour of the pre-exercise meal for each subject was determined based on

the time the investigators and equipment were available for the exercise test (see exercise protocol in a subsequent section). The pre-exercise meal was prepared by the principal investigator. The foods and the partial nutrient composition of the pre-exercise meal are presented in Table 3. Nutrient composition of the pre-exercise meal was calculated from the food composition tables of the Agricultural Handbook No. 456 (1975) and the Home Economics Research Report No. 36 (Orr 1969). The quantity of both margarine and jam or jelly was determined at breakfast by each subject. All subjects were instructed to consume nothing but water for the period beginning after breakfast and ending at the time of exercise testing.

Carbohydrate Dose

Crystalline α-D(+)glucose and β-D(-)fructose (Sigma Chemical Co., St. Louis, MO) were dissolved in sterile, redistilled water to prepare concentrated stock solutions of glucose and fructose, respectively. The glucose stock solution was 1.98 M and the fructose stock solution was 2.78 M. Both solutions were prepared by the principal investigator on a day preceding an exercise test and stored at 0 °C. A person not involved in this research formulated the fluids, coded the fluid containers and assigned the fluids to the subjects. The carbohydrate (CHO) dose for each subject was calculated using baseline body weight (BW), and therefore was constant throughout the study. The

Food	Serving	Protein	Fat	Carbohydrate	Vitamin B-6
	g	g	g	g	mg
Cereal, shredded wheat	40	4.0	0.8	32.0	0.098
Milk, 1% fat	250	9.8	2.6	13.9	0.017
Melon, cantelope or honeydew, fresh	150	1.1	0.3	11.4	0.028
Orange juice, frozen, reconstituted	250	1.8	0.3	26.8	0.070
Toast, whole wheat	58	6.1	2.1	27.7	0.104
Margarine	8*	--	6.5	--	--
Jam or jelly	21†	0.1	--	14.7	0.010
TOTAL		22.9	12.6	111.8	0.327
TOTAL KILOCALORIES		91.6	113.4	447.2	
PERCENT OF TOTAL KILOCALORIES		14.0	17.4	68.6	

* Range: 0.0 to 12.4 g; mean ± SD: 7.8 ± 2.5 g.
† Range: 9.0 to 25.3 g; mean ± SD: 21.1 ± 4.1 g.

TABLE 3
The pre-exercise meal.

exception was the use of the new baseline body weights of the two returning subjects for calculations of carbohydrate dose in the later study. A simple formula was used for calculating the CHO dose:

$$\text{CHO dose} = 1\ \text{g CHO} \times \text{kg}^{-1}\ \text{BW} \times \text{L}^{-1}\ \text{water}$$

The carbohydrate dose assigned to each subject is presented in Table 4.

A non-nutritive sweetener, saccharin, marketed as Nutradiet (S&W Fine Foods, Inc., San Mateo, CA), was used to disquise the control (0.019 mL of sweetener per mL of water) and glucose (0.005 mL of sweetener per mL of solution) fluids from that of the fructose. Neither the investigators nor the subjects knew what fluid type the subjects were consuming under the double-blind conditions. Each subject consumed 800 mL of the unknown fluid during exercise testing described below in the next section.

Exercise Protocol

The Human Performance Laboratory (HPL) was the site of all exercise tests. Due to a limited amount of equipment and number of investigators, only two subjects could be accommodated at one time. Depending on the particular test day, a total of two to six subjects completed exercise tests. In all cases, two subjects were tested at one time. Regardless of the designated times for pairs of subjects to report to the HPL for exercise testing, all subjects were

Subject	Body Weight	Carbohydrate Dose		
#	kg	g/200 mL	mmol/200 mL	% solution
1	62.0	12.4	68.8	6.2
2	58.3	11.7	64.7	5.8
3	80.0	16.0	88.8	8.0
4	80.8	16.0	88.8	8.0
5	84.3	16.9	93.6	8.4
6	74.1	14.8	82.3	7.4
7	71.8	14.4	79.7	7.2
8	91.8	18.4	101.9	9.2
Mean ± SD		15.1 ± 2.2	82.3 ± 11.8	7.5 ± 1.1

TABLE 4
Carbohydrate doses for the subjects.

approximately four hours postprandial. Five minutes after arrival, each subject was weighed and then prepared for electrocardiograph monitoring using either a three-lead or a five-lead system. Upon resting approximately 5 minutes, supine blood pressure and heart rate were determined. At this time, each subject moved and sat in a chair for a pre-exercise blood draw. Each subject was assigned one of two Monark cycle ergometers. Once seated, blood pressure and heart rate were again determined.

The exercise test protocol is depicted in Figure 6. At the time of initiation of the exercise, subjects were 4 hours 6 minutes ± 29 minutes postprandial (range 3 hours 34 minutes to 4 hours 43 minutes). At 0-time of exercise, each subject consumed 200 mL of an assigned fluid. Then, each subject warmed up by cycling at approximately 1 kp for 5 minutes and at increased work loads thereafter until 60 percent of VO2 max was reached. This workload was achieved at approximately 15 minutes of exercise. Periodic heart rate measurements were obtained during exercise testing to ensure that a predetermined target heart rate (equalling 60 percent of VO2 max) was achieved and maintained by each subject. Additionally, relative perceived exertion tests were administered to each subject in conjunction with heart rate measurements that involved asking the subject to point to a number on the Borg scale (1973) that described the intensity of exertion. At 30, 60, and 90 minutes of exercise, the work load was decreased to 1 kp to facilitate

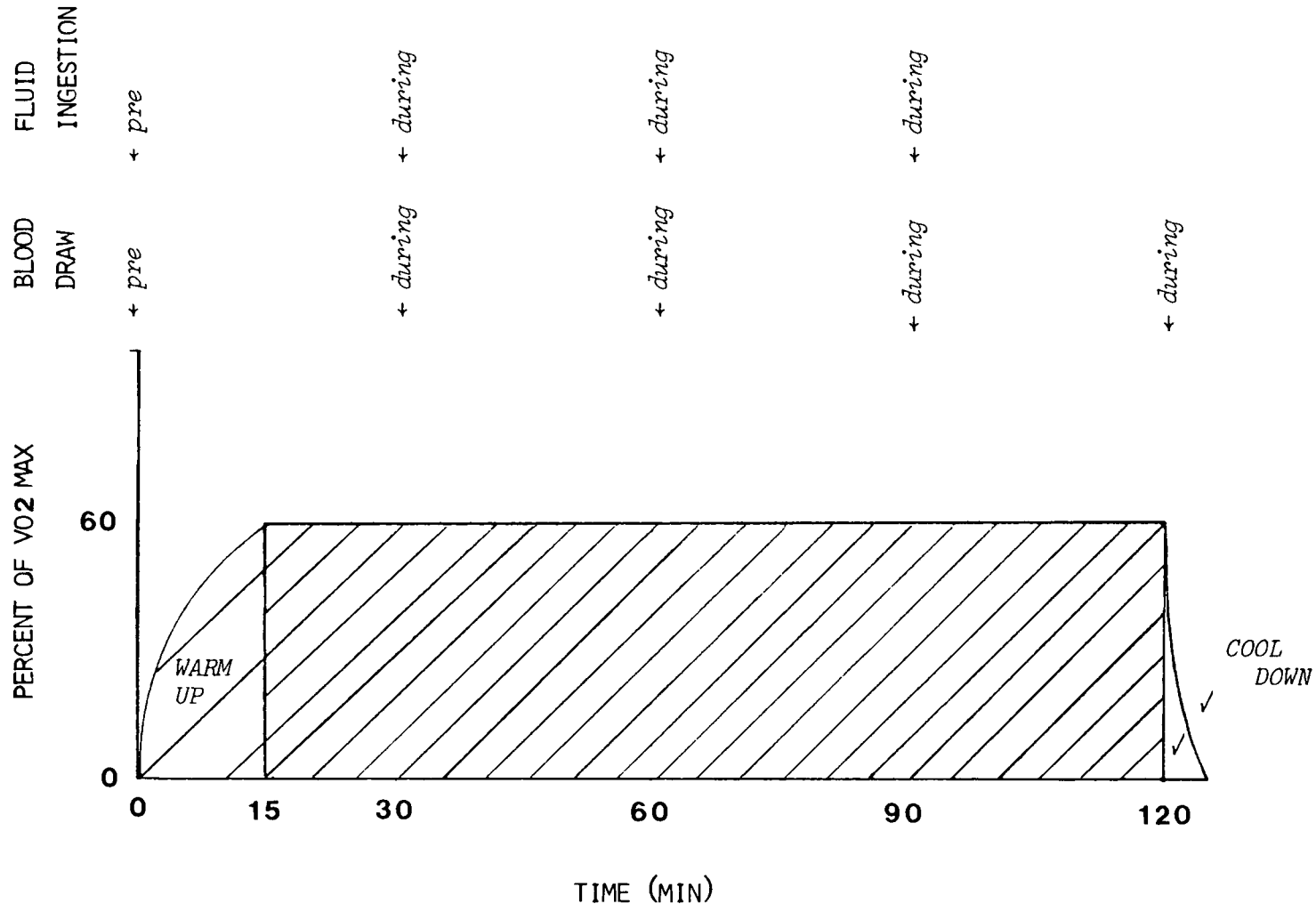

FIG 6. Exercise test protocol: times for blood sampling and fluid ingestion.

blood drawing. Immediately following this, 200 mL of fluid was consumed after which the workload was increased to the previous value. At 120 minutes of exercise, the workload was decreased to 1 kp for active recovery and for the final blood draw. After approximately 3 minutes of active recovery, each subject was weighed and provided with drinking water prior to leaving the laboratory.

Blood Sample Collection

Two medical technologists were responsible for all blood drawn during the study. Ten to 20 mL samples were drawn from the antecubital vein into heparinized tubes. Plasma was separated by centrifugation at 1860 g for 15 minutes at 4 °C. The plasma was divided into smaller aliquots and stored at -40 °C for future analyses.

Blood Sample Analyses

Hematocrit and Hemoglobin

Hematocrit (Hct) and hemoglobin (Hgb) were determined immediately after each pre-exercise blood draw. In the initial study, blood draws at 30, 60, 90, and 120 minutes of exercise were assayed for Hct only. For the later study, both Hct and Hgb measurements were obtained for all blood draws. Hct was determined in triplicate using the microhematocrit method. Hgb was determined in triplicate using the cyanomethemoglobin method.

Plasma Glucose

A microprocedure utilizing a Technicon Autoanalyzer (Technicon Corp., Terry Town, New York) was used to determine plasma glucose. All samples were determined in duplicate. The method is based on the reduction of ferricyanide to colorless ferrocyanide by reducing sugars (Hoffman 1937).

Plasma Lactate

An enzymatic procedure for plasma lactate utilized a kit from Sigma Chemical Company (technical bulletin no. 726-UV/826-UV). The enzyme lactate dehydrogenase catalyzes the formation of pyruvate and NADH from lactate and NAD. The amount of lactate originally present in blood plasma is determined by the increased absorbance at 340 nm due to NADH formation in the presence of excess NAD. Absorption was measured using a Beckman DU Quartz Spectrophotometer with a Gilford UV monitor (National Technical Laboratories, South Pasadena, CA). All samples were run in duplicate. There was a mean variation of 3.8 percent for duplications of a control sample run with each assay (n=7). The inter-assay coefficient of variation for the control was 11.3 percent (n=7).

Plasma Pyridoxal 5'-Phosphate

An enzymatic, radioisotope method of Chabner and Livingston (1970) modified in this laboratory was used to

determine plasma pyridoxal 5'-phosphate (PLP). The modifications included the use of 5 M potassium acetate as a buffering solution, incubation flasks with sidearms, and the composition of the liquid scintillation fluor. The method is based on the conversion of 1-^{14}C-L-tyrosine to tyramine and ^{14}C-carbon dioxide (CO2) in the presence of tyrosine decarboxylase, a PLP-dependent enzyme. NCS (Nuclear Chicago Solubilizer by Amershan Corp., Arlington Heights, IL) was used to trap the released radioactive CO2. All samples were counted in a Beckman liquid scintillation counter (Model LS-3133P). All samples of each subject were determined in duplicate in the same assay, except when repeats were necessary. There was a mean variation of 4.9 percent for duplication of a control sample run with four assays. The inter-assay coefficient of variation for the control was 7.9 percent (n=6).

Data Reduction

Two methods were used to calculate plasma volume (PV) changes. The method by van Beaumont (1972) uses only Hct measurements in the equation for calculating percent PV change:

$$\% \text{ change PV} = 100/(100 - \text{Hct1}) \times 100[(\text{Hct1} - \text{Hct2})/\text{Hct2}]$$

The "1" and "2" suffixes refer to initial and final measurements, respectively. The proportionality factor corrects for tne underestimation of PV change that occurs

when percent change in Hct is used. The method by Dill and Costill (1974) uses both Hct and Hgb measurements to calculate the percent change in PV:

$$\% \text{ change PV} = (Hgb1/Hgb2) \times [(100 - Hct2)/(100 - Hct1)] - 1$$

This method differs from the one previously described in that estimations of plasma volume changes are not distorted by alterations in the volume of the red blood cells. Calculations of PV changes were made for each blood sample at 30, 60, 90, and 120 minutes of exercise relative to pre-exercise.

The percent changes in plasma constituents (Co) was calculated from Hct and solute concentration (Cn) using an equation by van Beaumont et al. (1973):

$$\% \text{ change Co} = \frac{(Cn2) - [Hct2(100 - Hct1) \times (Cn1)] / Hct1(100 - Hct2)}{[Hct2(100 - Hct1) \times (Cn1)] / Hct1(100 - Hct2)}$$

The "1" and "2" suffixes refer to initial and final measurements, respectively.

Statistical Analyses

The design of this research study involved seven dependent variables: hematocrit, hemoglobin, plasma glucose, plasma lactate, plasma PLP, and plasma volume changes (by two calculations). The two independent variables were the times of measurement (five) and the

experimental conditions or treatments (three). It was appropriate then to perform seven separate 5 X 3 repeated measures ANOVAs, as well as descriptive statistics, on the data. Identical statistical analyses were performed on the first five dependent variables stated above after transformations to changes from pre-exercise were made. When significant interactions occurred, Newman-Keuls multiple comparisons (Sachs 1982) were performed to determine which data points were statistically different at the 0.05 level of significance. An additional ANOVA was performed on percent of VO2 max values by subject and treatment to determine if the relative work rates by the subjects varied significantly. Null hypotheses were rejected at the 0.05 level of significance.

IV. Results

This chapter presents the results of the 2-hour, submaximal exercise tests performed by subjects ingesting control, glucose, or fructose fluids during exercise. The data of each subject for all exercise tests can be found in the appendix.

VO2

The average percent of the VO2 max values during exercise were not statistically different between treatments ($p = 0.301$). These values were 60.3 ± 10.2, 58.0 ± 4.2, and 58.3 ± 6.3 percent for the control, glucose, and fructose treatments, respectively. The average percent of the VO2 max values among subjects were, however, significantly different ($p = 0.006$), although the percent of VO2 max values within a subject were similar. Every subject's data for all variables was included for presentation in this report because the overall average of 58.0 ± 7.2 percent of VO2 max approximated the target of 60 percent of VO2 max.

Plasma Glucose

Table 5 lists the mean plasma glucose concentrations during exercise and the differences from pre-exercise for all three treatments. Figures 7a & 7b illustrate the effect of exercise on mean plasma glucose concentrations

Time of Exercise	Plasma Glucose Control n=8	Treatment Glucose n=7	Fructose n=7
min	mM	mM	mM
0	5.22 ± 0.34	5.63 ± 1.01	5.25 ± 0.20
30	5.04 ± 0.62 -0.19	5.72 ± 0.99 +0.09	5.33 ± 0.32 +0.08
60	4.75 ± 0.72 -0.48	5.69 ± 0.58 +0.06	5.23 ± 0.29 -0.02
90	4.46 ± 0.75 -0.76	5.00 ± 0.39 -0.63	5.46 ± 0.26 +0.21
120	4.27 ± 0.75 -0.95	4.92 ± 0.59 -0.71	5.47 ± 0.39 +0.22

All plasma glucose concentrations are the mean ± standard deviation. The value below the mean is the difference from the pre-exercise value.

TABLE 5
Mean plasma glucose concentrations during exercise and differences from pre-exercise for all treatments.

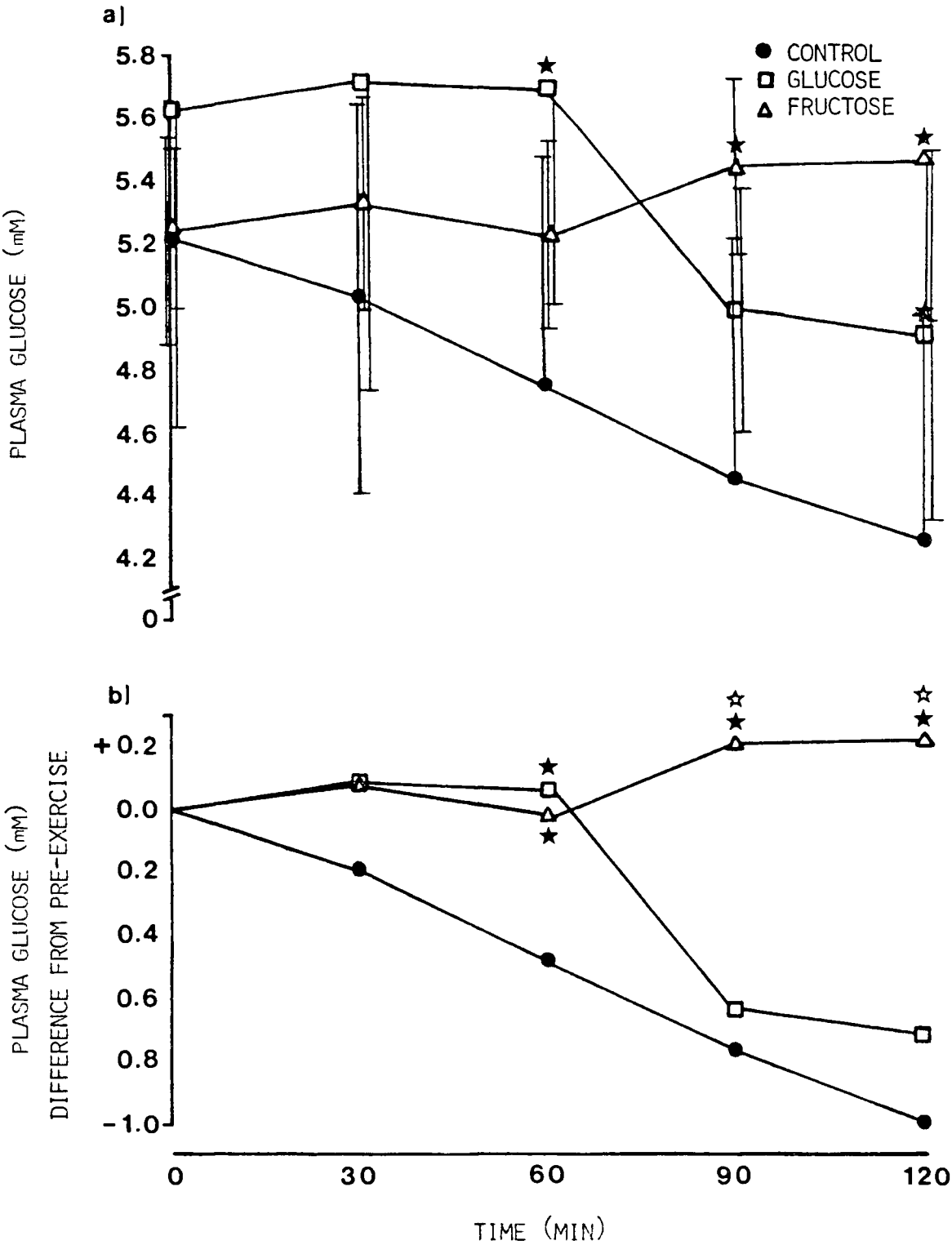

FIG 7. Effect of exercise on plasma glucose concentrations: a) means and b) means transformed to differences from pre-exercise. ★ significantly different from control ($p < 0.05$). ☆ significantly different from glucose ($p < 0.05$).

and indicate the statistically significant differences among treatments at points in time. Significant time ($p = 0.0002$) and treatment ($p = 0.0017$) effects, and a time by treatment ($p = 0.0148$) interaction existed for plasma glucose values. Transformations of the data to difference from pre-exercise, however, revealed only a significant time ($p = 0.00001$) effect and time by treatment ($p = 0.00006$) interaction. The treatment effect ($p = 0.1786$) for the difference from pre-exercise was nonsignificant ($p > 0.05$).

The mean plasma glucose concentration fell progressively during exercise for the control treatment. At 90 and 120 minutes of exercise, the mean plasma glucose concentrations were significantly less than both mean pre-exercise and 30-minute concentrations ($p < 0.05$). Decreases in mean plasma glucose from pre-exercise of 3.4, 9.0, 14.6 ($p < 0.05$), and 18.2 ($p < 0.05$) percent at 30, 60, 90, and 120 minutes of exercise, respectively, occurred when subjects consumed only water. Variability of the measurements as indicated by standard deviations was of the same magnitude for all times except for pre-exercise; the variability of the pre-exercise glucose value was half that of the variabilities of plasma glucose values at 30, 60, 90, and 120 minutes of exercise.

The glucose treatment produced a rise in blood glucose that peaked at 30 minutes of exercise and then fell below pre-exercise at 90 minutes of exercise. By 120 minutes,

the mean plasma glucose level was significantly less than the 30- and 60-minute values ($p < 0.05$). Glucose ingestions resulted in increases from pre-exercise of 1.6 and 1.1 percent at 30 and 60 minutes of exercise, respectively, and decreases from pre-exercise of 11.2 percent and 12.6 percent ($p < 0.05$) at 90 and 120 minutes, respectively. The variability of the pre-exercise and 30-minute mean plasma glucose values for the glucose treatment was approximately equal and twice that of the 60-, 90-, and 120-minute values. Plasma glucose values of subject #2 at pre-exercise (7.88 mM) and at 30 minutes of exercise (5.4 mM) contributed most highly to the large standard deviations. Due to nonsignificant differences ($p > 0.05$) among treatments at pre-exercise (basal levels), subject #2's data was not excluded from the statistical analyses. The inclusion of this data most likely was the reason that the rise in plasma glucose at 30 minutes of exercise was not significant.

The fructose treatment produced a relatively constant level of plasma glucose. Fructose ingestions produced an increase of 1.5 percent, a decrease of 0.4 percent, and increases of 4.0 and 4.2 percent from the pre-exercise level at 30, 60, 90, and 120 minutes of exercise, respectively. No significant difference between the levels at the four times were noted ($p > 0.05$). Variability of plasma glucose values was lowest at pre-exercise and highest (twofold difference) at 120 minutes of exercise.

On the average, variability of plasma glucose values was much lower in the fructose treatment than in the control or glucose treatments.

The elevated mean plasma glucose value and difference from pre-exercise at 60 minutes of exercise during the glucose treatment, was significantly greater than that for the control ($p < 0.05$). Additionally, the difference from the pre-exercise level in the fructose treatment at 60 minutes of exercise was significantly greater than that for the control ($p < 0.05$), although the actual (i.e. untransformed) plasma glucose value at 60 minutes was nonsignificant ($p > 0.05$). At 90 minutes of exercise, the fructose treatment produced a significantly greater mean plasma glucose value and difference from pre-exercise value than control. The difference from pre-exercise was also significantly greater than that for the glucose treatment ($p < 0.05$). At 120 minutes of exercise, the mean plasma glucose value and difference from pre-exercise for the fructose treatment was significantly greater than control and the difference from pre-exercise was also significantly greater than glucose ($p < 0.05$). At 120 minutes of exercise for the glucose treatment, only the actual plasma glucose value obtained was significantly greater than that for the control ($p < 0.05$).

Plasma Lactate

Table 6 lists the mean plasma lactate concentrations

Time of Exercise	Plasma Lactate Control n=8	Treatment Glucose n=7	Fructose n=7
min	mM	mM	mM
0	1.05 ± 0.20	1.60 ± 0.36	1.33 ± 0.63
30	2.28 ± 1.13 +1.23	2.35 ± 0.73 +0.75	2.22 ± 0.97 +0.90
60	1.86 ± 0.62 +0.81	2.03 ± 0.98 +0.42	1.99 ± 0.66 +0.66
90	1.88 ± 0.66 +0.83	1.63 ± 0.51 +0.02	1.67 ± 0.51 +0.34
120	2.06 ± 0.82 +1.01	1.89 ± 0.49 +0.29	1.78 ± 0.61 +0.45

All plasma lactate concentrations are the mean ± standard deviation. The value below the mean is the difference from the pre-exercise value.

TABLE 6
Mean plasma lactate concentrations during exercise and differences from pre-exercise for all treatments.

during exercise and the differences from pre-exercise for all three treatments. Figures 8a & 8b illustrate the effect of exercise on mean plasma lactate concentrations and indicate the statistically significant differences among times when data for all treatments was averaged. Significant time effects existed for both the actual values obtained ($p = 0.0000003$) and the transformed data (i.e. difference from pre-exercise) ($p = 0.0016$). The transformed data additionally exhibited a significant treatment effect ($p = 0.0388$); however multiple comparisons failed to produce significant differences among treatments ($p > 0.05$).

For all treatments, the mean plasma lactate concentration rose sharply at 30 minutes of exercise and then fell progressively for 60 minutes afterwhich lactate levels increased. In the control situation, variability of measurements was lowest for pre-exercise and highest (five- to sixfold difference) at 30 minutes of exercise. Values at 60 and 90 minutes of exercise were associated with variabilities of approximately the same magnitude, and the value at 120 minutes was only somewhat higher than at 60 and 90 minutes. In the glucose situation, variability of measurements was again lowest at pre-exercise but highest (two- to threefold difference) at 60 minutes of exercise. Lactate values at 90 and 120 minutes of exercise varied approximately the same amount, with the value at 30 minutes being somewhat higher than at 90 and 120 minutes. In the

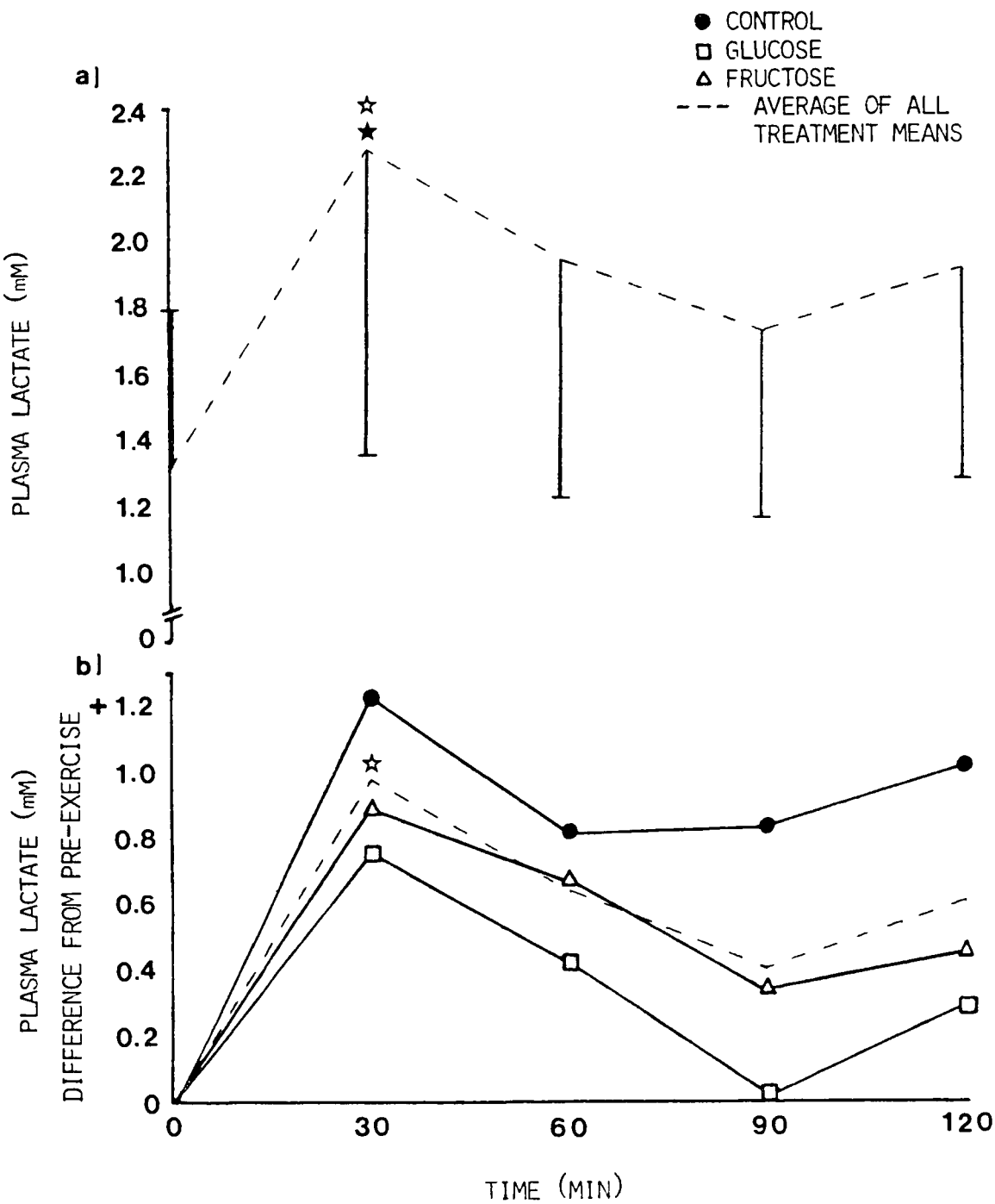

FIG 8. Effect of exercise on plasma lactate concentrations: a) average of means for all treatments and b) means and average of means transformed to differences from pre-exercise. ★ average significantly different from average pre-exercise ($p < 0.05$). ☆ average significantly different from all other averages ($p < 0.05$).

fructose situation, variability of plasma lactate was lowest at 90 minutes of exercise and highest (onefold difference) at 30 minutes of exercise. Other lactate values at pre-exercise, 60 and 120 minutes of exercise had similar standard deviations.

On the average, for all treatments there was a significant increase in plasma lactate at 30 minutes of exercise relative to the pre-exercise value, and this peak was significantly higher than other values at 60, 90, and 120 minutes of exercise ($p < 0.05$). At 120 minutes of exercise, the control situation resulted in the highest plasma lactate concentrations relative to pre-exercise; the subjects receiving the fructose treatment were associated with about half as much lactate by 120 minutes of exercise, while the glucose treatment produced slightly lower levels of lactate as compared to the fructose treatment.

Plasma Pyridoxal 5'-Phosphate

Table 7 lists the mean plasma PLP concentrations during exercise and the differences from pre-exercise for all three treatments. Figures 9a & 9b illustrate the effect of exercise on mean plasma PLP concentrations, and indicate the statistically significant differences among times when all treatments are averaged. Significant time effects existed for the actual values obtained ($p = 0.0049$) but not for difference from pre-exercise ($p > 0.05$). Treatment effects and time by treatment interactions were

Time of Exercise	Plasma Pyridoxal 5'-Phosphate Treatment Control n=8	Glucose n=7	Fructose n=7
min	nM	nM	nM
0	50.3 ± 14.4	44.9 ± 14.6	45.4 ± 14.1
30	59.0 ± 18.0 +8.7	48.7 ± 15.9 +3.8	51.4 ± 15.1 +6.0
60	52.1 ± 20.2 +1.8	49.7 ± 15.3 +4.8	51.1 ± 17.2 +5.7
90	55.8 ± 19.1 +5.5	47.0 ± 12.5 +2.1	50.7 ± 16.6 +5.3
120	52.8 ± 18.0 +2.5	46.9 ± 13.9 +2.0	49.5 ± 16.9 +4.1

All plasma pyridoxal 5'-phosphate concentrations are the mean ± standard deviation. The value below the mean is the difference from the pre-exercise value.

TABLE 7
Mean plasma pyridoxal 5'-phosphate concentrations during exercise and differences from pre-exercise for all treatments.

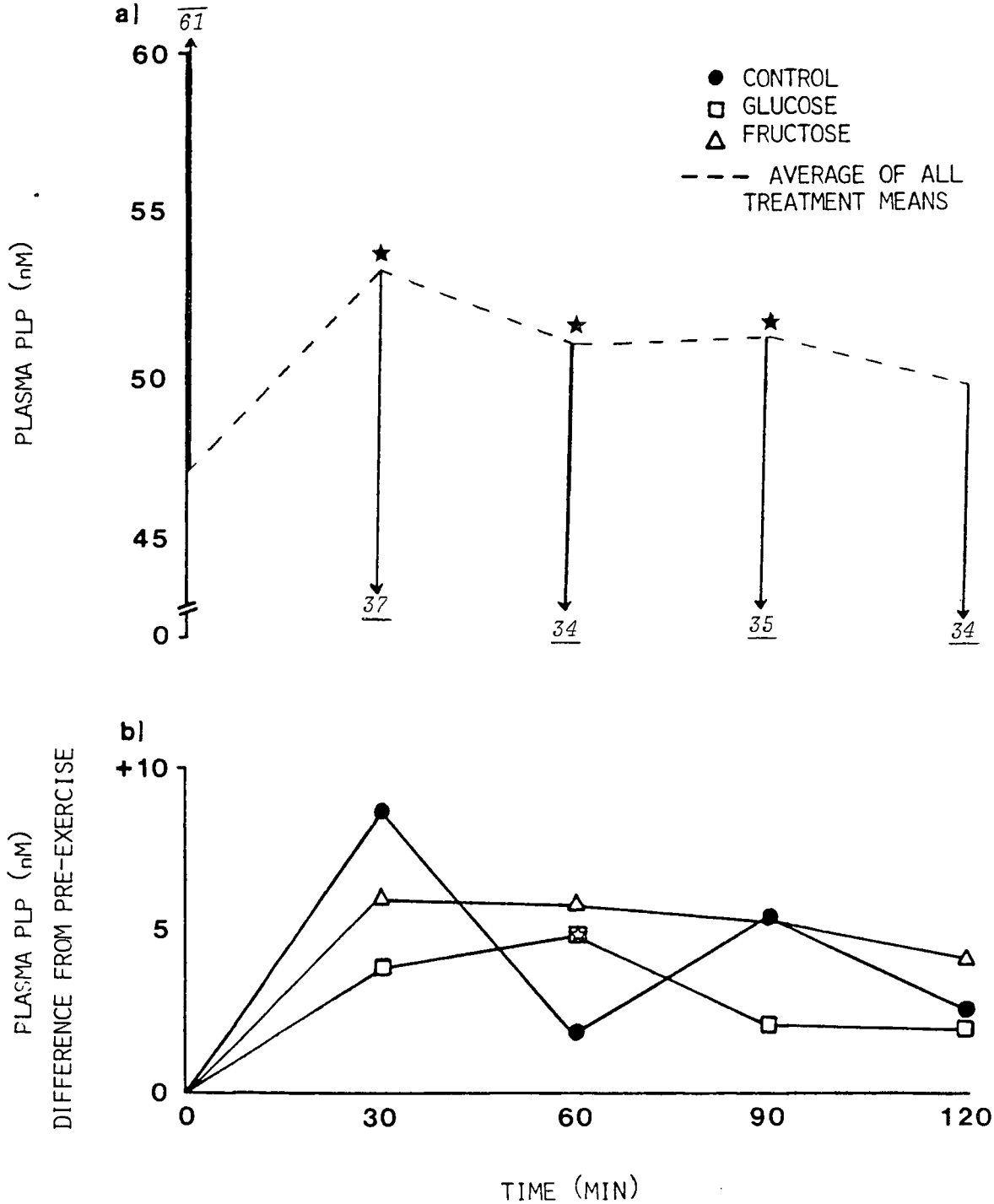

FIG 9. Effect of exercise on plasma pyridoxal-5'-phosphate concentrations: a) average of means for all treatments and b) means transformed to differences from pre-exercise. ★ average significantly different from average pre-exercise ($p < 0.05$). ✯ 60-minute control mean if Subject #6's data was excluded.

nonsignificant in both the untransformed and transformed data ($p > 0.05$).

With the exception of the glucose treatment, the mean plasma PLP concentration rose at 30 minutes of exercise and fell by 60 minutes. Excluding the control situation, the mean plasma PLP concentration was lower at 90 minutes of exercise than at 60 minutes, and by 120 minutes was lower than the 90-minute value. Variabilities of all mean plasma PLP values were approximately the same across the treatments.

The highest increase in mean plasma PLP concentration at 30 minutes was observed for the control situation. The glucose treatment exhibited half the increase in mean plasma PLP concentration than did the control treatment. At 60 minutes of exercise, the mean plasma PLP concentration decreased sharply for the control treatment but was associated with the highest variability of all PLP means. In the control situation, the 60-minute plasma PLP value of subject #6 (32.1 nM) contributed largely to this high variability and sharp drop in plasma PLP. At 90 minutes of exercise, the difference from the mean pre-exercise value for control had increased to approximate the slightly decreased fructose pre-exercise difference by 90 minutes. On the other hand, difference from mean pre-exercise plasma PLP level in the glucose situation decreased by half from 60 to 90 minutes of exercise, and the 90-minute mean pre-exercise difference was much less

than for control and fructose. By 120 minutes of exercise, mean plasma PLP pre-exercise differences decreased the most for control which approximated the glucose 120-minute mean pre-exercise difference that had decreased only very slightly. Fructose was associated with the highest mean plasma PLP pre-exercise difference at 120 minutes: this value was two-times that of glucose and slightly less for control.

On the average, all treatments produced significantly different mean plasma PLP values at 30, 60, 90, and 120 minutes of exercise as compared to the mean pre-exercise value ($p < 0.05$).

Hematocrit, Hemoglobin, and Plasma Volume Changes

Table 8 lists the mean hematocrit (Hct) and hemoglobin (Hgb) values during exercise and the mean plasma volume (PV) changes as calculated by the method of van Beaumont et al. (1972). Since Hgb values during exercise were not obtained during the initial study, PV changes as calculated by the method of Dill and Costill (1974) are not included in this report.

Significant time effects existed for the actual Hct ($p = 0$) and Hgb ($p = 0$) values obtained and for the pre-exercise differences in Hgb ($p = 0.00003$) but not for Hct ($p > 0.05$). Treatment effects and time by treatment interactions were nonsignificant ($p > 0.05$) for the actual values and for the difference from pre-exercise values of

Time of Exercise	Hematocrit			Hemoglobin			Plasma Volume Change*		
	Treatment								
	C† n=8	G† n=7	F† n=7	C n=4	G n=6	F n=6	C n=8	G n=7	F n=7
min	%			g/100 mL			%		
0	44.5	45.6	44.8	15.4	15.8	15.4			
	± 2.2	2.7	0.9	0.7	0.9	0.7			
	(45.0)			(15.5)					
30	46.8	46.6	45.5	16.3	16.0	16.0	-8.7	-3.6	-2.2
	± 2.6	2.6	2.1	0.9	0.6	0.5	2.5	4.2	9.4
	(46.3)‡			(16.1)‡					
60	46.4	46.2	46.1	16.2	16.0	16.2	-7.2	-4.1	-4.6
	± 1.9	3.1	1.4	0.7	0.7	0.5	2.9	4.4	6.5
	(46.2)‡			(16.1)‡					
90	46.2	46.2	46.2	16.4	16.2	16.4	-6.4	-2.4	-5.2
	± 1.8	2.5	1.6	0.9	0.7	0.6	2.8	4.7	6.9
	(46.2)‡			(16.3)‡					
120	46.6	46.7	46.3	16.5	16.5	16.4	-8.0	-4.3	-5.6
	± 2.3	2.4	1.1	1.2	0.8	0.6	2.4	5.6	5.5
	(46.5)‡			(16.5)‡§					

All values are mean and below each is the standard deviation; values in ( ) are the overall average. When a "±" precedes a row of values, the notation applies to all values.

* Plasma volume change calculated by the method of van Beaumont et al. (1972).

† Control (C), glucose (G), and fructose (F) respectively.

‡ Significantly different from pre-exercise value ($p < 0.05$).

§ Significantly different from 30- and 60-minute values ($p < 0.05$).

TABLE 8

Mean hematocrit and hemoglobin values during exercise and plasma volume change.

Hct and Hgb individually. The method of van Beaumont et al. (1972) which predicts PV changes from Hct measurements yielded nonsignificant time effects ($p = 0.20588 > .05$) and a significant time by treatment interaction ($p = 0.04998$) for PV changes.

Mean Hct increased at 30 minutes of exercise for all treatments and, with the exception of the fructose treatment, decreased very slightly thereafter at 60 and 90 minutes of exercise. At 120 minutes of exercise, the mean Hct value had increased to above the 30-minute value. Variability among the pre-exercise Hct means was two to three times lower for the fructose treatment as compared to the control and glucose treatments, and for all other times of exercise the variability of Hct means was consistently lower for the fructose situation. Hct rose progressively throughout exercise, however only slightly from 60 to 120 minutes, when subjects ingested fructose. On the average, Hct values at 30, 60, 90, and 120 minutes of exercise for all treatments were significantly greater than pre-exercise ($p < 0.05$).

Mean Hgb rose at 30 minutes of exercise for all treatments. The 60-minute mean Hgb value was essentially the same as the 30-minute mean Hgb value. With the exception of the fructose treatment, all 60-minute Hgb means had increased by 90 minutes of exercise, and continued to do so by 120 minutes. Variability among all Hgb means were approximately equal (although fructose

variability was always lower) except for the control at 120 minutes which varied one and one-half to two times more. This large variability can be attributed to subject #5's high Hgb value (17.7 g/100 mL). On the average, the actual Hgb values obtained at 30, 60, and 90 minutes of exercise were significantly greater than pre-exercise, and the 120-minute value was significantly greater than pre-exercise, 30 and 60 minutes of exercise ($p < 0.05$). Differences from pre-exercise Hgb, however, revealed that 30 and 90 minutes of exercise were significantly different and that the mean value at 120 minutes was different from the mean 90-minute value ($p < 0.05$).

PV changes, as calculated by the method of van Beaumont et al. (1972), were lower at specific time points during exercise when glucose or fructose was ingested as compared to water. At 30 minutes of exercise, both glucose and fructose ingestions were associated with significantly lower PV changes as compared to the control fluid ($p < 0.05$). At 90 and 120 minutes of exercise, glucose ingestion resulted in significantly lower PV changes as compared to control fluid ingestion ($p < 0.05$).

PV changes during exercise can influence the concentration of plasma constituents. By way of comparison, Table 9 presents the mean percent PV changes and the <u>actual</u> and <u>predicted</u> percent changes from pre-exercise for plasma glucose, lactate, and PLP over all treatments. The <u>predicted</u> percent changes in total plasma

Time of Exercise	PV Change*			Plasma Constituent Change								
				Glucose			Lactate			PLP		
	Treatment											
	C† n=8	G† n=7	F† n=7	C n=8	G n=7	F n=7	C n=8	G n=7	F n=7	C n=8	G n=7	F n=7
min	%			%			%			%		
30	- 8.7	3.6	2.2	-3.4	+1.6	+1.5	+ 117.1	46.9	66.9	+ 17.3	8.5	13.2
				-12.0	-2.4	-1.3	+ 97.9	41.1	62.3	+ 6.9	4.2	8.1
60	- 7.2	4.1	4.6	- 9.0	+1.1	-0.4	+ 77.1	26.9	49.6	+ 3.6	10.7	12.6
				-15.7	-1.3	-5.5	+ 64.1	23.8	42.0	-4.1	+8.0	+6.8
90	- 6.4	2.4	5.2	-14.6	-11.2	+4.0	+ 79.0	1.9	25.6	+ 10.9	4.7	11.7
				-20.2	-13.3	-1.7	+67.2	-0.6	+18.7	+ 3.6	2.2	5.5
120	- 8.0	4.3	5.6	-18.2	-12.6	+4.2	+ 96.2	18.1	33.8	+ 5.0	4.5	9.0
				-24.8	-16.4	-1.9	+ 80.3	13.0	26.0	-3.6	+0.1	+2.6

Actual percent change is given on the first line of each time of exercise; predicted percent changes from percent hematocrit changes are given below each actual percent change value.
All values are mean. When a "+" or a "-" precedes a series of values, the notation applies to all values unless a value is otherwise designated.
* Plasma volume (PV) change calculated by the method of van Beaumont et al. (1972).
† Control (C), glucose (G), and fructose (F) respectively.

TABLE 9
Mean percent plasma volume changes and selected plasma constituents' actual and predicted percent changes from pre-exercise for all treatments.

constituents were calculated from the pre-exercise values and the 30-, 60-, 90-, or 120-minute values of corrected Hct measurements and solute concentrations according to an equation derived by van Beaumont et al. (1973). For all mean plasma glucose values, the predicted percent changes due to PV decreases (hemoconcentration) were always more negative and thus greater than the actual percent changes. Likewise, plasma PLP predicted percent changes were all consistently lower but less than the actual percent changes. Plasma lactate predicted percent changes due to hemoconcentration were not all consistently higher than the actual percent changes. At 90 minutes and at 90 and 120 minutes of exercise in the glucose and fructose treatments, respectively, the predicted percent lactate changes were lower than the actual percent changes.

V. Discussion

This investigation of carbohydrate fluid metabolism during exercise is unique in that plasma levels of the major B-6 vitamer, pyridoxal 5'-phosphate (PLP) were determined. PLP is the cofactor for glycogen phosphorylase which catalyzes the breakdown of glycogen for energy release. Measurements of plasma PLP in addition to those of two other plasma constituents, glucose and lactate, can provide insight into the fuel metabolism of working muscles. The following discussion will focus upon carbohydrate metabolism by muscle during exercise. This chapter is organized into dicussions of the research findings in relation to the three objectives of the study.

The first objective of this study was to provide more knowledge on the effect of type and concentration of a carbohydrate drink on fuel metabolism when the carbohydrates are consumed during exercise. While other investigators have administered the same amount of carbohydrate to all subjects within a study, this research used a modification of a unique dose prescription by Coyle et al. (1983). The subjects in this investigation consumed 200 mL of water (control) and 7.5 ± 1.1 percent solutions of glucose or fructose fluids at 0, 30, 60, 90, and 120 minutes of exercise. The range in concentration of the CHO fluid was 5.8 to 9.2 percent.

At no time during exercise did any subject experience

gastro-intestinal (G-I) distress. This is in contrast to the glucose/fructose/exercise study by Fruth and Gisolfi (1983) in which all subjects consuming the 10 percent fructose solutions while exercising at 70 percent of VO2 max experienced G-I distress. Perhaps the lower concentrations of the fructose solutions and the lower exercise intensity (60 percent of VO2 max) in this investigation contributed to the prevention of G-I disturbances. It has been shown that exercise below 70 percent of VO2 max has no effect on gastric emptying, but that gastric emptying is delayed when exercise exceeds 70 percent of VO2 max (Costill and Saltin 1974). In a study by Bjorkman et al. (1984), it was not reported whether subjects consuming fructose during exercise at 68 percent of VO2 max experienced G-I distress. It is assumed therefore that the subjects in the study by Bjorkman et al. (1984) did <u>not</u> experience G-I distress. It is likely that the combination of the 10 percent fructose solution and the exercise at 70 percent of VO2 max in the Fruth and Gisolfi (1983) study promoted the G-I distress in every subject. This speculation seems accurate when considering that in the study by Bjorkman et al. (1984) a lower fructose fluid concentration (7 percent) in combination with exercise at approximately the same VO2 max was not associated with G-I disturbances.

The carbohydrate fluids administered to subjects in this investigation provided a total of 240.9 ± 35.8

kilocalories (kcal). Prolonged cycle exercise at 60 to 72 percent of VO2 max has been reported to use 13.8 kcal per minute in subjects of weight (72.8 kg), height (179 cm), and VO2 max (58.7 mL/ kg/ min) (Costill et al. 1973) similar to the subjects of this investigation. The duration of exercise was 120 minutes. Thus, if 13.8 kcal per minute was the caloric cost, a total of 1,656 kcal was expended over the course of the exercise. Potentially, the total amount of kcal ingested as carbohydrate could account for 14.5 percent of caloric needs during exercise. Because the average percent of VO2 max value was 59 in this study, the estimated 14.5 percent contribution of ingested carbohydrates to caloric requirement is probably low. That is, the ingested carbohydrates could have provided greater than 15 percent of the energy required for the cycle exercise. This maximal contribution is true if all of the carbohydrate ingested was oxidized and the ATP generated was used in muscle contraction.

Results from this investigation revealed that glucose and fructose ingestions during exercise at approximately 60 percent of VO2 max produced dissimilar patterns of changes in plasma glucose concentration. Additionally, the investigation revealed similar patterns of changes in plasma lactate concentration (although magnitudes differed) and similar patterns of plasma PLP concentration changes (with magnitude differences) when glucose or fructose was ingested during exercise. Water ingestions as compared to

glucose and fructose ingestions produced dissimilar patterns of changes in plasma glucose concentration, and similar patterns of changes in plasma lactate and PLP concentrations.

Exercise-induced changes in the three plasma constituents measured--glucose, lactate, and PLP--will be discussed at this point as three separate events occurring under the three experimental treatments: control, glucose, and fructose. Later, hypotheses of fuel metabolism by working muscle for each of the three treatments will be presented.

The plasma constituent glucose in the fasting state (i.e. at least 10 hours postprandial) at rest is normally within the range of 3.89 to 6.11 mM (Henry et al. 1974). The subjects in this investigation were, however, only 4 hours postprandial. Thus, plasma glucose values could be at the upper end of the normal range. One subject did exhibit a plasma glucose value outside the normal range: subject #2 (7.88 mM) in the glucose treatment. The pre-exercise value of 7.88 mM for subject #2 in the glucose situation was highly unusual and uncharacteristic for the subject when considering the subject's pre-exercise measurements for the other two situations: 5.27 and 5.55 mM. Subject #2's 30-minute glucose treatment value matches more closely these two pre-exercise values. An explanation is that, perhaps, the plasma aliquots were switched when processing the blood. It is unlikely that error was

produced during the laboratory assay for plasma glucose, since the assay was run twice on the uncharacteristically high (7.88 mM) sample, and similar results were obtained. As mentioned in the Results chapter, data was not changed or eliminated because all three treatments exhibited nonsignificant differences in pre-exercise plasma glucose levels ($p > 0.05$).

Resting blood glucose levels are altered by physical activity. With exercise, blood glucose is taken up by muscles at a greater rate than it is at rest. Glucose uptake by leg muscle has been shown to increase sevenfold that of the resting uptake rate after 40 minutes of cycling at 400 kpm per minute and ten- to twentyfold when the cycling intensity was 800 - 1200 kpm per minute (Wahren et al. 1971). During prolonged submaximal exercise, blood glucose can account for 30 to 40 percent of the total oxidative metabolism of leg muscle. The larger contribution of blood glucose to total oxidative metabolism in prolonged exercise is accomplished by increased hepatic glucose mobilization, mainly by glycogenolysis. When a glucose load is ingested during the course of exercise, blood-borne glucose may account for 50 to 60 percent of total oxidative fuel metabolism (Ahlborg et al. 1974).

The fructose treatment resulted in a more constant plasma level of glucose than either the control or glucose treatments. At 60 minutes of exercise, both the glucose and fructose treatments produced a significantly higher

mean plasma glucose level as compared to the control ($p < 0.05$). At 90 and 120 minutes of exercise, however, the fructose ingestions produced a significantly higher mean plasma glucose level than either the control or glucose treatments when comparing differences from pre-exercise ($p < 0.05$). An explanation for the relatively constant plasma glucose level during the fructose treatment as compared to the glucose treatment is that the fructose may have been absorbed more slowly (Dehmel 1969) and was largely converted into glycogen in the liver (Cori 1926, Nilsson and Hultman 1974); a larger storage of liver glycogen when fructose was consumed as compared to glucose likely resulted in a more steady entry of glucose into the blood.

Similar patterns of response of plasma glucose to control (water) and glucose ingestions during exercise to the present investigation have been reported by Felig et al. (1982). These investigators administered 5 percent glucose solutions to subjects exercising at 60 to 65 percent of VO2 max. In contrast to the results of Felig et al. (1982) in which 37 percent of the control subjects experienced hypoglycemia (blood glucose < 2.5 mM) by 60 to 150 minutes of exercise, no subject in the present investigation experienced abnormally low blood sugar levels at any time points.

The patterns of response of plasma glucose to all treatments (control, glucose, and fructose) observed during exercise in this investigation were not equivalent to those

reported by Fruth and Gisolfi (1983). These investigators administered 10 percent CHO solutions to trained subjects at 0-minute and at 20-minute intervals throughout exercise at 70 percent of VO2 max. The mean serum glucose level during exercise was greater than the pre-exercise value at 30, 60, 90, and 120 minutes of exercise in increments (est.) of 36, 40, 26, and 29 percent, respectively, during the glucose treatment; and 28, 37, 27, and 21 percent, respectively, during the fructose treatment. Even the ingestion of water (control fluid) was associated with an elevated mean serum glucose level which was 25, 29, 25, and 26 percent above pre-exercise at 30, 60, 90, and 120 minutes of exercise, respectively.

The subjects of the Fruth and Gisolfi (1983) study were not prone to blood glucose lowering during exercise as evidenced by the response of serum glucose to exercise during the control treatment. Coyle et al. (1983) hypothesized that the effects of glucose supplementation may depend on the subject's susceptibility to a lowering of blood glucose concentrations. These investigators found that glucose polymer feedings during prolonged exercise at 70 to 80 percent of VO2 max produced elevated blood glucose levels for all trained subjects. Higher levels, however, were demonstrated in those less prone to blood glucose lowering during exercise. When time to fatigue was observed, glucose feedings significantly delayed fatigue only in those subjects who had a decline in blood glucose

during exercise in the control situation as compared to subjects whose blood glucose levels were maintained at the normoglycemic level. Subjects in the Fruth and Gisolfi (1983) study did not benefit from carbohydrate feedings in terms of run time to exhaustion. This may be related to the finding by Coyle et al. (1983) that fatigue can be postponed through the ingestion of carbohydrates by those subjects prone to a decrease in blood glucose during exercise.

In the glucose/fructose/exercise study by Bjorkman et al. (1984), seven of the eight subjects were prone to blood glucose lowering during exercise when water was ingested. (In the eighth subject, blood glucose was maintained at the normoglycemic level.) When glucose or fructose was ingested, blood glucose was maintained at the normoglycemic level which was significanly higher than when water was ingested. There were no significant differences between blood glucose levels after 20 minutes of exercise when glucose was consumed as compared to fructose. Interestingly, the glucose and not the fructose ingestions promoted a significantly longer time to exhaustion than water ingestions. The investigators indicated that the improved performance and the glycogen-conserving effect with glucose feedings is related to differences in availability of ingested glucose.

In both glucose/fructose/exercise studies (Fruth and Gisolfi 1983, Bjorkman et al. 1984), in the glucose

polymer/exercise study (Coyle et al. 1983), and in the glucose/exercise study (Felig et al. 1982), subjects exercised after an overnight fast. In this investigation, subjects exercised 4 hours after a breakfast meal. Liver and muscle glycogen levels are likely to be higher in subjects 4 hours postprandial than after an overnight fast because of more recent replenishment of glycogen stores. Possible higher pre-exercise liver glycogen stores in the subjects in this investigation could have influenced the entrance of glucose from the liver into the blood after fructose or glucose was consumed.

A second explanation for the different patterns of changes in blood glucose concentrations after fructose ingestion as compared to glucose in this investigation and the glucose/fructose/exercise studies by Fruth and Gisolfi (1983) and Bjorkman et al. (1984), is that a greater insulin response to glucose ingestions occurred in the subjects who were 4 hours postprandial as opposed to 10 to 12 hours fasting. It is possible that the subjects in this investigation were in a different metabolic state prior to exercise than subjects in the studies by Fruth and Gisolfi (1983) and Bjorkman et al. (1984).

Nuttall and co-workers (1985) examined plasma insulin responses to isocaloric, high carbohydrate (63 percent of kcal) breakfast, lunch, and dinner meals, and found an increased plasma insulin response to the second meal (lunch) as compared to the first meal (breakfast) and an

even higher plasma insulin response to the third meal (dinner). These results are not identical to those of Aparico et al. (1974): the consumption of oral glucose loads were associated with a trend toward lower afternoon and night plasma insulin concentrations, however, at noon post-consumption plasma insulin levels peaked. The present study in which carbohydrate was ingested around noon and in the early afternoon might have shown elevated plasma insulin levels which resulted in low plasma glucose values as exercise continued. In the study by Nuttall et al. (1985), plasma glucose levels were similar after all meals. In the study by Aparico et al. (1974), plasma glucose levels were lowest after the first glucose load in the early morning, and highest after the third glucose load in the early evening. It appears, then, that in the present investigation, the falling plasma glucose level in the glucose treatment may not be a result of an increased glucose uptake secondary to a higher plasma level of insulin (speculated--not measured) associated with this treatment as compared to the other two treatments. Rather, the falling plasma glucose level is more likely due to other factors related to the metabolism of ingested glucose in the liver (e.g. conversion to liver glycogen for later delivery of glucose into the blood).

In this research investigation, subjects were prone to a blood glucose decrease during exercise at 60 percent of VO2 max, and exhibited higher plasma glucose levels when

glucose and fructose fluids were ingested as compared to the control fluid. Since respiratory exchange ratios (RER) for all treatments were not significantly different ($p > 0.05$) in the studies by Coyle et al. (1983) and Fruth and Gisolfi (1983), total carbohydrate oxidation was unchanged. It has been shown that glucose uptake by skeletal muscle is nearly proportional to blood glucose concentrations (Berger et al. 1975). Therefore, it is reasonable that the ingested carbohydrates probably spared muscle glycogen stores. The higher blood glucose concentrations could then have stimulated glucose uptake by exercising muscle. RER was not determined in this investigation, so the percentage of total oxidation attributed to carbohydrates is unknown. As previously mentioned, the amount of energy provided by the ingested carbohydrates could potentially account for 14.5 percent or more of the total caloric cost of the exercise.

A second plasma constituent observed was lactate. Venous plasma lactate levels in the fasting state at rest is normally within the range of 0.60 to 2.35 mM (Henry et al. 1974). One subject exhibited a slightly higher plasma lactate value: subject #2 (2.38 mM) in the fructose situation. The mean values of 1.05, 1.60, and 1.33 mM plasma lactate for the control, glucose, and fructose treatments, respectively, all were within the normal range of pre-exercise plasma lactate levels.

When physical activity is begun, pre-exercise plasma

lactate levels increase as a result of anaerobic carbohydrate metabolism. When oxygen supplies increase, plasma lactate is then reoxidized to glucose (gluconeogenesis) for energy release. Ahlborg et al. (1974) have reported that after 40 minutes of prolonged exercise at 30 percent of VO2 max, liver uptake of lactate, pyruvate, and alanine doubles, and glycerol utilization increases ten times. During exercise beyond 40 minutes, the rate of hepatic production and peripheral utilization of glucose are not in balance. Ahlborg et al. (1974) report that the relative contribution from gluconeogenesis to overall hepatic glucose output increases 25 percent from pre-exercise to 45 percent during prolonged exercise.

In this investigation, plasma lactate levels increased dramatically at 30 minutes of exercise (a 77.0 percent increase over all treatments), and then decreased (a 51.2 percent decrease from pre-exercise) by 60 minutes. These findings indicate that either one or both of two phenomena may be occurring: one, the liver is increasing lactate uptake for increased hepatic gluconeogenesis; and two, the muscle is decreasing its lactate output because of decreased muscle glycogenolysis. "Averages" are presented here since no significant differences were found between treatments ($p > 0.05$). Felig et al. (1982) also found no treatment differences in plasma lactate when control and glucose were compared; neither did Bjorkman et al. (1984) when control, glucose, and fructose were compared. Fruth

and Gisolfi (1983), however, found that at 30 minutes of exercise, fructose ingestions were associated with significantly higher lactate levels ($p < 0.05$). It is interesting to note in this investigation that in the control situation, lactate levels at 120 minutes of exercise were higher (although nonsignificant) than lactate levels at 120 minutes for either the glucose or fructose situation. In the control situation, there was an average of a 2 percent greater VO2 max than either glucose or fructose. Since lactate is a metabolic intermediate in fructose metabolism (Lehninger 1982), it could be expected that the ingested fructose would result in somewhat higher blood lactate as compared to control and glucose, but this was not evidenced in this investigation.

Lactate, formed through anaerobic carbohydrate metabolism, is indicative of glycolysis but can only indirectly indicate muscle glycogen breakdown. During exercise of 40 to 60 percent of VO2 max, Type IIa and some Type IIb fibers are recruited (Essen 1977). Increased ATP utilization decreases the inhibitory effect of citrate on PFK activity and thus enhances the rate of glycolysis (Astrand and Rodahl 1977). The increasing accumulation of plasma lactate after 90 minutes of exercise at 60 percent of VO2 max in this investigation may indirectly indicate increased muscle glycogen utilization. Plasma lactate levels at 120 minutes of exercise were 2.1, 1.9, and 1.8 mM for control, glucose, and fructose, respectively. Skinner

and McLellan (1980) have reported that blood lactate levels between 2 and 4 mM indicate a primary dependency on carbohydrate utilization.

A third plasma constituent measured was PLP. Plasma PLP determinations provide information not only for the first objective of this investigation, but also (and more directly) for the second objective: to provide more knowledge of vitamin B-6 metabolism during exercise. Plasma PLP levels in the fasting state at rest are normally greater than 39 nM for men (Shultz and Leklem 1981). Three subjects exhibited low values: subject #1 (26.7 and 25.8 nM in glucose and fructose, respectively), subject #5 (35.2 nM in control), and subject #8 (31.2, 25.0, and 33.8 nM in control, glucose, and fructose, respectively). The mean values of 50.3, 44.9, and 45.4 nM plasma PLP for control, glucose, and fructose, respectively, were normal. A wide range in pre-exercise plasma PLP levels, as seen in this investigation (25.0 to 69.6 nM), is not uncommon and has been reported in adolescent males by Leklem and Shultz (1983).

Plasma levels of B-6 vitamers represent the dynamic state of tissue uptake, release, and metabolism of these vitamers. A change can be explained by a modification of tissue uptake or release or both, and metabolism. Physical activity alters plasma levels of PLP. Prolonged exercise is likened to starvation because it alters blood glucose homeostasis through depletion of local muscle glycogen and

liver glycogen stores (Felig and Wahren 1975). In starvation, increased concentrations of aminotransferases in liver and decreased concentrations of phosphorylases have been observed (Black et al. 1978). PLP is required by these enzymes for their specific functions: to catalyze gluconeogenic and glycogenolytic reactions, respectively. Black et al. (1978) demonstrated a decrease in the PLP content of phosphorylase when a calorie-deficient diet was fed to rats. If strenuous exercise is viewed as acute starvation, the PLP released from glycogen phosphorylase could be the source of increasing plasma PLP levels. Hatcher (1983) reports that PLP is quantitatively the major B-6 vitamer changing with exercise. Leklem and Shultz (1983) hypothesize that the exercise-induced increase in plasma PLP is related to an increased need for cofactor for gluconeogenesis.

In this investigation, plasma PLP levels significantly increased from pre-exercise to 30 minutes of exercise by 13 percent on the average for all treatments ($p < 0.05$). Plasma PLP levels, on the average, remained significantly elevated over pre-exercise at 60, 90, and 120 minutes of exercise ($p < 0.05$). "Averages" are presented here because no significant treatment effects were exhibited ($p < 0.05$). With regard to the individual treatments, glucose ingestions generally were associated with lower plasma PLP levels than either water or fructose ingestions, while water ingestions generally promoted the highest plasma PLP

levels of all three treatments. The high plasma PLP level when water is ingested as compared to carbohydrate suggests that more PLP is needed for gluconeogenesis in the liver.

Increased plasma PLP levels with exercise has been seen by others from this laboratory (Wozenski 1977, Munoz 1983, Hatcher 1983, Leklem and Shultz 1983, and Walter 1985). The conclusion from these studies was that the increased PLP most likely comes from muscle glycogen phosphorylase. Black et al. (1977) has demonstrated in rat that muscle glycogen phosphorylase serves as a storage depot for B-6. If strenuous exercise is viewed as an acute form of starvation, then the PLP released from glycogen phosphorylase could be the major source of the increased plasma PLP levels. Although the liver is the primary site of PLP synthesis (Lumeng et al. 1974), it is unlikely that the this organ would release PLP when PLP is needed for gluconeogenesis. Other possible PLP sources are red blood cells, albumin released from interstitial fluid, and PLP-dependent enzymes released from tissues, however, each are less likely to be the major contributor to the increased plasma PLP (Leklem and Shultz 1983).

The third objective of this investigation was to determine the possible effects of glucose and fructose ingestions during exercise on blood volume changes, and consequently, on tissue hydration. Information obtained for this objective is related to both of the prior two objectives. Since plasma constituent levels are influenced

by plasma volume (PV) changes, it is pertinent to determine if the changes in plasma constituents are due solely to PV changes. In this investigation, all actual changes in plasma constituents were of greater magnitude than predicted changes; therefore, other factors must explain the differences.

PV is known to decrease in proportion to work intensity (Convertino et al. 1981, Wilkerson et al. 1977). The decrease results primarily from intravascular fluid filtering into working skeletal muscle for its increased metabolic needs. Costill and Fink (1974) report that during 2 hours of running exercise at 60 to 75 percent of VO2 max, PV changes fell 12 to 14 percent in the first 10 minutes of work and decreased 2 to 4 percent more in the next 110 minutes of exercise.

The exercise intensity used in this investigation (60 percent of VO2 max) does not delay gastric emptying (Costill and Saltin 1974) nor does prolonged severe exercise in general interfere with the intestinal absorption of glucose (Fordtran and Saltin 1967). Hyperosmolar solutions, however, delay stomach emptying. In this investigation, water absorption did not appear to be delayed during ingestion of the 7.5 percent (average) CHO solutions as evidenced by PV decreases (est. using method of van Beaumont et al. 1972) that were <u>not</u> than with the control treatment. On the contrary, PV changes by either method were less in every case when

glucose or fructose was ingested as compared to when water was ingested. The significant time by treatment interactions noted for PV changes cannot be explained. Following the ingestions of hyperosmolar solutions, the carbohydrate leaves the stomach in small amounts while gastric volume is kept constant through gastric secretions and some emptying into the small intestine (Costill and Saltin 1974). It therefore was expected that PV would decrease more when glucose or fructose was ingested as compared to water due to intravascular fluid entering the stomach to dilute its contents. This was not shown. PV changes for comparison in the studies by Fruth and Gisolfi (1983) and Bjorkman et al. (1984) were not reported.

Owing to the above findings, hypotheses can be formed with regard to fuel metabolism by working muscles under the three treatments. When water is ingested during prolonged exercise at 60 percent of VO2 max, more liver and muscle glycogen may be used. Liver glycogen levels are presumed to be low, resulting in the low plasma glucose levels. The elevated and increasing plasma lactate level near the end of exercise could reflect decreasing muscle glycogen stores as well as less reoxidation of the lactate produced in glycogenolysis. Although plasma lactate can only indirectly detect increased glycogenolysis, it appears that the increasing plasma lactate level indicates an increased reliance on muscle glycogen for energy. The decreasing

plasma PLP level with exercise suggests an increased liver uptake of PLP for gluconeogenesis. These trends in plasma lactate and PLP were observed during all treatments, however, differences (although nonsignificant) in the plasma constituents measured may reflect differences in fuel metabolism.

Glucose ingestions during the same exercise could be sparing muscle glycogen as evidenced by low lactate levels, or the low lactate levels could mean an elevated lactate turnover accompanied by a proportional increase of the rate of gluconeogenesis in the liver from lactate (Issekutz et al. 1976). Blood glucose is unlikely the major source of fuel during this treatment since plasma glucose levels were low compared to the normoglycemic level. Blood glucose is taken up by muscle in relation to its concentration (Berger et al. 1975). Since plasma PLP levels were low also, it could be that the PLP was not being released by the muscle, possibly because the PLP was needed in muscle for an increased activity of glycogen phosphorylase.

There is more convincing evidence that fructose ingestions during exercise were promoting less muscle glycogen utilization. This is supported by constant plasma glucose levels, and lower plasma lactate levels (amounts of which may even be products of fructose metabolism and not glycogenolysis) that show a smaller increase near the end of exercise as compared to glucose and water ingestion. Higher plasma PLP levels during exercise when fructose is

consumed (although decreasing also) could indicate mobilization of PLP from storage in muscle glycogen phosphorylase. A decrease in muscle pH because of one, an increased lactate production in the latter part of exercise, could result in PLP release from glycogen phosphorylase and release into the blood. This is unlikely since the variation in enzyme activity in the pH region is small and is probably not important for regulating the glycogenolytic rate (Hultman and Sahlin 1980).

In conclusion, it specifically appears that fructose ingestions produce important differences between 90 and 120 minutes of exercise. During cycle exercise at 60 percent of VO2 max, fructose ingestions maintain constant blood glucose levels which are hypothesized to be the preferred substrate for working muscles over glycogen, despite an increased rate of glycogen utilization indirectly evidenced by slightly increased plasma lactate and PLP decreases near the end of exercise. Short of obtaining muscle biopsies which reveal actual muscle glycogen stores, speculations of carbohydrate metabolism during this exercise can most be enhanced by investigations of fat metabolism. Determinations of plasma FFA will provide more insight into the fuel metabolism of exercising muscle in this research study. The findings as of this date, indicate that for the long-term exerciser, consumption of fluids containing 5.8 to 9.2 percent fructose may promote less muscle glycogen utilization than either water or glucose, thereby possibly

increasing endurance.

VI. Summary and Conclusions

This study was designed to obtain an indirect understanding of muscle glycogen utilization during prolonged, continuous exercise in adult males when either glucose, fructose, or water is ingested during exercise. The objectives of this study were threefold: 1) to provide more knowledge of the effect of type and concentration of a carbohydrate drink on fuel metabolism when the carbohydrates are consumed during exercise; 2) to demonstrate changes, if any, in vitamin B-6 metabolism during exercise when carbohydrates are consumed as compared to water; and 3) to determine the possible effects of glucose and fructose ingestions during exercise on blood volume changes and, consequently, on tissue hydration.

Eight trained healthy volunteers exercised on a cycle ergometer at 58 ± 7 percent of VO2 max for 2 hours on 2 to 4 occasions with at least 4-day intervals between exercise tests. At 0 minutes of exercise and at 30-minute intervals throughout the exercise, the subjects ingested 200 mL of fluid containing either glucose, fructose, or plain water in a double-blind, randomized fashion. The carbohydrate (CHO) fluid concentration was based on each subject's body weight (BW): 1 g CHO X kg^{-1}BW X L^{-1}water; and ranged in concentration from 5.8 to 9.2 percent (average = 7.5 percent) of BW. No subject experienced gastro-intestinal distress with any fluid. Blood samples were collected at

rest and immediately prior to fluid ingestion during exercise and analyzed for hematocrit, hemoglobin, and plasma levels of glucose, lactate, and pyridoxal 5'-phosphate (PLP). Statistical methods used to determine the significance of results were 5 X 3 repeated measures ANOVAs and Newman-Keuls multiple comparisons.

The results of this study indicate that changes in the plasma levels of lactate and PLP with exercise are not affected by the composition of fluid (glucose vs. fructose vs. plain water) consumed during exercise ($p > 0.05$). On the average, all treatments were associated with a peak plasma lactate level at 30 minutes of exercise which decreased until 90 minutes of exercise and then increased, and a peak plasma PLP level at 30 minutes of exercise which decreased for the remainder of the exercise. Although not statistically significant, plasma levels of lactate and PLP tended to be lower when glucose was consumed as compared to fructose or water.

Notably, this study demonstrates different changes in the plasma level of glucose with exercise when a fructose fluid is consumed versus a glucose fluid or plain water ($p < 0.05$). At the start of exercise, all subjects were approximately 4 hours postprandial and exhibited normoglycemic levels. During exercise, the plasma glucose level decreased, and there was a higher plasma glucose level when glucose and fructose fluids were ingested as compared to water. At 60 minutes of exercise, this

difference was evident for both glucose and fructose fluid ingestion ($p < 0.05$). At 90 and 120 minutes of exercise, fructose ingestion produced a significantly higher mean plasma glucose level than either water or glucose ingestion ($p < 0.05$). Berger et al. (1975) have shown that glucose uptake by exercising skeletal muscle is nearly proportional to blood glucose concentrations. It is hypothesized, then, that the higher plasma glucose levels observed at 90 and 120 minutes of exercise when fructose was ingested as compared to glucose or water, were accompanied by higher glucose uptakes by exercising muscle, thereby sparing muscle glycogen stores.

Plasma volume changes with exercise do not account for all of the changes in levels of the plasma constituents measured. Plasma volume decreased with exercise during all treatments, however, decreases were lower when carbohydrates were consumed as compared to water. Although nonsignificant, these lower plasma volume decreases in conjunction with the absence of G-I distress during exercise when the carbohydrates were consumed do not indicate detrimental effects of carbohydrate fluid consumption on athletic performance.

This study did not measure circulating levels of insulin and free fatty acids or muscle glycogen. While the determinations of plasma insulin and free fatty acids would add to the indirect understanding of muscle glycogen utilization during exercise, only muscle biopsies would

provide direct evidence on the amount of muscle glycogen used during exercise. A subjective measurement of muscle glycogen depletion is exercise duration time, for it is known that the amount of glycogen in muscle is a major determinant of muscular endurance (Bergstrom et al. 1967, Gollnick et al. 1972). The inclusion of these additional measurements would enhance a future study by providing a more complete picture of fuel metabolism.

The present data indicate important differences in fuel metabolism by working muscle at and beyond 90 minutes of exercise when a fructose fluid is consumed as compared to a glucose fluid or water. The consumption of a 5.8 to 9.2 percent fructose fluid during prolonged, continuous exercise appears to promote less muscle glycogen utilization than either a glucose fluid or water. Additionally, this dilute fructose fluid appears to promote and not hinder tissue hydration. It light of the findings, a 5.8 to 9.2 percent fructose fluid may be recommended to the performance-conscious athlete to possibly increase endurance.

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Appendix

APPENDIX TABLE 1
Health questionnaire.

Dept. Foods & Nutrition
Oregon State University

CONFIDENTIAL
Nutrition Project

*** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** *** ***

Name: _____ Date: _____
Last First Middle

Address: _____ Home Phone: _____

Age: _____ Birth Date: _____ State or County of Birth: _____

Predominate State of Residence: _____ City: _____ No. of Years: ____

Present Employment: _____

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

Marital Status (circle one): a. single c. divorced/separsted
b. married d. widowed

*** *** *** *** *** *** *** *** *** *** *** *** ***

HEIGHT/WEIGHT: Height (feet and inches) _____ Present weight _____
Most ever weighed _____ What year _____
Length of time you have maintained your current weight _____

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 disease)
_____f. osteoporosis
_____g. hepatitis
_____h. cirrhosis
_____j. kidney stones
_____k. nephritis
_____l. cystitis
_____m. high blood pressure
_____n. angina
_____o. mental depession requiring medication
_____p. insomnia requiring frequent medication
_____q. ulcers
_____r. pancreatitis
_____s. ulcerative colitis
_____t. spastic colon/ diverticulitis
_____u. recurring gastritis
_____v. allergies
_____w. hesrt problems (specify) _____
_____x. cancer (specify type) _____

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

Do any of your close relatives have diabetes? _____yes _____no
If yes, please check who of the relatives listed below had diabetes:
_____a. mother _____c. sister _____e. cousin _____g. uncle _____i. grandfather
_____b. father _____d. brother _____f. aunt _____h. grandmother

---

APPENDIX TABLE 1
Health questionnaire (continued).

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

_____ a. sleeping tablets
_____ b. barbitustes
_____ c. tranquilizers
_____ d. blood pressure tablets
_____ e. diuretics
_____ f. antibiotics
_____ g. oral contraceptives
_____ h. estrogens (female hormones)
_____ i. thyroid (thyroxin)
_____ j. insulin
_____ k. cortisone
_____ l. isoniazid
_____ m. other steroids (specify) _____

SURGICAL HISTORY (Please specify any type of surgery which 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 that 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? _____
Type _____ Amount _____ How long? _____
Type _____ Amount _____ How long? _____

Are you presently taking vitamins? _____yes _____no
If yes, what type, what amount and how often?

Please list all foods which you refuse to eat, cannot eat or prefer not to eat.

_____

_____

EXERCISE LEVEL:

Do you have a daily fitness program? _____ yes _____ no

Pleaae describe:

APPENDIX TABLE 2
Physical activity questionnaire.

NAME _____ DATE _____

PHYSICAL ACTIVITY HISTORY
(CONFIDENTIAL)

Please carefully review tbe following questions and answer tbem as accurately as possible. Place a check next to the appropriate answer for eacb question.

Occupational Activity

_____ I. Predominately sedentary--sitting position (desk worker, typist, light goods assembly line work, etc.)

_____ II. Ligbt activity--some standing and walking (casbier, student, general office work, ligbt factory work, police officer, etc.)

_____ III. Moderate activity--walking and material handling (waiter/waitress, mail carrier, construction worker, beavy factory work, etc.)

_____ IV. Heavy activity--heavy manual labor (beavy construction laborer, lumberjack, farm laborer, long sboreman, etc.)

Recreational/Leisure Activity

Rate your current level of leisure activity on tbe following scale.

_____ Totally sedentary
_____ Very ligbt
_____ Light
_____ Very moderate
_____ Moderate
_____ Somewbat abrd
_____ Hard
_____ Very hard

Which, if any, of tbe following exercises are you currently doing?

_____ No exercise
_____ Calisthenics
_____ Lift weights
_____ Walk for exercise
_____ Jog/Run
_____ Ride a bicycle
_____ Swim
_____ Otber (please describe)

How many days per week do you exercise?

_____ None
_____ One
_____ Two
_____ Three
_____ Four
_____ Five
_____ Six
_____ Seven

How much time do you spend on exercise eacb exercise day?

_____ None
_____ Less than 15 minutes
_____ 15-30 minutes
_____ 30-45 minutes
_____ 45-60 minutes
_____ 60-75 minutes
_____ 75-90 minutes
_____ More tban 90 minutes

APPENDIX TABLE 2
Physical activity questionnaire (continued).

If you exercise, please rate the intensity of your exercise on the following scale:

_____ 6
_____ 7 very, very light
_____ 8
_____ 9 very light
_____ 10
_____ 11 fairly light
_____ 12
_____ 13 somewhat hard
_____ 14
_____ 15 hard
_____ 16
_____ 17 very hard
_____ 18
_____ 19 very, very hard
_____ 20

How would you rate your current general state of physical fitness?

_____ very, very good
_____ very good
_____ good
_____ neither good nor poor
_____ poor
_____ very poor
_____ very, very poor

How long have you maintained your current level of physical fitness?

_____ < 6 months
_____ 6 months - 1 year
_____ 1 -2 years
_____ 2 -3 years
_____ > 3 years

Briefly outline a normal week's activities : (Include both occupational and recreational activities)

DAY	ACTIVITY	MINUTES ENGAGED IN ACTIVITY	INTENSITY

APPENDIX TABLE 3
Subject Expectations.

Subject Expectations

Prior to beginning the study:

1. complete health and physical activity history questionnaires.
2. record dietary intake for three days prior to each test day.
3. maintain weight and training program.
4. consume no vitamin or other nutritional supplements three weeks prior to testing.
5. have a VO2 max test conducted prior to testing.

Day of testing:

1. come to Milam 105 for breakfast at designated time (either 8:00 a.m. or 10:15 a.m.).
2. may leave Milam 105 and report to the Human Performance Laboratory at the designated time (either 11:45 a.m. or 2:00 p.m.).
3. weight measurement taken followed by a 10-minute rest period.
4. blood pressure measurement taken while supine
5. connection to ECG for continual moitoring of heart rate.
6. 10 mL blood sample drawn from antecubital vein.
7. ingest 200 mL of assigned fluid.
8. begin exercise on a cycle ergometer
   a. conditions: two hour ride at 60 percent of maximal heart rate.
   b. RPE test periodically throughout exercise
   c. 10 mL blood sample drawn at exercise times (minutes): 30, 60, 90, and 120.
   d. ingest assigned fluid in 200 mL amounts after blood sampling during exercise but not at end (total = 800 mL).
9. weight measurement after completion of exercise testing.
10. subject is free to leave the Human Performance Laboratory.

APPENDIX TABLE 4
Subject Consent Form.

Subject Consent Form

I, _____, give my consent to participate in this study. The study has been explained to me and all my questions have been answered. I agree to record a diet record for the three days prior to each test period. I agree to participate in the exercise testing outlined in my "Subject Expectations" paper. I understand the physical stress the exercises will place on me. I understand that with any physical exercise there is a risk of a heart attack. I further understand that facilities are available at the site to contact personnel to treat heart attack should that occur. I agree to allow 60 to 120 mL of blood to be drawn throughout the experimental period on each test day. I understand that there is a minimal risk of infection when blood is drawn and that sterile procedures will be followed to minimize this risk. I understand that I am free to withdraw from the study at any time. I also understand that the investigators reserve the right to withdraw me from this study at any time.

I am assured all information concerning me will be kept confidential. All data will be coded, and health and physical activity histories will be kept in one place under the supervision of the principal investigator.

Name_____Date_____

Witness_____Date_____

APPENDIX TABLE 5
Dietary record.

INSTRUCTIONS FOR RECORDING FOOD

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

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

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

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

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

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

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

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

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

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

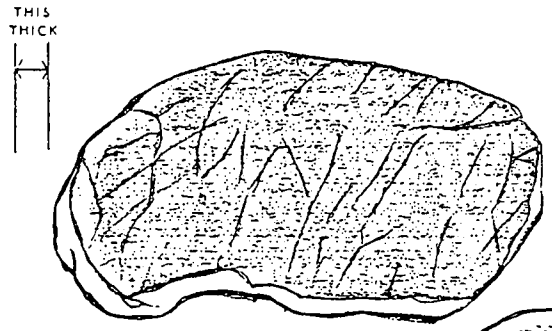
10. Indicate if milk is whole, skim, 2% or dry non-fat milk.

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

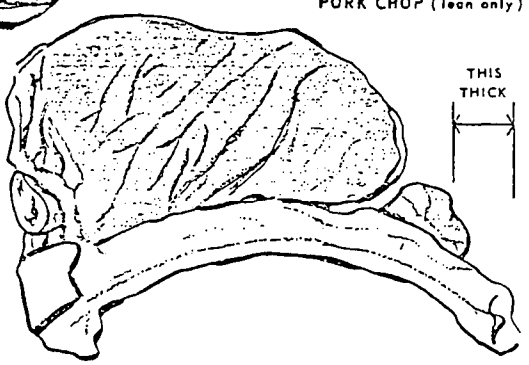
APPENDIX TABLE 5
Dietary record (continued).

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

ROAST BEEF ROUND (lean only)



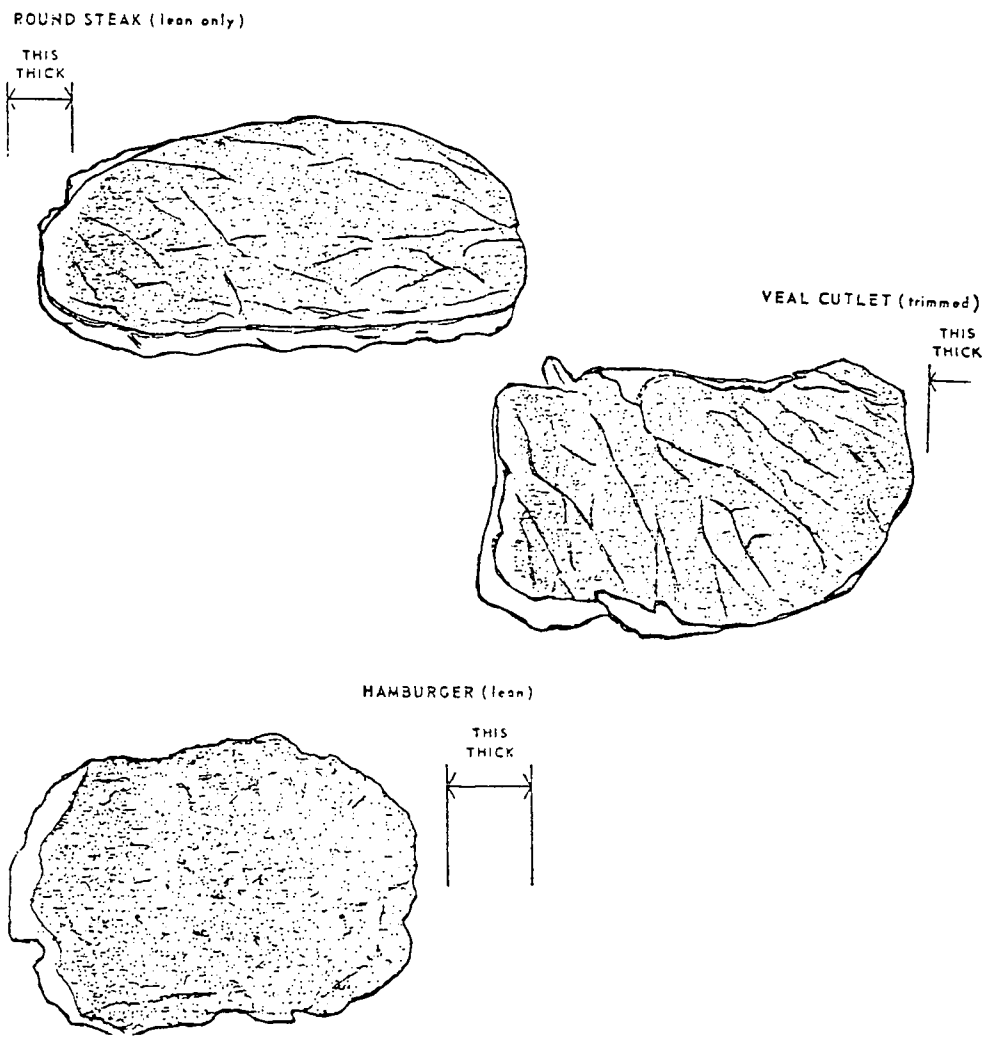
PORK CHOP (lean only)



ROAST TURKEY

APPENDIX TABLE 5
Dietary record (continued).

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



DIET RECORD SHEET

CODE NO. _____ DATE CONSUMED: _____

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

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

APPENDIX TABLE 5
Dietary record (continued).

CODE NO. _____ DATE CONSUMED: 5/30/79

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

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

APPENDIX TABLE 5
Dietary record (continued).

Subject	Treatment: Control		Glucose		Fructose	
	Measurement: HR	VO2	HR	VO2	HR	VO2
#	b/min†	% of max	b/min	% of max	b/min	% of max
1	134 ± 7	60.9	127 ± 7	56.1	126 ±17	55.2
2	141 ±15	62.1	143 ± 4	62.9	142 ± 9	62.4
3	136 ± 5	66.6	--‡	--	130 ± 4	58.0
4	120 ± 5	47.5	130 ± 7	51.7	--	--
5	160 ±16	72.1	144 ± 7	59.8	142 ± 6	59.3
6	151 ±13	42.9	161 ±24	54.8	153 ±10	44.4
7	139 ± 6	61.3	141 ± 6	62.9	141 ± 6	63.0
8	148 ±10	68.7	134 ± 9	57.5	139 ± 6	61.3
mean ± SD		60.3 ±10.2		58.0 ± 4.2		57.6 ± 6.4

* Averages for HR and percent of VO2 max include all measurements from 5 min to 120 min of exercise.
† b/min, beats per minute.
‡ Dashes indicate missing data.

APPENDIX TABLE 6
Individual averages* for heart rate (HR) and percent of VO2 max (VO2) measurements during exercise for all treatments.

Treatment	Time of Exercise	Subject 1*	2*	3	4	5	6	7	8	Mean ± SD
	min				mM†					mM
Control	0	5.33	5.27	5.66	5.44	4.66	4.83	5.11	5.50	5.22 ± 0.34
	30	5.05	5.22	6.22	5.44	4.27	4.55	5.05	4.50	5.04 ± 0.62
	60	4.66	5.00	5.94	5.44	4.00	3.83	4.83	4.27	4.75 ± 0.72
	90	4.38	4.66	5.66	5.38	3.72	3.55	4.16	4.16	4.46 ± 0.75
	120	4.27	4.94	5.16	5.16	3.66	3.11	3.94	3.94	4.27 ± 0.75
Glucose	0	5.22 5.16	7.88	--‡	5.22	5.11	5.33	5.11	5.61	5.63 ± 1.10
	30	5.83 5.33	5.38	--	6.49	4.44	7.49	5.61	5.27	5.72 ± 0.99
	60	5.33 5.38	5.83	--	5.27	5.44	6.94	5.38	5.55	5.69 ± 0.58
	90	4.94 4.94	4.83	--	4.88	4.61	5.72	4.72	5.33	5.00 ± 0.39
	120	5.00 4.88	4.55	--	4.72	4.72	5.72	4.16	5.72	4.92 ± 0.59
Fructose	0	5.27 5.88 5.55		5.11	--	4.94	5.16	5.38	5.33	5.25 ± 0.20
	30	4.88 5.55 5.22		5.88	--	5.16	5.38	5.55	5.22	5.33 ± 0.32
	60	4.83 5.50 5.38		5.50	--	5.27	4.83	5.55	5.22	5.23 ± 0.29
	90	5.44 5.44 5.27		5.66	--	5.22	5.44	5.94	5.27	5.46 ± 0.26
	120	5.22 5.88 5.66		5.61	--	4.72	5.88	5.72	5.50	5.47 ± 0.22

* The data in the first column are from the first exercise test in the given treatment; the data in the second column are from the second or "repeat" exercise test of that treatment. Only the data in the second column were included in statistical analyses.
† 1 mM = 180 mg/L = 18 mg/100 mL.
‡ Dashes indicate missing data.

APPENDIX TABLE 7
Individual plasma glucose concentrations during exercise for all treatments.

Treatment	Time of Exercise	Subject 1*	2*	3	4	5	6	7	8	Mean ± SD
	min				mM†					mM
Control	0	1.09	1.16	1.01	1.37	1.02	0.78	1.16	0.80	1.05 ± 0.20
	30	1.33	3.44	1.50	2.92	1.35	1.17	2.34	4.17	2.28 ± 1.13
	60	0.94	2.64	1.36	1.88	1.68	2.00	1.60	2.78	1.86 ± 0.62
	90	1.44	2.29	1.30	1.45	2.86	1.58	1.32	2.78	1.88 ± 0.66
	120	2.11	2.14	1.14	1.50	3.52	1.57	1.53	2.98	2.06 ± 0.82
Glucose	0	1.12 1.32	1.93	--‡	1.61	1.68	1.72	2.01	0.96	1.60 ± 0.36
	30	1.51 1.21	3.39	--	1.68	2.93	2.26	2.52	2.47	2.35 ± 0.73
	60	1.04 1.14	3.26	--	1.50	3.56	1.38	1.42	1.92	2.03 ± 0.98
	90	1.05 1.11	2.30	--	1.07	2.20	1.40	1.37	1.94	1.63 ± 0.51
	120	1.47 1.16	2.37	--	1.76	2.49	2.12	1.41	1.92	1.89 ± 0.49
Fructose	0	0.88 1.04	2.38	0.74	--	1.39	2.00	0.99	0.92	1.33 ± 0.63
	30	1.18 2.46	3.18	1.50	--	1.67	2.18	1.98	3.88	2.22 ± 0.97
	60	1.19 2.15	2.19	1.71	--	1.76	2.47	1.48	3.13	1.99 ± 0.66
	90	1.32 2.26	2.20	1.07	--	1.48	1.95	1.27	2.38	1.67 ± 0.51
	120	1.33 1.67	2.22	1.17	--	1.80	1.80	1.27	2.88	1.78 ± 0.61

* The data in the first column are from the first exercise test in the given treatment; the data in the second column are from the second or "repeat" exercise test of that treatment. Only the data in the second column were included in statistical analyses.
† 1 mM = 90 mg/L = 9 mg/100 mL.
‡ Dashes indicate missing data.

APPENDIX TABLE 8
Individual plasma lactate concentrations during exercise for all treatments.

Treatment	Time of Exercise	Subject 1*	2*	3	4	5	6	7	8	Mean ± SD
	min				nM†					nM
Control	0	39.5	69.6	55.1	48.0	35.2	68.2	55.8	31.2	50.3 ± 14.4
	30	38.3	73.9	78.4	66.7	38.4	73.8	65.8	37.0	59.0 ± 18.0
	60	30.6	76.3	73.3	66.9	36.5	30.7	66.3	36.4	52.1 ± 20.2
	90	40.6	77.9	74.4	57.4	29.4	67.9	66.1	32.8	55.8 ± 19.1
	120	36.7	74.8	68.8	56.6	34.0	62.2	62.9	26.3	52.8 ± 18.0
Glucose	0	33.3 26.7	45.5	--‡	63.6	49.7	45.8	58.1	25.0	44.9 ± 14.6
	30	33.2 30.5	51.7	--	64.6	52.6	46.9	68.5	26.2	48.7 ± 15.9
	60	25.5 31.1	48.1	--	62.8	56.2	48.8	70.9	30.0	49.7 ± 15.3
	90	32.7 32.1	46.8	--	63.8	55.2	45.9	55.5	29.7	47.0 ± 12.5
	120	34.0 29.0	52.8	--	57.7	56.0	45.5	60.8	26.7	46.9 ± 13.9
Fructose	0	25.8	77.5 68.0	43.8	--	43.0	45.6	57.8	33.8	45.4 ± 14.1
	30	28.2	73.5 73.4	58.0	--	44.6	51.6	63.1	40.6	51.4 ± 15.1
	60	28.0	72.8 76.1	61.8	--	43.2	46.6	65.4	36.5	51.1 ± 17.2
	90	27.3	77.4 74.9	60.4	--	43.9	43.3	65.0	40.0	50.7 ± 16.6
	120	27.3	76.7 72.4	64.8	--	43.3	40.3	62.4	36.3	49.5 ± 16.9

* The data in the first column are from the first exercise test in the given treatment; the data in the second column are from the second or "repeat" exercise test of that treatment. Only the data in the second column were included in statistical analyses.

† 1 nM = 247.2 ng/L = 0.247 ng/mL.

‡ Dashes indicate missing data.

APPENDIX TABLE 9

Individual plasma pyridoxal 5'-phosphate concentrations during exercise for all treatments.

Treatment	Time of Exercise	Subject 1*	2*	3	4	5	6	7	8	Mean ± SD
	min				%					%
Control	0	43.1	43.3	45.2	50.5	46.5	40.8	44.0	45.3	44.5 ± 2.2
	30	44.4	45.3	47.7	48.5	50.0	43.2	45.5	47.5	46.8 ± 2.6
	60	44.3	45.3	47.6	48.2	48.8	43.8	45.2	47.3	46.4 ± 1.9
	90	44.7	45.2	47.2	49.0	48.5	43.2	46.2	46.0	46.2 ± 1.8
	120	45.2	46.0	47.8	47.8	49.3	42.0	46.2	47.0	46.6 ± 2.3
Glucose	0	41.5 40.5	44.0	--†	47.8	47.0	48.5	45.0	46.5	45.6 ± 2.7
	30	42.7 41.8	44.0	--	49.3	47.5	47.8	47.5	48.0	46.6 ± 2.6
	60	42.3 41.7	43.2	--	49.0	47.5	--	46.7	49.0	46.2 ± 3.1
	90	42.3 42.0	43.5	--	48.2	47.5	47.3	46.7	48.5	46.2 ± 2.5
	120	42.8 43.0	44.0	--	48.9	48.7	47.0	47.0	48.5	46.7 ± 2.4
Fructose	0	45.2	46.2 45.7	43.8	--	45.5	45.7	44.0	44.0	44.8 ± 0.9
	30	41.5	46.5 45.5	45.1	--	46.5	46.8	45.0	48.0	45.5 ± 2.1
	60	43.8	47.4 45.5	45.3	--	47.5	46.5	46.0	47.8	46.1 ± 1.4
	90	43.7	46.8 46.5	45.2	--	47.5	46.3	45.8	48.5	46.2 ± 1.6
	120	44.2	46.8 46.0	46.0	--	47.7	46.8	46.5	47.0	46.3 ± 1.1

* The data in the first column are from the first exercise test in the given treatment; the data in the second column are from the second or "repeat" exercise test of that treatment. Only the data in the second column were included in statistical analyses.
† Dashes indicate missing data.

APPENDIX TABLE 10
Individual hematocrit values during exercise for all treatments.

Treatment	Time of Exercise	Subject 1*	2*	3	4	5	6	7	8	Mean ± SD
	min	g/100 mL								g/100 mL
Control	0	16.2	15.3	16.2	15.1	15.2	14.1	15.2	16.0	15.4 ± 0.7
	30	--†	--	--	--	16.9	15.1	16.2	17.0	16.3 ± 0.9
	60	--	--	--	--	16.6	15.2	16.3	16.7	16.2 ± 0.7
	90	--	--	--	--	17.3	15.1	16.6	16.7	16.4 ± 0.9
	120	--	--	--	--	17.7	14.8	16.5	16.9	16.5 ± 1.2
Glucose	0	15.8 14.7	15.2	--	17.1	16.0	17.0	15.1	15.7	15.8 ± 0.9
	30	-- 15.3	15.3	--	--	16.4	16.7	16.2	16.4	16.0 ± 0.6
	60	-- 15.2	15.4	--	--	16.2	--	16.1	17.0	16.0 ± 0.7
	90	-- 15.5	15.1	--	--	16.6	16.9	16.1	16.8	16.2 ± 0.7
	120	-- 15.5	15.5	--	--	17.1	17.1	16.7	17.2	16.5 ± 0.8
Fructose	0	14.3	15.3 16.0	16.7	--	15.2	15.3	15.4	15.2	15.4 ± 0.7
	30	15.4	-- 16.0	--	--	15.8	15.9	16.2	16.8	16.0 ± 0.5
	60	15.4	-- 16.2	--	--	16.1	15.9	16.5	17.1	16.2 ± 0.5
	90	15.6	-- 16.3	--	--	16.4	15.9	16.9	17.3	16.4 ± 0.6
	120	15.9	-- 16.3	--	--	16.2	16.0	16.6	17.7	16.4 ± 0.6

* The data in the first column are from the first exercise test in the given treatment; the data in the second column are from the second or "repeat" exercise test of that treatment. Only the data in the second column were included in statistical analyses.
† Dashes indicate missing data.

APPENDIX TABLE 11
Individual hemoglobin concentrations during exercise for all treatments.

Treatment	Time of Exercise	Subject 1†	2†	3	4	5	6	7	8	Mean ± SD
	min				%					%
Control	30	-5.2	-7.8	-9.6	-10.4	-13.1	-9.4	-5.9	-8.5	-8.7 ± 2.5
	60	-4.8	-7.8	-9.2	-2.8	-8.8	-11.6	-4.7	-7.7	-7.2 ± 2.9
	90	-6.3	-7.4	-7.7	-1.6	-7.7	-9.4	-8.5	-2.8	-6.4 ± 2.8
	120	-8.2	-10.4	-9.9	-4.7	-10.6	-4.8	-8.5	-6.6	-8.0 ± 2.4
Glucose	30	-4.8 -4.6	0	--‡	-5.8	-2.0	+2.8	-9.6	-5.8	-3.6 ± 4.2
	60	-3.2 -4.8	+3.3	--	-4.7	-2.0	--	-6.6	-9.5	-4.1 ± 4.4
	90	-3.2 -6.0	+2.1	--	-1.6	-2.0	+4.9	-6.6	-7.7	-2.4 ± 4.7
	120	-5.2 -9.8	0	--	-4.3	-6.6	+6.2	-7.7	-7.7	-4.3 ± 5.6
Fructose	30	+16.3	-1.2 +0.8	-5.1	--	-4.0	-4.3	-4.0	-14.9	-2.2 ± 9.4
	60	+5.8	-4.7 +0.8	-5.9	--	-7.7	-3.2	-7.8	-14.2	-4.6 ± 6.5
	90	+6.3	-2.4 -3.2	-5.5	--	-7.7	-2.4	-7.0	-16.6	-5.2 ± 6.9
	120	+4.1	-2.4 -1.2	-8.5	--	-8.5	-4.3	-9.6	-11.4	-5.6 ± 5.5

* Plasma volume change calculated by the method of van Beaumont et al. (1972).
† The data in the first column are from the first exercise test in the given treatment; the data in the second column are from the second or "repeat" exercise test of that treatment. Only the data in the second column were included in statistical analyses.
‡ Dashes indicate missing data.

APPENDIX TABLE 12
Individual plasma volume change values* during exercise for all treatments.