AN ABSTRACT OF THE THESIS OF

Ann deVos for the degree of Master of Science

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Title: Carbohydrate Loading, Vitamin B-6 Supplementation, and Fuel Metabolism During Exercise of Differing Intensity in Post-Absorptive Man

Abstract approved: Dr. James Leklem

Four young trained men were studied during 50 min of continuous bicycle ergometer exercise [30 min at 60%, 15 min at 80%, and 5 min at 90% maximal heart rate (MHR)] to elucidate changes in fuel metabolism resulting from a glycogen depletion-repletion regimen, and to determine the effect of supplemental vitamin B-6 (B6). The diets were: Week 1, 40% CHO normal diet (NC); Week 2, days 1-3 CHO 11% (LC), days 4-7 CHO 71% (HC); Week 3, same as week 2 but with an 8 mg B6 supplement each day (LC+B6, HC+B6). The men exercised after an overnight fast on days 4 and 7, upon completing the depletion or repletion phase. Blood was collected before exercise (PRE), during the 80% MHR work (DURING), immediately after completion of the 90% MHR work (POST), and 30 min and 60 min after exercise (30 MIN POST, 60 MIN POST).

Plasma FFA concentrations were from 30% to 75% higher ($p<0.05$) after the LC and LC+B6 diets than after the NC-1, NC-2, HC, or HC+B6 diets, and B6 did not appear to affect plasma FFA levels. Plasma glucose values were from 3% to 4% lower ($p<0.01$) for DURING HC and HC+B6 than DURING NC-1. Since plasma HC and HC+B6 lactate values were 57% higher ($p<0.05$) than DURING control values, the simultaneously low glucose and high lactate levels indicate that glucose was primarily derived from muscle glycogen in the HC and HC+B6 conditions. Addition of B6 to the HC diet resulted in elevated POST lactate levels, but this difference was not significant. LC glucose and lactate values did not differ significantly from control values. However, PRE LC+B6 glucose values were 12% lower than PRE control values ($p<0.02$) and continued to be lower during exercise. POST and 30 MIN POST LC+B6 values were 47% lower than the LC values ($p<0.005$ and $p<0.01$, respectively). The glucose and lactate data indicate that B6 supplementation does alter CHO metabolism when added to a glycogen depletion-repletion regimen. Due to the possible role of glycogen phosphorylase as an expanding depot for B6 storage, supplementation with B6 may cause a more rapid emptying of muscle glycogen stores and a reduction of athletic endurance.

Carbohydrate Loading, Vitamin B-6 Supplementation, and Fuel Metabolism During Exercise of Differing Intensity in Post-Absorptive Man

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LIST OF ABBREVIATIONS

FFA	Free fatty acids
PLP	Pyridoxal 5'-phosphate
ATP	Adenosine triphosphate
ADP	Adenosine diphosphate
CP	Creatine phosphate
VO_2max	Maximum aerobic capacity
W	Watt
kp	Kilopond
HR	Heart rate
MHR	Maximal heart rate
rpm	Revolutions per min
CHO	Carbohydrate
NC	Normal carbohydrate diet
LC	Low carbohydrate diet
HC	High carbohydrate diet
LC+B6	Low carbohydrate diet plus vitamin B-6 supplementation
HC+B6	High carbohydrate diet plus vitamin B-6 supplementation
A-V difference	Arteriol venous difference
ECG	Electrocardiogram
PV	Plasma volume
Hct	Hematocrit
ml	Milliliter
g	Gram
R.C.F.	Relative centrifugal force
µU	Micro unit
SD	Standard deviation
CV	Coefficient of variation

Carbohydrate Loading, Vitamin B-6 Supplementation, and Fuel Metabolism During Exercise of Differing Intensity in Post-Absorptive Man

I. INTRODUCTION

Our understanding of the physiology of exercise has expanded considerably in the last fifteen years, as has the development of nutritional science. A desire to improve the competitive edge of trained athletes has sparked experimentation with dietary manipulations since ancient times. Work done in the 1960's by Scandinavian investigators first quantified the relationship between muscle glycogen stores and improved endurance performance. The process of a sequential depletion of muscle glycogen stores while on a low carbohydrate diet, followed by glycogen supercompensation while eating a high carbohydrate diet, is now widely publicized and practiced among endurance athletes as a means of improving performance.

The role of vitamin B-6 in the production of energy has been studied by numerous researchers in the past. It is known that pyridoxal 5'-phosphate (PLP) is required in the functioning of the enzyme glycogen phosphorylase, which initiates the conversion of glycogen to glucose-1-phosphate. It has also been shown in rats that supplementation with vitamin B-6 increases the quantity of muscle glycogen phosphorylase, which acts as a storage depot for PLP (Black et al. 1977). Black et al. found that in rats the muscle glycogen phosphorylase level decreased under the stress of starvation, presumably with the release of PLP.

The expansion of our understanding of physiological interrelationships is often due to the unexpected relationships uncovered in metabolic studies. During a metabolic study in this laboratory involving varying doses of vitamin B-6, an unexplained rise in plasma PLP was reported for one subject. It was discovered that the subject had exercised following a test dose of vitamin B-6 and prior to the blood sampling. Follow-up studies in this laboratory confirmed that there is a positive significant increase in plasma PLP levels resulting from exercise.

It is postulated that the utilization of glycogen occurring in exercise resembles that seen in starvation, and may also trigger the release of PLP from its storage depot. If PLP is stored with glycogen phosphorylase in man as it is in rats, the vitamin may have an effect on the fuel metabolism of exercise, a phenomenon which has been previously unsuspected. Glycogen phosphorylase is the rate limiting enzyme in the control of muscle glycogenolysis. It is possible that a larger quantity of this enzyme could speed the glycogenolytic process, increasing the percentage of energy derived from muscle glycogen during exercise.

The investigation of the relationship between supplemental vitamin B-6 and the fuel metabolism of exercise was the purpose of this study. Healthy young men were studied during exercise after consuming a normal mixed diet, during a glycogen depletion-repletion regimen, and during a glycogen depletion-repletion regimen with supplemental vitamin B-6 added to the diet. Blood

samples were collected during the standardized exercise tests and analyzed for plasma free fatty acids, glucose, and lactate to monitor changes in the pattern of fuel metabolism under the different experimental circumstances.

II. REVIEW OF THE LITERATURE

Parameters of Exercise

Muscular activity and exercise physiology may be approached as a transformation of chemically bound energy into mechanical energy. Muscle can vary its metabolic rate to a greater degree than any other tissue in the human body, increasing its oxidative processes to more than 50 times the resting level (Åstrand & Rodahl 1977). In the process adenosine triphosphate (ATP) is hydrolyzed to adenosine diphosphate (ADP) and inorganic phosphate to provide the energy needed for the contraction of muscle fibers.

Aerobic and Anaerobic Metabolism

In the absence of oxygen (anaerobic conditions) ATP can be synthesized from two sources (Havel 1974). Creatine phosphate serves as a reserve store of high energy phosphate which can regenerate ATP from ADP, and this mechanism can support hard exercise for 5-8 seconds (Katch & McArdle 1977). Glycogenolysis and anaerobic glycolysis will produce two ATP molecules per glucose molecule metabolized, with lactic acid as an end product (Lehninger 1975). This provides a rapid but limited energy supply for muscular contraction and releases about 5% of the energy contained within the glucose molecule.

When exercise is continued for more than a few minutes, anaerobic supplies are rapidly depleted and energy needs must be met by oxidative phosphorylation (Havel 1974). This requires the

utilization of large quantities of oxygen for the complete combustion of glucose and free fatty acids (FFA) to CO_2 and H_2O for the resynthesis of ATP. Fuel selection and energy utilization should be visualized as a continuum of aerobic and anaerobic metabolic pathways. During MEL activities (muscular exertion of low intensity and long duration) the oxygen supply is ample and fatty acids are primarily utilized for fuel. Examples of such activities are long distance cycling or running. With increasing work intensity of MEM activities (muscular exertion of medium intensity and duration), the amount of oxygen available to the muscle will limit its ability to use fatty acids as fuel. A greater proportion of the energy of exercise will then be provided by anaerobic glycolysis and the ATP-Phosphocreatine system, which are used in conjunction with oxidative phosphorylation. Examples would be a 220 yard sprint, or the activities of a football player. Strictly anaerobic exercise is termed MES (muscular exertion of a short duration and strong intensity). Examples would be a 100 yard dash or weight lifting.

Oxygen Consumption

All energy-releasing reactions in the body depend ultimately on a continued supply of oxygen, and by measuring a person's oxygen consumption it is possible to gain an indirect estimate of energy metabolism. Open circuit spirometry is the most widely used technique to measure oxygen consumption during exercise. The subject inhales ambient air and exhaled air is collected for

analysis. Volume and composition of expired air as compared to inspired air provides a relatively simple means of calculating oxygen consumption (Katch & McArdle 1977, Taylor et al. 1955).

The oxygen uptake of muscle cells increases linearly with increasing exercise intensity, reflecting greater combustion of fuels for ATP production. When an exercising body has reached its maximum capacity to extract energy by aerobic means, oxygen consumption fails to increase further even though the work performed continues to increase in difficulty. At this point of maximal oxygen consumption (VO_2max) the body has reached its maximum capacity to deliver O_2 to the exercising muscles (Åstrand 1976). At work intensities exceeding VO_2max, anaerobic ATP generating reactions must supply the additional energy for exercise.

To meet muscular demand for increased oxygen consumption, the heart pumps progressively more rapidly during increasing exercise intensities in order to deliver greater quantities of oxygen loaded hemoglobin to the working muscles. As in the case of VO_2max, there is a limit to the speed at which the heart can pump. Beyond this maximal heart rate (MHR) a further increase in work load will not increase the rate of pumping or oxygen consumption of the individual. The MHR can be determined directly by palpation of pulse during all-out exertion, such as running as fast as one can up a hill. There is a gradual linear decline in this MHR with advancing age of about 1 beat/min/year (Åstrand et al. 1959, Åstrand et al. 1973). For the population at large, MHR

can therefore be indirectly calculated at 220 beats per minute minus the age of the individual (Åstrand et al. 1959).

Work Load

The intensity of exercise, or work load, is measured as a force exerted over a distance per unit of time. Such a unit used for the calibration of work load in bicycle ergometry is a watt, and is quantified as kilopond meters/min ($kpm \cdot min^{-1}$). A kilopond is the force acting on the mass of 1 kilogram at normal acceleration of gravity (Åstrand & Rodahl 1977). The distance in meters over which the force is exerted is controlled by pedal length and wheel diameter of the bicycle ergometer. Speed at which the distance is covered is controlled by cycle cadence. A work load of 1 watt is equal to 6.12 $kpm \cdot min^{-1}$, so 50 watts is approximately 300 $kpm \cdot min^{-1}$.

A linear increase in exercise work load will result in a correspondingly linear increase in both heart rate and oxygen consumption until maximal levels are reached (see Fig. 1). Thus an indirect measure of oxygen consumption can be made based on heart rate during light to moderately heavy exercise. This has been found to be accurate to ± 15% (Åstrand & Rodahl 1977, Rodahl et al. 1974).

The efficient utilization of energy for each of the three categories of exercise (MES, MEM, & MEL) is increased by training for that specific activity. Aerobically trained individuals, such as long distance cyclers or cross country skiers, utilize FFA more efficiently than do untrained individuals (Holloszy 1975).

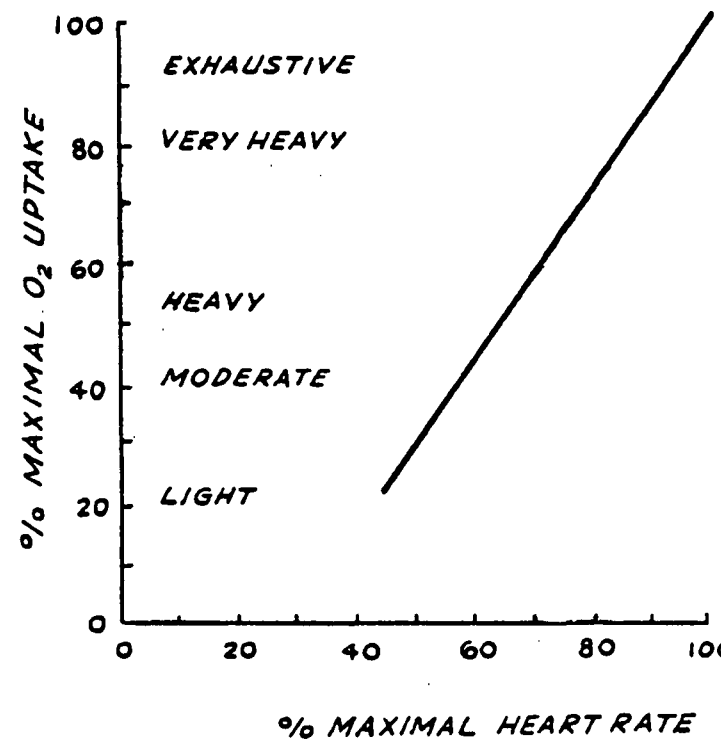


FIG. 1. Relationship between percent maximal heart rate, percent maximal oxygen uptake, and relative work load.

Adapted from Hellerstein et al., 1973.

Training affects many biochemical parameters involved in energy metabolism such as oxygen delivery, the utilization of FFA and blood glucose, the storage and utilization of muscle glycogen, and enzyme activity.

Fuel Metabolism of Exercise

Free Fatty Acids

The largest energy store of the human body is the triglyceride depot of adipose tissue. In the "average" healthy 70 kg man, expected values of triglyceride stores are from 10 to 20% of total body weight. Upon oxidation this would yield about 63,000 to 126,000 kcalories, while carbohydrate stores of muscle and liver glycogen can be expected to contribute only approximately 2,000 kcalories (Felig & Wahren 1975). Triglycerides are hydrolized to free fatty acids (FFA) and glycerol in response to various hormonal and nervous stimuli. The FFA's are then transported through the plasma bound to albumin and thus are available to oxidizing tissues (Hagenfeldt 1979).

The utilization of FFA by skeletal muscle during work is determined by the level of plasma FFA concentration, with an increased concentration always meaning an increased rate of utilization (Newsholme 1977). In studies with trained and untrained dogs, it was shown that plasma FFA concentration rose throughout the moderately hard exercise in the trained dog, and that FFA turnover rose simultaneously. The untrained dog, performing at

the same absolute work load, had a sharp rise in blood lactate. For reasons which will be explained later, this elevated lactate level inhibits FFA release from adipose tissue, and plasma concentrations of FFA decreased during exercise in the untrained dog. Concomitant with the decreased concentration of FFA there was a decreased turnover of FFA (Issekutz et al. 1965).

There is also an increased fractional turnover of plasma FFA which appears to be related to work intensity, the increment being 60% at a work load of 65 watts as compared to 95% at 160 watts (Hagenfeldt & Wahren, 1975). The quantity of FFA mobilized from adipose tissue in the post absorptive state at rest is more than enough to meet the needs of oxidative metabolism. During even light exercise, however, this rate of mobilization becomes inadequate (Havel 1974). Arterial plasma levels of FFA fall abruptly upon onset of moderate exercise, due to the increased rate of removal by working muscle, as demonstrated in Fig. 2. This initial decrease in plasma FFA is followed by a gradual rise as mobilization into the blood from adipose tissue increases, and usually exceeds resting values after 15-20 minutes (Wahren et al. 1975, Havel 1974). At moderately heavy work loads of 50-60%, VO_2max a steady level is reached after 30-40 minutes of exercise. A continuing rise of arterial FFA concentration is seen throughout exercise of lower work intensities which can be maintained for longer periods (Hagenfeldt 1979, Havel 1974).

Increased activity of the sympathetic nervous system appears to be the major mechanism responsible for the increased mobilization

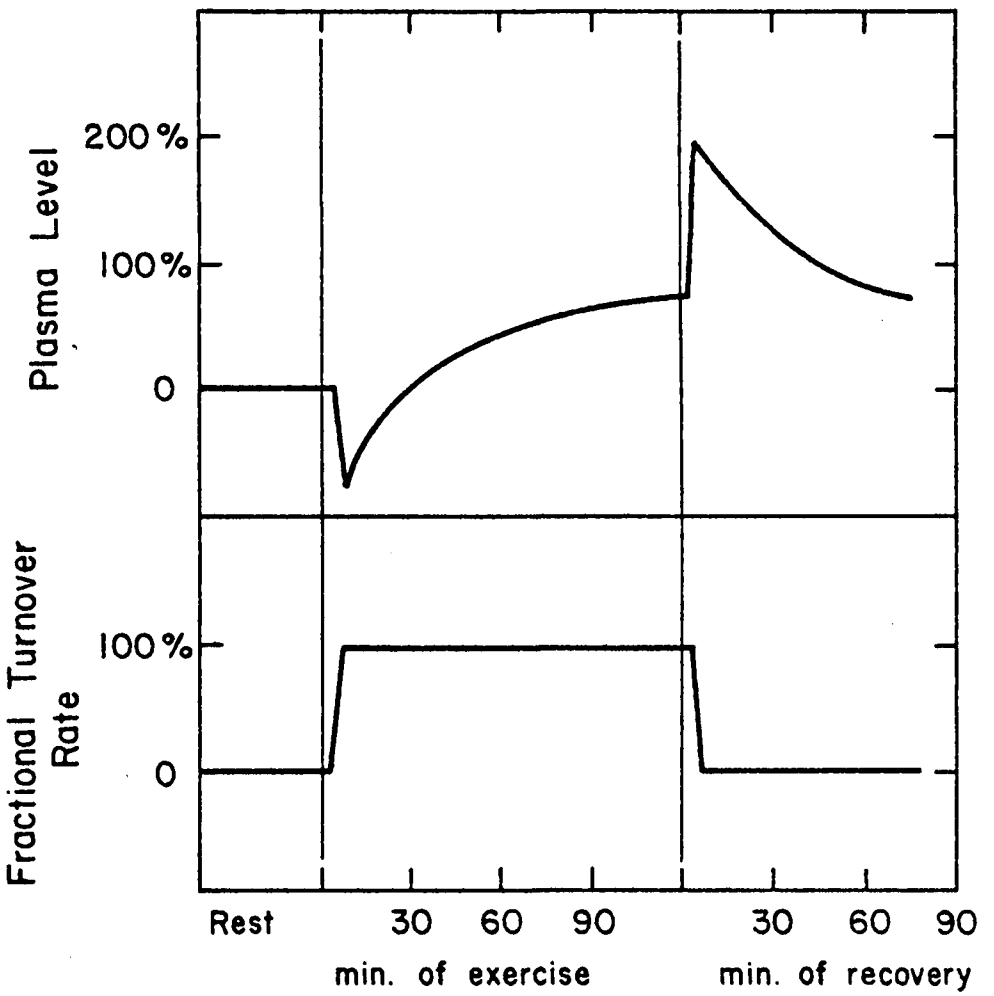


FIG. 2. Schematic representation of changes in transport of plasma FFA during exercise at moderate work load in the post absorptive state.

Adapted from Havel, 1974.

of FFA during exercise (Havel 1974, Fredholm 1969). Norepinephrine, whose release at sympathetic nerve endings is increased during exercise, is a potent stimulus of fat mobilization. It activates the adenyl cyclase system which in turn catalyzes the conversion of ATP to adenyl cyclophosphate (Cyclic 3',5'-AMP). Cyclic 3',5'-AMP in turn activates hormone-sensitive lipase in the adipose tissue cell which catalyzes the hydrolysis of triglycerides (Havel 1974).

Insulin normally inhibits hormone-sensitive lipase. Exercise is characterized by a fall in plasma insulin concentrations, creating an environment favorable to lipolysis (Havel 1974, Wahren 1979). Within ten minutes of onset of moderate leg exercise in men, Wahren (1979) recorded a 30% drop of arterial insulin levels which remained low until the cessation of the exercise. These alterations are more pronounced when the exercise is heavy or severe (Ahlborg et al. 1974). During exercise insulin appears to be released from non-specific receptors of muscle capillaries, making it more available for the specific receptors. This increases insulin's effect in promoting uptake of both glucose and FFA by the skeletal muscle, even in a situation of decreased insulin concentration (Rennie et al. 1976).

FFA mobilization will be inhibited, as mentioned earlier, by the accumulation of lactic acid during strenuous skeletal muscle work (Issekutz et al. 1975). FFA mobilization in isolated adipose tissue preparations, induced by sympathetic nerve stimulation, can be counteracted by lactate infusion in physiological concentrations (Fredholm 1969). It appears that a re-esterification

of FFA due to metabolic acidosis (Hjemdahl and Fredholm 1976) is the major mechanism of the lactate effect (Boyd et al. 1974). The accumulation of plasma lactate during work of high intensity may prevent the mobilization of FFA during the work itself, but permit a subsequent rise after work when the lactate levels have dropped (Rodahl et al. 1964). Using ^{14}C-labeled oleic acid infusion during and after 40 minutes of exercise at 60% VO_2max in adult men, Hagenfeldt and Wahren (1975) determined that the post exercise rise is a consequence of an augmented influx of FFA from adipose tissue. As mentioned before, increased sympathetic activity in subcutaneous tissue during exercise has been shown to stimulate triglyceride hydrolysis. Some of this FFA may be trapped, however, due to sympathetic vasoconstriction and resultant reduction of blood flow to the adipose tissue. With termination of exercise, sympathetic vasoconstrictor tone is released, subcutaneous blood flow increases, and large quantities of previously trapped FFA can be washed out (Hagenfeldt & Wahren 1975). This post exercise FFA elevation peaks within 10 minutes of recovery, but arterial concentration and the turnover of FFA remain above the resting value for about 60 minutes after exercise.

Blood Glucose

While there is a significant extraction of glucose by the skeletal muscle at rest, the relative contribution of blood glucose to the total oxidative metabolism of the muscle is only about 5% (Wahren et al. 1975). The primary resting muscular energy

substrate is FFA, and blood glucose is reserved mainly for the energy needs of the central nervous system (Hultman 1978).

During increasing physical exertion there is a rise in arterial glucose concentration, correlated to the increasing work load. Wahren et al. (1971) found that when healthy young men exercised on a bicycle ergometer at 400, 800, or 1200 kpm/min for 40 min, there was a significant rise in the arterial glucose level at both 800 and 1200 kpm/min (see Fig. 3). Thus while blood glucose concentration changes little during rest or brief exercise of mild intensity, it rises by 15-20% in response to more strenuous work (Wahren 1979).

Along with an increase in arterial concentration, determinations of arterial-venous differences indicate an increase in the extraction of glucose by skeletal muscle during exercise. In the same study by Wahren et al. (1971), the A-V difference of glucose across the leg rose gradually during exercise in the 400 and 800 kpm/min groups, and rose sharply in the 1200 kpm group (see Fig. 3). With this increase in blood glucose concentration and a concomitant increase in extraction rate by skeletal muscle, the rate of turnover of the glucose pool must expand considerably during physical exertion to allow for greater glucose oxidation. The proportion of the total energy for exercise derived from glucose increases with increasing work intensity in exercise of 40 minutes duration (Wahren 1979).

Exercise of long duration shows adaptations in fuel utilization over that of short duration. When a group of healthy men

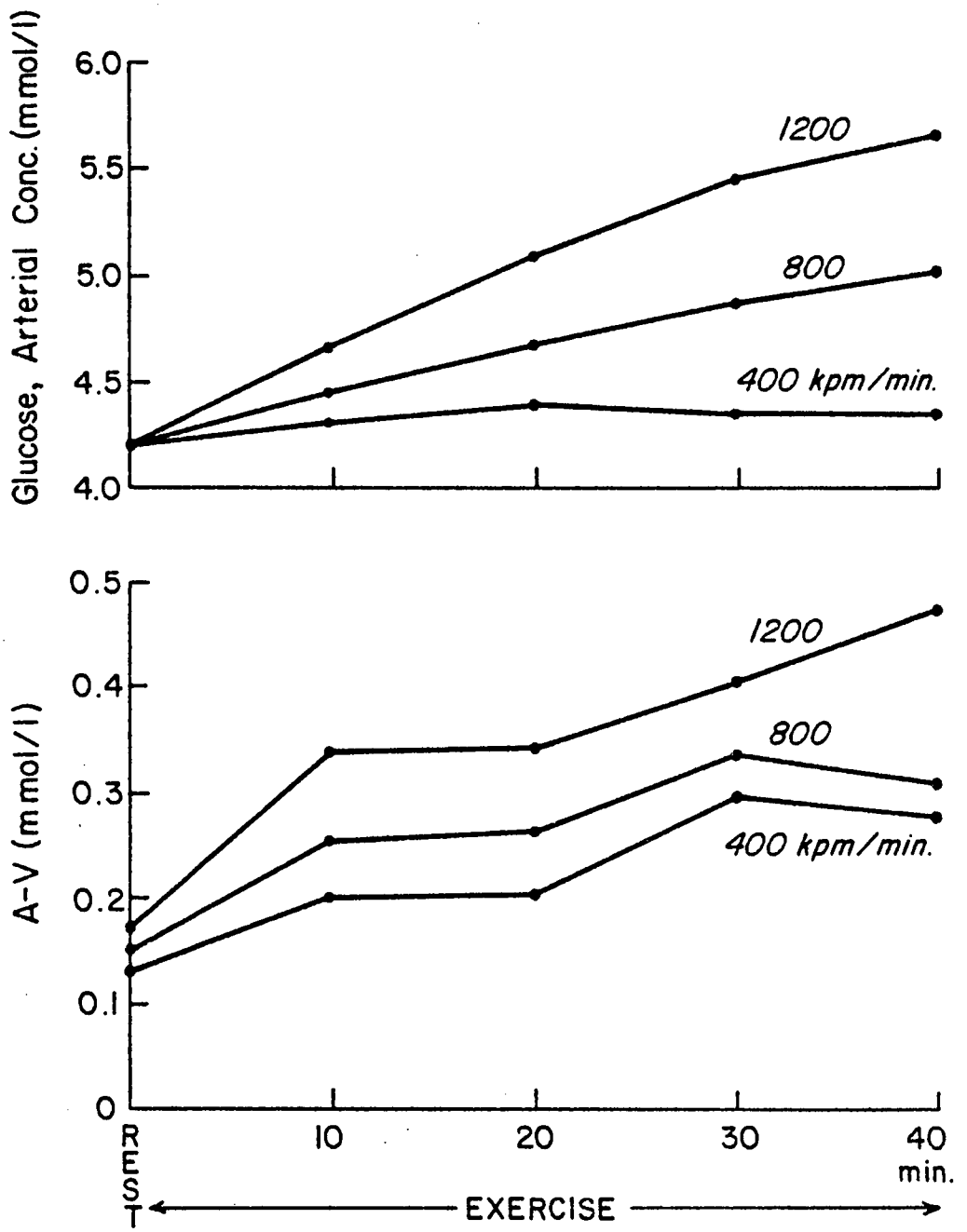


FIG. 3. Arterial concentration and arterial-venous (A-V) difference for glucose during exercise at 400, 800, and 1200 kpm/min.

Adapted from Wahren et al., 1971.

exercised on bicycle ergometers for 4 hours at a workload of approximately 30% of their tested VO_2max (65-105 W), arterial glucose concentration remained constant during the first 40 minutes at about 4.5 mmol/l, but then fell progressively to levels 30% below basal (Wahren et al. 1975). The peak rise in glucose uptake, however, at 17 times the basal value, did not occur until after 90 min of exercise. This was due to the continuing rise in the extraction rate by the leg muscles during long term, low intensity exertion. After the 90 min peak, falling blood glucose levels outweighed the augmented extraction rate and there was a gradual decline in glucose oxidation for the rest of the exercise period (see Fig. 4). Even so, after 4 hours of light exercise glucose uptake was still 12 times the basal value due to the tremendous overall increase in muscular extraction and metabolic rate.

The elevated rate of blood glucose uptake by skeletal muscle during exercise must be balanced by an augmented splanchnic glucose output. The liver is the dominant and probably the only source of increased blood glucose. Because of the absence of glucose-6-phosphatase in skeletal muscle tissue, muscle glycogenolysis cannot contribute to general blood glucose supplies. Muscle glycogen can provide fuel for the muscle fibers in which it is contained, but its contribution to glucose homeostasis is limited to the Cori cycle (Wahren 1979).

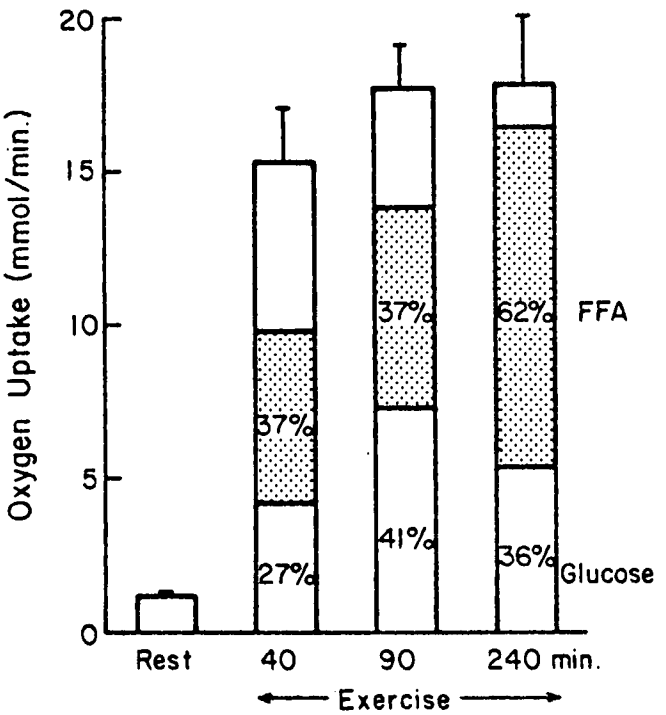


FIG. 4. Leg uptake of oxygen, glucose and FFA in the basal state and during exercise at 30% maximum oxygen consumption. The percent values represent the proportion of total oxygen uptake contributed by oxidation of glucose and FFA.

Adapted from Alhborg et al., 1974.

Hepatic Glucose Homeostasis

Splanchnic production of glucose may derive from hepatic gluconeogensis, hepatic glycogenolysis, or both. The rate of hepatic gluconeogenesis may be estimated from the splanchnic uptake of the gluconeogenic precursors lactate, glycerol, pyruvate, and alanine (Wahren et al. 1975). The splanchnic uptake of these glucogenic precursors increased from 2 to 10 times the basal value after four hours of light bicycle exercise. During such long, low intensity exercise, precursor uptake can account for an increase in gluconeogenesis from 25% of the glucose released by the liver in the resting state, to 45% after 4 hours of exercise (Ahlborg et al. 1974). Thus as glycogen stores are gradually depleted by prolonged exercise, at low intensity it is possible to accelerate the gluconeogenic process. In contrast, in the studies of cycling for 40 min at 400, 800, or 1200 kpm/min, hepatic gluconeogenesis could account for only 19, 12, and 9% of glucose production (Wahren 1979). It can therefore be concluded that during short term exercise the major part of hepatic glucose production is provided by glycogenolysis, especially during exercise of high intensity.

Not all amino acid uptake by the liver can be attributed to utilization of alanine for gluconeogenesis. Rats were found to oxidize carbon-14 labeled alanine and leucine directly when exercising at both low and high intensity (White and Brooks 1981). The peak of alanine decarboxylation occurred before the decarboxylation of glucose, and so did not reflect conversion of all

alanine to glucose prior to decarboxylation. It is becoming more accepted that alanine and the branched chain amino acids do contribute directly to the oxidative energy metabolism of exercise (Lemon & Nagle 1981, White & Brooks 1981), but they do so to a far lesser extent than either carbohydrate or lipid resources.

During prolonged exercise the blood borne substrates, especially FFA, play an increasingly important role in the supply of fuel to exercising muscle. Through comparisons of A-V difference of energy substrates and total oxygen consumption, it is shown that while 65% of the total metabolism of exercise can be accounted for by blood borne glucose and FFA after 40 min of exercise, 90% of the energy comes from these sources after 4 hours (see Fig. 4) (Ahlborg et al. 1974). This shift reflects the stimulatory effect lowered insulin levels have on FFA release and on hepatic glucose production. The finite stores of muscle glycogen make increasing availability of blood borne fuel essential, in order to spare glycogen as long as possible.

Muscle Glycogen

While the liver of the average 70 kg man will contain about 80-90 g of glycogen, skeletal muscle tissue can be expected to contain about 350 g (Lemon & Nagle 1981). Most of the glucose used for increased glycolysis of exercise are derived from these muscle glycogen stores (Toews et al. 1979). Beginning with the glycogen polymer stored in the muscle cell, a phosphorolytic cleavage catalyzed by glycogen phosphorylase will yield an

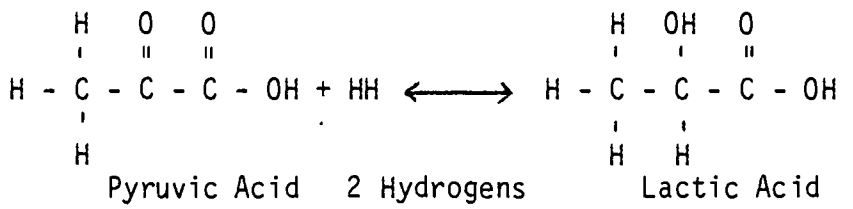
phosphorylated glucose moiety, which isomerizes to glucose-6-phosphate. From this point on the regular pathway of glycolysis can be followed, just as is the case with blood-born glucose (Leninger 1975). As mentioned earlier, the glycogen of skeletal muscle fibers is used locally for the energy of contraction, but cannot diffuse out of the cell for systemic glucose homeostasis once it has been phosphorylated.

By needle biopsy, small bits of skeletal muscle tissue (10-20 mg) can be sampled from humans and analyzed for its glycogen content (Bergström 1962), in order to monitor the glycogen status of muscle by serial analysis during exercise. At heavy workloads which can only be tolerated for 1½ hours or so, exhaustion leading to termination of the exercise appears to coincide with depletion of the muscle glycogen stores (Bergström et al. 1967). Bergström and co-workers showed that at a workload of about 75% VO_2max, a larger initial store of skeletal muscle glycogen allowed a subject to continue exercising longer at that workload. This increased endurance is the conceptual basis for the desire to enlarge glycogen stores by dietary and/or exercise manipulations, and will be addressed in detail in the section on carbohydrate loading.

Lactic Acid

During anaerobic glycolysis there is a buildup of $NADH_2$ which is generated in the cytoplasm by the dehydrogenation of triose-phosphate glycolytic intermediates in the evolution of pyruvate (Åstrand & Rodahl 1977). This NADH pool can donate hydrogen ions

to pyruvate to form lactic acid. Thus NAD is freed to continue anaerobic glycolysis when there is insufficient oxygen to act as a hydrogen acceptor (Leninger 1978, Åstrand 1977). Lactic acid acts therefore as a hydrogen sump, postponing the moment of



reckoning when there will be insufficient NAD for the continuation of glycolysis. Accumulation of lactic acid, although partly buffered by the carbonic acid system, causes a decrease in metabolic pH and a stimulation of respiration to increase the intake of needed oxygen (Kuel et al. 1967).

The conversion of pyruvate to lactic acid is a reversible reaction, controlled by lactate dehydrogenase (LDH). There are several isozymes of LDH in muscle tissue. H-LDH, the isozyme most prevalent in heart muscle, favors the conversion of lactate to pyruvate, while the isozyme designated M-LDH (muscle lactate dehydrogenase) favors the conversion of pyruvate to lactate (Sjödin 1976). The relative prevalence of the H-LDH or M-LDH form of enzyme will therefore influence the rate of lactate formation.

Within skeletal muscle there are various types of muscle fiber, referred to as Type I and Type II (Essen 1977). Type I muscle fiber (slow twitch, oxidative) has a predominance of the H-LDH isozyme, a slow conversion of pyruvate to lactate, and an

abundance of mitochondria with the enzymes for oxidative phosphorylation. These fiber types favor aerobic metabolism and are preferentially recruited for low intensity exercise (Essen 1977, Skinner & McLellan 1980).

At exercise of greater intensity,the Type II fibers are preferentially recruited. These fibers (fast twitch, glycolytic) have mainly the M-LDH isozyme, favor the rapid formation of lactate from pyruvate, and have a predominance of the enzyme for anaerobic metabolism. Needle biopsy of leg skeletal muscle has shown that after maximal intensity exercise, Type II fibers will be depleted of glycogen while the unrecruited Type I fibers have only modest glycogen loss (Secher & Jensen 1976).

Lactic acid produced during exercise can be reoxidized to pyruvate if exercise of aerobic intensity is initiated, and can then enter the Krebs cycle, sparing glycogen as fuel. Thus mild exercise performed as a "cool down" after hard exercise is more efficient for the rapid removal of lactate than is immediate rest (Belcastro & Bonen 1975). Once lactate has built up to a certain extent it will perfuse out of the muscle tissue into the blood stream, to be taken up by the liver for gluconeogenesis (Wahren et al. 1975, Hultman 1978). Lactic acid accumulation in skeletal muscle can be inferred from venous blood lactic acid levels (Di Prampero et al. 1973), but this can not be used to definitively quantify anaerobic metabolism. During early recovery there is a significantly higher concentration of lactic acid within muscle tissue than in the venous blood, with about a 2 min diffusion time

lag (Mader et al. 1978). Blood lactate levels are a dynamic reflection of factors of production (influenced by fiber recruitment), diffusion, and elimination, and thus there is wide individual variation in LA accumulation (Mader et al. 1978). While training results in a lower blood lactate level at a given workload, the total accumulation at maximal work capacity can be much higher for trained than for untrained individuals (Skinner & McLellan 1980), with values of over 20 mmole/l recorded.

Upon completion of exercise, there is a rapid conversion of lactate into glycogen to replenish the glycogen stores. It is assumed that about 85% of that lactate released from muscle into the blood is carried to the liver for conversion into glycogen (Tergin et al. 1974, Cohen & Little 1976), and stores can be expected to be fully replete within 24 hours (Bergström 1967). Findings of a rapid lactate disappearance in skeletal muscle tissue during recovery, along with a rapid glycogen resynthesis and almost no glucose uptake by muscle, indicate there is probably a direct conversion of lactate into glycogen within skeletal muscle (Larsen et al. 1976, Vaage et al. 1978). Much of the process is poorly understood, however, and the fate of lactic acid during recovery is still an unanswered question.

Model of Exercise Metabolism

The information discussed thus far now allows the construction of a model of exercise metabolism, divided into three phases in Fig. 5 (Skinner & McLellen 1980). Phase I entails low intensity

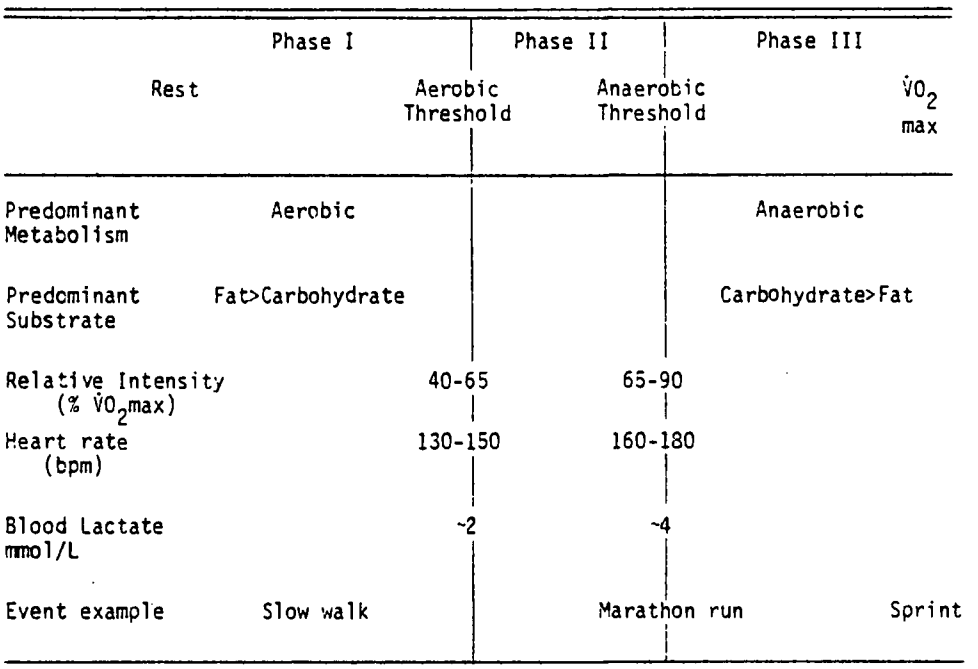


FIG. 5. Exercise intensities and predominant type of metabolism, energy substrates, heart rate, oxygen consumption and event examples.

Adapted from Skinner and McLellan, 1980.

exercise which is aerobic in nature. In this phase there will be preferential recruitment of Type I muscle fibers, so that any lactate produced can be oxidized back to pyruvate for use in the Krebs cycle, with a minimal increase in blood lactate levels. Recruitment of oxidative muscle fibers promotes FFA oxidation. After the first few minutes of low intensity exercise increased release of norepinephrine augments lipolysis for a consequent elevation of plasma FFA concentration. Blood glucose levels remain unelevated with the low intensity work load, and FFA is the predominant fuel. This level of exercise could be continued for hours; blood born substrates provide an ever increasing proportion of the energy of metabolism through increasing lipolysis and hepatic gluconeogenesis, which offsets the gradual depletion of glycogen stores.

Phase II is exercise of moderate intensity, and can be visualized as a transition between aerobic and anaerobic metabolism. In this case there will be a recruitment of Type II muscle fibers along with Type I. The M-LDH of the Type II fibers favors anaerobic glycolysis, with lactate as an end product. In this phase the moderate acidosis caused by a buildup of from 2 to 4 mmol/l of blood lactate probably acts to slightly inhibit lipolysis and increase the utilization of carbohydrate fuels.

In Phase III, intensity of exercise is high and metabolism is primarily anaerobic. Type II fibers are predominately recruited, although some slight Type I activity continues. There is a buildup of lactate to more than 4 mmol/l with an inhibitory effect

on lipolysis, so that carbohydrate serves as the main source of fuel. At this level of metabolism anaerobic glycolysis must provide the energy needed for work, and exercise at these intensities can only be maintained for a few minutes.

Carbohydrate Loading

To a certain extent we are a product of what we eat. It has been known since prehistoric times that the lack of an adequate food supply lead to reduced physical performance and ultimate death. The question of what an athlete should eat in order to achieve superior performance is a logical extension of that knowledge. Much work in recent years has dealt with the proportion of carbohydrates in the diet, and the relationship of these nutrients to athletic prowess.

In 1967 Hermansen et al. showed by needle biopsy that there was a close relationship between actual glycogen utilization and total carbohydrate oxidation during exercise at 77% VO_2max on a bicycle ergometer, and that the local muscle glycogen store is probably the most important source of carbohydrate during heavy exercise. Because exhaustion coincided with the complete depletion of glycogen stores, it was postulated that the size of glycogen stores may be the limiting factor in this kind of exercise.

As early as 1939, Christensen and Hansen had shown that men on a high carbohydrate (CHO) diet for 3 days could perform heavy work for more than twice as long as men on a high-fat diet (Forgac

1979). Bergstrom et al. (1967) performed endurance experiments on men under dietary manipulation. They first depleted their subjects' glycogen stores by bicycle ergometer exercise to exhaustion at about 75% of VO_2max. Some of the subjects then ate a high protein, high fat diet for three days, while others ate a high CHO diet for 3 days. All exercised again at the end of the diet phase. Those on the high CHO diet were able to exercise significantly longer than those on the low CHO diet, and muscle biopsy showed they had higher initial stores of glycogen in the quadriceps muscles.

Karlsson and Saltin (1971) found that after a depletion run, subjects on a high CHO diet for 3 days stored twice the glycogen in leg muscle as subjects on a mixed diet. When the subjects then ran a 19 mile race, finishing times were 5% shorter for those on the high CHO diets. Åstrand (1968) conducted a similar experiment utilizing a normal mixed diet, a low CHO, or high CHO diet for three days. He found by needle biopsy that the lowest glycogen stores were accumulated on the low CHO diet, and the largest stores during the high CHO diet. Work time to exhaustion at moderately heavy workloads was 57, 114, and 167 minutes on the low CHO, the mixed, and the high CHO diets, respectively.

Thus there is considerable evidence that the capacity to perform prolonged work is influenced by the amount of glycogen stored in the muscle, and that the amount of stored glycogen is influenced by the diet. It does not appear that additional glycogen stores will be of benefit in exercise of short duration,

since this exercise ends long before normal glycogen stores are depleted. In the study by Karlsson and Saltin (1971) of a 19 mile race, the initial speeds of the runners were similar no matter which diet had been administered. After the mixed diet, performance began to drop off compared to the performance following the high carbohydrate diet only after about 40 to 95 minutes of running. It was the maintenance of speed at the end of a long race which improved the running time of those on the high carbohydrate diet, rather than an improvement of speed throughout.

There appears to be large individual variation in the ability to supercompensate with glycogen storage. Karlsson and Saltin (1971) found that some subjects were able to increase muscle glycogen storage to 65 g/kg wet muscle weight after a high CHO diet, while others could only store 21 g/kg wet muscle weight. Those with greater storage capacities showed the greatest improvement in running times during the 19 mile race. Perhaps this can be explained on the basis of a genetic endowment with a certain percentage of Type II (glycolytic) versus Type I (oxidative) muscle fibers, with a higher percentage of Type II being conducive to greater glycogen storage. It has been shown that glycogen utilization is extremely local, and that recruitment patterns of fiber types influence the depletion pattern of glycogen (Secher & Jensen 1976). There is also a large individual variation in lactate production with exercise (Skinner & McLellan 1980), which may be linked with glycogen utilization patterns in the muscle,

which in turn are linked with genetic endowment of fiber types (Åstrand & Rodahl 1977). This is a poorly understood aspect of fuel metabolism which warrants further research.

The rate limiting step in the formation of glycogen is controlled by the enzyme glycogen synthetase (Leloir et al. 1959). Total glycogen synthetase activity is one of the parameters improved in muscle by training (Jeffress et al. 1968). More importantly, muscle contraction with glycogen depletion results in an increase in the level of the active form of glycogen synthetase (Terjung et al. 1974). Kochan et al. (1979) had six healthy men exercise only one leg on a bicycle ergometer at 75% VO_2max for one hour. After the exercise, the men consumed a low CHO diet for 3 days, exercised once more, and then consumed a high CHO diet for 4 days. Glycogen synthetase activation was strikingly increased in the exercised leg after exercise, but only somewhat elevated in the unexercised leg. Glycogen in the exercised leg returned to normal on the high CHO diet within 24 hours, and to 193% of normal levels within 48 hours. The unexercised leg did not show the supercompensation effects. Therefore, diet alone cannot be enough to trigger the loading effect-exercise leading to glycogen depletion is an essential part of the mechanism.

The full method of carbohydrate loading as used in competition, outlined by Forgac (1979), consists of a seven day routine. Since glycogen loading is such a localized phenomenon, the specific muscles to be used in the exercise event should be

exercised to exhaustion during the depletion phase. This is done 6 to 7 days before the day of the competition, and is followed by a diet high in fat and protein (and therefore low in carbohydrates) for 3 days. Training may be continued during this phase. Three days before the competitive event the high CHO diet is initiated. Exercise is not recommended during this phase, as it depletes the glycogen stores. The last phase is the day of the event, when the athlete eats as desired and then competes. Diets should be adequate in calories and balanced nutritionally in both the depletion and repletion phase. During the depletion phase, at least 100 g of carbohydrate should be consumed daily to prevent the development of ketosis.

There are some concerns to be considered during a carbohydrate loading regime. With every gram of glycogen stored there are about 3 grams of water stored (Karlsson & Saltin 1971). The extra weight (approximately 5 lb) so acquired by glycogen supercompensation may be considered a disadvantage by some, and the volume occupied in the muscle can cause an unpleasant stiffness or bloated feeling. In a sport such as wrestling, in which glycogen depletion is not a significant factor limiting performance, the added weight and stiffness would present a distinct disadvantage. In long distance running, however, the extra water helps to compensate for evaporative water losses, and benefits of the extra energy from glycogen stores offset the additional weight (Forgac 1979).

When diets low in carbohydrates are consumed, there is

physical discomfort experienced by some. Symptoms such as depression, dizziness, and general malaise are reported, probably due to low blood sugar. Sherman et al. (1981) have recently reported that by using the repletion phase only, supersaturation can still be achieved without the unpleasantness of the low carbohydrate phase. Healthy athletes who exercised to exhaustion and then had a high carbohydrate diet for three days achieved glycogen stores of 203 mmoles/kg body weight, compared to 208 mmoles/kg wt in those who underwent the entire routine. For all practical purposes, these glycogen values can be considered to be equal.

An additional concern is illustrated by a report in the literature that a 40 year old man, who had originally taken up running due to high blood cholesterol, developed chest pains and ECG abnormalities during the loading phase of a complete 7 day regimen (Mirkin 1973). This does not prove cause and effect, but would indicate that caution should be exercised in the case of individuals who are older or who are not in excellent health, especially until further research can delineate some of the physiological stresses involved in this routine.

It is advised that even for those in top condition the entire technique should be used only sparingly, two or three times a year, due to possible stress on the muscles involved (Forgac 1979, Karlsson & Saltin 1971).

Vitamin B-6 in Exercise and Fuel Metabolism

Vitamin B-6 Metabolism

Vitamin B-6 is active as pyridoxal 5'-phosphate (PLP). This coenzyme can be formed in the body from any one of the three forms found in food: pyridoxine, pyridoxal, or pyridoxamine (Guthrie 1975). The pathways by which these substances can form PLP are shown in Fig. 6. Liver is the only site capable of synthesizing PLP for subsequent release into the blood. After uptake into other tissues as pyridoxal, B-6 may be trapped by phosphorylation (Fig. 7).

Vitamin B-6 is primarily involved as a cofactor in the reactions involving protein metabolism; in transamination, deamination, decarboxylation, and desulfhydration (Sauberlich 1980). It is also involved in carbohydrate metabolism, in the production of antibodies, in nucleic acid metabolism, at least secondarily in lipid metabolism, and is implicated in regulation of hormone action (Sauberlich 1980). It is the role of vitamin B-6 in glycogen phosphorylase which directly relates it to glycogen utilization in exercise.

Glycogen Phosphorylase

In carbohydrate metabolism, PLP is an integral part of glycogen phosphorylase (Leninger 1975). Glycogen phosphorylase facilitates the release of glucose moieties from their polymerous form in order that the storage form of carbohydrate may be

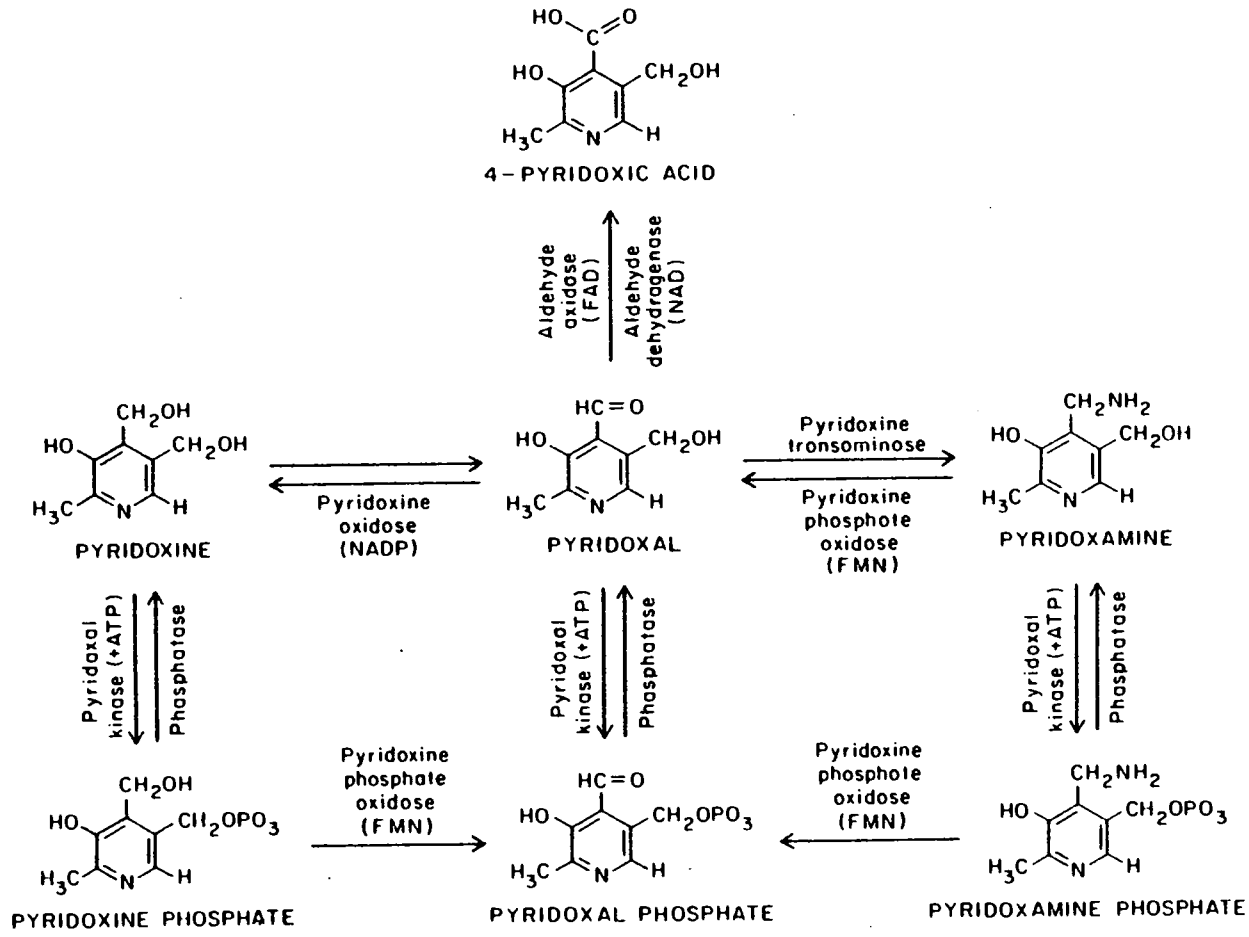


FIG. 6. Interconversions of vitamin B-6 compounds.

Adapted from Snell and Haskell, 1971.

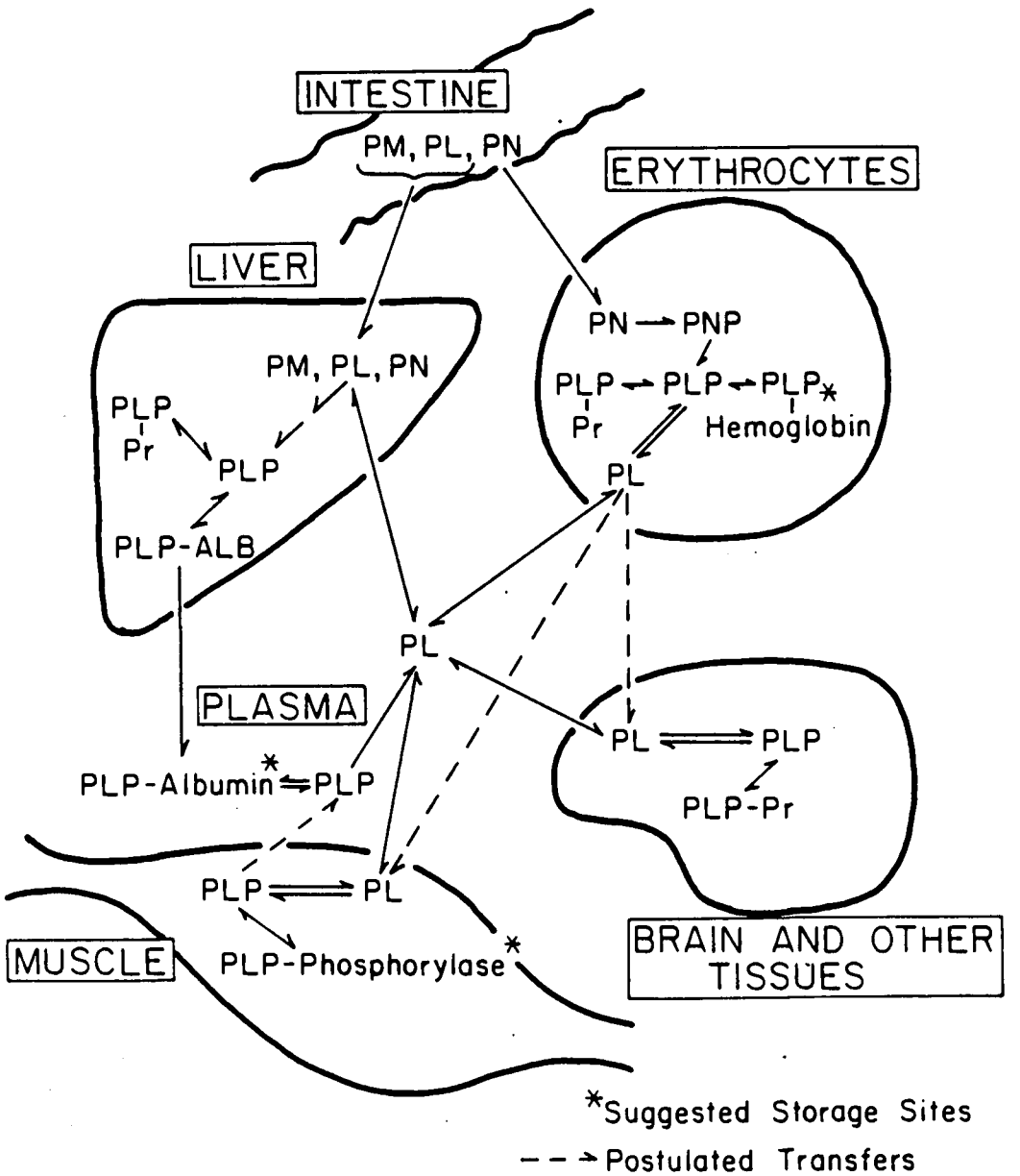


FIG. 7. Postulated interrelationships of the vitamin B-6 compounds in various compartments of the body. Abbreviations: PL, pyridoxal; PM, pyridoxamine; PN, pyridoxine; PLP, pyridoxal 5'-phosphate; PNP, pyridoxine phosphate; PR, protein.

available for glycolysis. Glycogen phosphorylase appears to be the enzyme which is rate limiting for glycogenolysis (Toews et al. 1979).

Studies in rats indicate that when excess vitamin B-6 is fed, glycogen phosphorylase accumulates in muscle in conjunction with bound PLP (Black et al. 1978). This is surprising, as one usually thinks of water soluble vitamins as having very limited tissue storage, with the excess being eliminated through the renal system. The quantity of vitamin B-6 in the phosphorylase reservoir is potentially quite large since phosphorylase constitutes 5% of muscle protein and muscle is 40% of body mass (Black et al. 1977).

It is shown in these rat studies that the phosphorylase stores accumulated by excessive vitamin B-6 intake were not later reduced upon consumption of a diet deficient in vitamin B-6 (Black et al. 1978). This too is confounding, as a storage depot should presumably allow access to the vitamin in time of need. It was found that when the rats were so vitamin B-6 deficient that they became anorectic and subsequently lost weight, phosphorylase was finally depleted and vitamin B-6 released.

Black and co-workers have postulated comparison of phosphorylase with adipose tissue as a possible explanation of this confusing phenomenon. Adipose tissue acts, undoubtedly, as a storage depot for triglycerides. In the case of an essential fatty acid deficiency, specific fatty acids are not released to cover demand for this nutrient. Yet, during a calorie deficit, adipose tissue will release FFA for maintenance of energy

homeostasis. So, too, in the case of vitamin B-6 needs and starvation in rats. Phosphorylase is then depleted with release of vitamin B-6 for the enzymes involved in gluconeogenesis from amino acids, such as liver alanine aminotransferase and liver aspartate aminotransferase (Black 1978).

In its enzymatic role, phosphorylase responds acutely to prevent hypoglycemia through liver glycogenolysis, or to supply local energy needs through muscle glycogenolysis. In addition, as a storage site for vitamin B-6, it may provide a prolonged capability for glucose homeostasis during starvation by sustaining the gluconeogenic enzymes involved in providing glucose precursors from amino acids (Black et al. 1978).

Vitamin B-6 in Exercise

In research performed in this laboratory, a sharp rise in plasma vitamin B-6 values was noticed in only one subject out of five two hours after 0.5 mg of pyridoxine had been administered. Upon questioning, the subject reported having run two miles just before the blood sample was taken. It appeared that strenuous exercise might have resulted in the high plasma B-6 levels (Wozenski 1977).

Subsequently, seven male adolescent cross country runners were monitored by Leklem et al. (1979) for plasma PLP changes following exercise. The boys were tested at the beginning and end of the training season. In both instances exercise produced significant increases in plasma PLP, with the increase being

greatest after training.

Also in this laboratory, Munoz (1982) compared plasma PLP changes with exercise in adolescent versus adult males, trained versus untrained, and bicycle ergometer exercise versus running. She found significant increases in plasma PLP in each exercise situation. PLP response was similar between trained and untrained, and between bicycle exercise and running. But adult athletes showed a 22% increase in plasma PLP after 20 minutes of bicycle ergometer exercise, which was a significantly greater increase than seen in the adolescents. Perhaps this is due to the larger muscle mass and consequent greater PLP storage capacity of the adults.

In summary, it has been shown that excessive intake of vitamin B-6 results in the marked buildup of glycogen phosphorylase in rats, and hence its integral constituent, PLP. During starvation the depletion of phosphorylase is initiated, with consequent release of PLP into the blood stream. That a similar release of PLP may occur with exercise is indicated by a marked rise in plasma PLP after exertion in human subjects. Perhaps these two states, starvation and exercise, which are similar in many metabolic parameters, are each activating the release of PLP in response to glycogen depletion.

The effects which a buildup of glycogen phosphorylase may have on exercise are, as yet, unknown. As glycogen phosphorylase is believed to control the rate limiting step of glycogenolysis (Toews et al. 1979), it is conceivable that an increase in this enzyme may speed the breakdown of glycogen and increase its rate

of utilization as the fuel of exercise. A single blind study was performed on young swimmers over a period of 6 months to see if either supplemental vitamin E or vitamin B-6 would effect an improvement in endurance (Lawrence et al. 1975). Although no difference in endurance was evident between experimental groups at the end of the study, the investigators were startled to discover a significantly elevated serum lactate level in the group supplemented with vitamin B-6. They could offer no explanation for this elevation.

If vitamin B-6 supplementation in man results in storage of PLP as elevated levels of glycogen phosphorylase, then the swimmers could be expected to have substantially increased their phosphorylase levels by the 51 mg daily supplements they were receiving. This increased quantity of the enzyme which regulates glycogenolysis could conceivably make glycogen more readily available as fuel for exercise. An increased lactic acid production could thus result from a greater proportional dependence upon muscular glycogenolysis than on blood borne FFA. In this situation of elevated PLP storage with glycogen phosphorylase, it is possible that glycogen stores could be more rapidly emptied than with the non supplemented diet, leading to an actual decrease in exercise endurance. Because the 1,000 meter test swims were of insufficient duration to seriously tax glycogen stores, it was not possible to assess endurance capability in this study design. The study which was the basis for this thesis utilized well controlled dietary manipulations and a series of monitored exercise tests to further evaluate the effects of supplemental vitamin B-6 on exercise.

III. METHODS AND MATERIALS

Subject Selection

Five healthy young men ranging in age from 20 to 23 years volunteered to take part in the study. The subjects were recruited from a university cycling class and so were specifically trained in bicycle exercise. As one volunteer left school and was able to participate only in the control week, statistical analysis was performed on the four subjects who completed the entire protocol. These subjects received a small stipend upon completion of the study.

Criteria for subject selection included:

1. a normal routine physical examination.

2. normal resting, backlying, and post-hyperventilation ECG as evaluated by a staff physician at Oregon State University Student Health Center.

3. normal hemoglobin and hematocrit.

4. normal blood chemistry as determined by automated analysis at Good Samaritan Hospital (Corvallis, OR) (see appendix).

5. avoidance of drugs, alcohol, and caffein during the experimental weeks.

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

Consent forms approved by the Human Subjects Committee of Oregon State University were signed by all subjects before beginning the study (see appendix). The subjects' physical

characteristics are shown in Table 1. Weights were recorded each morning before breakfast, and both immediately before and after exercise. Percent body fat was determined by Lauren Hatcher using the method of Sloan (1967). Mean skinfold measurements were taken at the beginning of the study, following the 2nd study week, and following the third study week. The equation of Sloan uses thigh and subscapular skinfolds to predict body density.

Experimental Procedure

Calculation of Diets

The experimental design is summarized in Fig. 8. Three isocaloric test diets were developed for use in the experimental procedure. These were designated as Normal CHO (NC), Low CHO (LC), and High CHO (HC). The NC diet, which served as the control condition, was consumed for the entire first week of the experimental procedure. This latter diet was patterned after the normal American diet with 40% of its kcalories from carbohydrates, 18% from protein, and 42% from fat. The percentage of kcalories from carbohydrates were 11% for the LC diet and 71% for the HC diet, as outlined by Forgac (1979) for a glycogen depletion-repletion regimen. The diets were calculated from the food composition tables of the Agricultural Handbook No. 456 (1975) and the Home Economics Research Report No. 36 (Orr 1969) to provide 3500 kcalories per day and 2 mg of vitamin B-6. The partial nutrient composition of the three diets is shown in Table 2, and the complete menus are shown in Tables 3 through 5.

TABLE 1
Subject descriptions

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

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

† Determined by method of Sloan(1967) from mean values of skinfolds of three separate determinations.

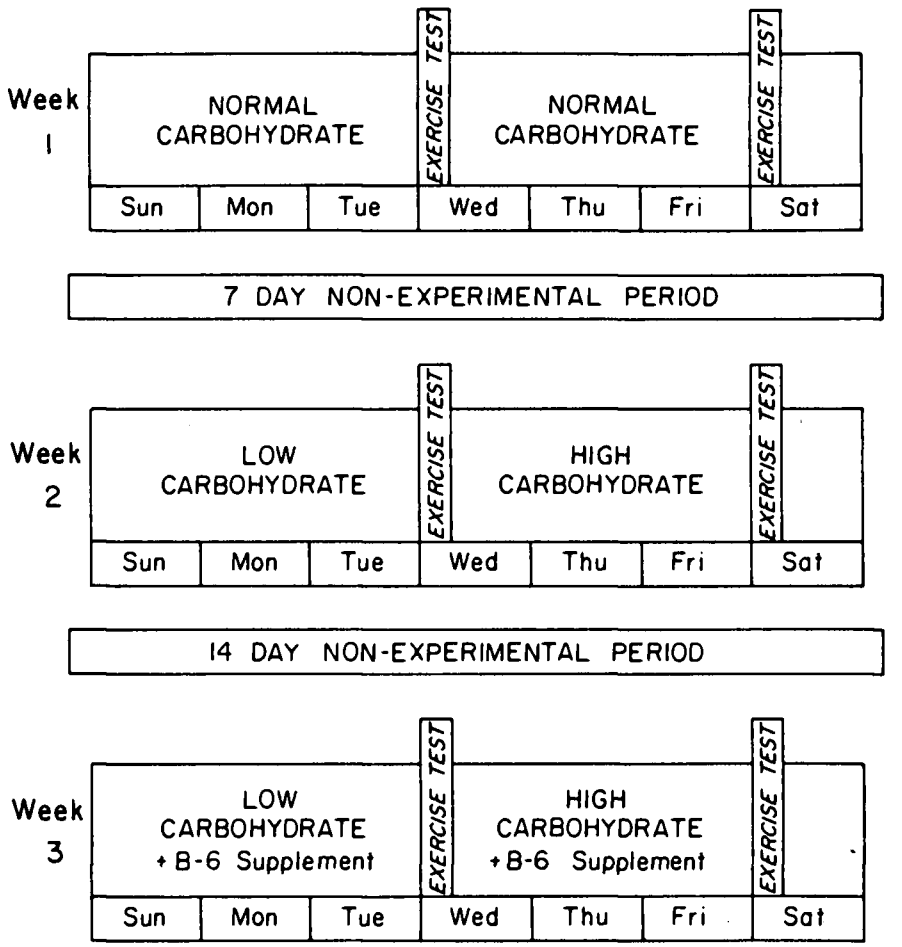


FIG. 8. Experimental procedure. Subjects exercised on Wednesday and Saturday mornings after an overnight fast. A full explanation is given in the text.

TABLE 2
Experimental Diet Compositions

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

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

Table 3.
Experimental diet compositions:
Normal Carbohydrate Diet (NC)

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

Table 4.
Experimental diet compositions;
Low Carbohydrate Diet (LC)

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

Table 5
Experimental diet compositions:
High Carbohydrate Diet (HC)

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

Supplemental vitamin B-6 was added as pyridoxine hydrochloride to achieve the LC+B6 and the HC+B6 conditions. The pyridoxine hydrochloride was prepared in a 0.5% acetic acid solution in one batch and separated into aliquots for storage at -20°C. Each morning an aliquot was thawed, protected from light, and pipeted into sample cups. Subjects were instructed to drink the cup contents, rinse the cup with water, and drink the rinse. A 5 ml aliquot was given at breakfast and 5 ml at dinner to provide a total of 8 mg of pyridoxine.

When possible,foods were purchased in bulk amounts from a local supermarket in order to minimize variation in nutrient content. For example, hamburger, an important source of vitamin B-6, was purchased in one lot, separated into pre-weighed individual servings, and frozen for consumption throughout the study. Foods were weighed to the tenth of a gram during preparation in the metabolic kitchen of Rm 105, Milam Hall, Oregon State University. The subjects consumed their meals in the metabolic kitchen and were instructed to eat no other foods, alcohol, or caffeinated beverages. On certain occasions of schedule conflicts, sack lunches were prepared for consumption elsewhere.

Experimental Time Line

During the three test weeks the subjects were instructed to perform their customary weekly exercise within the first three days of the week to facilitate the glycogen depletion effect of the diet. The high carbohydrate diet began after the exercise

test on the morning of the 4th day (see Fig. 8), and subjects were then instructed to refrain from all but the most minimal of daily exercise in order to facilitate glycogen supercompensation. Subjects recorded the type and duration of exercise engaged in during these periods. As summarized in Table 6, primary modes of exercise were found to be running, trampolining, and cycling for Subject 1, cycling and running for Subjects 2 and 3, and swimming and cycling for Subject 4.

As indicated in Fig. 8, there was a non-experimental period of 7 days between the first and second experimental weeks, and a period of 14 days between the second and third experimental weeks. During these non-experimental days the subjects ate and exercised according to their normal patterns, recording their dietary intakes and their exercise activities.

Exercise Procedure

To determine the effects of dietary modifications on fuel metabolism during exercise, each subject exercised on a Monarch bicycle ergometer (Quinton Instruments, Seattle, WA) while blood samples were drawn for laboratory analysis. The standardized exercise procedures were conducted in the exercise physiology laboratory of Langton Hall, Oregon State University. Subjects reported for the exercise test in the early morning following a 12 to 14 hour fast, having consumed one of the test diets for 3 days. Each subject rode the same bicycle ergometer during each test session. The exercise tests were performed at an ambient

Table 6.
Summary of subjects' individual exercise during the study weeks

Diet	Day	Subject 1	Subject 2	Subject 3	Subject 4
		min	min	min	min
NC-1	Sun	cycle 10	cycle 90	run 40	cycle 30
	Mon	cycle 60	cycle 60	volleyball 60	swim 60
	Tue	cycle 50, trampoline 45	cycle 60	run 40	---
NC-2	Wed	slow dance 60	cycle 10	run 20	swim 45
	Thu	cycle 10	cycle 30	run 20	---
	Fri	slow dance 60	cycle 10	cycle 20	---
LC	Sun	cycle 44	cycle 60	basketball 90	cycle 20
	Mon	cycle 15, run 45	swim 50	run 40	cycle 17, swim 30, stairs 30
	Tue	run 60	run 5	run 40	---
HC	Wed	slow dance 50	---	volleyball 60	water polo 45
	Thu	cycle 15	slow dance 120	---	---
	Fri	slow dance 50	---	volleyball 60	---
LC+B6	Sun	cycle 50	cycle 75	run 50, basketball 60	cycle 30
	Mon	run 50, trampoline	cycle 60	volleyball 60	cycle 70
	Tue	run 50, cycle 15	cycle 90	run 30	---
HC+B6	Wed	cycle 10	run 5	volleyball 60	---
	Thu	cycle 20	slow dance 90	run 15	---
	Fri	cycle 15	---	---	---

temperature of from 22°-25°C.

A resting ECG was performed before initiating the exercise session. The monitored exercise for 50 minutes of continuous cycling consisted of: 30 min at 60%, 15 min at 80%, and 5 min at 90% of age-adjusted calculated maximum heart rate (MHR). Subjects rode at a rate of 50 pedal rpm, and workloads were continuously adjusted to maintain the target heart rates. Heart rates were monitored by ECG tracings utilizing a multiple Graded Exercise Test (GXT) exercise lead system. A 12 minute monitored recovery session succeeded the exercise, and subjects rested for one hour after completion of the test before leaving the exercise room. Workload, HR, and ECG tracings were evaluated by and are presented in the Masters Theses of Frank Goulard (1982) and Art Sieman (1982) of the Oregon State University Department of Health and Physical Education.

Fig. 9 shows a schematic representation of the blood sampling time line. Blood samples were drawn on 5 occasions during the exercise tests: before the exercise began (PRE), from 1 to 7 min before the increase to 90% MHR (DURING), from 2 to 5 min after cessation of the exercise (POST), thirty minutes after cessation (30 MIN POST), and one hour after cessation of the exercise (60 MIN POST). Twenty ml of blood was drawn from the anti-cubital vein of the forearm into heparinized tubes, and was transfered on ice to a refrigerated centrifuge and spun for 20 min at an R.C.F. of 1860. The plasma was divided into aliquots for later analysis of glucose, lactate, and FFA, and stored at -70°C.

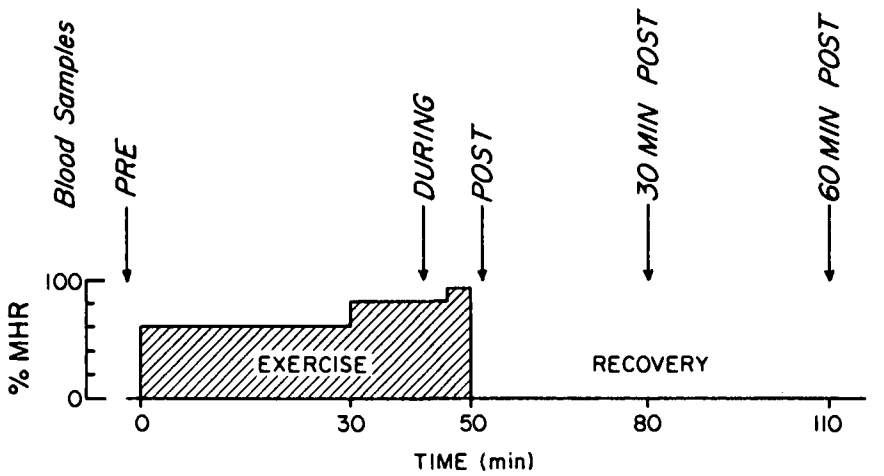


FIG. 9. Time line for blood sampling during the exercise text. Abbreviation: % MHR, percent maximal heart rate.

Assays

Diet Composites

Composites were made of one day's meals during each of the five dietary manipulations. The diet composite was prepared by blending together each item of known weight in a blender, separated into animal and vegetable fractions. The total weight of the composites were noted and a well homogenized sample was frozen at -20°C for later analyses. Aliquots were acid hydrolyzed and analyzed for vitamin B-6 by a microbiological method using S. uvarum (AOAC, 1980[1]). Vitamin B-6 contents of the composites are given in Table 7. Diets were determined to have 1.65 mg, 1.55 mg, and 1.82 mg of pyridoxine in the NC, LC, and HC conditions, respectively.

Hemoglobin and Hematocrit

Hemoglobin and hematocrit were determined immediately following the exercise.[2] Hematocrit was determined in duplicate in the fresh blood using the micro hematocrit method. Hemoglobin was measured in triplicate utilizing the cyano-methemoglobin method.

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1. Hossein Kabir performed the vitamin B-6 analysis on the diet composites.

2. Karin Hardin and Linda Barstow performed the hemoglobin and hematocrit calculations.

Plasma Glucose

Plasma glucose was determined in duplicate on a Technicon Autoanalyzer (Technicon Corporation, Terry Town, New York). The method is a modification of the procedure of Hoffman (1937) and depends on the reaction of ferricyanide ions with reducing sugars to form colorless ferrocyanide.

Plasma insulin values were determined PRE and POST for each exercise day by another investigator, using an Insulin Radioimmunoassay Kit [^{125}I] (Benton Dickinson & Co., Orangeburg, NY).

Plasma Lactate

Plasma lactate was enzymatically determined by ultraviolet absorption using a Beckman DU Quartz Spectrophotometer (National Technical Laboratories, South Pasadena, CA). In the presence of the enzyme lactate dehydrogenase and of excess NAD, pyruvic acid is formed from lactic acid (Hohorst 1963). The increased absorbance at 340 nm due to NADH formation becomes a measure of the lactic acid originally present. A kit from Sigma Chemical Company (technical bulletin no. 726-UV/826-UV) was used for this analysis. The interassay coefficient of variation was 5%, n=6.

Plasma FFA

Plasma FFA concentrations were determined in duplicate by titration with alkali, using a modification of the method of

Trout et al. (1960). Plasma was extracted by isopropanol-heptane containing 1 N Sulfuric acid, and washed again with 0.05% sulfuric acid to remove lactate. Titration with 0.02 N NaOH was accomplished with a micro pipet (Manostat Digi-pet, New York, NY). Nile blue was the color indicator, and comparisons were made with a standard of palmitic acid. The interassay coefficient of variation was 4%, n=9.

Plasma Volume Changes

Plasma volume changes were calculated by the method of Beaumont (1972). This method involves multiplication with a proportionality factor since the % change in hematocrit (Hct) with exercise is known to underestimate the actual plasma volume (PV) change. The equation is:

$$\% \text{ change PV} = \frac{100}{100-Hct_1} \times 100 \frac{(Hct_1 - Hct_2)}{Hct_2}$$

Statistical Analysis

The statistical significance of differences between any two dietary conditions was assessed by using a Student's t-test for paired values. Because of the great individual variation among subject responses, natural log conversions of the raw data values were employed for some t-tests to minimize large standard deviations. Those instances in which natural logarithm conversions

have been incorporated are designated by placing the abbreviation "ln" after the statement of probability. A Hewlett Packard Model 10 programable calculator (Hewlett Packard Calculator Products Division, Loveland, CO) was utilized in the statistical analyses.

IV. RESULTS

As outlined in the previous section, each exercise test was performed after consuming one of the controlled diets and performing either heavy or light personal exercise for a period of 3 days (see Fig. 8). In the following discussion these test conditions will be referred to as: NC-1 (normal CHO diet and heavy personal exercise), NC-2 (normal CHO diet and light personal exercise), LC (low CHO diet and heavy personal exercise), HC (high CHO diet and light personal exercise), LC+B6 (low CHO diet plus supplemental vitamin B-6 with heavy personal exercise) and HC+B6 (high CHO diet plus supplemental vitamin B-6 with light personal exercise). In the control work periods of NC-1 and NC-2, the diet itself was identical for the entire week. In the first part of the week, however, subjects exercised heavily on their own time, while during the latter half of the week they refrained from heavy exercise. In this manner it was expected to see a slight glycogen loading effect resulting from the above exercise patterns alone. The various data for NC-1 and NC-2 (Figs. 10, 12, and 14) indicate that such a trend is evident, although not reaching the level of significance.

Plasma Glucose

Figs. 10 and 11 indicate the pattern of glucose values throughout the 6 exercise tests, and Table 7 lists the individual glucose concentrations and points of statistically significant

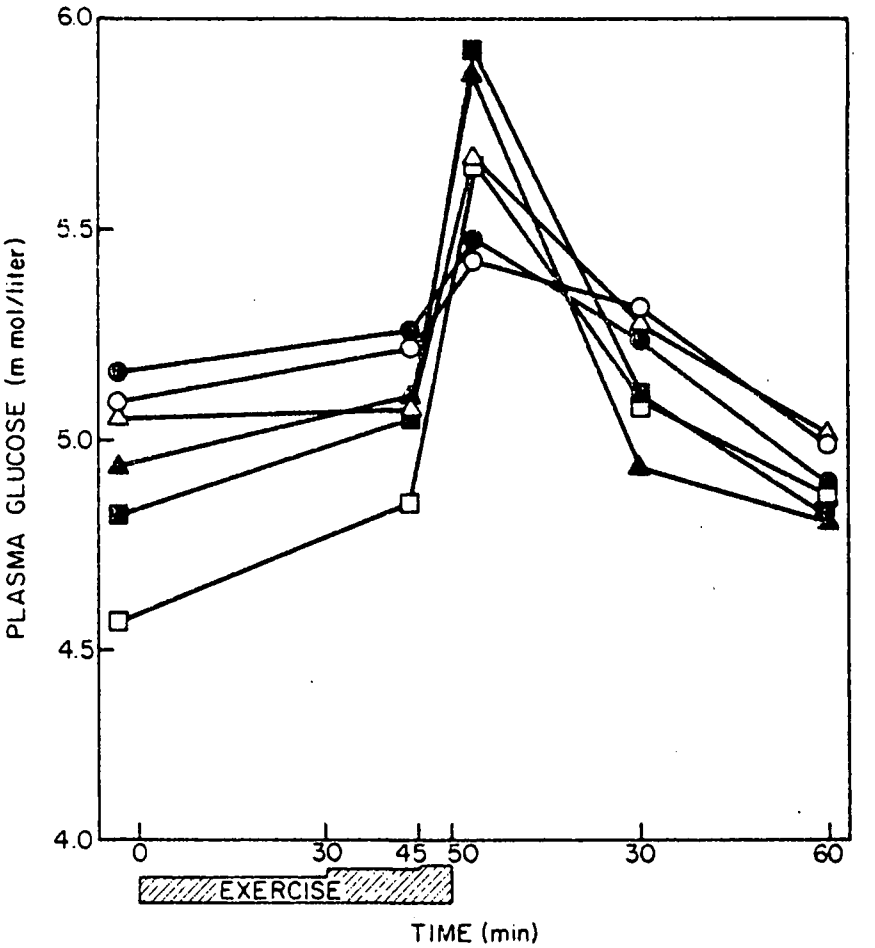

FIG. 10. Plasma glucose concentrations. Values are means of four subjects during exercise and recovery after NC-1 (○—○), NC-2 (●—●), LC (△—△), HC (▲—▲), LC+B6 (□—□), or HC+B6 (■—■) diets. Refer to the text for an explanation of the diets.

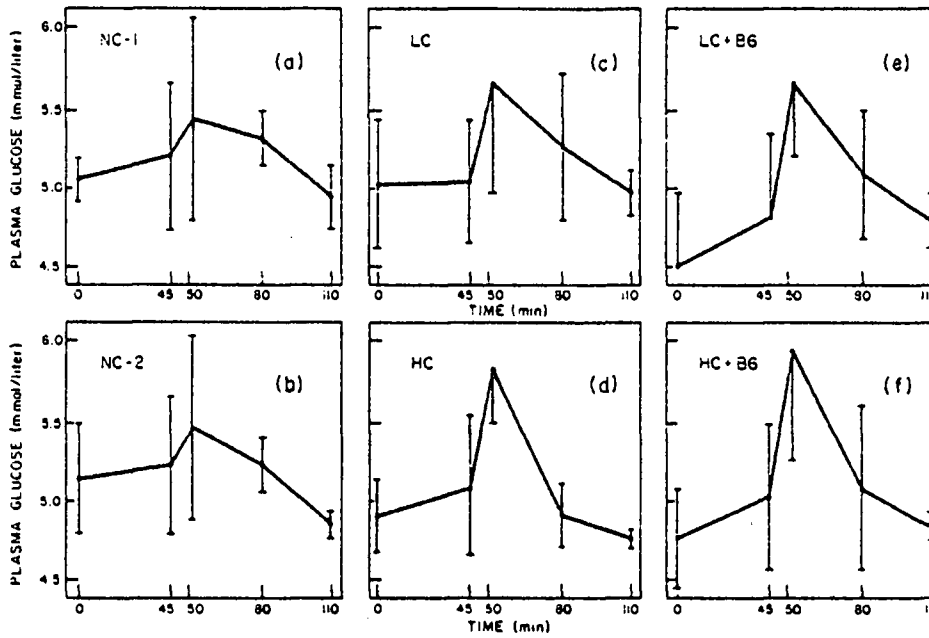

FIG. 11. Sequential plasma glucose concentrations. Values are means of four subjects during 50 min exercise and 60 min recovery after (a) NC-1, (b) NC-2, (c) LC, (d) HC, (e) LC+B6, or (f) HC+B6 diets. Vertical lines represent 1 standard deviation from the mean. Refer to the text for an explanation of the diets.

Table 7.
Plasma glucose concentrations during rest, exercise, and recovery after six diet regimens.

Diet	Sample	Plasma Glucose	Diet	Sample	Plasma Glucose
		mmol/l			mmol/l
NC-1	PRE	5.09±0.13*[a]	NC-2	PRE	5.16±0.35[m]
	OURING	5.22±0.48[b]		OURING	5.26±0.42[e,h,n]
	POST	5.43±0.63[c]		POST	5.48±0.55[i]
	30 MIN POST	5.32±0.18[d]		30 MIN POST	5.24±0.16[f]
	60 MIN POST	4.99±0.20		60 MIN POST	4.90±0.08[j]
LC	PRE	5.05±0.39	HC	PRE	4.94±0.22
	OURING	5.07±0.38		OURING	5.10±0.44[e]
	POST	5.67±0.67		POST	5.82±0.32[d,f]
	30 MIN POST	5.28±0.44[k]		30 MIN POST	4.93±0.20[k]
	60 MIN POST	5.01±0.14		60 MIN POST	4.81±0.06
LC+B6	PRE	4.57±0.46[a,m]	HC+B6	PRE	4.82±0.30[h,l]
	OURING	4.85±0.52[b,l,n]		OURING	5.06±0.45[c,i]
	POST	5.65±0.45		POST	5.93±0.67
	30 MIN POST	5.11±0.40		30 MIN POST	5.10±0.52[j]
	60 MIN POST	4.82±0.18		60 MIN POST	4.87±0.09

*values are given as means ± standard deviations

[a-n]values sharing the same suprascript are significantly different ($p<0.05$).

Abbreviations are: normal carbohydrate (NC-1, NC-2); low carbohydrate (LC); high carbohydrate (HC); low carbohydrate plus vitamin B-6 (LC+B6); and high carbohydrate plus vitamin B-6 (HC+B6).

differences between diets. Plasma glucose concentration rose during exercise after each dietary condition, returning to near PRE values by 60 MIN POST. Fig. 10 shows, however, that the magnitude and pattern of the increased concentration was altered by the experimental conditions. At no time during the experimental procedure was there a significant difference between the control conditions of NC-1 and NC-2, each of which had an increased glucose concentration of about 2% from PRE to DURING and of about 6.5% from PRE to POST. Under each of the dietary manipulations, however, values were lower DURING than for the control diets, followed by a more radical rise of from 12-16% between the DURING and POST samples for the depletion-repletion regimens as compared to a 4% rise for the control diet regimen.

Although fasting glucose values were within the range of normal in all cases, there were differences seen among dietary groups. PRE LC+B6 values were lower by 11% and by 13% than were the PRE NC-1 ($p<0.02$, ln) or the PRE NC-2 ($p<0.01$) values, respectively, and LC+B6 continued to be lower for both DURING NC-1 and NC-2 ($p<0.005$, ln). POST LC+B6 values did not vary significantly from the POST controls, nor did the respective values for the recovery periods.

With both the DURING HC and DURING HC+B6 conditions, glucose values were from 3% to 4% lower than for the DURING NC-2 condition ($p<0.01$, ln). However, POST HC+B6 values rose more sharply than controls and reached an 8% to 9% higher value than either POST control ($p<0.05$ versus NC-1, ln; and $p<0.02$ versus

NC-2, ln). While the POST HC value showed a similar trend, as compared to POST controls with an increase of about 6%, this was not significant. Both the 30 MIN POST HC and HC+B6 values dropped to a lower level than that seen in other 30 MIN POST conditions, with the HC value being about 7% lower than either control value ($p<0.01$, ln).

LC and HC plasma glucose concentrations did not vary significantly from each other for any other times except 60 MIN POST, when LC values were 4% higher than HC values ($p<0.01$, ln). With the addition of vitamin B-6, however, there was a 4% lower value for DURING LC+B6 than for DURING HC+B6 ($p<0.05$). As mentioned previously, this DURING LC+B6 value was also significantly lower than either control value ($p<0.005$, ln).

Plasma Lactate

Figs. 12, 13, and Table 8 show the plasma lactate values determined throughout the exercise tests. As expected with exertion of the intensities used in this study, plasma lactate rose with exercise after all dietary conditions and returned to pre-exercise values during recovery. The magnitude of the increase was 135% to 350% from PRE to DURING, with an even greater increase of 800% to 950% from PRE to POST. Individual lactate responses to the exercise and to the diets were variable, as is expected for this metabolite (Skinner and McLellan 1980).

PRE lactate values were within the normal range during each dietary condition, and showed very little variation between diets.

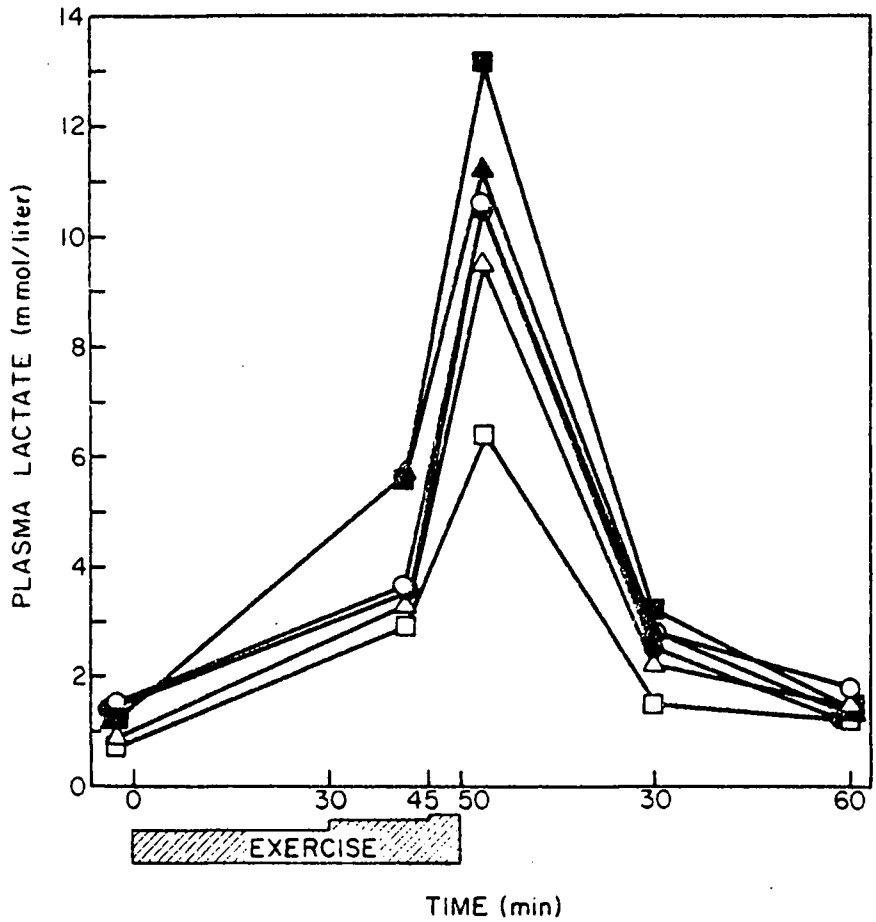

FIG. 12. Plasma lactate concentrations. Values are means of four subjects during exercise and recovery after NC-1 (○—○), NC-2 (●—●), LC (△—△), HC (▲—▲), LC+B6 (□—□), or HC+B6 (■—■) diets. Refer to the text for an explanation of the diets.

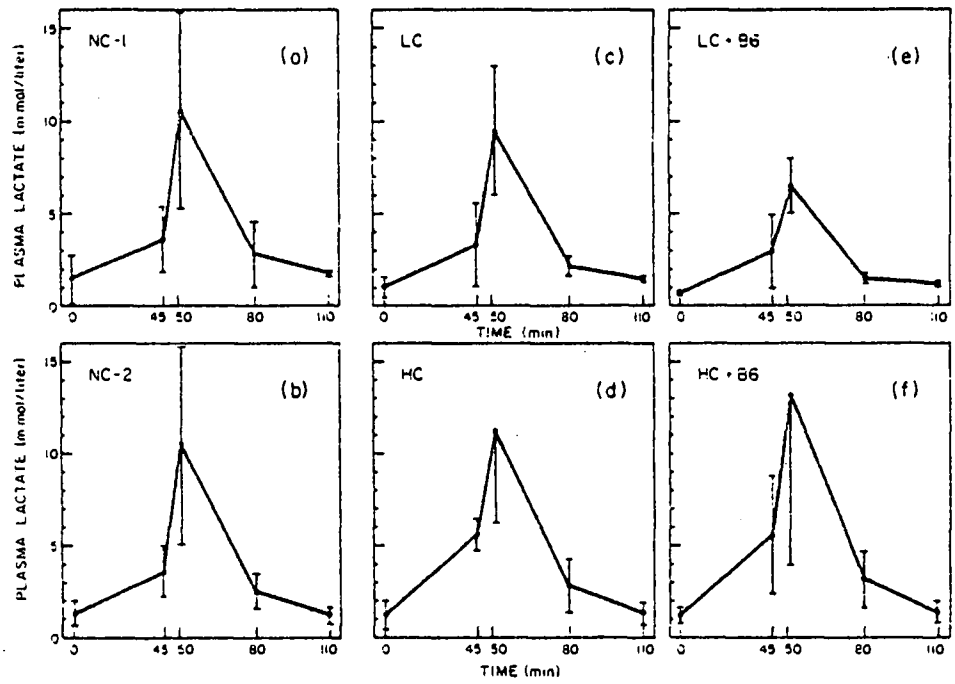

FIG. 13. Sequential plasma lactate concentrations. Values are means of four subjects during 50 min exercise and 60 min recovery after (a) NC-1, (b) NC-2, (c) LC, (d) HC, (e) LC+B6, or (f) HC+B6 diets. Vertical lines represent 1 standard deviation from the mean. Refer to the text for an explanation of the diets.

Table 8.
Plasma lactate concentrations during rest, exercise, and recovery after six diet regimens.

Diet	Sample	Plasma Lactate	Diet	Sample	Plasma Lactate
		mmol/l			mmol/l
NC-1	PRE	1.53±1.32*	NC-2	PRE	1.30±0.74[d,e]
	DURING	3.59±1.89[a,b]		DURING	3.59±1.44[d,e]
	POST	10.60±5.33		POST	10.52±5.51
	30 MIN POST	2.83±1.93		30 MIN POST	2.55±1.01
	60 MIN POST	1.79±0.12[c]		60 MIN POST	1.23±0.54
LC	PRE	0.98±0.52	HC	PRE	1.25±0.80
	DURING	3.28±2.32[f]		DURING	5.66±0.92[a,e,i]
	POST	9.47±3.46[g]		POST	11.18±5.07[h]
	30 MIN POST	2.17±0.46		30 MIN POST	2.84±1.47
	60 MIN POST	1.54±0.36		60 MIN POST	1.32±0.65
LC+B6	PRE	0.72±0.17[i]	HC+B6	PRE	1.26±0.40[b,d]
	DURING	2.92±1.99[f]		DURING	5.64±3.23
	POST	6.46±1.54[g,h,j]		POST	13.16±9.18[j]
	30 MIN POST	1.47±0.32		30 MIN POST	3.21±1.46
	60 MIN POST	1.23±0.17[c]		60 MIN POST	1.45±0.59

*values are given as means ± standard deviation

[a-j]values sharing the same suprascript are significantly different ($p<0.05$).

Abbreviations are: normal carbohydrate (NC-1, NC-2); low carbohydrate (LC); high carbohydrate (HC); low carbohydrate plus vitamin B-6 (LC+B6); and high carbohydrate plus vitamin B-6 (HC+B6).

Lactate values were 57% higher DURING HC and DURING HC+B6 than for either of the control conditions ($p<0.05$, ln). There was no effect on lactate values by addition of vitamin B-6 to the HC diet as compared to the HC diet alone, nor was there a significant difference between lactate values of HC or HC+B6 and control values during the rest of the experimental procedure.

While LC conditions alone did not lead to significantly different lactate values as compared to controls, the HC or the HC+B6 diets, addition of vitamin B-6 to the LC regimen did produce a decrease in lactate concentrations. DURING HC values were 93% greater than the corresponding LC+B6 values ($p<0.02$, ln) and were again 93% higher at 30 MIN POST ($p<0.05$, ln). The POST and 30 MIN POST LC values were 47% higher than for LC+B6 ($p<0.005$, ln and $p<0.01$, ln, respectively). Finally, 30 MIN POST HC+B6 values were 118% higher than LC+B6 values ($p<0.005$, ln) and 60 MIN POST NC-1 values were 45% higher than LC+B6 values ($p<0.001$, ln).

Plasma FFA

Plasma FFA concentrations are plotted in Figs. 14 and 15, and Table 9 shows the plasma FFA values determined throughout the exercise tests. During both control tests of NC-1 and NC-2, there appears to be a sampling error in the PRE plasma values. The PRE values of NC-1 were roughly twice expected levels in two of the subjects, exceeding even maximum exercise values for that day. Similarly, the PRE NC-2 sample of a third subject was about twice expected values. While it is possible that these elevated

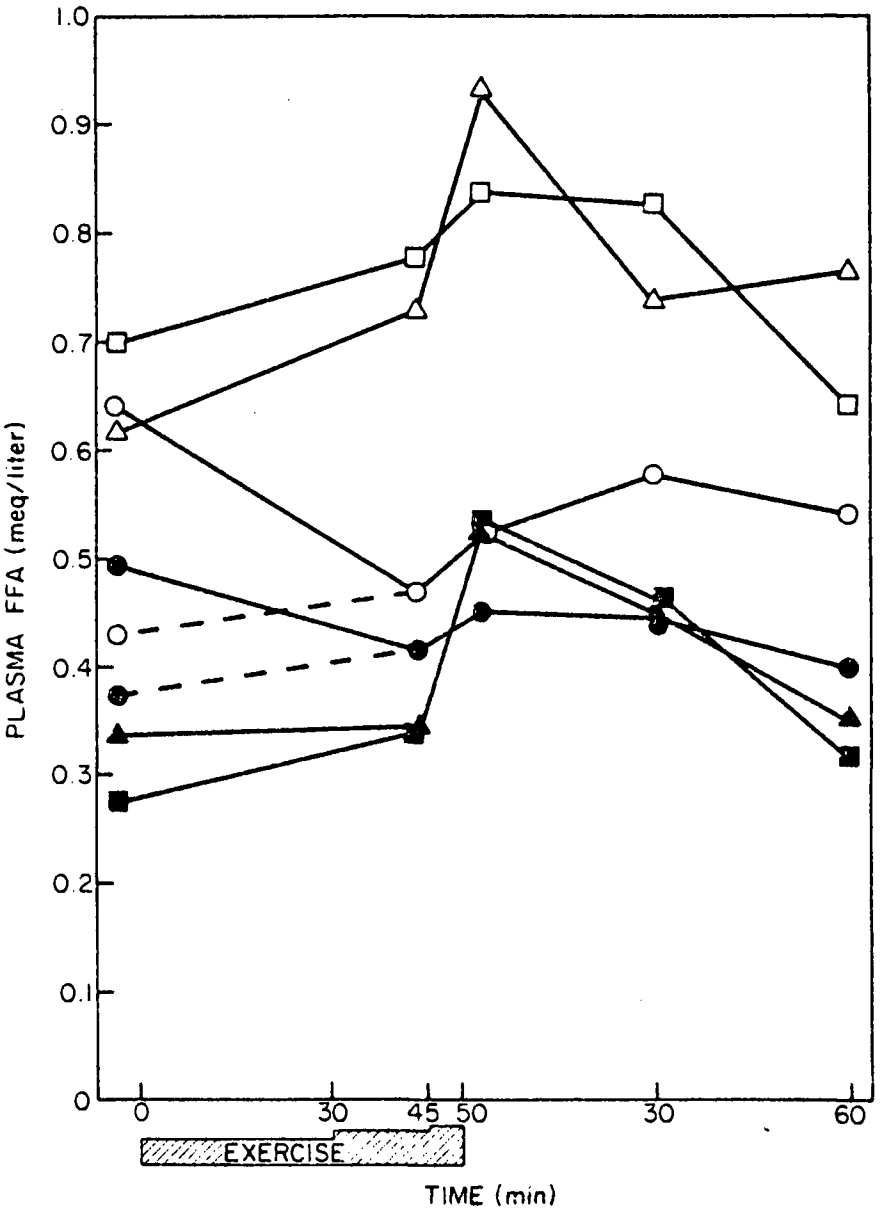

FIG. 14. Plasma FFA concentrations. Values are means of four subjects during exercise and recovery after NC-1 (○—○), NC-2 (●—●), LC (△—△), HC (▲—▲), LC+B6 (□—□), or HC+B6 (■—■) diets. Values representing data results of all four subjects are indicated with solid lines. Values obtained after exclusion of unreliable readings for subjects 1, 2 (○), and subject 3 (●) are represented by dotted lines. Refer to the text for an explanation of the diets.

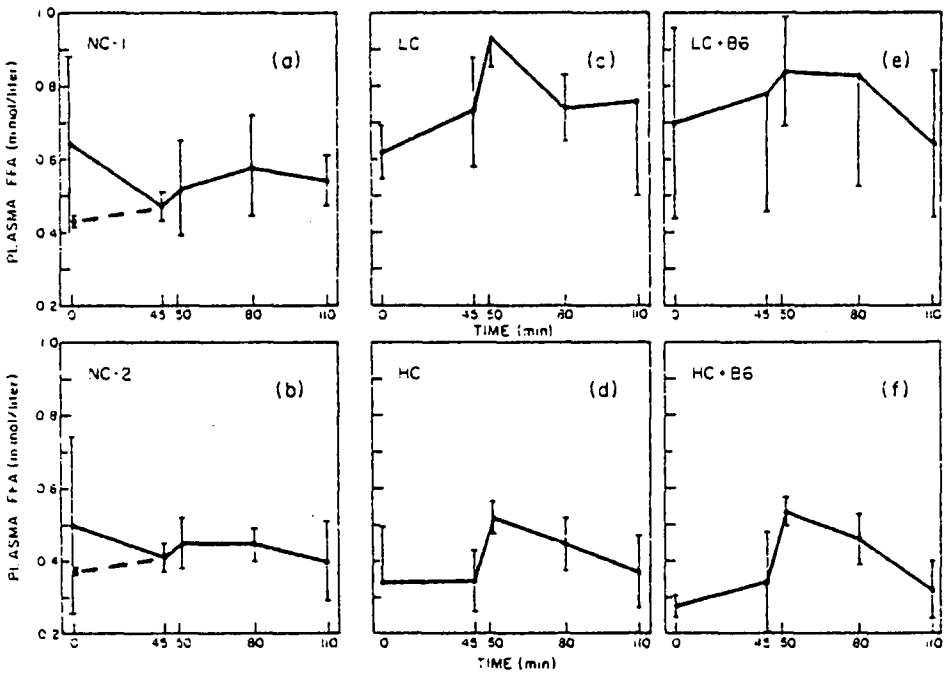

FIG. 15. Sequential plasma FFA concentrations. Values are means of four subjects during 50 min exercise and 60 min recovery after (a) NC-1, (b) NC-2, (c) LC, (d) HC, (e) LC+B6, or (f) HC+B6 diets. Vertical lines represent 1 standard deviation from the mean. Values representing data results of all four subjects are indicated with solid lines. Values obtained after exclusion of unreliable readings for subjects 1, 2 (a), and subject 3 (b) are represented by dotted lines. Refer to the text for an explanation of the diets.

Table 9.
Plasma FFA concentrations during rest, exercise, and recovery after six diet regimens.

Diet	Sample	Plasma FFA	Diet	Sample	Plasma FFA
		mEq/l			mEq/l
NC-1	PRE	0.639±0.240*	NC-2	PRE	0.494±0.246[f]
	DURING	0.468±0.038[a]		DURING	0.414±0.042[f]
	POST	0.524±0.127[b,d]		POST	0.450±0.069[g,l,t]
	30 MIN POST	0.576±0.140[c]		30 MIN POST	0.446±0.048[h,q]
	60 MIN POST	0.542±0.074[e]		60 MIN POST	0.396±0.114[r]
LC	PRE	0.616±0.076[i,k]	HC	PRE	0.336±0.157[i]
	DURING	0.727±0.150[a,f]		DURING	0.341±0.087[a]
	POST	0.932±0.080[b,g]		POST	0.525±0.046[g,l]
	30 MIN POST	0.738±0.093[c,h,j]		30 MIN POST	0.446±0.072[j,m]
	60 MIN POST	0.764±0.260		60 MIN POST	0.352±0.104
LC+B6	PRE	0.698±0.261	HC+B6	PRE	0.276±0.033[k]
	DURING	0.776±0.318[d,l,n]		DURING	0.338±0.141[n,t]
	POST	0.837±0.154[m,o,q]		POST	0.535±0.039[o]
	30 MIN POST	0.825±0.300		30 MIN POST	0.459±0.071[r]
	60 MIN POST	0.640±0.207		60 MIN POST	0.317±0.084

*values are given as means ± standard deviations

[a-t]values sharing the same suprascript are significantly different ($p<0.05$).

Abbreviations are: normal carbohydrate (NC-1, NC-2); low carbohydrate (LC); high carbohydrate (HC); low carbohydrate plus vitamin B-6 (LC+B6); and high carbohydrate plus vitamin B-6 (HC+B6).

values reflect the mobilization of FFA during the walk from the subjects' homes to the exercise site, glucose and lactate data did not support the theory that the subjects had just completed an exercise session. Such a pronounced effect on plasma FFA levels would be expected to influence these other metabolites as well.

The most probable explanation for these discrepancies is that an error occurred during assay. There may have been contamination of the sample, but more likely an aliquot was sampled twice for one determination. In Figs. 14 and 15, two values have been graphed for each of these PRE determinations. In one of these the erroneous value has been deleted from calculation of the mean. Neither suspect PRE value has been included in the statistical analysis.

PRE exercise FFA values were within the range of normal except for the HC and HC+B6 conditions, when the values were slightly low. This is consistent with a greater dependence on carbohydrate fuels in a state of high glycogen storage, and resembles the values reported by Maughan et al. (1978) after a similar carbohydrate loading.

There was an increase in plasma FFA concentration with exercise in all of the experimental conditions. LC and HC had similar increases in PRE to POST values of 51% and 53%, respectively. However, with the vitamin B-6 supplementation, LC+B6 and HC+B6 values increased PRE to POST by 20% and 94%, respectively.

At no time was there a significant difference between the

NC-1 and the NC-2 plasma FFA values. Also, there was no significant difference between plasma values for the LC and LC+B6 conditions, nor between values for the HC and HC+B6 conditions.

There was a substantial difference between the LC and HC values, and between the LC+B6 and HC+B6 values. LC FFA values were higher than the corresponding HC values: 80% higher than PRE HC ($p<0.02$, ln); more than twice as high as DURING HC ($p<0.05$, ln); 80% higher than POST HC ($p<0.01$, ln); and 65% higher than 30 MIN POST HC values ($p<0.02$, ln).

Similarly, POST LC+B6 FFA values were 55% higher than POST HC+B6 values ($p<0.02$, ln) and 80% higher than 30 MIN POST HC+B6 values ($p<0.05$, ln).

The LC FFA values were significantly elevated compared to the control values. DURING LC values were 55% higher than NC-1 values ($p<0.05$) and 75% above NC-2 values ($p<0.01$, ln), the POST LC values were 78% above NC-1 values and 107% above NC-2 values ($p<0.005$, ln), and the 30 MIN POST LC values were 28% above NC-1 values ($p<0.05$) and 65% above NC-2 values ($p<0.01$, ln).

FFA levels for LC+B6 were also elevated throughout the experimental procedure compared to the other dietary conditions, but reached significance only for the POST samples. POST LC+B6 values were 60% higher than the NC-1 values, 86% higher than the POST NC-2 values, and 85% higher than the 30 MIN POST NC-2 values ($p<0.05$, ln).

Plasma Volume Changes

The mean plasma volume changes during the exercise tests are listed in Table 10. The PRE hematocrit value was used as a base for calculating changes. The greatest changes in plasma volume values were seen immediately POST exercise, ranging from -9% to -11%. Values at 60 MIN POST ranged from -3½% to +9%, with the mean values for 5 of the six diet conditions showing there was a net hemodilution (i.e., a positive change).

TABLE 10
Mean percent change in plasma volume for all exercise tests by method of van Beaumont (1972).

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

All values are reported as mean ± 1SD.

V. DISCUSSION

It was found under well controlled dietary conditions that a carbohydrate depletion-repletion regimen resulted in clear alterations in fuel utilization during standardized exercise tests. After the carbohydrate depletion phase of the study, plasma FFA's contributed a significantly greater proportion of the energy for exercise than after carbohydrate repletion. Plasma glucose and lactate values support this conclusion, with the relative contribution of carbohydrate fuels being greater after the carbohydrate repletion regimen than after carbohydrate depletion. Supplemental vitamin B-6 added to the diet resulted in further alterations in carbohydrate utilization, indicating that glycogen may have been more rapidly depleted in the presence of excessive vitamin B-6.

In the study of plasma concentrations of fuel metabolites during and after muscular exertion, it is important to recall that there are a myriad of parameters changing concomitantly with exercise. One such parameter is the plasma volume (PV) change in which fluid is lost from the plasma compartment during exercise and returns to normal values during recovery from exercise (Beaumont et al. 1972). Pre to post decreases of PV ranged from 9 to 11% while FFA concentrations increased by a magnitude of up to 95%, glucose concentrations up to 24%, and lactate concentrations up to 950%. Clearly hemoconcentration could not account for these increases observed in fuel metabolites.

The dietary modifications imposed on the subjects altered the

interrelationship of fat and carbohydrate metabolism during exercise. While we did not directly measure muscle glycogen, it appears that there was an inverse relationship between the muscle glycogen content and the plasma FFA concentration. As in the carbohydrate depletion-repletion studies of Janssen (1980) and Maughan et al. (1978), plasma FFA concentrations were higher following the low CHO diets than after the high CHO diets or the control diets. The rate of lipolysis, as reflected in the greater plasma FFA concentration, appears to have been accelerated following the period on the low CHO diets. Muscle glycogen levels would be expected to be quite reduced at this time, as previously reported (Bergstrom et al. 1967, Karlson and Saltin 1971). It is known that under normal conditions the rate of FFA utilization by working skeletal muscle is proportional to the plasma FFA concentration (Hagenfeldt and Wahren 1975). In this case, when a low CHO diet has reduced the quantity of muscle glycogen, the higher plasma FFA concentration increased the capacity of skeletal muscle for fat oxidation. Following the high CHO diets, when there should be an abundant supply of muscle glycogen, FFA values were depressed.

Metabolism of carbohydrate fuel is partially reflected by plasma lactate concentrations. As was seen by Janssen (1980) and by Maughan et al. (1978), lactate values in the present study were significantly higher during exercise on the high CHO regimens than with the control diets. The trend, although not significant, was for lactate values to be higher throughout the exercise procedure when subjects consumed the high CHO diets. This, together

with the FFA results, emphasizes that a greater portion of the energy of metabolism was supplied by carbohydrate metabolism after the high CHO diets than after the normal or low CHO diets.

Conversely, the lowest lactate values were obtained after the low CHO diets, when FFA values were at their highest. This was significant after the LC+B6 diet, but not after the LC diet, implying that vitamin B-6 was having an effect on carbohydrate metabolism.

Along with values of the other metabolites, plasma glucose can provide evidence of a dietary effect. Plasma glucose values were higher on control diets both before and during exercise than they were on either the low CHO or high CHO diets. After exercise the high CHO and low CHO diets' glucose values were greater than the control diet values, but this was significant only when the high CHO diet was supplemented with vitamin B-6. Again, this indicates that vitamin B-6 was having an effect on energy metabolism. Maughan et al. (1978) found no difference in resting plasma glucose values between high CHO and control diets. They also found glucose values during exercise at 50% $\dot{V}O_2max$ to be slightly higher after the high CHO than after the low CHO diets. In that study, however, subjects selected their own diets from written instructions which explained sources of dietary carbohydrates. Total calorie intake was not controlled, and may have introduced a bias to the results. In the present study, significantly elevated lactate values during exercise with the HC and HC+B6 diets indicated that carbohydrate was indeed making a major contribution to the energy metabolism of the exercising skeletal

muscle at the time that decreased plasma glucose values were observed. Glucose for lactate formation may have been derived directly from muscle glycogen stores rather than from hepatic glucose production. Since glucose released from muscle glycogen would be phosphorylated, it can only be used locally within the muscle cell, and will not contribute to general blood glucose homeostasis (Wahren 1979).

Plasma glucose values were lower before and during exercise after the LC+B6 diet, as expected in a condition of glycogen depletion. However, low values were not obtained after the LC diet, primarily due to the elevated values of Subject 1 on the LC test day. Subject 1 experienced nausea and discomfort during the exercise test, symptoms which can be indicative of hypoglycemia. However, blood analysis indicated that his plasma glucose was actually elevated at the time of his discomfort.

Plasma insulin data for this study, analyzed by another investigator, indicate that Subject 1 had pre-exercise insulin concentrations which were dramatically lower than those of any other 3 subjects on the LC test day (15 μU/ml versus 25.0 ± 2.0 μU/ml). This pre-exercise LC insulin concentration was also notably lower than Subject 1's values while consuming any other diet (15 μU/ml versus 24.4 ± 3.0 μU/ml). Although Subject 1's plasma glucose values were slightly elevated, these very low insulin levels indicate that the plasma glucose was not as readily available for skeletal muscle metabolism. As insulin inhibits hormone sensitive lipase (Havel 1974), markedly reduced insulin values would be expected to release inhibition of

lipolysis. Indeed, Subject 1 had the highest FFA values on the LC test day, indicating that fat was probably his major fuel at this time. The cause of his very low insulin values or his nausea are undertermined at this time.

In all subjects, insulin values were higher after exercise with the high CHO diets (26.4 ± 4.5 μU/ml) than they were with control diets (19.4 ± 7.0 μU/ml). These data are in line with the more radical drop seen in plasma glucose values after exercise with the high CHO diets.

Another index of carbohydrate utilization is the level of lactic acid. Plasma lactate concentrations during exercise were significantly higher after the high CHO diets than after the control diets, and continued to show this trend throughout the exercise protocol. Maughan et al. (1978) reported similar increases in lactate after high versus low carbohydrate diets. However, as the exercise was of only 50% VO_2max in that study, there was not a strong lactate response and these differences did not reach the level of significance seen in our study. The elevated lactate values after the high CHO diets may be directly related to the decreased FFA values. It is known that lactate is a potent inhibitor of lipolysis in adipose tissue (Skinner & McLellan 1980, Issekutz et al. 1965). Issekutz et al. (1965) found a lactate concentration of 5 mmol/l effective in inhibiting lipolysis in dogs, and Skinner and McLellan (1980) reported that 4 mmol/l was effective in humans. In the present study, lactate values of 5.6 mmol/l recorded during exercise after the high CHO diets should therefore have been more than adequate to inhibit FFA release from

adipose tissue. The surge of FFA observed post exercise in all dietary regimens could occur despite the expected flux of lactate from muscle tissue to plasma, due to release of the norepinephrine induced vaso-constriction of the subcutaneous capillary bed (Hagenfeldt and Wahren 1975). This would permit the release of FFA's which were trapped by decreased blood perfusion of the adipose tissue.

On the other hand, with the low CHO regimens, decreased plasma glucose values seen during exercise occurred in conjunction with lactate values which were not elevated over control values. These simultaneously low glucose and lactate values indicated that CHO was not as major a fuel source as it was with the high CHO diets. High plasma FFA values seen at this time support the conclusion that both muscle and liver glycose production were decreased and that FFA's supplied the major portion of the oxidative energy of metabolism.

The alterations in fuel metabolism which occur after dietary modifications of carbohydrates appear to be a result of both changes in FFA availability and in the glycogen content of the muscle. The addition of supplemental vitamin B-6 to the low and high CHO diets did not seem to further affect the mobilization of FFA. While the FFA values during the low CHO diets were markedly elevated as compared to control and high CHO conditions, no trends in FFA levels were evident to suggest that the two low CHO conditions differed from each other with addition of vitamin B-6. The FFA values during the two high CHO conditions were even more

strikingly similar to one another. That supplemental vitamin B-6 did not change FFA concentration is not particularly surprising, as the vitamin is not directly involved in lipid metabolism. Any effect on FFA mobilization would have to occur secondarily to the effect exerted on carbohydrate metabolism.

If supplemental vitamin B-6 had produced an increase in muscle glycogen phosphorylase in these men as has been seen in rats (Black et al. 1978), this enlarged pool of the rate controlling enzyme of muscle glycogenolysis could result in a more rapid breakdown of muscle glycogen. As evidenced by the change in levels of the metabolites of carbohydrate utilization, addition of supplemental vitamin B-6 to the low CHO diet did alter carbohydrate metabolism. Lactate values tended to be lower throughout the exercise protocol with the LC+B6 diet than with the LC diet or the control diet. A further effect of supplemental B-6 was evident in that glucose values for the LC+B6 diet were lower than for other dietary conditions. This difference was particularly significant in comparison to the control diets.

If vitamin B-6 stored in muscle glycogen phosphorylase increased the rate of breakdown of muscle glycogen, it would be expected that glycogen depletion would occur more rapidly with vitamin B-6 supplementation. In this study design, the subjects were to exercise hard on their own while receiving the low CHO diets for 3 days, in order to arrive for the exercise test in a state of glycogen depletion. Lower plasma glucose and lactate values indicated that subjects were in a more glycogen depleted

state after the LC+B6 diet than they were after the LC diet. Therefore, less glucose would have been released from the liver, and less lactate would have been formed in the muscle from carbohydrate metabolism.

Further, evaluation of exercise diaries kept by the subjects while depleting for the exercise test did not provide support for the theory that more complete glycogen depletion occurred as a result of more personal exercise during the LC+B6 days than during the LC days. Indeed, Subject 3 actually exercised more during the LC days than during the LC+B6 days, yet displayed the most marked decrease in lactate values with vitamin B-6 supplementation.

As mentioned before, an increased level of muscle glycogen phosphorylase may cause a direct reduction in muscle glycogen stores via a more rapid rate of muscle glycogenolysis. Hepatic glycogen depletion would be expected to occur secondarily, as blood borne glucose of hepatic origin would be used both for energy and to replenish the muscular glycogen depots. The major portion of hepatic glucose output comes from glycogenolysis during short term exercise, especially during higher intensities (Wahren et al. 1975). Therefore, the significantly lowered plasma glucose and lactate values during LC+B6 diets would indicate that less hepatic glycogen was available for mobilization during the exercise test.

In summary, addition of supplemental vitamin B-6 to a glycogen depletion regimen did appear to cause less utilization of carbohydrate fuel sources during exercise than did the glycogen

depletion regimen without supplemental vitamin B-6. It is postulated that this results from a more thorough depletion of muscle glycogen stores before the exercise test day due to an increased level of glycogen phosphorylase.

When vitamin B-6 was added to the high carbohydrate diet during the glycogen supercompensation regimen, no significant change was detected between the HC and the HC+B6 plasma values for glucose, lactate, or FFA. The mean values immediately after the 90% MHR exercise were slightly lower after the HC diet than after the HC+B6 diet for both glucose and lactate, indicating a greater utilization of carbohydrate fuels with vitamin B-6 supplementation. Although the mean plasma lactate values were elevated, only two of the subjects showed such an increase immediately post exercise with vitamin B-6 supplementation. The values of the other two subjects either stayed the same or decreased at that point.

The difference in the pattern of lactate response among the 4 subjects is also of interest. Subject 1 had very low lactate values and showed little variation in lactate response after the different dietary regimens. He also had lower glucose and insulin values than did the other subjects. Subject 1's carbohydrate metabolites very often would fluctuate slightly in one direction while the other 3 subjects showed a strong trend in the opposite direction. Subject 3, on the other hand, was at the other extreme for the group. He had quite high lactate values and displayed the most marked effects of changing the carbohydrate content of the

diets. His glucose and insulin values were the highest of the group. Subject 3's fuel patterns displayed a strong effect with the addition of vitamin B-6.

There are several explanations that could account for the patterns of these subjects. Subject 1 may simply have been more aerobically trained than Subject 3, and hence would have had a decreased lactate response to exercise at submaximal workloads (Skinner and McLellan, 1980). All subjects were recruited from a cycling class to help prevent such a bias. Personal exercise recorded in daily exercise diaries by the subjects during the study weeks indicated that while Subject 1 reported doing somewhat more voluntary exercise, differences were not great enough to account for such wide variations in carbohydrate metabolism.

Another possible explanation is that Subject 1 may have a greater proportion of Type I muscle fibers than does Subject 3. Type I fibers (slow twitch, oxidative), with their predominance of the H-LDH isozyme and plentiful mitochondria, would favor aerobic metabolism and have a low rate of lactate production (Essen 1977). If Subject 3 had primarily Type II fibers (fast twitch, glycolytic) with mainly the M-LDH isozyme and the enzymes for anaerobic metabolism, a rapid formation of lactate from pyruvate would be expected in this individual (Skinner and McLellan 1980).

If vitamin B-6 is indeed stored by increasing muscle glycogen phosphorylase, it would be expected that supplemental vitamin B-6 would have the most pronounced effect on the

carbohydrate metabolism of individuals who had the greatest dependence on glycogen stores for the production of energy. Conversely, individuals with primarily oxidative muscle fiber types and a reliance on FFA metabolism would show less response to alterations in the glycolytic pathway. Such may be the difference between Subject 1 and Subject 3. In the absence of more direct measures, such as needle biopsy of muscle tissue for analysis of fiber type and glycogen content, this explanation can only be conjectural.

In a study by Lawrence et al. (1975), teenaged swimmers were found to have elevated post-exercise lactate levels after a diet supplemented with vitamin B-6 for six months. Their subjects ate self selected diets. Although data is not available concerning their dietary habits, it is probably safe to assume that these teenaged athletes consumed an abundance of carbohydrates and were exercising with adequate glycogen stores.

In the present study, the HC+B6 regimen would most closely approximate the condition of the vitamin supplemented swimmers in Lawrence's study. As mentioned previously, the group mean lactate value was highest at the post exercise HC+B6 sample. Although not statistically significant, these results are similar to the findings of Lawrence et al. Lawrence and co-workers were studying the effects of vitamin B-6 supplementation on swimming endurance and had not expected to discover elevated lactate values. An increase of glycogen phosphorylase in the supplemented group of swimmers would explain this increased lactate production.

While the subjects of this present cycling study received 8 mg of supplemental vitamin B-6/day, the swimmers received 51 mg/day. Also, the cyclists were supplemented for one week compared to 6 months for the swimmers, providing less time for glycogen phosphorylase to accumulate. Finally, the swimmers were exercised by 1,000 meter test swims in which they were competing for the best times. Such a competitive maximal exertion would be a more anaerobic test than was our controlled exercise protocol, and one would therefore expect a more pronounced lactate response after the race. Perhaps the elevation of the lactate values seen post exercise in the supplemented cyclists would also have reached the level of statistical significance if vitamin B-6 had been supplemented at a higher level or for a longer period of time, or if the exercise had presented a greater anaerobic stress on the subjects.

Lactate is only produced by anaerobic carbohydrate metabolism and thus gives some indication that glycolysis is occurring. However, this can only indirectly implicate muscle glycogen breakdown. Since heavy rhythmic exercise such as cycling has been shown to be limited by the emptying of muscle glycogen stores (Bergstrom et al. 1967), controlled exercise to exhaustion could give a valid comparison of rates of glycogen utilization. A study measuring the length of time for which a given workload could be performed under vitamin B-6 supplemented and non-supplemented conditions, in conjunction with plasma metabolite data, would be valuable in evaluating the role of glycogen phosphorylase as it

relates to vitamin B-6 storage and exercise.

Vitamin supplementation is very popular among some segments of the American population. Among athletes in particular there is a keen interest in any dietary modifications which could provide the all important competitive edge. In the case of vitamin B-6, however, it is well to note that there may be an alteration in fuel metabolism which actually results in a competitive disadvantage. There was an indication in this study that cyclists performing with vitamin B-6 supplementation of 8 mg/day depleted their glycogen stores more rapidly than those not receiving supplementation. A more rapid emptying of glycogen stores would probably result in a reduced endurance performance, since some glycogen is always necessary in the fuel mix to perform heavy exercise. If a change is apparent in this study on a dosage of 8 mg vitamin B-6, as was indicated in the low carbohydrate regimen, commonly available supplements of 50 mg vitamin B-6 or more may cause even more pronounced alterations.

While the results of the present study do not yield conclusive proof of an increase in glycogen phosphorylase activity, at this time pharmacological supplementation of vitamin B-6 should be approached with the understanding that there may be less than desirable consequences on some parameters of athletic performance.

VI. SUMMARY AND CONCLUSIONS

The purpose of this thesis was to test if there is a relationship between supplemental vitamin B-6 and the fuel metabolism of exercise. The objectives were to:

1. observe the fuel metabolism of healthy male athletes performing high intensity endurance exercise after consuming a normal mixed diet with adequate vitamin B-6.

2. compare the fuel metabolism of these same athletes during a glycogen depletion-repletion regimen with adequate vitamin B-6.

3. evaluate any changes in fuel metabolism of exercise when supplemental vitamin B-6 is added to a glycogen depletion-repletion regime.

Four young men trained in bicycle exercise were recruited for the study, which consisted of three non-consecutive study weeks separated by non-experimental periods. Subjects consumed meals which were calculated to provide: a normal mixed diet with adequate vitamin B-6 to serve as a control study week, a glycogen depletion-repletion regimen with adequate vitamin B-6 during the second study week, and a glycogen depletion-repletion regimen plus supplemental vitamin B-6 during the final study week.

In addition to the dietary manipulations, subjects were requested to exercise heavily on the first three days of each study week in order to facilitate glycogen depletion, and to refrain from exercise during the last three days to facilitate glycogen repletion.

A standardized exercise test was performed on the 4th and

7th days of each study week. Each subject performed a 50 min graded exercise test on a bicycle ergometer at workloads adjusted to achieve 60%, 80%, and 90% of his calculated age-adjusted maximal heart rate. They were monitored by an ECG throughout the exercise and blood was sampled 5 times: prior to exercise (PRE), during 80% maximal heart rate exercise (DURING), immediately after exercise (POST), 30 min after exercise (30 MIN POST), and 60 min after exercise (60 MIN POST). Blood analysis included plasma FFA, glucose, lactate, hemoglobin and hematocrit levels for all samples collected.

The data collected were statistically evaluated using paired t-tests to determine if there were significant differences between the six test days. Group means of plasma FFA, glucose, and lactate values were compared at each sampling point during exercise and recovery.

Plasma FFA values increased with exercise and gradually returned to near PRE levels by 60 MIN POST. Concentrations throughout the exercise were higher during the two glycogen depletion regimens than during the control diet days or the glycogen-repletion regimens. The glycogen repletion FFA values tended to be lower than values of the control regimen, but for the most part this difference was not significant. There were no significant differences in plasma FFA values between the two glycogen depletion-repletion weeks with addition of supplemental vitamin B-6.

Concentrations of plasma glucose generally increased during

exercise and then fell after exercise. With the glycogen repletion regimens plasma glucose concentrations were slightly lower DURING exercise than control values. While both glycogen repletion regimens had higher mean POST plasma glucose values than POST control values, this was statistically significant ($p<0.05$, ln) only for the vitamin B-6 supplemented glycogen-repletion condition. Glycogen depletion plasma glucose values without vitamin B-6 supplementation did not differ from the control values. However, the glycogen depletion regimen with vitamin B-6 supplementation had glucose concentrations which were significantly lower than control values both PRE ($p<0.02$) and DURING ($p<0.005$, ln) exercise.

Plasma lactate values rose sharply with exercise and dropped rapidly during recovery. Values were 57% higher during exercise after the glycogen repletion regimens than after control or glycogen depletion regimens ($p<0.05$, ln). Plasma lactate control values did not differ from the values after glycogen depletion alone, but glycogen depletion with vitamin B-6 supplementation resulted in the lowest lactate values seen in the study.

It is concluded from the increased plasma glucose and lactate values with the HC+B6 diets and the decreased plasma glucose and lactate values with the LC+B6 diets that addition of vitamin B-6 to a glycogen depletion-repletion regimen does alter fuel utilization during exercise. This may be mediated by the storage of vitamin B-6 as muscle glycogen phosphorylase. It appears that glycogenolysis may occur more rapidly after vitamin B-6 supple-

mentation due to a greater concentration of the rate limiting enzyme of glycogenolysis.

Since a more rapid breakdown of muscle glycogen for exercise metabolism would lead to an earlier depletion of muscle glycogen stores, excessive amounts of vitamin B-6 supplementation could lead to reduced athletic endurance. Further research is warranted in this area, and it would be especially revealing to incorporate controlled exercise to exhaustion as a measure of endurance capacity.

Technological advances have made it possible to achieve high intakes of nutrients, such as vitamin B-6, which greatly surpass the human body's evolutionary expectations. It behooves the responsible individual to respect and attempt to understand the consequences of radically manipulating the somatic mileu.

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APPENDIX

Consent Form

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

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

All information concerning me will be kept confidential.

Name _____ Oate _____

Witness _____ Oate _____

Form I

VITAMIN B_6

NAME _____ TELEPHONE NO. _____

LOCAL ADDRESS _____

DATE OF BIRTH _____ HEIGHT _____ WEIGHT _____

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

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

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

Describe briefly your daily physical activities.

Do you smoke? _____ How long? _____

What is your class and work schedule?

Thank You.

INSTRUCTIONS FOR RECORDING FOOD

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

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

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

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

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

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

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

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

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

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

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

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

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

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

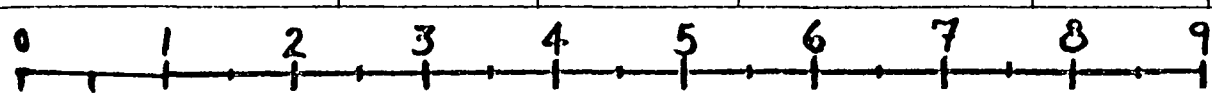
FOOD specify each food or beverage on a separate line	SOURCE canned, dried fresh etc.	BRAND be specific	PREPARATION fried, baked, raw etc.	AMOUNT measure in cups inches etc.	FOR OFFICE USE AMT. code	FOR OFFICE USE WT. code
Egg	Chicken	—	scrambled	1 med.		
Orange juice	frozen	Flavorpac	diluted with water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

DIET RECORD SHEET

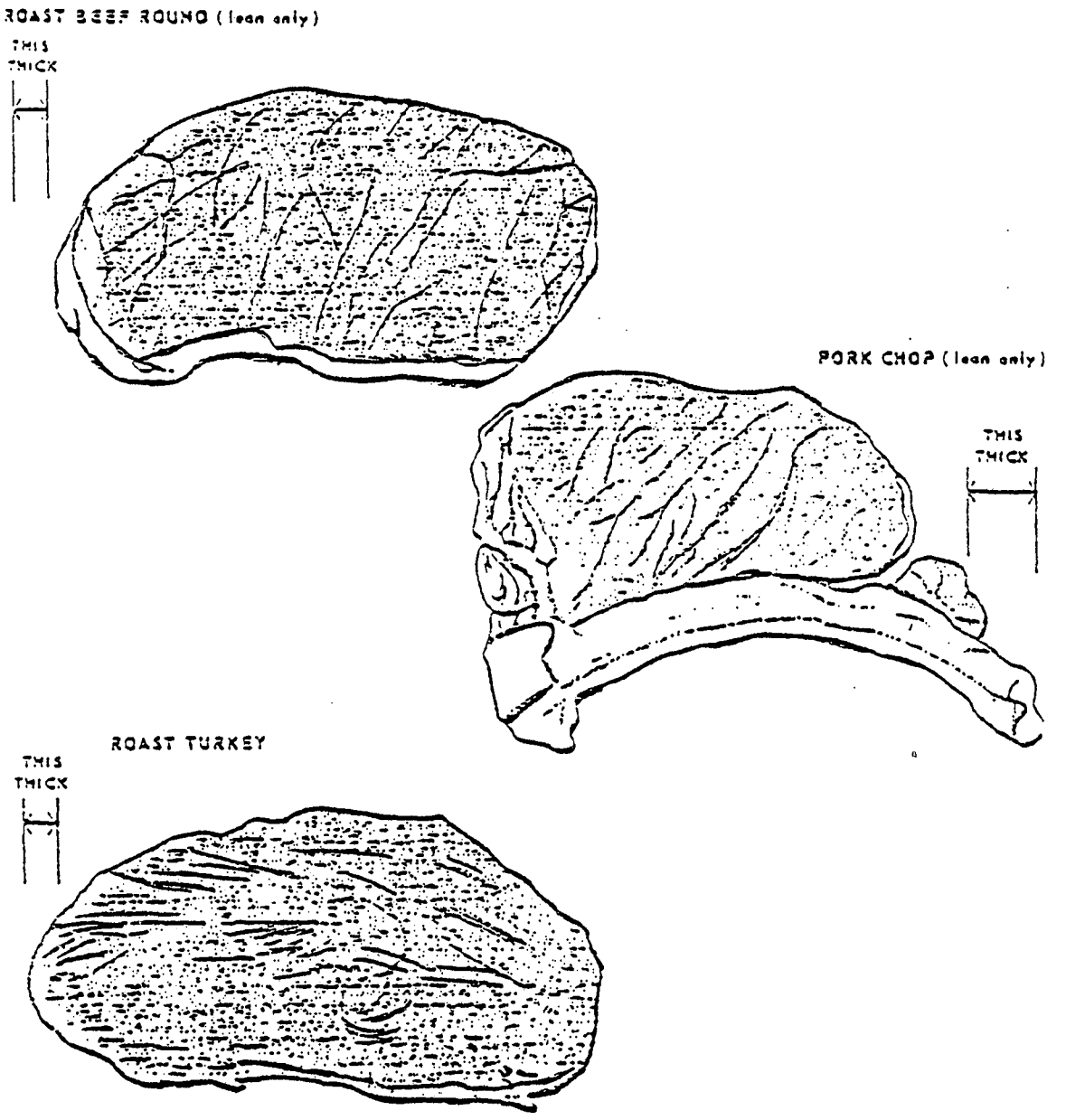
CODE NO. _____ DATE CONSUMED: _____

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

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

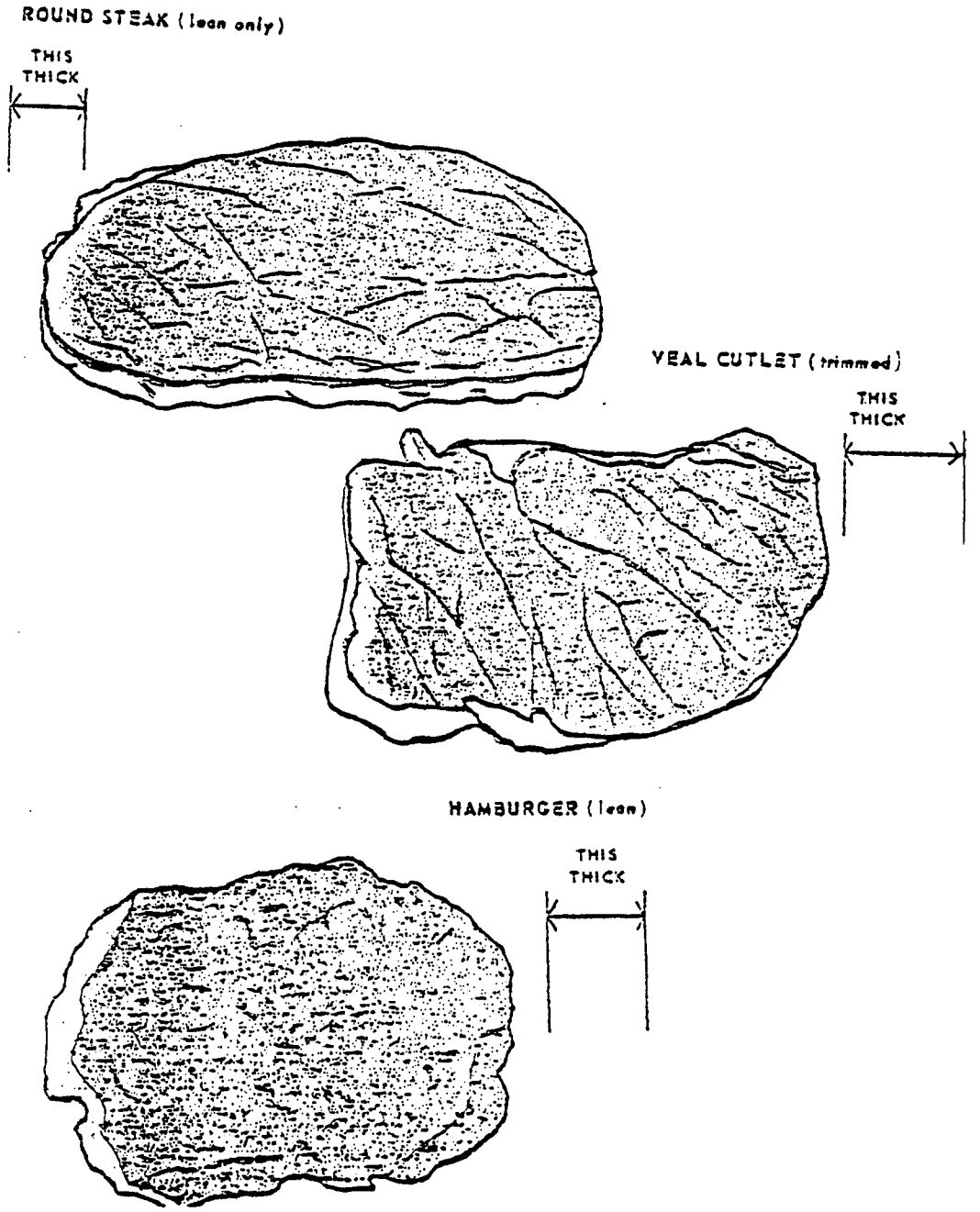


It takes 2 piece of cooked meat without bone of the size pictured to equal 3 ounces.



Reduced 74% for Reproduction

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



Reduced 74% for Reproduction

Name _____

Date _____

DAILY ACTIVITY SHEET

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

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

2. Record all "free" foods

Decaffinated Coffee (cups) _____

Herbal Tea (cups) _____

Sugar free beverages (cups) _____

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

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

5. Unusual events - exams! and other.

6. Other comments.

Appendix Table 1
Individual blood chemistry screen results

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

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

† Methods were different for values in parentheses.

Appendix Table 2.
Individual plasma FFA values for all exercise tests.

Subject	Diet	Plasma FFA mEq/l					Subject	Diet	Plasma FFA mEq/l				
		PRE	DURING	POST	30 MIN POST	60 MIN POST			PRE	DURING	POST	30 MIN POST	60 MIN POST
1	NC-1	0.883	0.508	0.679	0.723	0.528	1	NC-2	0.324	0.422	0.492	0.411	0.332
2		0.812	0.492	0.568	0.662	0.546	2		0.432	0.363	0.404	0.407	0.268
3		0.419	0.432	0.391	0.492	0.637	3		0.857	0.463	0.379	0.512	0.497
4		0.440	0.438	0.457	0.426	0.457	4		0.364	0.409	0.526	0.452	0.486
1	LC	0.665	0.944	1.028	0.838	1.148	1	HC	0.201	0.261	0.463	0.348	0.237
2		0.545	0.646	0.919	0.744	0.630	2		0.240	0.269	0.532	0.506	0.299
3		0.696	0.708	0.947	0.755	0.693	3		0.552	0.424	0.530	0.436	0.399
4		0.556	0.611	0.833	0.614	0.586	4		0.350	0.408	0.575	0.492	0.472
1	LC+B6	0.569	0.621	0.851	0.666	0.569	1	HC+B6	0.296	0.330	0.557	0.385	0.241
2		1.041	0.767	0.713	1.271	0.627	2		0.242	0.252	0.509	0.522	0.388
3		0.744	1.222	1.049	0.716	0.927	3		0.254	0.229	0.495	0.516	0.247
4		0.436	0.492	0.735	0.645	0.438	4		0.310	0.539	0.578	0.411	0.390

Appendix Table 3.
Individual plasma glucose values for all exercise tests.

Subject	Diet	Plasma Glucose mg%					Subject	Diet	Plasma Glucose mg%				
		PRE	DURING	POST	30 MIN POST	60 MIN POST			PRE	DURING	POST	30 MIN POST	60 MIN POST
1	NC-1	91.5	86.2	86.7	91.8	90.3	1	NC-2	85.7	84.5	86.2	91.0	86.7
2		90.8	88.9	93.4	97.2	89.7	2		90.5	94.2	95.0	96.6	90.0
3		89.7	95.4	97.9	94.9	94.1	3		95.1	97.3	106.6	97.1	89.2
4		94.9	105.9	113.6	99.5	85.4	4		100.5	102.7	106.7	93.1	87.5
1	LC	99.5	91.8	98.3	91.8	91.3	1	HC	84.7	80.9	100.9	85.2	85.7
2		89.2	84.9	94.5	89.2	86.8	2		93.4	91.8	100.1	93.7	86.5
3		82.6	88.1	95.7	92.7	90.5	3		86.4	95.1	105.8	88.9	88.0
4		92.3	100.5	120.2	106.9	92.5	4		90.9	99.5	112.5	87.2	85.9
1	LC+B6	70.6	76.9	98.6	88.5	85.7	1	HC+B6	86.2	81.2	95.6	84.7	86.5
2		83.3	83.3	92.1	84.4	84.4	2		79.3	89.4	97.4	90.8	89.5
3		85.6	91.1	105.8	94.1	90.5	3		89.7	93.5	119.9	105.2	88.9
4		89.9	98.4	110.4	101.1	----	4		91.7	100.5	114.1	86.4	86.2

Appendix Table 4.
Individual plasma lactate values for all exercise tests.

Subject	Diet	Plasma Lactate mg%					Subject	Diet	Plasma Lactate mg%				
		PRE	DURING	POST	30 MIN POST	60 MIN POST			PRE	DURING	POST	30 MIN POST	60 MIN POST
1	NC-1	5.1	13.8	35.7	8.4	16.8	1	NC-2	2.9	21.1	36.6	10.5	4.3
2		30.9	27.3	96.6	49.4	15.0	2		14.2	29.5	151.0	28.4	15.9
3		6.2	54.6	153.4	24.6	15.8	3		18.7	51.1	116.5	30.9	11.8
4		13.0	33.5	96.2	19.5	17.1	4		11.0	27.7	75.0	22.1	12.2
1	LC	7.1	23.5	63.5	17.3	12.1	1	HC	10.1	49.6	46.9	22.7	3.6
2		15.9	14.1	67.1	24.4	18.4	2		20.4	57.5	135.6	44.5	17.3
3		6.0	60.4	130.9	21.3	13.9	3		2.9	57.1	141.5	20.5	13.3
4		6.5	20.1	79.6	15.0	11.2	4		11.8	39.8	79.0	14.7	13.6
1	LC+B6	5.8	19.2	54.0	10.3	9.9	1	HC+B6	8.9	19.1	42.3	18.7	6.0
2		8.7	12.8	51.3	14.2	10.1	2		8.3	44.1	91.7	24.6	12.2
3		5.4	52.8	78.8	16.8	11.2	3		16.3	89.3	235.9	48.2	18.0
4		6.1	20.6	48.8	11.8	13.2	4		12.1	50.7	104.4	24.2	15.9

Appendix Table 5
Individual hematocrit values for all exercise tests

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

Appendix Table 6
Individual hemoglobin values for all exercise tests

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