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The Effect of Various Feedings on Skeletal Muscle and Liver Glycogen in the Rat Following High Intensity Exercise

by
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ABSTRACT

Carbohydrate feeding has been found to improve muscle glycogen repletion following exercise. The depleted muscle provides a stimulus for repletion. It has been suggested that the simple sugars glucose and fructose give different rates of glycogen repletion. Glucose enters the bloodstream rapidly and it was though this result in rapid muscle glycogen repletion. Fructose must first be converted in to glucose in the liver and then is transported to the muscle by the blood; therefore, it was thought that a fructose feeding would result in slower but progressive muscle glycogen repletion.

Glucose, fructose, a glucose and fructose combination and regular rat chow were fed to the animals following high intensity,intermittent, treadmill running. A 10% solution of glucose, fructose or glucose and fructose was fed to the animals by gavage needle. Blood was sampled at the following intervals to monitor blood glucose concentration: immediate post-exercise, 0.5 hours post-exercise, one hour post-exercise, two hours post-exercise, three hours post-exercise and five hours post-exercise. Animals were sacrificed immediately post-exercise, one hour post-exercise, two hours post-exercise and five hours post-exercise to determine plantaris muscle and liver glycogen concentrations. Pre-exercise blood glucose and muscle glycogen samples were also taken for comparison with the post-exercise data.

The glucose-feeding resulted in the highest blood glucose values at 0.5 hours post-exercise while the fructose-feeding resulted in the lowest blood glucose values at this time. The muscle glycogen values at one hour also showed that the glucose-fed group had the highest concentrations and the fructose-fed group the lowest, but the muscle glycogen values were not significant. Blood glucose concentrations decreased after one hour in all the experimentally fed groups and the muscle glycogen concentrations rose non-significantly in these groups as well. In the chow-fed group the blood glucose concentrations continued to rise post-exercise (significantly above the other groups) resulting in higher muscle glycogen concentrations (non-significantly above the other groups). Liver glycogen rose significantly in the chow-fed group at five hours post-exercise.

The continuous ad-lib feeding resulted in increasing blood glucose and muscle glycogen concentrations. A criterion blood glucose level was reached which resulted in muscle glycogen repletion. Attaining this level appeared to be critical to the repletion process. Further investigation as what this level actually is would be very important for repletion research.

Chapter I

THE PROBLEM

1.1 INTRODUCTION

Most sporting events have rigorous training schedules which, to some extent, deplete muscle and liver of glycogen. These muscles are required for the following day's practice; therefore, they require glycogen for fuel. At present the dietary regimen needed to optimize glycogen repletion in the athlete is not known. It is known that muscle glycogen is important to the activity performed. The amount of muscle glycogen determines, in part, the rate and volume of work that can be done by the athlete (Maehlum et al, 1978; MacDougall et al, 1977; Costill, et al, 1971; Saltin and Karlsson, 1971; Hermansen, et al, 1967).

Carbohydrates are used to supply the body with energy and are the main source for the repletion of the glycogen stores (MacDougall et al, 1977; Bergstrom, et al, 1967; Bergstrom and Hultman, 1966). However, recent research on diabetic diets indicates that different carbohydrates have different effects when ingested. It was once thought that complex carbohydrates were more slowly absorbed into the bloodstream as simple sugars and therefore resulted in a tempered hyperglycemia. Conversely,

simple sugars rapidly elevate blood glucose levels and within approximately one hour were rapidly depressed following elevations of serum insulin. Consequently, the hypothesis was that complex carbohydrates would supply the muscles with higher concentrations of fuel over a longer period of time (Jenkins, 1982).

It has since been found that not all the complex carbohydrates react as previously described. In fact some complex carbohydrates give blood glucose responses like some simple sugars and vice versa. A glycemic index was then developed in which a known quantity of carbohydrate was compared to the same quantity of glucose. The sugars are plotted on a blood glucose response curve in which glucose is assigned a glycemic index of 100 (100%) while other sugars are assigned indices pertaining to that portion of the curve in which they are located after a criterion absorption time (Jenkins,1982).

The simple sugars, specifically the monosaccharides, have different indices. As previously stated, glucose has an index of 100; whereas, fructose has an index of 20. Sucrose, a disaccharide combination of glucose and fructose, has a glycemic index of 59 (Jenkins, 1982). These indices suggest that an ingested glucose load would rapidly be transported by the bloodstream to the muscle while an ingested fructose load would be slowly transported by the bloodstream to the muscle; sucrose would probably have an intermediate rate of transport. Which sugar repletes muscle glycogen at the fastest rate and to the greatest extent is yet to be determined.

The Costill et al (1983) study comparing ingested solutions of 20% glucose or fructose following exhaustive exercise in rats found that both sugars repleted the muscles to similar degrees at three hours post-exercise. Results of pilot work for the study described in this report, using 5 and 10% glucose and fructose solutions, showed that fructose gave higher amounts of muscle glycogen after five hours; however, there was not a large enough number of rats to perform a statistical analysis on these values.

The paths that these absorbed monosaccharides take to get to the muscle can be quite different which may account for some or all of the variability in repletion rates and volumes. Ingested glucose (G) can go directly to the blood and muscle to be converted to glycogen. Fructose (F) must first be converted in the liver to glucose before proceeding to the muscle. Thus the necessity of fructose (F) conversion in the liver may result in the further promotion of a slow rise and more continuous maintenance of serum glucose than glucose (G) ingestion (Costill, et al, 1983).

Additional studies have shown that not only does blood glucose fluctuate less with F ingestion, but hormonal responses also follow a similar, more stable pattern. In a study performed by Bohannon et al (1980) the insulin response was not increased with F as it was with G, glucagon was not suppressed following F ingestion as compared with G and blood glucose did not rise as sharply with F as with G. Studies of normal and impaired glucose tolerance groups gave similar findings (Crapo et al, 1980).

1.2 RATIONALE

Given the relatively high glycemic index of glucose as compared to fructose, as well as the more direct delivery of glucose to the muscle, it is possible that glucose and fructose feedings at concentrations which reflect a relatively normal feeding habit would illicit different insulin-induced responses. Studies done to date using much higher concentrations (20 to 50%) have shown that glucose feedings produced hyperglycemia which, if sampling was continued long enough, was followed by insulin-induced hypoglycemia (Bohannon et al, 1980). Conversely, fructose feedings have not been seen to induce an insulin response and more commonly result in normoglycemia (Bohannon et al, 1980; Crapo et al, 1980; Conlee et al, 1978). When glucose and fructose were given in combination a blood glucose response between that of glucose and fructose was seen. It was found that fructose gave rise to the release of insulin when some glucose was also present, but this did not occur in the absence of glucose (Curry et al, 1972; Bohannon et al, 1980). Consequently, it would seem that, if there was a prolonged normoglycemia seen following fructose ingestion, then fructose should be a superior fuel for muscle glycogen repletion.

In the present study high intensity exercise was used to deplete the glycogen stores to the maximum extent and was followed by feedings of glucose, fructose, a glucose and fructose combination or regular rat chow provided ad libitum. The concentrations of simple sugars in all feedings was selected to

be representative of levels normally found in rat chow. Measurements were designed to determine which fuel would result in the greatest and most rapid glycogen repletion.

1.3 PURPOSE

The purpose was to study the repletion process of muscle and liver glycogen following different feedings after high intensity exercise. Glucose, fructose, a glucose and fructose combination and regular rat chow were compared on the basis of the resulting muscle and liver glycogen levels.

1.3.1 Subpurpose

The subpurpose was to monitor blood glucose concentrations at 0.5, 1, 2, 3, 4 and 5 hour times following post-exercise gavage feedings of fructose, glucose and a glucose and fructose combination as well as an ad libitum feed of regular rat chow.

1.4 OVERVIEW OF DESIGN

Rats were used for the study to facilitate the control of diet and exercise preceding the experiment. As well, the requirement for biochemical determination of muscle glycogen makes the use of the animal model a more acceptable alternative to multiple muscle biopsy sampling.

The experimental exercise protocol was of a high intensity, intermittent type. Pilot results showed that the plantaris muscle, a mixed, fast-twitch muscle became quite depleted with this type of activity and was one of the fastest muscles to replete following the exercise. The plantaris is a prime ankle plantar flexor; therefore, is used in treadmill running although it may not be called upon during less forceful plantar flexion. This muscle is a large muscle in the rat and its fibre typing as a mixed, fast-twitch muscle has shown little variation between litter mates in this species. Consequently, it appeared to be an appropriate muscle to investigate.

Following the exercise the animals were fed glucose, fructose, a glucose and fructose combination or regular rat chow ad libitum. Pilot work indicated that there were variations in blood glucose and muscle glycogen after glucose and fructose feedings which extended to at least three hours post-exercise. The animals' progress in repleting their glycogen stores was therefore monitored for five hours post-exercise.

1.5 LIMITATIONS

This study was conducted on rats; therefore, extrapolations to humans can be made only in general terms.

The control group fed on rat chow ad-lib following the exercise and this feeding was not controlled for volume. As well, the rat chow varies in carbohydrate composition from batch to batch; thus precision is reduced in comparisons between

experimental and control groups.

1.6 DEFINITIONS

G	:	ingested glucose
F	:	ingested fructose
ad-lib	:	ad libitum
AMP	:	Adenosine Monophosphate
cAMP	:	cyclic Adenosine Monophosphate
ADP	:	Adenosine Diphosphate
ATP	:	Adenosine Triphosphate
G6P	:	glucose 6-phosphate
F6P	:	fructose 6-phosphate
FDP	:	fructose 1,6-diphosphate
PFK	:	phosphofructokinase
DHAP	:	dihydroxyacetone phosphate
GADP	:	glyceraldehyde 3-phosphate
GAD	:	glyceraldyhyde
UDP-glucose	:	uridine diphosphate glucose
F1P	:	fructose 1-phosphate
kpm	:	kilopond meter
rpm	:	revolutions per minute
g	:	gram
m	:	meters
FG	:	fast glycolytic
FOG	:	fast oxidative glycolytic
SO	:	slow oxidative

Chapter II

REVIEW OF LITERATURE

2.1 INTRODUCTION

The repletion of muscle glycogen involves a number of factors which are grouped and clarified in the following review of literature. A review of saccharide metabolism involves glucose, fructose, sucrose and glycogen metabolism. This is followed by some pertinent exercise specifics such as muscle fibre characteristics, muscle glycogen depletion and exercise fuel supply. The final section deals with actual muscle glycogen repletion studies. This includes preferential glycogen repletion, the effects of exercise as a stimulus, carbohydrate refeeding regimens, glucose or fructose infusion and ingestion studies and the hormonal influence of repletion.

2.2 SACCHARIDE METABOLISM

2.2.1 Introduction

Carbohydrates are broken down into simple sugars before they are absorbed into the blood. If the simple sugars, glucose and fructose, are ingested, they are absorbed into the blood from the small intestine; however, from this point onward their

metabolic pathways differ. One of the metabolic pathways that the simple sugars could take is that of glycogen formation. Both glycogen breakdown and synthesis involve a complex set of reactions that are under hormonal control.

2.2.2 Glucose Metabolism

The ingested monosaccharide, glucose, reaches the stomach intact and continues to the small intestine from which it is absorbed into blood. The large surface area of the intestine provided by the villi and microvilli permits rapid absorption of the glucose into the blood for transport to the liver (Lehninger, 1982).

Once in the liver the glucose is phosphorylated primarily by the enzyme, glucokinase (E.C.2.7.1.2), producing glucose 6-phosphate (G6P). At this point the metabolism of glucose proceeds to either glycogenesis or glycolysis.

Assuming that the substrate is required for energy producing reactions in the liver, glycolysis of the absorbed glucose would continue. The aldose, G6P is converted into the ketose, fructose 6-phosphate (F6P) by the reversible enzyme phosphoglucoisomerase. In the next step, F6P is phosphorylated to produce F 1,6-diphosphate (FDP) by one of the main regulatory enzymes in glycolysis, phosphofructokinase (PFK). PFK can be activated by AMP, ADP, fructose 1,6-diphosphate, phosphate and potassium ions as is the case when the body requires energy. This enzyme is inhibited by ATP, citrate, magnesium ions and calcium

ions when the body is in a resting state (Lehninger, 1982).

An aldolase cleaves the (FDP) giving dihydroxyacetone phosphate (DHAP) and glyceraldehyde 3-phosphate (GADP) which are interconverted by triose phosphate isomerase. The conversion to GADP is favoured as glycolysis proceeds from this molecule. GADP is oxidized and phosphorylated by the enzyme glyceraldehyde 3-phosphate dehydrogenase forming 1,3-diphosphoglycerate. ATP is generated in the next reaction as 3-phosphoglycerate is formed by 3-phosphoglycerate kinase. Within this molecule the phosphoryl group is transferred to the second carbon by phosphoglycerate 2,3-mutase. 2-Phosphoglycerate then becomes phosphoenol-pyruvate due to the enzymatic reaction of an enolase (Lehninger, 1982).

The next reaction involves the second regulatory enzyme, pyruvate kinase. This irreversible reaction is again activated when the ATP concentrations of the body are low and inhibited when the ATP concentrations are high. When the reaction is favoured, pyruvate is formed. Pyruvate can then be reduced to form lactate under anaerobic conditions by the enzyme lactate dehydrogenase. Under aerobic conditions, pyruvate is oxidized to acetyl-CoA and further oxidized in the citric acid cycle to CO_2 and H_2O (Lehninger, 1982).

When the amount of glucose absorbed by the liver is in excess of that required by the body, it is converted to glycogen for storage. From G6P there is a conversion to glucose 1-phosphate by phosphoglucomutase and then to uridine diphosphate glucose (UDP-glucose) by UDP-glucose pyrophosphorylase. Using the existing terminal branch of glycogen as a primer, the glucose

molecule becomes attached to the terminal glycogen branch and UDP is liberated. Glycogen synthase is the enzyme responsible for the extension of the glycogen. The glucose is added to the glycogen in a 1,4 linkage. Once at least seven residues have accumulated, the glycogen branching enzyme transfers glucosyl residues, to a neighbouring branch creating a 1,6 linkage, four residues away from an existing 1,6 linkage (Lehninger, 1982).

If large amounts of glucose reach the liver, but are not needed for energy at that moment they are stored as glycogen. The liver has the capacity to store large amounts of glycogen (approximately 50 g/kg in postabsorptive man (Hultman and Nilsson, 1971)) which can be mobilized for distribution via the central blood volume through reactions to be discussed in section 2.2.5 (Lehninger, 1982).

In the muscle, hexokinase (E.C.2.7.1.1) catalyzes the phosphorylation of glucose by ATP. Unlike glucokinase in the liver, hexokinase is inhibited by G6P if G6P is not being metabolized as fast as it is formed. The hexokinase appears to balance the formation of G6P with its rate of utilization. (Lehninger, 1982).

Glycolysis proceeds from G6P, in the muscle, as it does in the liver. The final products are lactic acid under anaerobic conditions and CO_2 and H_2O under aerobic conditions (Figure 1).

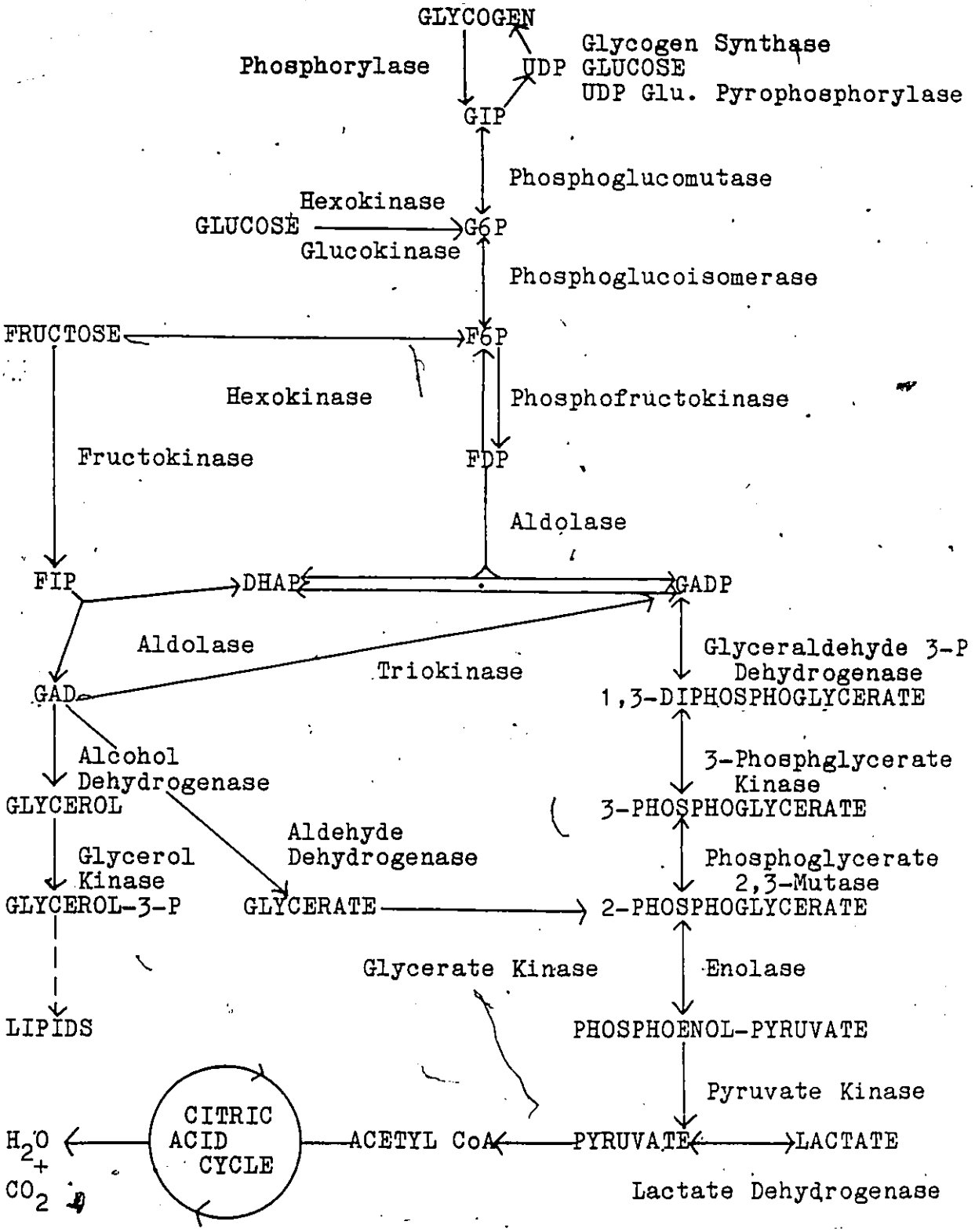

Figure 1. Glucose, fructose and glycogen metabolism (Lehninger, 1982)

2.2.3 Fructose Metabolism

Once fructose is ingested it travels down the esophagus to the stomach and then to the small intestine. Up to 10% of the fructose is metabolized within the intestinal mucosa forming glucose using the same enzymes that will be discussed in conjunction with the metabolism in the liver (Chen and Whistler, 1977).

Evidence points to active transport of the unmetabolized fructose from the small intestine into the blood (Vrana and Fabry, 1983). The fructose is slowly absorbed from the small intestine as no abrupt changes in serum fructose levels are noted following fructose meals (Chen and Whistler, 1977; Bergstrom and Hultman, 1967). Via the portal blood vessel the fructose is transported to the liver, which is the principal site of metabolism. The first step involves the phosphorylation of the first fructose carbon. Fructose plus ATP, catalyzed by the enzyme fructokinase (E.C.2.7.1.4), produces fructose 1-phosphate (F1P) and ADP. The phosphorylation of glucose is catalyzed principally by glucokinase in liver and by hexokinase in skeletal muscle. Hexokinase is also present in the liver and does phosphorylate glucose at this site. As well, hexokinase has been found to catalyze the phosphorylation of fructose, forming fructose 6-phosphate. However, the activity of fructokinase has been found to be greater than the activities of the other phosphorylating enzymes in the liver by a factor of eleven (Zakim, 1967) resulting in a higher degree of conversion of

fructose than glucose to its metabolic products (Vrana and Fabry, 1983; Periera and Jangaard, 1971). That the rate of conversion is variable under different conditions can be seen from the results of Heinz et al (1968) who reported that fructose was phosphorylated in the liver four times faster than glucose while Vestling et al (1950) reported that fructose was phosphorylated ten times faster and Pereira and Jangaard (1971) found that fructose was metabolized forming glycogen seven to fifteen times more rapidly than glucose.

The affinity of fructokinase for fructose is greater than that of the other phosphorylating enzymes'. Fructokinase has a Km for fructose of 0.5mM while hexokinase has a Km for fructose of 2 to 6mM. The presence of glucose has also been found to inhibit the phosphorylation of fructose by hexokinase (Vrana and Fabry, 1983; Heinz et al, 1972; Adelman, 1972; Pereira and Jangaard, 1971).

Following phosphorylation, the FIP is cleaved by an aldolase (E.C.4.1.2.7 ketose 1-phosphate aldolase) forming dihydroxyacetone phosphate (DHAP) and glyceraldehyde (GAD). This liver aldolase is also responsible for the cleavage of FDP forming DHAP and GADP and the reverse reaction or the formation of FDP. This aldolase is specific to the liver, differing from muscle aldolase in composition, physical, kinetic and immunochemical properties (Adelman, 1972; Heinz et al, 1972). Subsequently the DHAP can be isomerized forming GADP and continue in glycolysis to be further broken down to form pyruvate, fatty acids or CO_2 and H_2O (Chen and Whistler, 1977; Vrana and

Fabry, 1983).

GAD is further metabolized via three possible routes. The main pathway involves the phosphorylation of GAD by a specific liver triokinase (E.C.2.7.1.28) forming glyceraldehyde 3-phosphate (GADP). GADP could then continue in the glycolytic cycle. GADP and DHAP could also combine to form fructose 1,6-diphosphate by aldolase catalysis. The phosphate from the first carbon of fructose is then cleaved by the enzyme fructose 1,6-disphosphatase (E.C.3.1.3.11) producing fructose 6-phosphate (F6P). An additional isomerase produces the aldose G6P. Then glucose could be produced or anabolism could continue, producing glycogen depending on the body's requirements at the time (Chen and Whistler, 1977).

The second route from GAD involves a reduction by alcohol dehydrogenase to form glycerol. This is followed by a phosphorylation forming glycerol 3-phosphate. From here either DHAP is formed or fat metabolism continues. Glycerol takes longer to be metabolized than does GADP which is another reason why more fructose is channelled to GADP (Chen and Whistler, 1977; Vrana and Fabry, 1983).

Oxidation of GAD by aldehyde dehydrogenase (E.C. 1.2.1.2.) produces glycerate, the third pathway. This is followed by a phosphorylation, catalyzed by glycerate kinase (E.C.2.7.1.31). Glycerate 2-phosphate (or 2-phosphoglycerate) is the product of this reaction. From here glycolysis, glucogenesis or glycogenesis proceeds (Figure 1) (Chen and Whistler, 1977; Vrana and Fabry, 1983).

Although the metabolism of fructose occurs mainly via the pathway to GADP, products from glycerate and glycerol can enter the glycolytic pathway for further catabolism or the products could be rerouted towards the formation of glucose or glycogen. If the body needed energy, the glycolytic pathway would be the predominant pathway; whereas, if the body had an energy surplus, the glucogenic or glycogenic pathways would predominate. However, if fructose was the substrate given and another tissue such as the skeletal muscle was deficient of energy, glucose would be formed, transported in the blood and further metabolized by the muscles (Chen and Whistler, 1977; Vrana and Fabry, 1983).

The liver has been found to metabolize up to 85% of an orally administered fructose load (Adelman, 1972; Chen and Whistler, 1977). In experiments performed on resting humans, 30% of a labelled fructose infusion was found as glucose in the blood while only a small proportion of the fructose was found to be directly oxidized, forming CO_2 and H_2O. As well, a high concentration of F1P, resulting from a fructose infusion, inhibits glycogen breakdown promoting glycogen formation (Sestoft, 1979).

The important controlling enzymes in fructose metabolism are fructokinase, aldolase and triokinase. These enzymes are also found in the intestine permitting a small amount of fructose metabolism to occur. Fructokinase and triokinase are not found in skeletal muscle; therefore, it is unlikely that the metabolism of fructose takes place in skeletal muscle. Some suggestion that fructose could be taken up directly from the bloodstream by

skeletal muscle to be converted to glycogen was made by Bergstrom et al (1972) although the actual metabolic pathway was undefined.

Insulin levels increase following glucose ingestion, but this does not appear to be the case following fructose ingestion. Lack of insulin does not appear to affect the activity of fructokinase as it does that of glucokinase which is insulin-dependent (Zavaroni et al, 1980, Chen and Whistler, 1977, Vrana and Fabry, 1983; Macdonald et al, 1978; Curry et al, 1972). However, laboratory rats ingesting a diet containing 66% of the calories from fructose have shown increased insulin levels with increased insulin resistance (Zavaroni et al, 1980). This effect was seen after one week on the high fructose diet and has not been found with acute ingestion (Chen and Whistler, 1977, Vrana and Fabry, 1983).

2.2.4 Sucrose Metabolism

Sucrose is a disaccharide consisting of glucose and fructose. When it is ingested, it is broken down by sucrase into its constituent monosaccharides. Animal experiments have shown that there is no significant amount of transport from the small intestine to the blood of sucrose itself. This enzyme is produced by the mucosal cells of the small intestine and is localized in the brush border membrane of the mucosal epithelia. This location is ideal; the enzyme digests the sucrose and due to the proximity of the plasma membrane rapid absorption into the blood is facilitated. Some diffusion backward into the lumen does take

place. Following hydrolysis of sucrose, 10% of the glucose and 50% of the fructose have been found in the lumen. The concentration of fructose in the lumen increases when glucose and fructose are given as opposed to sucrose, due to the splitting of the sucrose at the brush border near the transport site. However, the absorption of glucose does exceed the absorption of fructose. Glucose and fructose are actively transported into the bloodstream, but there appears to be a specific carrier for the glucose that is released from sucrose during the hydrolysis. This carrier is different from the carrier for free glucose and gives the glucose released from sucrose a kinetic advantage over free glucose promoting faster absorption and metabolism. Fructose from sucrose is also transported by a different carrier than free fructose, but it is not yet known whether an advantage from this different carrier is accrued (Dehmel, et al 1969; Dalquist, 1972; Chen and Whistler, 1977; Vrana and Fabry, 1983).

Following the absorption of glucose and fructose into the bloodstream, the monosaccharides are taken up by the liver (Glu and Fru) or muscle (Glu) and continue to be metabolized as previously described.

2.2.5 Glycogen Metabolism

Glycogen is stored in skeletal muscle and in liver. The synthesis of glycogen from glucose has been discussed briefly; however, glycogen synthesis and degradation are carefully controlled by hormones and enzymes. The covalent regulation of

glycogen metabolism is based on a series of phosphorylation/dephosphorylation reactions. Phosphorylation promotes glycogenolysis while dephosphorylation promotes glycogen synthesis.

For example, if the body needed energy, adrenalin would be released. The hormone would interact with the cell membrane activating adenylate cyclase. This would cause an increase in intracellular cyclic AMP (cAMP) activating cAMP-dependent protein kinase (cAMP-PrK). cAMP-PrK phosphorylates phosphorylase kinase, causing activation and glycogen synthase, causing inhibition. The preceeding phosphorylations result in the activation of glycogen phosphorylase, again by phosphorylation, promoting glycogenolysis. This overview indicates the complexity of the regulation of glycogen metabolism (Cohen, 1978).

The hormone adrenalin interacts with β-receptors on the outer surface of the plasma membrane to initiate glycogenolysis in the muscle. Glucagon interacts with α-receptors to promote glycogenolysis in the liver, with the proceeding steps similar to those in the muscle. Insulin binds to β-receptors of both liver and muscle once activated by increased blood glucose. Insulin causes an increase in glycogen synthesis. It does this by either decreasing the activity of glycogen synthase kinase (an enzyme which phosphorylates glycogen synthase) or by increasing the activity of glycogen synthase phosphatase (an enzyme which dephosphorylates glycogen synthase) (Cohen, 1983).

Hormones initiate glycogen metabolism by activating enzymes which cause phosphorylation or dephosphorylation. Before

this can be done the hormones actually function by triggering the action of adenylate cyclase on the outer surface of the plasma membrane. This facilitates the conversion of cAMP from ATP. The increase in intracellular cAMP activates cAMP-PrK. cAMP-PrK catalyzes the phosphorylation of phosphorylase kinase, inhibitor 1 and glycogen synthase (Figure 2) (Cohen, 1978).

Phosphorylase kinase is an enzyme with four subunits ($\alpha,\beta,\gamma,\delta$). Eighty-five per cent of the molecule is made up of the α and β subunits which provide a regulatory function. The α and β subunits are phosphorylated, with the β subunit being phosphorylated first. The α subunit takes four to five times longer to phosphorylate. This is important for the dephosphorylation process as well. The phosphorylation takes place in the presence of cAMP-PrK, ATP, magnesium ions and calcium ions. In response to adrenalin, two sites on the α and β subunits become phosphorylated (Cohen, 1978).

Calcium ions are needed in quantities similar to those required for muscular contraction. The calcium that is released from the sarcoplasmic reticulum to initiate muscle contraction also activates phosphorylase kinase. Calcium interacts with the δ' subunit, which is identical to the calcium binding protein calmodulin, and interacts with a δ'' subunit, which is a second molecule of calmodulin. The δ subunit is complexed with the catalytic γ subunit of the intact molecule. The δ subunit interacts with the α and β subunits (Cohen, 1983).

The α subunit may play an important role in the dephosphorylation of phosphorylase kinase (conversion of

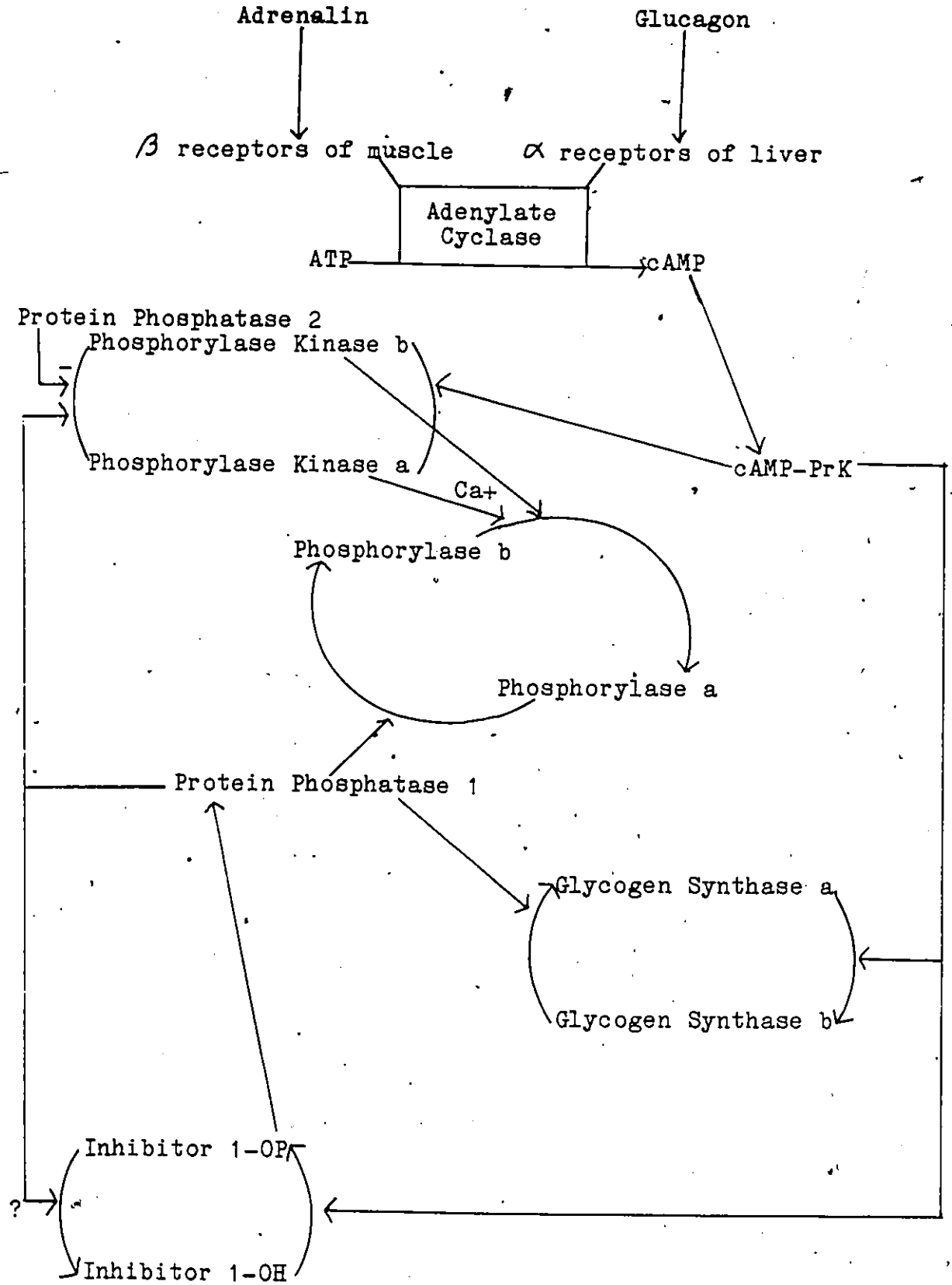

Figure 2. Specific enzymes of glycogen metabolism (Cohen, 1983)

phosphorylase a to b). The α subunit takes longer to phosphorylate; however, once two α subunits are phosphorylated the conformation of the enzyme is altered. The conformational change facilitates the action of phosphorylase kinase phosphatase on the β subunit. Therefore, once the α subunits are phosphorylated , dephosphorylation could begin in the β subunits, although rephosphorylation of the β subunits is not prevented by the α subunit phosphorylation. As well, there does appear to be a time when the kinase and phosphatase do not compete when the subunits are not phosphorylated completely. The reconversion to the inactive phosphorylase kinase, once the hormonal stimulus is removed, is assisted by the phosphorylation of the α subunits. The exact rate of phosphorylation and dephosphorylation of the subunits is not yet clarified (Cohen, 1978).

Once cAMP-PrK has activated phosphorylase kinase in the correct cellular milieu, glycogen phosphorylase becomes activated. This is the final step in glycogen degradation . At the same time the phosphorylation inactivates glycogen synthase.

Glycogen synthase is a complex enzyme, consisting of four subunits of uniform size and seven phosphorylation sites. These sites are phosphorylated by different enzymes and all are almost fully phosphorylated during glycogenolysis. Glycogen synthase a or I is the active or independent form which promotes glycogen synthesis. Glycogen synthase b or D is the less active or dependent form because it depends on G6P for activity. Glycogen synthase b is the phosphorylated form of the enzyme which predominates in glycogen degradation (Cohen, 1978; Cohen, 1983).

It has been found in muscle that during glycogenolysis the activity of glycogen synthase a is in the range of 15 to 25%. There would be no glycogen synthesis at this time. The amount of glycogen synthase in the a form depends on the amount of phosphate bound to it. If a stimuli were present increasing the activity of glycogen synthase a to 40%, changes in glycogen synthesis would be great (Roach and Larner, 1976).

Adrenalin inactivates glycogen synthase in two ways. It does this by activating 1. cAMP-PrK and 2. glycogen synthase kinase 3. cAMP-PrK catalyses the phosphorylation of sites 1a, 1b, and 2 directly of glycogen synthase. Activated by cAMP-PrK, phosphorylase kinase catalyses the phosphorylation of site 2, indirectly. The other protein kinase, glycogen synthase kinase 3, catalyses the phosphorylation of sites 3a+3b+3c. Site 5 appears to be phosphorylated under all conditions. The phosphorylation of site 5 was recently found to be a prerequisite for phosphorylation of site 3 (Picton et al, 1982).

The phosphorylation of sites 3a+3b+3c catalysed by glycogen synthase kinase 3 is shown to decrease the activity of glycogen synthase to a greater extent than the phosphorylations catalysed by cAMP-PrK or phosphorylase kinase. Although glycogen synthase kinase 3 inhibits the action of glycogen synthase in response to adrenalin, its action is unaffected by cAMP. It appears that the phosphorylation of sites 3a+3b+3c and perhaps site 2, is a consequence of the inhibition of protein phosphatase 1, through the phosphorylation of inhibitor 1. Protein phosphatase activates glycogen synthase by dephosphorylation.

Inhibitor 1 prevents the action of protein phosphatase 1 (Cohen, 1983).

Another possible reason for the inhibition of glycogen synthase could be that as the rate of phosphorylation of glycogen phosphorylase increases, the rate of dephosphorylation of glycogen synthase decreases. This could be possible due to the higher concentrations of glycogen phosphorylase (70 uM) compared to glycogen synthase (2.5 uM) (Cohen, 1983).

When glycogen synthase is activated, or dephosphorylated, glycogen synthesis occurs. Upon activation by insulin, glycogen synthase is dephosphorylated at sites 3a+3b+3c. Sites 1a, 1b and 2 are unaffected by insulin. This could be because insulin does not act by decreasing the levels of cAMP or inhibition of cAMP-PrK. As previously stated, insulin either decreases the activity of glycogen synthase kinase or increases the activity of protein phosphatase. It is suggested that the former hypothesis is correct (Cohen, 1983). Both protein phosphatases 1 and 2 are capable of dephosphorylating sites 3a+3b+3c, but they also dephosphorylate site 2 and are specific for sites 3a+3b+3c. Glycogen synthase kinase 3 could be inhibited by a second messenger triggered by insulin. It is postulated that the insulin receptor is a transmembrane protein which includes a binding site for insulin on the outer surface and the activity of the protein kinase occurs on the inner surface. The insulin/receptor interaction would then release the second messenger to inhibit glycogen synthase kinase 3 (Cohen, 1983).

Although the decrease in activity of glycogen synthase

kinase 3 may increase the activity of glycogen synthase, the protein phosphatases are also responsible for increased glycogen synthesis. There are four protein phosphatases present in skeletal muscle and other tissues; the phosphatases are 1, 2a, 2b and 2c. Their actions result in the dephosphorylation of the phosphoproteins (Cohen, 1983).

Protein phosphatase 1 catalyses the dephosphorylation of the β subunit of phosphorylase kinase, sites 1a, 2 and 3a+3b+3c of glycogen synthase and glycogen phosphorylase. Its actions are inhibited by inhibitors 1 and 2. Protein phosphatase 1 is one of the main regulatory enzyme promoting glycogen synthesis or turning off glycogen degradation; however, the dephosphorylations of phosphorylase kinase, glycogen synthase and glycogen phosphorylase can be regulated independently. 5'AMP activates phosphorylase b allosterically. The state of phosphorylation of the α subunit of phosphorylase kinase influences the dephosphorylation of the β subunit. As well the amount of glycogen present influences the dephosphorylation of glycogen synthase (Cohen, 1983; Cohen, 1978).

Protein phosphatase 2A is the major phosphorylase kinase phosphatase of the α subunit of phosphorylase kinase, and has some glycogen synthase phosphatase activity and a small proportion of phosphorylase phosphatase and phosphorylase kinase phosphatase (β subunit) activity. In resting skeletal muscle, protein phosphatase 2A functions in the absence of calcium ions. Protein phosphatase 2B depends on calcium ions; therefore, it is functional during muscular contractions also acting on the α

subunit of phosphorylase kinase. Protein phosphatase 2C has been identified in liver and cardiac muscle as a glycogen synthase phosphatase, but in negligible quantities (Cohen, 1983).

Inhibitor 1 and 2 have been identified in liver and skeletal muscle. Inhibitor 1 becomes active after phosphorylation by cAMP-PrK; whereas, inhibitor 2 is active without phosphorylation. The phosphorylated form of inhibitor 1 inhibits the action of protein phosphatase 1. It inhibits the dephosphorylation of phosphorylase kinase, phosphorylase and glycogen synthase. It is also possible that protein phosphatase 1 dephosphorylates inhibitor 1; however, while it is being dephosphorylated it still has some ability to act on protein phosphatase 1. It would seem that the role of inhibitor 1 is to make the metabolism of glycogen sensitive to changes in cAMP levels (Cohen, 1983;Cohen, 1978).

Although inhibitor 2 does not need to be phosphorylated to be active, it may be present in quantities sufficient to inhibit only a small amount of protein phosphatase 1. It may act at specific locations to prevent protein phosphatase 1 from acting on proteins that are dephosphorylated by other enzymes (Cohen, 1978).

In summary, glycogen can be synthesized from glucose and fructose. The synthesis can occur directly from glucose in the muscle. Fructose is first metabolized in the liver producing glucose which is then taken by the blood to the muscle where it is converted into glycogen. The conversion of glucose to

glycogen is promoted through the release of insulin which activates glycogen synthase by increasing the activity of protein phosphatase or decreasing the activity of glycogen synthase kinase. As will be seen in section 2.4, exercise also promotes glycogen synthesis with or without insulin.

2.3 EXERCISE SPECIFICS

2.3.1 Introduction

Exercise involves the contraction of muscles. The fibres of muscles are specific for the type of exercise they perform. In addition, as different fuels are metabolized at different rates, exercise will affect the choice of fuel. The characteristics of the muscle fibres determine the rate and extent of glycogen depletion and repletion.

2.3.2 Muscle Fibre Characteristics

Three fibre types have been identified in the skeletal muscles of rats. These types are fast-twitch white, fast-twitch red and slow-twitch red. The fast-twitch white fibres have a high glycolytic capacity, a low respiratory capacity and high myosin ATPase activity; hence they are often called fast glycolytic or FG fibres. The fast-twitch red fibres have a high glycolytic capacity, a high respiratory capacity and high myosin ATPase activity. They are also referrred to as fast oxidative

glycolytic or FOG fibres. The slow-twitch red fibres have a low glycolytic capacity, a moderate respiratory capacity and low myosin ATPase activity. They are referred to as slow oxidative or SO fibres (Baldwin et al, 1973a; Baldwin et al, 1973b; Terjung et al, 1974; Conlee et al, 1978; Baldwin et al, 1972; Barnard et al, 1971).

The fibre types are, in part, determined by contractile characteristics. Both red and white fast-twitch fibres demonstrate similar times to peak tension and half-relaxation times, of approximately 20 msec. The slow muscle times are approximately 80 msec and 110 msec for time to peak tension and half-relaxation time respectively. A correlation between contraction time and myosin ATPase activity has also been found to exist. The faster the contraction time the higher the myosin ATPase activity. This appears logical due to the enzyme's involvement in contraction (Barnard et al, 1971).

The characteristics of the fibres are also determined by their predominant metabolic traits. The slow red fibres obtain the energy they require from aerobic metabolism. The fast red fibres also obtain energy from aerobic sources, but are able to obtain energy from anaerobic metabolism due to their high glycolytic capacity. White muscle has a high glycolytic capacity and its contractile energy is supplied primarily by anaerobic metabolism.

Hexokinase activities appear to be different between red and white fibres which may result in differences in the synthesis of glycogen. Following acute or chronic exercise, it has been

found that hexokinase activity is elevated (Peter et al, 1968; Conlee et al, 1978), with values for hexokinase activity having been seen to be twice as high in red skeletal muscle as in white muscle (Conlee et al, 1978). However, no differences were found between fast red and slow red fibres regarding hexokinase activity (Peter et al, 1968; Conlee et al, 1978).

Upon examination of the activities of glycogen synthase, following acute exercise, it was found that there were differences among the three types of fibres. Glycogen synthase a activity was highest in fast red muscle, lowest in white muscle and between these two levels in slow red muscle. The actual muscles representing the fibres were red vastus lateralis (FOG), white vastus lateralis (FG) and soleus (SO). The rate of replenishment of muscle glycogen stores following exercise has been shown to correlate linearly with the activity of glycogen synthase a. As might be expected, fast-twitch red muscle had the highest rate of repletion followed by slow red and then fast white (Conlee et al, 1978).

As will be explained later, the type of activity affects the metabolic characteristics of the different fibres. This in turn would affect depletion and repletion rates. However, it does appear that the fast red fibres have higher repletion rates than the other two types under a variety of exercise conditions (Baldwin et al, 1973a; Terjung et al, 1974; Conlee et al, 1978; Barnard et al, 1971; Poland et al, 1980; Armstrong and Ianuzzo, 1977; Peter et al, 1968; Tan et al, 1984).

2.3.3 Exercise Fuel Supply

Any type of exercise depletes muscle and liver glycogen stores to some extent, but it is the intensity and duration of the activity that determines the predominant fuel that will be used to supply the energy. Both fat and carbohydrate supply fuel to the working muscle. The contribution of each substrate is determined by the above conditions.

The results of many experiments indicated that during prolonged light to moderate aerobic exercise, of the type that can be maintained for up to three hours, fat metabolism provides up to 70% of the fuel used (Christensen and Hansen, 1939; Basu et al, 1960; Carlson et al, 1963; Friedberg et al, 1963; Rodahl et al, 1964). Brief, heavy exercise, which must draw on anaerobic mechanisms, relies on carbohydrate sources for energy (Christensen and Hansen, 1939; Friedberg et al, 1960; Issekutz and Miller, 1962; Rodahl et al, 1964). The greater the anaerobic contribution, the higher the carbohydrate utilization.

In experiments in which diet was manipulated in humans to provide high fat or high carbohydrate, the high carbohydrate diet resulted in the continuation of a standard work load, of indeterminate intensity, for up to three times as long as with the high fat diet (Christensen and Hansen, 1939).

As well, the conditioning of the subject plays an important role in fuel supply. In experiments done comparing trained to untrained dogs, it was found that the fatty acid utilization increased during the exercise period in the trained

dog while the opposite occurred in the untrained dog. (The trained dogs were conditioned to run on a treadmill for 30 to 40 minutes at a speed of 100 m/min at a grade of 15 .) At the same time as the free fatty acid levels decreased in the untrained dog, the lactic acid levels increased. This indicates that the activity could no longer be maintined by aerobic metabolism (Issekutz et al, 1965). Similar findings have been shown in studies performed on rats (Barakat et al, 1982).

The extent of the depletion depends on the duration, intensity and type of activity. Following depletion of glycogen the body works to replete these stores.

2.3.4. Muscle Glycogen Depletion

Assuming that a person or animal is in a recently fed, rested state, the liver and muscle glycogen stores would be full. These stores can be emptied in two ways, by decreasing nutritional supply and by increasing utilization. Fasting has been shown to deplete liver glycogen considerably in rats (Brooks, 1973; Conlee et al, 1976) and evidence indicates that almost complete depletion of liver glycogen occurs after a twenty-four hour fast (Agren and Jorpes, 1931; Leveille and Chakrabarty, 1967; Russell and Bloom, 1956). As well, a twenty-four hour fast has been shown to deplete muscle glycogen stores in rats (Conlee et al, 1976). In Conlee's study, the soleus was depleted by 8% in the afternoon and by 49% the following morning; the red vastus lateralis by 23% in the

afternoon and 47% the following morning and the gastrocnemius by 11% in the afternoon and 23% the following morning. These figures are derived from a comparison of fed and fasted rats at the same time of day, indicating not only fasting effects but variance in glycogen content throughout the day. During the time of Agren's, Leveille's and Russell's studies, it was thought that only liver glycogen was depleted during a fast. It is now understood that although liver is depleted first during a fast, muscle is also depleted.

Under exercise conditions, muscle tissue is depleted prior to liver tissue. Although liver glycogen contributes fuel, in the form of glucose, to the work performed, it is mainly the muscle glycogen which determines the working capacity or duration of the exercising animal (Bergstrom and Hultman, 1967b). In a study performed by Bergstrom and Hultman (1967b) the characteristics of muscle glycogen in the quadriceps femoris of men performing work on a bicycle ergometer were determined. In one phase of the experiment the men pedalled at a workload of 950 kpm/min for as long as the pedalling rate of 60 rpm was maintained. In another phase the exercise intensity was reduced to 400 to 500 kpm/min to permit catheterization of the veins of the liver. Through these experimental phases it was found that muscle glycogen was utilized triphasically. Initially there was a rapid disappearance of glycogen. This was followed by a second phase of constant fall which was predominant and possibly indicated steady state. The third phase showed a slower disappearance of the glycogen during which there was an increase

in the output of glucose from the liver.

It was mainly the glycogen stored in the muscles that was used for energy. Although not measured, it was assumed that the muscular work raised the levels of adrenalin which in turn stimulated glycogenolysis. As well, the rise in AMP stimulated phosphofructokinase, increasing glycolysis; therefore, it was implied that the rate of glycogenolysis and glycolysis would increase with hard muscular work. This rate was highest during the initial phase, but was still higher than basal level throughout the work period (Bergstrom and Hultman, 1967b).

During the third period, the rate of glycogenolysis was slower. The glycogen store at this stage was greatly reduced and this deficiency could probably explain the increased glucose uptake from the blood. However, the glucose production from the liver was minimal compared to the total amount of carbohydrate metabolized. Thus the store of muscle glycogen was felt to be the major determinant of the length of the work period.

Wahren et al (1971) also exercised males on a bicycle ergometer to determine the contribution of glucose from the liver during exercise. The subjects cycled for 40 minutes at workloads of either 400, 800 or 1200 kg-m/min. At each workload the uptake of blood glucose increased with time. The heaviest work load showed the highest increase in blood glucose uptake. Again it was concluded that as the muscle glycogen stores became depleted the oxidation of the blood glucose contributed more to maintaining the workload. Wahren et al stated that 75 to 90% of the carbohydrate metabolism and 28 to 37% of the total oxidative

metabolism could be sustained by blood glucose at 40 minutes of exercise. These figures are estimates based on the complete oxidation of the blood glucose. Regardless of the actual percentage of blood glucose oxidation, the increasing importance of this substrate was seen.

The increase in liver glucose output was noted for all intensities of exercise. As well, decreases in insulin concentration were observed. Despite the hypoinsulinemia, glucose uptake by the muscle was detected. There may have been an influence by glucagon, but it was not measured in this study.

In summary, muscle glycogen is the main determinant of the duration of exercise. When these stores become reduced, liver glycogen contributes to the maintenance of the activity. This would be the case if the activity was supported by carbohydrate metabolism as opposed to fat metabolism. Exercise of a high enough intensity would ensure this. If the activity was extreme enough intensity the FG fibres and possibly some FOG fibres would be recruited. If it was of a longer duration the SO fibres would be involved. Often a combination of all the fibres are involved, but at different times during the activity. As was seen, each fibre type has specific depletion and repletion times.

2.4 MUSCLE GLYCOGEN REPLETION

2.4.1 Introduction

Muscle glycogen repletion occurs following depletion of

the muscle by fasting or exercise. It will be seen that repletion of muscle glycogen occurs before repletion of liver glycogen especially if the muscles are depleted by exercise. The depleted muscle itself is a potent stimulus for repletion of the glycogen stores. Repletion can occur without substrates, but it is enhanced by simple and complex carbohydrates. Studies on humans reveal greater repletion rates with carbohydrates as opposed to other foodstuffs. Studies which limit the substrates to simple sugars have been conducted on laboratory rats to demonstrate the differences between glucose or fructose feedings. Some studies have involved infusion techniques while more recent studies involved ingestion techniques. The latter technique appears to elicit a hormonal response that more closely corresponds to what would happen following exercise.

2.4.2 Preferential Repletion

Studies conducted on laboratory rats have looked into the effects of exercise on glycogen repletion. Rats were chosen, as muscle and liver samples were required for analysis.

One predominant finding, following exercise induced glycogen depletion, was that muscle glycogen repletion was favoured over liver glycogen repletion (Blawacka, et al, 1978; Fell et al, 1980; Poland et al, 1980; Armstrong and Ianuzzo, 1977; Costill et al, 1983; Maehlum et al, 1978). Regardless of the intensity, duration or type of activity or the amount of training, the muscle glycogen stores repleted more rapidly. It

was felt that the repletion of liver glycogen was a continuous process; whereas, the repletion of muscle glycogen was a dual-phased procedure involving a fast insulin independent phase and a slow insulin dependent phase (Blawacka, 1978).

Perfusion studies have begun to clarify the two phases of glycogen repletion in muscle (Garetto et al, 1984; Richter et al, 1984). Rats were exercised at a high intensity (36 m/min for 50 minutes - 5 runs of 10 minutes with 2 to 3 minutes rest between) producing depletion from 60 to 70% in the muscles extracted. During the first thirty minutes post- exercise, the muscles repleted their glycogen stores in the presence or absence of insulin which was added to the perfusion medium. In two hours and thirty minutes the muscle glycogen stores approached the resting control levels, but glycogen continued to be synthesized in the presence of insulin, to levels reaching and exceeding those of the resting controls. It was concluded that Phase I occurred when muscle glycogen was depleted and was due to increased glucose utilization and glycogen synthesis in the absence or presence of insulin. Phase II occurred as the glycogen stores reached resting levels and glycogen synthesis was due to increased insulin sensitivity. The second phase occurred only in the presence of insulin. Comparisons of glycogen repletion in electrically stimulated muscle during phase II, showed that in those muscles stimulated to contract, physiological concentration of insulin resulted in greater glucose transport and glycogen synthesis than in those muscles not stimulated to contract (Garetto et al, 1984; Richter et al,

1984).

2.4.3 Exercise as a Stimulus

An additional potent stimulus for muscle glycogen repletion appeared to be the exercise itself. In another perfusion study, exercised animals compared to non-exercised animals showed a ten-fold increase in glucose uptake in the absence of insulin (Ivy and Holloszy, 1981). Other studies have also shown increased glucose uptake, followed by increased muscle glycogen repletion after exercise (Richter et al, 1982; Fell et al, 1982; Conlee et al, 1978; Terjung et al, 1974).

It is generally agreed that the rate-limiting step in glucose utilization in resting rat skeletal muscle is the transport of glucose across the cell membrane (Tan et al, 1984; Richter et al, 1984; Richter et al, 1982; Fell et al, 1982). Under exercise conditions this has not always been agreed upon. Richter et al (1984) qualified this statement by saying that glucose transport was the rate-limiting step under most conditions. Fell et al (1982) hypothesized that when the glucose transport was high the rate-limiting step in glucose utilization was the ability to synthesize glycogen from glucose. It was theorized that if the glycogen content of the muscle was high G6P might accumulate when additional glucose was given. G6P's inhibition of hexokinase may result in glucose phosphorylation being the rate limiting step for glucose uptake at the muscle. This would occur only when the muscles had a high glycogen

content and had continuing exposure to high levels of glucose and insulin (Fell et al, 1982).

It was then hypothesized that the muscle would take up glucose and convert it to glycogen as long as the glycogen content in the muscle was low. In the Fell study, rats swam to exhaustion (approximately three hours) and were fed either regular chow (60% carbohydrate) or lard following the exercise. It was undetermined whether or not the rats were weighted during the swim. Perfusion of the hindquarters of these rats was done the following day. The animals fed the lard showed higher glucose uptake than those fed the chow when perfused with varying concentrations of glucose and insulin. This lends support to the glucose utilization hypothesis. As well, of the glucose taken up by the muscles, 82% was converted to glycogen in the lard fed animals. The lactate production was higher in the chow fed group (28% lactate in chow fed; 12% lactate in lard fed of the glucose taken up) and the G6P content was higher in the muscles from the chow fed animals. This study indicated that chow feeding following exercise raised muscle glycogen to higher levels than lard feeding. When the muscles contained high levels of glycogen it was the ability to synthesize glycogen, as glucose was available for conversion, that determined further glycogen repletion. It was also found that perfusion with the same insulin concentrations caused an increased rate of glucose uptake in the muscles with low glycogen levels compared to the muscles with high glycogen levels. The findings from this study give credence to the theory that when glucose is available, it is the ability

to synthesize glycogen that is the rate-limiting step. Additional studies using humans and rats help to clarify glycogen repletion under various refeeding conditions.

2.4.4 Carbohydrate Refeeding

Repletion of glycogen stores appear to be enhanced by the addition of certain substrates following exercise (Bergstrom and Hultman, 1966; Bergstrom et al, 1967; Conlee et al, 1978; Fell et al, 1982; Costill et al, 1983; Costill et al, 1981; Terjung et al, 1974; Maehlum et al, 1978; Piehl, 1973), but repletion without substrates may also occur (MacDougall et al, 1977; Poland et al, 1980).

Studies that have looked at muscle glycogen repletion following exercise in humans, have revealed different results. Early studies by Bergstrom et al (1967) found that high carbohydrate diets did enhance muscle glycogen repletion. In this study men were exercised to exhaustion on a bicycle ergometer at a work load approximating 75% of their maximum oxygen uptake. Prior to the first exercise they ate an uncontrolled mixed diet (M). For three days after the exercise they were given either a high fat/protein (F/P) diet or a high carbohydrate (C) diet. Then they repeated the exercise. Following this they were fed the other diet (F/P or C) for three days and repeated the exercise.

Biopsies of the lateral part of the quadriceps femoris showed wide ranges of glycogen concentrations upon repletion

although glycogen per fibre type was not identified. The F/P diet repleted the muscle glycogen stores to approximately 50% of initial values; whereas, the carbohydrate diet raised the muscle glycogen levels to above normal ranges (0.95 to 2.0 g/100g, normal, raised to 3.31 g/100g). A good correlation between initial glycogen concentration and length of work time was also found. The glycogen values and work times from the C diet were the highest.

Table 1. Glycogen concentrations and work times as a result of different diets (Bergstrom et al, 1967, pp. 144-145)

	M	F/P	C
Muscle Glycogen (g/100g)	1.75	0.63	3.31
Work Times (min)	114	57	167

The biopsies in the previous study were taken immediately before and after the exercise; therefore, data on the time course of repletion following exercise was not taken. A study done by Piehl (1973) examined this time course. She exercised male subjects for two hours. The first hour involved endurance activities (running, skiing, swimming and bicycling) and the second hour involved repeated maximal efforts on a bicycle ergometer to exhaustion. Following the exercise the subjects ate a controlled, 4000 kcal/day diet consisting of 60% carbohydrate for two days. Biopsies were taken immediately pre-exercise, immediately post-exercise, 5 hours post, 10 hours post, 22 hours

post and 46 hours post with the following results:

Table 2. Muscle glycogen concentrations before and after exercise (Piehl, 1973, p. 300)

	Muscle Glycogen (mmol/kg)
Pre-exercise	125
Post-exercise	23
5 Hours Post	64
10 Hours Post	86
22 Hours Post	95
46 Hours Post	124

It can be seen that approximately 60% of the muscle glycogen had been repleted after 10 hours. Of the 2600 mmol of glucose supplied during both days, the muscles showed increases of 72 mmol/kg and 29 mmol/kg on the first and second days respectively. Piehl concluded that in a 20 kg muscle mass most of the carbohydrate intake was initially converted to muscle glycogen. This would be in agreement with the theories of Garetto et al (1984) and Richter et al (1984).

Costill et al (1981) also looked at muscle glycogen repletion in humans following moderate intensity endurance exercise and high intensity interval exercise. These investigators varied the type, amount and frequency of the carbohydrate diet. Comparing diets of simple carbohydrates to complex carbohydrates (70% of the diet as carbohydrate), needle

biopsies of the gastrocmenius muscle showed that the repletion after 24 hours was similar to normal pre-exercise values for both diets, while the repletion after the second 24 hours was greater with the complex carbohydrate diet (22.1 mmol/kg increase with complex and 7.8 mmol/kg increase with simple for second 24 hours). Comparing the amount of carbohydrates, it was found that a low carbohydrate diet decreased the muscle glycogen stores (71.3 to 66.6 mmol/kg in 24 hours), while a high carbohydrate diet increased the muscle glycogen stores (55.3 to 125.6 mmol/kg in 24 hours).

The frequency of feeding compared a high carbohydrate diet taken in two meals to one taken in seven meals. More frequent meals did not enhance muscle glycogen repletion, but exercise following the feeding showed that the seven feedings produced increases in carbohydrate oxidation over the two feedings. This may have been due to the shorter time that had elapsed following the last meal (8 hours for seven feeding regime; 15 hours for two feeding regime).

In the experiments comparing the type of carbohydrates, 648 grams of carbohydrate were ingested in the first 24 hours and in the experiments comparing the number of feedings, 525 grams of carbohydrate were ingested. Repletion to normal values occurred after 24 hours with both amounts. It was then suggested that between 525 and 648 grams of carbohydrate would replete muscle glycogen to normal levels.

MacDougall et al (1977) also found that complete recovery following exercise could occur within 24 hours in humans.

However, the results of this study indicated that the rate of repletion could not be enhanced by additional carbohydrates, above a normal intake of 50%. This study involved high-intensity intermittent work on a bicycle ergometer (140% of their maximum oxygen uptake in a 1:3 work to rest ratio until exhaustion). Following exercise, the subjects ate either a mixed diet consisting of 3,100 kcal, 50% carbohydrate, or the same diet plus 2,500 kcal of carbohydrate. No difference in repletion rates could be seen between the two groups. The intense but brief exercise may have altered repletion rates as the men were hyperglycemic post-exercise. This is in contrast to the other experiments where the subjects were hypoglycemic (Bergstrom and Hultman, 1966; Piehl, 1973; Costill et al, 1981). Although not measured, it was felt that liver glycogen was not depleted during the exercise. Consequently, it was thought that glucose levels would rise rapidly following exercise as would insulin levels contributing to immediate repletion. In fact at 2 hours post-exercise glycogen levels had risen 11% from post-exercise levels without the addition of substrate.

As well muscle glycogen measurements were not made after 24 hours. The increased carbohydrate may or may not have caused increases in muscle glycogen levels in the subsequent 24 hours as in the Costill et al (1981) study comparing simple to complex carbohydrates.

2.4.5 Glucose or Fructose Infusion

It would seem apparent that the ingestion of carbohydrates following exercise does affect muscle glycogen repletion. Although Costill et al (1981) did look at simple sugars as a carbohydrate source the specifics as to the effects of these sugars were not made clear.

As previously stated, it is felt that the effects of the monosaccharides, glucose and fructose would be quite different. Studies done in the late 60's and early 70's infusing the two substrates did show different results.

Bergstrom and Hultman (1967) showed that the infusion of a 20% glucose or fructose solution produced a similar, moderate rise in muscle glycogen in resting muscle of humans. In previously exercised muscle, greater increases in muscle glycogen levels were noted after both infusions. However, glucose infusion resulted in twice the levels of muscle glycogen as the fructose infusion in the working muscle.

Table 3. Muscle glycogen content before and after exercise following glucose or fructose infusion (Bergstrom and Hultman, 1967a, pp. 97-98)

	Mean Muscle Glycogen Content (g/100g)			
	Glucose		Fructose	
	Before Inf	After Inf	Before Inf	After Inf
No Exercise Before Test	1.39	1.74	1.37	1.70
Exercise Before Test	0.44	1.04 2h	0.09	0.55 2h
		1.57 4h		0.89 4h

The rate of repletion of muscle glycogen, which has been depleted by heavy exercise, favours glucose as a substrate upon infusion.

As well, additional experiments using humans showed that liver glycogen repletion favours fructose as a substrate upon infusion. In these experiments, liver biopsies were taken following an overnight fast and during glucose or fructose infusion. Increases in liver glycogen with both substrates were detected, but the increases were higher with fructose (Nilsson and Hultman, 1970).

2.4.6 Glucose or Fructose Ingestion

The studies cited using infusion techniques may not give a true indication of repletion rates with glucose or fructose. Infusion does not seem to promote the same hormonal responses as

oral ingestion does and for this reason more recent studies have involved ingestion of these substrates (Costill et al, 1983; Neiwoehner et al, 1984).

In a study done by Conlee et al (1978) rats were fed 0.5 grams of glucose by gavage following an exhaustive swim (25% solution). The swimming group also had a restricted food intake for 15 hours prior to the swim. The animals were then sacrificed at 0.5, 1, 2 or 4 hours post-exercise. The results were compared to those of a non-exercised group sacrificed at the times the run began (8 a.m.). Samples of the soleus, red vastus lateralis and white vastus lateralis muscles were taken to determine muscle glycogen concentrations. The red vastus repleted the most rapidly followed by the soleus and then the white vastus. In four hours the glycogen content of the red vastus reached pre-exercise levels; whereas, the white vastus had increased to only 60% of the pre-exercise level.

Upon completion of the exercise blood glucose reached a mean of 50.7 mg/100ml. The animals were hypoglycemic compared to the resting, fed controls (blood glucose of 115.7 mg/100ml); however, the blood glucose surpassed the control values within half an hour (133.6 mg/100ml), increased further at one hour (168.1 mg/100ml) and approximated the control values after 4 hours (123.3 mg/100ml).

Liver glycogen was significantly depleted following exercise (0.55 mg/g compared to 31.59 mg/g pre-exercise). Within 4 hours following the exercise, liver glycogen increased to approximately 60% of resting levels.

Terjung et al (1974) also conducted a study using twice the amount of glucose. 0.5 grams were administered immediately post-exercise and an additional 0.5 grams were administered one hour later. As well, rat chow and water were permitted ad libitum following exercise. Prior to exercise, the rats were fasted for 24 hours.

Again the rats were hypoglycemic following the swim to exhaustion (58 mg/100ml immediate post; 140 mg/100ml resting fed). The blood glucose then rose to 118 mg/100ml in 30 minutes which is still below resting levels, but returned to resting levels after 2 hours. Some hyperglycemia was observed at the 4 and 24 hour times, although not to the same degree that was observed by Conlee et al. The red vastus repleted most rapidly, followed by the soleus and then the white vastus. The muscle glycogen content of the red vastus returned to resting levels within 2 hours following the exercise, while the muscle glycogen content of the soleus returned to resting levels between 2 and 4 hours. Some supercompensation was noted in the red vastus at 4 and 24 hours post-exercise and in the soleus at 4 hours post. The glycogen in the white vastus reached resting levels by 24 hours with some supercompensation at this time as well.

As in the Conlee et al study, liver glycogen was depleted following exercise (0.60 mg/g immediate post; 42.0 mg/g resting fed). At 30 minutes post-exercise, liver glycogen levels reached only 4% of the fed controls and after 4 hours 47% of control levels rather than 60% as in Conlee's study. Supercompensation was noted in the liver glycogen values after 24 hours. The

overall rate of liver glycogen repletion was 13.12 mg/g/h.

It was suggested that during the first 30 minutes post-exercise the glucose was not yet available for conversion to glycogen and this was the limiting factor. During the next 30 minutes the highest rates of conversion were found. These overall rates were comparable to those reported for sugar transport. The rates of conversion of glucose to glycogen for the red vastus lateralis were 7.48 mg/g/h, for the white vastus lateralis 2.64 mg/g/h and for the soleus 3.92 mg/g/h. Studies measuring sugar transport induced by insulin in the rat diaphragm (60% fast red; 20% slow red; 20% white) revealed rates between 5.93 and 8.27 mg/g/h. Response of frog sartorius (white) to contraction or insulin showed rates between 2.88 and 4.32 mg/g/h (Kipnis and Cori, 1959; Buse et al, 1964).

The ingestion of glucose did appear to increase the rates of muscle glycogen repletion. This influence of glucose ingestion was compared to the influence of fructose ingestion in a study by Costill et al (1983). In this study all rats were starved for 14 hours prior to the experiment. Five groups of animals were then studied. The groups were as follows:

1. control, sacrificed at rest
2. control, exercised until exhaustion, then sacrificed
3. control, exercised until exhaustion, sacrificed 3 hours later
4. experimental, exercised to exhaustion, fed 2ml, 20% fructose, sacrificed 3 hours later
5. experimental, exercised to exhaustion, fed 2ml, 20% glucose, sacrificed 3 hours later.

As well, the fructose and glucose solutions contained radioactive labelled carbon.

The exercise consisted of running on a treadmill until exhaustion and the average time to exhaustion was 98.7 minutes, which suggests that the exercise was of a moderate intensity, but the animals were not previously trained.

Blood glucose results indicated that the animals were hypoglycemic following exercise. The blood glucose dropped from 133.2 mg/100ml, pre-exercise, to 39.6 mg/100ml, post-exercise. Without substrate the levels of blood glucose rose to 93.6 mg/100ml in 3 hours and with substrate the values were similar to the pre-exercise group.

Taking average values from the plantaris, soleus, white vastus and red vastus, muscle glycogen concentrations were 61% lower than resting values following the exhaustive run (3.90 mg/g resting control to 1.54 mg/g immediate post). The muscle glycogen levels rose in the three hours following exercise with and without substrates to 2.95 mg/g, no substrate, 3.95 mg/g, fructose and 4.05 mg/g, glucose. These average values show no significant differences between the substrates, but more carbon from the glucose feed was found in the muscle than from the fructose feed. However, in the plantaris glycogen repletion from the glucose feeding was significantly greater than from the fructose feeding (5.82 mg/g glucose, 4.49 mg/g fructose).

Liver glycogen values declined during the exercise and rose 3 hours later (pre 3.98 mg/g, post 1.31 mg/g, 3 hour post no substrate 1.81 mg/g, glucose 6.74 mg/g, fructose 6.97 mg/g).

Table 4. Comparison of results of the Conlee et al (1978), Terjung et al (1974) and Costill et al (1983) studies

		Conlee	Terjung	Costill
Muscle Glycogen (mg/g)				
Pre-Exercise	RVas	7.52	8.33	
	WVas	6.87	8.38	3.90 average
	Sol	6.09	5.84	with plantaris
	Liv	31.59	42.00	3.98
Post-Exercise	RVas	1.33	2.01	
	WVas	1.73	2.47	1.54 average
	Sol	1.74	1.55	with plantaris
	Liv	0.55	0.60	1.31
4 Hours Post	RVas	7.27	11.28	3 Hours Post Control 2.95
	WVas	4.34	6.24	(average Glucose 4.05
	Sol	5.65	6.68	for muscle) Fructose 3.95
	Liv	18.79	19.94	Control 1.81
				Glucose 6.74
				Fructose 6.97
Blood Glucose (mg/100ml)				
Pre-Exercise		115.74	~140	133.2
Post-Exercise		50.7	~ 58	39.6
0.5 Hours Post		133.56	~118	-
1 Hour Post		168.12	~120	-
2 Hours Post		135.18	~140	-
3 Hours Post		-	-	Control 93.6
				Glucose ~130
				Fructose ~130
4 Hours Post		123.3	~150	-

- no values given ~ approximate values

There was a greater increase after the glucose or fructose feedings but there was no significant difference between the glucose or fructose feedings. Although these differences were not significant more of the carbon label from the fructose was found in the liver. As well, when the stomach and small intestine were examined more carbon label from fructose was found than from glucose.

The absolute repletion of glycogen in the muscle and liver showed little difference between the two feedings. However, when looking at the carbon label some differences were detected. More labelled glucose appeared in the muscle and more labelled fructose appeared in the liver. The labelled glucose also appeared to be more evenly distributed between the two tissues.

2.4.7 Hormonal Influence

In the glucose ingestion studies the rates of repletion appeared to slow down after one hour when glycogen levels approached resting levels. This would lend support to the idea that higher levels of muscle glycogen retard conversion of glucose to glycogen although the influence of insulin does permit continued glycogen synthesis. The time factors are somewhat different than in the perfusion studies during the first 30 minutes post-exercise. This may have been due to the perfusion itself, the type of activity or some other unknown; however, there was rapid repletion followed by slower repletion in both studies.

The rates were not determined in the Costill et al (1983) study; however, this study did show that ingestion of carbohydrate improved repletion in the muscle. The reasons for the improvement could be multi-faceted.

In rats, it has been found that immediately following exhaustive exercise plasma insulin was below resting levels (Conlee et al, 1978; Fell et al, 1980). The levels of insulin in the plasma do rise following the exercise, but the time course has not been established. It would appear to vary depending on the intensity and duration of the exercise as well as the substrate given post-exercise (Koivisto et al, 1981; Fell et al, 1980; Conlee et al 1978).

In both the Fell et al (1980) study and the Conlee et al (1978) study, plasma insulin levels were extremely low immediately post-exercise (0.08 ng/mg Fell et al, not measurable Conlee et al). In the former study the animals were exhausted on the treadmill by a moderate-intensity endurance run followed by a high-intensity interval run. In the latter study the animals swam to exhaustion. It has been found that a swim to exhaustion does not deplete the muscle glycogen stores to the same extent as does a treadmill run to exhaustion (Armstrong et al, 1974). Three hours after exercise, in the Fell et al study, the plasma insulin values rose to approximately 9% of fed, resting controls. These animals had not been given any substrate. 24 hours later the chow fed group showed increases in plasma insulin to 60% of control. Four hours after the exercise in the Conlee et al study the rats demonstrated increased levels of plasma insulin to half

of the control values.

Before considering the glycogen repletion despite low insulin levels it is necessary to look at the effects of other hormones. In both studies discussed above, plasma glucagon was elevated immediately following exercise (Fell et al, 9 times above resting fed controls; Conlee et al, 5 times above resting fed controls). Following three hours (Fell et al, 1980) and four hours (Conlee et al, 1978) post-exercise the glucagon levels remained twice as high as the resting fed control. At 24 hours post-exercise, glucagon levels were still above control levels, but were higher in the fat fed group than in the chow fed group. Adrenalin was elevated 7 times above resting levels immediately following exercise and remained 3 times above controls three hours later (Fell et al, 1980). Noradrenalin was elevated 3 fold immediately post-exercise and remained this way for the four hours of monitoring (Conlee et al, 1978).

The hormonal milieu found following exercise was one which would normally promote glycogenolysis and inhibit glycogenesis; however, this was not seen. Muscle glycogen was rapidly repleted following exercise, especially during the first 30 minutes of recovery (Conlee et al, 1978). The amount of glycogen in the muscle appeared to determine the rate of glycogen repletion.

This has been further supported by studies comparing repletion rates in diabetic and normal rats. Rats that were not previously trained were run on a treadmill for 30 minutes at 23 m/min, 5% incline, a moderate intensity. Glycogen depletion was

evident and almost total repletion was noted within 2 hours following the exercise. Similar results were noted for both the normal and diabetic animals (Armstrong and Ianuzzo, 1977).

Similar findings were noted in a study conducted by Tan et al (1984). The rats in this study were exhausted by a treadmill run at a speed of 30 m/min, 8% incline. In one group of animals 3 g/kg of glucose was given immediately following exercise. The ingestion of glucose resulted in faster repletion in all muscles looked at. With the glucose or without, the rates of repletion in the diabetic and normal, untrained rats showed no significant differences.

These diabetic animals were insulin deficient, but were still able to replete their glycogen stores to the same degree as the rats who were able to secrete insulin. It appeared that the diabetic animals were able to activate glycogen synthase promoting glycogenesis.

In summary, it can be seen that muscle glycogen is preferentially repleted over liver glycogen when the muscle is depleted by exercise. Upon refeeding it appeared that the conversion of glucose to glycogen was the rate-limiting step. Refeeding with carbohydrates appeared to give the most rapid repletion. The type of food for the greatest repletion was being investigated, in part, by this study. Studies infusing glucose or fructose showed that muscle glycogen repletion favoured glucose as a fuel; whereas, liver glycogen repletion favoured fructose as a fuel. Ingested glucose also facilitated rapid

repletion of muscle glycogen, especially in fast red muscle. When comparing ingested glucose to ingested fructose, muscle glycogen repletion rates were similar, but more labelled glucose was found in the muscle while more labelled fructose was found in the liver. Due to the preliminary metabolism of fructose by the liver it might be speculated that the corresponding glucose release would be gradual permitting greater glycogen repletion over a longer period of time; an hypothesis which requires examination.

Chapter III

METHODOLOGY

3.1 DESCRIPTION OF SUBJECTS

Eighty-four male Wistar rats were used for the study. Rats were chosen to facilitate the control of diet and exercise preceding the experiment. As well, the incorporation of continuous blood samples and muscle tissue samples for biochemical analysis was made more feasible through the use of an animal model. Upon arrival they were approximately 12 to 14 weeks of age, weighing 200 to 225 grams.

3.2 ACCLIMATIZATION PROCEDURE

Upon arrival the rats were housed two or three to a cage and fed Purina Rat Chow and water ad libitum. They were maintained on a 12 hour dark / 12 hour light cycle. The dark cycle was from 9 am to 9 pm and the light cycle was from 9:01 pm to 8:59 am.

The rats were exercised on a motor driven rodent treadmill (Quinton Model 42-15). During the first week they ran at a constant speed of 27 m/min for 10, 15, 20, 25 and 30 minutes on successive days. The rats were exercised five consecutive days a week. The treadmill incline was set at 10% at all times.

The second week consisted of intermittent running. An

intermittent training protocol was chosen because 1. glycogen is the prime fuel at higher intensities, requiring the recruitment of fast tissue (Dallaire, 1977); 2. the purpose was to assure the ability to deplete tissue glycogen rather than to induce improvement in glycogen repletion chemistry; 3. after only three weeks of similar training, large depletions of muscle glycogen were evident following the high intensity protocol chosen for this study (30 to 40% of pre-exercise values, pilot results). During the second week the speed of the treadmill was set at 35, 40, 45, 50 and 55 m/min on successive days. Ten seconds of work was followed by 10 seconds of rest for a total of 15 minutes.

During the third week the running speed was maintained at 55 m/min and the work to rest was maintained at 10 seconds. The first 10 minutes of intermittent work was followed by 5 minutes rest and a second, 10 minute series of intermittent work. Pilot results indicated that the rats were capable of following this exercise protocol in that all animals were able to complete the work period in the third week of acclimatization.

3.3 EXPERIMENTAL GROUPS

Prior to experimentation all animals were assigned to one of 14 groups with a minimum of six animals per group. The groups were as follows:

Pre-exercise

Immediate post-exercise

Glucose-fed 1 hour post-exercise

Glucose-fed 2 hours post-exercise

Glucose-fed 5 hours post-exericse

Fructose-fed 1 hour post-exercise

Fructose-fed 2 hours post-exercise

Fructose-fed 5 hours post-exercise

Glucose&fructose-fed 1 hour post-exercise

Glucose&fructose-fed 2 hours post-exercise

Glucose&fructose-fed 5 hours post-exercise

Control 1 hour post-exercise

Control 2 hours post-exercise

Control 5 hours post-exercise

Pre-exercise sacrifices were done immediately prior to the exercise session on each experimental day. The immediate post-exercise group was sacrificed at the termination of exercise. The glucose-fed, fructose-fed and glucose&fructose-fed groups were given the appropriate sugar at the end of the exercise and the control group was permitted rat chow ad libitum, and all groups were allowed water ad-lib. The rat chow consisted of approximately 58% carbohydrate, but direct communication with the supplier indicated that there is some variability in the composition at least with respect to percentages and to some degree with the components themselves. For a more precise composition see Appendix F. Representatives of all four feeding groups were sacrificed at the designated times following the exercise (one hour, two hours and five hours). Muscle and liver samples were obtained from the sacrificed animals (Figure 3).

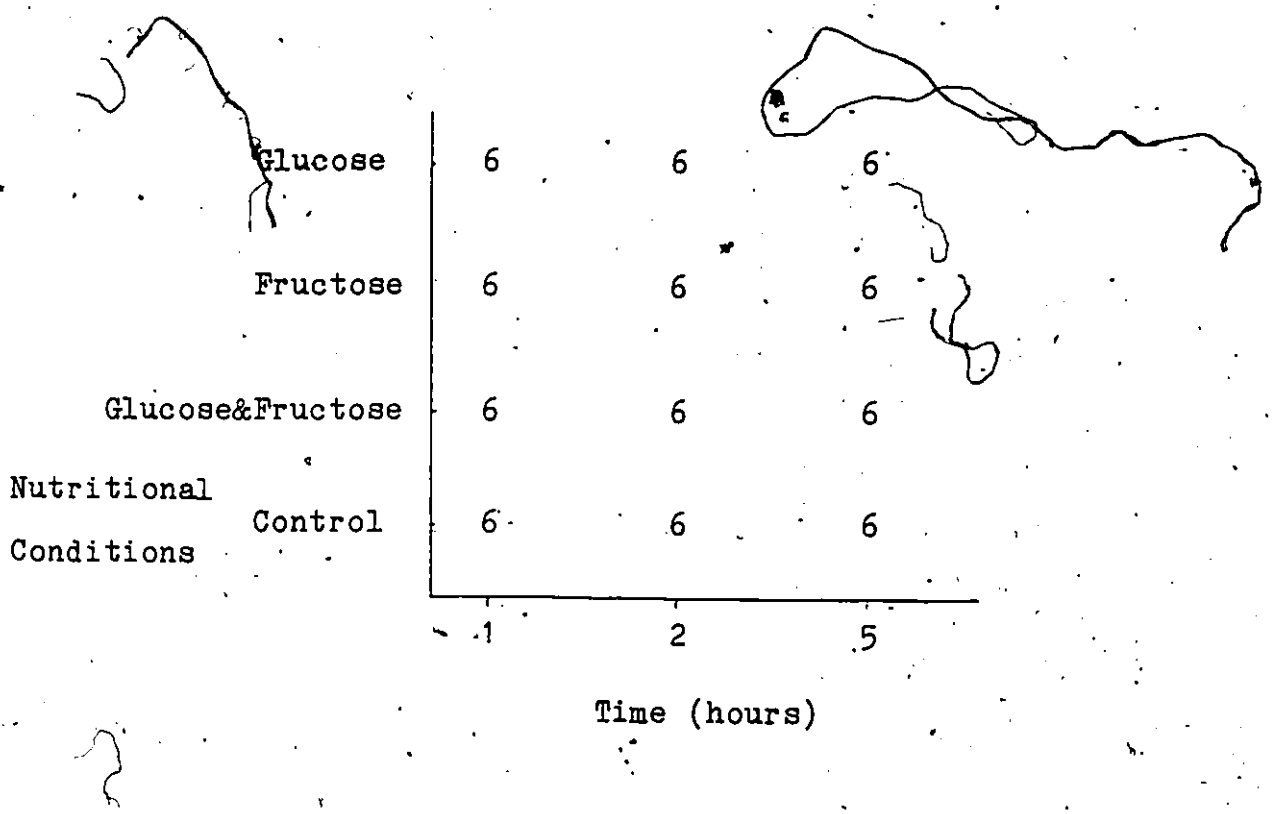

Figure 3. Number of rats sampled for muscle and liver glycogen

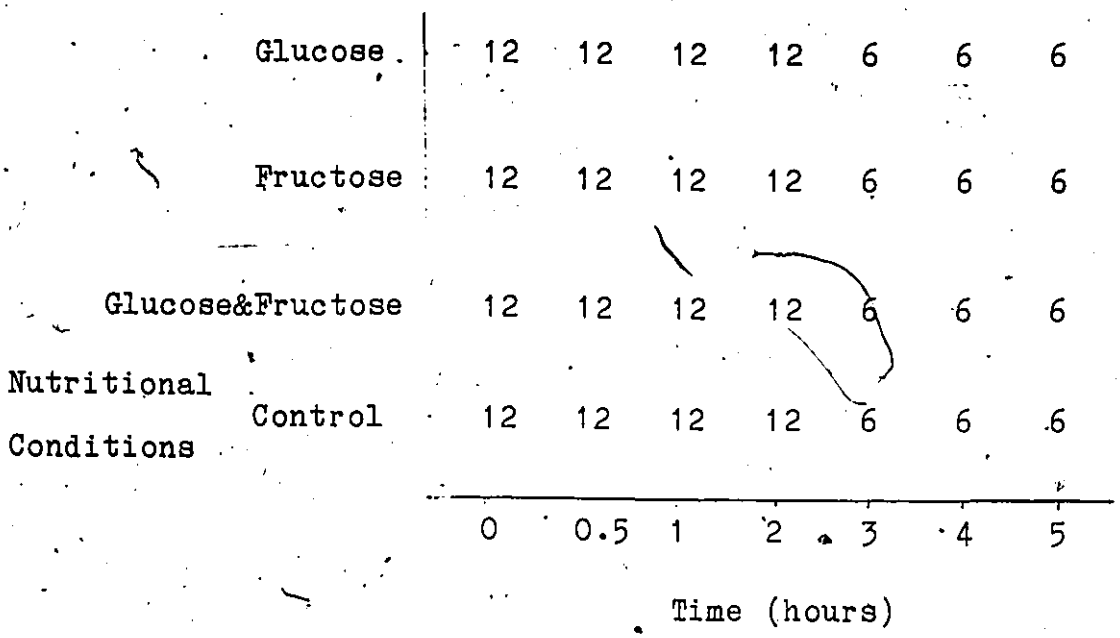

Figure 4. Number of rats sampled for blood glucose

Blood samples were taken at designated times following exercise. A random selection of 20 animals provided pre-exercise and immediate post-exercise blood samples. Additional blood samples were obtained at the following times: immediate post-exercise, 0.5 hours post-exercise, one hour post-exercise, two hours post-exercise, three hours post-exercise, four hours post-exercise and five hours post-exercise (Figure 4). Blood samples were taken only from animals designated to be sacrificed at the two hour and five hour times. Blood glucose data was available on twice as many animals for the first two post-exercise hours than during the remaining three hours. This constituted a "two hour" comparison group for blood glucose with a cellular N=12 and a "five hour" comparison group with a cellular N=6.

Pilot results indicated that blood glucose values increased rapidly within one hour following substrate ingestion and then increased slowly (Appendix A). Thus, hourly monitoring following the first hour post-exercise should adequately indicate blood glucose fluctuations.

The time course selected for glycogen repletion was based on findings of previous research. Garetto et al (1984) and Richter et al (1984) have shown that there are two stages to musclefollowed by a second slow repletion stage. In these studies the first hour showed rapid repletion with a levelling off at approximately two hours. Additional studies have monitored muscle glycogen patterns for three, four or five hours (Costill et al, 1983; Richter et al, 1982; Blawacka, 1978; Terjung et al,

1974). The five hour time was chosen as there was considerable repletion with ad-lib feeding at five hours post-exercise, making comparisons at this time useful (Blawacka, 1978).

3.4 EXPERIMENTAL DAY

At 11 pm prior to the experimental day, the rats' food was taken away to ensure a post-absorptive state for the experiment. Each experimental day began at 9 am. Rats exposed to 12 hours of light and 12 hours of dark have shown gradual increases in muscle glycogen during the dark period (Conlee et al, 1976). The increases in skeletal muscle glycogen were directly related to the increases in food consumption during these times. Following the ingestion of food, blood glucose values rose somewhat as did insulin levels. Glucagon levels remained steady throughout the 24 hours. Animals that had been fasted for 24 hours and sacrificed at the beginning of the light cycle showed marked decreases in skeletal muscle glycogen (FOG -47%, FG -29%, SO -49%) whereas, animals sacrificed at the beginning of the dark cycle did not show the dramatic reductions (FOG -23%, FG ~-15%, SO -8%) (Conlee et al, 1976).

Six rats representing a random selection from all groups were treated on each experimental day. Pre-exercise blood samples were taken and pre-exercise rats were sacrificed and muscle and liver samples excised. The other animals were then placed on the treadmill. The rats ran at a speed of 55 m/min with work and rest times of 10 seconds as during the third week

of acclimatization. After 10 minutes there was a five minute rest. Following the rest, the same protocol of intermittent work was used and this cycle was repeated for one hour at which time the rest period was taken out. The exercise continued until the rats could no longer maintain the velocity. When the rats could not remain on the running belt for three consecutive 10 second work bouts the exercise was terminated. The rats were then fed according to their experimental group allocation. Those rats receiving glucose (G), fructose (F) or glucose and fructose (G&F) were fed 2 ml of the appropriate, 10% solution by gavage needle. The animals in the control (Con) group were placed in a cage where they had free access to rat chow. All post-exercise animals were allowed free access to water.

At the end of the exercise, post-exercise blood samples were taken from the pre-exercise sampled group. Additional blood samples were taken as previously stated. The animals were sacrificed and muscle and liver samples obtained according to previous experimental allocation.

3.5 EXPERIMENTAL PROCEDURES

3.5.1 Blood Samples

The rats' tails were slightly nicked with scissors and the blood was collected in heparinized capillary tubes. It was then centrifuged and the plasma transferred to non-heparinized capillary tubes and frozen for future analyses (Appendix B).

3.5.2 Gavage Feeding

The gavage feeding consisted of 2 mls of a 10% solution. The 10% solution was glucose, fructose or glucose&fructose. The glucose&fructose combination was a 10% solution of which half was made up of glucose and the other half was made up of fructose.

A 10% concentration was chosen for several reasons. Pilot results with a 50% solution revealed a supercompensation of muscle glycogen within one to two hours. No clear pattern of repletion could be detected due to extremely high glycogen values in the muscles. Values for the red vastus (RVas) and soleus (Sol) exceeded pre-exercise values at the one hour time when the animals were fed glucose (Pre RVas 4.14 mg/g, One Hour Post RVas 4.39 mg/g; Pre Sol 7.38 mg/g, One Hour Post Sol 9.22 mg/g). At two hours the glucose fed animals had muscle glycogen concentrations 50% higher than pre-exercise levels in the RVas and 75% higher than pre-exercise levels in the Sol.

In Costill et al's (1983) study muscle glycogen concentrations exceeded pre-exercise levels after three hours with glucose and fructose feedings of 20% solutions. In this study the average glycogen concentrations of the Sol, RVas, white vastus (WVas) and Plantaris (Plan) were given and only the concentrations three hours post-exercise were reported. In comparison to the pilot study using the 5 and 10% concentrations it was felt that the 20% concentration was too high and did not represent a normal feeding following exercise.

Pilot results using 5 and 10% solutions did not show the

same high muscle glycogen concentrations after five hours. It was found that the 5 and 10% fructose-fed groups had higher muscle glycogen concentrations in RVas after five hours than the glucose-fed group. However, the 10% solutions did not show as dramatic a decrease in blood glucose after two hours as the 5% solution. In light of this finding it was felt that the 5% solution was too dilute for the purposes of extended post-exercise blood glucose monitoring (Appendix A).

On a day to day basis it was found that rats normally consume approximately 3.3 to 3.8% carbohydrate per waking hour (Correspondence with Dr. Keeney, University of Ottawa Veterinary and Animal Services). If the rat was glycogen depleted, a 5% solution would not appear to be sufficient to restore the glycogen as the average intake over two hours would be below normal while a 10% solution closely approximated normal conditions. Therefore, it was decided that a 10% solution would be a better concentration with which to contrast glucose and fructose induced glycogen repletions under relatively "normal" feeding conditions.

3.5.3 Sacrifice Procedure

At the allocated time, the animals were given an intraperitoneal injection of 0.4 ml of sodiumpentobarbitol. Pilot results indicated that after four or five minutes the animals were anesthetized at which time the abdominal and thoracic cavities and ventricles were incised resulting in immediate

death.

3.5.4 Muscle and Liver Dissection

Immediately following the sacrifice of the animal, a small amount of liver tissue was excised, (approximately 50 mg) via the abdominal opening for the purpose of comparing the depletion and repletion of the liver to that of muscle.

Pilot results indicated that the plantaris, a mixed muscle, consisting of 94% fast-twitch fibres (53% red and 41% white) and 6% slow-twitch fibres in rodents (Ivy and Holloszy, 1981; Fell et al, 1980) repleted rapidly. As well, the selection of the intermittent running protocol facilitated recruitment of this muscle and its fast-twitch fibres.

Following liver tissue excision, the skin from the animals' right hindlimb was reflected to reveal the superficial lower leg muscles. The Achilles tendon was cut and reflected to separate the gastrocnemius and plantaris from the deeper soleus (Armstrong and Laughlin, 1983). The plantaris was removed and two samples, weighing approximately 35 to 50 mg were taken. The samples were immediately frozen in liquid nitrogen and stored frozen in scintillation vials in the laboratory freezer at -50°C. The muscle and liver samples were later assayed for glycogen (Appendix C).

3.6 STATISTICAL ANALYSES

The statistical analyses consisted of comparisons of the animals' weights and run times, muscle and liver glycogen and blood glucose concentrations at different times and under different nutritional conditions. The analyses were conducted in four phases.

The first phase compared the animals' weights and run times between groups. A one-way analysis of variance was used (ANOVA). In the second phase a one-way ANOVA was also used to compare pre-exercise and immediate post-exercise muscle and liver glycogen and blood glucose concentrations.

The third phase of analyses involved first the muscle glycogen values as the dependent variable and then the liver glycogen values. The independent variables were time (one, two and five hours) and nutritional treatment (G, F, G&F and Con). A two-way ANOVA was used to analyse this data.

In the fourth phase of analysis the blood glucose concentrations constituted the dependent variable. Nutritional treatments and time were the independent variables. In the first part of the analysis the blood glucose levels at the following times were used: immediate post-exercise, 0.5 hours post-exercise, one hour post-exercise and two hours post-exercise. In the second part of the analysis the following times were used: immediate post-exercise, 0.5 hours post-exercise, one hour post-exercise, two hours post-exercise, three hours post-exercise, four hours post-exercise and five hours post-exercise.

Chapter IV

RESULTS AND DISCUSSION

4.1 RESULTS

The purpose of this study was to compare glycogen repletion in the plantaris muscle and liver following feedings of glucose, fructose, glucose&fructose and regular rat chow after exhaustive exercise. Blood glucose was monitored during this time to support the results of glycogen repletion.

The following data was used as the basis for comparison: a) the mean weights and run times for each group b) the pre-exercise and post-exercise concentrations of glycogen in plantaris muscle and liver and blood glucose c) the glycogen concentrations of the plantaris muscle and liver and blood glucose concentrations at specific times during a five hour recovery period.

4.1.1 Weights and Run Times

A three week period of acclimatization was directed at training running skill to a degree which would allow a significant plantaris muscle and hepatic glycogen depletion on the experimental day. All rats, including the pre-exercise group, could perform the exercise task as for the third week of acclimatization.

Prior to the experimental day the rats were randomly assigned to experimental groups. To determine whether or not any group had different characteristics from other groups, a one-way ANOVA was conducted comparing the animals' mean weights and mean run times per group on the experimental day. No significant differences between any groups were found (Tables 5 and 6) indicating that the experimental groups were similar with respect to weight and run times within and between experimental days.

Table 5 Mean Body Weights of Groups on Experimental Day

Experimental Group	N	Mean (g)	+/- SD	SE
Pre Exercise	6	303	+/- 22	9
Immediate Post Exercise	6	313	+/- 13	5
Glucose 1 Hour Post	6	302	+/- 10	4
Glucose 2 Hours Post	6	304	+/- 18	7
Glucose 5 Hours Post	6	320	+/- 24	10
Fructose 1 Hour Post	6	306	+/- 16	6
Fructose 2 Hours Post	6	308	+/- 9	4
Fructose 5 Hours Post	6	312	+/- 23	9
Glucose&Fructose 1 Hour Post	6	311	+/- 10	4
Glucose&Fructose 2 Hours Post	6	298	+/- 9	4
Glucose&Fructose 5 Hours Post	6	311	+/- 18	7
Control 1 Hour Post	6	319	+/- 23	9
Control 2 Hours Post	6	305	+/- 14	6
Control 5 Hours Post	6	306	+/- 16	6
Grand Mean	84	308	+/- 16	6

Table 6. Mean Run Times of Groups on Experimental Day

Experimental Group	N	Mean (min)	+/- SD	SE
Immediate Post Exercise	6	65	+/- 24	10
Glucose 1 Hour Post	6	79	+/- 27	11
Glucose 2 Hours Post	6	86	+/- 27	11
Glucose 5 Hours Post	6	79	+/- 25	10
Fructose 1 Hour Post	6	73	+/- 13	5
Fructose 2 Hours Post	6	86	+/- 16	7
Fructose 5 Hours Post	6	81	+/- 18	7
Glucose&Fructose 1 Hour Post	6	83	+/- 32	13
Glucose&Fructose 2 Hours Post	6	81	+/- 15	6
Glucose&Fructose 5 Hours Post	6	79	+/- 10	4
Control 1 Hour Post	6	61	+/- 17	7
Control 2 Hours Post	6	76	+/- 18	7
Control 5 Hours Post	6	79	+/- 13	5
Grand Mean	78	78	+/- 20	8

4.1.2 Pre-Exercise and Post-Exercise Comparisons

The effects of the exercise and feedings on the blood glucose concentrations is shown in Tables 7 and 8. The mean post-exercise blood glucose concentration was reduced to 60% of the mean pre-exercise value ($p<0.05$).

Table 7. Mean Blood Glucose Concentrations - Pre and Post Exercise

Experimental Group	N	Mean (mg/100ml)	+/- SD	SE
Pre Exercise	10	109.6	+/- 7.6	2.4
Post Exercise	10	63.5	+/- 21.0	6.6

Table 8. ANOVA of Blood Glucose Concentrations Pre and Post Exercise

Source of variation	Sum of Squares	df	Mean Square	FObs
Between Groups	21270.67	1	21270.67	85.52*
Residual	9451.36	38	248.72	

*significant at the $p<0.05$ level

Similarly, the glycogen concentrations in the plantaris muscle and in the liver (Tables 9 and 10) were approximately 20% of the mean pre-exercise values ($p<0.05$). This implies that the exercise was of sufficient intensity, duration and specificity to reduce glycogen reserves in these locations.

Table 9. Mean Glycogen Concentrations for the Plantaris Muscle - Pre and Post Exercise

Experimental Group	N	Mean (mg/g)	+/- SD	SE
Pre Exercise	6	4.93	+/- 1.74	0.71
Post Exercise	6	0.96	+/- 0.49	0.20

Table 10. Mean Glycogen Concentrations for the Liver - Pre and Post Exercise

Experimental Group	N	Mean (mg/g)	+/- SD	SE
Pre Exercise	6	5.31	+/- 4.37	1.79
Post Exercise	6	1.12	+/- 0.23	0.09

Table 11. ANOVA of Plantaris Muscle Glycogen Concentrations - Pre and Post Exercise

Source of variation	Sum of Squares	df	Mean Square	FObs
Between Groups	47.283	1	47.283	29.080*
Residual	16.260	10	1.626	

*significant at the $p<0.05$ level

Table 12. ANOVA of Liver Glycogen Concentrations - Pre and Post Exercise

Source of variation	Sum of Squares	df	Mean Square	FObs
Between Groups	52.459	1	52.459	5.474*
Residual	95.833	10	9.583	

*significant at the $p<0.05$ level

4.1.3 Plantaris Muscle and Liver Glycogen and Blood Glucose Comparisons

From Table 13 and Figure 5 it can be seen that the plantaris muscle repleted considerably after one hour of recovery under all conditions. At one hour post-exercise the glucose-fed group repleted the most while the fructose-fed group repleted the least. However, the differences between the feedings were not statistically significant (Table 14 and Appendix E).

Blood glucose concentrations (Tables 15 and 16 and

Figures 7 and 8) at 0.5 hours and muscle glycogen concentrations (Table 13 and Figure 5) at one hour appeared to be related. The blood glucose concentrations were statistically significant at 0.5 hours, except between the fructose-fed group and the control group (Tables 17 and 18, Appendix E).

Table 13. Mean Plantaris Muscle Glycogen Concentrations of Experimental Groups

Experimental Group	N	Mean (mg/g)	+/- SD	SE
Pre Exercise	6	4.93	+/- 1.74	0.72
Immediate Post Exercise	6	0.96	+/- 0.49	0.20
Glucose 1 Hour Post	6	4.40	+/- 0.90	0.37
Glucose 2 Hours Post	6	3.43	+/- 1.46	0.60
Glucose 5 Hours Post	6	4.11	+/- 1.51	0.62
Fructose 1 Hour Post	6	2.73	+/- 1.04	0.42
Fructose 2 Hours Post	6	2.69	+/- 0.73	0.30
Fructose 5 Hours Post	6	4.48	+/- 1.24	0.51
Glucose&Fructose 1 Hour Post	6	3.37	+/- 1.71	0.70
Glucose&Fructose 2 Hours Post	6	4.01	+/- 1.29	0.53
Glucose&Fructose 5 Hours Post	6	3.88	+/- 1.01	0.41
Control 1 Hour Post	6	3.91	+/- 2.06	0.84
Control 2 Hours Post	6	3.98	+/- 1.33	0.54
Control 5 Hours Post	6	5.67	+/- 1.61	0.66

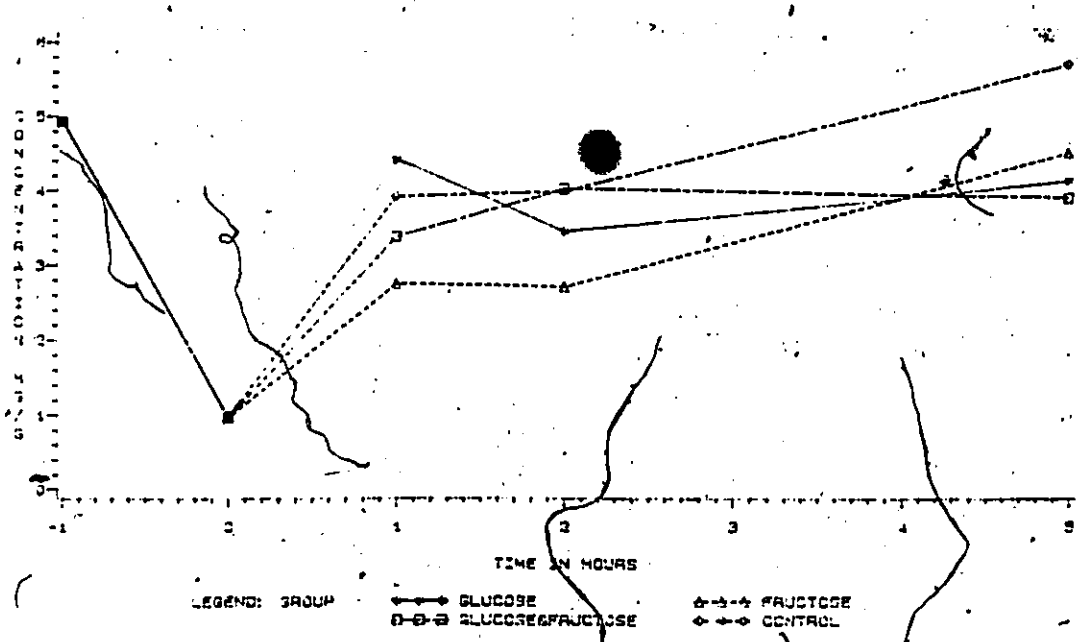

FIGURE 5. PLANTARIS MUSCLE GLYCOGEN CONCENTRATIONS OVER TIME

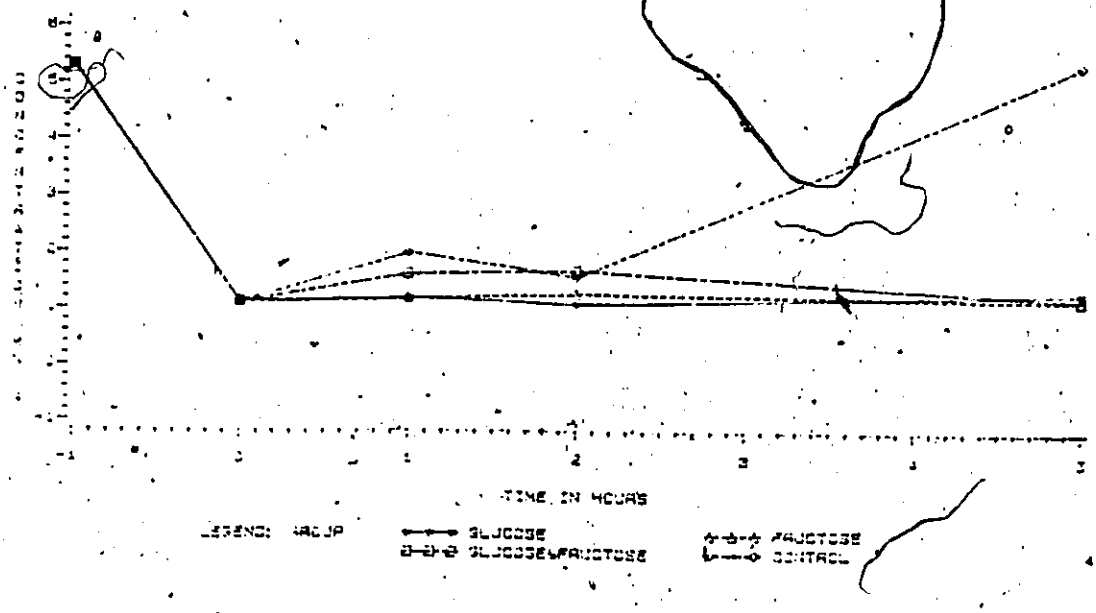

FIGURE 6. LIVER GLYCOGEN CONCENTRATIONS OVER TIME

Table 14. ANOVA of Plantaris Muscle Glycogen Concentrations

Source of variation	Sum of Squares	df	Mean Square	FObs
Fuel (A)	13.795	3	4.598	2.442
Time (B)	15.163	2	7.582	4.025*
B at G	2.998	2	1.499	0.796
B at F	12.530	2	6.265	3.327*
B at G&F	1.375	2	0.687	0.365
B at Con	11.972	2	5.986	3.179*
Two way interaction				
A x B	13.706	6	2.284	1.213
Residual	113.007	60	1.883	

* significant at $p<0.05$ level

Table 15. Mean Blood Glucose Concentrations (mg/100ml) Over Two Hours +/- SD and SE (N=12)

Time	0	0.5	1	2
Experimental Group				
Glucose	62.1	187.7	134.0	105.7
SD	16.7	32.8	20.2	10.9
SE	4.8	9.5	5.8	3.1
Fructose	67.2	127.9	134.9	130.4
SD	15.2	18.6	11.9	33.6
SE	4.4	5.4	3.4	9.7
Glucose & Fructose	65.7	157.5	132.7	120.3
SD	19.5	31.1	26.8	19.9
SE	5.6	9.0	7.7	5.7
Control	69.6	123.1	153.1	152.7
SD	23.9	16.7	17.1	15.9
SE	6.9	4.8	4.9	4.6

Table 16. Mean Blood Glucose Concentrations (mg/100ml) Over Five Hours +/- SD and SE (N=6)

Time	0	0.5	1	2	3	4	5
Experimental Group							
Glucose	63.4	205.0	144.4	102.3	90.1	84.8	84.7
SD	17.4	25.3	8.5	12.7	13.0	8.7	5.9
SE	7.1	10.3	3.5	5.2	5.3	3.6	2.4
Fructose	62.4	126.1	133.0	111.2	97.1	85.4	86.3
SD	12.8	18.3	16.7	12.0	10.3	13.4	15.9
SE	5.2	7.5	6.8	4.9	4.2	5.5	6.5
Glucose & Fructose	60.1	153.1	124.9	110.9	94.2	87.8	88.4
SD	21.5	30.6	22.0	14.2	16.2	12.9	16.3
SE	8.8	12.5	9.0	5.8	6.6	5.3	6.7
Control	64.0	131.1	160.9	151.1	134.6	125.5	124.9
SD	22.6	11.7	11.3	11.6	11.3	9.0	22.4
SE	9.2	4.8	4.6	4.7	4.6	3.7	9.1

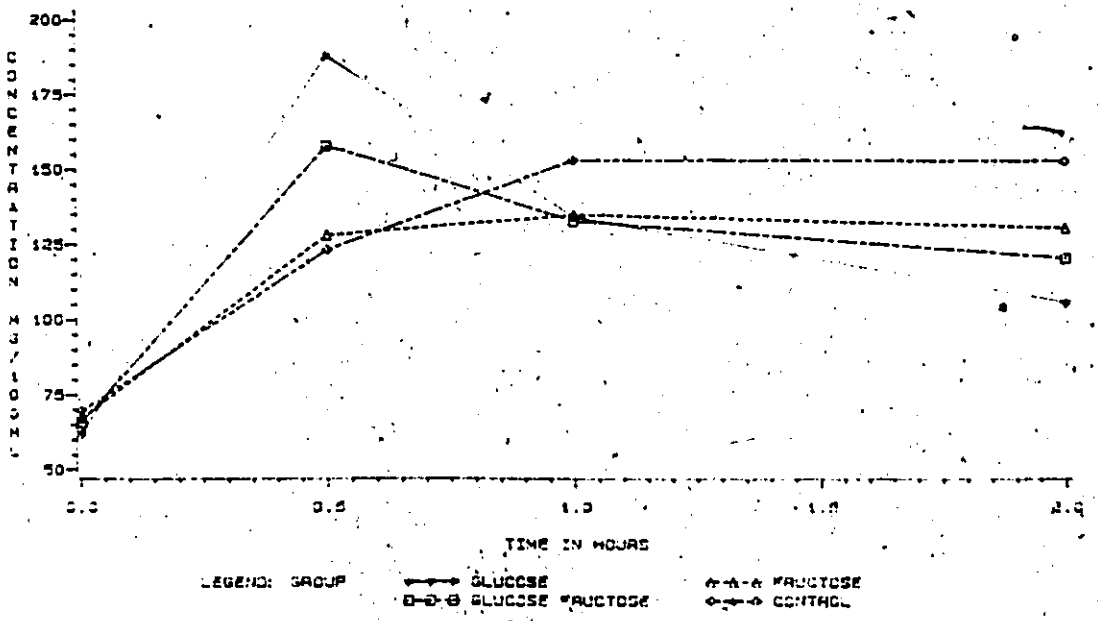

FIGURE 7. BLOOD GLUCOSE CONCENTRATIONS OVER TWO HOURS

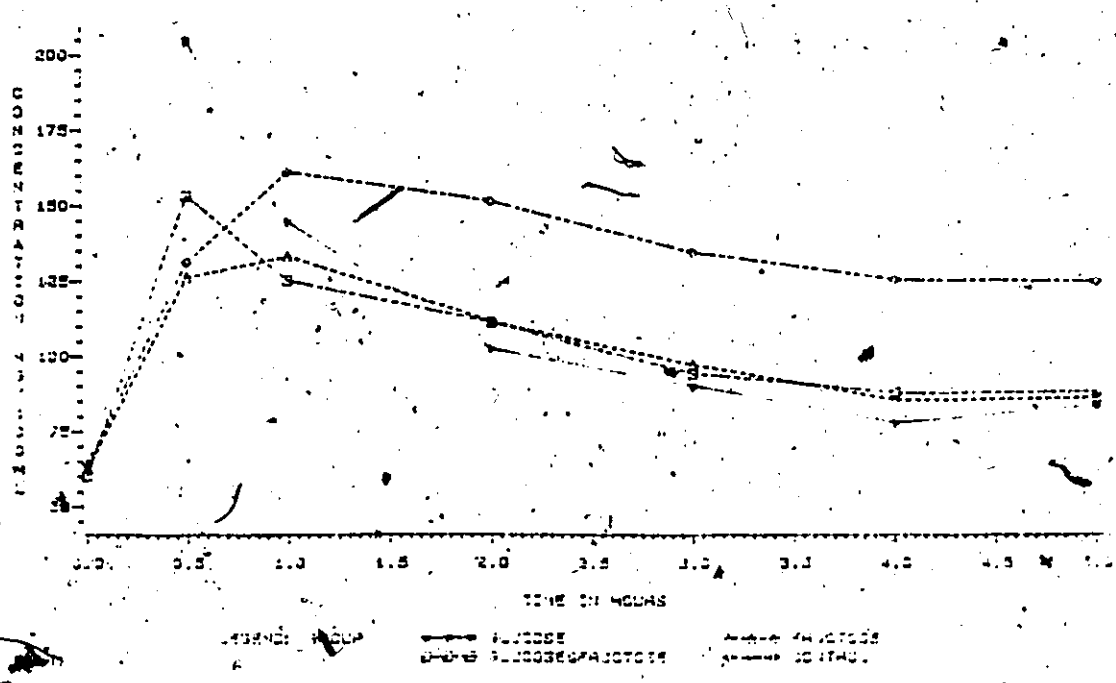

FIGURE 8. BLOOD GLUCOSE CONCENTRATIONS OVER FIVE HOURS

Table 17. Two Way ANOVA with Repeated Measures for Blood Glucose Concentrations Over Two Hours

Source of variation	Sum of Squares	df	Mean Square	FObs
Fuel (A)	2482.15	3	827.38	<1.00
A at Oh	362.20	3	120.73	<1.00
A at 0.5h	32373.87	3	10757.62	21.45*
A at 1 h	3356.40	3	1118.80	2.23
A at 2 h	14075.55	3	4691.85	9.35*
Residual Between	46188.08	44	1049.73	
Residual Within'	88278.41	176	501.58	
Time (B)	199001.19	3	66333.73	208.03*
B at G	99841.81	3	33280.60	104.37*
B at F	36954.82	3	12318.60	38.63*
B at G&F	54258.26	3	18086.09	56.72*
B at Con	55538.52	3	18512.84	58.06*
Two way interaction A x B	47591.68	9	5287.96	16.58*
Residual Within	42090.33	132	318.87	

* significant at $p<0.05$ level

Table 18. Two Way ANOVA with Repeated Measures for Blood Glucose Concentrations Over Five Hours

Source of variation	Sum of Squares	df	Mean Square	FObs
Fuel (A)	18995.91	3	6331.97	7.37*
A at 0 h	52.21	3	17.40	0.067
A at 0.5 h	23377.19	3	7792.40	29.83*
A at 1 h	4387.58	3	1462.53	5.60*
A at 2 h	8624.25	3	2874.75	11.01*
A at 3 h	7631.85	3	2543.95	9.74*
A at 4 h	7033.70	3	2344.57	8.98*
A at 5 h	6700.16	3	2233.39	8.55*
Residual Between	17177.25	20	858.86	
Residual Within'	36567.47	140	261.20	
Time (B)	135319.99	6	22553.33	139.58*
B at G	84629.57	6	14104.93	87.29*
B at F	22359.13	6	3726.52	23.06*
B at G&F	32440.90	6	5406.82	33.46*
B at Con	34693.53	6	5782.26	35.78*
Two way interaction A x B	38826.49	18	2157.03	13.35*
Residual Within	19390.22	120	161.59	

* significant at $p<0.05$ level

At two hours post-exercise muscle glycogen had not yet reached pre-exercise levels. There did not appear to be a relationship between one hour blood glucose concentrations and two hour muscle glycogen concentrations. However, at these times the control group showed a significant rise in blood glucose compared to the glucose-fed, fructose-fed and glucose-fructose-fed groups; an increase which was paralleled by rising muscle glycogen concentrations in the control group.

There was some discrepancy between Figures 7 and 8 as to the differences or similarities between the glucose-fed group and the control group at one hour post-exercise. In Figure 8 the blood glucose concentrations of the glucose-fed group and the control group appear to be somewhat close together. This does not appear to be the case in Figure 7 where all three experimentally-fed groups are similar. Further statistical examination showed that the glucose-fed group was different from the control group at one hour, in the animals monitored over two hours, but was similar in the animals monitored over five hours (G vs Con -4.08 * N=12; G vs Con -3.65 N=6 (Appendix E)). The larger sample size of the blood glucose comparisons over two hours is a result of combining the data from the animals monitored for five hours with the animals monitored for two hours; therefore, the larger sample size makes the comparison over two hours more powerful suggesting that there was a difference in blood glucose between the glucose-fed group and the control group at one hour post-exercise.

The continuing decline in blood glucose after one hour of recovery appeared to restrict repletion, which did not progress to any great extent after two hours. The fructose-fed group and the control group did show some trend toward continued repletion; but their values were not significantly different from the other two groups (Appendix E). The two, three, four and five hour blood glucose concentrations rose progressively only within the control group and this was reflected in the highest blood glucose concentrations and muscle glycogen concentrations at five hours

being measured in this group (Appendix E).

The liver glycogen following experimental feedings did not increase above post-exercise values until five hours post-exercise (Tables 19 and 20, Figure 6). However, values in the control group had regained near pre-exercise values at five hours post-exercise and were significantly different from the five hour values following the other experimental feedings as well as the one and two hour control group values (Appendix E). Although sequential repletions favouring one tissue over another were not planned for in this experiment, the retarded hepatic repletions suggest a predisposition toward regeneration of muscle glycogen prior to a significant hepatic repletion.

In summation, muscle glycogen repletion occurred following intake of all substrates, approaching pre-exercise levels at five hours post-exercise. This occurred to the greatest extent in the control group although there were no significant differences between any of the feedings on the basis of comparing repletions at one, two and five hours. It appeared from graphical comparisons that a weak relationship between blood glucose concentration and muscle glycogen. Even though there appeared to be faster initial loading with a single glucose feed, the repletion in the muscle was not significantly higher than with the other feedings and was not progressive after one hour. Consequently, as there was a lack of significance in the muscle glycogen values and because the blood glucose data at 0.5 hours and the muscle glycogen data at one hour came from different

animals, no correlational statistics were attempted.

Table 19. Mean Liver Glycogen Concentrations of Experimental Groups

Experimental Group	N	Mean (mg/g)	+/- SD	SE
Pre Exercise	6	5.31	+/- 4.37	1.78
Immediate Post Exercise	6	1.12	+/- 0.23	0.09
Glucose 1 Hour Post	6	1.19	+/- 0.26	0.11
Glucose 2 Hours Post	6	1.08	+/- 0.30	0.12
Glucose 5 Hours Post	6	1.32	+/- 0.29	0.12
Fructose 1 Hour Post	6	1.18	+/- 0.30	0.12
Fructose 2 Hours Post	6	1.27	+/- 0.28	0.11
Fructose 5 Hours Post	6	1.18	+/- 0.29	0.12
Glucose&Fructose 1 Hour Post	6	1.62	+/- 0.59	0.24
Glucose&Fructose 2 Hours Post	6	1.68	+/- 0.44	0.18
Glucose&Fructose 5 Hours Post	6	1.19	+/- 0.40	0.16
Control 1 Hour Post	6	1.99	+/- 1.54	0.63
Control 2 Hours Post	6	1.57	+/- 0.92	0.38
Control 5 Hours Post	6	5.34	+/- 6.02	2.46

Table 20. ANOVA of Liver Glycogen Concentrations

Source of variation	Sum of Squares	df	Mean Square	FObs
Fuel (A)	38.353	3	12.784	3.771*
A at 1h	2.675	3	0.892	0.263
A at 2h	1.349	3	0.450	0.133
A at 5h	76.150	3	25.383	7.488*
Time (B)	10.648	2	5.324	1.570
Two way interaction A x B	41.843	6	6.974	2.057
Residual	203.425	60	3.390	

* significant at $p<0.05$ level

4.2 DISCUSSION

The statistical analyses indicated a specific inter-group homogeneity in that the groups did not differ with respect to the body weights and run times on the experimental day. Therefore, there can be some confidence that glycogen repletion would not have been affected by the grouping of the animals.

The high intensity exercise depleted the plantaris muscle and liver glycogen (plantaris post-exercise=19% of pre-exercise; liver post-exercise=21% of pre-exercise). Blood glucose concentrations were reduced to 60% of pre-exercise values. Depletion of glycogen and reduction of blood glucose concentrations were evident in previous studies although absolute values were different (Conlee et al, 1978; Terjung et al, 1974; Costill et al, 1983). In both Conlee's and Terjung's studies the animals swam to exhaustion, the pre-exercise values were from resting, fed controls and three homogeneous muscle types were analysed. Costill used moderate intensity treadmill running and averaged the glycogen values from the three more homogeneous muscles and the plantaris. In the present study treadmill running continued at a high intensity until the animals could no longer maintain the running velocity, pre-exercise values were taken from rats on the experimental day after an overnight fast and only the plantaris muscle was examined.

The aforementioned experimental differences would account for the discrepancies between this study and the others in the following ways. It has previously been noted that swimming load-

not deplete muscle glycogen to the extent that treadmill running does (Armstrong et al, 1974). This coupled with the finding that higher intensity exercise relies primarily on muscle glycogen, would explain why the animals' muscles in the present study were depleted to a greater extent than in the Conlee, Terjung and Costill studies. Furthermore, as the high intensity exercise of this study was discontinued when the animals could no longer maintain the speed, the contribution from the liver should not have been to the extent that would be involved in exhaustive activity. Similarly, the exhaustive activities would account for the lower blood glucose concentrations. As well, the pre-exercise levels that were used for comparing pre-exercise and post-exercise values were taken from rats that were fed and rested rather than following an overnight fast as performed by the present study. Finally, comparing three homogeneous muscles, or the average of the three, with a mixed muscle (plantaris) permits only rough comparisons. However, highly significant depletions were detected in the present study and verify the effectiveness of this exercise protocol in reducing muscle glycogen.

The literature has noted that low muscle glycogen itself favours glycogen repletion (Garetto et al, 1984; Richter et al, 1984). When the muscle is being repleted it does so in two stages. First there is a rapid short phase of usually less than one hour and then a slower long phase from one hour until the glycogen reaches or surpasses pre-exercise values (Garetto et al, 1984; Richter et al, 1984). In this study the 0.5 hour blood

glucose values with glucose feeding seem to demonstrate the first phase. To some extent this was carried over to the plantaris muscle as glycogen was higher following the glucose feeding at one hour. However, no significant differences between groups were found after one hour of recovery.

The lack of significant differences in muscle glycogen between groups in the face of differences in blood glucose suggested that repletion was not sensitive to blood glucose providing minimum blood levels were attained. This lack of sensitivity of muscle repletion to blood glucose concentration beyond a minimum level was supported by the lack of any trend between blood glucose values at one hour, and glycogen values at two hours.

However continuous ad-lib feeding resulted in a progressive rise in glucose throughout recovery and higher muscle glycogen concentrations in the control group at five hours. As previously stated, the rat chow was composed of approximately 58% carbohydrate, but the carbohydrate components and percentages were variable. It would therefore appear that the final breakdown of carbohydrates could have resulted in the release of some percentage of glucose and fructose as well as other sugars into the bloodstream. Prior to the release of the simple sugars into the blood, the complex carbohydrates were digested which would increase the time that was taken for these sugars to be absorbed in the intestine and released into the blood. As well, the additional fibre, fat and protein would have increased the gastric ejection and absorption time resulting in slower

increases in blood concentrations. The actual volume of consumption was unknown, but it is known that the rats fed continuously. This continuous feeding combined with the slower absorption may have been the explanation for the improved repletion which was observed.

It was originally hypothesized that fructose ingestion would result in slower but progressive muscle glycogen increases; however, a slow persistent increase in blood glucose concentration was also anticipated. The increase in muscle glycogen repletion of the fructose-fed group from two to five hours was not identifiable in this study as the single, post-exercise bolus of all of the substrates resulted in what was presumed to be an insulin induced decline in blood glucose after two hours. Even without the distinct differences between the glucose-fed and fructose-fed groups, the fructose-fed group did closely resemble the control group. Absolute values for the control and fructose groups were different, but because of the similarities between them, benefits of fructose as a fuel for repletion cannot be ignored.

Glycogen values reached pre-exercise levels only in the control group five hours after exercise. The repletion process was not characteristic of the pattern seen by some previous studies (Garetto et al, 1984; Richter et al, 1984; Terjung et al, 1974; Conlee et al (1978). Garetto et al (1984) noted the rapid rise followed by the slow steady rise in glycogen in their perfusion studies. Terjung et al (1974) noted supercompensation in the red vastus in two hours post-exercise and in the soleus in

four hours post-exercise, but did use five times the concentration of glucose. Conlee et al (1978) reported muscle glycogen repletion to pre-exercise levels in the red vastus, almost 90% repletion in the soleus and 60% repletion in white vastus four hours post-exercise with two and one half times the concentration of glucose. He also noted that the rate of repletion was rapid in the first hour following exercise. There was, however, agreement with Costill's (1983) study, where one type of feeding did not give better repletion than another. It appeared that the substrate involved was not as important as the maintenance of blood glucose at some criterion level; a level which is not identifiable under these experimental conditions.

Glycogen repletion in a mixed muscle such as the plantaris does not occur in all fibre types identically. It was not possible to distinguish the rate at which the specific fibre types were repleted in this study, but it can be speculated that in accordance with the observations of Terjung et al (1974) and Conlee et al (1978) (supra pp. 25-27) the fast-twitch red fibres repleted first followed by the slow-twitch red and then the fast-twitch white. Thus, while the use of whole muscle homogenates as has been done in this study, does not show an effect of substrate on repletion times, the possibility of differential repletion times as a function of fibre type still exists as a topic for future study.

It was observed that the glucose feeding promoted a rapid rise in blood glucose at 0.5 hours in this and other studies (187.7 mg/100ml, present study; 120 mg/100 ml Terjung et al,

1974; 133.6 mg/100ml Conlee et al, 1978). It would seem that if the lower concentration of glucose feeding (10%) could elicit a rapid rise in blood glucose concentrations, then this feeding concentration would be sufficient. The problems of gastric distress with the high sugar concentrations would be reduced (Foster, et al, 1980) and the maintenance of the blood glucose levels by additional high concentration feedings may prove less stressful to the organism, especially in humans where repletion is carried out over extended periods.

The control group fed as often as they wanted, resulting in slower but progressive increases of blood glucose values ultimately exceeding those following other feedings and, subsequently, resulted in higher muscle glycogen values. Fell et al (1982) has stated that under exercise conditions when glucose transport was high, the rate-limiting step in glucose utilization was the ability to synthesize glycogen from glucose. Under most conditions the transport of glucose across the cell membrane was the rate-limiting step (Tan et al, 1984; Richter et al, 1984; Richter et al, 1982; Fell et al, 1982). Under the conditions of this experiment the glucose did not appear to be available for conversion and thus hindered the repletion of glycogen.

The only notable change in liver glycogen was in the control group at five hours. The liver repletes continuously (Blawacka, 1978) and it too appeared to require the maintenance of blood glucose concentrations to attain pre-exercise levels. The liver glycogen values of this study cannot be compared with the values in other studies as higher concentrations of

substrates were ingested. Costill's study did show statistically similar liver glycogen repletion with glucose and fructose after three hours although the absolute values are quite different due to the 20% concentrations of the substrates ingested (6.74 mg/g glucose, 6.97 mg/g fructose at three hours post-exercise, Costill; 5.34 mg/g control at five hours, present study).

In summary, the type of substrate did not appear to affect glycogen repletion but the maintenance of a criterion level of blood glucose did affect repletion. Any superiority of feeding type at thirty minutes was not reflected in glycogen repletion.

The selection of a 10% concentration for feeding was arrived at by an estimation of carbohydrate intake in a rat's daily diet. In an average "waking" hour a rat ingests approximately a 3.5% carbohydrate solution (rat chow and water) (correspondence with Dr. M. Keeney, University of Ottawa Veterinary and Animal Services). In a severely depleted state, such as following exercise, a larger concentration of glucose than normal would be required. Glucose, a simple sugar, enters the bloodstream rapidly and is directly taken up by the muscle. Fructose must first be transported to the liver for conversion to glucose before being taken up by the muscle forming glycogen. A glucose and fructose feed should span the time period achieved by the two separate substrates. Initially low muscle glycogen promotes glycogen synthesis itself. Then insulin regulates glycogen synthesis especially by promoting the conversion of

glycogen synthase to its active form. When blood glucose is increased, insulin works to reduce the glucose concentration of the blood thereby increasing the muscle and/or liver glycogen content. It would seen reasonable to deduct, from this information and from the results found in this study, that subsequent feedings with a 10% sugar concentration would progressively elevate muscle glycogen. The 10% solution used did not produce either a severe hyperglycemia or a subsequent hypoglycemia following the highest increase at 0.5 hours and would appear to be less stressful to the animal's digestive and hormonal system.

Generalizing to humans, it would seem that large amounts of simple sugars following a training session or game may not be required to replete glycogen stores. A usual well-balanced diet with some supplementation, particularly with regard to short interval, intermittent feedings, may therefore be adequate compensation for the athlete and avoid any problems associated with high concentration feeding. It is also possible that the immediate blood concentration response to glucose ingestion followed by the slower and more progressive response to fructose and rat chow point to the benefits of some sort of "phasic" feeding. With this type of feeding the "pure" substrate is beneficial only at the outset and then only in modest concentrations while the second phase is promoted through more "normal", intermittent nutritional processes and compositions. However, specific research on human glycogen repletion will be necessary to test these speculations.

Chapter V

CONCLUSIONS AND RECOMMENDATIONS

5.1 CONCLUSIONS

The purpose of this study was to investigate plantaris muscle and liver glycogen repletion following different feedings after high intensity exercise. Blood glucose concentrations were also monitored for five hours following the exercise.

It was hypothesized that the glucose feedings would reveal high blood glucose concentrations soon after the feeding was administered and that this would be reflected in rapid muscle glycogen repletion. It was further hypothesized that the fructose would produce less dramatic but continuous increases in blood glucose favouring continuous repletion of muscle glycogen. The results from the glucose&fructose feeding would be between those of glucose and fructose.

Conclusions

The results lead to the following conclusions, for the conditions of this study:

(1) No experimental feeding in this study resulted in significantly different levels of muscle glycogen repletion at two and five hours;

(2) Liver glycogen was significantly more repleted by ad-lib rat chow feedings at five hours.

(3) The post-exercise glucose feeding gave higher blood glucose at 0.5 hours than fructose, glucose&fructose or rat chow;
(4) Glucose, fructose and glucose&fructose combined were not different in their effects on blood glucose after 0.5 hours;
(5) Rat chow consumed ad-lib in total concentrations similar to the other feedings resulted in stable blood glucose at significantly higher levels than the other feedings;

It is however worth noting that there was a non-significant trend toward higher muscle glycogen repletion at five hours parallel to the continued increase in blood glucose following continuous ad-lib rat chow feeding; a trend that deserves further examination with regard to the effect of maintaining minimum criterion blood glucose concentration.

5.2 RECOMMENDATIONS

The following recommendations for future studies were based on the findings of this study.

1) Maintenance of blood glucose concentrations appeared to be critical for muscle glycogen repletion; therefore, the examination of this criterion level of blood glucose, following feedings of any of the substrates used in this study, would be important to the continued research in this field.

2) The superiority of the glucose feeding for the rapid increase in blood glucose concentration and muscle glycogen repletion was

seen with a 10% solution of glucose. This concentration was much lower than those used experimentally to date and it may be more beneficial to the experimental animals as the problems with high concentrations of solutions (stomach upset, loss of water, etc.) would not occur. A 10% feeding concentration would be recommended.

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Appendix A

RESULTS FROM PILOT STUDIES

Pilot Study Using 50% Feeding Concentration (N=1)

Muscle Glycogen Concentrations (mg/g)

	WVas	RVas	LGas	MGas	Sol	Plan	Liver
Pre	4.77	4.14	6.96	8.11	5.91	7.38	1.26
Post	0.35	0.38	0.47	0.36	0.91	0.47	1.24
G 1 hr	2.74	4.39	7.85	5.01	4.93	9.22	1.38
G 2 hr	3.82	6.24	10.71	10.32	7.62	12.15	1.23
G 3 hr	no data						
F 1 hr	0.71	1.99	4.45	3.66	3.03	6.25	0.88
F 2 hr	1.19	1.97	4.78	3.79	3.65	5.20	1.46
F 3 hr	1.09	1.74	5.46	4.47	3.99	5.47	1.96

Pilot Study Using 10% Feeding Concentration

Muscle Glycogen Concentrations (mg/g)

	RVas	Liver	
Pre	5.44	4.81	(N=1)
Post	1.64	1.35	(N=1)
G 5 hr	2.50	1.33	(N=3)
F 5 hr	4.67	1.21	(N=3)

Blood Glucose Concentrations (mg/100ml)

Time	Pre	Post	1 hr	2 hr	3 hr	4 hr	5 hr
	115.7	96.9					
N	8	6					
Glucose			126.2	108.2	95.3	78.1	75.9
N			3	3	2	3	3
Fructose			123.2	87.5	85.3	86.4	76.6
N			3	2	3	3	3

Pilot Study Using 5% Feeding Concentration

Muscle Glycogen Concentrations (mg/g)

	RVas	Liver
Pre	5.51	14.19 (N=1)
Post	2.52	1.38 (N=1)
G 5 hr	3.64	1.50 (N=3)
F 5 hr	4.92	1.67 (N=3)

Blood Glucose Concentrations (mg/100ml)

Time	Pre	Post	1 hr	2 hr	3 hr	4 hr	5 hr
	110.5	65.5					
N	8	7					
Glucose			115.1	99.1	89.7	84.6	90.6
N			3	2	3	3	3
Fructose			93.1	72.6	89.1	89.3	90.5
N			2	2	2	2	2

Appendix B

BLOOD GLUCOSE ASSAY

Reagents:

PGO enzyme capsules - each capsule contains 500 Units of glucose oxidase, 100 Purpurogallin units of peroxidase and buffer salts

one capsule of enzyme was added to 100 ml of distilled H_2O in an amber bottle

to this enzyme solution 1.6 ml of the Colour Reagent Solution was added

o-Dianisidine dihydrochloride - preweighed vial containing 50 mg, this is the Colour Reagent

the vial was reconstituted with 20 ml of distilled H_2O, this is the Colour Reagent Solution

Glucose standard solution - 100 mg/dl in benzoic acid, 0.1%

Procedures

1. 0.5 ml of distilled H_2O was added to a tube labelled blank.
2. 25 ul of glucose standard was added to 0.475 ml of distilled H_2O. This tube was labelled standard.
3. 25 ul of plasma was added to 0.475 ml of distilled H_2O.
4. To each tube 5.0 ml of Combined Enzyme-Colour Reagent Solution was added and mixed thoroughly.

5. All tubes were then left at room temperature for 45 minutes.

6. After the incubation period the samples were read on the Ultraspectrophotometer at 450 nanometers, using the blank as the reference. All readings were completed within 30 minutes.

The results were calculated using the following formula:

$$\text{Serum Glucose (mg/100ml)} = \frac{\text{ATest}}{\text{AStandard}} \times 100$$

Appendix C

MUSCLE GLYCOGEN ASSAY

Reagents:

30% KOH solution saturated with Na_2SO_4

300 g KOH pellets were dissolved in 1 liter distilled H_2O and saturated with Na_2SO_4

95% ethanol

5% phenol

50 g phenol crystals dissolved in 1 liter distilled H_2O

96% - 98% H_2SO_4

50 mg of glycogen powder dissolved in 10 ml distilled H_2O

working standard solutions: 0.5, 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 ml of stock solution diluted to 10 ml in a volumetric flask to obtain working standard solutions of 10, 20, 40, 60, 80, 100 and 120 ug glycogen/ml

Na_2So_4 saturated aqueous solution

Procedure:

1. After removal from frozen storage, the muscle samples were kept on solid CO_2 until they were weighed on a Mettler H balance.
2. Samples were transferred individually to stoppered tubes containing 0.5 ml of 30% KOH saturated Na_2SO_4.
3. The capped tubes were than placed in a boiling water bath for

30 minutes. They were agitated periodically.

4. The tubes were removed from the water bath and cooled in ice for 10 minutes.

5. One drop of aqueous Na_2SO_4 was added to the muscle samples.

6. 0.6 ml of 95% ethanol was added to the samples to precipitate the glycogen from the alkaline digestate.

7. After the samples were vortexed they were cooled on ice for 60 minutes.

8. The samples were then centrifuged at 4 C and 3500 X g for 60 minutes.

9. The supernatants were then aspirated carefully using disposable Pasteur pipettes.

10. The excess fluid was drained from the inverted tubes by gravity for 15 minutes.

11. The precipitated glycogen was then redisolved by adding 3 ml of distilled H_2O.

12. Two one ml aliquots of the glycogen solution were pipetted into clean test tubes.

13. One ml of 5% phenol solution was added to the aliquots.

14. Five ml of 96 -98% H_2SO_4 were rapidly added to the aliquots.

15. The tubes were allowed to cool for 10 minutes and then vortexed.

16. The tubes were placed in a water bath at 25 - 30 C for 30 minutes.

17. The tubes then stood at room temperature for 10 minutes before reading.

18. Blanks were prepared by using one ml of distilled H_2O instead of the glycogen solution.

19. The absorbance was read on an Ultraspectrophotometer at 490 nanometers.

Whenever samples were analyzed, two samples of standard glycogen solution were subjected to the same procedure.

To calculate the glycogen content of the tissue the following equation was used:

$$\text{mg glycogen/g wet tissue weight} = \frac{(A\ 490 - A)}{B} \times \frac{V}{v} \times \frac{1}{R} \times \frac{1}{W}$$

where: A 490 = Absorbance of sample at 490 nonometers

A = Y intercept

B = slope of standard curve

V = total volume of glycogen solution

v = volume of aliquot used in the colour reaction

W = weight of muscle sample in mg

R = recovery

Appendix D

RAW DATA

Glycogen Results (mg/g)

Animal Number	Plantaris Muscle	Liver
Pre-Exercise		
1R	6.140	13.674
10	2.082	1.300
15	6.329	3.581
23R	3.497	5.810
28	5.567	2.897
37	5.964	4.566
Immediate Post-Exercise		
2	0.937	1.387
9R	0.270	0.899
17R	0.686	1.208
28R	1.632	1.135
31	1.387	1.300
38R	0.838	0.798
Glucose 1 Hour Post-Exercise		
1	5.632	1.158
8	3.791	1.529
16R	4.118	1.292
25R	4.069	1.368
30R	3.408	0.935
37R	5.400	0.849
Glucose 2 Hours Post-Exercise		
3R	4.769	1.237
10R	2.479	1.598
17	1.939	0.997
26R	5.656	1.037
32R	3.133	0.853
38	2.594	0.767
Glucose 5 Hours Post-Exercise		
4	4.587	1.226
19R	2.243	1.752
23	3.290	1.443
33	3.344	1.466
39	4.619	1.009
43	6.580	1.030

Animal Number	Plantaris Muscle	Liver
Fructose 1 Hours Post-Exercise		
5	1.499	1.283
13	3.089	1.481
21R	2.942	1.377
30	2.324	1.048
35R	4.501	1.258
40	2.053	0.638
Fructose 2 Hours Post-Exercise		
8R	2.830	0.964
15R	2.375	1.061
22	1.837	1.636
29	2.056	1.175
36	3.612	1.216
41	3.428	1.568
Fructose 5 Hours Post-Exercise		
2R	3.734	1.520
9	5.438	1.346
16	3.202	1.426
24R	3.936	1.020
31R	4.079	0.877
38RR	6.497	0.884
Glucose&Fructose 1 Hour Post-Exercise		
3	5.866	2.603
11R	3.650	1.791
18R	1.502	1.551
44	1.804	1.593
32	4.723	1.384
39RR	2.656	0.793
Glucose&Fructose 2 Hours Post-Exercise		
5R	4.647	1.587
12	2.083	1.682
19	5.233	1.333
27	2.718	1.295
35	4.368	2.509
40RR	4.994	1.690
Glucose&Fructose 5 Hours Post-Exercise		
7R	2.687	1.403
14R	4.499	1.224
21	4.292	1.556
24	4.119	1.534
34R	5.084	0.889
41RR	2.594	0.549

Animal Number	Plantaris Muscle	Liver
Control 1 Hour Post-Exercise		
4R	6.893	2.022
11	2.794	1.496
18	5.620	1.454
27R	4.292	5.020
33R	1.679	1.099
39R	2.165	0.817
Control 2 Hours Post-Exercise		
6R	4.604	1.687
13R	3.219	3.274
20R	1.924	1.476
25	4.990	0.889
34	3.583	1.372
40R	5.543	0.680
Control 5 Hours Post-Exercise		
7	4.301	1.103
14	6.986	11.910
22R	6.909	3.169
29R	7.370	14.065
36R	4.859	0.979
41R	3.587	0.833

Blood Glucose Results (mg/100ml)

Animal Number	0 h	0.5 h	1 h	2 h	3 h	4 h	5 h
Glucose 2 Hours Post-Exercise							
3R	47.6	138.8	113.6	95.2			
10R	64.3	152.6	88.4	108.8			
17	44.9	162.5	128.5	103.1			
26R	87.1	195.7	121.1	117.2			
32R	46.2	151.4	129.1	114.7			
38	74.6	225.4	161.3	115.7			
Glucose 5 Hours Post-Exercise							
4	58.9	185.6	135.9	79.3	64.7	73.7	76.1
43	42.6	213.3	155.1	112.9	100.8	77.7	90.5
19R	68.9	200.8	144.0	101.5	90.5	88.3	79.4
23	81.6	248.4	152.3	108.6	91.8	98.4	87.0
33	45.5	177.4	133.9	112.8	98.4	87.3	90.2
39	82.8	200.8	145.4	98.5	94.4	83.7	84.8

Animal Number	0 h	0.5 h	1 h	2 h	3 h	4 h	5 h
Fructose 2 Hours Post-Exercise							
8R	81.2	132.9	133.3	134.9			
15R	47.8	120.5	141.0	222.3			
22	96.3	166.0	131.7	157.0			
29	70.5	111.6	133.3	113.2			
36	59.3	111.8	143.9	135.4			
41	77.5	135.5	137.0	134.4			
Fructose 5 Hours Post-Exercise							
2R	56.4	129.2	110.4	97.2	80.4	76.0	81.6
9	65.1	99.2	123.1	106.3	96.9	87.5	88.9
16	50.0	122.3	151.6	130.9	112.1	110.2	108.7
31R	52.7	116.3	136.4	108.0	93.9	73.5	64.8
38RR	85.2	153.1	152.0	119.5	98.4	86.6	98.4
42R	64.9	136.6	124.8	105.6	101.1	78.5	75.2
Glucose&Fructose 2 Hours Post-Exercise							
5R	62.2	125.6	94.4	98.5			
12	54.2	120.9	122.1	117.7			
19	86.0	194.3	144.2	118.1			
27	87.0	199.2	186.6	148.0			
35	87.1	176.6	150.4	153.5			
40RR	50.6	155.3	145.5	142.4			
Glucose&Fructose 5 Hours Post-Exercise							
7R	50.4	138.1	112.2	97.3	67.5	69.0	65.1
14R	32.5	130.2	110.1	119.0	110.1	95.5	98.8
21	86.4	170.6	132.1	98.5	94.3	93.5	106.1
34R	57.8	183.0	148.1	122.7	106.8	97.2	95.0
41RR	85.0	185.0	150.4	128.9	102.8	98.0	94.3
44R	48.6	111.7	96.5	99.2	83.6	73.8	71.3
Control 2 Hours Post-Exercise							
6R	49.4	147.0	160.2	185.5			
13R	55.6	06.0	133.7	126.3			
20R	100.8	160.8	170.2	142.2			
25	77.6	106.1	155.7	164.6			
34	111.3	101.6	125.8	159.0			
40R	56.8	114.4	126.1	148.6			
Control 5 Hours Post-Exercise							
7	63.5	142.4	152.5	151.4	123.5	110.2	89.4
14	86.8	144.2	150.2	138.8	129.3	123.6	119.8
22R	49.2	126.4	167.1	165.1	141.5	122.9	130.2
29R	94.7	122.4	171.6	161.0	153.3	136.2	149.2
36R	53.1	136.3	174.4	136.3	125.2	127.7	114.1
44RR	36.7	115.1	149.8	154.2	134.7	132.3	146.8

Animal Number	Pre-Exercise	Post-Exercise
2	108.4	45.6
4R	100.7	47.4
6R	108.1	55.6
9	97.6	65.1
11	109.6	31.7
17R	106.9	49.4
18	102.7	50.4
20R	127.3	60.2
23	103.8	68.9
25	110.9	85.2
31R	106.3	87.1
32R	107.8	42.2
30	124.0	77.6
34	113.0	87.0
35	123.0	111.3
27	112.5	50.6
40RR	106.9	77.5
41	105.6	37.8
38RR	104.4	51.6
42	112.1	87.1

Appendix E

TUKEY'S PAIRWISE COMPARISONS

Comparisons of Blood Glucose Concentrations Over Two Hours

Comparisons of Feedings at Specific Times

0 h		0.5 h	
G vs F	- 1.10	G vs F	12.79*
G vs G&F	< 1.00	G vs G&F	6.45*
G vs Con	- 1.62	G vs Con	13.83*
F vs G&F	< 1.00	F vs G&F	- 6.33*
F vs Con	< 1.00	F vs Con	1.04
G&F vs Con	< 1.00	G&F vs Con	7.37*

1 h		2 h	
G vs F	< 1.00	G vs F	- 5.28*
G vs G&F	< 1.00	G vs G&F	- 3.13
G vs Con	- 4.08*	G vs Con	-10.06*
F vs G&F	< 1.00	F vs G&F	2.15
F vs Con	- 3.90*	F vs Con	- 4.78*
G&F vs Con	- 4.36*	G&F vs Con	- 6.94*

Comparisons of Times for Specific Feedings

Glucose		Fructose	
0 h vs 0.5 h	-48.75*	0 h vs 0.5 h	-23.55*
0 h vs 1 h	-27.93*	0 h vs 1 h	-26.24*
0 h vs 2 h	-16.92*	0 h vs 2 h	-24.51*
0.5 h vs 1 h	20.83*	0.5 h vs 1 h	- 2.70
0.5 h vs 2 h	31.83*	0.5 h vs 2 h	< 1.00
1 h vs 2 h	11.01*	1 h vs 2 h	1.74

Glucose&Fructose		Control	
0 h vs 0.5 h	-35.66*	0 h vs 0.5 h	-20.73*
0 h vs 1 h	-26.03*	0 h vs 1 h	-32.39*
0 h vs 2 h	-21.21*	0 h vs 2 h	-32.25*
0.5 h vs 1 h	9.63*	0.5 h vs 1 h	-11.66*
0.5 h vs 2 h	14.44*	0.5 h vs 2 h	-11.52*
1 h vs 2 h	4.81*	1 h vs 2 h	< 1.00

* significant at $p<0.05$ level

Comparisons of Blood Glucose Concentrations Over Five Hours

Comparisons of Feedings at Specific Times

0 h			0.5 h		
G	vs F	< 1.00	G	vs F	17.44*
G	vs G&F	< 1.00	G	vs G&F	11.47*
G	vs Con	< 1.00	G	vs Con	16.33*
F	vs G&F	< 1.00	F	vs G&F	- 5.97*
F	vs Con	< 1.00	F	vs Con	- 1.11
G&F	vs Con	< 1.00	G&F	vs Con	4.86*
1 h			2 h		
G	vs F	2.52	G	vs F	- 1.99
G	vs G&F	4.32*	G	vs G&F	- 1.92
G	vs Con	- 3.65	G	vs Con	-10.80*
F	vs G&F	1.80	F	vs G&F	< 1.00
F	vs Con	- 6.17*	F	vs Con	- 8.82*
G&F	vs Con	- 7.97*	G&F	vs Con	- 8.89*
3 h			4 h		
G	vs F	- 1.55	G	vs F	< 1.00
G	vs G&F	< 1.00	G	vs G&F	< 1.00
G	vs Con	- 9.84*	G	vs Con	- 8.98*
F	vs G&F	< 1.00	F	vs G&F	< 1.00
F	vs Con	- 8.28*	F	vs Con	- 8.87*
G&F	vs Con	- 8.93*	G&F	vs Con	- 8.32*
5 h					
G	vs F	< 1.00			
G	vs G&F	< 1.00			
G	vs Con	- 8.90*			
F	vs G&F	< 1.00			
F	vs Con	- 8.55*			
G&F	vs Con	- 8.07*			

Comparisons of Times for Specific Feedings

Glucose				Fructose			
0	h vs 0.5	h	-54.55*	0	h vs 0.5	h	-24.56*
0	h vs 1	h	-31.23*	0	h vs 1	h	-27.23*
0	h vs 2	h	-14.99*	0	h vs 2	h	-18.83*
0	h vs 3	h	-10.30*	0	h vs 3	h	-13.39*
0	h vs 4	h	- 8.27*	0	h vs 4	h	- 8.86*
0	h vs 5	h	- 8.20*	0	h vs 5	h	- 9.21*
0.5	h vs 1	h	23.33*	0.5	h vs 1	h	- 2.67
0.5	h vs 2	h	39.58*	0.5	h vs 2	h	5.73*
0.5	h vs 3	h	44.27*	0.5	h vs 3	h	11.47*
0.5	h vs 4	h	46.29*	0.5	h vs 4	h	15.70*
0.5	h vs 5	h	46.36*	0.5	h vs 5	h	15.36*
1	h vs 2	h	16.25*	1	h vs 2	h	8.40*
1	h vs 3	h	20.94*	1	h vs 3	h	13.84*
1	h vs 4	h	22.96*	1	h vs 4	h	18.37*
1	h vs 5	h	23.03*	1	h vs 5	h	18.03*
2	h vs 3	h	4.69*	2	h vs 3	h	5.44*
2	h vs 4	h	6.71*	2	h vs 4	h	9.97*
2	h vs 5	h	6.78*	2	h vs 5	h	9.63*
3	h vs 4	h	2.02	3	h vs 4	h	4.53*
3	h vs 5	h	2.09	3	h vs 5	h	4.18
4	h vs 5	h	< 1.00	4	h vs 5	h	< 1.00

Glucose&Fructose				Control			
0	h vs 0.5	h	-35.83*	0	h vs 0.5	h	-25.87*
0	h vs 1	h	-24.96*	0	h vs 1	h	-37.35*
0	h vs 2	h	-19.58*	0	h vs 2	h	-33.58*
0	h vs 3	h	-13.13*	0	h vs 3	h	-27.20*
0	h vs 4	h	-10.68*	0	h vs 4	h	-23.68*
0	h vs 5	h	-10.91*	0	h vs 5	h	-23.48*
0.5	h vs 1	h	10.87*	0.5	h vs 1	h	-11.48
0.5	h vs 2	h	16.25*	0.5	h vs 2	h	- 7.71*
0.5	h vs 3	h	22.71*	0.5	h vs 3	h	- 1.33
0.5	h vs 4	h	25.15*	0.5	h vs 4	h	2.18
0.5	h vs 5	h	24.92*	0.5	h vs 5	h	2.39
1	h vs 2	h	5.38*	1	h vs 2	h	3.78
1	h vs 3	h	11.84*	1	h vs 3	h	10.15*
1	h vs 4	h	14.29*	1	h vs 4	h	13.66*
1	h vs 5	h	14.05*	1	h vs 5	h	13.88*
2	h vs 3	h	6.45*	2	h vs 3	h	6.38*
2	h vs 4	h	8.90*	2	h vs 4	h	9.89*
2	h vs 5	h	8.67*	2	h vs 5	h	10.10*
3	h vs 4	h	2.45	3	h vs 4	h	3.51
3	h vs 5	h	2.22	3	h vs 5	h	3.72
4	h vs 5	h	< 1.00	4	h vs 5	h	< 1.00

* significant at $p<0.05$ level

Comparisons of Plantaris Muscle Glycogen Concentrations

Comparisons of Feedings at Specific Times

1 h

G	vs F	2.98
G	vs G&F	1.85
G	vs Con	< 1.00
F	vs G&F	-1.13
F	vs Con	-2.10
G&F	vs Con	< 1.00

2 h

G	vs F	1.31
G	vs G&F	-1.03
G	vs Con	< 1.00
F	vs G&F	-2.35
F	vs Con	-2.29
G&F	vs Con	< 1.00

5 h

G	vs F	< 1.00
G	vs G&F	< 1.00
G	vs Con	-2.79
F	vs G&F	1.08
F	vs Con	-2.12
G&F	vs Con	-3.20

Comparisons of Times for Specific Feedings

Glucose

1	h vs 2	h	1.74
1	h vs 5	h	< 1.00
2	h vs 5	h	-1.22

Fructose

1	h vs 2	h	< 1.00
1	h vs 5	h	-3.12
2	h vs 5	h	-3.20

Glucose&Fructose

1	h vs 2	h	-1.14
1	h vs 5	h	< 1.00
2	h vs 5	h	< 1.00

Control

1	h vs 2	h	< 1.00
1	h vs 5	h	-3.15
2	h vs 5	h	-3.03

Comparisons of Liver Glycogen Concentrations

Comparisons of Feedings at Specific Times

1 h

G	vs F	< 1.00
G	vs G&F	< 1.00
G	vs Con	-1.06
F	vs G&F	< 1.00
F	vs Con	-1.07
G&F	vs Con	< 1.00

2 h

G	vs F	< 1.00
G	vs G&F	< 1.00
G	vs Con	< 1.00
F	vs G&F	< 1.00
F	vs Con	< 1.00
G&F	vs Con	< 1.00

5 h

G	vs F	< 1.00
G	vs G&F	< 1.00
G	vs Con	-5.35*
F	vs G&F	< 1.00
F	vs Con	-5.54*
G&F	vs Con	-5.52*

Comparisons of Times for Specific Feedings

Glucose

1	h vs 2	h	< 1.00
1	h vs 5	h	< 1.00
2	h vs 5	h	< 1.00

Fructose

1	h vs 2	h	< 1.00
1	h vs 5	h	< 1.00
2	h vs 5	h	< 1.00

Glucose&Fructose

1	h vs 2	h	< 1.00
1	h vs 5	h	< 1.00
2	h vs 5	h	< 1.00

Control

1	h vs 2	h	< 1.00
1	h vs 5	h	-4.47*
2	h vs 5	h	-5.02*

* significant at $p<0.05$ level

Appendix F

RAT CHOW COMPOSITION

Carbohydrate 58% *	Protein 22%
Ash 8%	Fibre 5%
Fat 4%	Minerals 3%

Actual Ingredients

ground extruded yellow corn	soybean meal
fish meal	wheat germ meal
dried beet pulp	ground oats
wheat midlings	dehydrated alfalfa meal
cane molasses	soybean oil
dried brewer's yeast	DL-methionine
Vit B12 supplement	calcium pantothenate
choline chloride	riboflavin supplement
thiamine	niacin
Vit A supplement	D-actified sterol
Vit E supplement	calcium carbonate
dicalcium phosphate	calcium iodate
salt	zinc oxide
manganous oxide	iron oxide
cobalt carbonate	copper oxide

* There was considerable variability in the specific carbohydrates so that the percentages of simple sugars were not recorded on the packaging, as verified by the supplier.