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I am submitting herewith a dissertation written by James Bailey Angel entitled "Metabolic Responses to Various Strength Glucose Solutions Ingested Prior to Exercise." I have examined the final copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Education.

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METABOLIC RESPONSES TO VARIOUS STRENGTH GLUCOSE SOLUTIONS INGESTED PRIOR TO EXERCISE

A Dissertation

Presented for the

Doctor of Philosophy

Degree

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James Bailey Angel

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Dedicated to the

four most important ladies

in my life

my mother, LoEmma
for 38 years of love and support

my wife, Linda
for her encouragement and devotion

my daughters, Holly and Heather
for loving me through it all.

Dedicated to

Jesus Christ

for guiding and directing my life

and

making everything worthwhile.

Isaiah 40:31

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ABSTRACT

The effects of pre-exercise glucose ingestion on the changes in blood glucose, free fatty acid, lactic acid, and insulin induced by 30 minutes of exercise at about 60% VO_{2max} were investigated. Four male and two female subjects were studied while riding a bicycle ergometer, 30 minutes after ingestion of water, 0.36 $g \cdot kg^{-1}$bw of glucose or 0.54 $g \cdot kg^{-1}$bw of glucose. When compared to the water trial, there was no difference in blood lactate levels as a result of pre-exercise glucose ingestion. Plasma glucose increased 39% as a result of the low glucose load, while the ingestion of the moderate glucose load caused a 41% increase. These increases caused significant differences in the glucose concentrations at rest between both the low glucose ($P < .001$), the moderate glucose ($P < .01$), and water. No significant differences were noted during the subsequent time periods. Plasma free fatty acid levels increased from rest and then decreased slightly at minute 30. There was no difference noted due to the pre-exercise ingestion of either of the glucose solutions. Serum insulin increased to coincide with the rise in glucose at rest and then declined during exercise. Significant differences in the serum insulin levels were measured at rest for both the moderate and low glucose trials. The results of this study indicate that a low strength glucose solution ingested 30 minutes prior to exercise will not impair the mobilization of free fatty acids, while the ingestion of a moderate strength glucose solution may impair the mobilization of free fatty acids.

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CHAPTER I

INTRODUCTION

The importance of muscle and liver glycogen stores to endurance type exercise is well recognized and accepted. Ingestion of carbohydrates during exercise, usually in the form of a glucose solution, has been shown to provide an alternate source of substrate for muscular work and to spare the use of stored glycogen (Gordon et al., 1924; Christensen and Hansen, 1939; Rodahl et al., 1964; Ahlborg et al., 1967; Staff and Nilsson, 1971; Costill et al., 1973; Benade et al., 1973; Brooke et al., 1975; Ahlborg and Felig, 1976; Pirnay et al., 1977; Pirnay et al., 1977; Ivy et al., 1979). This alternate source of energy is most important since both the size of the glycogen stores at the beginning of exercise and the rate of glycogen utilization combine to determine when muscle glycogen stores become exhausted.

Research examining the metabolic responses to pre-exercise carbohydrate ingestion and its effects on performance has been somewhat limited. Issekutz et al. (1963) measured an increased carbohydrate utilization during exercise following pre-exercise ingestion of 100 g of glucose. Orava et al. (1974) used a mean 65 g glucose load in a submaximal running test and found antilipolytic activity due to an elevated serum insulin. Ahlborg and Felig (1977) investigated the effect of pre-exercise ingestion of 200 g of glucose and measured a decrease in lipolysis mediated in part by hyperinsulinemia. Luyckx et al. (1978) found that the FFA level of experimental subjects was reduced about 60% following the pre-exercise ingestion of 100 g of

glucose compared to control subjects. One hour after ingestion of 100 g glucose, Ravussin et al. (1979) measured a decrease in FFA levels during a subsequent 2-hour exercise period. Foster et al. (1979) showed a decrease in exercise time to exhaustion at 84% VO_{2max} as a result of a pre-exercise feeding of 75 g of glucose. The study supported the earlier findings of Costill et al. (1977) who demonstrated that a 75 g glucose feeding 45 minutes prior to exercise at 70% VO_2max, decreased work time to exhaustion due to the increased rate of carbohydrate utilization and the restriction of FFA mobilization.

Generally, these studies are mutually supportive concerning the metabolic responses to pre-exercise glucose feedings. Although the exercise intensities varied in the studies, all recorded or suggested the same basic sequence of events: (1) a rapid rise in blood glucose and plasma insulin after glucose ingestion, followed by a decrease of both insulin and glucose during exercise (although insulin decreased less rapidly) and (2) a depressed or unchanged FFA level after glucose ingestion, which remained depressed or below normal levels during exercise.

Although the glucose loads in the above studies were intentionally large to produce a large insulin response and maximize the experimental results, they do not give a true representation of the type and amount of glucose ingested by athletes prior to competition (15-30 g). The primary purpose of this study was to determine: (1) how selected glucose solutions modify the blood glucose concentration at rest and (2) how they modify the plasma FFA and insulin concentrations during exercise.

CHAPTER II

REVIEW OF RELATED LITERATURE

The fact that nitrogen excretion is not significantly increased during muscular work in the fed individual shows that protein is not used as fuel to any appreciable extent as long as the energy supply is adequate. Therefore, the choice of fuels for muscular work is narrowed to either carbohydrate or fat. The percentage participation of these two fuels is dependent on several factors: (1) Whether the muscular work is continuous, prolonged, light, or heavy, (2) Whether the individual is trained or untrained, (3) Whether the diet is high or low in carbohydrates and if the diet is manipulated in combination with exercise, and (4) Whether the utilization of fat or carbohydrate is influenced by other physiological factors.

The primary focus of the present study is on the metabolic responses to pre-exercise glucose ingestion. Therefore, its influence on the utilization and availability of substrates for muscular work, as well as its effect on performance, will be the primary emphasis of this review of literature. This review will be divided into the following sections: (1) research dealing with diet manipulation and exercise to increase glycogen stores, (2) studies dealing with the effect of exercise intensity (% VO_{2max}) on fuel preference, (3) the influence of insulin and lactic acid on the mobilization of FFA and lipolysis and hence the utilization of carbohydrate, and (4) the metabolic responses resulting from glucose ingestion prior to and during exercise.

I. DIET AND GLYCOGEN STORES

Glycogen stored in the muscle can be metabolized to lactic acid through anaerobic glycolysis or to pyruvic acid and utilized in the Krebs cycle via the aerobic system. In the anaerobic system, lactic acid diffuses into the blood, is transported to the liver and either converted to glucose, stored as liver glycogen, or returned to the blood.

The fact that glycogen stores have a direct effect on endurance performance is well known. The fact that these stores can be altered by a combination of diet manipulation (carbohydrate loading) and exercise, makes this aspect of fuel availability most important. It is especially critical since the glucose production from the liver is increased during heavy exercise (Hultman, 1966; Hultman, 1967; Rowell et al., 1966; Rowell et al., 1965).

As shown in Table 1, a mixed diet, a protein plus fat diet and a high carbohydrate diet were the regimens used to test the effect of diet on muscle glycogen modification. Bergstrom et al. (1967) found that the mean pre-exercise muscle glycogen content and mean maximal work time were significantly increased after first depleting the muscle glycogen through exhausting exercise followed by 3 days of a high carbohydrate diet. Saltin and Hermansen (1967), used nine subjects in their study to determine if experimental variation in muscle glycogen content would affect the capacity to perform prolonged work. Following the same regimen as Bergstrom et al. (1967), the average work time for 6 of the subjects was 189 minutes and muscle glycogen content was 4.7%. This compared to work times of 126 and 59 minutes and glycogen

Table 1. Original research design for carbohydrate loading

Monday	Tuesday	Wednesday/Thursday	Friday	Saturday/Sunday	Monday
Mixed diet	Work	Protein 1500 Kcal Fat 1300 Kcal	Work	Carbohydrates 2300 Kcal Protein 500 Kcal	Work

contents of 2.5% and 1.3% for the mixed and protein plus fat diets, respectively.

After administering the above protocol involving exercise to exhaustion followed by diet manipulation, Bergstrom and Hultman (1972) determined the relationship between glycogen content and work time. Again, the carbohydrate rich diet regimen showed an increased work time to exhaustion and increased pre-exercise muscle glycogen content compared to the protein plus fat and the mixed diets.

To test the influence of high muscle glycogen content on performance during heavy prolonged exercise, Karlsson and Saltin (1971) 10 subjects who twice ran a 30 Km race within a 3 week interval. Six of the subjects performed the first race following work to exhaustion and a high carbohydrate diet, while the remaining four subjects followed a normal mixed diet after muscle glycogen depletion. Before the second race, the subjects reversed the diets. Prior to the races the mean muscle glycogen content of the subjects who followed the carbohydrate regimen was approximately double the level found after the mixed diet. Additionally, the mean race time for the subjects on the high carbohydrate treatment was significantly faster than for the subjects on the mixed diet treatment.

Bergstrom and Hultman (1966) exercised two subjects, one on either side of a bicycle ergometer, to determine the effects of exercise and diet manipulation on muscle glycogen stores. Exercise was performed with one leg while the other leg rested completely. Both subjects worked simultaneously, one with the left leg, the other with the right leg. The vastus lateralis was exercised to exhaustion to deplete glycogen stores. This was followed by 3 days of a high

carbohydrate diet with the subjects at rest. The muscle glycogen stores in the exercised leg showed a 2 to 3 times increase above that of the resting leg.

In two studies, Gollnick et al. (1972, 1973) investigated the glycogen content of trained subjects. Their findings showed that (1) glycogen content was highest in the muscle that was used most extensively in the sport activity (this was in agreement with the study of Bergstrom and Hultman (1966), and (2) trained subjects have muscle glycogen stores approximately 2 to 3 times the untrained muscle and about equal to levels reported due to carbohydrate loading regimens. Costill (1978) determined that the muscle glycogen content could be increased well above the normal levels by first emptying the glycogen from muscles by strenuous exercise and then ingesting a high carbohydrate diet for 3 or 4 days.

Hultman et al. (1971) determined that the feeding of a carbohydrate-free diet for 10 days resulted in a decrease of 30-40% of muscle glycogen stores. Additionally, they found that carbohydrate rich food increased the glycogen stores, but only within the normal range if not preceded by glycogen depletion.

From this review of the literature, it can be readily seen that carbohydrate loading may effectively increase glycogen stores and therefore enhance endurance performance. While the effects of this diet manipulation may be quite apparent, i.e., a threefold increase in glycogen stores; the method used to affect these increases is being critically questioned by knowledgeable people in the field of work physiology. The primary reason for this new analysis of carbohydrate

loading stems from the original research design used by Bergstrom et al. (1967) and Saltin and Hermansen (1967), (Table 1).

Upon close examination of the studies reported above, a period of exhaustive work followed each different diet period in the experimental phase of the research. It naturally follows that the protein plus fat diet and the high carbohydrate diet followed a period of exhaustive muscle glycogen depleting work. However, when the carbohydrate loading regimen was schematically reported in the Saltin and Hermansen study and alluded to in the other studies, there was no indication of the exhaustive work period between the protein plus fat diet and the high carbohydrate diet. This was in contrast to the diet regimen used in the research design of each study. It is, however, this schematically adopted design that most exercise physiology texts and athletic magazines publish (Figure 1). Most researchers now warn that the high protein plus fat diet depicted in texts as a part of carbohydrate loading can be both physiologically and psychologically damaging (Costill, 1978; Vitousek, 1979). There has even been one documented case of an abnormal EKG and angina reported following an overindulgence of bread in a carbohydrate loading diet regimen (Mirkin, 1973).

Table 2 presents a summary of carbohydrate loading research.

II. FUEL FOR MUSCULAR WORK

In general, as exercise intensity increases and duration decreases the predominate fuel shifts toward carbohydrates. Conversely, as exercise intensity decreases and duration increases the predominate fuel shifts toward fat. Specifically, several studies

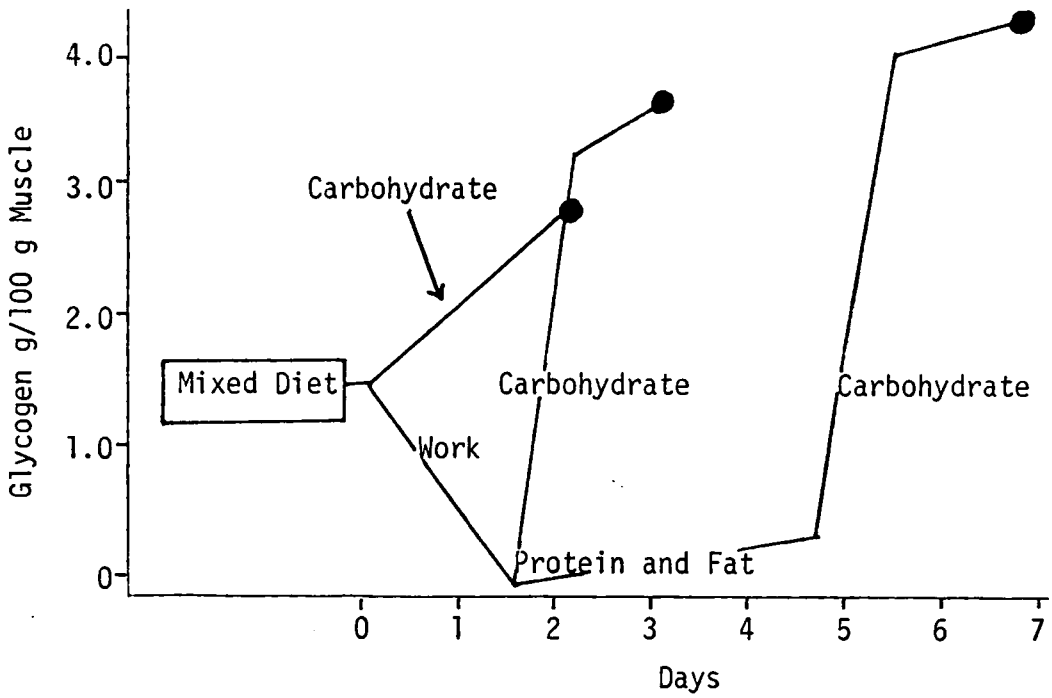

Figure 1. Carbohydrate loading as schematically presented in texts. (Notice the absence of work following the Protein/Fat diet.)

Table 2. Summary of carbohydrate loading research

Study	Number of Subjects	Training (T) Special Regimen (SR)	Glycogen Stores		
			$\bar{X}$	High g/kg	Low g/kg
Saltin and Hermansen (1967)	6**	SR	37.0	47.0	27.0
Bergstrom et al. (1967)	6**	SR	37.0	46.8	26.6
Karlsson and Saltin (1971)	10	SR	35.2	64.8	20.9
Bergstrom and Hultman (1972)	6	SR	37.0	47.0	27.0
Gollnick et al. (1972)	6*	T	33.8	34.7	32.7
Gollnick et al. (1973)	N.A.	T	N.A.	40.14	22.9

*Only three subjects measured pre and post for muscle glycogen stores.

**Mean glycogen content equal to 35.0 g/kg if highest subject measured is eliminated.

have identified a critical level that separates the use of fat and carbohydrates as the fuels of preference for muscle contraction.

In discussion the study by Hultman (1967), Dr. D. Bruce Dill recounted one of his studies undertaken 30 years prior. His subjects performed four 2-hour bouts over a 24-hour period utilizing oxygen at about 2 liters/minute. He measured the RQ at the beginning of work to be 0.85 and during the last 2-hour bout at 0.72. This indicated that fat was being burned almost exclusively during this period. Dr. Dill used a dog in another study to assess fuel utilization. Work was done at a rate of 10 Km/hr, on a grade of 17.6%, which in man would require about 5 to 6 liters of oxygen per minute. At the end of about 5 hours, the blood sugar level was down to about 50 mg% and he was approaching exhaustion. After glucose ingestion, the dog recovered and continued running for an additional 12 hours. From these studies and others, Dill felt that there exists a critical level for man and dog above which they must have glycogen or glucose and below which they can butn fat almost exclusively.

At about 50% VO_{2max}, Pruett (1971) found that the RQ dropped from 0.84 to 0.81 during 4 1/2 hours of work (with 15 minutes rest each hour). During this prolonged exercise, it was determined that fat accounted for as much as 60% of the energy. At 70% VO_{2max} during exercise of the same duration, the RQ went from 0.89 to 0.84 and fat accounted for about 50% of the fuel for energy.

Wahren et al. (1975) found an increase in the RQ, from 0.77 at rest to 0.87 as the exercise work rate increased from 65 W to 200 W during a 40 minute ride on a bicycle ergometer. This indicated a

diminished use of fat as the substrate for muscular activity as work rate increased.

In the discussion of the work by Hultman previously cited, Saltin summarized the critical levels of energy metabolism (Hultman, 1967). Initially he discussed his own group of fasting subjects who walked from Goteborg to Stockholm. He reported that while walking at about 35 to 40% VO_{2max} they burned mainly fat during the 10 days needed to complete the trip. Reviewing the study of Christensen and Hansen (1939), Saltin calculated that their subjects worked at about 60 to 65% VO_{2max}. At that level a definite decrease in RQ occurred. Another study by Saltin using prolonged exercise at a level of 70 to 80% VO_{2max} showed only a slight decrease in the RQ of the subjects. In Hultman's study where subjects worked at 75 to 80% VO_{2max}, it was impossible for them to continue at that intensity to the point of a decrease in RQ.

It is quite evident from the discussion and studies presented above that endurance work performed up to approximately 75% VO_{2max} uses fat for at least 50% of the fuel. At a critical range of about 70 to 80% VO_{2max}, the primary fuel shifts from fat to carbohydrate. As the percentage of VO_{2max} increases beyond this critical range, carbohydrate is used increasingly as the fuel to support muscular work and apparently, the work rate cannot be maintained if there is a depletion of carbohydrate.

Based on the critical range described above, fatigue in endurance work can be attributed to several factors depending on the percentage of VO_{2max} at which the work is performed: (1) at work loads up to about 75% VO_{2max}, perceived fatigue due to a hypoglycemic

condition is generally the limiting factor, (2) at approximately 75 to 80% VO_{2max}, fast twitch fibers are depleted of muscle glycogen resulting in fatigue, and (3) at heavy work rates (above 75 to 80% VO_{2max}), Adams and Welch (1980) concluded that the buildup of H^+ caused fatigue, even though glycogen was still available.

It is well established that training increases the maximal oxygen uptake and as a result increases the utilization of fat as an energy source, thereby sparing the use of glycogen. In many of the studies discussed above the subjects were highly trained and highly motivated for the work they performed. Therefore, these results dealing with the different levels of VO_{2max} and fuel utilization would have to be used with caution when relating them to the normal population or even moderately trained individuals.

III. EFFECTS OF INSULIN AND LACTIC ACID ON CARBOHYDRATE UTILIZATION AND FFA UTILIZATION

As described earlier in this review, when a fasting individual continues to perform hard work for several hours, the RQ gradually declines indicating that fat is increasing in proportion as the fuel for muscular work (Dill et al., 1935). In prolonged work, it is a distinct advantage for the fasting individual to utilize fat as the fuel of preference, since fat stores are much larger than those of carbohydrates. In a well fed man of average size, fat stores amount to approximately 10^5 Kcal while stored glycogen amounts to only 2,000 Kcal. The ability to utilize fat as the substrate for muscular work while sparing the more limited carbohydrate reserves, makes the availability of FFA of prime importance for physical activity.

Insulin

Adipose tissue responds to insulin administration with an almost instantaneous curtailment of FFA release (Bierman et al., 1957). This is accomplished in one of two ways: (1) the increased entry of glucose into the fat cell permits rapid formation of α glycerol phosphate which reesterfies the FFA, produced by lipolysis, to triglycerides, (2) insulin has an antilipolytic effect and seems to decrease the activity of lipase, which is hormone sensitive. This is caused by increased activity of cyclic nucleotide phosphodiesterase, which degrades cyclic AMP. The increased phosphodiesterase favors the production of 5' AMP, thereby increasing the degradation of cyclic AMP and hence impeding the mobilization of FFA (Figure 2).

Since insulin promotes the conversion of glycogen synthetase to its active form, it thereby increases the formation of glycogen in muscle tissue. Again, this may be caused by an increased activity of cyclic nucleotide phosphodiesterase which results in a decrease of cyclic AMP. Not only does cyclic AMP promote the formation of phosphorylase a, but it triggers the conversion of the synthetase to its inactive form, thus inhibiting glycogen formation (Figure 3). By decreasing phosphorylase a, insulin may also remove the inhibition of the activation of glycogen synthetase (Hers, 1976).

Insulin decreases the production and secretion of glucose by the liver. This occurs rapidly and results from a decrease in cyclic AMP caused by activation of cyclic nucleotide phosphodiesterase. The decrease in cyclic AMP favors the formation of glycogen and decreases hepatic gluconeogenesis (Mountcastle, 1980).

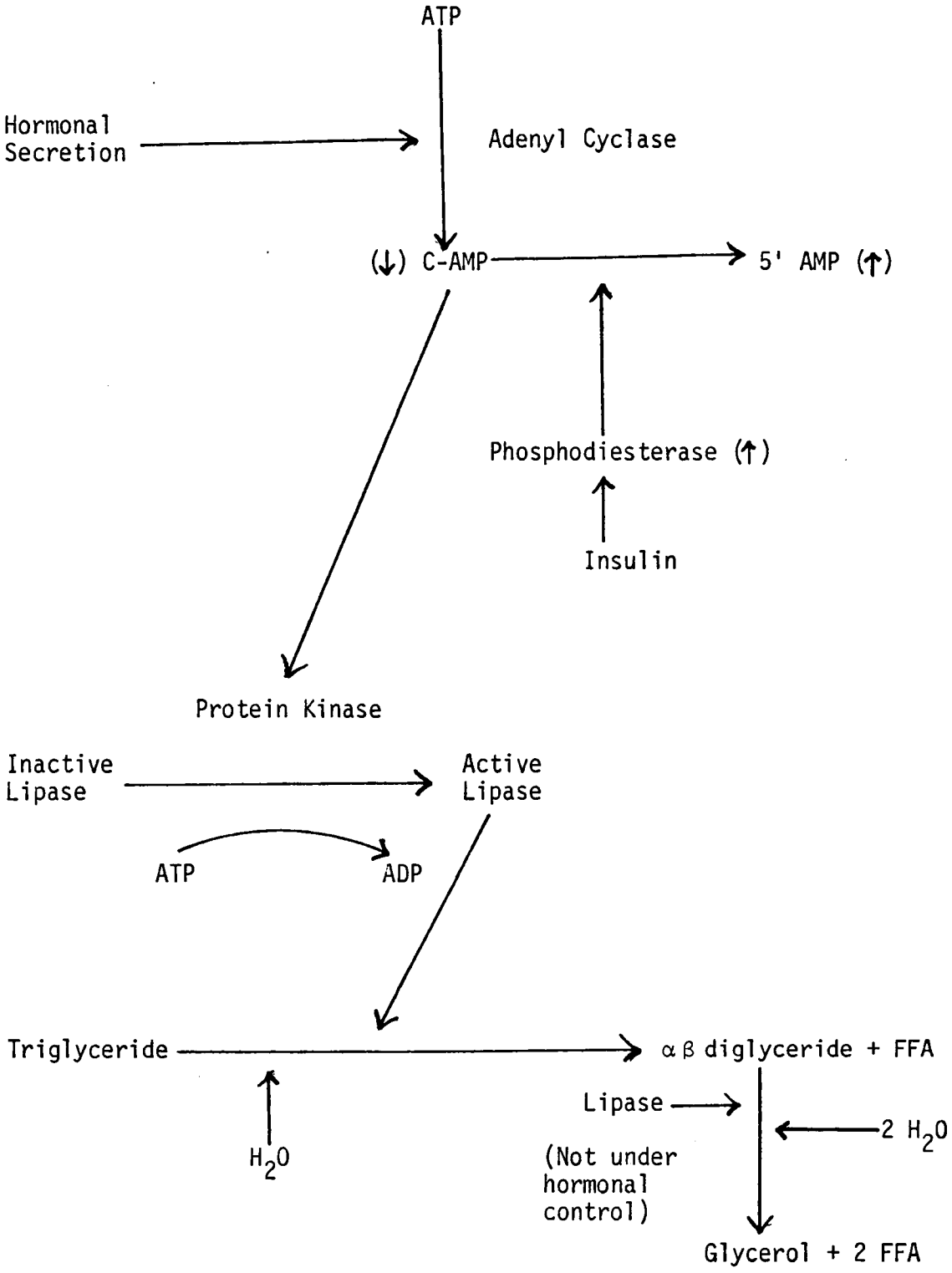

Figure 2. The limiting effect of insulin on cyclic AMP.

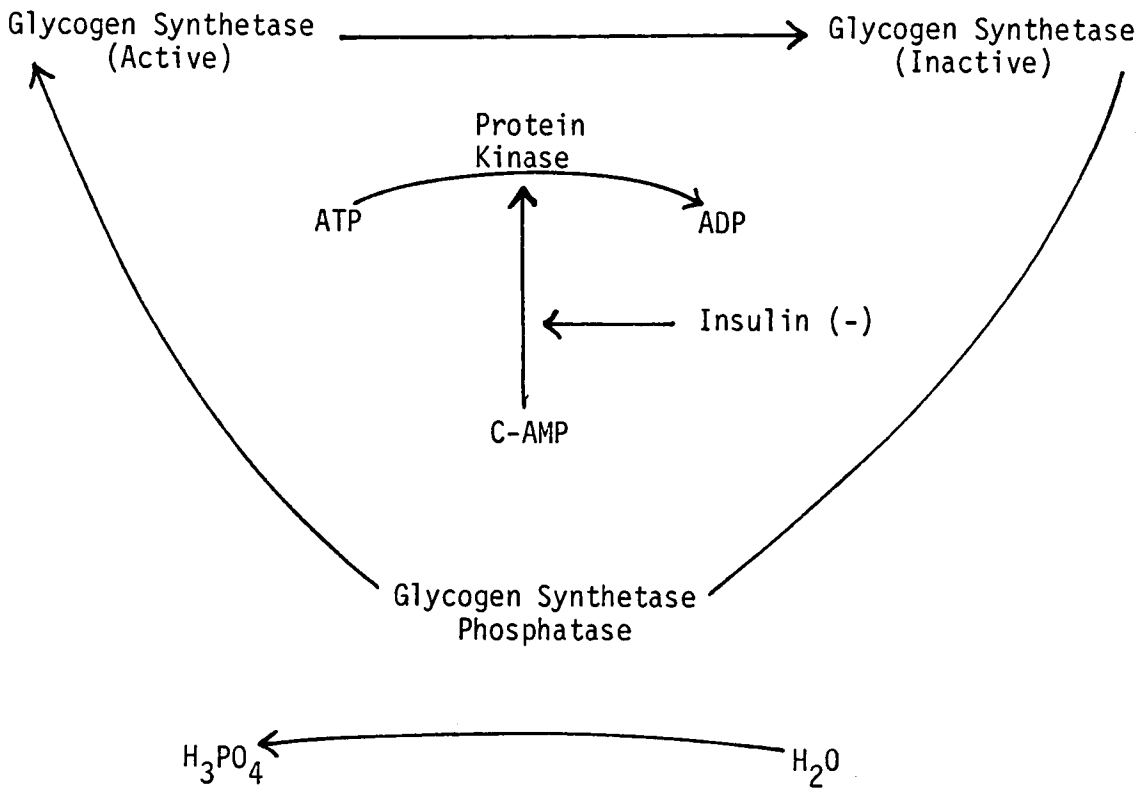

Figure 3. Increased glycogen synthesis due to C-AMP inhibition by insulin.

Dole (1956) found that insulin facilitated the uptake of glucose by the muscle and also decreased the release of FFA in adipose tissue. He also reported a decrease in the level of FFA after the ingestion of oral glucose. Issekutz et al. (1963) speculated from this conclusion that a diet low in carbohydrates brings about a depressed insulin production and thereby a high rate of release of FFA. They subsequently determined that the actual carbohydrate intake rather than the amount of fat in the diet was the decisive factor determining whether FFA or glucose was the preferred fuel.

Hunter and Sukkar (1968) reported a decrease in plasma insulin during 2 hours of treadmill walking at 6.4 Km/hr. They felt that this fall in the insulin level was important as it prevented blood glucose from falling to hypoglycemic levels and enhanced the effects of other hormones on fat mobilization.

Following pre-exercise ingestion of glucose, Orava et al. (1974) measured no increase in FFA levels as compared to an increased level after placebo ingestion. They determined that this was due to the antilipolytic activity of elevated serum insulin.

In their study, Ahlborg and Felig (1977) showed that the hyperinsulinemia which accompanied a pre-exercise glucose feeding was the major factor responsible for increased glucose utilization resulting from decreased lipolysis. Costill et al. (1977) concluded that the elevation of plasma glucose and insulin prior to exercise increased the rate of carbohydrate metabolism due to a decrease in the mobilization of FFA. Foster et al. (1979) reported depressed FFA levels during exercise as a result of pre-exercise ingestion of glucose. They

determined that this depressed level of FFA was caused by the increased plasma insulin following a carbohydrate challenge.

One hundred grams of glucose ingested at the beginning of a 240 minute walk showed metabolic results quite similar to those of pre-exercise glucose ingestion. Blood glucose and plasma insulin appeared to peak after approximately 30 to 40 minutes of exercise with a resulting decrease in the plasma FFA concentration. Since exercise was performed at 50% VO_{2max}, lactate levels did not have a depressing effect on FFA levels. It was therefore concluded that glucose availability at the beginning of exercise reduced the mobilization of FFA (Luyckx et al., 1978).

As a part of their study, Ravussin et al. (1979) measured the metabolic response resulting from the ingestion of 100 g of glucose 1 hour prior to exercising on a bicycle ergometer at approximately 40% VO_{2max}. They found the FFA concentration in normal subjects was only 1/3 to 1/2 of the FFA concentration in glycogen depleted subjects, possibly due to the hyperinsulinemia mediated by glucose ingestion.

Eight male subjects who combined exercise on the bicycle ergometer with treadmill walking were twice fed (high carbohydrate meals) during the 6 hours of work. The results showed a depressed rate of release of FFA due to an elevated insulin level (Rodahl et al., 1964).

Related research dealing with the relationship between the insulin concentration and FFA mobilization is shown in Table 3.

Table 3. Summary of research--insulin concentrations and FFA mobilization

Study	Number of Subjects	Dosage	Type/Intensity of Exercise	Results
Dole (1956)	82	50 g glucose 0.1 /kg B.W. of insulin	None	Decrease in concentration of FFA
Issekutz et al. (1963)	6 male	Decrease CHO-463 g to 100 g	Cycling 300 kpm	High rate of FFA release
Rodahl et al. (1964)	8 male	116 g glucose	600 kpm 3.5 MPH treadmill	Depressed rate of release of FFA
Hunter and Sukkar (1968)	6	None	Treadmill 2 hrs.	Fall in insulin level--enhanced fat metabolism
Orava et al. (1974)	20 male	65 g glucose	Submaximal running	Decreased lipolysis
Ahlborg and Felig (1977)	12 male	200 g glucose	30% VO_{2max} 4 hrs.	Decreased lipolysis
Costill et al. (1977)	7 male	75 g glucose	70% VO_{2max} 30 mins. treadmill	Increased CHO utilization--decreased FFA mobilization
Luyckx et al. (1978)	8 male	100 g glucose	50% VO_{2max} 240 min. walk	Reduced FFA mobilization
Foster et al. (1979)	8 male 8 female	75 g glucose	80% VO_{2max} cycling	Increased CHO utilization--decreased FFA mobilization--reduced endurance
Ravussin et al. (1979)	10 male	100 g glucose	40% VO_{2max} 2 hrs. cycling	Reduced FFA levels

Lactic Acid

During light exercise, the oxygen provided as respiration and circulation adapt to the work plus the oxygen stored in the muscle completely meet the required oxygen need. During exercise of moderate intensity, anaerobic processes supply the necessary energy until the aerobic process can meet the energy needs. Any lactic acid produced diffuses into the blood and can be traced in venous blood draining the muscle. During heavier exercise, blood lactate concentrations are higher and remain so during the total exercise. During very severe exercise the oxygen deficit and blood lactate increase as the work is predominantly anaerobic.

Pruett (1970) found that at work loads up to 70% VO_{2max} plasma FFA levels increased significantly during exercise, while blood lactate levels increased only slightly. At 85 to 90% VO_{2max}, plasma FFA levels fell and remained low until work stopped, whereas lactate levels rose.

As discussed above and as generally agreed upon by most knowledgeable researchers in the exercise physiology field, blood lactate levels begin to increase when individuals reach approximately 60 to 70% of their VO_{2max}. This percentage would increase to about 75 to 85% VO_{2max} if the individual were trained. This steady increase over the course of the exercise has been shown to decrease the mobilization of FFA. Investigations in recent years have focused on the decreased availability of this most important fuel source.

In order to determine the effects of exercise on FFA and to learn more about the role of FFA in muscular work, Issekutz and Miller (1962) carried out treadmill experiments with dogs. After infusion of 10 or 20% lactic acid solution, they determined that a slight rise

in blood lactate from 7 to 20 mg/100 ml caused a considerable drop in FFA.

Miller et al. (1964) infused sodium lactate into unanesthetized pancreatectomized dogs to determine its effect on FFA levels. They found an inverse correlation between FFA concentrations and blood lactate levels, while glucose infusion had no significant effect on plasma FFA. It was concluded that lactic acid had a direct effect on the release of FFA which did not require the presence of insulin and was independent of blood glucose concentration.

After infusing sodium lactate into exercising dogs, Issekutz et al. (1965) determined that the elevation of blood lactate levels to between 58 and 80 mg/100 ml in 30 minutes, decreased both FFA turnover as well as plasma FFA levels. The measurements were not depressed in trained dogs due to the lower lactate concentrations. They concluded that the rise of blood lactate during exercise, which reflects the state of aerobic work capacity, was an important factor affecting the utilization of FFA in exercise metabolism.

To define the role of increasing lactate levels on FFA levels Boyd et al. (1974), infused 6 meq/kg of sodium lactate into six exercising normal males. A mild work load was used in order to increase arterial FFA concentration, but not the lactate concentration. Controls were infused with sodium bicarbonate and sodium chloride of equal volume. They determined that a rise in arterial lactate to a level of 8 μm/ml resulted in a significant inhibition of the increase of plasma FFA and glycerol compared to controls. Further, they stated that this decrease was not caused by the "insulin effect" as the concentration of insulin in arterial blood decreased during exercise.

Lipolysis in dogs was stimulated both by moderate treadmill exercise and by the infusion of norepinephrine, followed by the infusion of sodium lactate. Issekutz et al. (1975) found that an inverse correlation developed between the logarithms of FFA and plasma lactate, while a linear correlation was obtained between glycerol and plasma lactate. They concluded that (1) physiological concentrations of lactate inhibited FFA release stimulated by exercise, (2) the increase in NADH/NAD ratio leads to the formation of glycerol via α-glycerolphosphate, and (3) the effect of lactate on FFA was caused by increased reesterification and/or possibly lipase inhibition.

Green et al. (1979) examined the lactate and FFA concentrations in two groups of subjects: (1) cycling subjects who performed intermittent supramaximal arm work,and (2) control subjects who only cycled. Both groups of subjects exercised at the same intensity (59% VO_{2max}) and for the same duration (120 minutes). They measured a progressive rise in plasma lactate from 2.1 to 7.2 mM following arm work, which coincided with a significant decrease in FFA levels during the same period. Their findings indicated an increased glycogen utilization during the combined arm-leg exercise mediated by a decrease in muscle oxidation of FFA caused by a lactate-induced inhibition of lipolysis.

Bagby et al. (1978) found in rats, that running time to exhaustion at a speed of 21 m/min on a treadmill at 10% incline was prolonged by glucose infusion but shortened by lactate or pyruvate infusion. Following saline infusion, at the point of exhaustion plasma FFA levels had risen to 3 times pre-exercise levels. In contrast, plasma FFA with glucose and lactate infusions were consistently depressed to pre-exercise concentrations. This implied that

the depression in plasma FFA following lactate and glucose infusions resulted from reesterification.

In order to increase the arterial lactate level to values associated with heavy exercise, Fredholm (1971) stimulated the nerve to the subcutaneous adipose tissue of dogs for 5 to 10 minutes with 4 Cps. The amount of FFA and glycerol released during stimulation were compared for two treatments: (1) during an infusion of lactate and (2) during an infusion of saline. He found that the effect of lactate on FFA was dose dependent; 2mM lactate had no effect on FFA, 5 mM lactate had a borderline effect on FFA, while 10 mM lactate or more caused an approximate 70% inhibition of FFA mobilization.

Rodahl et al. (1964) investigated the effects of exercise of different intensities and durations on FFA, blood glucose, and lactate. In heavy 10 minute work, an increased lactate level of approximately 6 to 8 mg/100 ml resulted in a FFA decrease.

One investigation that was not in agreement with the above studies on the effect of lactate on FFA was reported by Ahlborg et al. (1976). They determined that infusion of sodium lactate had no effect on mobilization of FFA from adipose tissue. Arterial lactate levels were 3 to 5 mM during the infusion. As reported by Issekutz and Miller (1962), Issekutz et al. (1965), Boyd et al. (1974), Green et al. (1979), and Fredholm (1971), lactate levels had a critical level of at least 5 mM/l before there was an adverse effect on the mobilization of FFA. It appears that this study by Ahlborg et al. did not have a lactate level high enough to meet this critical level and therefore would not be applicable during periods of heavy exercise.

The summary of the relationship of lactate concentration and FFA mobilization is shown in Table 4.

IV. GLUCOSE INGESTION DURING EXERCISE

It is generally agreed that ingestion of a glucose solution during endurance type exercise will help spare muscle glycogen and delay or prevent hypoglycemia. Gordon et al. (1924) found a marked improvement in marathon runners after a year of moderately high carbohydrate diet. This experimental group of runners was also encouraged to eat glucose candy, as well as tea with large amounts of sugar taken at designated stations, during a 25-mile run. The blood studies showed normal blood glucose levels in this group of runners and most had improved times and finished the race in higher positions. It was concluded that the symptoms of hypoglycemia following prolonged effort may be prevented by the adequate and timely ingestion of glucose.

In a study where subjects exercised to exhaustion, Christensen and Hansen (1939) found that if 200 g of glucose was ingested when symptoms indicated the presence of hypoglycemia, blood sugar levels increased and work could be continued for an additional hour.

Rodahl et al. (1964) studied the effects of carbohydrate feedings on the blood glucose level of five subjects during light work of long duration. They found a definite rise of blood sugar in contrast to the stable levels of blood sugar of subjects who exercised while fasting.

After infusing glucose into working subjects, Ahlborg et al. (1967) determined that the metabolism of the added glucose reduced the

Table 4. Summary of research--lactate concentration and FFA mobilization

Study	Number of Subjects	Dosage	Type/Intensity of Exercise	Results
Issekutz and Miller (1962)	6 dogs	Lactate 16-34 mg/kg/min. for 80 mins.	100 m/min., 15-19% grade, 20-25 mins.	Increased lactate caused a decrease in FAA
Miller et al. (1964)	4 dogs	Lactate 7-15 mg/kg/min. for 30-40 mins.	None	Inverse correlation between lactate and FFA
Rodahl et al. (1964)	8 male	None	Heavy cycling 10 mins.	FFA decrease
Issekutz et al. (1965)	Dogs	C^{14} palmitate 12-18 μ C/kg/min. for 190-200 mins.	100 m/min. 15% grade, 30-40 mins.	Elevated lactate above 58 mg/100 ml in 30 mins. decreased FFA turnover rate to below resting
Fredholm (1971)	Dog	Lactate 2-10 mM+	Tissue stimulation for 5-10 mins. at 4 cps.	5-10 mM of lactate caused inhibition of FFA mobilization
Boyd et al. (1974)	6 male	Na-lactate 6 meq/kg	Mild, 90 mins.	Lactate rise to 8 mm/ml inhibited FFA
Issekutz et al. (1975)	Dogs	Na-lactate	100 m/min., 10% grade, 150 mins.	High lactate level inhibited FFA release
Ahlborg et al. (1976)	10 males	Lactate 5-12 mM/min. for 30 mins.	None	No effect on FFA mobilization
Bagby et al. (1978)	49 rats	Lactate and glucose--2.2 mM/hrs. for 4 hrs.	21 m/min., 1, 2, 3 hrs. or exhaustion	Running time to exhaustion increase by glucose, decreased by lactate
Green et al. (1979)	4 males	None	Suprarm, cycling 50% VO_{2max}	Lactate rise from 2.1 to 7.2 mM coincided with a decrease in FFA concentration

fat combustion during work, but could not replace the energy derived from the muscle glycogen.

Studies by Staff and Nilsson (1971) showed that ingestion of 225 ml of a 5% glucose solution every 15 minutes was sufficient to prevent hypoglycemia in subjects working at 70% VO_{2max} for 3 hours.

In exercise at 60 to 72% VO_{2max}, Costill et al. (1973) determined that orally ingested glucose had only a minor role in carbohydrate oxidation during exercise. However, they did hypothesize that since the hepatic contribution to blood glucose was reduced following glucose ingestion, that these feedings may prevent liver glycogen depletion during prolonged severe exertion.

Subjects who exercised on a bicycle ergometer for 50 minutes of each hour, for 6 hours at approximately 47% VO_{2max}, ingested 100 g of sucrose at the beginning of the fourth hour. Blood glucose levels of the exercising subjects compared favorably to those of the resting subjects during the first 3 hours of exercise, but were significantly higher than the group of exercising subjects who ingested no sucrose ingestion (Benade et al., 1973).

Brooke et al. (1975) studied the effects of different dietary ingestions taken during a bicycle ergometer ride to exhaustion at approximately 67% VO_{2max}. They found that both glucose syrup drink and rice plus sucrose diet delayed the onset of exhaustion by providing more combusted carbohydrate. This was shown by an elevated RQ and blood glucose levels, compared to the low energy drink and restricted food intake regimens which showed little or no change.

Ahlborg and Felig (1976) determined the effect of the ingestion of 200 g of glucose at the 90th minute of exercise during a 4 hour

exercise period at 30% VO_{2max}. Although they did not determine the effect of this feeding on performance, they did find a significant rise in blood glucose and greater dependence on this substrate.

Pirnay et al. (1977) studied the effects of 100 g of carbon-13 glucose ingested during an uphill walk on a treadmill at about 50% VO_{2max}. Within 15 minutes of this ingestion, the expired CO_2 became rapidly enriched in carbon-13 and reached a maximum in 1 to 2 hours. At this time, the glucose accounted for as much as 55% of the carbohydrate metabolized and 24% of the energy expenditure.

The effects of the ingestion of 100 g of exogenous glucose during exercise were measured by Pirnay et al. (1977). They determined that exogenous glucose contributed markedly to total carbohydrate oxidation and decreased the percentage of energy derived form lipids. Additionally, the ingestion of glucose spared the use of glycogen stores and increased endurance.

Ivy et al. (1979) studied the results of the ingestion of approximately 90 g of glucose polymer during the first 90 minutes of a 2-hour exercise bout. Although there was no significant improvement in performance or perceived exertion, they did observe that the glucose ingestion appeared to reduce the onset of fatigue during the last 30 minutes of exercise.

Table 5 is a summary of research dealing with glucose ingestion during exercise.

V. GLUCOSE INGESTION PRIOR TO EXERCISE

While studies dealing with the ingestion of glucose during exercise show a beneficial effect on glycogen utilization and hence

Table 5. Summary of research--glucose ingestion during exercise

Study	Number of Subjects	Dosage	Type/Intensity of Exercise	Results
Gordon et al. (1924)	17 male	Unknown, candy/ sugar tea	Vigorous, 25 mile run	Symptoms of hypoglycemia prevented
Christensen and Hansen (1939)	10 male	Glucose 200 g	60-65% VO_{2max} to exhaustion, cycling	Additional work capacity
Rodahl et al. (1964)	8 male	Glucose 84-116 g	Light prolonged work on treadmill	Increase in blood sugar
Ahlborg et al. (1967)	2	Glucose 330 g/ 180 g	60% VO_{2max} to exhaustion, cycling	Replaced fat as fuel
Staff and Nilsson (1971)	6 male	Glucose 130 g	70% VO_{2max} for 3 hrs.	Hypoglycemia prevented
Costill et al. (1973)	7 male	Glucose 32 g	60-72% VO_{2max} for 90 mins., cycling and running	Prevented liver glycogen depletion
Benade et al. (1973)	6 male	Glucose 100 g	47% VO_{2max} for 6 hrs., cycling	Higher blood glucose levels vs. controls
Brooke et al. (1975)	8 male	Glucose syrup, 250 ml	67% VO_{2max} to exhaustion, cycling	Delayed onset of exhaustion
Ahlborg and Felig (1976)	6 male	Glucose 200 g	30% VO_{2max} for 4 hrs., cycling	Increase in level and utilization of blood glucose
Pirnay et al. (1977)	7 male	Glucose 100 g	50% VO_{2max} for 4 hrs., treadmill	Increased utilization of blood glucose
Pirnay et al. (1977)	7 male	Glucose 100 g	50% VO_{2max} for 2/4 hrs., treadmill	Spared glycogen and increased endurance
Ivy et al. (1979)	7 males 2 females	Glucose 90 g	80 rpm for 2 hrs., cycling	Reduced onset of fatigue

performance, studies dealing with glucose ingestion prior to exercise indicate an impairment of performance.

Issekutz et al. (1963) studied the effects of the ingestion of 100 g of glucose, 1.5 hours prior to light exercise, on bicycle ergometry performance. The study lasted for approximately 70 days and included four different diets. They found a significant increase in carbohydrate utilization during exercise following this pre-exercise ingestion of glucose. Furthermore, they determined that the amount of carbohydrate rather than the amount of fat in the diet was the decisive factor determining whether FFA or glucose was the preferred fuel.

A 30 minute submaximal running test was used to examine the influence of carbohydrate rich liquid on energy metabolism. Orava et al. (1974) used a 65 g glucose load and Coca-Cola® (23 g) ingested 15 minutes prior to exercise and analyzed blood samples for glucose, FFA, and insulin. They measured a clear increase in the serum FFA level after the intake of Coca-Cola®, but FFA levels did not differ from the initial value after the intake of the 65 g glucose solution. They reported that this depressed level of FFA was obviously due to the antilipolytic activity of elevated serum insulin.

Costill et al. (1977) measured a 3.3 fold increase in plasma insulin and a 38% rise in plasma glucose 45 minutes after ingesting 75 g of glucose. During subsequent exercise for 30 minutes at 70% VO_{2max} on a treadmill, FFA levels remained at the resting level for the duration of the work period. They concluded that the elevation of plasma glucose and insulin prior to exercise increased the rate of carbohydrate metabolism.

The effect of ingestion of 200 g of glucose 50 minutes prior to the initiation of 4 hours of exercise at 30% VO_{2max} was investigated by Ahlborg and Felig (1977). Compared to control subjects, glucose fed subjects showed a 30 to 40% increase in arterial glucose levels and a two- to threefold increase in plasma insulin during exercise. Coinciding with these rises, FFA showed a steady decline of approximately 50% from rest to 140 minutes of exercise. It was concluded that glucose ingestion prior to exercise resulted in a diminution in lipolysis caused in part by the hyperinsulinemia which accompanied the glucose load.

Luyckx et al. (1978) submitted healthy subjects to one of three procedures: (1) ingestion of 100 g of glucose at rest (controls), (2) an exercise test without glucose, and (3) an exercise test with the ingestion of 100 g of glucose. Exercise was performed at about 50% VO_{2max} and continued for 4 hours. They found that although there was a slight exercise induced increase in plasma FFA, it was markedly delayed and reduced by about 60% compared to the exercise-no glucose group. They felt that glucose availability, as in pre-exercise glucose ingestion, reduced exercise-induced glucagon secretion and possibly FFA mobilization.

Ravussin et al. (1979) studied five control and five glycogen depleted subjects after the ingestion of 100 g of glucose. One hour after ingestion, each subject pedaled a bicycle ergometer for 2 hours at 40% of their VO_{2max}. They determined that the controls used mainly carbohydrates (65%) and the glycogen depleted subjects used mainly lipid (70%) during the exercise. Control subjects also exhibited a FFA level 1/3 to 1/2 lower throughout the total exercise period.

In their study, which utilized 75 g of glucose ingested 30 minutes prior to exercise to exhaustion on a bicycle ergometer, Foster et al. (1979) found significant differences in performance compared to water and milk ingestion. Compared to the endurance ride (80% VO_{2max}) in the water treatment, endurance time was reduced 19% due to the pre-exercise glucose ingestion. Serum FFA was depressed during the total glucose trial and indicated an impaired lipid mobilization which led to a reduced exercise time to exhaustion.

A summary of the research of glucose ingestion prior to exercise is listed in Table 6.

VI. SUMMARY

Since the size of the glycogen stores and their rate of utilization have an extremely important role in endurance performance, this review attempted to identify several of the factors which influenced these physiological processes.

Diet manipulation, especially carbohydrate loading prior to exercise, has been shown to increase glycogen stores two to threefold and thereby improve performance. However, several drawbacks have been associated with the regimen of carbohydrate loading described in this review. The physiological and psychological aspects of the high fat plus protein diet, the weight gain with accompanying stiffness, and the possible EKG abnormalities are several factors that have been identified. Two studies by Gollnick et al. (1972, 1973) have determined that training can increase glycogen stores to levels approaching those of carbohydrate loading. It is currently recommended that

Table 6. Summary of research--glucose ingestion prior to exercise

Results	Number of Subjects	Dosage	Type/Intensity of Exercise	Results
Issekutz et al. (1963)	6 male	Glucose 100 g	Very light (300 kpm/ min.) for 30 mins., cycling	Increased carbohydrate utilization
Orava et al. (1974)	20 male	Glucose 65 g/ 24 g	Submaximal for 30 mins., running	Depressed FFA due to hyperinsulinemia
Costill et al. (1977)	7 male	Glucose 75 g	70% VO_{2max} for 30 mins., treadmill	Increased carbohydrate metabolism
Ahlborg and Felig (1977)	6 male	Glucose 200 g	30% VO_{2max} for 4 hrs., cycling	Decrease in lipolysis
Luyckx et al. (1978)	8 male	Glucose 100 g	50% VO_{2max} for 4 hrs., treadmill	Decrease in FFA levels
Ravussin et al. (1979)	10 male	Glucose 100 g	40% VO_{2max} for 2 hrs., cycling	Increased carbohydrate utilization and depressed FFA levels
Foster et al. (1979)	8 male 8 female	Glucose 75 g	80% VO_{2max} to exhaustion, cycling	Reduction in endurance

athletes "naturally load" through a diet high in complex carbohydrates and adherence to their normal workout routine.

A critical range of exercise intensity has been determined, above which carbohydrate is increasingly used as fuel to support muscular work, and below which fat is the primary fuel for working muscles. This range for trained subjects is 70 to 80% of their VO_{2max}. The upper limit of this range can be increased as fitness improves, thereby allowing a greater percentage of fat to be used as fuel and consequently sparing the glycogen stores.

Both high lactate and high insulin levels have been shown to impede the mobilization of FFA, thereby increasing the use of carbohydrates (glycogen) and decreasing performance. Lactate levels induced by exercise or electrical stimulation that approach 5 mM have a significant depressing effect on FFA mobilization. Glucose ingested during exercise tends to prevent hypoglycemia, increase blood sugar levels, and allow additional work to be accomplished. However, glucose ingested prior to exercise has been shown to cause hyperinsulinemia and as described above, impedes FFA mobilization.

Most factors reviewed are well researched and the conclusions are generally accepted. However, one factor, that of pre-exercise glucose ingestion, needs further study. Previous research has used a glucose load between 65 g and 200 g prior to exercise and found detrimental results regarding its effect on performance. This investigator questions the practicality and soundness of applying these conclusions to lower level glucose solutions commonly used by most athletes.

The purposes of the present study were to determine (1) how selected glucose solutions modify the blood glucose concentration at rest and (2) how they modify the plasma FFA and insulin concentrations during exercise.

CHAPTER III

METHODS

I. SUBJECTS

Four well-conditioned males and two well-conditioned females, all volunteers, took part in the research study. Each subject was given a thorough explanation of the procedures, including any stresses and discomfort that might occur before giving his or her written consent to participate (see Table 7 for physical characteristics of subjects). For all phases of the study, the subjects were at least 4 hours post absorptive and the glucose load was administered according to body weight, dissolved in 300 ml of water (.36 g/kg bw or .54 g/kg bw).

II. TRAINING PROGRAM AND VO_{2max} TEST

The training program was used to acquaint the subjects with the equipment and procedures prior to the actual experimental sessions. The training consisted of 30 minute bicycle ergometer rides, 4 days per week, for 3 weeks at a work rate equal to or greater than 60% of their VO_{2max}. Following the training program, each subject was tested to determine their VO_{2max} during an incremental ride to exhaustion on a bicycle ergometer. The findings of this test were used to compute each subject's work load during the experimental rides. All submaximal and maximal tests used a Monark bicycle ergometer calibrated prior to each ride. During the VO_{2max} test and all experimental rides, the respiratory exchange ratio was determined.

Table 7. Physical characteristics of subjects

Subject	Age (Years)	Height (cm)	Weight (kg)	VO_{2max} ml $(kg\text{-}Min.)^{-1}$
1	27	188.3	88.5	41.8
2	21	183.5	63.6	57.7
3	31	185.6	84.4	41.7
4	23	168.2	45.4	43.8
5	23	163.4	56.8	38.2
6	37	177.6	74.9	40.0

III. PROCEDURES

Resting Glucose Test

The first phase of the present study determined the amount of glucose to be ingested, 30 minutes prior to exercise, to cause an increase in the blood glucose level slightly above the normal resting range of 70 to 100 mg% (Table 8). Once this base was established, one solution containing 50% more glucose in addition to the base was used as a test solution. An equal volume of water was used as the control solution.

Experimental Work Tests

Immediately upon reporting to the laboratory, a catheter was placed in a vein of the dorsal aspect of the hand and 10 ml of blood were drawn while the subject rested. Following this procedure each subject ingested 300 ml of one of the test solutions or an equal volume of water. In order to eliminate an ordering effect of the treatments, the subject was assigned to 1 of the 6 possible treatment sequences. To protect against investigator and subject bias this experimental phase was conducted as a double blind study.

Thirty minutes after the glucose or water ingestion the subject began the exercise test. An incremental increase of 0.5 kg/min. was used to reach a work rate for each subject that was approximately 60% of his/her VO_{2max}. All subjects pedaled at 60 rpm for a total of 30 minutes.

Blood samples were drawn at 0, 10, 20, and 30 minutes during exercise (Table 9). A 2-minute gas sample was collected during each

Table 8. Resting glucose protocol

Subject	Pre Blood Glucose mg%	Post Blood Glucose mg%	Glucose Load* Administered
1	85	112	31
2	88	114	23
3	83	114	31
4	82	108	16
5	75	99	21
6	90	116	27

*Glucose load determined to be .36 g glucose · kg body wt^{-1}.

Table 9. Experimental work test protocol

Test Phase	Sampling Time (Minutes)	Physiological Measurements
Rest	-30*	Blood
Exercise test	0	Blood, VO_2, VCO_2, HR
	10	Blood, VO_2, VCO_2, HR
	20	Blood, VO_2, VCO_2, HR
	30	Blood, VO_2, VCO_2, HR

*Thirty minutes prior to exercise.

blood withdrawal period for purposes of oxygen uptake measurement and determination of the respiratory exchange ratio.

The experimental work tests, using the assigned test solutions, were administered to all subjects on 3 separate days, over a 2 to 3 week period. On each testing day the laboratory was between 20 and 30°C and the relative humidity was moderate (30 to 65%). Subjects were allowed to dress according to their own comfort and followed the same procedure during each of the 3 testing days.

IV. INSTRUMENTS AND TECHNIQUES OF MEASUREMENT

Catheterization

The following catheterization sequence was utilized for each test.

A 21-gauge disposable needle was placed on a sterile 20 ml disposable plastic syringe (Becton, Dickinson and Co., Rutherford, N.J.) and 4 ml of sodium heparin (Heparin Sodium, 1000 U/ml, Lilly Co., Indianapolis, Ind.) was drawn from the heparin vial. This volume was added to a vial containing 30 ml of sterile saline (Bacteriostatic Sodium Chloride, Cutter, Berkeley, Ca.).

A Velcro tourniquet was wrapped around the forearm and the dorsal aspect of the hand was examined for a vein to be punctured. After cleansing the skin with alcohol, a catheter (Deseret Minicath-PRN Catheter, 3/4 inch, 21-gauge, Deseret Pharmaceutical Co., Sandy, Utah) was removed from its sealed container and inserted about 1/2 inch into the vein. If blood could be withdrawn from the syringe, the catheterization was considered acceptable and the catheter was inserted.

The catheter was then secured in place by tape and blood was withdrawn into the syringe to be certain the needle was still properly placed. Approximately 1 to 2 ml of heparinized saline was then infused into the vein to keep the catheter patent during the test.

The minute before blood collection, a disposable 30 ml plastic syringe was used to clear the catheter of its heparin and saline. A small amount of blood was withdrawn and a new sterile 20 ml syringe was put in its place for sampling. A 15 ml blood sample was then taken. The syringe containing the blood sample was removed from the catheter and replaced by the syringe containing the heparinized saline, after which 0.5 to 1.0 ml of the solution was again infused to keep the catheter patent. This sequence was repeated for each sampling period.

Ventilation and Gas Exchange

Oxygen uptake was measured by the open circuit method utilizing meteorological balloons and a Daniels valve. Expired gases were measured by an Applied Electrochemistry model S-3A O_2 analyzer and a Beckman model LB2 CO_2 analyzer. The analyzers were calibrated prior to each experiment and between each sample collection time with standardized gases previously measured by the Scholander method (Scholander, 1947).

Inspired minute ventilation (V_I) was measured by a Parkinson-Cowan CD4 dry meter and recorded with a Narco Biosystems DMP-4A recorder. The dry gas meter was calibrated against a 120 liter Tissot gasometer. Oxygen uptake was calculated according to Consolazio et al. (1963).

Heart Rate

Heart rate was recorded with surface electrodes and a Narco Biosystems DMP-4A recorder.

Blood Samples

Blood samples were taken via an indwelling catheter previously placed in the hand. During each period, approximately 15 ml of blood was withdrawn for determination of plasma glucose, FFA, lactic acid, and insulin. About 1 ml of the blood used for lactic acid analysis was placed on a spot plate containing 50 µl of 4% sodium fluoride that had been dried. The blood and sodium fluoride were mixed and 50 µl of the sample was placed in 2 ml of perchloric acid, thoroughly mixed and put in an ice bath.

The remaining blood samples to be used for glucose and FFA analysis were placed in specifically prepared heparinized tubes and put in an ice bath. The blood sample for the insulin analysis was put into a tube and allowed to clot.

At the completion of the 30-minute exercise test, the glucose and FFA samples were centrifuged at 4000 rpm for 10 minutes. The tubes containing percholoric acid and 50 µl of blood were centrifuged and then decanted into another chilled tube and frozen for later analysis. The plasma for glucose and FFA analysis was aspirated into a Pasteur pipette, delivered into plastic tubes, capped, and frozen to be analyzed at a later date. The serum for the insulin analysis was also aspirated into a Pasteur pipette, delivered into a plastic tube, capped, and frozen for later analysis.

Blood Analysis

The blood lactate concentration was determined by the enzymatic method of Gutmann and Wahlefeld (1974), modified for use in the fluorometer. The FFA analysis followed the titration method of Dole and Meinertz (1960) and Trout et al. (1960). Each FFA sample was analyzed in duplicate by two independent technicians and the standards were reanalyzed periodically during the assay procedure. The glucose assay was a modification of the Lowry and Passonneau (1972) glucose analysis. The insulin was analyzed using a radioimmunoassay (RIA) procedure according to Corning Insulin ^{125}I Radioimmunoassay Test System (See Appendix A, B, C, and D for detailed descriptions of each assay procedure.)

Statistics

Since the major area of interest in the present study was the response of FFA levels as a result of pre-exercise glucose ingestion, this aspect was specifically addressed.

A paired t-test was used to determine the difference between the control solution and each of the two experimental loads (low and moderate glucose load). An overall significance of 0.10 was set, but a level of 0.05 was used for each of the two a priori tests selected.

In addition to the paired t-test used above, a one-sample t-test was used to compare results of the present study to results reported from studies by Costill et al. (1977), Ravussin et al. (1979), Foster et al. (1979), and Orava et al. (1974).

CHAPTER IV

RESULTS

Since the present study is similar to protocols used in several other studies, this chapter will be divided into two sections. The first section will contain the data of the present study, while the second section will deal with a comparison of the present study with similar studies.

I. PRESENT STUDY

Glucose

The ingestion of the low glucose solution (.36 g/kg body weight) produced a 39% increase in plasma glucose, while the ingestion of the moderate glucose solution (.54 g/kg body weight) produced a 41% increase. This rapid rise in plasma glucose from rest produced a significant difference between the water trial and both the low glucose trial ($P < .001$) and the moderate glucose trial ($P < .01$) at the beginning of exercise. These differences dissipated by minute 10 and remained nonsignificant ($P > .05$) for the duration of the exercise period (Table E-1). As shown in Table 10, the plasma glucose concentrations for both experimental trials exhibited a slow steady decline and then leveled off at minute 20. The lowest plasma glucose value recorded on any subject was 48 mg% in minute 30 of the low glucose trial.

Table 10. Plasma glucose concentration at rest and during the exercise work test

	Rest		Exercise		
Time (Min.)	-30	0	10	20	30
Control	79 ± 3	76 ± 5	76 ± 4	77 ± 3	78 ± 3
Low glucose	80 ± 2	111 ± 2*	96 ± 8	67 ± 3	67 ± 4
Moderate glucose	83 ± 1	117 ± 6*	91 ± 6	70 ± 6	71 ± 5

*Significantly different from control trial.

Note: Values are glucose in mg% expressed as $\bar{X}$ ± SE.

Blood Lactate

Data on the blood lactate concentrations are listed for both the experimental conditions and the control in Table 11. Blood lactic acid levels showed an incremental increase over the total exercise period, however no significant differences were noted (P at least > .05) due to either of the glucose treatments. See Table E-2 for the statistical results of blood lactate.

Free Fatty Acid

The free fatty acid concentration for both the experimental and control trials increased from rest until approximately minute 20 of exercise (Figure 4). Although the free fatty acid level was depressed during the moderate glucose trial, the difference was not statistically significant (P at least > .10). From minute 20 to minute 30, a slight nonsignificant decrease in the free fatty acid concentration was measured in all three trials. See Table E-3 for the statistical results.

Insulin

The ingestion of glucose solutions prior to exercise increased the serum insulin levels at the beginning of exercise as expected. The serum insulin concentration increased from 8.0 μU/ml to 27.5 μU/ml and from 5.0 μU/ml to 42.0 μU/ml at the beginning of exercise for the low glucose and moderate glucose solutions, respectively. These values then decreased over the course of the exercise period until near resting levels were reached at minute 30 (Figure 5). There were significant differences noted at rest as a result of both the low

Table 11. Blood lactate concentration at rest and during the exercise work test

	Rest		Exercise		
Time (Min.)	-30	0	10	20	30
Control	.58 ± 0.1	.72 ± 0.2	1.27 ± 0.3	2.00 ± 0.3	2.27 ± 0.5
Low glucose	.60 ± 0.1	.57 ± 0.1	1.67 ± 0.3	2.36 ± 0.3	2.52 ± 0.5
Moderate glucose	.65 ± 0.3	.70 ± 0.2	1.88 ± 0.2	2.67 ± 0.2	2.68 ± 0.4

Note: Values are lactic acid in millimoles per liter expressed as $\bar{X}$ ± SE.

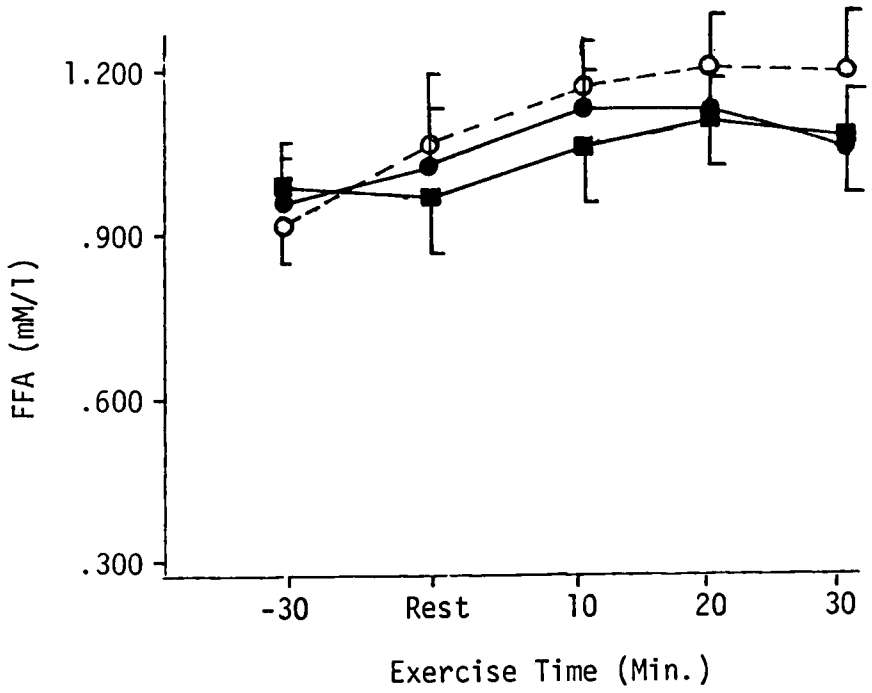

Figure 4. Comparison of plasma FFA concentration in response to the different experimental solutions and the control trial ingested prior to exercise. Values are $\bar{X} \pm SE$.

_____ ● = low glucose

_____ ■ = moderate glucose

------- o = control

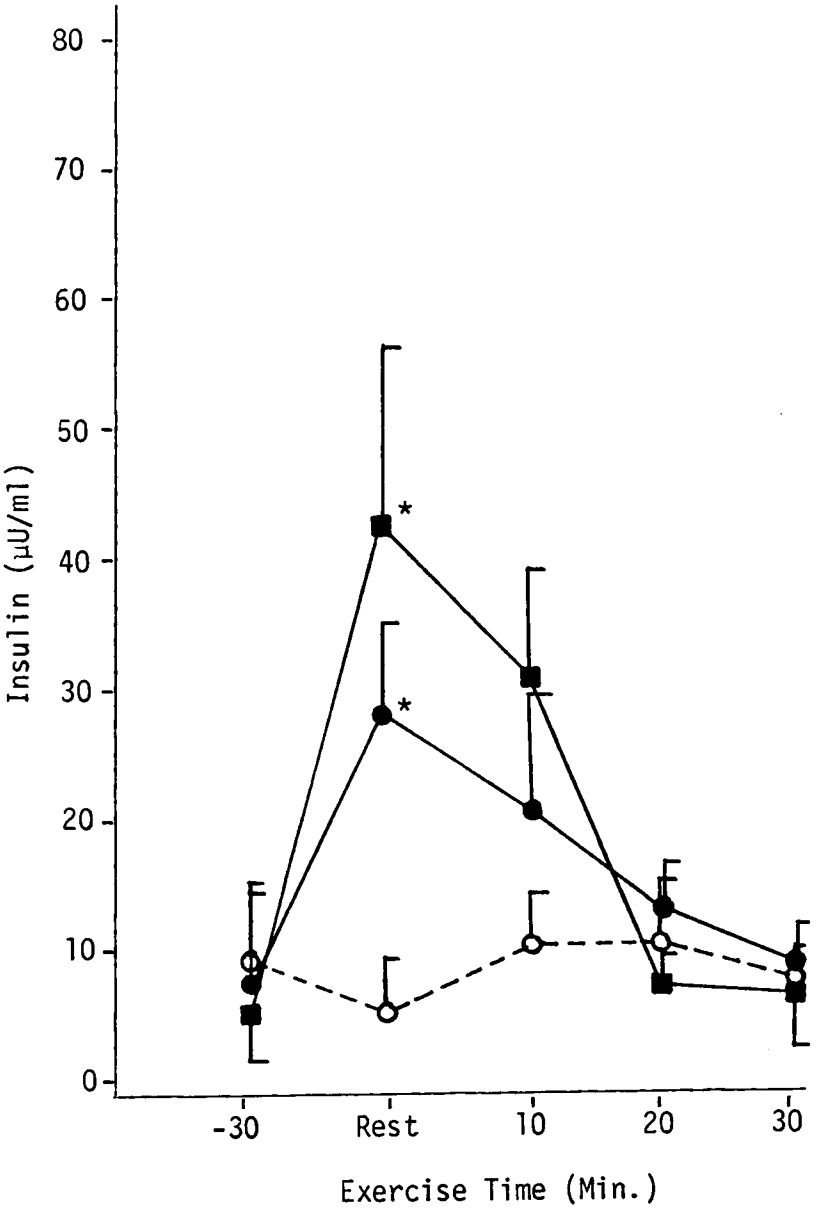

Figure 5. Comparison of serum insulin concentration in response to the different experimental solutions and the control trial ingested prior to exercise. Values are $\bar{X} \pm SE$.

_____ ● = low glucose

_____ ■ = moderate glucose

------- o = control

*Significantly different from control trial (n = 4).

glucose treatment ($P < .01$) and the moderate glucose treatment ($P < .02$) (Table E-4).

Respiratory Exchange Ratio (R)

The ratio of exchanged carbon dioxide and oxygen (R) was significantly greater at all times ($P < .02$ at 10', 20', $P < .05$ at 30') during the moderate glucose trial as compared to the water trial (Figure 6). Based on the mean respiratory exchange data it was determined that carbohydrate accounted for approximately 81% of the fuel during the moderate glucose trial, 77% during the low glucose trial and 67% during the water trial (Table E-5).

Oxygen Consumption

As indicated in Figure 6, oxygen consumption during the 30 minute trials showed a gradual increase and represented approximately 60% of each subject's VO_{2max}. There were no significant differences ($P > .20$) noted at any of the data collection times due to the treatments (Table E-6).

II. OTHER STUDIES

In the present study, three different solutions were ingested prior to exercise; water, low glucose ($\bar{X}$ = 25 g), and moderate glucose ($\bar{X}$ = 37 g). Although the protocols of other studies did not always include these three solutions, meaningful comparisons were possible.

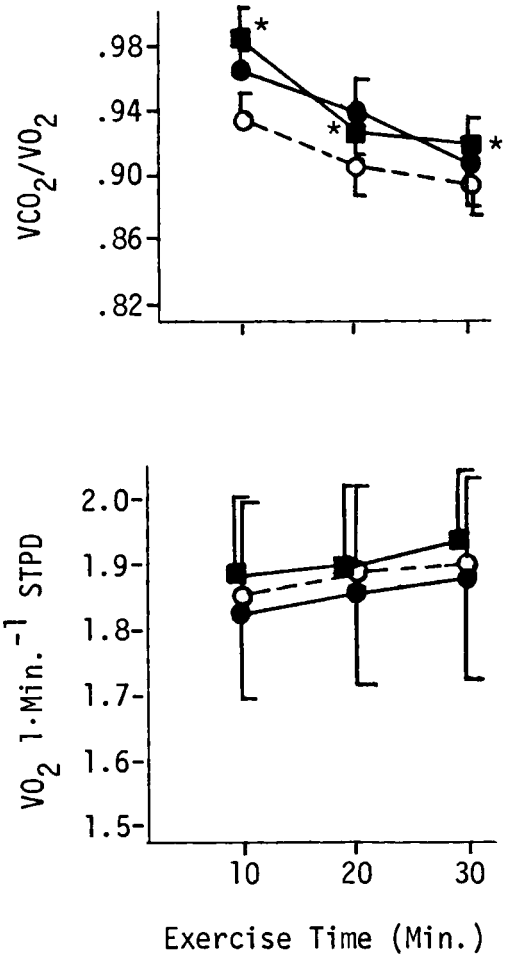

Figure 6. Comparison of VO_2 and R changes across time between the experimental solutions and the control. Values are $\bar{X}$ ± SE.

_____ ● = low glucose

_____ ■ = moderate glucose

------- o = control

*Significantly different from control trial.

Glucose

Figure 7 shows a comparison of plasma glucose concentrations of the present study with similar studies following pre-exercise glucose ingestion. Included is the exercise intensity and the amount of glucose ingestion for the various studies.

The plasma glucose concentration measured in the present study following the pre-exercise ingestion of water was compared to studies by Foster et al. (1977) and Costill et al. (1979). The concentrations in the present study compared most favorably with the Foster study where nonsignificant differences ($P > 0.5$) were noted at all data collection times. However, significant differences were recorded at minute 10 ($P < .05$), minute 20 ($P < .01$), and minute 30 ($P < .01$) when compared to the Costill study.

Ingestion of the low glucose solution prior to exercise elicited a lower plasma glucose concentration and resulted in significant differences ($P < .05$) between the present study and both the Foster and Costill study at minutes 0 and 10. This significance was eliminated in the later stages of exercise. When the low glucose trial of the present study was compared to that of Ravussin et al. (1979), the reverse was true in that no significance ($P > .05$) was exhibited at minutes 0 and 10, while a significant difference was apparent at minute 20 ($P < .002$) and minute 30 ($P < .05$).

When the moderate glucose solution of the present study was ingested prior to exercise, the plasma glucose concentration at the beginning of exercise increased to approximately the same concentrations as reported in studies by Foster ($P > 0.8$), Costill ($P > 0.1$), and Ravussin ($P > 0.2$). The higher glucose concentration during

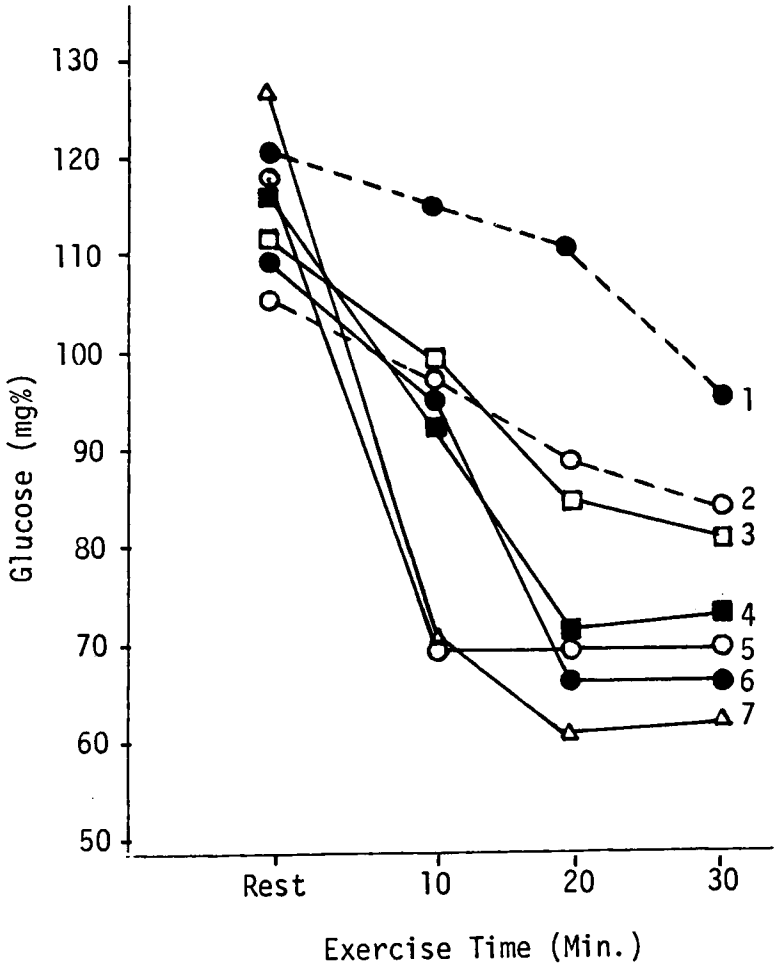

Figure 7. Comparison of plasma glucose concentrations of the present study with other similar studies during exercise. Values are $\bar{X}$.

1 = Orava (65 g)--submax
2 = Orava (23 g)--submax
3 = Ravussin (100 g)--40% VO_{2max}
4 = Present study (moderate)--60% VO_{2max}
5 = Foster (75 g)--80% VO_{2max}
6 = Present study (low)--60% VO_{2max}
7 = Costill (75 g)--70% VO_{2max}

minute 10 produced significant differences ($P < .02$) between the present study and both the Foster and Costill studies. However, these differences dissipated in minutes 20 and 30. The comparison with Ravussin's study showed a slightly different result, as a significant difference ($P < .05$) in the concentration did not appear until minute 20 and then became nonsignificant ($P > 0.1$) by minute 30.

Table E-7 shows the single sample t-test results of the comparisons of the glucose levels of the present study to those of similar studies at various times during exercise following a glucose ingestion.

Free Fatty Acid

In comparing the free fatty acid concentration of the present study with the results of studies by Orava et al. (1974), Foster et al. (1977), Costill et al. (1979), and Ravussin et al. (1979) care in interpretation was taken due to the different methods used to analyze the FFA concentrations and the difference in the fasting period prior to data collection.

When the free fatty acid concentrations of the present study were compared to those of the above studies following the ingestion of similar pre-exercise solutions, there were significant differences at all times for all solutions ($P < .002$). The free fatty acid levels of the present study were consistently higher than in the other studies. As mentioned above, this may have been due to the method of analysis and the fasting period. The present research used the titration method, the standard against which all other methods are based, while the other studies used colormetric methods. The length of the

fasting period prior to each experimental trial may also have contributed to the higher FFA concentrations of the present study.

A comparison of the free fatty acid levels of the present study following the glucose ingestions are compared to similar studies in Figure 8. In addition to the higher FFA levels of the present study, these concentrations due to the glucose solutions tended to increase during the exercise period, similar to the increase resulting from the water trial. This was in contrast to the depressed FFA levels reported in all but the Orava study, which measured a slight FFA increase. Statistical results for these comparisons are shown in Table E-8.

Insulin

When comparing the serum insulin levels of the present study to those of other studies (Costill et al., 1977; Ravussin et al., 1979), the results were not surprising. Since lower glucose loads were ingested prior to exercise, it followed that lower insulin levels were produced.

It was determined that the insulin level, as a result of the water trial, remained near resting levels throughout the exercise period and was consistent with the levels reported in the Costill study ($P > .10$).

When the insulin levels of the low glucose trial of the present study were compared to similar studies, significant differences ($P < .05$) were found at all time periods in the Ravussin study and at all times except minute 10 ($P > .10$) in the Costill study. These significant differences were a result of the consistently lower insulin levels of the present study.

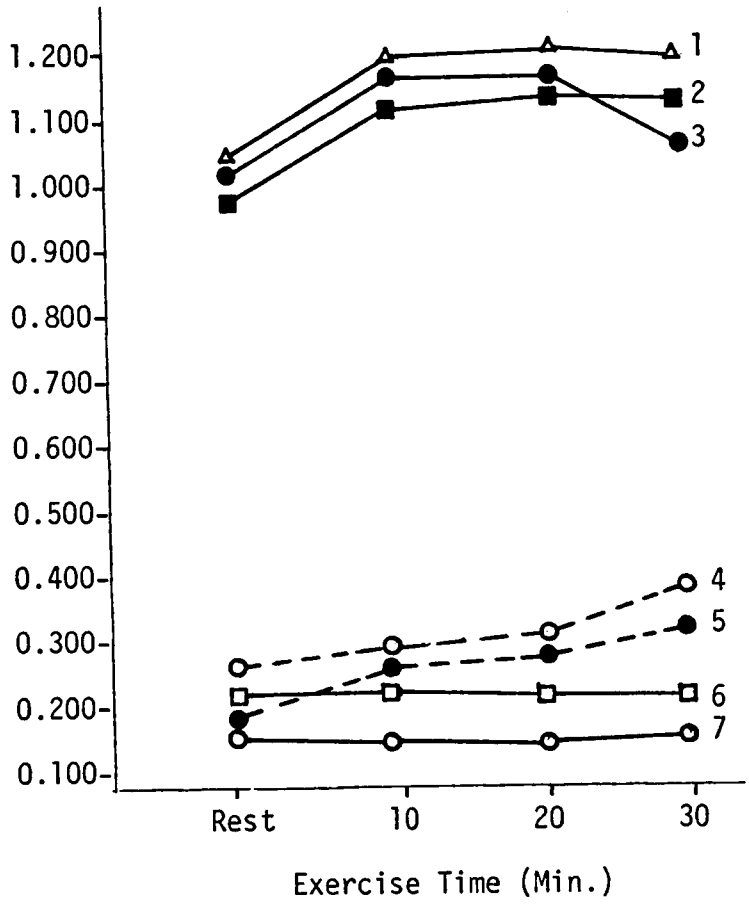

Figure 8. Comparison of plasma free fatty acid concentrations of the present study with other similar studies during exercise. Values are $\bar{X}$.

1 = Present study (water)--60% VO_{2max}
2 = Present study (moderate)--60% VO_{2max}
3 = Present study (low)--60% VO_{2max}
4 = Orava (23 g)--submax
5 = Orava (65 g)--submax
6 = Ravussin (100 g)--40% VO_{2max}
7 = Foster (75 g)--80% VO_{2max}

The comparison of the insulin levels due to the moderate glucose load produced slightly different results when compared to the above same studies. These comparisons showed no significant differences ($P > .05$) until minute 10 of exercise, but significant differences ($P < .01$) appeared in the later stages.

See Table E-9 for all insulin comparisons.

Respiratory Exchange Ratio

The respiratory exchange ratios (R) of the present study were compared to the (R) of studies by Foster et al. (1977) and Costill et al. (1979). The only significance ($P < .02$) found was at minute 10 of the moderate glucose trial when compared to both the Foster and Costill studies. This significance was not apparent during the later stages of the exercise periods (Table E-10).

CHAPTER V

DISCUSSION

The depletion of muscle glycogen stores has been shown to be the major contributor to fatigue during endurance performance (Ahlborg et al., 1967; Hultman, 1967; Karlsson and Saltin, 1971).

Studies by Hickson et al. (1977) and Rennie et al. (1976) determined that enhanced free fatty acid mobilization and utilization was responsible for a glycogen sparing effect and enhanced performance. Therefore, the free fatty acid responses to various strength glucose solutions ingested prior to exercise were of primary interest in the present study. Previous studies in this area (Issekutz et al., 1963; Orava et al., 1974; Costill et al., 1977; Ahlborg and Felig, 1977; Luyckx et al., 1978; Ravussin et al., 1979; and Foster et al., 1979) which showed depressed free fatty acid concentrations, used glucose loads (65 to 100 mg) significantly greater than would normally be ingested prior to exercise by the average competitor. However, results of these studies have been used to suggest dietary practices that should be followed by endurance athletes.

Therefore, the present study was undertaken to determine whether or not lower glucose loads (25 to 50 g) ingested prior to exercise diminished free fatty acid levels during exercise.

Glucose

In the present study, the plasma glucose concentrations increased significantly at the beginning of exercise after ingestion of both the moderate and low glucose solutions. The ingestion of water prior to

exercise did not cause an increase. These results were not only consistent with the findings of other investigators (Orava et al., 1974; Costill et al., 1977; Luyckx et al., 1978; Foster et al., 1979; and Ravussin et al., 1979) but were intuitively obvious.

During the initial phases of exercise in the present study, the reason for the gradual decrease in the plasma glucose concentrations became apparent when compared to the reports of concentrations of plasma glucose during exercise of similar studies. Costill reported a decrease in the glucose concentration from 126 mg% at the beginning of exercise to 70 mg% at minute 10 of exercise following a 75 g glucose ingestion while exercising at 70% VO_{2max}. This was consistent with the drop in the glucose concentration reported in the Foster study. It showed a decrease from 117 mg% to 70 mg% during the same time period following a 75 g glucose ingestion prior to exercising at 80% VO_{2max}. However, when the intensity was reduced to 40% VO_{2max} as reported in the study by Ravussin, the glucose concentration at minute 10 was 99 mg% following the ingestion of 100 g of glucose. This was consistent with the findings of the Orava study which reported a decrease in plasma glucose concentration from about 120 mg% at the beginning of exercise to 108 mg% in minute 10. This study required submaximal running following the ingestion of a 65 g glucose solution. Both of these studies compared favorably with the present study where plasma glucose concentrations in minute 10 were 96 mg% for the low glucose and 91 mg% for the moderate glucose.

From the above information it seems reasonable to conclude that the initial rate of decrease of the plasma glucose concentration during exercise, following the ingestion of a glucose solution, was most dependent on the intensity of the exercise performed.

This was further emphasized in the later stages of exercise when the low glucose solution of the present study was compared to the studies in which the exercise intensity was relatively moderate (Ravussin, 40% VO_{2max}; Orava, submaximal). During minute 20, the plasma glucose concentration was 85 mg% and 90 mg% for the Ravussin and Orava studies, respectively. These concentrations decreased slightly to 80 mg% and 84 mg% respectively in minute 30. These levels are even higher than the present study (60% VO_{2max}) for the same time periods and further reinforced the claim that the rate of decrease of the plasma glucose concentration was greatly influenced by exercise intensity.

Blood Lactate

The finding that elevated blood lactate concentrations (> 5mM) decreased free fatty acid mobilization (Issekutz and Miller, 1962; Issekutz et al., 1965; Boyd et al., 1974; Fredholm, 1971; and Green et al., 1979) influenced the protocol of the present study. In order to eliminate the depressing influence of elevated lactate concentrations on lipolysis and to isolate the effects of glucose induced hyperinsulinemia, the work intensity was limited to approximately 60% of each subject's VO_{2max}. Consequently, the lactate concentrations remained relatively low (< 3mM) and of little consequence in their relationship to free fatty acid mobilization.

Free Fatty Acid

The focus of the present study was centered around the changes in free fatty acid levels as a result of various strength glucose solutions ingested prior to exercise. The reason for the interest

in the free fatty acid levels became apparent when research dealing with the effects of pre-exercise glucose ingestion on these levels was reviewed. Through experimental free fatty acid elevation, Costill et al. (1977) and Hickson et al. (1977) demonstrated enhanced endurance performance. Conversely, the findings of impaired performance due to depressed lipid mobilization following large pre-exercise glucose ingestions further reinforced the importance of free fatty acids in endurance performance (Orava et al., 1974; Costill et al., 1977; Foster et al., 1979; and Ravussin et al., 1979).

The important point in the previous paragraph was the fact that large glucose loads were ingested prior to exercise. In the present study, three solutions were ingested prior to exercise: water, a low glucose load, and a moderate glucose load. The results showed that both the low glucose load as well as the moderate glucose load ingested 30 minutes prior to exercise did not have a depressing effect on free fatty acid concentrations during exercise. Although there was a slight decline in the free fatty acid concentration at the end of the exercise period, there were no statistical differences found between either of the glucose trials and the water trial during minute 20 or minute 30. This gradual decrease in free fatty acid level in the later stages of the exercise period may have indicated an impending depression in the free fatty acid level. However, this would seem rather unlikely as the insulin level had declined to approximately the resting level at this point. A more logical explanation would be that these were transitory decreases experienced in all three trials and similar to the transient decreases reported in

studies using a heavy glucose load (Ravussin et al., 1979; Foster et al., 1979).

When the insulin levels of the present study were compared with insulin levels of similar studies, it became obvious what the main factor was that influenced free fatty acid concentrations and their changes during exercise. As can be seen in Figure 8, page 56, the free fatty acid levels of the present study were markedly higher when compared to the other studies cited. As discussed earlier, this vast difference could be either due to the method of analysis used (titration versus colormetric), the length of the fasting period prior to exercise or a combination of the two. Nevertheless, the studies which showed the greatest fre fatty acid depression were those in which an extremely large glucose load was ingested prior to exercise. Again the summary in Figure 8, page 56, outlines the various precedures of the studies compared.

The concluding remarks of both the Orava and Foster study contain a recommendation that a smaller amount of glucose be ingested prior to exercise; in essence to prevent the depression of free fatty acid.

The results of the present study support this recommendation with facts, i.e., a low glucose solution ingested prior to exercise did not cause a depression in the free fatty acid levels during exercise.

Insulin

As stated previously, the serum insulin level in the present study was mediated by the ingestion of various strength glucose loads.

The relatively low strengths of these glucose solutions were intentionally chosen to keep the responding insulin concentrations at a lower level than reported in similar studies (55 to 60 μU/ml). Since high concentrations of insulin are known to suppress the mobilization of free fatty acids and hence inhibit performance, a smaller amount of glucose was ingested to determine if the resulting insulin level had a similar effect.

As discussed in the results section of this paper, the glucose loads chosen for this study produced lower serum insulin levels when compared to other studies. These insulin levels in addition to being lower, returned to near resting levels more rapidly. As a result of the plasma insulin levels of the present study remaining elevated for a shorter period, an impairment of lipid mobilization was not experienced as reported in similar studies.

Since blood lactic acid remained low (< 3 mM) during the exercise period of the present study, the serum insulin levels were isolated to determine their effect on FFA levels. It appears from the results of the present study that if the pre-exercise glucose loads are moderate enough, the responding insulin levels will remain low enough so as not to suppress free fatty acid levels during exercise.

Respiratory Exchange Ratio (R)

Changes in the plasma concentration of glucose, free fatty acids or lactate could be due to changes in the rate of influx into or efflux from the plasma. These changes alone do not adequately describe changes in whole body metabolism. On the other hand, during

stable conditions, the ratio of CO_2 production to oxygen consumption does. In fact, this measure, the respiratory exchange ratio (R), is used as the primary piece of evidence describing relative changes in the rate of carbohydrate and fat metabolism. Related to this, the accuracy of the methods used to measure the R permits the evaluation of small changes in this matter.

The higher R as a result of the moderate glucose load was indicative of the increased use of carbohydrate as the fuel to support the exercise. However, this was in contrast to the nonsignificant differences of the FFA concentrations between the moderate glucose trials and the water trial found through blood analysis. Ideally, a reduced rate of FFA oxidation would be consistent with this reported increase in carbohydrate oxidation. Although nonsignificant differences were reported in the FFA concentrations due to the glucose treatments, Figure 4, page 48, reveals that the ingestion of the moderate glucose load mediated the lowest FFA concentration. This trend was consistent with the elevated R as a result of the same treatment. This lower FFA concentration was especially noticeable and the difference became significant when only five of six subjects were used due to the inconsistent data of one subject.

CHAPTER VI

CONCLUSIONS AND RECOMMENDATIONS

Glucose

1. Plasma glucose concentrations increased significantly after ingestion of both low and moderate strength glucose solutions.

2. Plasma glucose concentrations dissapate gradually across time during exercise and the rate of this decrease is highly related to the intensity of the exercise.

Insulin

1. Serum insulin concentrations increased significantly at rest in response to the ingestion of both glucose solutions prior to exercise.

2. The rate of decrease of serum insulin during exercise is greatly influenced by the initial insulin concentration resulting from the pre-exercise glucose ingestion.

Free Fatty Acid

1. The ingestion of a low glucose load, (.36 g/kg body weight) or less, prior to exercise did not result in a FFA depression during a 30-minute exercise test.

2. The ingestion of a moderate glucose load, .54 g/kg body weight or more, may result in a FFA depression during a 30-minute exercise test.

A follow-up study to determine the effects of these glucose solutions on endurance performance is both appropriate and planned for the immediate future.

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APPENDICES

APPENDIX A

ENZYMATIC LACTIC ACID ANALYSIS

Lactic Acid Procedure for Turner 450 Fluorometer

$$\text{(L+) Lactic acid + NAD} \xleftrightarrow{\text{LDH}} \text{Pyruvic acid + NADH}$$

In the presence of LDH, lactic acid and NAD are converted to pyruvic acid and NADH respectively. As this reaction will not go to completion unless the pyruvic acid is removed from the mixture, a hydrazine-glycine buffer is added in order that the hydrazine will complex with the pyruvic acid.

Reagents

1. 70% perchloric acid ($HClO_4$)
2. Hydrazine-glycine buffer
   a. Hydrazine sulfate --Sigma No. H-3376
   b. Glycine --Sigma No. G-7126
3. Lactic dehydrogenase (LDH)--Sigma No. L-2625
4. Nicotinamide adenine dinucleotide, OX. Form
   --Sigma No. N-7004
5. Lithium L + lactate standard-stock
   a. Sigma L + lithium
   b. Lactate (L2250)
   c. 20 mM = 480 mg up to 250 ml with H_2SO_4 - H_2O solution

(0.25 ml concentrated H_2SO_4 made up to 500 ml)

6. 4% NaF (W/V)

Daily Working Solutions

1. Deproteinizing agent--7% perchloric acid

a. Add 6.0 ml of 70% $HClO_4$ to a flask and add water up to 100 ml. (Refrigerate)

ml 7% $HClO_4$	100	200	300	400	500	1000
$HClO_4$	6	12	18	24	30	60
H_2O	94	188	282	376	470	940

2. Hydrazine glycine buffer (2 ml/assay). Example for 100 ml of buffer:

a. 0.4 molar hydrazine and 0.5 molar glycine. Add 3.76 gm glycine and 5.20 gm hyerazine sulfate to about 80 ml of water.

b. Adjust pH to 9.0 with concentrated NaOH and bring up to 100 ml with water.

c. Stable for about 1 week.

Assays	24	37	50	75	100	250	500
ml buffer	50	75	100	150	200	500	1000
gm hydrazine sulfate	2.60	3.90	5.20	7.80	10.40	26.00	52.00
gm glycine	1.88	2.82	3.76	5.64	7.52	18.80	37.00
ml H_2O	35.00	60.00	80.00	120.00	170.00	400.00	800.00
Final volume	50.00	75.00	100.00	150.00	200.00	500.00	1000.00

*3. Lactic dehydrogenase

a. To a given volume of buffer add enough LDH so that the final concentration is 1.0 U LDH/ml buffer. Approximate values are:

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*Should be added to buffer only before use. This will be after all samples and standards have been pipetted into fluorometer tubes.

Assays	25	37	50	75	100
ml buffer	50	75	100	150	200
ml LDH (5900 U/ml LDH)	0.0085	0.0127	0.0169	0.0254	0.0339

*4. B-nicotinamide adenine dinucleotide, oxidized form

a. Add enough NAD to a given volume of buffer so that the final concentration is .25 mg NAD/ml buffer.

Assays	25	37	50	75	100
ml buffer	50	75	100	150	200
mg NAD	12.5	18.8	25.0	37.5	50.0

Working Standards

Add water--H_2SO_4 solution to all volumes of stock standard to make up correct standards.

0 = H_2SO_4 solution = Blank

1 mM = 5 ml stock up to 100 ml

5 mM = 25 ml stock up to 100 ml

10 mM = 50 ml stock up to 100 ml

15 mM = 75 ml stock up to 100 ml

Sample Collection

1. 0.05 ml of 4% NaF is added to spot plate and dried at 80°C overnight.

2. Put blood sample on spot plate containing the 4% NaF. Mix.

3. Using 50 µl micropipettes, transfer 50 µl of the sample to a cold test tube containing 2.0 ml of 7% $HClO_4$. Mix and either put on ice or refrigerate.

4. Centrifuge at 4000 RPM for 5 minutes at 0-5°C.

5. Decant supernatant into another chilled tube.

6. Refrigerate or put on ice until analysis.

Standard Curve

1. Add 50 µl of each working standard to 2.0 ml cold 7% $HClO_4$. Mix thoroughly (Vortex) and treat as blood sample.

Analytical Procedure

1. Standards and unknowns should be analyzed in duplicate. Add 0.1 ml of Blank, Standards, and Samples to fluorometer tubes.

2. Treat all of the above tubes as follows:

   a. Add 2.0 ml of buffer enzyme solution to each tube.

   b. Mix thoroughly.

   c. Incubate in water at 30°C for 30 minutes and cool to room temperature.

Fluorescence Determination

Read fluorescence at 360/460 with 60 nm excitation slit, and 60 nm emission slit. Use the blank to correct for all readings.

Calculations

Due to quenching by the fluorometer, the Standard Curve is curvilinear, not linear. In order to correctly calculate concentrations, a graph of the Standard Curve must be used.

References

Gutmann, I. and A. W. Wahlefeld. L-(+)-Lactate. Determination with lactate dehydrogenase and NAD. In: Methods of enzymatic analysis. Edited by H. U. Bergmeyer. Second English Edition. Academic Press, Inc., New York, pp. 1464-1468, 1974.

APPENDIX B

PLASMA GLUCOSE ANALYSIS

Plasma Glucose Procedure for Turner 450 Fluorometer

$$\text{Glucose} + \text{ATP} \longrightarrow \text{Glucose-6-P} + \text{ADP}$$

$$\text{Glucose-6-P} + \text{NADP}^{+} \longrightarrow \text{6-P-Gluconolactone} + \text{NADPH} + \text{H}^{+}$$

Stock Glucose Standard

Dissolve 2.00 gm pure anhydrous glucose (Dextrose) in 1 liter of water containing 1.5 gm benzoic acid = 200 mg/100 ml. Stable for 2 years in refrigerator.

Daily Working Standards

1. Stock standard = 200 mg/100 ml.
2. 75 ml stock standard up to 100 ml = 150 mg/100 ml.
3. 50 ml stock standard up to 100 ml = 100 mg/100 ml.
4. 25 ml stock standard up to 100 ml = 50 mg/100 ml.

Reagent

Buffer. Add 0.303 gm THAM (TRIS) F.W. 121.14 to about 80 ml water and stir with magnetic stirring bar under pH electrode. Add drop by drop dilute HCL to reach pH = 8.1 (under hood). Then dilute to 100 ml with water using a 100 ml volumetric flask = 25 mM TRIS base.

To this 100 ml buffer, add the following:

1. 20.3 mg of $MgCl_2 - 6H_2O$ (F.W. = 203.31). Yields a 1 mM solution relative to $MgCl_2$.

2. 7.7 mg of Dithiothreitol (F.W. = 154.3). Yields a 0.5 mM solution relative to this compound.

3. Add 16.5 mg of ATP (M.W. = 551.2). Yields a 300 μM solution relative to ATP.

4. 2.3 mg of $NADP^+$. Yields a 30 μM solution relative to $NADP^+$.

5. Add 0.1 mg of Hexokinase (15 U per mg). Yields 1 μl/ml.

6. Add 5 μl (2 U) of glucose-6-P dehydrogenase. This package contains 0.4 ml of enzyme, containing 150 U. Yields 0.02 U/ml.

Procedure

1. Take 50 μl of each sample, working standard and water into a sample tube and add 2.00 ml of water. Vortex.

2. Take 100 μl of each of the tubes into a fluorometer tube.

3. Add 2.0 ml of reagent buffer to each tube. Vortex.

4. Wait 10 minutes at room temperature.

5. Read at 360/460 with 60/60 slits.

6. Calculate net fluorescence.

References

Lowry, O. and Passoneau, J. A Flexible System of Enzymatic Analysis. Academic Press, New York, 1972.

APPENDIX C

PLASMA FFA DETERMINATION

Reagents

1. "Extraction mixture"--for first extraction step

Isopropanol:	78% = 400 ml	800 ml	1600 ml	2400 ml
n-Heptane:	20% = 100 ml	200 ml	400 ml	600 ml
In H_2SO_4: (14 ml conc. up to 500 ml)	2% = 10 ml	20 ml	40 ml	60 ml

2. 0.05% aqueous sulfuric acid.

   a. Take 0.5 gm concentrated H_2SO_4 up to 1 liter with distilled water.

   b. Store in a re-pipet bottle.

3. Nile Blue A

   a. Stock: add 20 mg Nile Blue A to 100 ml water, dissolve.

   b. Extract with heptane in separatory funnel, repeatedly, until heptane phase is no longer green.

   c. Working solution: add 10 ml stock to 90 ml absolute ethanol on day of analysis.

4. Titration alkali

   a. Prepare saturated NaOH by adding NaOH pellets to water until precipitation occurs. Do not heat while adding NaOH.

   b. Take 0.05 ml saturated NaOH up to 75 ml H_2O on day of analysis.

   c. Keep NaOH covered during test.

5. Standards

a. Dissolve approximately 50 mg palmitic acid in 250 ml heptane. Determine concentration by dividing the actual amount by (256.4 mg X .25). For example, 53.6 mg ÷ (256.4 mg X .25) = .837 mM or 837 µM.

b. Making working standards:

1. 1 ml stock + 9 ml heptane = 83.7 µM/l.
2. 2 ml stock + 8 ml heptane = 167.4 µM/l.
3. 4 ml stock + 6 ml heptane = 334.8 µM/l.
4. 5 ml stock + 5 ml heptane = 418.5 µM/l.

6. Gas washing solution

1N NaOH with 1 drop of phenophtalene (1% in ethanol).

Procedure

Use acid washed separatory funnels (60 ml). Set them up on wrist action shaker and follow directions.

Blank	Standard	Sample
3 ml water	3 ml H_2O	3 ml plasma
15 ml ext. mixture	15 ml ext. mixture	15 ml ext. mixture
SHAKE 5 min.	SHAKE 5 min.	SHAKE 5 min.
STAND 5 min.	STAND 5 min.	STAND 5 min.
9 ml heptane	9 ml STANDARD	9 ml heptane
6 ml water	6 ml water	6 ml water
SHAKE 2 min.	SHAKE 2 min.	SHAKE 2 min.
STAND 5 min.	STAND 5 min.	STAND 5 min.
Drain lower layer	Drain lower layer	Drain lower layer
Add 12 ml 0.05% H_2SO_4	Add 12 ml 0.05% H_2SO_4	Add 12 ml 0.05% H_2SO_4
SHAKE 2 min.	SHAKE 2 min.	SHAKE 2 min.
STAND 5 min.	STAND 5 min.	STAND 5 min.
Drain lower layer	Drain lower layer	Drain lower layer

Take 3 ml for titration _____ _____

Add 1 ml of working solution of Nile Blue A _____ _____

Place tube on titrator assembly _____ _____

Titrate until clear, then go to pink end point.

Before placing the tube on the Digi-Pet assembly and starting the

titration, re-set the counter and advance to 1.0 on far right scale. Wipe tip with moist kim wipe.

Calculation

Assume standard = 837 μM/l = .837 μM/ml; so 7.53 μM were added to funnel (since 9 ml stock standard was used)

FFA concentration (μ Eq or μM per ml) =

$$\mu M/ml = \frac{T \text{ sample} - T \text{ blank}}{T \text{ standard} - T \text{ blank}} \times 7.53 \ \mu M \times \frac{1 \text{ ml plasma}}{3 \text{ ml plasma}}$$

References

Dole, V. P. and H. Meinertz. Microdetermination of long-chain fatty acids in plasma and tissue. J. Biol. Chem., 235:2595-2599, 1960.

Trout, D. L. E. H. Estes and S. M. Friedberg. Titration of free fatty acids of plasma: A study of current method and a new modification. J. Lipid Research, 1:199-202, 1960.

APPENDIX D

INSULIN I^{125} RADIOIMMUNOASSAY ANALYSIS

Materials

1. Insulin antibody bulk: 96 ml per container
2. 3 vials iodinated insulin tracer
3. 3 vials insulin standard, 0 μU/ml
4. 3 vials insulin standard, 400 μU/ml
5. 3 vials insulin reference control I
6. 3 vials insulin reference control II

Instructions for Reconstitution of Reagents

All lyophilized reagents should be reconstituted with distilled or deionized water in the following amounts. After addition of the water, the reagents should be swirled for 2 to 3 seconds and allowed to stand at room temperature for 30 minutes with intermittent mixing.

To Reconstitute:	Add:
Insulin tracer	5.0 ml distilled water
Insulin standard, 0 μU/ml	8.0 ml distilled water
Insulin standard, 400 μU/ml	3.0 ml distilled water
Insulin controls	3.0 ml distilled water

Dilution of Standards[1]

After reconstitution, the supplied insulin standards must be serially diluted to give the following levels of insulin concentration: 0, 6.25, 12.50, 25, 50, 100, 200, and 400 µU/ml.

1. Set up a series of 6 text tubes, either glass or plastic, approximately 12 X 75 mm in size.

2. Pipette 1 ml of the reconstituted 0 µU/ml standard into each tube.

3. Add 1 ml of the reconstituted 400 µU/ml standard to the first tube, and vortex 2 to 3 seconds. Label this tube as the 200 µU/ml standard.

4. Using a clean pipette tip, transfer 1 ml from the first tube to the second tube. Mix well, and label this tube as the 100 µU/ml standard.

5. Using clean pipette tips, continue transferring 1 ml from tube to tube until all of the above standards are prepared. Care should be taken to label these standards correctly.

Procedure

1. Label polystyrene test tubes for each standard, control and patient sample to be assayed.

2. Pipette 0.30 ml (300 µl) of the appropriate standard, control or patient serum into appropriately marked test tubes.

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[1]0.3 ml of each diluted standard, as well as 0.3 ml of the 0 and 400 standards, will be transferred respectively to the antibody tubes marked with the appropriate concentration.

3. Add 0.10 ml (100 µl) of the radioiodinated insulin tracer to each tube.

4. Pipette 0.8 ml (800 µl) of the bulk antibody solution into each test tube.

5. Incubate for 2 hours at room temperature (determine total counts added by counting several tubes during incubation period).

6. Centrifuge at 1400-1600 g's for 10 minutes. Either a room temperature or refrigerated centrifuge may be used but consistency should be observed for quality control.

7. Decant the supernatant of each tube into a suitable radioactive waste container. Care should be taken to decant all tubes in the same manner.

8. Count all tubes in a gamma scintillation counter for a time sufficient to remove counting statistics as an important source of variability, and record the data in counts per minute (cpm) bound.

Reference

Corning Medical. Insulin ^{125}I Radioimmunoassay Test System. Corning Glass Works, Medfield, Mass., 1981.

APPENDIX E

STATISTICAL ANALYSIS

Table E.1. Repeated measures t-test for plasma glucose between the two experimental glucose solutions and the control solution during exercise

Trial	Measurement Period (Min.)	df	ΣX	$\Sigma(X^2)$	t	P
Low	0	5	205	7399	9.64	< .001
	10	5	105	4673	2.06	> .05
	20	5	-67	1243	2.46	> .05
	30	5	-58	1686	1.79	> .10
Moderate	0	5	240	11410	5.28	< .01
	10	5	68	3152	1.68	> .10
	20	5	-46	1988	0.90	> .10
	30	5	-28	1676	0.90	> .10

Table E.2. Repeated measures t-test for blood lactate between the two experimental glucose solutions and the control solution during exercise

Trial	Measurement Period (Min.)	df	ΣX	$\Sigma(X^2)$	t	P
Low	0	5	-0.90	1.09	0.84	> .20
	10	5	2.40	7.22	0.87	> .20
	20	5	2.20	5.80	0.88	> .20
	30	5	1.60	4.54	0.73	> .20
Moderate	0	5	0.10	0.13	0.31	> .20
	10	5	3.70	4.61	2.26	> .05
	20	5	4.00	7.12	1.74	> .20
	30	5	2.60	4.00	1.39	> .20

Table E.3. Repeated measures t-test for plasma free fatty acid between the two experimental glucose solutions and the control solution during exercise

Trial	Measurement Period (Min.)	df	ΣX	$\Sigma(X^2)$	t	P
Low	0	5	79	108681	0.23	> .20
	10	5	104	67859	0.11	> .20
	20	5	112	48393	0.50	> .20
	30	5	470	142986	1.31	> .20
Moderate	0	5	392	88924	1.11	> .20
	10	5	355	55943	1.76	> .20
	20	5	230	81868	0.79	> .20
	30	5	422	94942	1.34	> .20

Table E.4. Repeated measures t-test for serum insulin between the two experimental glucose solutions and the control solution during exercise

Trial	Measurement Period (Min.)	df	ΣX	$\Sigma(X^2)$	t	P
Low	0	3	- 79	1703	5.93	< .01
	10	3	- 29	525	1.42	> .10
	20	3	- 12	380	0.67	> .20
	30	3	- 6	20	1.56	> .10
Moderate	0	3	- 137	5227	5.12	< .02
	10	3	- 70	2886	1.49	> .10
	20	3	25	535	1.11	> .10
	30	3	6	78	0.63	> .20

Table E.5. Repeated measures t-test for respiratory exchange ratio between the two experimental glucose solutions and the control solution during exercise

Trial	Measurement Period (Min.)	df	ΣX	$\Sigma(X^2)$	t		P
Low	10	5	.150	.019	1.34	>	.05
	20	5	.210	.023	1.77	>	.05
	30	5	.130	.013	0.90	>	.05
Moderate	10	5	.270	.018	3.62	<	.02
	20	5	.190	.008	3.41	<	.02
	30	5	.240	.014	3.23	<	.05

Table E.6. Repeated measures t-test for oxygen uptake between the two experimental glucose solutions and the control solution during exercise

Trial	Measurement Period (Min.)	df	ΣX	$\Sigma(X^2)$	t	P
Low	10	5	0.08	.008	0.65	> .20
	20	5	0.06	.012	1.81	> .10
	30	5	0.18	.014	1.77	> .20
Moderate	10	5	0.21	.050	1.06	> .20
	20	5	0.02	.009	0.00	> .20
	30	5	0.05	.014	0.47	> .20

Table E.7. Single sample t-test for plasma glucose comparing the experimental trials of the present study with other similar studies

Trial	Measurement Period (Min.)	Study							
		Foster et al.		Costill et al.		Ravussin et al.		Orava et al.	
		t	P	t	P	t	P	t	P
Water	0	0.20	> 0.80	2.00	> 0.20	N.A.		N.A.	
	10	0.50	> 0.50	3.25	< 0.05				
	20	0.33	> 0.50	4.67	< 0.01				
	30	0.33	> 0.50	5.33	< 0.01				
Low glucose	0	3.00	< 0.05	7.50	< 0.002	0.50	> 0.50	2.50	> 0.05
	10	3.25	< 0.05	3.25	< 0.05	0.38	> 0.50	0.50	> 0.50
	20	0.67	> 0.50	2.00	> 0.10	6.00	< 0.002	7.80	< 0.002
	30	0.25	> 0.80	1.00	> 0.20	3.25	< 0.05	4.25	< 0.01
Moderate glucose	0	0.00	> 0.80	1.50	> 0.10	0.83	> 0.20	0.50	> 0.50
	10	3.50	< 0.02	3.50	< 0.02	1.30	> 0.20	3.83	< 0.02
	20	0.20	> 0.80	1.50	> 0.10	3.20	< 0.05	6.00	< 0.002
	30	0.60	> 0.50	1.60	> 0.10	1.80	> 0.10	4.50	< 0.01

Table E.8. Single sample t-test for plasma free fatty acid comparing the experimental trials of the present study with other similar studies

Trial	Measurement Period (Min.)	Study: Foster et al. t	Foster et al. P	Costill et al. t	Costill et al. P	Ravussin et al. t	Ravussin et al. P	Orava et al. t	Orava et al. P
Water	0	6.20	< .002	6.20	< .002	N.A.		N.A.	
	10	9.09	< .002	9.09	< .002				
	20	8.91	< .002	8.91	< .002				
	30	10.61	< .002	10.61	< .002				
Low glucose	0	6.95	< .002	N.A.		6.82	< .002	6.45	< .002
	10	8.32	< .002			7.95	< .002	7.27	< .002
	20	8.25	< .002			8.75	< .002	9.94	< .002
	30	9.42	< .002			8.89	< .002	7.36	< .002
Moderate glucose	0	6.88	< .002	N.A.		6.74	< .002	6.88	< .002
	10	8.42	< .002			7.97	< .002	7.41	< .002
	20	10.72	< .002			9.87	< .002	8.98	< .002
	30	14.10	< .002			13.35	< .002	11.60	< .002

Table E.9. Single sample t-test for serum insulin comparing the experimental trials of the present study with other similar studies

Trial	Measurement Period (Min.)	Study			
		Costill et al.		Ravussin et al.	
		t	P	t	P
Water	0	2.30	> .10	N.A.	
	10	0.27	> .50		
	20	0.12	> .80		
	30	0.73	> .50		
Low glucose	0	3.59	< .05	4.10	< .05
	10	2.18	> .10	2.82	< .05
	20	3.95	< .05	5.12	< .02
	30	3.33	< .05	3.33	< .05
Moderate glucose	0	0.93	> .20	1.20	> .20
	10	1.64	> .10	2.47	> .05
	20	10.40	< .01	12.60	< .01
	30	6.25	< .01	6.25	< .01

Table E.10. Single sample t-test for respiratory exchange ratio comparing experimental trials of the present study with other similar studies

Trial	Measurement Period (Min.)	Study: Foster et al. t	Foster et al. P	Costill et al. t	Costill et al. P
Water	10	1.490	> 0.20	1.490	> 0.20
	20	1.500	> 0.80	0.500	> 0.80
	30	0.700	> 0.80	0.000	> 0.80
Low glucose	10	2.396	> 0.05	2.396	> 0.05
	20	1.790	> 0.20	2.396	> 0.05
	30	0.646	> 0.50	0.646	> 0.50
Moderate glucose	10	3.870	< 0.02	3.870	< 0.02
	20	2.000	> 0.20	2.000	> 0.20
	30	1.000	> 0.50	1.000	> 0.50

APPENDIX F

SUBJECT DATA

Table F.1. Subject data for plasma glucose

Trial	Measurement Period (Min.)	Subjects					
		1	2	3	4	5	6
Water	-30	70	84	82	73	88	82
	0	60	72	87	70	90	76
	10	60	90	85	73	80	81
	20	67	81	86	74	75	83
	30	73	83	87	71	74	69
Low glucose	-30	82	77	83	73	86	80
	0	100	113	114	112	111	110
	10	105	105	102	100	55	107
	20	70	80	64	56	62	67
	30	72	74	48	72	73	60
Moderate glucose	-30	83	80	80	80	87	84
	0	122	88	122	118	135	120
	10	97	71	90	98	77	114
	20	62	57	54	79	76	92
	30	82	61	58	65	84	77

Note: Values are in mg%.

Table F.2. Subject data for blood lactate

Trial	Measurement Period (Min.)	Subjects					
		1	2	3	4	5	6
Water	-30	0.3	0.9	0.5	0.5	1.0	0.3
	0	0.4	0.9	0.7	0.5	1.5	0.3
	10	1.2	2.1	1.4	0.6	1.8	0.5
	20	1.4	2.2	3.1	1.4	2.2	1.7
	30	1.3	2.9	4.3	1.5	1.7	1.8
Low glucose	-30	0.4	1.3	0.5	0.5	0.5	0.4
	0	0.4	1.0	0.5	0.5	0.5	0.5
	10	0.6	2.1	1.6	1.5	1.3	2.9
	20	1.0	3.1	2.3	3.1	1.9	2.8
	30	1.0	3.0	3.8	3.4	1.4	2.5
Moderate glucose	-30	0.3	1.0	0.5	0.5	1.2	0.4
	0	0.4	1.1	0.5	0.5	1.3	0.4
	10	1.3	2.2	1.3	1.9	2.6	2.0
	20	2.1	3.2	2.3	3.5	2.5	2.4
	30	1.4	3.8	3.7	3.1	2.2	2.9

Note: Values are in mM.

Tablf F.3. Subject data for free fatty acid

Trial	Measurement Period (Min.)	Subjects					
		1	2	3	4	5	6
Water	-30	1.125	0.885	1.020	0.915	1.320	0.420
	0	0.885	1.395	0.750	0.912	1.575	0.810
	10	1.005	1.245	0.840	1.071	1.605	1.209
	20	1.065	1.245	0.915	0.945	1.611	1.410
	30	1.140	0.960	1.170	1.020	1.617	1.209
Low glucose	-30	0.924	0.690	0.645	1.065	1.575	0.645
	0	1.275	0.915	0.450	1.260	1.050	1.140
	10	1.230	1.065	0.660	1.260	1.065	1.620
	20	1.230	0.990	0.570	1.200	1.245	1.620
	30	0.936	0.855	0.465	1.230	1.245	0.975
Moderate glucose	-30	0.555	1.335	0.675	0.894	1.245	1.185
	0	0.960	0.975	0.540	0.744	1.050	1.005
	10	1.290	0.885	0.690	0.735	1.230	1.980
	20	1.650	0.855	0.816	0.795	1.230	1.155
	30	1.680	0.708	0.816	0.744	1.005	1.020

Note: Values are in mM.

Table F.4. Subject data for serum insulin

Trial	Measurement Period (Min.)	Subjects 1	2	3	4
Water	-30	13	26	0	0
	0	5	8	10	8
	10	17	13	0	14
	20	17	11	0	18
	30	6	12	0	13
Low glucose	-30	25	7	0	0
	0	20	25	40	25
	10	13	15	12	33
	20	8	14	17	19
	30	8	12	0	17
Moderate glucose	-30	9	3	5	3
	0	30	45	33	60
	10	20	50	38	6
	20	0	10	7	4
	30	5	6	5	9

Note: Values in μU/ml.

Table F.5. Subject data for respiratory exchange ratio and VO_2

Trial	Measurement Period (Min.)	Respiratory Exchange Ratio						VO_2					
		Subjects											
		1	2	3	4	5	6	1	2	3	4	5	6
Water	10	1.0	.86	.95	.90	.90	.99	2.36	2.37	2.26	1.07	1.28	1.71
	20	.96	.85	.89	.89	.89	.92	2.43	2.34	2.26	1.13	1.35	1.80
	30	.92	.84	.85	.87	.87	.92	2.39	2.46	2.27	1.13	1.37	1.84
Low glucose	10	.93	.93	.98	.98	.94	.99	2.32	2.39	2.19	1.10	1.26	1.71
	20	.90	.92	.94	.98	.89	.98	2.37	2.40	2.28	1.11	1.35	1.74
	30	.87	.87	.92	.94	.87	.93	2.34	2.41	2.20	1.17	1.37	1.79
Moderate glucose	10	1.03	.95	.99	.96	.96	.98	2.41	2.45	2.14	1.05	1.36	1.85
	20	.96	.91	.94	.92	.92	.94	2.38	2.37	2.22	1.15	1.35	1.86
	30	.94	.91	.93	.90	.91	.92	2.33	2.54	2.23	1.14	1.38	1.89

Note: Values for VO_2 in l/min.

VITA

James Bailey Angel was born in Miami, Florida on March 5, 1943. He attended public schools there and was graduated from Miami Senior High School in June 1961.

In June 1966, he completed a Bachelor of Science degree in Engineering at the United States Naval Academy, Annapolis, Maryland. He then entered the United States Navy as an Ensign and served at various duty stations until discharged in 1968. Following discharge from the Navy, he was in private business until 1977 when he entered graduate school. He completed his Masters degree in Health and Physical Education in March 1979 at the University of Alabama, Birmingham.

After completion of the Master's degree, he was accepted in the Doctoral program at The University of Tennessee, Knoxville. His program of study was concentrated in the area of exercise physiology with a collateral area of study in nutrition. His degree program was completed in 1981 and he was graduated on August 24, 1981. Following graduation, he accepted a faculty position at Liberty Baptist College, Lynchburg, Virginia.