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Effect of Concentrated Carbohydrate-Electrolyte Gel on Moderate-Intensity Intermittent Exercise

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**EFFECT OF CONCENTRATED CARBOHYDRATE-ELECTROLYTE GEL
ON MODERATE-INTENSITY INTERMITTENT EXERCISE**

by

Sarah M. Handlogten

**A Thesis
Submitted to the
Faculty of The Graduate College
in partial fulfillment of the
requirements for the
Degree of Master of Arts
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and Recreation**

**Western Michigan University
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EFFECT OF CONCENTRATED CARBOHYDRATE-ELECTROLYTE GEL ON MODERATE-INTENSITY INTERMITTENT EXERCISE

Sarah M. Handlogten, M.A.

Western Michigan University, 1998

The researcher compared the effects of a carbohydrate-electrolyte (CE) gel and water ingested 5 min before and after 20 min of moderate-intensity running for 1 hour. Six dependent variables were measured: heart rate (HR), VO_2 , blood glucose, respiratory exchange ratio (R), rating of perceived exertion (RPE), and timed exercise performance. Independent variables were condition, trials (2), and sample time. On 4 days, subjects completed a 60-min training run at 65% of maximal oxygen consumption in 20-min bouts with 3-min rests between bouts. A timed 200-m sprint followed each run. Two trials were completed with each condition. Water was given after 40 and 60 min of exercise for all trials. HR was taken every 5 min during the 60-min intermittent run. Blood samples were taken preexercise, during each 3-min rest, and postsprint. R and VO_2 were measured every 20 s. RPE was determined the last 30 s of each 20-min bout. Significant differences existed: (a) in blood glucose levels, which were higher with the gel at preexercise, after 40 min, and postsprint; (b) in blood glucose for the gel condition between preexercise and both 40 min and postsprint; (c) among the times for R and 20 and 60 min for RPE; and (d) between trials, with levels greater for Trial 2 for R and Trial 1 for RPE and sprint time. The ingestion of a gel may enhance glycogen reserves for exercise, although R decreased progressively across time in all trials, indicating a greater reliance on fat utilization. Sprint performance was not improved with supplementation.

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CHAPTER I

INTRODUCTION

Carbohydrate-electrolyte supplements have been highly researched in sports nutrition and fitness, and recommendations continue to evolve. It has been the consensus over the past several years that sports drinks were appropriate and beneficial in moderate- to high-intensity activities lasting longer than 90 min (Coggan & Coyle, 1991; Coyle, Coggan, Hemmert, & Ivy, 1986; Coyle & Montain, 1992a, 1992b). During endurance exercise, the body loses fluid and electrolytes through sweat. Fluid replacement is essential to prevent hyperthermia and dehydration. Fluids containing electrolytes and carbohydrates (CHO) also can prevent hyponatremia, by providing sodium, and hypoglycemia, by maintaining blood glucose levels and sparing muscle glycogen stores. Recently, several researchers have suggested that carbohydrate-electrolyte (CE) beverages may improve performance of moderate- to high-intensity cycling exercise lasting approximately 60 min (Anantaraman, Carmines, Gaesser, & Weltman, 1995; Below, Mora-Rodriguez, Gonzalez-Alonso, & Coyle, 1995; Jackson, Davis, Broadwell, Query, & Lambert, 1995; Jeukendrup, Brouns, Wagenmakers, & Saris, 1997; Neuffer et al., 1987).

Problem Statement

The problem of this study was to determine the effects of concentrated CE gels on heart rate (HR), VO_2 , blood glucose levels, respiratory exchange ratio (R),

ratings of perceived exertion (RPE), and timed 200-m sprint performance in moderate-intensity intermittent aerobic exercise lasting 1 hr.

Need for the Study

Further research is needed in the area of fluid and CHO supplementation as elite and recreational athletes, as well as exercise and nutrition professionals, seek safe, ethical, and effective means to enhance performance. Although there is extensive research related to fluid, CHO, and electrolyte replacement with endurance events, there are limited studies on the benefit of fluid replacement and CHO supplementation in exercise lasting less than 90 min. The few studies that have been done on exercise lasting about 1 hr involved cycling and did not address the needs of runners and athletes in sports that involved running. This activity would be common in many sports such as soccer, football, racket sports, and field events, in which moderate- to high-intensity exercise are required in bouts lasting less than 1 hr. There is little information related to supplementation and the intermittent exercise most commonly associated with sports training and competition, and more research is needed on the effects of fluid replacement and CHO supplementation related to performance lasting about 1 hr.

Delimitations

The following delimitations were identified for the study:

1. Subjects ($n = 8$) were male and female volunteers between the ages of 18 and 30 years.
2. The subjects were well-trained recreational runners, who ran a minimum of 35 miles per week.

3. Maximal oxygen consumption (VO_2 max) and R were measured using a metabolic cart.

4. The subjects rated perceived exertion using the original Borg's Rating of Perceived Exertion (RPE) scale of 6–20 (Manore & Ryan, 1992).

5. The subjects performed two VO_2 max trials and two trials each of concentrated CE gel supplementation and of the control condition, plain water (C).

6. A minimum of 24 hr was required between consecutive exercise sessions.

7. For each trial, subjects performed a timed 200-m run in the shortest time possible after completing three 20-min bouts of running at 65% VO_2 max on the treadmill.

8. Subjects ran on a treadmill for the VO_2 max tests and intermittent exercise bouts. The 200-m timed exercise performance test was performed on an indoor track.

Limitations

The following limitations that may affect the interpretation of the results were identified for this study :

1. Subjects were selected opportunistically rather than randomly, and therefore the study may lack heterogeneity and external validity.

2. Subjects completed only two trials of each condition, and a greater number of trials might improve the internal validity.

3. The number of subjects was limited to 8, and a larger sample might increase the external validity of the study.

Assumptions

It was assumed that:

1. Subjects fasted a minimum of 4 hr prior to the tests following a meal providing 1 g of carbohydrate per pound of body weight.
2. Subjects avoided exhaustive exercise 48 hr prior to the test day and did not exercise the day of the test.
3. Dietary intake was consistent on the days immediately preceding all tests, and normal dietary intake was maintained throughout the study period.
4. Training levels were kept constant throughout the study period.
5. Subjects performed the graded exercise test and timed exercise performance test with all-out effort.

Hypotheses

It was hypothesized that:

1. Subjects would experience a smaller reduction in blood glucose levels for the CE condition than for the C.
2. Subjects would experience a higher value of R for the CE condition than for the C.
3. Subjects would identify a lower mean RPE (RPE taken the last 30 s of each 20-min bout and at the end of the timed exercise performance tests) for the CE condition than for the C.
4. Subjects' mean sprint times would be lower for the CE condition than for the C.

Definition of Terms

The following definitions were pertinent to this study:

1. *Borg's Rating of Perceived Exertion (RPE)*: A rating scale from 6 to 20 that is used to determine exercise intensity by measuring subjective exertion or effort level (Manore & Ryan, 1992).

2. *Gluconeogenesis*: A process that occurs primarily in the liver and kidneys in which glucose is synthesized from noncarbohydrate sources when carbohydrate is not available in sufficient amounts in the diet (Anderson & Anderson, 1994).

3. *Glycogen*: A complex carbohydrate consisting of branched chains of glucose units linked together. It serves as the storage form of carbohydrates in humans. Glycogen is stored in the liver and muscles and may be readily broken down to glucose (Anderson & Anderson, 1994).

4. *Glycogenolysis*: A nonoxidative process that occurs primarily in the liver and muscle in which glycogen is broken down to glucose (Anderson & Anderson, 1994).

5. *Glycolysis*: A process in which body cells metabolize glucose to lactic acid without oxygen. It provides energy for short bursts of intense muscular activity when oxygen consumption exceeds demands (Anderson & Anderson, 1994).

6. *Hyperthermia*: An exceptionally high body temperature, usually 41 °C or higher. Symptoms include flushed skin, skin that is warm to the touch, increased respiratory rate, tachycardia, and seizures or convulsions (Anderson & Anderson, 1994).

7. *Hyponatremia*: An abnormally low concentration of sodium in the blood; normal is 136–143 mmol/L (Barr & Costill, 1989).

8. *Maximal Oxygen Consumption (VO₂ max)*: Oxygen consumption increases as workload increases until a plateau is reached. This plateau is referred to as VO₂ max and is the product of maximum cardiac output and maximum

arteriovenous oxygen difference. It is the best measure of the capacity of the cardiovascular system (Brooks, Fahey, & White, 1996).

9. *Percentage of Maximal Oxygen Consumption (%VO₂ max)*: The amount of oxygen consumed relative to VO₂ max. It is useful for determining how stressful the exercise is with respect to maximum capacity (Manore & Ryan, 1992).

10. *Respiratory Exchange Ratio (R)*: The ratio of the volume of expired carbon dioxide in a subject to the volume of consumed oxygen. It is expressed by the formula $R = VCO_2/VO_2$, where VCO₂ = the value of expired carbon dioxide (Brooks et al., 1996).

CHAPTER II

REVIEW OF RELATED LITERATURE

Introduction

Fluid and blood glucose are of primary importance during exercise and can directly affect performance. Manipulation of the diet and the use of CHO, electrolytes, and fluids as ergogenic aids are common in athletics. Until recently, however, supplements were deemed necessary or beneficial only for endurance events lasting over 90 min. It was believed that a well-balanced diet, along with adequate fluid intake, would be adequate to sustain the body through activities lasting less than 90 min, and little benefit could be gained through supplementation. Most training and many competitive events involve a shorter duration of activity. In this chapter, the role of fluid and carbohydrates in the body during exercise, factors affecting fluid and CHO intake, supplements available, and current findings on CE supplements in exercise lasting about 1 hr are examined.

Fluid

Adequate fluid intake is essential before and during exercise to prevent dehydration and hyperthermia and to optimize performance. Fluid balance is difficult for many athletes to maintain, and many suffer the consequences of dehydration including physical symptoms and poor exercise performance.

Fluid Balance

Normally, the body's balance of fluid and sodium is well-regulated by the hormones aldosterone and antidiuretic hormone (ADH). Once exercise begins, body water shifts into exercising muscles. This decreases the plasma volume, which stimulates the release of ADH and the reabsorption of water as well as a decrease in renal blood flow. During exercise, there is also an increase in catecholamines, which stimulates the release of renin from the kidneys, secretion of aldosterone, and reabsorption of sodium. Water balance can be maintained with 1.5 to 20 L per day. This large range of acceptable fluid intake demonstrates that the capacity to ingest fluid is met by the capacity to excrete fluids under normal circumstances (Barr & Costill, 1989).

Dehydration

The hypothalamus stimulates thirst when the body experiences a 1% loss of body weight due to dehydration. The response is delayed and generally does not keep up with fluid losses. Many athletes do not replace fluids adequately and may lose up to 10% of body weight through dehydration (Gisolfi & Duchman, 1992). A body weight loss of 5% is not uncommon in sports such as soccer, football, tennis, and running (Brooks et al., 1996). Many factors inhibit adequate hydration during exercise, including the priority given to performance time over time required to consume fluids, the rules of competition (number of time outs), gastrointestinal (GI) discomfort that may result in some athletes when they ingest the large volume of fluid needed, and the availability of fluids.

Heat produced by the working muscles during exercise is transported from the body's core to the skin's surface, where sweating allows heat to dissipate through the process of evaporation. Inadequate fluid replacement during exercise leads to dehydration, which decreases stroke volume, cardiac output, and skin blood flow, which, in turn, increases the body's core temperature, heart rate, and RPEs (Coyle & Montain, 1992b). The most serious consequence of dehydration is hyperthermia, which can lead to heat exhaustion, heat stroke, and death (Coyle, 1994).

Sweat is generally more hypotonic than most body fluids, and water losses generally exceed sodium losses. Hyponatremia is rare except in cases of extreme sweat losses or excessive fluid intake and retention of low-sodium fluids (Barr & Costill, 1989). Too much fluid ingestion can also result in GI discomfort and can impair performance due to the time required to consume a large amount of fluid. For most endurance athletes, sweat rate does not generally exceed 2–3 L/hr, and sodium losses are not generally greater than 60–120 mEq (Gisolfi & Duchman, 1992). Coyle and Montain (1992a) found that although sweat rate usually exceeded 1 L/hr during prolonged continuous exercise at 50%–80% VO_2 max, athletes preferred to drink only 500 ml/hr. It was stated in the American College of Sports Medicine (ACSM) position stand (1996) that most athletes in running, cycling, and swimming allow themselves to dehydrate up to a loss of 2–3 kg of their body weight. The ACSM (1996) recommended the consumption of approximately 500 ml of liquid 2 hr before exercise, the maintenance of a large, but tolerable, volume of fluid (400–600 ml) during exercise, and an attempt to consume fluids at a rate to replace water lost in sweat. The fluid replacement recommended to attenuate hyperthermia is that which most closely matches the athlete's rate of sweating, up to 80% of sweat rate (Coyle & Montain, 1992b).

Carbohydrate Metabolism

Glycogen

Glycogen reserves in liver and muscle are exceedingly important as fuel for exercise. Once exercise starts, CHO contributes as the primary fuel source. This dependence increases as workload increases. As the duration of exercise increases, the relative utilization of fat increases.

Glycogen reserves in liver and muscle tissue are directly affected by diet. In a 150-lb male, muscle glycogen reserves provide approximately 1500 kcal of fuel, the liver provides 320 kcal, and blood glucose provides the smallest amount, 80 kcal. The combined glycogen and glucose pool can last approximately 2 hr for an endurance athlete, and the body's storage capacity for muscle glycogen is enhanced by endurance training (Manore & Ryan, 1992).

After the first few seconds and up to 50 s of exercise, muscle glycogen and blood glucose are the main energy sources. These immediate sources are referred to as nonoxidative or glycolytic energy sources and involve the process of glycolysis and glycogenolysis. Intense exercise longer than approximately 30 s cannot be sustained without oxidative metabolism. After about 2 min of exercise, muscle and liver glycogen reserves, as well as blood glucose, free fatty acids, and amino acids, contribute as fuel sources. Free glucose in skeletal muscle is very low, and most potential energy during endurance exercise is provided by glycogenolysis. Glycogen reserves can be depleted in 8–24 hr of fasting, and over 80% of carbon for glycolysis in muscle must come from glycogen and not blood glucose. Therefore, if glycogen reserves are depleted, fatigue will occur (Brooks et al., 1996).

Respiratory Exchange Ratio

R is the amount of CO₂ produced compared to the amount of O₂ consumed by the body. R values represent substrate utilization during exercise. During hard exercise, R approaches 1.0, although during prolonged exercise R will decrease due to an increase in fat utilization and a decrease in CHO utilization. At 80% of VO₂ max, approximately 95% of fuel utilized is supplied by carbohydrates and 5% by fat (Brooks et al., 1996). During intense exercise, oxygen intake can be a limiting factor. However, a greater amount of energy per unit of oxygen can be produced using CHO rather than fats. In prolonged exercise, glycogen reserves are more limiting than oxygen availability, and fat utilization plays a greater role as a fuel source. R is represented as a nonprotein respiratory quotient because its value has been adjusted to take into account the contribution of protein to energy expenditure. R generally exceeds 1.0 when VO₂ max is reached.

Blood Glucose

During and after exercise, blood glucose levels are maintained by gluconeogenesis in the liver and kidneys using the primary precursor, lactate. Fasting gluconeogenesis, along with glycogenolysis, takes place in the liver to maintain adequate blood glucose. Normal fasting blood glucose is approximately 100 mg/dl. During exercise, skeletal muscle must switch from a low glucose uptake to a much higher uptake. Maintaining blood glucose levels requires hepatic glucose metabolism to greatly increase. When exercise begins, blood glucose rises slightly due to hormone feed-forward mechanisms. Feed-forward mechanisms include factors that increase glucose 6-phosphate, which stimulates glycolysis. Epinephrine and muscle

contractions stimulate glycogenolysis, and muscle contractions and insulin stimulate glucose uptake. Metabolites from glycolysis and muscle contractions, as well as a decline in blood glucose, serve as feedback mechanisms. Over time, as the body becomes dependent on liver glycogen reserves, amino acids, and free fatty-acids, blood glucose levels may fall, but the body will maintain levels within 10% of normal limits until exercise stops (Brooks et al., 1996).

Exercise after an extended fast will still elicit a slight rise in blood glucose in the beginning, although blood glucose will then fall due to depleted glycogen stores and the inability of gluconeogenesis to compensate. Blood glucose levels below 65 mg/dl indicate hypoglycemia and results in fatigue. Exercise draws on blood glucose reserves from previously digested meals in the gut, the liver, and the kidneys. As blood glucose is taken up by working muscles, insulin is inhibited by catecholamines (primarily epinephrine), and less glucose is taken up by nonactive tissues to spare glucose for the brain, muscle, and nervous tissue. The body may also draw on free fatty acids or amino acids. Blood glucose may actually rise slightly with increased gluconeogenesis and glycogenolysis. In prolonged exercise, insulin secretion decreases to spare glucose and muscle glycogen by enhancing lipolysis and making free fatty acids available (Brooks et al., 1996).

Diet

Diet affects training and performance primarily through its effects on glycogen reserves. Muscle glycogen contributes 3–5 times as much fuel for exercise as blood glucose in prolonged submaximal exercise (Brooks et al., 1996). Maintaining glucose levels for the brain and nervous tissues is critical because it relies

almost exclusively on glucose. Dietary manipulation has been used for thousands of years in an attempt to enhance performance.

Diet and precompetition meals should be designed to replete and maintain liver glycogen in order to maintain blood glucose and leave muscle glycogen to fuel exercise. After 8 hr of sleep, liver glycogen reserves can be depleted, and blood glucose production will rely primarily on protein catabolism and gluconeogenesis. Eating before exercise is important to prevent hypoglycemia, absorb gastric secretions, settle an athlete's stomach, provide fuel for the body, and provide the psychological benefit of knowing the body is properly fueled. Ideally, an athlete should begin training or enter competition neither hungry nor overly full, with blood glucose within normal limits.

A diet consistently high in complex CHO (60%–70% of total calories) is recommended for athletes, and preexercise foods should consist primarily of carbohydrates. The consistency, timing, and amount of food, however, vary based on the activity, availability of food, and individual food preferences and tolerances. Some athletes choose not to eat at all prior to or during training or competition due to nerves, fear of GI discomfort, or timing of a very early morning training or competition. Common GI complaints include heartburn, nausea, vomiting, bloating, stomach pain, gas, intestinal cramps, loose stool, and diarrhea. Runners tend to have more GI complaints than athletes involved in activities such as cycling, in which the stomach remains fairly stationary.

Carbohydrate Supplementation and Exercise

Endurance exercise can result in a decrease in blood glucose and a depletion of glycogen stores. The addition of CHO and electrolytes to fluid serves many

purposes including the provision of substrate and electrolytes and improved palatability, which results in increased intake, thereby preventing dehydration (Wilk & Bar-Or, 1996). The addition of sodium prevents hyponatremia in some individuals (ACSM, 1996), although sodium typically can be replaced with a normal diet. The addition of CHO enhances reabsorption of fluids because CHO promotes absorption from the glucose-stimulated sodium absorption and increased water transport from the intestinal lumen to the blood (Gisolfi & Duchman, 1992). The primary purpose of the addition of CHO to sports beverages is to maintain blood glucose and enhance CHO oxidation in exercise lasting longer than 1 hr, especially when muscle glycogen is low, thereby preventing fatigue and improving athletic performance (ACSM, 1996).

Blood Glucose

Glucose taken 45 min before exercise may increase blood glucose, but the blood glucose level will fall if no further glucose is provided during exercise because of an increase in insulin secretion. When glucose is taken during exercise, the fuel can enter the circulation without causing an insulin response. The insulin response also appears to be inhibited if glucose is taken within 15 min of the onset of exercise (Gisolfi & Duchman, 1992). In one study, glucose ingested 15 min prior to exercise at 80% of VO_2 max maintained blood glucose, but no glucose ingestion resulted in a drop in blood glucose (Wright, Sherman, & Dernbach, 1991). Coyle et al. (1983) found that blood glucose was 20%–40% higher when a glucose polymer was administered than when no supplement was given, and other studies have reported blood glucose levels were significantly higher with than without the consumption of a

5%–7% concentration CHO drink (Millard-Stafford, Sparling, Roszkopf, & DiCarlo, 1992; Mitchell et al., 1988; Murray et al., 1987).

Performance

Sufficient CHO must be available for energy production to prevent fatigue. CE solutions are recommended for activities lasting over 1 to 2 hr to maintain adequate blood glucose, prevent dehydration, and prevent hyperthermia and have been shown to enhance performance and time to exhaustion during exercise lasting longer than 2 hr at 50%–70% of VO_2 max (Coyle et al., 1986; Coyle et al., 1983; Wright et al., 1991). Coggan and Coyle (1991) showed that the addition of CHO to beverages may improve performance by maintaining blood glucose levels and CHO oxidation later in exercise when endogenous CHO stores may be compromised. Coyle et al. (1986) found that when cyclists were fed CHO during exercise, plasma glucose concentrations were maintained and the subjects exercised an additional hour before fatiguing, with little reliance on muscle glycogen stores.

A more recent study showed cyclists biking to exhaustion at 70% of VO_2 max improved performance with the ingestion of CHO before or during exercise and CHO ingestion both before and during exercise further improved performance (Wright et al., 1991). Fielding et al. (1985) also demonstrated that sprint performance was enhanced following prolonged exercise with the frequent ingestion of CHO. Neuffer et al. (1987) found that during 1 hr of exercise after a 12-hr fast cyclists performed significantly better when supplemented with 45 g of liquid or solid carbohydrate than when not supplemented. Performance was further enhanced when exercise was preceded by a meal containing 200 g of carbohydrate 4 hr before exercise in addition to the 45-g supplement.

In most studies, CHO supplementation of 25–60 g/hr of exercise was sufficient to maintain blood glucose oxidation, delay fatigue in later stages of exercise, and improve performance (Anantaraman et al., 1995; Coggan & Coyle, 1991; Coyle & Montain, 1992a; Millard-Stafford et al., 1992; Murray, Paul, Seifert, & Eddy, 1991). Two studies, however, did not result in significant improvement in performance when CHO supplementation was provided before exercise (Hargreaves, Costill, Fink, King, & Fielding, 1987) or during 2 hr of endurance exercise (Flynn et al., 1987).

R Value

Coyle et al. (1986) found that initial R values were maintained throughout exercise when the athletes were fed CHO. Other researchers demonstrated that the R value was significantly greater when glucose was ingested before or during exercise than when no glucose was ingested (Bonen, Malcolm, Kilgour, MacIntyre, & Belcastro, 1981). These studies indicated that a considerable amount of the glucose was metabolized during exercise, thereby sparing muscle glycogen reserves. Hargreaves et al. (1987) found no difference in R between subjects who received supplements and those who did not.

Gastric Emptying Rate

Several factors may influence the gastric emptying (GE) rate of a substance including the glucose or energy content, osmolality, pH, and temperature (Rehrer, Brouns, Beckers, Ten Hoor, & Saris, 1989). It was previously believed that the addition of CHO to solutions impaired fluid replacement because the addition of CHO slowed the GE rate. Several researchers, however, have demonstrated that the

volume of fluid primarily affects GE rate (Coyle & Montain, 1992a, 1992b; Millard-Stafford et al., 1992; Mitchell et al., 1989; Rehrer, Beckers, Brouns, Ten Hoor, & Saris, 1990; Rehrer, Brouns, et al., 1989) and that solutions up to 8% CHO concentration have little effect on the rate of GE or GI discomfort (Coyle & Montain, 1992b; Houmard et al., 1991; Mitchell et al., 1988). Maintaining larger gastric volumes appears to be beneficial for rehydration because it promotes increased GE rates, but it can lead to large gastric residues resulting in GI discomfort and impaired performance (Mitchell & Voss, 1991). The addition of CHO also increases fluid osmolality, which helps maintain body fluids. Rehrer et al. (1990) found that dehydration of 3.5%–4% body weight before exercise can increase GI distress. Exercise intensity may also affect GE rate and fluid consumption.

Gisolfi and Duchman (1992) demonstrated that although GE does not appear to be affected by exercise up to 70%–75% of VO_2 max, it is significantly reduced at greater intensities. Rehrer, Beckers, Brouns, Ten Hoor, & Saris (1989) found that there was a trend toward slower GE rate as exercise intensity increased up to 70% of VO_2 max. High-intensity exercise, as well as CHO supplementation greater than 8%, delays GE, which may lead to GI discomfort and impaired performance.

Running results in more movement of the stomach and its contents, which produces more GI complaints, than activities that allow the stomach to remain relatively stable. There was no difference in GE rate between the prolonged intense exercise modes of running or cycling, even when substantial volumes of fluid and a 7% CHO solution were consumed (Houmard et al., 1991). Rehrer et al. (1990) also found no important difference in GE rate between runners and cyclists and concluded that the increased complaints of GI upset in runners could not be explained by a difference in GE rate.

Exercise Lasting Less Than 1 Hr

Gisolfi and Duchman (1992) recommended that although 30–50 g CHO, or 300–500 ml of 6%–10% CHO solution, is beneficial for preventing glycogen depletion, if taken 0–15 min prior to high-intensity exercise lasting less than 1 hr, only water at half of the athlete's sweat rate (approximately 500–1000 ml) is recommended during high-intensity exercise greater than 75% of VO_2 max. The researchers found that carbohydrate ingestion delays GE, and in exercise less than 1 hr CHO consumption during exercise had little benefit on exercise performance. Armstrong and Maresh (1996) found little evidence that CHO supplementation in fluids affected performance when exercise lasts less than 60 min regardless of the intensity. However, Gisolfi and Duchman (1992) stated that there was more than sufficient evidence to support the rationale of replacing fluids during exercise lasting less than 1 hr to control core temperature.

Endurance exercise can result in a decrease in blood glucose and depletion of glycogen stores in high-intensity exercise lasting less than 1 hr. Recently, several studies have suggested that the consumption of CE beverages may improve performance of moderate- to high-intensity exercise lasting about 60 min. Neuffer et al. (1987) found that ingesting 45 g of liquid or solid CHO 5 min prior to intense cycling (80% of VO_2 max) enhanced performance when glycogen stores were less than optimal. The researchers believed that the timing of the preexercise feeding may have partially inhibited the insulin response due to the exercise-induced rise in counter-regulatory hormones. Further improvement in exercise performance was noted when a CHO meal was consumed 4 hr prior to exercise in combination with the solid CHO feeding immediately before exercise. Jeukendrup et al. (1997) found

that consumption of a 7.6% CE solution produced a greater cycling performance of approximately 1 hr than a placebo. Jackson et al. (1995) reported that for 1 min cycling bouts at 120–130% VO_2 max performance time to fatigue was greater, blood glucose and insulin during exercise were higher, and RPEs of the leg were lower with the consumption of an 18% CHO beverage than with a placebo. Other researchers showed that the preexercise ingestion of a 10% CHO beverage resulted in a smaller decline in power output during an hour of high-intensity (90% of VO_2 max) exercise performance, although no further benefit was noted from the additional consumption of CHO during exercise (Anantaraman et al., 1995). Below et al. (1995) found that cycling performance times were improved equally as well by fluid and by CHO consumption and that the effects were additive.

Carbohydrate-Electrolyte Solutions

CHO in most sports supplements are provided by fructose, sucrose, or glucose, alone or in combination. Whether the supplement is a liquid or a solid does not appear to be a factor in maintaining blood glucose levels or enhancing exercise performance (Mason, MacConell, & Hargreaves, 1993).

Glucose and lactate polymers, or maltodextrins, are becoming increasingly popular because they can deliver high concentrations of CHO without being excessively sweet. The recent use of glucose polymers has allowed CHO concentrations of greater than 10% to remain palatable (Coyle & Montain, 1992a). Polymers are also convenient and lightweight, because they are concentrated; however, it is recommended that they be taken with adequate water. According to Gisolfi and Duchman (1992), glucose and glucose polymers empty from the stomach at the same rate, although they have very different osmolalities, but if glucose

polymers are “rapidly hydrolyzed in the duodenum before they reach the presumed osmoreceptors in this organ, the similarity in gastric emptying rate between glucose polymers and glucose would be explained” (p. 680). Murray, Paul, Seifert, Eddy, and Halaby (1989) found no advantage to the ingestion of glucose polymers alone rather than ingesting a combination of sucrose and glucose or a combination of glucose polymer and fructose.

Fructose is not commonly used, because it often causes gastric upset and discomfort. Fructose is not actively absorbed in humans when it is ingested in large quantities (Gislofi & Duchman, 1992). Murray et al. (1989) reported that a 6% fructose beverage elicited greater GI distress, more compromised physiological responses, and produced a lower exercise capacity than a 6% sucrose and glucose beverage. Coyle and Montain (1992b) found little difference between the gastric emptying rates of beverages with any of the substrates and found that concentrations up to 8% did not affect fluid reabsorption.

Summary

The literature demonstrated that carbohydrates and fluid are vital aspects of sports training and athletic competition. It has been a long-held belief that supplements were necessary only for activities lasting longer than 90 min, but recommendations continue to evolve. Recent studies suggest that the benefit of supplements may extend to activities lasting about 1 hr. The ingestion of fluids improves GE rate and helps cool the body, thereby reducing complications related to hyperthermia. The addition of CHO and electrolytes to fluid does not appear to affect GE rate or GI discomfort in most situations, and there is no apparent difference in GE rate or GI in runners and cyclists.

The limited research that has been conducted on the effects of CHO and fluid supplementation on activities lasting about 60 min suggests a benefit to athletes participating in moderate- to high-intensity events of this length. These studies have been limited to cycling, although most sports lasting less than 90 min involve walking and running activities. Most research has also been limited to beverages and does not address the desire of many runners to carry lightweight and convenient supplements. This lack of research on supplementation in running events lasting 1 hr indicates a need for more research to establish appropriate recommendations. As recreational and competitive athletes and nutrition and fitness experts seek safe, ethical, and effective means to enhance exercise performance, there is a need to investigate CE gels and their effect on running performance to determine appropriate recommendations.

CHAPTER III

DESIGN AND METHODOLOGY

Introduction

The problem of this study was to determine the effect of preexercise supplementation of a CE gel during moderate- to high-intensity intermittent exercise on HR, VO_2 , blood glucose, R, RPE, and timed exercise performance. HR, VO_2 , blood glucose, R, and RPE were measured during four experimental conditions involving preexercise supplementation with a CE gel or C and running at 65% VO_2 max on a treadmill. A timed 200-m sprint exercise test, to simulate a sprint finish, was completed following an endurance run of 60 min, which was divided into three 20-minute exercise bouts separated by 3-min rest periods. Subjects completed two VO_2 max tests in order to calculate the work intensity subjects were to maintain during each experimental condition. This chapter includes information on the following: subjects, experimental design, instrumentation, experimental conditions, and statistical analysis.

Subjects

Approval to conduct this study was granted by the Human Subjects Institutional Review Board at Western Michigan University (see Approval Letter, Appendix A). Subjects ($n = 8$) were recruited from health, physical education, and recreation classes at Western Michigan University (see Recruitment Script,

Appendix B). The subjects were screened for cardiovascular disease and other risk factors that may have affected their participation in the study (see Screening Form, Appendix C). Individuals with symptoms or diagnosis of cardiovascular disease were not allowed to participate, in accordance with ACSM (1995) guidelines. Pregnant women were also excluded from participation in the study. The 8 well-trained, apparently healthy subjects gave written consent prior to participation in the study (see Consent Form, Appendix D).

Each subject was required to be well-conditioned, defined as running a minimum of 35 miles per week. Subjects were instructed to maintain regular training throughout the study period, although exhaustive exercise was prohibited 24 hr prior to test days and subjects were not allowed to exercise on the day of testing. The subjects were instructed to keep dietary intake consistent throughout the study period. Subjects were instructed to consume a meal consisting of 1 g CHO per pound of body weight 4 hr before their scheduled trial run followed by a 4-hr fast. The 4-hr fast was required prior to each exercise test session but not prior to the VO_2 max tests. Liberal water intake was encouraged up to 2 hr before the exercise tests. Consumption of alcohol, caffeine, and nicotine was discouraged during the study period.

Experimental Design

A repeated measures experimental design was used in this study. The order of experimental conditions was randomly assigned. Subjects completed two trials on each of two experimental conditions including CE gel supplementation and C. HR, VO_2 , blood glucose, R, RPE, and timed exercise performance were the six dependent variables measured.

Instrumentation

Subjects ran on a Quinton programmable treadmill, model Q65, Quinton Instrument Company, Seattle, WA. Oxygen consumption and respiratory exchange ratio were measured during the VO_2 max tests and exercise tests using a Quinton Q-Plex metabolic cart, model Q-Plex 1, Quinton Instrument Company, Seattle, WA. Heart rate was monitored during VO_2 max and exercise tests using a Polar Heart Rate monitor, model 61210, County Technology, Inc., Gay Mills, WI. The electrocardiogram (ECG) during the VO_2 max test was monitored with an oscilloscope, Bosch 501A and ECS 502, Germany. Blood samples were collected prior to beginning exercise, during each 3-min rest period, and prior to and after the timed exercise performance test, using the procedure described in Appendix E. Blood glucose was analyzed using the Medisense II blood glucose analyzer. The subjects' exercise tolerance was assessed using Borg's original RPE scale of 6 to 20 (Manore & Ryan, 1992).

Experimental Procedures

Initial Procedures

All testing was completed at the Exercise Physiology Laboratory in the University Recreation Center at Western Michigan University, Kalamazoo. Prior to participation in the study, each subject signed and dated a consent form in which testing procedures and known possible risks were explained. Subjects were asked to wear appropriate running attire.

VO₂ Max Test

VO₂ max was determined on a motorized treadmill. Subjects were familiarized with the treadmill and breathing equipment prior to the test. Following a 3-min warm-up, the treadmill speed was adjusted every 2 min, and the grade was adjusted every minute using the Lamb protocol for well-conditioned athletes (Lamb, 1984). Maximum heart rate (MHR) was calculated using the age-predicted maximum heart rate formula (220 – age). Subjects' VO₂, HRs, and ECGs were monitored continuously throughout the tests. An RPE value was determined during the last 30 s at the end of each 20-min stage. The test was terminated at volitional fatigue or when HR or VO₂ plateaued. Each subject completed two maximal exercise tests on two separate occasions a minimum of 24 hr apart, and the better VO₂ max of the two trials was used as a basis for determining the required percentage of VO₂ max sustained during the exercise test sessions.

Exercise Tests

Subjects were instructed to consume 1 g CHO per pound of body weight and then fast for 4 hr before each test. Exhaustive exercise was prohibited 24 hr prior to the tests. Subjects were told not to exercise the day of the tests. Subjects were also instructed to eat a consistent diet throughout the study period. At 5 min prior to beginning the first 20-min exercise bout, subjects consumed either 260 or 360 ml CE (21 or 28 g CHO gel followed by 260 or 360 ml of water to make an 8% solution) or 260 or 360 ml plain water based on body weight of less than or greater than 150 pounds. The order that subjects received these treatments was determined at random. Plain water (120 ml) was consumed during each 3-min rest period and prior to

completing the timed exercise performance. Following a 5-min warm-up on the treadmill at approximately 40% of VO_2 max, subjects were asked to perform three 20-min bouts of running at a 3% grade with 3-min rest periods between bouts. Workload was adjusted to maintain a VO_2 of approximately 65% of VO_2 max. HR was assessed every 5 min during each 20-min exercise bout, and the last three readings in each bout were averaged. VO_2 was measured every 20 s and was analyzed by averaging the reading obtained every minute for the last 15 min of each 20-min exercise bout. Blood samples were taken to analyze blood glucose before the start of each test, during each 3 min rest, and after completion of the 200-m sprint. RPE was measured in the last 30 s of each 20-min exercise bout, and R was determined by averaging the 15 measures taken in the last 5 min of each 20-min bout. Each condition was performed by each subject twice. After completing the three 20-min exercise bouts, subjects completed a timed exercise performance test on an indoor track. The performance test required subjects to run 200 m in as short a time as possible.

Statistical Analysis

A randomized-block factorial ANOVA was calculated for each of the dependent variables measured: HR, VO_2 , blood glucose, R, RPE, and timed exercise performance. Simple main effects and Tukey HSD tests were completed for HR, blood glucose, R, and RPE among times if a significant difference was found.

CHAPTER IV

RESULTS AND DISCUSSION

Introduction

The problem of this study was to compare the effects of CE gel and C on intermittent moderate-intensity exercise lasting 1 hr. HR, VO_2 , blood glucose, R, RPE, and exercise performance were assessed during a 60-min intermittent run of moderate intensity followed by a 200-m sprint. HR was taken every 5 min during the intermittent moderate-intensity run and averaged for each 20-min bout. VO_2 was analyzed every minute for the last 15 min of each 20-min exercise bout and averaged. A blood sample was collected using a finger stick prior to exercise, at 20 min, 40 min, and 60 min of exercise, and postsprint to analyze blood glucose. R was measured every 20 s for the last 5 min of each exercise bout and averaged. RPE was taken the last 30 s of each of the three 20-min exercise bouts. This chapter was organized into the following result sections: (a) subject characteristics, (b) HR, (c) VO_2 , (d) blood glucose, (e) R, (f) RPE, and (g) exercise performance. The discussion follows the results.

Results

Subject Characteristics

Subject characteristics are described in Table 1. A total of 8 subjects, 4 females and 4 males, ages 20–27 years completed this study. The subjects were well-

trained endurance athletes who ran a minimum of 35 miles per week. All subjects completed two VO_2 max tests and the four trial runs within a 4-week period to avoid significant changes in fitness levels. The best VO_2 max was used to determine the maintained work intensity for each subject. A mean of 66.47% of VO_2 max was maintained by all the subjects during their endurance runs. Subjects were supplemented with either a concentrated CE gel along with plain water prior to exercise and after the first 20-min exercise bout or the same amount of water alone. Plain water was given after 40 min and 60 min of exercise across the two trials for each of the two conditions.

Table 1
Descriptive Characteristics of Subjects

Characteristic	Male ($n = 4$)		Female ($n = 4$)	
	<u>M</u>	<u>SD</u>	<u>M</u>	<u>SD</u>
Age (yr)	24.00	2.24	21.50	1.12
Height (in.)	72.75	1.30	66.75	3.56
Weight (lb)	170.25	15.16	131.75	14.53
VO_2 max (ml/kg/min)	65.68	4.44	52.78	6.44

The concentrated CE gel consisted of maltodextrin (glucose polymer); water; fructose; citric acid; natural flavor; amino acid blend (leucine, valine, isoleucine); potassium chloride, salt, sodium citrate, sodium benzoate, and potassium sorbate (to retard spoilage); and antioxidants vitamins C (ascorbic acid) and E (alpha-tocopherol acetate). To provide an 8% solution, the 1.4-oz gel packet (28 g of CHO) was taken

with 360 ml of water if the subject weighed over 150 lb, and $\frac{3}{4}$ of a gel packet (21 g of CHO) was taken with 260 ml of water if the subject weighed under 150 lb. Each packet provided 110 calories, 50 mg sodium, 40 mg potassium, 9 mg of vitamin C, and 1.5 mg of vitamin E. Subjects consumed either the supplement or plain water 5 min prior to beginning each exercise trial and after 20 min of exercise. They also ingested 120 ml of plain water during each 3-min rest period.

The gel was selected because it was a relatively new product and it was important to assess its effect on VO_2 , HR, blood glucose, R, RPE, and exercise performance. Also, a gel was selected to compare the tolerance of runners ingesting CHO as a gel followed by water to the same volume of water alone. The amount of CHO (42–56 g) and CHO concentration (8%) along with the volume of fluid was selected in accordance with ACSM (1996) fluid replacement guidelines and recommendations by Coyle and Montain (1992a, 1992b). Coyle and Montain (1992b), Houmard et al. (1991), and Mitchell et al. (1988) reported that solutions up to 8% CHO concentration had little effect on GE rate or GI discomfort in exercise at less than 70% VO_2 max. Rehrer et al. (1990) reported no difference in GE rate between runners and cyclists and that the common complaints of GI discomfort by runners must be explained by other factors. In the present investigation, only 2 subjects complained of fullness or discomfort associated with the consumption of the gel and water.

A randomized-block factorial ANOVA was calculated for each of the dependent variables: HR, VO_2 , blood glucose, R, RPE, and timed exercise performance. The randomized-block factorial ANOVA was calculated for the conditions with two levels, gel and water, and the trials with two levels, on 2 separate

trial days. Time was analyzed using three levels each for HR, VO_2 , R, and RPE; five levels for blood glucose; and one level for the timed exercise performance.

HR

The ANOVA summary for HR is shown in Table 2. The results were as follows:

1. There was no significant difference between conditions, CE, $\underline{M} = 157.37$ bpm, and C, $\underline{M} = 158.27$ bpm.

Table 2
Summary ANOVA for Heart Rate

Source	<u>SS</u>	<u>df</u>	<u>MS</u>	<u>F</u>
Subjects	9659.86	7	1379.98	89.15*
Condition (C)	19.44	1	19.44	1.26
Trial (T)	306.02	1	306.02	19.77*
Time (TI)	584.34	2	292.17	18.87*
C × T	7.71	1	7.71	0.50
C × TI	20.37	2	10.19	0.66
T × TI	3.15	2	1.58	0.10
C × T × TI	6.70	2	3.35	0.22
Error	1192.19	77	15.48	

*Significant at the .05 level.

2. A significant difference was found between the trials, $F(1, 77) = 19.77$, $p < .05$. The means for Trial 1 and Trial 2 were 159.61 bpm and 156.04 bpm, respectively.

3. A significant difference was found among the times, $F(2, 77) = 18.87$, $p < .05$. The means for 20 min, 40 min, and 60 min of exercise were 154.60 bpm, 158.27 bpm, and 160.60 bpm, respectively. The Tukey test, $HSD = 4.60$, indicated a significant difference between 20 min and 60 min.

4. All first- and second-order interaction effects were not significant.

VO₂

The ANOVA summary for VO₂ max is shown in Table 3. The main effects, conditions, trial, and time, were not significant. Also, no significant first- or second-order interactions were found.

Blood Glucose

The ANOVA summary for blood glucose is shown in Table 4. The results were as follows:

1. A significant difference was found between the CE, $\underline{M} = 89.35$ mg/dl, and the C, $\underline{M} = 85.05$ mg/dl, conditions, $F(1, 133) = 4.64$, $p < .05$.

2. No significant differences were found between Trial 1, $\underline{M} = 88.48$ mg/dl, and Trial 2, $\underline{M} = 85.93$ mg/dl.

3. A significant difference was found among the times, $F(4, 133) = 3.63$, $p < .05$. The means for preexercise; 20 min, 40 min, and 60 min of exercise; and postsprint blood glucose levels were 81.84 mg/dl, 89.06 mg/dl, 90.06 mg/dl, 83.53 mg/dl, and 91.50 mg/dl, respectively.

Table 3
Summary ANOVA for VO₂

Source	<u>SS</u>	<u>df</u>	<u>MS</u>	<u>F</u>
Subjects	1922.46	7	274.64	150.90
Conditions (C)	0.67	1	0.67	0.37
Trial (T)	3.95	1	3.95	2.17
Time (TI)	1.17	2	0.58	0.32
C × T	0.98	1	0.98	0.54
C × TI	1.04	2	0.52	0.29
T × TI	0.53	2	0.26	0.15
C × T × TI	0.90	2	0.45	0.25
Error	140.34	77	1.82	

4. A significant first-order interaction effect, conditions by time, was found, $F(4, 133) = 4.35, p < .05$. The simple main effect test showed significant differences between the CE and C conditions: (a) $\bar{M} = 76.00$ mg/dl and $\bar{M} = 87.69$ mg/dl, respectively, at Time 1 (preexercise), $F(1, 133) = 6.85, p < .05$; (b) $\bar{M} = 95.88$ mg/dl and $\bar{M} = 84.25$ mg/dl, respectively, at Time 3 (40 min), $F(1, 133) = 6.78, p < .05$; and (c) $\bar{M} = 96.57$ mg/dl and $\bar{M} = 86.44$ mg/dl, respectively, at Time 5 (postsprint), $F(1, 133) = 5.14, p < .05$. No significant difference was found between conditions for Time 2 or Time 4. The simple main effect for times at the gel condition was significant, $F(4, 133) = 7.23, p < .05$. The Tukey test, $HSD = 17.23$, indicated a significant difference between the following times: (a) preexercise, $\bar{M} = 76.0$ mg/dl,

and 40 min, $\bar{M} = 95.88$ mg/dl; and (b) preexercise, $\bar{M} = 76.0$ mg/dl, and postexercise, $\bar{M} = 96.57$ mg/dl.

5. All other first- and second-order interaction effects were not significant.

Table 4
Summary ANOVA for Blood Glucose

Source	<u>SS</u>	<u>df</u>	<u>MS</u>	<u>F</u>
Subjects	10553.40	7	1507.63	9.45*
Condition (C)	739.60	1	739.60	4.64*
Trials (T)	260.10	1	260.10	1.63
Time (TI)	2313.66	4	578.42	3.63*
C × T	40.00	1	40.00	0.25
C × TI	2777.71	4	694.43	4.35*
C at TI ₁	1093.25	1	1093.25	6.85*
C at TI ₂	264.50	1	264.50	1.66
C at TI ₃	1082.06	1	1082.06	6.78*
C at TI ₄	259.01	1	259.01	1.62
C at TI ₅	820.13	1	820.13	5.14*
TI at C _{CE}	4615.18	4	1153.80	7.23*
TI at C _C	477.61	4	119.40	0.75
T × TI	418.46	4	104.62	0.66
C × T × TI	69.31	4	17.33	0.11
Error	21215.36	133	159.51	

*Significant at the .05 level.

R

The ANOVA summary for R is shown in Table 5. The results were as follows:

1. No significant difference was found between the conditions, CE, $\underline{M} = 0.903$, and C, $\underline{M} = 0.911$.
2. A significant difference was found between Trial 1, $\underline{M} = .900$, and Trial 2, $\underline{M} = .914$, $\underline{F}(1, 77) = 7.24$, $p < .05$.
3. A significant difference was found among the times, $\underline{F}(2, 77) = 3.26$, $p < .05$, although the Tukey test, $HSD = 0.0174$, showed no significance among the pairwise comparisons. The means for the times 20, 40, and 60 min were 0.916, 0.906, and 0.900, respectively.
4. All first- and second-order interaction effects were not significant.

RPE

The ANOVA summary for RPE is shown in Table 6. The results were as follows:

1. There was no significant difference between conditions, CE, $\underline{M} = 11.38$, and C, $\underline{M} = 11.50$.
2. A significant difference was found between trials, $\underline{F}(1, 77) = 16.76$, $p < .05$. The means for Trial 1 and Trial 2 were 11.71 and 11.17, respectively.
3. A significant difference was found among the times, $\underline{F}(2, 77) = 16.98$, $p < .05$. The means at 20-min, 40-min, and 60-min of exercise were 10.94, 11.50, and 11.88, respectively. The Tukey test, $HSD = 0.76$, indicated a significant difference between 20 min and 60 min.

Table 5
Summary ANOVA for R

Source	<u>SS</u>	<u>df</u>	<u>MS</u>	<u>F</u>
Conditions	0.00153	1	0.00153	2.32
Trial (T)	0.00478	1	0.00478	7.24*
Time (TI)	0.00430	2	0.00215	3.26*
C × T	0.00152	1	0.00152	2.30
C × TI	0.00002	2	0.00001	0.02
T × TI	0.00025	2	0.00012	0.18
R × S × T	0.00028	2	0.00014	0.21
Error	0.05076	77	0.00066	

*Significant at the .05 level.

4. A significant first-order interaction effect of conditions, CE gel and C, by trials, 1 and 2, was found, $F(1, 77) = 4.86$, $p < .05$. The simple main effects test showed significant differences between: (a) conditions, CE and C, at Trial 1, $F(1, 77) = 4.95$, $p < .05$, but not at Trial 2; and (b) trials at Condition 2, C, $F(1, 77) = 19.69$, $p < .05$, but not at Condition 1, CE gel.

5. All other first- and second-order interaction effects were not significant.

Exercise Performance

The ANOVA summary for exercise performance is shown in Table 7. The results were as follows:

Table 6
Summary ANOVA for RPE

Source	<u>SS</u>	<u>df</u>	<u>MS</u>	<u>F</u>
Subjects	48.46	7	6.92	16.48*
Condition (C)	0.38	1	0.38	0.90
Trial (T)	7.04	1	7.04	16.76*
Time (TI)	14.25	2	7.13	16.98*
C × T	2.04	1	2.04	4.86*
C at T ₁	2.08	1	2.08	4.95*
C at T ₂	1.33	1	0.33	0.79
T at C ₁	1.73	1	0.73	1.74
T at C ₂	8.27	1	8.27	19.69*
C × TI	1.00	2	0.50	1.19
T × TI	0.08	2	0.04	0.10
C × T × TI	0.33	2	0.17	0.40
Error	32.05	77	0.42	

*Significant at the .05 level.

1. No significant difference in sprint time was found between conditions, CE gel, $\bar{M} = 33.90$ s, and C, $\bar{M} = 33.97$ s.

2. A significant difference was found between Trial 1, $\bar{M} = 34.49$ s, and Trial 2, $\bar{M} = 33.39$, $F(1, 21) = 10.21$, $p < .05$.

3. No significant interaction effect was found between conditions, CE gel and C, and trials, 1 and 2.

Table 7
Summary ANOVA for Exercise Performance

Source	<u>SS</u>	<u>df</u>	<u>MS</u>	<u>F</u>
Subjects	398.97	7	57.00	60.00*
Condition (C)	0.04	1	0.04	0.04
Trial (T)	9.70	1	9.70	10.21*
C × T	0.60	1	0.60	0.63
Error	20.05	21	0.95	

*Significant at the .05 level.

Discussion

The results of this study were compared to information obtained from recent similar studies related to supplementation and exercise lasting approximately 1 hr. The studies completed thus far focused on cycling. Running was chosen for this study to mimic conditions found in sports requiring bouts of running lasting less than 1 hr, such as soccer, basketball, football, racket sports, and field events. The concentrated CE gel was selected over CE beverages because glucose polymers are a relatively new method of supplementation and little research is available concerning their effects on exercise performance. The discussion is organized into the following sections: (a) HR and VO_2 , (b) blood glucose, (c) R, (d) RPE, and (e) exercise performance.

HR and VO₂

The mean HR across times increased steadily (\underline{M} = 154.60 bpm, 158.27 bpm, and 160.60 bpm, respectively), although workload was adjusted to maintain approximately 65% of VO₂ max. This may be related to a phenomenon, described in Brooks et al. (1996), called cardiovascular drift. During prolonged exercise, a decrease in stroke volume causes a progressive increase in HR at the same workload. This may be caused by a breakdown in the sympathetic blood flow control mechanism, an increase of blood flow to the skin for cooling, or both. This process is necessary to maintain cardiac output and blood pressure.

Blood Glucose

For this study, subjects consumed 1 g CHO per pound of body weight 4 hr prior to their scheduled trial run in an attempt to equalize glycogen stores among all the subjects and to prevent subjects from performing a trial in a state of glycogen depletion, which can occur after an 8-hr fast. The 2 g of CHO per pound of body weight recommended by Clark (1997) and Sherman (1989) 4 hr before exercise exceeded what was considered normal intake for most of the runners who participated in the study; therefore, a smaller amount was used. Despite the same consumption prior to all trials, the mean blood glucose prior to the C trials (\underline{M} = 87.69 mg/dl) was higher than that prior to the CE gel trials (\underline{M} = 76.00 mg/dl), although one subject skipped his preexercise meal and completed a water trial with a preexercise blood glucose below normal. Both of the means for the water and gel trials were within normal limits of 70–140 mg/dl.

During the CE trials, blood glucose increased steadily through 40 min of exercise and then dropped. For the C trials, blood glucose dropped steadily through all 60 min of exercise. Blood glucose was significantly different between the CE and C trials at Time 1 (preexercise), Time 3 (40 min), and Time 5 (postexercise). Blood glucose rose after the sprint in both the CE and C trials. This was probably due to the anaerobic effect of the high-intensity exercise. There was a significant difference in blood glucose in the CE trials between preexercise and 40 min, and pre- and postexercise times. Jackson et al. (1995) reported that both blood glucose and insulin during 1-min cycling bouts to fatigue at 120%–130% VO_2 were higher in the CHO group than in the placebo group of cyclists when the CHO subjects were supplemented with 4 ml/kg body weight of an 18% CHO drink. This effect may be explained because the concentrated source of CHO may provide a fuel source for glycogen synthesis and allow for rapid glycogen synthesis during rest periods.

R

Although no significant difference in R was found between the conditions, there was a significant difference between the trials. A significant difference was also observed among the times, although the Tukey HSD did not show a significant difference among the pairwise comparisons. The other researchers specifically assessing CHO and exercise lasting approximately 1 hr did not assess the effects of CHO supplementation on R. Coyle et al. (1986) and Bonen et al. (1981), who studied exercise lasting longer than 1 hr, found that R was the same as or significantly higher than for the placebo group when CHO was consumed before or during cycling to fatigue.

RPE

An increase in RPE was observed as the exercise time progressed despite adjustments in workload to maintain approximately 65% of VO_2 max. The mean RPE values for 20 min, 40 min, and 60 min of exercise were $\bar{M} = 10.94$, 11.50, and 11.88, respectively. The Tukey HSD indicated a significance between 20 min and 60 min of exercise. A significant difference between Trials 1 ($\bar{M} = 11.71$) and 2 ($\bar{M} = 11.17$) was observed, which may be attributed to a learning effect. A significant first-order interaction effect was observed between the conditions, CE and C, for Trial 1 but not Trial 2. A significant first-order interaction effect was also observed between Trials 1 and 2 for the C condition, but not for the CE condition. No significant difference was observed between the gel and water trials, although Jackson et al. (1995) showed a significantly lower RPE for the cyclists' legs for the CHO group than for the placebo group cycling at 120%–130% VO_2 max in 1-min bouts to fatigue. They also showed increased time to fatigue with supplementation, which was attributed to an increase in glycogen resynthesis during the rest intervals. Ball, Headly, and Vanderburgh (1995) showed RPE was significantly lower during a 50-min simulated time trial when supplemented with a 7% CE than with a flavored-water placebo at 10, 20, 30, and 40 min of exercise.

Exercise Performance

Although no significance was found between the timed exercise performance (200-m sprint) for the conditions, CE and C, there was a significant difference between Trials 1 and 2, possibly due to a learning effect. Jackson et al. (1995), however, found exercise time to fatigue was significantly longer with CHO

supplementation than with placebo supplementation, and Below et al. (1995) showed a 6.3% faster performance time (designed to simulate a finishing sprint following a 40-k time trial) with supplementation with a 6% CE beverage than with a placebo. Murray et al. (1989) showed that ingestion of a 6% solution resulted in significantly higher performance times (13.02 min) when compared to a placebo (13.62 min) and a 10% solution (13.57 min). During 115 min of intermittent cycling at 65%–80% of VO_2 max, Neuffer et al. (1987) showed that cyclists produced significantly greater total work during the final 15 min of 1 hr of exercise after a 12-hr fast when supplemented with 45 g of liquid or solid CHO. Performance was further enhanced by the ingestion of a meal containing 200 g of CHO 4 hr prior to exercise. Jeukendrup et al. (1997) also showed the time to complete a set amount of work was significantly faster with the consumption of a carbohydrate-electrolyte drink when compared to a placebo.

Researchers in two studies looked at power output and supplementation. Ball et al. (1995) found that peak, mean, and minimum power output were all significantly higher with CE supplementation than with a placebo. Anantaraman et al. (1995) also demonstrated that preexercise ingestion of a 30-g glucose polymer in a 10% solution caused a smaller drop in power output during 1 hr of high-intensity exercise when compared to a placebo. Little difference between treatments was observed until 40–60 min of exercise, when power output was significantly greater with the glucose polymer. The drop-off in power output was significantly smaller and the total amount of work completed was greater with the glucose polymer than without the glucose polymer. No further benefit was observed when the same amount of supplement was consumed every 15 min.

CHAPTER V

SUMMARY, FINDINGS, CONCLUSIONS, AND RECOMMENDATIONS

Summary

The problem of this study was to compare the effects of a concentrated CE gel to plain water in intermittent moderate-intensity exercise lasting 1 hr. Eight subjects, 4 males and 4 females, performed two VO_2 max tests followed by four trial runs, during which they maintained an average intensity of 65% VO_2 max. The subjects consumed 1 g CHO per pound of body weight 4 hr prior to the scheduled trial runs followed by a 4-hr fast. Liberal water consumption was allowed until 2 hr prior to the scheduled trial run. Based on body weight, subjects were supplemented with either 21 g or 28 g CHO in the form of a concentrated CE gel along with 260 or 360 ml water or 260 or 360 ml of plain water 5 min prior to the trial run and again after running 20 min. Plain water (120 ml) was provided after 40 and 60 min of running.

Findings

Blood Glucose

A significant difference was found between the gel, $\bar{M} = 89.35$ mg/dl, and the water, $\bar{M} = 85.05$ mg/dl, conditions, $F(1, 133) = 4.64$, $p < .05$. A significant first-order interaction effect was found, conditions by time, $F(4, 133) = 4.35$, $p < .05$. The simple main effect test showed differences between the CE gel and C conditions:

(a) before exercise, $\underline{M} = 76.00$ mg/dl and $\underline{M} = 87.69$ mg/dl, respectively, $\underline{F}(1, 133) = 6.85$, $p < .05$; (b) after 40 min, $\underline{M} = 95.88$ mg/dl and $\underline{M} = 84.25$ mg/dl, respectively, $\underline{F}(1, 133) = 6.78$, $p < .05$; and (c) postsprint, $\underline{M} = 96.57$ mg/dl and $\underline{M} = 86.44$ mg/dl, respectively, $\underline{F}(1, 133) = 5.14$, $p < .05$. The simple main effect for time was significant for the gel condition, $\underline{F}(4, 133) = 7.23$, $p < .05$, and the Tukey test, $HSD = 17.23$, indicated a significance between preexercise and 40 min and between pre- and postexercise.

The significant difference between the CE and C conditions prior to exercise cannot be explained, because the 4-hr pretrial CHO meal was designed to prevent this difference. Intake prior to the 4-hr meal and the CHO content of the subject's daily diet was not controlled. Fitness level may have also affected the body's ability to maintain glycogen reserves. Blood glucose increased in the gel trials through 40 min of exercise with a drop after 60 min, but blood glucose dropped steadily through 60 min of exercise in the water trials. Blood glucose increased after the sprint, which was probably related to the anaerobic nature of the exercise. At all times, mean blood glucose was consistently higher in the CE trials than in the C trials. This was probably due to the glucose supplementation and possible glycogen sparing. This finding was supported by the results of related studies that showed higher blood glucose with supplementation.

R

No significant difference was found between the gel and water conditions, which was not expected. A higher R was expected with CHO supplementation, due to the body's ability to utilize available blood glucose and glycogen stores, and a lower R was expected with water intake alone, due to greater relative fat utilization

as endurance exercise duration increased. This effect may have explained the significant difference found among the times, $F(2, 77) = 3.26$, $p < .05$. The means for the times were 0.916, 0.906, and 0.900, respectively, for 20, 40, and 60 min of time. A significant difference was found between Trials 1 and 2 ($M = 0.900$ and 0.914 , respectively), $F(1, 77) = 7.24$, $p < .05$, which may be explained by the effect of learning on the comparison of the gel and water conditions.

RPE

No significant difference was found between the conditions, gel and water, which contradicts studies that showed lower RPE with supplementation than with a placebo. A significant difference was found between trials, $F(1, 77) = 16.75$, $p < .05$. The means for Trial 1 and 2 were 11.71 and 11.17, respectively. This may also be explained by a learning effect. A significant difference was found among the times, $F(2, 77) = 16.98$, $p < .05$. The means for 20 min, 40 min, and 60 min of exercise were 10.94, 11.50, and 11.88, respectively. The Tukey test, $HSD = 0.76$, indicated significance between 20 min and 60 min of time. The gradual increase in RPE as exercise time increased was probably related to fatigue.

Exercise Performance

No significant difference was found in sprint time between conditions, gel and water, $M = 33.90$ s and $M = 33.97$ s, respectively. The lack of significance between CE supplementation and plain water intake contradicts the findings of other studies that demonstrated better exercise performance, lower time to fatigue, and greater power output with supplementation. A significant difference was found between the trials, which may be related to a learning effect.

Conclusions

The hypothesis of this study was that the ingestion of a concentrated CE gel would maintain blood glucose, result in a smaller decline in R, lower RPE, and enhance exercise performance when compared to the ingestion of water alone. Results of this study demonstrated that ingestion of 42 or 56 g of CHO, based on body weight, before and after 20 min of endurance exercise at 65% VO_2 max resulted in the better maintenance of blood glucose during exercise than did water alone. This implies that the supplement provides a readily available source of blood glucose and enhanced glycogen reserves for exercise. No significant difference was observed between conditions for R or RPE, although the mean for R decreased significantly as the duration of the trial run progressed, indicating greater relative reliance on fat utilization for fuel. RPE also increased significantly as the duration of the activity increased, indicating increased fatigue, despite adjustments in the workload to maintain a steady percentage of VO_2 max. No significant difference was found in sprint exercise performance between the conditions, although the faster time with supplementation may be practically significant in a competitive situation. Significant differences in R, RPE, and exercise performance were observed between trials, which may be attributed to a learning effect.

Recommendations

After completion of this study, several recommendations can be made for future research:

1. Increase the number of subjects to improve external validity.
2. Increase the number of trials to improve internal validity.

3. **Maintain greater control of subjects' diets 24–36 hr before data collection to enhance internal validity by ensuring similar glycogen reserves, carbohydrate intake, and fluid status prior to all trials.**

Appendix A

Human Subjects Institutional Review Board Approval

Human Subjects Institutional Review Board



Kalamazoo, Michigan 49008-3899

WESTERN MICHIGAN UNIVERSITY

Date: 20 November 1997

To: Roger Zabik, Principal Investigator
Sarah Handlogten, Student Investigator

From: Richard Wright, Chair

A handwritten signature in black ink that reads "Richard A. Wright".

Re: HSIRB Project Number 97-10-05

This letter will serve as confirmation that your research project entitled "Effect of Carbohydrate-Electrolyte Solutions on Exercise Performance in High-Intensity Intermittent Exercise Lasting 1 Hour" has been **approved** under the **full** category of review by the Human Subjects Institutional Review Board. The conditions and duration of this approval are specified in the Policies of Western Michigan University. You may now begin to implement the research as described in the application.

Please note that you may **only** conduct this research exactly in the form it was approved. You must seek specific board approval for any changes in this project. You must also seek reapproval if the project extends beyond the termination date noted below. In addition if there are any unanticipated adverse reactions or unanticipated events associated with the conduct of this research, you should immediately suspend the project and contact the Chair of the HSIRB for consultation.

The Board wishes you success in the pursuit of your research goals.

Approval Termination: 20 November 1998

Appendix B
Recruitment Script

**Western Michigan University
Department of Health, Physical Education, and Recreation
Recruitment Script**

Sarah Handlogten, an HPER graduate student, is looking for volunteers to participate in her master's thesis research. The study is titled, "The Effect of Concentrated Carbohydrate-Electrolyte Gels on Exercise Performance in Moderate-Intensity Intermittent Exercise Lasting 1 hr." The study requires 12 well-trained volunteers, between the ages of 18 and 30 years, who run a minimum of 35 mi per week.

Volunteers will be asked to complete a health history questionnaire to determine if they qualify to participate. Coronary artery disease, diabetes, pregnancy, or other health conditions that may put a participant at risk will result in exclusion from the study. If accepted, the subject must read and sign a consent form prior to participating.

Participants will be asked to commit to six sessions. All sessions will take place in the Exercise Physiology Laboratory, room 1055, at the Student Recreation Center. The first 2 exercise sessions will be tests to determine maximal oxygen consumption (VO₂ max). The other 4 sessions will require running on a treadmill at 65% VO₂ max for three 20-min exercise bouts with 5-min rests between bouts. The 70 min will be followed by a timed exercise performance test in which you will be required to run 200 m on the indoor track in the shortest time possible. Subjects will be given at least 48 hrs of rest between successive exercise sessions.

Subjects will be required to consume either a concentrated carbohydrate-electrolyte gel or plain water prior to each test session and plain water during each 5-min rest. All test sessions will take place following a 4-hr fast. Water may be taken until 2 hrs before each test. A random selection process will be established to determine which supplement each subject will receive first.

All of the sessions involve breathing through an apparatus that will collect and analyze expired air. The VO₂ max tests will involve walking/jogging on a treadmill with increasing workloads according to the pre-established test protocol. During the VO₂ max tests and all exercise test sessions, VO₂, respiratory exchange ratio, heart rate, blood pressure, electrocardiogram (ECG), and perceived exertion will be recorded. Blood glucose will also be monitored during each of the 6 exercise test conditions; this will require blood collection using a finger prick.

At any time, voluntary termination of involvement in the study is permitted for any reason. Participation or performance during the study will not affect academic standing or evaluations. All test information is strictly confidential, although copies of your individual test results will be provided to you upon your request. If you are interested in getting more information or volunteering for the study, please fill out the information below or contact Sarah Handlogten by phone at (616)388-4336.

Thank you.

Name

Phone number

Appendix C
Subject Screening Form

SUBJECT SCREENING FORM

- _____ 1. Have you been diagnosed with cardiac disease (angina, heart attack, congestive heart failure, coronary artery disease, or vascular disease), diabetes, or pulmonary disease (chronic obstructive pulmonary disease, emphysema)?
- _____ 2. Is there any possibility that you are pregnant?
- _____ 3. Has a member of your immediate family (parent or sibling) suffered a heart attack before the age of 55 for men or 65 for women?
- _____ 4. Do you smoke?
- _____ 5. Have you been diagnosed with high blood pressure, or do you take medication for high blood pressure?
- _____ 6. Have you been diagnosed with high blood cholesterol?
- _____ 7. Do you exercise regularly (run > 35 miles per week)?
- _____ 8. Have you ever experienced pain or tightness in your chest, neck, arms, or jaw?
- _____ 9. Have you ever felt short of breath or unusually fatigued with normal activities?
- _____ 10. Have you ever experienced dizziness or fainting spells?
- _____ 11. Do your ankles or lower legs swell?
- _____ 12. Do you ever have a rapid heart beat or have you been told you have a heart murmur?
- _____ 13. Do you suffer from leg pain or do you have varicose veins?
- _____ 14. Do you have a bone or joint problem that could be made worse by physical activity?
- _____ 15. Do you have arthritis?
- _____ 16. Do you have asthma?
- _____ 17. Are you taking any of the following drugs: Alpha or Beta Blockers, Angiotensin-Converting Enzyme, Antiadrenergic Agents, Antiarrhythmic Agents, Antihyperlipidemic Agents, Blood Thinners, Calcium-Channel Blockers, Digitalis, Diuretics, Nitrates and Nitroglycerin, Peripheral Vasodilators, or Sympathomimetic agents?
- _____ 18. Have you been seriously ill within the past 6 weeks?
- _____ 19. Do you use illicit drugs? (marijuana, cocaine, amphetamines)?
- _____ 20. Do you take any medication, prescribed or over the counter? If so, what are you taking? _____

Failure to answer any of the above questions will result in elimination from the study. Only those subjects who qualify as "apparently healthy" individuals according the American College of Sports Medicine (ACSM, 1995) will be allowed to participate in the study. Individuals who answered "yes" to items 1 or 2 will not be allowed to participate. Potential subjects who answered "yes" to more than one item for cardiovascular risk factors 3-7, or subjective symptoms 8-13, will not qualify as "apparently healthy" and will also not be allowed to participate in the study. An individual judgment will be made concerning participation of potential subjects answering "yes" to items 14-20, based on the impact exercise may have on the particular individual.

Appendix D
Consent Form

Western Michigan University
Department of Health, Physical Education, and Recreation

Principal Investigator: Dr. Roger Zabik

Research Associate: Sarah M. Handlogten

WESTERN MICHIGAN UNIVERSITY
Approved for use for one year from this date.
H. S. I. R. B.
NOV 20 1997

HSIRB Chair

I have been invited to participate in a research project entitled "The effect of concentrated carbohydrate-electrolyte gels on exercise performance in moderate-intensity intermittent exercise lasting 1 hr". I understand that this research is intended to determine what effects carbohydrate-electrolyte gels have on perceived exertion, blood glucose, respiratory exchange ratio, and exercise performance during moderate-intensity intermittent exercise lasting 1 hr. I further understand that this project is a master's thesis for Sarah Handlogten in the Department of Health, Physical Education, and Recreation at Western Michigan University.

By consenting to participate in this study, I am indicating that I will participate in six exercise sessions. These sessions will involve 2 maximal oxygen consumption tests (VO₂ max) using the Lamb protocol for well-conditioned athletes and 6 additional exercise sessions at 65% VO₂ max. I will be familiarized with the testing procedures and equipment prior to the first VO₂ max test. I will be asked to breathe through an apparatus that collects and analyzes expired air and I will be monitored by an electrocardiogram (ECG) during the 2 VO₂ max tests. I understand the blood glucose analysis requires several blood samples during each exercise session. Each of the 4 exercise sessions will involve running for three 20-min bouts on a treadmill with 5 min rests following each bout, followed by a timed exercise performance test which involves running 200 m on the indoor track in the shortest time possible. All exercise sessions, not including the timed sprint, will take place in the Exercise Physiology Laboratory, room 1055, in the Student Recreation Center.

I will be required to fast for 4 hrs before each of the 4 exercise sessions but water may be taken until 2 hrs before the tests. I will consume either a concentrated carbohydrate-electrolyte gel with 360 ml of water or 360 ml of plain water prior to the 4 sessions and drink 120 ml of water during each 5 min rest. There will be at least 48 hrs between consecutive exercise sessions. I understand that I will need to refrain from exhaustive exercise 48 hrs prior to each session.

I may benefit from participation by developing a better understanding of the procedures and equipment used to determine VO₂ max and by determining my individual max on the treadmill. I may also learn more about the content, dose, tolerance, and possible benefits of carbohydrate-electrolyte supplementation in relation to my training and exercise performance.

As in all research, there may be unforeseen risks to me. If an accidental injury occurs, appropriate emergency measures will be taken; however, no compensation or treatment will be made available to me except as otherwise stated in this consent form. I understand that there are potential risks involved such as muscle soreness or heart attack and that all appropriate measures will be taken to

minimize these risks. The investigators and assistants involved in the study are CPR and First Aid trained and emergency procedures are posted in the Exercise Physiology Laboratory.

I understand that I may terminate my involvement with this research at any time without prejudice or penalty. I also understand that all information is confidential. My name will not appear in any reports or presentations other than on a list of identification codes and on this form which will be handled only by those involved in administering the exercise tests. All data will be retained for a period of 3 yrs in a locked file controlled by the principal investigator. At the conclusion of the study, a copy of my results will be made available upon my request.

If any questions or concerns arise during the course of this study, I may contact Sarah Handlogten at (616) 388-4336 or Dr. Zabik at 387-2720. I may also contact the Chair, Human Subjects Institutional Review Board at 387-8293 or the Vice-President for Research at 387-8298. My signature below indicates that I understand the purpose and requirements of this study and I agree to participate.

Signature

Date

Appendix E

Western Michigan University Blood Collection Procedures

**Western Michigan University
Procedures for Collection of Blood and Blood Products**

1. COLLECTION OF THE BLOOD PRODUCT:

Blood will be collected in the HPER exercise physiology laboratory in the University Recreation Center. Blood samples by finger prick are taken each semester in laboratory activities associated with exercise physiology classes taught by the department. These samples are collected by Dr. Roger M. Zabik and Dr. Mary L. Dawson, faculty members in the department who work in the lab facility.

Samples are taken by the finger prick method. The sample site is first cleaned with a disposable alcohol prep pad. A disposable, Microtainer safety flow lancet is then used to produce a prick on the finger pad. The initial blood drop is wiped away with a disposable alcohol prep pad. The next drop is collected using a disposable heparinized blood capillary tube. The sample site is then cleaned with a disposable alcohol prep pad and a band-aid is then applied.

Because the investigators do not have access to confidential medical information about participating subjects, extreme caution will be taken in the handling of the collected samples.

No direct contact should occur between the blood sample and the investigator collecting the sample. The investigators collecting the samples will wear a lab coat, disposable rubber gloves, and safety glasses. **The disposable rubber gloves will be changed between subjects.** Blood samples will be taken only by Dr. Zabik and Dr. Dawson. Both have been trained by Western's environmental safety division in the handling of blood products and the Sindecuse Health Center medical lab was consulted concerning procedures for taking the blood sample.

Any accidental direct contact with the blood sample by a subject with another subject's blood will be reported to the Sindecuse Health Center staff and HSIRB. If, during blood collection, an accidental wound to the subject occurs, emergency first aid will be administered and the subject then taken to the Sindecuse Health Center for further evaluation. The incident will be reported to HSIRB.

The blood samples will be transferred from the blood capillary tube to the glucose test blots and the blood lactate analyzer immediately after they are taken using a microsyringe. No blood samples will be stored.

2. TREATMENT OF THE MATERIAL USED TO DRAW THE BLOOD:

The blood capillary tube, glucose test strip, microtainer lancet, alcohol prep pads, rubber gloves and any other disposable materials that come in contact with blood will be placed in a Sharps container for disposal by the Sindecuse Health Center. The blood lactate instrument contains a waste collection container internally. When full, this container will be emptied by Dr. Zabik into a plastic container with a

secure lid. The plastic container will then be transported to Sindecuse Health Center for disposal. If an accidental spill of the waste occurs, the spill will be absorbed using cotton pads with a plastic backing and the spill area cleaned using a bleach solution. The cotton pads any other materials used in the clean-up will be placed in a Sharps container. The waste collection container will then be sterilized using a Cydex solution. Dr. Zabik will wear a lab coat, rubber gloves, and safety glasses during these procedures. If contaminated, the lab coat will be washed and bleached. The rubber gloves will be placed in a Sharps container.

3. STORAGE AND DISPOSITION OF THE BLOOD SAMPLE:

No blood will be stored in the lab after collection. As each sample is taken, it will be immediately analyzed and then disposed of using procedures previously described and recommended by Sindecuse Health Center.

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