



Effects of caffeine, fructose, and glucose ingestion on muscle glycogen utilization during exercise  
by Mark Alan Erickson

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in  
Physical Education

Montana State University

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Abstract:

Five competitive cyclists (four male and one female) were studied during 95 min of bicycle ergometer exercise (approx. 65%  $V_{O2max}$ ) to determine the effects of ingesting caffeine before exercise (CAF) (5mg/kg body weight), fructose before exercise (FRU) (1g/kg), glucose during exercise (GLU) (1g/kg), a combination of caffeine/fructose before plus glucose during exercise (CFG) (same quantities as other trials), and a control (CON) on muscle glycogen utilization during exercise. Each subject performed all trials with not less than seven days and not more than fourteen days between trials. Preexercise ingestion occurred one hour prior to exercise and ingestion during exercise began fifteen minutes into the ride. Muscle biopsies were performed before initial ingestion (BIM) and following exercise (FEM).

Muscle glycogen levels were similar in all five trials, both before ingestion (CON = 152.0  $\mu\text{mol/gr}$  W.w., CAF = 144.6, CFG = 135.7, FRU = 146.5, GLU = 138.2) and following exercise (CON = 60.66, CAF = 81.44, CFG = 68.72, FRU = 79.86, GLU = 76.40). Muscle glycogen utilization, however, was greater ( $P < 0.05$ ) during trial CON than trials CAF and GLU. Although not statistically significant, there was a trend ( $P < 0.1$ ) towards lower glycogen utilization in trials CFG and FRU when compared with trial CON. No significant differences were observed between trials CAF, CFG, FRU, and GLU. These data indicate that caffeine ingestion before exercise and glucose ingestion during exercise can decrease muscle glycogen utilization.

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APPROVAL

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Mark Alan Erickson

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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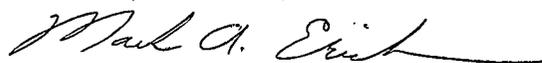
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## ABSTRACT

Five competitive cyclists (four male and one female) were studied during 95 min of bicycle ergometer exercise (approx. 65%  $\dot{V}O_{2max}$ ) to determine the effects of ingesting caffeine before exercise (CAF) (5mg/kg body weight), fructose before exercise (FRU) (1g/kg), glucose during exercise (GLU) (1g/kg), a combination of caffeine/fructose before plus glucose during exercise (CFG) (same quantities as other trials), and a control (CON) on muscle glycogen utilization during exercise. Each subject performed all trials with not less than seven days and not more than fourteen days between trials. Preexercise ingestion occurred one hour prior to exercise and ingestion during exercise began fifteen minutes into the ride. Muscle biopsies were performed before initial ingestion (BIM) and following exercise (FEM).

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## CHAPTER 1

## INTRODUCTION

Endurance athletes and coaches at all levels of competition are interested in increasing performance. One factor limiting endurance performance is muscle glycogen depletion. In an attempt to increase performance and decrease glycogen usage some athletes ingest carbohydrates and/or caffeine before and/or during exercise. Uninformed or misinformed ingestion of these substances could result in a possible increase in endurance performance and glycogen sparing (9,21,35,36,44), increased glycogen depletion (28,41,44), physical discomfort (6,54,41,44), physical harm (6,54), or even death (6). To gain optimal benefit and reduce the risk of complications, the relative effects of these substances should be known before use in competition. This study will attempt to clarify these effects.

### Problem Statement

What are the relative effects of: 1) fructose ingestion before exercise, 2) glucose ingestion during exercise, 3) caffeine ingestion before exercise, 4) fructose/caffeine ingestion before exercise plus glucose ingestion during exercise, and 5) control ingestion on muscle glycogen usage and other measured parameters?

### Hypothesis

There will be no significant difference in muscle glycogen utilization or the other measured parameters while using the tested dietary manipulations.

### Delimitations

This study was delimited to a group of five selected competitive cyclists living in the Bozeman area, 1984-1985.

Limitations

1. One subject was female and four subjects were male.
2. Subjects were highly trained competitive cyclists.
3. A Schwinn Bio-Dyne bicycle ergometer provided the exercise work load.
4. All tested substances and solutions were administered according to body weight.
5. Only one concentration of each tested substance was used.
6. Exercise started sixty minutes following the initial ingestion of solution.
7. The blood sample taken following exercise was taken within three minutes after exercise stopped.
8. The muscle biopsy taken following the ninety minute exercise period was taken within ten minutes after exercise stopped.

Definitions

Caffeine : a bitter alkaloid, usually obtained from coffee or tea: used chiefly as a stimulant and diuretic (50).

Carbohydrate : a food substance that includes various sugars and starches and is found in the body in the form of glucose and glycogen (57).

Endurance : the ability to resist fatigue.

Includes muscular endurance, which is local or specific endurance, and cardiovascular endurance, which is a more general, total body endurance (57).

Ergometer : a device for exercising the subject in a manner in which the physical work performed can be measured, e.g., bicycle ergometer (57).

Fatigue : inability to continue work, due to any one or a combination of factors (57).

Fatty Acid : along with glycerol, the product of the breakdown of fats (57).

Fructose : a monosaccharide, sometimes known as fruit sugar (56).

Glucose : a simple sugar which is transported in the blood and metabolized in the tissues (57).

Glycogen : the storage form of carbohydrates (CHO) in the body, found predominately in the muscles and liver (57).

Highly trained competitive cyclist : (for the purpose of this study) males with a  $VO_{2max}$  greater than 60 ml/kg x min or females with a  $VO_{2max}$  greater than 50 ml/kg x min, and having competitive experience at the state level.

Hypoglycemia : abnormally low blood glucose, or sugar, levels (57).

Maximal oxygen uptake ( $VO_{2max}$ ) : the best physiological index of total body endurance. Also referred to as aerobic power, maximal oxygen intake, maximal oxygen consumption, and cardiovascular endurance capacity (57).

Respiration : the exchange of gases at both the level of the lung and tissue (57).

Respiratory exchange ratio (R or RER) : the ratio of carbon dioxide ( $CO_2$ ) expired to oxygen consumed, at the level of the lungs (57).

Respiratory quotient (RQ) : the ratio of the  $CO_2$  produced in the tissues to the oxygen consumed by the tissues (57).

## CHAPTER 2

## REVIEW of RELATED LITERATURE

Competition has probably been around as long as mankind itself. With competition man has wanted to go faster and farther. This has invited the attention of scientists for many years concerning the effects of diet and other factors on athletic performance. Over one hundred years ago vonLeibig (45) suggested that protein might be the fuel for muscular work. However, Cathcart (11) showed urinary excretion of nitrogen was not affected by prolonged strenuous exercise. This has been confirmed repeatedly (12,30) indicating that protein was not used as a fuel for muscular work to any great extent.

Krogh and Lindhard (42), using <sup>1920</sup> respiratory exchange ratio (RQ) measurements, found that fat and carbohydrate (CHO) were used as energy sources for muscular work. The proportion of these substrates depended on both the intensity and duration of exercise (4,13,52). The importance of CHO in improving work capacity was demonstrated by Dill, Edwards, and Talbott

(24). They administered CHO to dogs, resulting in considerably increased work times before the animals were exhausted. By varying the diet prior to work, Christensen and Hansen (15) produced marked improvements in work times, a CHO rich diet giving work times two to three times longer than a high fat diet.

More recent studies using direct methods, involving needle biopsy of the working muscle, have shown that glycogen stores decreased during work (13,17,38,52) and were almost emptied during exhaustive work (38). Further, the initial glycogen content was closely correlated to work time to exhaustion (38), the more glycogen stored in the muscle the longer the time to exhaustion. This fact prompted the practice of CHO loading, which induced elevated glycogen levels in the tissues and enhanced performance time (38). Foster, et al. (28) reported that endurance performance could be determined by the rate of muscle glycogen utilization along with the preexercise muscle glycogen content.

To further increase work time, athletes have sought dietary manipulations that could decrease the rate of muscle glycogen utilization. Through dietary manipulations athletes have attempted to spare glycogen by supplementation with exogenous CHO, and increased free fatty acid (FFA) availability and metabolism with

caffeine. Manipulations of diet have included: 1) CHO ingestion before exercise, 2) CHO ingestion during exercise, 3) caffeine ingestion before and/or during exercise and, 4) combinations of the preceding.

The effects of CHO ingestion before exercise on endurance performance and its relationship to glycogen sparing are varied. In an attempt to provide CHO to contracting muscles, it has been observed that glucose ingestion before exercise resulted in increased insulin secretion. This hyperinsulinemia was followed by an exercise induced rapid decrease in blood glucose concentration and greater depletion of muscle glycogen (18,44). Several studies that have compared fructose ingestion with that of glucose or sucrose have noted significantly lower elevations in plasma glucose and little or no elevation in insulin after fructose ingestion. The normal hypoglycemic effect after glucose ingestion was also avoided with fructose (5,23,41,44). Because of these findings, it was proposed that fructose ingestion before submaximal exercise could have a glycogen sparing effect (44).

To examine the effects of various carbohydrates on the metabolic and hormonal response to exercise, Koivisto, et al. (41) administered 75 grams glucose, fructose, or placebo to nine well trained males

( $\text{VO}_2\text{max}=60 \text{ ml/kg} \times \text{min}$ ) forty-five minutes before cycle ergometer exercise performed at 75% maximal oxygen uptake ( $\text{VO}_2\text{max}$ ) for thirty minutes. After glucose ingestion, the rise in plasma glucose was three times greater ( $P<0.005$ ) and in plasma insulin 2.5 times greater ( $P<0.01$ ) than after fructose. During exercise, after glucose administration plasma glucose fell from 5.3 to 2.5 millimole/liter (mM/l) ( $P<0.001$ ) and after fructose from 4.5 to 3.9 mM/l ( $P<0.05$ ). The fall in plasma glucose was closely related to the preexercise levels of plasma insulin ( $r=0.82, P<0.001$ ) and glucose ( $r=0.81, P<0.001$ ). Both glucose and fructose ingestion decreased the FFA levels by 40-50% ( $P<0.005$ ) and during exercise they remained 30-40% lower after CHO than placebo administration ( $P<0.02$ ). The researchers suggested that glucose ingestion prior to exercise resulted in hypoglycemia during vigorous exercise; this rapid fall in plasma glucose was mediated, at least in part, by hyperinsulinemia. Fructose ingestion, in contrast, was associated with a modest rise in plasma insulin and did not result in hypoglycemia during exercise.

Levine, et al. (44) examined substrate utilization after fructose, glucose, or water ingestion in four male and four female subjects during three treadmill

runs at approximately 75%  $VO_{2max}$ . Each test was preceded by three days of a CHO rich diet. The runs were thirty minutes long and at least one week apart. Exercise began forty-five minutes after ingestion of 300 milliliters (ml) of randomly assigned 75 gram fructose(F), 75 gram glucose(G), or control(C) solution. Muscle glycogen depletion determined by preexercise and postexercise muscle biopsies (gastrocnemius muscle) was significantly ( $P < 0.05$ ) less during the F trial than during the C or G trials. Venous blood samples revealed significant increases in serum glucose ( $P < 0.05$ ) and insulin ( $P < 0.01$ ) within forty-five minutes after the G drink, followed by a significant decrease ( $P < 0.05$ ) in serum glucose during the first fifteen minutes of exercise. These changes were not observed in the C or F trials. R.Q. was higher ( $P < 0.05$ ) during the G than the C or F trials for the first five minutes of exercise and lower ( $P < 0.05$ ) during the C trial compared with the G or F trials for the last fifteen minutes of exercise. The higher R.Q. values indicated an increased CHO utilization. Using these results, Levine suggested that fructose ingestion before thirty minutes of submaximal exercise maintains stable blood glucose and insulin concentrations, which may lead to the observed sparing of muscle glycogen.

Replenishment of fluid and nutrients during exercise has been reported to be mainly limited by gastric emptying, which was controlled by many independent factors (16,27). In order to further analyze some of these factors, Costill, et al. (19) undertook a series of studies to determine the effects of solute volume (200,400,600,800 ml), temperature (5,15,25,35C), and glucose concentration (139,278,556,834 mM) on the rate of gastric emptying. Additional observations were made to assess the effects of exercise intensity on the rate at which 400 ml of a glucose (139 mM) solution was removed from the stomach. At rest the addition of even small amounts of glucose (>139 mM) induced a marked reduction in the rate of gastric emptying. The volume of fluid remaining in the stomach fifteen minutes after ingestion was somewhat greater when the solution was thirty-five degrees centigrade as opposed to colder fluids ( $P < 0.05$ ). The rate of gastric emptying increased in proportion to the volume ingested, with a maximal rate of emptying attained at a volume of 600 ml. In general, exercise had no effect on gastric emptying until the working intensity exceeded 70%  $V_{O2}$  max. Costill emphasized the importance of minimizing the glucose content of solutions ingested in order to obtain an optimal rate

of fluid replacement, since in combination with high intensity exercise even small amounts of carbohydrate could slow gastric emptying.

By the use of naturally enriched carbon-13 glucose as a metabolic tracer, Pirnay, et al. (48) investigated the utilization of exogenous glucose ingested during muscular exercise. Four subjects walked on an uphill treadmill for two hours, and three others for four hours. The energy expenditure, close to 50% of the individual  $\text{VO}_2\text{max}$ , varied from 1.9 to 2.1 liters of oxygen/minute, while the heart rate ranged between 142 and 165 beats/minute. The subjects, who were on a mixed diet and had fasted overnight, were given 100 grams of naturally labeled glucose. Following this intake, the expired  $\text{CO}_2$  became rapidly enriched in carbon-13. The increase was observed as early as fifteen minutes after the oral intake, and reached a maximum within one to two hours, when utilization of exogenous glucose varied between 500 and 650 mg/min, representing as much as 55% of the carbohydrate metabolism and 24% of the total energy expenditure.

Oral glucose administration was studied by Costill, et al. (18) at rest and during prolonged physical activity (60-72%  $\text{VO}_2\text{max}$ ). Seven male subjects ingested an aqueous solution of glucose (31.8 grams in

300 ml) that was tagged with 40 microcuries of uniformly labeled glucose carbon-14 ( $^{14}\text{C}$ ). In the exercise test the men ran or cycled for thirty minutes before and sixty minutes after glucose feeding. In both the exercise and resting experiments venous blood samples were taken at: 0, 3, 5, 7, 9, 11, 13, 15, 20, 30, 40, 50, 60, 90, and 180 minutes after the glucose administration. Expired air was sampled and analyzed for  $\text{O}_2$ ,  $\text{CO}_2$ , and  $^{14}\text{CO}_2$ . Compared to the control studies (resting) the serum glucose- $^{14}\text{C}$  and expired  $^{14}\text{CO}_2$  data suggested that exercise had little or no effect on intestinal glucose absorption. From thirty to seventy-five minutes after the glucose ingestion, the liver appeared to decrease its contribution to blood glucose. The initial appearance of  $^{14}\text{C}$  in the serum and expired air occurred five to seven minutes after the glucose feeding both at rest and during exercise. The glucose  $^{14}\text{C}$  was utilized 6.5 times faster during exercise than at rest. During the final 20 minutes of exercise, the ingested glucose was found to comprise about 5% of the total carbohydrate oxidation.

Brooke, et al. (9) conducted a study to determine the effects of normal and glucose work diets on the performance of racing cyclists. After preliminary trials, eight male racing cyclists performed rides at

loads to elicit approximately 67%  $\dot{V}O_2$ max in the laboratory on four dietary treatments during work: 1) glucose syrup drink, (2) isocaloric "normal" feeding containing rice pudding and sucrose, (3) low energy (< 4kcal) isovolumetric drink, and (4) no food. Both high energy diets delayed the onset of exhaustion, providing more combusted carbohydrate as shown by elevated RQ and blood glucose levels. More efficient work was performed when the glucose syrup drink was taken in comparison to the other diets. The researchers concluded that CHO ingestion during prolonged exercise increased performance, as measured by time to exhaustion.

Coyle, et al. (22) conducted a study to determine whether CHO feeding during exercise could delay the development of fatigue. Ten trained cyclists performed two bicycle ergometer exercise tests one week apart. The initial work rate required 74% of  $\dot{V}O_2$ max. The point of fatigue was defined as the time at which the exercise intensity the subjects could maintain decreased below their initial work rate by 10% of  $\dot{V}O_2$ max. During one exercise test the subjects were fed a glucose polymer solution beginning twenty minutes after the onset of exercise; during the other test they were given a placebo. Blood glucose concentration was

20-40% higher during the exercise after glucose ingestion than during the placebo trial. The RQ was unchanged by the glucose feeding. Fatigue was postponed by carbohydrate feeding in seven of the ten subjects. This effect appeared to be mediated by prevention of hypoglycemia in only two subjects. The exercise time to fatigue for the ten subjects averaged 134 minutes without and 157 minutes with glucose feeding. The increased time to fatigue with glucose feeding was significant ( $P < 0.01$ ). From these results Coyle concluded that CHO feeding during prolonged strenuous exercise could delay fatigue.

Observations with rats and humans have demonstrated a sparing of muscle glycogen and an enhanced capacity for endurance performance when FFA were elevated (20,32,51). In each of these studies the increased endurance performance and diminished rate of CHO metabolism followed an injection of heparin, which stimulated an increase in plasma FFA with a subsequent increase in fatty acid oxidation. Since caffeine was known to stimulate the mobilization of FFA (2), studies were undertaken to determine the effects of caffeine ingestion on endurance performance and metabolism (21,35).

In an effort to assess the effects of caffeine ingestion on metabolism and performance during prolonged exercise, Costill, et al. (21) had nine competitive cyclists (two females and seven males) exercise to exhaustion on a bicycle ergometer at 80%  $\dot{V}O_{2max}$ . One trial was performed one hour after ingesting decaffeinated coffee (D), while a second trial (C) required that each subject consume coffee containing 330 milligrams of caffeine sixty minutes before the exercise. Following the ingestion of caffeine (C), the subjects were able to perform an average of 90.2 minutes of cycling as compared to an average of 75.5 minutes in trial D. Measurements of plasma free fatty acids, plasma glycerol, and R.Q. were reported by the researchers as evidence of a greater rate of lipid metabolism during the caffeine trial as compared to the decaffeinated exercise treatment. Calculations of CHO metabolism from RQ data revealed that the subjects oxidized roughly 240 grams of CHO in both trials. Fat oxidation, however, was significantly higher ( $P < 0.05$ ) during the C trial (118 grams or 1.31 grams/minute) than in the D trial (57 grams or 0.75 grams/minute). On the average the participants rated (Perceived Exertion Scale) their effort during the C trial to be significantly ( $P < 0.05$ ) easier than the

demands of the D treatment. From their findings the researchers concluded that, the enhanced performance observed in the C trial was likely the result of the combined effects of caffeine on lipolysis and its positive influence on nerve impulse transmission.

Nine trained cyclists were studied by Ivy, et al. (35) to determine the effects of caffeine (CAF) and glucose polymer (GP) feedings on work production (kpm) during two hours of isokinetic cycling exercise (80 rpm). Ingestion of 250 milligrams of CAF sixty minutes prior to the ride was followed by ingestion of an additional 250 milligrams in divided doses fed at fifteen minute intervals over the first ninety minutes of the exercise. This treatment significantly increased work production by 7.4% and  $V_{O_2}$  by 7.3% as compared to control (C) while the subjects' perception of exertion remained unchanged. Ingestion of approximately ninety grams of GP during the first ninety minutes (12.8 g/15min) of the exercise had no effect on total work production or  $V_{O_2}$ . It was, however, effective in reducing the rate of fatigue over the last thirty minutes of cycling. Although GP maintained blood glucose and insulin levels ( $P < 0.05$ ) above those of the C and CAF trials, total CHO utilization did not differ between treatments. During

the last seventy minutes of the CAF trial, however, fat oxidation was elevated 31% and appeared to provide the substrate needed for the increased work production during this period of exercise. The researchers reported an enhanced rate of lipid catabolism and work production following the ingestion of caffeine.

In summary, the experimental findings of previous researchers indicate that glycogen sparing and/or endurance performance may be increased through the use of fructose ingestion before exercise (44), glucose ingestion during exercise (9,22,35,36), and caffeine ingestion before exercise (21,35). More testing is needed to determine the relative effectiveness of these substances and their combined effects on glycogen sparing and endurance performance.

## CHAPTER 3

## METHODOLOGY

Research Method

The experimental research design used for this study was a crossover single-blind protocol with five subjects, each acting as their own control. The study was designed to measure the relative effects of ingesting: 1) a fructose solution before exercise (FRU), 2) a glucose solution during exercise (GLU), 3) a caffeine solution before exercise (CAF), 4) a fructose/caffeine solution before plus a glucose solution during exercise (CFG), and 5) a control solution (CON) on muscle glycogen utilization, blood glucose and free fatty acid levels, and other measured parameters.

Sample

The sample consisted of one female and four male competitive cyclists that volunteered from the Bozeman area. All subjects completed a basic personal data questionnaire before participation in the study. A

sample subject questionnaire is found in Appendix A. All subjects had competition experience at the state level. Physical characteristics including:  $VO_2\max$ , age, height, weight, sex, and body composition of each subject by sex and for the total sample are included in Table 1. There were no reported cases of diabetes mellitus in the subjects' immediate families, and none of the subjects reported any sugar tolerance problems. None of the subjects reported taking any prescription drugs during the test period. Training levels remained constant throughout the test period. All subjects were informed of the nature of the study including: number and type of blood and muscle biopsy samples to be taken, intensity required for each test ride, time requirements, and the nature of the various ingested substances before giving their verbal and written consent to participate. A sample consent form is found in Appendix A. All subjects were told they could withdraw from the study at any time.

Table 1. Physical Characteristics of Subjects.

Subject	Sex	Age	VO <sub>2</sub> max	% Body Fat	Height	Weight
JW	M	24	73.3	4.1	178	75.5
BG	M	35	68.7	5.8	170	70.9
BO	F	25	57.8	12.5	166	60.0
SJ	M	29	63.5	5.6	190	79.3
RC	M	24	62.8	6.6	185	77.3
MEAN		27.4	65.2	6.9	178	65.2
SE (+ or-)		(2.1)	(2.7)	(1.4)	(4.5)	(3.4)

### Testing Battery

1. Maximal oxygen consumption (VO<sub>2</sub>max).
2. Anthropometric measurements.
  - A. Height (HT)
  - B. Body weight (BW)
  - C. Abdominal skinfold (AB), (males only).
  - D. Chest skinfold (CH), (males only).
  - E. Suprailiac skinfold (SI), (female only).
  - F. Thigh skinfold (TH).
  - G. Triceps skinfold (TR), (female only).
3. Blood samples.
  - A. Blood glucose levels (BGL).
  - B. Blood fatty acid levels (BFA).
4. Muscle glycogen levels.
5. Respiratory gas analysis.
  - A. Percent VO<sub>2</sub>max (%VO<sub>2</sub>max).
  - B. Respiratory Exchange Ratio (RER).

6. Heart Rate (HR).
7. Relative perceived exertion (RPE).
8. Control and experimental tests.
  - A. Control (CON).
  - B. Fructose ingestion before exercise (FRU).
  - C. Glucose ingestion during exercise (GLU).
  - D. Caffeine ingestion before exercise (CAF).
  - E. Caffeine/fructose ingestion before exercise plus glucose ingestion during exercise (CFG).

#### Maximal Oxygen Consumption

VO<sub>2</sub>max was measured using a standardized open circuit procedure patterned after Fox, et al. (29) on a Schwinn Bio-Dyne bicycle ergometer with a pedal frequency of 90 rpm. Gases were collected in a 350 liter Collins Tissot and analyzed with a Beckman E2 (Oxygen) and LB2 (Carbon dioxide) analyzer calibrated with standard gases prior to each test. Criteria for a maximal value was a plateau or decline in oxygen consumption at a subsequent higher intensity exercise bout and a RER greater than 1.0. The peak value will be used in the absence of attaining maximal criteria.

Standard calculation procedures were followed for computation of  $VO_{2max}$  (ml/kg x min, corrected for standard temperature and pressure dry (STPD) (46)). A sample calculation form is found in Appendix A.

#### Anthropometric Measurements

Height and body weight were determined using a Detecto-Medic. Height was measured with the subject bare foot and recorded in centimeters (cm). Body weight was measured with the subject wearing cycling shorts and a T-shirt, and recorded in kilograms (kg).

All skinfolds were measured by the same trained technologist using Lange skinfold calipers and recorded in millimeters (mm). The sites described by Behnke, et al. (1) were used to collect the skinfold data. Three measurements were taken at each site to the nearest 0.5 mm. The average of the three scores was recorded unless one deviated by more than 1.0 mm, in which case the site was remeasured. Percent body fat was then determined using the tables of Pollock (49).

#### Blood Samples

All blood samples were taken by the same medical technologist. Samples were drawn before ingestion of the initial solution (BIB), before exercise began

(BEB), and following exercise (FEB). They were allowed to clot for thirty minutes and were then centrifuged ten minutes and separated. The serum was then frozen in liquid nitrogen until analysis. The serum was analyzed, by a trained laboratory technologist, for glucose (BGL) using a hexokinase method (53) and free fatty acids (BFA) using a colorimetric method (43). BGL was reported as milligram percent (mg%) and BFA as micromoles per liter ( $\mu\text{mol/l}$ )

#### Muscle Glycogen Levels

Muscle glycogen levels were determined from muscle samples obtained by biopsy. The biopsies were performed by the same medical doctor. Biopsies were performed following the BIB (BIM) and FEB (FEM) blood samples. The "suction" method described by Evans, et al. (26), using a 5mm biopsy needle, was used to perform all biopsies. All samples were taken from the vastus lateralis. Samples were frozen in liquid nitrogen within thirty seconds of removal and stored in liquid nitrogen until analyzed.

Analysis of muscle glycogen levels was performed by the same trained laboratory technologist using an acid hydrolysis method described by Passonneau, et al. (47). Adipose tissue and blood were removed from the sample in a cold chamber ( $-40\text{C}$ ), and the sample was

then weighed on a torsion balance. After the muscle was hydrolyzed, centrifuged, and the supernate fluid neutralized to pH 6-7 with KOH, the glycogen concentration was measured as glucose using a hexokinase technique (53). Glucose concentration was reported as micromoles per gram wet muscle weight ( $\mu\text{mol/g w.w.}$ ).

#### Respiratory Gas Analysis

Respiratory gases were collected and analyzed every ten minutes during exercise using the same procedure used for  $\text{VO}_2\text{max}$ . Standard calculation procedures were used to calculate  $\% \text{VO}_2\text{max}$  and RER (46). A sample calculation form is found in Appendix A.

#### Heart Rate

Heart rate (HR) was monitored every ten minutes during exercise following the initial warmup using a Schwinn pulse monitor.

### Relative Perceived Exertion

A perceived exertion scale developed by Borg (8) was used to rate each subject's relative perceived exertion (RPE) every ten minutes during exercise following the initial warm up. A sample is found in Appendix B.

### Control and Experimental Tests

Each subject performed the control ride (CON), and the four experimental rides (FRU, CAF, GLU, and CFG) in a randomized order. A standardized procedure was followed for each test with a minimum of seven days and a maximum of fourteen days between tests. Preconditions necessary for controlled testing were followed by each subject prior to each test.

Each subject reported to the laboratory, between 6:30am and 8:00am, following a normal night's sleep and eight to twelve hours postabsorptive. Each subject was given recommendations concerning the quantity and content of food intake for the two days prior to testing. Balanced meals high in CHO and of slightly greater quantity than normal (for the subject) were recommended. Instructions were given to abstain from the ingestion of any foods or liquids (other than water) during the eight hours preceding each test.

Training was restricted the two days preceding each test. Two days prior to each test an easy workout was allowed. The subjects abstained from training the day before testing. This procedure of diet and exercise was an attempt to prevent low pretest muscle glycogen levels.

A blood sample (BIB) and muscle biopsy (BIM) sample were taken from each subject when he/she reported to the laboratory and before ingestion of the initial solution, a time schedule can be found in Appendix C. All solutions were administered as milliliters solution per kilogram body weight (ml/kg), glucose and fructose were administered as grams substance per kilogram body weight (g/kg), and caffeine was administered as milligrams caffeine per kilogram body weight (mg/kg). Following initial blood and biopsy samples, each subject was given a grape Kool-Aid solution (10ml/kg) containing either: 1) fructose (1g/kg) (FRU), 2) Nutra Sweet (CON and GLU), 3) caffeine (5mg/kg) (CAF), or 4) fructose (1g/kg) and caffeine (5mg/kg) (CFG) with instructions to ingest the solution within five minutes. All solutions were served cold. The subject was not informed of the contents of the solution. The subject was then placed in a quiet area for forty-five minutes. At this time

he/she returned to the laboratory and the second blood sample (BEB) was taken.

Exercise started sixty minutes following ingestion. A five minute warm up at approximately 50%  $\text{VO}_2\text{max}$  and ninety rpm was followed by ninety minutes of exercise at approximately 65%-70%  $\text{VO}_2\text{max}$  and ninety rpm. Respiratory gases were monitored every ten minutes during exercise. Heart rate and RPE were monitored every ten minutes during exercise, following the initial warm up. The subject was given a grape Kool-Aid solution (4ml/kg) containing Nutra Sweet (CON, FRU, CAF) or glucose (0.25g/kg) (GLU, CFG) with instructions to ingest the solution within two minutes. This solution was administered fifteen minutes, thirty minutes, forty-five minutes, and sixty minutes into exercise. Total fluid intake during exercise was twelve ml/kg and total glucose intake (GLU, CFG) was one g/kg. The subject then dismounted the Bio-Dyne and a blood sample (FEB) and muscle biopsy (FEM) were taken.

Statistical Treatment of Data

An analysis of variance with multiple factors (MSUSTAT AVMF) was used to assess differences with each subject receiving all combinations of "factors". Standard error (SE + or -) was used to indicate variation in individual means. Multiple comparison of factor means was performed using the Least Significant Difference by Student's T to locate differences between means and a probability level of 0.05 was chosen as the criterion for acceptance of statistical significance.

## CHAPTER 4

## RESULTS

The results will be presented in four sections. The first section will include HR, VO<sub>2</sub>, RPE, and RQ. Blood glucose, blood FFA, and muscle glycogen follow in separate sections.

HR, VO<sub>2</sub>, RPE, and RQ

No significant differences were observed between trials for heart rate (Table 2), or oxygen uptake (Table 3). There was a significant difference ( $P < 0.05$ ) in RPE after thirty minutes of exercise between the CAF and FRU trials (Table 4). Significant differences ( $P < 0.05$ ) in RQ were observed after twenty minutes of exercise between the FRU, and the CON, GLU, CAF rides (Table 5).

Table 2. Heart rate (HR) (SE + or -) in beats per minute versus time.

Time (min.)	CON	CAF	CFG	FRU	GLU
15	147.8 (6.9)	143.0 (6.0)	151.4 (5.1)	151.0 (4.8)	145.2 (3.9)
25	150.6 (7.3)	142.6 (6.4)	153.2 (5.2)	151.4 (5.0)	148.2 (4.4)
35	148.4 (7.7)	144.2 (7.1)	157.2 (4.8)	152.2 (4.8)	149.8 (4.7)
45	150.4 (7.8)	146.0 (7.1)	156.6 (4.4)	151.4 (5.2)	153.0 (4.3)
55	151.2 (7.5)	146.8 (6.8)	158.4 (4.3)	151.2 (4.8)	153.4 (4.0)
65	150.4 (7.8)	146.0 (6.7)	157.4 (4.4)	148.6 (5.4)	154.6 (4.0)
75	150.8 (8.5)	145.8 (6.3)	157.6 (4.5)	150.0 (5.1)	154.8 (3.4)
85	152.0 (7.9)	148.0 (6.2)	156.2 (5.5)	150.2 (5.4)	155.5 (3.8)
95	154.4 (8.2)	151.8 (6.8)	155.8 (5.5)	153.4 (5.8)	155.2 (3.3)

Table 3. Oxygen uptake ( $\dot{V}O_2$ ) (SE + or -) in liters per minute versus time.

Time (min.)	CON	CAF	CFG	FRU	GLU
10	2.996 (0.278)	3.032 (0.284)	3.026 (0.269)	3.058 (0.329)	2.874 (0.260)
20	3.128 (0.303)	3.198 (0.309)	3.114 (0.257)	2.996 (0.306)	2.994 (0.262)
30	3.112 (0.278)	3.092 (0.251)	3.172 (0.259)	3.110 (0.261)	3.076 (0.280)
40	3.156 (0.302)	3.178 (0.318)	3.114 (0.259)	3.110 (0.281)	3.116 (0.279)
50	3.138 (0.293)	3.234 (0.280)	3.144 (0.255)	3.150 (0.272)	3.088 (0.277)
60	3.148 (0.305)	3.200 (0.272)	3.160 (0.265)	3.176 (0.279)	3.142 (0.267)
70	3.148 (0.310)	3.282 (0.288)	3.220 (0.283)	3.152 (0.292)	3.150 (0.267)
80	3.258 (0.324)	3.262 (0.306)	3.200 (0.269)	3.162 (0.282)	3.170 (0.275)
90	3.260 (0.312)	3.288 (0.256)	3.200 (0.238)	3.240 (0.312)	3.194 (0.262)

Table 4. Relative perceived exertion (RPE)  
(SE + or -) versus time.

Time (min.)	CON	CAF	CFG	FRU	GLU
15	12.40 (0.51)	11.00 (0.45)	11.80 (0.37)	12.40 (0.60)	12.00 (0.45)
25	12.60 (0.51)	11.60 (0.24)	11.80 (0.37)	12.60 (0.51)	12.00 (0.45)
35	13.00 (0.63)	11.80 (0.37)	12.40 (0.40)	13.40 (0.75)	12.80 (0.37)
45	13.40 (0.81)	12.00 (0.45)	13.00 (0.55)	13.00 (0.45)	13.40 (0.40)
55	13.60 (0.93)	12.80 (0.37)	13.20 (0.49)	13.80 (0.58)	13.60 (0.51)
65	14.40 (0.81)	13.20 (0.20)	13.60 (0.51)	14.00 (0.55)	13.60 (0.60)
75	14.20 (0.73)	13.00 (0.32)	13.80 (0.37)	14.60 (0.60)	13.80 (0.73)
85	14.80 (0.97)	13.40 (0.40)	13.80 (0.37)	14.80 (0.58)	13.60 (0.68)
95	15.40 (1.03)	13.80 (0.49)	13.80 (0.58)	14.80 (0.58)	13.80 (0.58)

Table 5. Resriratory quotient (RQ) (SE + or -) versus time.

Time (min.)	CON	CAF	CFG	FRU	GLU
10	0.896 (0.012)	0.910 (0.025)	0.925 (0.014)	0.925 (0.007)	0.894 (0.016)
20	0.900 (0.007)	0.909 (0.015)	0.929 (0.009)	0.947 (0.015)	0.900 (0.012)
30	0.903 (0.010)	0.896 (0.014)	0.928 (0.010)	0.922 (0.007)	0.898 (0.011)
40	0.899 (0.008)	0.897 (0.015)	0.915 (0.010)	0.912 (0.007)	0.893 (0.012)
50	0.893 (0.009)	0.889 (0.019)	0.916 (0.010)	0.924 (0.009)	0.901 (0.009)
60	0.892 (0.008)	0.886 (0.016)	0.913 (0.015)	0.913 (0.015)	0.904 (0.010)
70	0.889 (0.010)	0.886 (0.015)	0.912 (0.015)	0.905 (0.009)	0.909 (0.010)
80	0.887 (0.013)	0.878 (0.016)	0.906 (0.015)	0.902 (0.013)	0.908 (0.013)
90	0.886 (0.012)	0.876 (0.017)	0.917 (0.015)	0.901 (0.017)	0.906 (0.009)

Blood Glucose

As illustrated in Table 6, there were no significant differences between the before ingestion blood glucose (BIBG) levels. Before exercise blood glucose (BEBG) levels for the CAF and CFG trials were significantly higher ( $P < 0.05$ ) than that of the FRU trial. This difference resulted from an increase in blood glucose in the CAF (7%) and CFG (9%) with a contrasting 9% decrease in blood glucose observed in the FRU trial. From BEBG to following exercise blood glucose (FEBG), blood glucose levels increased 29% for GLU, 21% for CFG, 12% for FRU and 9% for CON, and decreased 9% for CAF. This resulted in FEBG levels for the CFG and GLU trials that were significantly higher ( $P < 0.05$ ) than those for the CON, CAF, and FRU trials.

Table 6. Blood glucose (SE + or -) in mg%.

Sample	CON	CAF	CFG	FRU	GLU
BIBG	84.44 (3.20)	91.88 (4.66)	89.08 (3.95)	85.48 (3.60)	87.26 (2.10)
BEBG	84.38 (1.50)	98.04 (5.64)	96.82 (7.91)	77.42 (7.18)	84.42 (2.04)
FEBG	92.18 (3.27)	89.44 (3.41)	117.4 (8.6)	86.38 (3.39)	109.0 (3.58)

Blood FFA

No differences were observed between the before ingestion blood fatty acid (BIFA) levels, as illustrated in Table 7. Between the BIFA and the before exercise blood fatty acid (BEFA) samples, blood FFA levels decreased 30% for FRU, 15% for GLU, and 5% for CON. In contrast, FFA levels increased 24% for CFG and 8% for CAF. The BEFA levels of CFG were higher ( $P < 0.05$ ) than those of FRU. During exercise, FFA levels increased in each trial, producing a significant ( $P < 0.05$ ) time effect even though the amount of increase varied. Following exercise blood fatty acid (FEFA) levels for the CON, CAF, and FRU rides were significantly higher ( $P < 0.05$ ) than the FEFA levels for the CFG and GLU rides.

Table 7. Blood FFA (SE + or -) in  $\mu\text{mol/liter}$ .

Sample	CON	CAF	CFG	FRU	GLU
BIFA	476.2 (39.0)	417.2 (54.8)	434.2 (59.7)	474.2 (61.1)	454.4 (29.5)
BEFA	451.4 (18.9)	451.4 (14.7)	537.2 (147.4)	328.6 (10.1)	385.6 (28.9)
FEBF	1336 (229)	1126 (123)	737.2 (138.8)	1034 (129)	714.4 (51.5)

Muscle Glycogen

Muscle glycogen levels were similar in all five trials, both before ingestion (BIMG) and following exercise (FEMG), as illustrated by Table 8. Muscle glycogen utilization (BIMG-FEMG), however, was greater ( $P < 0.05$ ) during trial CON than trials CAF and GLU. Although not statistically significant, there was a trend ( $P < 0.1$ ) toward lower glycogen utilization in trials CFG and FRU when compared with trial CON. No significant differences were observed between trials CAF, CFG, FRU, and GLU.

Table 8. Muscle glycogen (SE + or -) in  $\mu\text{mol/g w.w.}$ 

Sample	CON	CAF	CFG	FRU	GLU
BIMG	152.0 (19.5)	144.6 (7.6)	135.7 (13.1)	146.5 (7.1)	138.2 (13.6)
FEMG	60.66 (17.72)	81.44 (7.21)	68.72 (12.76)	79.68 (8.39)	76.40 (14.09)
BIMG-FEMG	91.36 (10.01)	63.14 (7.90)	66.98 (10.78)	66.80 (12.05)	61.84 (5.25)

## CHAPTER 5

## DISCUSSION

It has been demonstrated that during prolonged exercise, the onset of fatigue is delayed if muscle glycogen is spared (20,28,32,51). With this in mind, the present investigation was undertaken to determine the relative effects of: 1) caffeine ingestion before exercise, 2) fructose ingestion before exercise, 3) glucose ingestion during exercise, and 4) caffeine/fructose ingestion before exercise plus glucose during exercise on muscle glycogen utilization and other measured parameters. Knowledge of the effects of the various treatments could help athletes at all levels of competition make a more educated decision as to their optimal use. The subjects used in this study were highly trained competitive cyclists, therefore the information is most directly applied to this group of athletes. However, athletes from other disciplines may also find the information relevant.

Caffeine

Caffeine has become popular as a potential ergogenic aid for endurance athletes based on evidence that caffeine increases lipid utilization in trained cyclists (21,35). In other studies, however, caffeine failed to increase lipid oxidation or inhibit carbohydrate utilization (10,40). In the present study muscle glycogen utilization was significantly lower during CAF than CON. However increased blood FFA levels or decreased RQ values, when compared to CON, were not present.

Several explanations are possible for the failure of caffeine to increase estimated FFA utilization in the present study. Based on past competition results, these subjects are capable of prolonged cycling using lipid as a significant energy source at approximately 65% of  $\dot{V}O_{2max}$ . Lipolysis is increased during exercise due to the release of epinephrine, at a rate proportional to exercise intensity (55). Bellet (3) has shown that caffeine also increases lipolysis by increasing epinephrine release. It is possible that exercise-induced release of epinephrine is a more influential mediator of plasma FFA concentration than caffeine-induced release in some well trained athletes. Caffeine may play a more important role in promoting

lipid oxidation at a higher exercise intensity when glycogen sparing is critical. For example, during cycling requiring approximately 80% of  $V_{O2max}$ , and presumably involving a significant anaerobic contribution to energy needs based on reported RQ-values, Essig et al. (25) observed increased lipid utilization and muscle glycogen sparing. The possibility that caffeine promotes lipid oxidation at higher exercise intensities is also indirectly supported by the knowledge that long-term training enhances lipid utilization due to increased lipolytic enzyme activity and mitochondrial size (33). Thus, it is possible that long-term endurance training confers optimal ability to utilize FFA during cycling at 65% of  $V_{O2max}$ , allowing little potentiation by caffeine. If the exercise intensity had been higher or the fitness level of the subjects lower, caffeine may have had a greater influence on FFA mobilization and utilization.

Blood glucose increased 7% from BIBG to BEBG, possibly due to caffeine's stimulatory effect on the adrenal glands (6), then dropped 9% between BEBG and FEBG. These results may indicate that the decreased utilization of muscle glycogen was at the expense of increased liver glycogen utilization.

Four of the five subjects reported feeling very

motivated during the first thirty minutes of exercise and the tester had to monitor pedal RPM to prevent excessive spinning. Although not significant (except at thirty minutes compared to FRU), CAF consistently had the the lowest RPE readings. These observations probably resulted from caffeine's stimulatory effect on the central nervous system.

### Fructose

Since preexercise glucose feedings have been shown to increase the rate of muscle glycogen utilization during exercise (44) and decrease exercise time to exhaustion (28), a source of CHO without these negative effects would be preferred as a preexercise feeding. Fructose has been considered as a possible source for preexercise CHO intake. Absorption of fructose from the gut occurs more slowly than does that of glucose and, in healthy humans, 70% - 90% of ingested fructose enters the portal circulation as fructose. In the fasted state most of the glucose formed in the liver is converted to glycogen and subsequently there is no significant rise in plasma glucose or insulin levels (5,23). In a previous study, Levine (44) reported a decrease in muscle glycogen utilization using fructose compared to a control.

The present study supports the glycogen findings of Levine. Although not significant, there was a trend toward lower glycogen utilization ( $P < 0.1$ ) with the FRU compared to CON. No significant difference was observed between GLU, CAF, CFG or FRU. Blood glucose decreased between BIBG and BEBG, while previous studies reported a slight increase in blood glucose following fructose ingestion (41,44,31). This variation may be due to a time factor, since this study measured blood glucose sixty minutes after ingestion while the previous studies took samples at forty five minutes following ingestion.

RQ remained above 0.9 during the entire exercise period. This indicates that CHO was available, and metabolized for fuel.

The high RPE (significantly higher than CAF after thirty minutes of exercise) values reported were due to gastric upset experienced by three of the five subjects. Because of its slower absorption from the intestine, it is not uncommon for osmotic diarrhea to occur after fructose in doses as large as used in the present study (14). Before an athletic event, fructose supplementation would likely be less than that employed experimentally.

Glucose

It has been demonstrated that the time of glucose ingestion is critical to its effects on physical performance (9,20,44), and that ingested glucose is present in the blood in as little as five minutes (18). It has been previously reported that ingested glucose is metabolized during prolonged exercise (18,48), may decrease the livers contribution to blood glucose (18), and that glucose ingested during prolonged exercise can delay fatigue (9,22). It was demonstrated in the present study that muscle glycogen utilization was significantly decreased when glucose (GLU trial compared to CON) was ingested during ninety minutes of exercise. FEBG levels were higher than BEBG levels and RQ levels remained very stable at approximately the 0.9 level through out exercise. FEFA levels for GLU, and CFG were significantly lower than the FEFA level of CON. This may indicate that the ingested glucose was used as a fuel source, thus saving the bodies fuel reserves. Also of interest was the finding that RPE levels remained stable from the thirty minute reading to the end of exercise. This finding was reinforced by comments from two subjects that the exercise felt very consistent during GLU and continuing would not be difficult.

Caffeine, Fructose, and Glucose

The CFG trial was included to explore the possibility that the various treatments were additive in regard to glycogen sparing. It was found that glycogen utilization tended to be less in comparison to CON, but not different from the remaining treatments. More variability was present between individual BEBG, FEBG, and BEFA with CFG than any other treatment, indicating that individual reaction to the ingestion of multiple substances is a complex problem. Blood glucose increased from BIBG to BEBG to FEBG, this along with the consistently high RQ readings indicate that CHO was readily available as a fuel source. Also of interest was the finding that the rise in blood FFA was modest even though caffeine was ingested, this was previously observed after caffeine and sucrose ingestion at rest (2). These findings raise many questions concerning the effects of ingesting multiple substances before and during exercise.

## CHAPTER 6

## SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

Summary

Five competitive cyclists (four male and one female) were studied during 95 min of bicycle ergometer exercise (approx. 65% VO<sub>2</sub>max) to determine the effects of ingesting caffeine before exercise (CAF) (5mg/kg body weight), fructose before exercise (FRU) (1g/kg), glucose during exercise (GLU) (1g/kg), a combination of caffeine/fructose before plus glucose during exercise (CFG) (same quantities as other trials); and a control (CON) on muscle glycogen utilization during exercise. Each subject performed all trials with not less than seven days and not more than fourteen days between trials. Preexercise ingestion occurred one hour prior to exercise and ingestion during exercise began fifteen minutes into the ride. Muscle biopsies were performed before initial ingestion (BIM) and following exercise (FEM). Blood samples were taken before ingestion (BIB), before exercise began (BEB), and following exercise (FEB). Blood samples were analyzed for

glucose and FFA.

There were no significant differences between BIB glucose levels. BEB glucose levels for the CAF and CFG trials were significantly higher ( $P < 0.05$ ) than that of the FRU trial. FEB glucose levels for the CFG and GLU trials were significantly higher ( $P < 0.05$ ) than those for the CON, CAF, and FRU trials.

No differences were observed between the BIB FFA levels. The BEB FFA levels of CFG were higher ( $P < 0.05$ ) than those of FRU. During exercise, FFA levels increased in each trial, producing a significant ( $P < 0.05$ ) time effect even though the amount of increase varied. FEB FFA levels for the CON, CAF, and FRU rides were significantly higher ( $P < 0.05$ ) than those for the CFG and GLU rides.

Muscle glycogen levels were similar in all five trials, both before ingestion and following exercise. Muscle glycogen utilization, however, was greater ( $P < 0.05$ ) during trial CON than trials CAF and GLU. Although not statistically significant, there was a trend ( $P < 0.1$ ) toward lower glycogen utilization in trials CFG and FRU when compared with trial CON. No significant differences were observed between trials CAF, CFG, FRU, and GLU.

These data indicate that caffeine ingestion before exercise and glucose ingestion during exercise can decrease muscle glycogen utilization. This decrease in muscle glycogen utilization could result in an increase in endurance performance.

### Conclusions

This study was conducted with a small group of competitive cyclists, therefore broad generalizations could not be drawn. Based on the limitations of this study the following conclusions appear justified.

1. Due to the variability among subjects, the use of any of the tested substances should be limited to training until the effects on the individual are known.
2. Glucose was a safe and consistent performer, providing a decreased utilization of muscle glycogen compared to CON. This could lead to an increase in endurance performance. The concentration ingested by an athlete should meet the needs of CHO supplementation and fluid replacement.
3. Caffeine ingestion decreased muscle glycogen utilization and received the lowest scores for RPE. These findings indicate that caffeine

ingestion may increase endurance performance. Individual reactions to caffeine varied and side effects are possible. Its use needs to be restricted to safe levels, and individual tolerance should be determined.

4. Fructose ingestion caused gastric upset at the levels ingested in this study, therefore use in competition would probably be less.

Individual tolerance levels and effects need to be determined before use in competition.

5. Ingestion of multiple substances is a complex problem requiring additional research.

#### Recommendations

Based on the results of this study, further research in the area of ingesting caffeine, fructose, glucose or combinations of the preceding seem warranted. The following recommendations are presented.

1. Strict control of dietary intake and training load during the testing period, to control preexercise muscle glycogen levels, would be beneficial for statistical reasons.

2. Varying the amount of ingestion to determine if a dose:response relationship exists for the individual substances would be valuable.
3. The time of ingestion prior to and during exercise should be investigated to determine the optimal time of ingestion.
4. The relation between percent  $V_{O2max}$ , fitness level, and substrate utilization should be examined to determine the role caffeine plays in glycogen sparing.
5. The relative effects the tested substances have on endurance performance as measured by time to exhaustion should be investigated.

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APPENDICES





















