2011

Effect of pre-exercise ingestion of modified amylomaize starch on endurance performance

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Effect of pre-exercise ingestion of modified amylomaize starch on endurance performance

by

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A thesis submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Diet and Exercise

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Iowa State University

Ames, Iowa

2011

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ABSTRACT

If exercise is initiated when insulin remains elevated from a pre-exercise meal, some individuals may experience a brief period of hypoglycemia 15-30 min into exercise. Rebound hypoglycemia is a consequence of increased glucose uptake by skeletal muscle and suppressed hepatic glucose production. The impact of pre-exercise carbohydrate ingestion on endurance exercise performance remains unclear. Amylomaize-7, a type of high amylose corn starch, evokes a smaller insulin response than dextrose and therefore could be a better pre-exercise carbohydrate. The purposes of this study were to 1) use a strategically timed high intensity cycling trial to detect possible performance impairment caused by a carbohydrate preload, and 2) compare metabolic response and exercise performance when amylomaize-7 versus dextrose is consumed in the hour before exercise.

Ten trained cyclists (VO_2max of 64.6 ± 1.8 ml/kg/min) were given 1 g/kg body mass of either dextrose or amylomaize-7 or a sugar-free flavored water placebo 45 min prior to exercise on a cycle ergometer. A 15 min ride at 60% Wmax was immediately followed by a self-paced time trial with a workload equivalent to 15 min at 80% Wmax (264 ± 12 KJ).

Time required to complete the performance trial was not significantly different between treatments ($p = 0.209$). In the dextrose trial, serum glucose concentrations increased from 5.8 ± 0.1 mM to 9.1 ± 0.6 mM before exercise. By the end of the TT, glucose had decreased to 5.5 ± 0.4 mM – significantly lower than placebo and starch ($p < 0.05$). The insulin concentration was also significantly elevated at pre-exercise, but was no different than placebo by the start of the TT. Serum insulin and glucose concentrations for the starch trial were the same as placebo for all time points. Affect scale ratings, RPE, and questionnaire responses did not reveal significant differences between treatments. In

conclusion, pre-exercise ingestion of amylomaize-7 instead of dextrose results in more stable serum glucose and insulin concentrations, but does not offer an additional performance advantage.

CHAPTER 1. INTRODUCTION

Carbohydrate ingestion during prolonged exercise has been shown to improve performance by maintaining blood glucose levels (4). Carbohydrate ingestion after exercise has been shown to speed recovery by promoting glycogen resynthesis (19). However, carbohydrate ingestion before exercise has shown mixed effects on endurance performance; in some studies it was improved (11), in others it was impaired (9,25), and in others it was unaffected (15,20,21,31). The conflicting results have been attributed to differences in study design such as the carbohydrate type, amount, or timing; exercise intensity or duration; subject training status; and performance testing method.

After a large carbohydrate load, blood glucose rises steeply, stimulating insulin secretion by the pancreas. Insulin promotes cellular uptake of glucose via GLUT4 translocation. Skeletal muscle contractions also promote GLUT4 movement to the cell membrane (39). When the start of exercise coincides with peaking insulin levels (30-60 min after carbohydrate consumption), the synergistic effect of insulin and exercise can cause a rapid decline in blood glucose to baseline levels or below (7). Rebound hypoglycemia, as it has been termed, has been a recent area of research because it is unclear why some individuals are more susceptible to the transient hypoglycemia and to what extent it affects endurance performance (20,21,22).

Low molecular mass carbohydrates such as sucrose, dextrose, and glucose polymers are often used in carbohydrate supplements because of their high digestibility and water solubility (41). However, rapid entry of glucose into the blood leads to a large insulin release, which may predispose an athlete to hypoglycemia at the onset of exercise. Alternative carbohydrate types, such as corn starch, have been proposed as a way

to moderate the metabolic response by providing a more slowly digested source of glucose (3). The rate of starch digestion is largely determined by the relative proportion of amylopectin and amylose it contains (12). Amylopectin has a highly branched chemical structure, making it more susceptible to enzymatic degradation. Amylose is more slowly digested because its linear configuration limits enzymatic access and permits hydrogen bonding between molecules (26). Most cereal starches are 80% amylopectin and 20% amylose (12). After Guezennec et al. (14) found that pure corn starch consumed before exercise offered no advantage over dextrose, Seewi et al. (34) determined that a minimum of 30% amylose content is necessary to attenuate postprandial insulin responses.

There are certain corn genotypes that naturally contain a higher percentage of amylose. Amylomaize has a 70% amylose content (41). It has been shown to lower insulin response by 42% (12), a desirable trait for a pre-exercise carbohydrate source. Unmodified high amylose corn starch is not regularly used in carbohydrate supplements because it is only 68% digestible (14) and insoluble in cold water (41). However, high amylose corn starch can be partially hydrolyzed with hydrochloric acid and alcohol, producing amylomaize-7, which is 92% digestible and readily soluble in water (41). With these physical characteristics and a chemical structure that suppresses insulin secretion, amylomaize-7 may be a better pre-exercise carbohydrate than rapidly absorbed carbohydrates like dextrose.

To test the effect of rebound hypoglycemia on exercise performance or detect benefits from substituting amylomiaze-7 for dextrose as a pre-exercise carbohydrate, an appropriate cycling time trial protocol must be selected. The majority of previous studies have utilized a time to exhaustion trial at a fixed workload (9,15,25,28) or a submaximal pre-load ride followed by a maximal time trial (1,11,21,22,29,30). Jeukendrup et al. (23) showed that time

to exhaustion trials have poor reproducibility, making this type of test insensitive to small changes caused by a nutritional intervention. Protocols with lengthy submaximal pre-load rides are also inappropriate for testing the effects of rebound hypoglycemia because the rebound hypoglycemia may be resolved by the time the performance task is begun. Hargreaves et al. found that after pre-exercise dextrose ingestion, blood glucose concentration declined significantly 15 min into exercise, but was no different from the placebo by 30 min (15). If a time trial is undertaken 60 min into exercise, counterregulatory hormones will have already normalized blood glucose levels, and observation of performance impairment would be unlikely. A better way to detect even a brief detrimental effect caused by rebound hypoglycemia is to introduce a high intensity time trial directly during the hypoglycemic period.

It is important to understand the impact of the hypoglycemia on performance and determine if amylomaize-7 can prevent it from occurring because if an athlete is required to exert a sudden burst of energy early in a competition (e.g., a cyclist encountering a hill), valuable seconds could be lost, which could ultimately decide the winner of the race. The purposes of this study are to 1) use a strategically timed high intensity cycling trial to detect possible performance impairment caused by a carbohydrate preload, and 2) compare metabolic response and exercise performance when amylomaize-7 versus dextrose is consumed in the hour before exercise. It is hypothesized that pre-exercise carbohydrate ingestion will not affect exercise performance, and that substitution of amylomaize-7 for dextrose as the carbohydrate source will result in a more stable plasma glucose concentration in the first 30 min of exercise.

CHAPTER 2. LITERATURE REVIEW

Introduction

Exercise physiology research literature contains numerous studies on carbohydrate ingestion and exercise performance. The results of studies on carbohydrate supplementation during and after exercise are mostly in agreement, whereas those on carbohydrate ingestion before exercise are not. This literature review first outlines the current knowledge concerning pre-exercise carbohydrate ingestion. Then, glucose and insulin kinetics are used to explain why rebound hypoglycemia may be observed in people who consume carbohydrate in the hour before exercise. The influence of starch structure on digestion and insulin response is discussed, along with the benefits of starch modification. Finally, the need for designing a specific cycling protocol for testing performance after a pre-exercise carbohydrate load is presented.

Carbohydrate and Exercise

Carbohydrate feedings during exercise have been shown to improve endurance performance by maintaining blood glucose (4). Consuming carbohydrate in the two h following exercise has been shown to speed glycogen resynthesis (19). Ingestion of carbohydrate in the hour before exercise has been shown to impair (9,25), improve (11), or have no effect on (15,20,21,31) endurance exercise performance.

Two older studies by Foster et al. (9) and Keller and Schwarzkopf (25) are the most commonly cited examples of impaired performance following a pre-exercise carbohydrate load. Foster et al. (9) observed a 19% reduction in time to exhaustion when subjects (8 males, 8 females) were given 75 g of dextrose 30 min before exercising at 80% VO_2max. Pre-exercise carbohydrate ingestion, and subsequent elevation of insulin levels during

exercise, resulted in a significantly lower free fatty acid concentration during the cycling bout. Blood samples collected 10 min into exercise revealed a period of transient hypoglycemia (<3.5 mM) in the carbohydrate group; however, plasma glucose concentrations at exhaustion were similar to those who consumed only water before exercise. Although three subjects reported symptoms of hypoglycemia just before stopping the ride, because blood lactate concentration was low and fatigue was primarily isolated to the quadriceps femoris, glycogen depletion rather than hypoglycemia was blamed for the performance decline.

Similarly, Keller and Schwarzkopf (25) found that subjects (five male collegiate distance runners) who consumed 100 g of dextrose 60 min before exercise on a cycle ergometer had a 25% shorter time to exhaustion compared to subjects who consumed a placebo. The exercise was continuously repeated high intensity intervals: 85% VO_2max for two min, separated by a one min rest. Post-exercise plasma glucose levels were similar for both groups, but blood lactate was 23% higher in the carbohydrate group. It was concluded that impaired fatty acid mobilization and increased glycogen utilization likely caused the premature fatigue in the subjects who consumed dextrose before exercise.

Glycogen depletion could not be confirmed in either of these studies because no muscle biopsies were taken. The exercise intensities of these trials were higher than those employed in more recent research. This may be one reason why few, if any, other studies have been able to detect performance impairment caused by pre-exercise carbohydrate.

A trial by Goodpaster et al. (11) found pre-exercise carbohydrate actually improved the endurance performance of ten male competitive cyclists. Subjects were provided 1 g/kg body mass of dextrose, waxy starch, resistant starch, or placebo 30 min before a 90 min

submaximal preload ride (66% VO_2max) and 30 min time trial. Dextrose improved time trial performance by 8% and waxy starch improved it 6%. Serum glucose and insulin were significantly higher in the dextrose group at the start of exercise, but after 15 min there were no differences between any of the treatments. The authors surmised that pre-exercise carbohydrate provided an ergogenic effect by providing additional carbohydrate for oxidation when glycogen stores were depleted by the lengthy preload ride.

Research by Hargreaves et al. (15), in which muscle biopsies were taken, showed that pre-exercise dextrose caused more fluctuations in plasma glucose and insulin levels early in exercise, but had no effect on endurance performance or glycogen use. Subjects in the study were given 75 g of dextrose, fructose, or placebo 45 min prior to a ride to exhaustion at 75% VO_2max. Blood glucose fell rapidly at the onset of exercise in the dextrose trial, and remained significantly different from the placebo until 15 min. However, there were no differences in plasma glucose at exhaustion, which was reached at approximately 90 min in all three treatments. The exercise-ending fatigue must have been caused by factors other than glycogen depletion because subjects had as much as 50 to 55 mmol/kg wet wt of glycogen remaining in their muscles.

One of the most extensive investigations of pre-exercise carbohydrate and performance was carried out in the Human Performance Laboratory at the University of Birmingham (1,20,21,22,31). In their systematic research series, the investigators showed that time trial performance was not affected by pre-exercise carbohydrate ingestion regardless of carbohydrate timing (15, 45, 75 min pre-exercise), load (0, 25,75, 200g dextrose), type (trehalose, galactose, dextrose), or exercise intensity (40%, 65%, 80% VO_2max). Unless otherwise specified, the protocol of each study was the same: a 75g

carbohydrate load was given to 8-10 endurance trained males 45 min prior to a 20 min submaximal preload ride (65-75% VO_2max), followed by a time trial. The lack of agreement in the scientific literature regarding pre-exercise carbohydrate ingestion has often been attributed to the wide variation in study designs. By standardizing the research protocol, researchers at the University of Birmingham were able to compare results between trials. However, if some aspect of their standard design (e.g. cycling protocol) prevented a performance impairment from being detected, it would have been prevented in all the studies.

Glucose and Insulin Kinetics

After Eating

Normal fasting blood glucose is 3.9-5.5 mM. When the concentration is elevated following a carbohydrate meal, the pancreas secretes insulin, a hormone stimulating cellular uptake of glucose. In adipose and skeletal muscle cells, insulin causes translocation of GLUT4, a glucose transport protein, from intracellular pools to the plasma membrane. High insulin levels also suppress lipolysis and hepatic glucose production. Plasma insulin peaks 30-60 min after a meal, depending on the amount and structure of the carbohydrate consumed (3,34). There appears to be a dose-response relationship between the carbohydrate load and the amount of insulin secreted (35,39).

During Exercise

Although exercise suppresses pancreatic insulin production, cellular uptake of glucose increases during exercise because muscle contractions also cause GLUT4 translocation (39). In the fasted state, hepatic glycogenolysis and gluconeogenesis maintain plasma glucose levels during exercise until glycogen stores are exhausted (4).

Eating and Exercising: Rebound Hypoglycemia

If exercise is undertaken when insulin levels are still elevated from a pre-exercise carbohydrate meal, some individuals may experience a rapid decline in plasma glucose to baseline levels or below during the first 10-30 min of exercise. This phenomenon, known as rebound hypoglycemia, does not occur in everyone, but appears to be more common at moderate intensities and in trained individuals (39). Although high insulin sensitivity, characteristic of trained individuals, would seem to be a logical predisposing factor for rebound hypoglycemia, Jentjens et al. (22) were unable to find an association between the two. Due to the actions of counter-regulatory hormones, rebound hypoglycemia is short-lived; plasma glucose concentration is usually normalized within the 20-30 min.

It is important to note that the term hypoglycemia is not associated with a specific plasma glucose concentration. In studies it has been defined as 2.5 mM (29), 3.5 mM (16,17), or anything below an individual's resting blood glucose concentration (39). Rebound hypoglycemia is not a reflection of depleted glycogen stores; it is a consequence of increased glucose uptake and suppressed hepatic glucose production caused by residual insulin (27).

Exercise and insulin appear to have a synergistic interaction, markedly increasing the rate of cellular glucose uptake, which can lead to the sharp decline in plasma glucose concentration at the start of exercise. One study showed that glucose uptake was 55% higher than predicted if the actions of insulin and exercise were purely additive; together they increased leg glucose uptake 3-6 times more than one or the other alone (7). In the study, DeFronzo et al. (7) first examined the effect of insulin by itself on glucose uptake. Ten subjects were given a continuous infusion of insulin to raise the plasma concentration by ~100μU/ml. Since that dose completely suppresses splanchnic glucose production, the rate

of glucose uptake could be determined based on the amount of glucose provided by a glucose infusion pump. Next, the effect of exercise alone on peripheral glucose uptake was measured. Subjects cycled at 40% VO_2max for 30 min while glucose concentrations were monitored using catheters inserted into the hepatic vein, femoral artery, and femoral vein. Then, the insulin infusion and exercise of the first two protocols were combined. The authors concluded that increased extraction (via GLUT4 translocation by both insulin and muscle contraction) and increased blood flow (newly opened capillary beds exposing nonperfused muscle cells to insulin) caused the significant increase in glucose uptake.

In the fasted state, a normal feed-forward activation of glucose production by the liver provides for the initial demands of exercise. When this mechanism is blunted by insulin, short term hypoglycemia can ensue. In an investigation by Marmy-Conus et al. (27), six trained men were given 75g of dextrose 30 min before an hour-long cycling bout at 71% VO_2max. At the onset of exercise, hepatic glucose production decreased in the carbohydrate group from ~12.5 μmol/kg to 1.0 ± 0.7 μmol/kg. Ten min into exercise, hepatic glucose production of the carbohydrate group began increasing. However, during the entire 60 min of exercise, hepatic glucose production never exceeded pre-exercise levels and was reduced by 62% compared to the placebo group.

Classic symptoms of hypoglycemia (e.g., headache, nausea, weakness, disorientation) have sometimes been reported by subjects experiencing rebound hypoglycemia (39), while others have been asymptomatic (20,21,30,35). In their study on multiple pre-exercise carbohydrate feedings, Short et al. (39) reported "it was of interest that 2-3 of the subjects in each CHO treatment indicated that their legs felt somewhat 'heavy' or 'fatigued' during the first 30 min of exercise. These individuals gradually regained their feeling of well-being and

were able to complete the entire ride." The cyclists' blood glucose concentrations at this time were 2.1-2.9 mM, some of the lowest reported in pre-exercise carbohydrate literature. Although these findings suggest rebound hypoglycemia could temporarily impair endurance performance, no studies to date have been able to objectively demonstrate it.

Type of Carbohydrate

Structure and Digestion

Low molecular mass carbohydrates such as sucrose, dextrose, and glucose polymers are often used in carbohydrate supplements because of their high digestibility and water solubility (41). However, a large insulin release due to rapid entry of glucose into the blood may predispose an individual to hypoglycemia at the beginning of exercise. Because of this, alternative carbohydrate types have been proposed as a way to moderate the metabolic response by providing a more slowly digested source of glucose (3).

The rate of starch digestion is largely determined by the proportion of amylopectin and amylose it contains (3,12,32). Amylopectin is a polysaccharide with a highly branched chemical structure, making it more susceptible to enzymatic degradation. Amylose, on the other hand, is more slowly digested because its linear configuration limits enzymatic access and permits hydrogen bonding between molecules (2,26). Most cereal starches are 80% amylopectin and 20% amylose (12).

Corn Starch

Guezennec et al. (14) tested corn starch as a pre-exercise carbohydrate, but found it offered no advantage over dextrose. Six male subjects were given isocaloric amounts (~380kcal) of either dextrose or corn starch one hour before exercising at 60% VO_2max for 120 min. Exogenous carbohydrate oxidation and glycemic/insulinemic responses during

exercise were the same for both carbohydrates. In a later study by Seewi et al. (34), 26 healthy volunteers consumed soups containing 30g of pea starch (34% amylose) or corn starch (24% amylose). The results showed that a minimum of 30% amylose content is necessary to attenuate postprandial insulin responses.

Modified High Amylose Corn Starch

Amylomaize starch, the product of a unique corn hybrid, has a 70% amylose content and could be an excellent pre-exercise carbohydrate source. Granfeldt et al. (12) prepared arepas (corn bread cakes) with either ordinary corn flour (25% amylose) or high amylose corn flour. When nine subjects consumed the high amylose arepas, mean glucose response was reduced by 57% and insulin response by 42%. Two different trials were conducted (meal mass matched for total starch or matched for digestible starch), so the researchers were able to conclude that the favorably low insulinemic response was caused by a slower rate of enzymatic digestion, not a lesser amount of potentially available starch. This brings up an important point; unmodified high amylose corn starch is not regularly used in carbohydrate supplements because it is only 68% digestible (13) and insoluble in cold water (41). However, high amylose corn starch can be partially hydrolyzed with hydrochloric acid and alcohol, producing amylomaize-7, which is 92% digestible and readily soluble in water (41,38).

Behall et al. (2) fed 25 subjects carbohydrate at 1g/kg body mass in the form of crackers made from regular (30% amylose) or high amylose corn starch. Glucose levels peaked lower and fluctuated less from fasting levels in response to the high amylose crackers. Consequently, the insulin levels were significantly lower in the hour after ingestion. The researchers confirmed that the two starches were equally digested because

total glucose observed under the curves were equivalent. In addition, Severijnen et al. (36) found that high amylose corn starch has good long-term stability and a gastric emptying rate comparable to maltodextrin. Because of these favorable chemical and physical properties, amylomaize-7 may be a better pre-exercise carbohydrate than rapidly absorbed carbohydrates like dextrose.

Cycling Protocols

Time to Exhaustion vs. Time Trial

Within-subject variability is inherent in exercise performance. This variability can be further accentuated when subjects are exposed to unfamiliar surroundings, spectators, muscle biopsies, and blood draws. Since carbohydrate ingestion before exercise has also been shown to have an unpredictable effect on exercise performance, selection of an appropriately sensitive performance measure is crucial for these trials. Time to exhaustion tests have been frequently used to study the effects carbohydrate supplementation, but research by Jeukendrup et al. (23) suggests these may not be the most reliable. Researchers compared the reproducibility of three different 60 min protocols (A: continuous ride at 75% VO_2max to exhaustion, B: 45 min preload at 75% VO_2max followed by a 15 min time trial, C: workload equivalent to one hour at ~75% VO_2max to be completed as fast as possible). Thirty well-trained subjects were divided into groups of ten, and assigned one protocol to complete six times. The exercise to exhaustion protocol displayed poor reproducibility – a coefficient of variation of 26.6%. In contrast, protocols B and C had coefficients of variation of ~3.5%. Work by McConell et al. (28) confirmed these findings, but found time to exhaustion trials for subjects cycling at approximately the same intensity was only 12.1%.

Open-ended protocols may place more emphasis on psychological factors. Indeed, Foster et al. (9) noted that compared to a short, fixed duration time trial, the point of exhaustion during an open-ended ride was "much more subjectively determined and was usually manifested as unwillingness on the part of the subject to continue the ride, rather than by a physical inability to turn the pedals." It appears a protocol with a fixed endpoint is less influenced by motivation or boredom, has a smaller coefficient of variation, and may be a more sensitive test for pre-exercise carbohydrate ingestion trials.

A mixed design (steady preload followed by a time trial) is a useful protocol for supplementation studies because it allows time to monitor physiological responses and then test exercise performance. Sewell et al. (37) showed that reproducibility of this kind of design is reasonably good: a coefficient of variation of 6.3% for recreationally active subjects. Furthermore, since the time trial takes place late in the exercise, endogenous fuel sources will be reduced and the benefits a nutritional supplementation on endurance performance may be most apparent.

Testing performance during rebound hypoglycemia

Protocols with lengthy submaximal pre-load rides are inappropriate for testing the effects of rebound hypoglycemia because the hypoglycemia may be resolved by the time the performance task is begun. After a pre-exercise carbohydrate load, blood glucose levels have been shown to decline significantly 15 min into exercise, but then return to baseline by 30 min (11,15,17). If a time trial is undertaken 60 min into exercise, when blood glucose has already been normalized, it is unlikely that any consequence of the rebound hypoglycemia would be detected. Thus, a more appropriate protocol for a pre-exercise carbohydrate study

would include a high intensity time trial introduced directly during the hypoglycemic period (15-30 min into exercise).

Conclusion

Conflicting results regarding the effect of pre-exercise carbohydrate ingestion on performance (9,11,20) are likely due to differences in study design such as the carbohydrate type, amount, or timing; exercise intensity or duration; subject training status; and performance testing method. Rebound hypoglycemia, a consequence of hyperinsulemia at the start of exercise, is commonly observed after pre-exercise carbohydrate ingestion, but has never been proven to impair performance. However, no study has initiated a short, high intensity performance time trial during the hypoglycemic period (15-30 min into exercise). Since elevated insulin at the start of exercise is the cause of rebound hypoglycemia, a slowly digestible carbohydrate such as amylomaize-7, which evokes a smaller insulin release, may prevent rebound hypoglycemia from occurring in the first place.

CHAPTER 3. METHODS

Subjects

Ten male trained cyclists and triathletes 18-35 y with a minimum VO_2max of 50 ml/kg/min were recruited for the study. Institutional Review Board approval and written informed consent were obtained before the trials.

Exercise Protocol

Each subject reported to the laboratory four times for the study. All visits were conducted after a 12 hour overnight fast and separated by approximately one week. They were told the purpose of the study was to observe how carbohydrate consumption affects endurance performance. During the first visit, an incremental test to exhaustion on a Load Excaliber cycle ergometer (Groningen, Netherlands) was used to determine VO_2max and Wmax. The max test was designed to be completed in 10-14 min and was conducted according to ACSM guidelines (8). The initial workload of 100 W was increased by either 50 W or 25 W every two min until the subject reached volitional exhaustion. Revolutions per min were recorded at each stage to determine the subject's preferred cycling cadence.

The following three visits were identical, except for the randomly assigned experimental beverage. When the subject arrived, body mass and resting heart rate/blood pressure were obtained. A flexible catheter was inserted into an antecubital vein and was kept patent with sterile isotonic saline after each blood draw. The subject ingested one of three carbohydrate solutions before resting quietly for 45 min prior to exercise.

For the first 15 min of the cycling bout, the Lode Excaliber was set to hyperbolical mode (RPM independent) at a workload equivalent to 60% Wmax. At min 15, the cycle ergometer was switched into linear mode (RPM dependent) for the performance time trial: a

workload equivalent to 15 min at 80% Wmax. During the time trial, the subject was not allowed to see the clock and did not receive any verbal encouragement other than a prompt at 50% completion. Thirty min of seated recovery followed the performance time trial.

Beverage Contents

The beverages provided 1g/kg body mass of either dextrose or amylomaize-7 starch dissolved in 400ml water. The placebo contained only water. The study was double-blind; subjects were told the beverages were different sports drinks. All three drinks had added sugar-free orange Kool-Aid flavoring and were given to the subject in a lidded, opaque cup. An additional 100ml chaser of water was used to rinse residual carbohydrate from the cup.

To prepare the amylomaize-7 starch, one kg of high amylose corn starch (Hylon VII – National Starch) was soaked in 1 L 100% ethanol and 100 ml hydrochloric acid for three days as described in the procedure of Sharp et al. (38).

Physiological and Psychological Measurements

Blood samples were collected pre-ingestion, pre-exercise, immediately before and after the performance time trial, and 30 min into recovery. The samples were kept on ice, centrifuged at 3,000 RPMs for 20 min, and stored at -80° C until analyzed. Serum glucose (Sigma HK assay, Beckman Spectrophotometer), insulin (Calbiotech ELISA, Fluostar plate reader), and lactate (Sigma colorimetric assay, Beckman Spectrophotometer) concentrations were measured in duplicate. Two psychological affect scales were administered at the time of the first four blood draws: a feeling scale ranging from -5 (very bad) to +5 (very good) and an arousal scale ranging from 1 (low arousal) to 6 (high arousal). The subjects' rating of perceived exertion (from 6 to 20) was assessed before and after the performance time trial. A

hypoglycemia symptom questionnaire from Hepburn et al. (16) was completed at baseline and immediately after the time trial.

Statistical Analysis

Serum variables were analyzed by two-way repeated measures analysis of variance (ANOVA) (time and treatment) using SigmaStat statistical software. Performance was analyzed by one-way repeated measures ANOVA; treatment order was also used as a covariate to test for a significant effect of trial order. Holm-Sidak post hoc tests were used to locate significant mean differences. Psychological data were averaged because no treatment differences were observed. All data are reported as mean ± SEM, $n = 10$. Significant differences were determined at $p < 0.05$.

Dietary and Exercise Control

Subjects were asked to keep a three d food log prior to the first carbohydrate ingestion trial and to duplicate it before the following two visits. They were asked to refrain from alcoholic beverages and strenuous exercise in the 24 h before each trial. Maintenance of consistent training at their usual volume was encouraged throughout the study.

CHAPTER 4. RESULTS

Subject Characteristics

Subjects completed the four lab visits with a minimum of four and maximum of nine d between each. Physical characteristics of the subjects are listed in Table 1.

Table 1. Subject characteristics

Age	21 ± 1 y
Height	177 ± 2 cm
Body Mass	71.8 ± 3.0 kg
Body Mass Index	22.9 ± 0.5 kg/m^2
VO_2max	64.6 ± 1.8 ml/kg/min
Wmax	366 ± 16 W

Values are mean ± SEM (n = 10)

The self-reported dietary intake for three d prior to each experimental trial is listed in Table 2. The percentage of energy coming from carbohydrate was slightly lower than the Acceptable Macronutrient Distribution Range recommended by the Institute of Medicine for 19 to 30 y males (45-60%, 10-35%, 20-35% for carbohydrate, protein, and fat, respectively) (18). This was primarily due to one subject who reported consuming a low-carbohydrate diet (14% of total kcal). Statistical analyses were conducted with and without this subject's data. All conclusions regarding significant differences remained the same; therefore, his data was retained.

Table 2. Self-reported mean dietary intake for the three d prior to each experimental trial

Macronutrient	***Daily Intake***	***% Total Kcal***
Energy	2810 ± 136 kcal	
Carbohydrate	292 ± 29 g	43%
Protein	165 ± 21 g	24%
Fat	101 ± 10 g	33%

Values are means ± SEM (n = 10)

Time Trial Performance

Time required to complete the workload of the performance trial was not significantly different between treatments ($p=0.21$). However, in comparison to the placebo trial (PL), there was a tendency for subjects to complete the dextrose trial (DEX) slower and the starch trial (AMY-7) faster (Figure 1). Analysis of performance with treatment order as a covariate revealed no significant effect of order.

Metabolic Responses

There was a significant treatment-by-time interaction for serum glucose concentration ($p < 0.001$). The pre-exercise and 30 min post-exercise concentrations within DEX were significantly higher than the pre-ingestion baseline. Compared to PL, glucose concentration in DEX was significantly higher pre-exercise and 30 min post-TT, but significantly lower immediately post-TT ($p < 0.05$). Glucose concentration in AMY-7 was not different than PL at any time point ($p < 0.05$) (Figure 2).

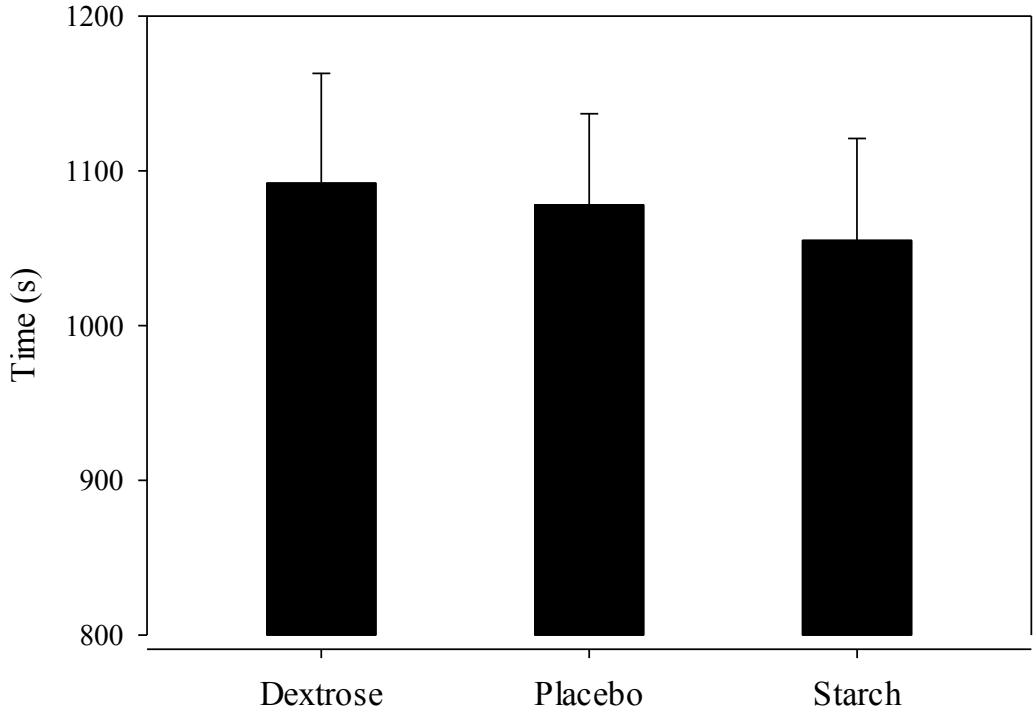

Figure 1. Time required to complete the workload of the cycling performance trial (mean ± SEM, n = 10). Time trial performance was not significantly different between treatments (p = 0.209).

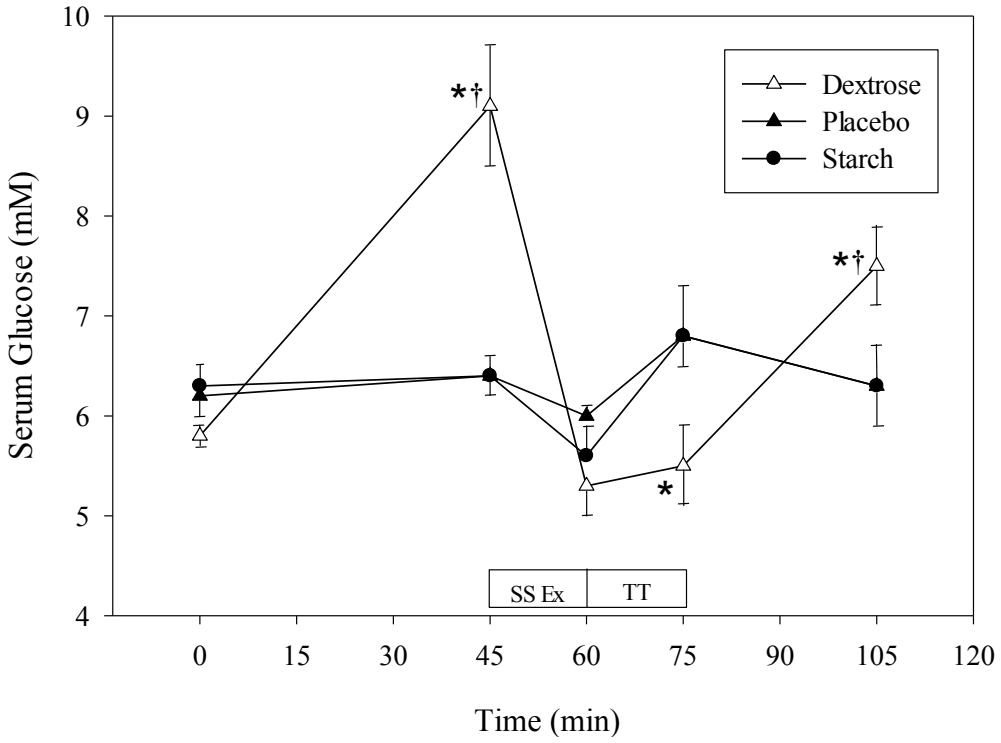

Figure 2. Change in serum glucose concentration after pre-exercise carbohydrate ingestion (mean ± SEM, n = 10). There was a main effect of time ($p < 0.001$) and treatment-by-time interaction ($p < 0.001$), but no main effect of treatment ($p = 0.164$). * indicates a significant difference between treatments ($p < 0.05$); † indicates a significant difference from baseline ($p < 0.05$).

Unsurprisingly, the pre-exercise serum insulin concentration was significantly higher in DEX, resulting in a main effect for treatment, time, and treatment-by-time interaction ($p<0.001$). Insulin responses (increase from baseline) ranged from 0.8 μIU/ml to 33.4 μIU/ml for individual subjects in the 45 min following dextrose ingestion. Peak insulin response for AMY-7 compared to DEX was 1.5 ± 0.1 μIU/ml and 11.7 ± 3.2 μIU/ml, respectively. By pre-TT, however, DEX was not different than PL (2.2 ± 0.3 μIU/ml and 1.3 ± 0.0 μIU/ml, respectively) ($p = 0.907$). Serum insulin concentrations in AMY-7 were no different from PL at any time point ($p < 0.05$) (Figure 3).

There was a significant effect of time ($p < 0.001$) on serum lactate concentration, as well as a treatment-by-time interaction ($p = 0.008$). Samples taken during exercise (pre-TT and post-TT) were significantly higher than non-exercising time points ($p < 0.05$). Lactate concentration in DEX was significantly higher than PL at pre-TT (5.6mM and 4.5mM, respectively) ($p < 0.05$). In AMY-7, lactate concentration was 10% higher than both DEX and PL post-TT ($p <0.05$) (Figure 4).

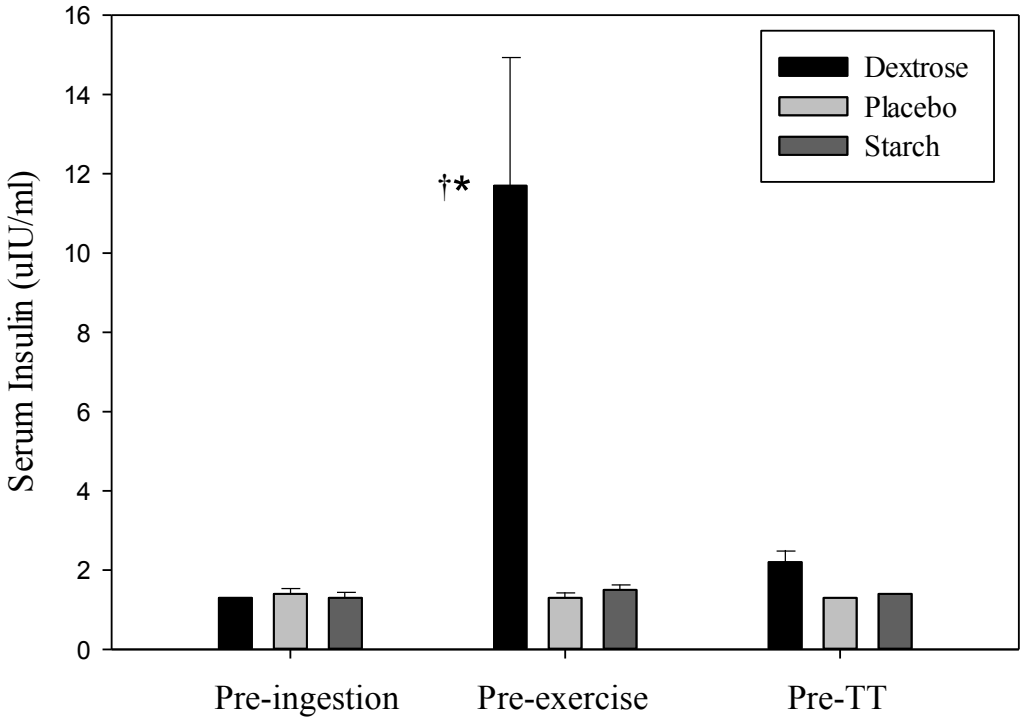

Figure 3. Change in serum insulin concentration after pre-exercise carbohydrate ingestion (mean ± SEM, n = 10). There were main effects of treatment ($p < 0.001$) and time ($p < 0.001$) and treatment-by-time interaction ($p < 0.001$). * indicates a significant difference between treatments ($p < 0.001$); † indicates a significant difference from all other time points ($p < 0.001$).

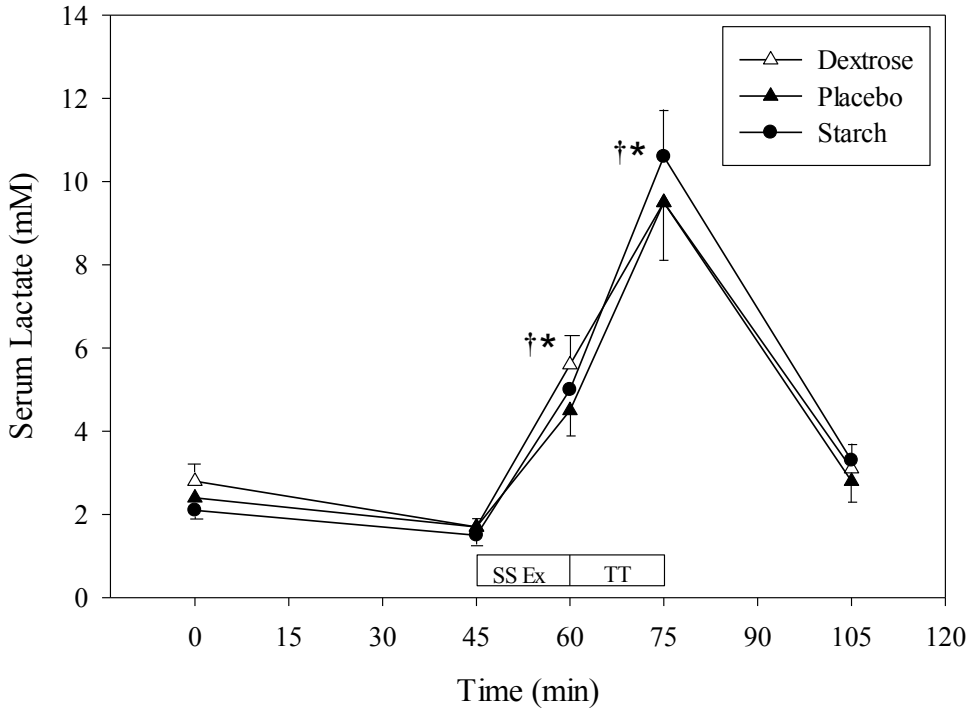

Figure 4. Change in serum lactate concentration after pre-exercise carbohydrate ingestion (mean ± SEM, n = 10). There was a main effect of time ($p < 0.001$) and trial-by-time interaction ($p = 0.008$), but no main effect of treatment ($p = 0.267$). * indicates a significant difference from placebo ($p < 0.05$); † indicates a significant difference from baseline ($p < 0.05$).

Psychological Indices

There were no differences in affect scale ratings between the treatments. Rating of perceived exertion was higher post-TT than pre-TT (Table 3).

Table 3. Psychological ratings

	Pre-ingestion	*Pre-exercise*	*Pre-TT*	*Post-TT*
Feeling				
Dextrose	3 ± 1	3 ± 1	3 ± 1	1 ± 1
Placebo	3 ± 0	3 ± 0	2 ± 0	1 ± 1
Starch	3 ± 0	3 ± 0	3 ± 0	1 ± 0
Arousal				
Dextrose	3 ± 0	3 ± 0	4 ± 0	4 ± 0
Placebo	2 ± 0	3 ± 0	4 ± 0	5 ± 0
Starch	3 ± 0	3 ± 0	4 ± 0	4 ± 0
RPE				
Dextrose			12 ± 0	17 ± 0
Placebo			12 ± 1	17 ± 0
Starch			12 ± 0	17 ± 1

Values are means ± SEM (n = 10)

For five common hypoglycemia symptoms, mean ratings (on a scale of 1 to 7) did not increase more than one point between baseline and post-TT nor exceed a rating of three at any time for any treatment. (Table 4).

Table 4. Hypoglycemia questionnaire responses

	Weakness	*Nausea*	*Headache*	*Dizziness*	*Inability to Concentrate*
Dextrose					
Baseline	2 ± 0	1 ± 0	1 ± 0	1 ± 0	1 ± 0
Post-TT	2 ± 0	1 ± 0	1 ± 0	1 ± 0	2 ± 0
Placebo					
Baseline	2 ± 0	1 ± 0	1 ± 0	1 ± 0	1 ± 0
Post-TT	3 ± 0	1 ± 0	1 ± 0	2 ± 0	1 ± 0
Starch					
Baseline	2 ± 0	1 ± 0	1 ± 0	1 ± 0	1 ± 0
Post-TT	3 ± 0	1 ± 0	1 ± 0	1 ± 0	2 ± 0

Values are means ± SEM (n = 10)

CHAPTER 5. DISCUSSION

As expected, cyclists began exercise hyperglycemic and hyperinsulinemic when they ingested dextrose 45 min prior to cycling. The observed serum glucose and insulin response (3 mM and 10 μIU/ml increases, respectively) were comparable to a previous investigation done in this laboratory with an identical carbohydrate load (24). After 15 min of exercise, the elevated glucose concentration returned to baseline due to enhanced glucose uptake and oxidation. Muscle contractions cause GLUT4 translocation to the cell membrane, facilitating glucose transport (39). Insulin acts synergistically with exercise to further increase cellular glucose uptake (7). Insulin also reduces hepatic glucose production (27), suppresses lipolysis (17), and increases the rate of carbohydrate oxidation (5,11,24). A 75 g pre-exercise dextrose load was shown to inhibit hepatic glucose production 62% during 60 min of exercise at 70% VO_2max (26). During a two hour cycling bout at 65% VO_2max, total carbohydrate oxidation (measured via ^{13}C isotopic ratio) was reportedly 21% higher when 1 g/kg body mass of dextrose was consumed 30 min prior to exercise (24). Carbohydrate oxidation was not directly assessed in the present study, but the significantly elevated serum lactate pre-TT in DEX (5.6 ± 0.7 mM DEX; 4.5 ± 0.6 mM PL), indicates increased glycolytic activity following hyperinsulinemia.

Post-TT glucose concentration was significantly lower in DEX compared to PL and AMY-7 even though insulin was the same for all treatments by pre-TT. The number of insulin receptors still bound or treatment differences in the rate of insulin disappearance during the first 15 min of exercise cannot be ascertained from that single measurement of serum concentration. Insulin's effects persist even after serum concentration has returned to

normal (29). Because it is well established that pancreatic production of insulin dramatically decreases during exercise, post-exercise samples were not analyzed for insulin in the present study (7,11,15,35,39).

Decreased metabolic demand from skeletal muscle and action of the counterregulatory hormones raised the glucose in DEX to above baseline and the other two treatments by 30 min into recovery. Similar overcompensation has been observed 90 min after pre-exercise carbohydrate ingestion even when cyclists continued cycling (70% VO_2max), suggesting glucagon, epinephrine, norepinephrine play primary roles in this occurrence (27).

The serum glucose fluctuations observed in the present study parallel those in reported in other investigations on pre-exercise carbohydrate ingestion and rebound hypoglycemia (9,14,20,30,39). However, glucose concentration did not drop low enough to meet the criteria for hypoglycemia. The mean DEX post-TT concentration, although lower than PL and AMY-7, was not lower than DEX pre-ingestion baseline. The lowest individual glucose concentration observed was 4.0 mM (pre-TT DEX) - still well above 2.5 mM or 3.5 mM cited as diagnostic of hypoglycemia (20,21,35). Several subjects verbally reported dizziness at the conclusion of the DEX TT, but the symptoms were not reflected in the RPE, affect scales, or hypoglycemia questionnaire responses. Short et al. (39) described similar complaints of "heaviness" and "fatigue" from subjects with glucose concentrations 2.1-2.9 mM in the first 30 min of cycling (65-70% VO_2max) after consuming 75g dextrose, but again RPE was not different from PL. Changes in glucose concentration on account of pre-exercise carbohydrate consumption appear to occur with mild to no symptoms (20,21,22,30,35).

Unlike DEX, ingestion of amylomaize-7 starch did not result in serum glucose or insulin concentrations different from baseline or PL at any time point. One could argue that a non-insulin-stimulating starch is simply indigestible. Unmodified high amylose corn starch is indeed only 68% digestible (13), but acid/alcohol modification disrupts the exterior of the starch granule, partially hydrolyzes glycosidic bonds in the interior, and improves digestibility to 92% (10,41). Work by Johannsen and Sharp (24) verified amylomaize-7 serves as a combustible substrate for exercise. During two h of cycling at 65% VO_2max, carbohydrate oxidation was equally elevated after pre-exercise ingestion of either dextrose or amylomaize-7. Likely due to its slow digestion, amylomaize-7 provided a more sustained fuel source; after 90 min, carbohydrate oxidation was higher with amylomaize-7 than dextrose. Unmodified high amylose starch was no different than placebo. Immediately post-TT in the present study, lactate concentration averaged 10% higher in the AMY-7 than the other two treatments (10.6 mM AMY-7; 9.5mM PL/DEX). The elevated concentration may be attributed to the sustained release of amylomaize-7 (supporting carbohydrate oxidation) or simply the higher intensity since the AMY-7 TT tended to be completed the fastest.

The linear structure of amylomaize-7 is the reason for the minimal insulin response. It slows enzymatic digestion, controlling the rate of glucose entry into the blood and reducing the amount of insulin needed (2,3,12). Not only is the insulin peak lowered, the total amount of insulin released is reduced. Granfeldt et al. (12) reported a 42% decrease in the area under the curve for insulin when subjects consumed cornbread cakes made from high amylose corn starch instead of ordinary corn meal. In the present study, the mean peak insulin response was 70% lower in AMY-7 compared to DEX. Although digested slowly in the small intestine, gastric emptying for amylomaize-7 has been reported to be similar to other

maltodextrins (36). Confirming previous findings (24), subjects did not experience bloating, nausea, or abdominal cramps during exercise after ingestion of amylomaize-7.

Despite the metabolic differences between treatments, endurance performance in the present study was unaffected by pre-exercise carbohydrate consumption. There was a tendency for subjects to complete AMY-7 24 s faster than PL and 37 s faster than DEX. However, due to variability in the performance trial times (inherent characteristic of human performance), the differences did not reach significance. Based on the statistical power, a sample size of 49 would be needed for the difference between to reach significance.

Rather than doing a TT fixed at 80% Wmax, subjects were allowed to work at a self-selected intensity to better replicate real-world athletic competition. Actual mean intensities of the TT were 66%, 67%, and 68% Wmax for the dextrose, placebo, and starch trials, respectively (range: 59% to 74% Wmax). In a study where cyclists worked at a higher intensity for a longer TT (~75% Wmax for ~40 min), the outcome was the same: performance was not significantly affected by pre-exercise carbohydrate ingestion, but the dextrose trial (~1 g/kg) averaged 43 s slower than placebo (43:07 ± 1:04 vs 42:34 ± 0:48) (20).

Very different cycling protocols were employed by the studies with conflicting conclusions regarding the effect of pre-exercise ingestion on endurance performance. In those finding carbohydrate ingestion to be detrimental (9,25), cyclists rode at a higher intensity to exhaustion (80% and 85% VO_2max). At near-maximal intensities, muscle substrate stores (i.e. glycogen) become the predominant fuel source (33). Elevated catecholamine levels stimulate glycolysis and inhibit lipolysis. Hyperinsulinemia further increases carbohydrate oxidation, potentially leading to earlier glycogen depletion (11,40).

Due to the brevity of the cycling bout in the present study, glycogen stores were not a limiting factor in performance.

A TT preceded by a 90 min submaximal pre-load ride was used in the study finding pre-exercise carbohydrate improved performance (11). Investigators concluded the exogenous glucose provided additional substrate for oxidation late in exercise. Rebound hypoglycemia was not a factor in the TT; serum glucose and insulin concentrations had been the same for the dextrose and placebo trials for more than 75 min prior to the TT.

Another investigation also found pre-exercise carbohydrate ingestion improved performance (40), but the carbohydrate dose given was five times greater than that of the previously mentioned studies (5 g/kg vs ~1 g/kg). Such variations in carbohydrate type, amount, or timing or in the method of performance assessment make it difficult to reach conclusions regarding pre-exercise carbohydrate and performance. In the current study, pre-exercise carbohydrate consumption did not offer a significant advantage or disadvantage during a brief early-exercise TT, compared to water alone.

In conclusion, rebound hypoglycemia is a consequence of increased glucose uptake and suppressed hepatic glucose production during exercise caused by residual insulin from pre-exercise carbohydrate ingestion (27). The present study was designed to maximize the chance of rebound hypoglycemia (i.e. carbohydrate dose, timing, exercise intensity) and to test performance during that period (brief TT early in exercise). Yet, clinical hypoglycemia was not observed and performance impairment could not be demonstrated. Pre-exercise ingestion of amylomaize-7 instead of dextrose results in more stable serum glucose and insulin concentrations, but does not offer an additional performance advantage.

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APPENDIX

Complete Data Tables

Key

Treatment

Name	*Treatment*
A	Dextrose
B	Placebo
C	Starch

Time

Number	*Name*	*Time from Ingestion*
1	Pre-ingestion	0
2	Pre-exercise	45
3	Pre-TT	60
4	Post-TT	75
5	30min post-exercise	105

Table I. Subject characteristics

Subject	*Height (cm)*	*Weight (kg)*	*BMI (kg/m^2)*	*Age (y)*
S1	165.5	58.4	21.3	21
S2	190.5	80.5	22.2	21
S3	172.5	70.6	23.7	22
S4	169.0	66.6	23.3	19
S5	183.0	76.1	22.7	20
S6	185.0	90.0	26.3	24
S7	177.0	74.0	23.6	21
S8	172.5	69.6	23.4	21
S9	179.0	72.2	22.5	20
S10	173.0	59.9	20.0	25

Table II. Self-reported dietary intake for the three days prior to each experimental trial

	Kcal	*CHO*	*% CHO*	*PRO*	*% PRO*	*FAT*	*% FAT*
S1	2448	271	40%	185	27%	70	23%
S2	2612	336	49%	113	17%	81	27%
S3	2427	251	37%	106	15%	44	14%
S4	3054	314	46%	183	27%	117	38%
S5	2926	405	59%	97	14%	106	35%
S6	2783	236	34%	169	25%	126	42%
S7	3756	275	40%	296	43%	152	50%
S8	2303	95	14%	256	37%	98	32%
S9	2676	329	48%	104	15%	107	35%
S10	3120	406	59%	145	21%	105	34%

Table III. Cycling data

Subject	*VO2max (ml/kg/min)*	*Wmax (W)*	*RPMs at 80%*	*L-Factor*	*TT KJ*	*Trial Order*
S1	66.4	300	108	0.0206	216	CBA
S2	68	400	108	0.0274	288	BAC
S3		325	93	0.0301	234	ACB
S4	68.2	350	110	0.0231	252	BAC
S5	63.6	400	111	0.026	288	CAB
S6	50.9	425	75	0.0604	306	CBA
S7	71.9	425	94	0.0385	306	CAB
S8	64.7	350	100	0.028	252	BAC
S9	63.9	400	100	0.032	288	CBA
S10	64	287.5	106	0.0205	207	ACB

Table IV. Time Trial Performance

Subject	*Treatment*	*50% Completion (s)*	*100% Completion (s)*
S1	A	529	1083
S1	B	497	1018
S1	C	513	1006
S2	A	531	1035
S2	B	537	1091
S2	C	506	1069
S3	A	535	1130
S3	B	572	1163
S3	C	548	1097
S4	A	590	1156
S4	B	530	1132
S4	C	586	1176
S5	A	565	1147
S5	B	559	1146
S5	C	545	1100
S6	A	545	1083
S6	B	551	1111
S6	C	528	1113
S7	A	511	1032
S7	B	517	1032
S7	C	493	1013
S8	A	493	1006
S8	B	478	999
S8	C	500	1017
S9	A	589	1226
S9	B	521	1073
S9	C	476	973
S10	A	491	1021
S10	B	485	1017
S10	C	475	985

Table V. Serum concentration glucose, insulin, and lactate

Subject	*Treatment*	*Time*	*Glucose (mM)*	*Insulin (μIU/ml)*	*Lactate (mM)*
S1	A	1	6.1	1.2	1.5
S1	A	2	10.5	15.0	2.1
S1	A	3	4.9	2.0	6.2
S1	A	4	4.7		9.6
S1	A	5	6.0		2.5
S1	B	1	6.7	1.4	0.9
S1	B	2	6.5	1.1	1.4
S1	B	3	6.2	1.1	3.4
S1	B	4	6.5		8.9
S1	B	5	5.5		2.3
S1	C	1	5.7	1.2	2.4
S1	C	2	5.5	1.4	1.2
S1	C	3	4.9	1.1	3.4
S1	C	4	7.2		10.3
S1	C	5	5.2		3.4
S2	A	1	6.0	1.0	1.4
S2	A	2	6.7	3.1	1.8
S2	A	3	5.9	1.5	2.3
S2	A	4	6.8		4.0
S2	A	5	5.7		1.7
S2	B	1	5.5	1.1	1.9
S2	B	2	6.9	1.1	2.2
S2	B	3	6.1	1.0	1.9
S2	B	4	5.4		3.2
S2	B	5	5.6		1.4
S2	C	1	7.4	1.2	1.2
S2	C	2	5.8	1.1	1.3
S2	C	3	6.7	1.4	3.1
S2	C	4	6.1		3.8
S2	C	5	6.2		1.4
S3	A	1	5.0	1.4	3.7
S3	A	2	9.5	11.7	2.1
S3	A	3	5.3	1.7	8.1
S3	A	4	5.1		10.8
S3	A	5	7.1		3.6
S3	B	1	6.3	1.4	1.9
S3	B	2	6.2	1.4	3.2
S3	B	3	6.3	1.4	4.4
S3	B	4	7.8		6.8
S3	B	5	6.8		1.9
S3	C	1	5.9	1.3	2.9
S3	C	2	6.1	2.0	2.0
S3	C	3	5.4	1.4	6.9
S3	C	4	6.6		9.4
S3	C	5	6.5		3.5
S4	A	1	5.8	1.4	3.4
S4	A	2	9.4	2.2	2.4

Subject	*Treatment*	*Time*	*Glucose (mM)*	*Insulin (μIU/ml)*	*Lactate (mM)*
S4	A	3	6.3	1.3	4.1
S4	A	4	7.9		10.6
S4	A	5	6.6		2.7
S4	B	1	5.4	1.3	3.7
S4	B	2	5.6	1.2	1.2
S4	B	3	5.5	1.3	4.3
S4	B	4	8.1		14.2
S4	B	5	5.2		4.3
S4	C	1	6.8	1.4	1.6
S4	C	2	7.2	1.5	0.9
S4	C	3	4.6	1.4	4.1
S4	C	4	6.2		10.6
S4	C	5	5.7		3.6
S5	A	1	5.7	1.4	4.2
S5	A	2	7.1	4.5	1.5
S5	A	3	4.9	1.9	6.4
S5	A	4	4.7		11.9
S5	A	5	8.4		4.5
S5	B	1	6.4	1.6	3.3
S5	B	2	6.9	1.3	1.4
S5	B	3	6.2	1.3	6.9
S5	B	4	7.6		13.5
S5	B	5	7.0		3.5
S5	C	1	6.1	1.4	2.5
S5	C	2	6.2	1.4	1.2
S5	C	3	6.2	1.2	6.0
S5	C	4	7.5		12.7
S5	C	5	7.7		3.8
S6	A	1	6.5	1.2	1.5
S6	A	2	6.0	2.1	2.2
S6	A	3	5.1	1.6	7.6
S6	A	4	5.3		10.6
S6	A	5	7.8		3.2
S6	B	1	6.8	1.2	1.2
S6	B	2	6.9	1.3	1.0
S6	B	3	5.9	1.3	6.1
S6	B	4	5.2		9.1
S6	B	5	5.5		1.9
S6	C	1	6.4	1.4	1.6
S6	C	2	6.7	1.4	1.8
S6	C	3	5.7	1.3	8.1
S6	C	4	5.6		10.8
S6	C	5	5.8		2.9
S7	A	1	5.2	1.4	2.2
S7	A	2	8.5	12.8	1.4
S7	A	3	5.0	1.6	2.8
S7	A	4	5.5		7.3
S7	A	5	7.9		2.2
S7	B	1	5.7	1.5	1.9

Subject	*Treatment*	*Time*	*Glucose (mM)*	*Insulin (μIU/ml)*	*Lactate (mM)*
S7	B	2	5.6	1.4	1.3
S7	B	3	5.7	1.4	2.1
S7	B	4	5.7		5.4
S7	B	5	5.9		2.1
S7	C	1	5.4	1.4	1.4
S7	C	2	5.6	1.6	1.4
S7	C	3	4.8	1.4	2.8
S7	C	4	7.4		8.6
S7	C	5	6.8		2.5
S8	A	1	5.8	1.4	4.5
S8	A	2	10.9	34.8	1.5
S8	A	3	4.0	2.1	9.2
S8	A	4	5.8		16.0
S8	A	5	7.3		6.3
S8	B	1	6.3	1.4	4.9
S8	B	2	6.3	1.4	1.8
S8	B	3	5.8	1.3	8.3
S8	B	4	9.8		17.5
S8	B	5	9.6		6.1
S8	C	1	6.2	1.4	2.4
S8	C	2	7.5	1.4	1.4
S8	C	3	5.1	1.4	8.5
S8	C	4	8.4		17.7
S8	C	5	7.4		6.6
S9	A	1	5.8	1.2	2.1
S9	A	2	12.1	11.5	1.5
S9	A	3	6.8	4.8	4.5
S9	A	4	4.3		5.1
S9	A	5	8.9		1.8
S9	B	1	6.5	1.2	2.5
S9	B	2	6.3	1.2	1.7
S9	B	3	5.6	1.4	3.3
S9	B	4	5.7		7.2
S9	B	5	5.8		2.1
S9	C	1	6.8	1.0	2.5
S9	C	2	6.7	1.3	2.1
S9	C	3	6.2	1.4	3.9
S9	C	4	6.3		11.8
S9	C	5	6.0		3.0
S10	A	1	6.3	1.5	3.1
S10	A	2	10.5	19.6	2.1
S10	A	3	4.6	3.4	4.5
S10	A	4	4.5		7.6
S10	A	5	9.6		2.8
S10	B	1	6.3	1.6	1.4
S10	B	2	6.5	1.6	1.2
S10	B	3	6.7	1.5	3.9
S10	B	4	6.3		9.3
S10	B	5	6.0		2.3

Subject	*Treatment*	*Time*	*Glucose (mM)*	*Insulin (μIU/ml)*	*Lactate (mM)*
S10	C	1	6.3	1.6	2.8
S10	C	2	7.0	1.8	1.4
S10	C	3	6.7	1.6	3.5
S10	C	4	6.5		10.6
S10	C	5	5.9		2.4

Table VI. Psychological scale responses

Subject	*Treatment*	*Time*	*Affect Scale*	*Feeling Scales*	*Rating of Perceived Exertion*
S1	A	1	1	0	
S1	A	2	1	0	
S1	A	3	1	-1	13
S1	A	4	2	0	16
S1	B	1	2	1	
S1	B	2	2	1	
S1	B	3	2	1	10
S1	B	4	3	1	17
S1	C	1	2	1	
S1	C	2	2	2	
S1	C	3	2	2	10
S1	C	4	4	3	16
S2	A	1	1	1	
S2	A	2	2	2	
S2	A	3	3	3	13
S2	A	4	4	1	18
S2	B	1	1	4	
S2	B	2	2	4	
S2	B	3	3	1	13
S2	B	4	5	-3	17
S2	C	1	2	2	
S2	C	2	3	2	
S2	C	3	3	1	13
S2	C	4	2	-1	20
S3	A	1	3	4	
S3	A	2	1	5	
S3	A	3	4	5	13
S3	A	4	3	3	17
S3	B	1	4	4	
S3	B	2	3	3	
S3	B	3	4	3	13
S3	B	4	5	2	19
S3	C	1	2	4	
S3	C	2	3	3	
S3	C	3	4	2	11
S3	C	4	4	1	16
S4	A	1	1	0	
S4	A	2	1	0	
S4	A	3	3	1	11
S4	A	4	4	-2	17
S4	B	1	2	0	
S4	B	2	3	0	
S4	B	3	4	1	13
S4	B	4	4	-3	17

Subject	*Treatment*	*Time*	*Affect Scale*	*Feeling Scales*	*Rating of Perceived Exertion*
S4	C	1	1	0	
S4	C	2	2	0	
S4	C	3	3	1	14
S4	C	4	4	-2	18
S5	A	1	2	3	
S5	A	2	2	4	
S5	A	3	3	4	8
S5	A	4	1	2	14
S5	B	1	2	4	
S5	B	2	3	4	
S5	B	3	3	4	8
S5	B	4	4	3	15
S5	C	1	2	3	
S5	C	2	2	4	
S5	C	3	3	3	11
S5	C	4	4	2	14
S6	A	1	3	4	
S6	A	2	3	3	
S6	A	3	4	3	13
S6	A	4	6	3	18
S6	B	1	2	3	
S6	B	2	2	3	
S6	B	3	4	3	13
S6	B	4	5	3	17
S6	C	1	2	4	
S6	C	2	2	4	
S6	C	3	5	4	13
S6	C	4	6	2	18
S7	A	1	3	4	
S7	A	2	3	4	
S7	A	3	4	3	12
S7	A	4	4	2	17
S7	B	1	3	3	
S7	B	2	4	3	
S7	B	3	4	3	13
S7	B	4	5	3	16
S7	C	1	1	3	
S7	C	2	3	3	
S7	C	3	4	3	13
S7	C	4	4	-1	16
S8	A	1	5	3	
S8	A	2	5	3	
S8	A	3	5	3	12
S8	A	4	5	-2	17
S8	B	1	4	3	
S8	B	2	4	3	

Subject	*Treatment*	*Time*	*Affect Scale*	*Feeling Scales*	*Rating of Perceived Exertion*
S8	B	3	5	1	12
S8	B	4	6	-2	17
S8	C	1	5	4	
S8	C	2	6	4	
S8	C	3	5	3	13
S8	C	4	5	1	17
S9	A	1	4	4	
S9	A	2	4	4	
S9	A	3	5	4	12
S9	A	4	5	1	17
S9	B	1	1	3	
S9	B	2	3	4	
S9	B	3	4	4	12
S9	B	4	5	3	17
S9	C	1	5	4	
S9	C	2	5	4	
S9	C	3	6	3	12
S9	C	4	6	1	18
S10	A	1	5	3	
S10	A	2	5	3	
S10	A	3	5	3	13
S10	A	4	4	0	16
S10	B	1	2	2	
S10	B	2	2	2	
S10	B	3	3	3	14
S10	B	4	4	1	17
S10	C	1	3	2	
S10	C	2	3	2	
S10	C	3	4	3	12
S10	C	4	5	0	16

Table VII. Hypoglycemia questionnaire responses

Subject	*Treatment*	*Time*	*Weakness*	*Hiccough*	*Bloating*	*Difficulty Speaking*	*Double Vision*	*Yellow Vision*	*Nausea*	*Headache*	*Constipated*	*Itching*	*Back Pain*	*Drowsiness*	*Tremor*	*Dizziness*	*Inability To Concentrate*	*Pounding Heart*	*Feeling Tearful*	*Pain In Legs*	*Shivering*	*Blurred Vision*	*Anxiety*	*Heartburn*	*Abdominal Cramps*	*Tingling Around Mouth*	*Difficulty Breathing*	*Hunger*	*Tiredness*	*Confusion*
S1	**A**	**1**	1	1	1	1	1	1	1	1	1	1	1	3	1	1	1	1	1	3	1	1	1	1	1	1	1	2	3	1
S1	**A**	**5**	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	3	1	1	1	1	1	1	1	3	2	1
S1	**B**	**1**	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	2	1	1	1	1	1	1	1	3	2	1
S1	**B**	**5**	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1	2	1
S1	**C**	**1**	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	2	1	2	1	1	2	1	1	1	1	3	1	1
S1	**C**	**5**	2	1	1	2	1	1	1	1	1	1	1	2	1	1	2	2	1	3	1	1	1	1	1	1	1	3	2	1
S2	**A**	**1**	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	4	3	1
S2	**A**	**5**	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	5	4	1
S2	**B**	**1**	1	1	1	1	1	1	1	1	1	1	1	3	1	1	1	1	1	2	1	1	1	1	1	1	1	3	2	1
S2	**B**	**5**	4	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	6	1	1	1	1	1	1	1	6	4	1
S2	**C**	**1**	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	5	3	1
S2	**C**	**5**	3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	6	5	1
S3	**A**	**1**	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
S3	**A**	**5**	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1
S3	**B**	**1**	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
S3	**B**	**5**	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	3	1	2	1	1	1	1	1	1	3	2	1	1
S3	**C**	**1**	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1
S3	**C**	**5**	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	2	1	1	1	1	1	1	2	1	2	1
S4	**A**	**1**	2	1	1	1	1	1	1	2	1	1	1	3	1	1	1	1	1	1	1	1	2	1	1	1	1	2	3	1
S4	**A**	**5**	3	1	1	2	2	1	2	1	1	1	1	2	1	2	2	1	1	3	2	2	1	1	1	3	2	3	3	1
S4	**B**	**1**	3	1	1	1	1	1	2	2	1	1	3	3	1	1	3	1	1	2	1	1	1	1	1	1	1	4	5	1
S4	**B**	**5**	4	2	2	4	2	1	4	4	1	1	1	4	1	5	3	1	1	5	1	3	1	1	1	4	1	6	5	3
S4	**C**	**1**	1	1	2	1	1	1	2	1	1	1	1	2	1	1	2	1	1	2	1	1	1	1	1	1	1	2	2	1
S4	**C**	**5**	3	1	2	3	3	1	3	3	1	1	2	4	1	3	3	3	1	3	1	3	1	2	1	4	2	4	3	2
S5	**A**	**1**	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	3	2	1
S5	**A**	**5**	2	1	1	1	1	1	2	1	1	1	1	1	1	1	1	3	1	1	1	1	1	1	1	1	2	1	2	1
S5	**B**	**1**	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
S5	**B**	**5**	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	3	1	1	1	1	1	1	1	1	2	1	1	1
S5	**C**	**1**	3	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	1
S5	**C**	**5**	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	4	1	2	1	1	1	1	1	1	4	1	2	1
S6	**A**	**1**	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	5	2	1
S6	**A**	**5**	4	1	1	3	1	1	1	1	1	1	1	1	1	2	3	5	1	2	1	1	1	1	1	1	5	2	2	1
S6	**B**	**1**	4	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	3	2	2	1
S6	**B**	**5**	4	1	1	3	1	1	1	1	1	1	1	1	1	2	2	4	1	2	1	1	1	1	1	1	5	1	3	1
S6	**C**	**1**	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
S6	**C**	**5**	5	1	1	3	1	1	1	1	1	1	1	1	1	1	3	5	1	2	1	1	1	1	1	1	5	1	3	1
S7	**A**	**1**	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	5	2	1
S7	**A**	**5**	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	3	1	1	1	1	1	1	2	5	2	1
S7	**B**	**1**	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	3	1	1

Subject	*Treatment*	*Time*	*Weakness*	*Hiccough*	*Bloating*	*Difficulty Speaking*	*Double Vision*	*Yellow Vision*	*Nausea*	*Headache*	*Constipated*	*Itching*	*Back Pain*	*Drowsiness*	*Tremor*	*Dizziness*	*Inability To Concentrate*	*Pounding Heart*	*Feeling Tearful*	*Pain In Legs*	*Shivering*	*Blurred Vision*	*Anxiety*	*Heartburn*	*Abdominal Cramps*	*Tingling Around Mouth*	*Difficulty Breathing*	*Hunger*	*Tiredness*	*Confusion*
S7	**B**	**5**	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	3	1	1	1	1	1	1	1	4	1	1
S7	**C**	**1**	3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	4	1	1
S7	**C**	**5**	4	1	1	3	1	1	1	1	1	1	1	1	1	1	1	4	1	4	1	1	1	1	1	1	2	4	1	1
S8	**C**	**1**	2	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	1
S8	**A**	**5**	3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	2	1	1	1	1	2	1	2	3	1	1
S8	**A**	**1**	2	1	1	1	1	1	1	1	1	1	1	4	1	1	1	1	1	1	1	1	1	1	1	1	1	3	3	1
S8	**B**	**5**	4	1	1	2	1	1	1	1	1	1	1	1	1	1	1	3	1	3	1	1	1	1	3	1	1	5	2	1
S8	**B**	**1**	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	3	2	1
S8	**C**	**5**	3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	3	1	1	1	1	1	1	1	4	2	1
S8	**C**	**1**	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	1
S9	**A**	**5**	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	2	1	1	1	1	1	1	2	2	1	1
S9	**A**	**1**	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	3	2	1
S9	**B**	**5**	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	2	1	1	1	1	1	1	1	2	2	1
S9	**B**	**1**	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	3	3	1
S9	**C**	**5**	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	4	1	2	1	1	1	1	1	1	1	2	2	1
S9	**C**	**1**	2	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	1
S10	**A**	**5**	3	1	1	3	1	1	3	1	1	1	1	3	1	1	5	2	1	1	1	1	1	3	1	1	1	3	3	1
S10	**A**	**1**	2	1	1	1	1	1	1	1	1	1	1	3	1	1	1	1	1	1	1	1	1	1	1	1	1	3	3	1
S10	**B**	**5**	3	1	1	1	1	1	2	1	1	1	1	2	1	1	2	1	1	3	1	1	1	2	1	1	1	2	2	1
S10	**B**	**1**	2	1	1	1	1	1	1	1	1	1	1	3	1	1	1	1	1	1	1	1	1	1	1	1	1	3	2	1
S10	**C**	**5**	4	1	1	2	1	1	3	1	1	1	1	1	1	1	3	1	1	1	1	1	1	3	1	1	1	2	1	1