ORIGINAL RESEARCH

Effects of Muscle Glycogen on Performance of Repeated Sprints and Mechanisms of Fatigue

Michelle Smith Rockwell, Janet Walberg Rankin, and Helen Dixon

This study investigated the effect of initial muscle glycogen on performance of repeated sprints and some potential mechanisms for an effect of glycogen on fatigue. Eight subjects performed 2 cycling trials (repeated 60-s sprints) following consumption of either a high carbohydrate (HC) or a low carbohydrate (LC) diet. Muscle biopsies and blood samples were collected at baseline, following a 15% (15% fatigue) and a 30% decline in sprint performance (30% fatigue), when exercise was terminated. Baseline muscle glycogen levels [346 ± 19 HC (*SEM*) vs. 222 ± 19 mmol/kg dw LC] and total exercise time to 30% fatigue were higher following HC than LC (57.5 ± 10.0 vs. 42.0 ± 3.6 min; $p < .05$). Similar significant ($p < .05$) decreases over the entire exercise bout were seen in muscle glycogen (43%), creatine phosphate (CP; 35%), and sarcoplasmic reticulum (SR) Ca^{2+}-uptake in isolated homogenized muscle (56%) for both trials ($p > .05$ between trials). The percentage decline in SR Ca^{2+}-release was less for HC than LC (36% and 53%, respectively), but this was not statistically different. In summary, HC delayed fatigue during repeated sprints. As the reductions in muscle glycogen, CP, and SR function during exercise were not different by dietary treatment, these data do not support a link between whole muscle glycogen and SR function or CP reduction during repeated sprint exercise.

Key Words: carbohydrate, creatine phosphate, calcium, sarcoplasmic reticulum, fatigue

Introduction

Although high muscle glycogen has been consistently considered ergogenic for prolonged aerobic exercise, research on the role of dietary carbohydrate and muscle glycogen in performance of high intensity exercise has produced conflicting results. Some laboratories comparing moderate or high (50–83%) to low (4–12%) carbohydrate intakes for 2 to 3 days report an increase in performance of single-bout high intensity tests (19, 21, 22) and intermittent high intensity tests (1, 4, 18) when subjects consumed the higher carbohydrate diets. For example, subjects could produce 9% more total power during a 30-s Wingate test when they had consumed a 50% versus 5% carbohydrate diet (19). Some research groups (14, 27), in contrast,

The authors are with the Department of Human Nutrition, Foods, and Exercise at Virginia Tech, Blacksburg VA 24061-0430.

did not observe an ergogenic effect of a higher compared to a lower carbohydrate diet on single-bout high intensity exercise tests.

The mechanism by which high carbohydrate diets (HC) could enhance high intensity exercise performance is unclear. In prolonged submaximal exercise, high levels of initial muscle glycogen may delay fatigue by maintaining glycogen stores for use in later stages of exercise, or by blunting the fall in blood glucose that commonly accompanies this type of exercise. However, since neither total muscle glycogen nor blood glucose typically achieve limiting concentrations during high intensity exercise, these factors are less obviously tied to performance of high intensity exercise of short duration.

An extensive amount of animal research points to diminished SR function as a precipitator of muscle fatigue. Specifically, Ca^{2+}-uptake, Ca^{2+}-release, and Ca-AT-Pase activity have been observed to be depressed following fatiguing bouts of exercise (12, 29). Several human studies have shown decreased Ca^{2+}-uptake following ~70 min of cycling at 70% VO_{2max} (2), several 30-s cycling sprints (15), 30 min of repeated submaximal isometric knee extensions (26), and ~3–7 min of isokinetic leg extension (11, 17).

Evidence for a structural and metabolic relationship between muscle glycogen and SR function exists. Glycogen molecules and glycogenolytic enzymes have been located near the SR membrane (31). Depletion of glycogen via incubation of skinned muscle fibers with amylase depressed SR function (3) and fatigued muscle fibers exhibited improved tetanic force and Ca^{2+}-handling when allowed to recover in a glucose bath as compared to a glucose-free bath (5). Glycogen may serve as a localized source of ATP for the Ca-ATPase pump, thereby enabling Ca^{2+}-uptake (6). Thus, beginning exercise with higher levels of muscle glycogen may prevent a critical depletion of glycogen specifically associated with the SR, slow the decline in SR function, and thus delay fatigue.

Availability of creatine phosphate (CP) may be a limiting factor with intense exercise, especially during repeated sprints. Febbraio et al. (8) measured a 73–77% reduction in muscle CP following four 1-min cycling sprints. Some research suggests that low initial muscle glycogen may accelerate the use of CP and thus precipitate more rapid fatigue during high intensity exercise (20, 25).

The purpose of this study was to determine whether initial muscle glycogen influences performance of repeated 60-s sprint exercise. In addition, we tested the hypothesis that lower initial muscle glycogen accelerates the decline in SR function and CP concentration during exercise, thus providing an explanation for the effect of diet on performance.

Methods

Subjects

Eight competitive male cyclists between the ages of 18 and 30 years volunteered to serve as subjects (Table 1). Three subjects were members of the university's Division I intercollegiate cycling team, and 5 were members of a local pro-am cycling team and competed in either the Mountain Bike EXPERT category or USCF Category 3 or 4 Road Racing category. All subjects were currently cycling 4–6 times per week for at least 2 hours each ride. The study took place approximately 1 month prior to the university cyclists' season and 1 month after the pro-am cyclists' season.

Table 1   Subject Characteristics

Variable	Mean ± *SEM*
Age (y)	23.9 ± 1.6
Weight (kg)	73.5 ± 1.6
Body fat (%)	8.5 ± 1.2
Relative VO_{2peak} (ml · kg^{-1} · min^{-1})	58.0 ± 1.6
Absolute VO_{2peak} (L/min)	4.25 ± 0.14

Subjects gave their written informed consent and were screened for contraindications to strenuous exercise, diet manipulation, and muscle biopsies. All methods and procedures were approved by the Institutional Review Board of Virginia Polytechnic Institute and State University.

Pre-testing

The schedule of dietary manipulation and testing is shown in Table 2. Each subject's body weight was recorded upon arrival to the laboratory for pre-testing and all cycling trials. On the first day of the experiment, subjects performed a VO_{2peak} test and a 30-s Wingate test. The VO_{2peak} test was performed as an incremental test to exhaustion on a Monark cycle ergometer. A MedGraphics cart was used for analysis of expired gases and was calibrated prior to each subject's test. Following a low intensity warm-up, subjects cycled for 2 min with a load of 2.0 kg at 90 rpm. The load was increased by 0.5 kg every 2 min, unless the subject's heart rate did not increase by more than 8 beats/min in the first minute of the stage. In that case, the 0.5-kg increase was made at 1 min. Exhaustion was determined as the point when subjects were not maintaining the required 80 rpm, when VO_2 failed to increase over a 60-s period, or when subjects volitionally terminated the test.

A modified Monark cycle ergometer with freely hanging weights was used for the Wingate test. After the subject pedaled to top speed without load, a load of 8.75% of body weight was dropped, and the subject cycled at maximal effort for 30 s. The number of pedal revolutions made during each 5-s segment was manually and mechanically counted and converted into power (Watts). Only subjects whose VO_{2peak} was greater than 52 ml · kg^{-1} · min^{-1} and whose ratio of power at VO_{2peak} to average power during the Wingate test was greater than 0.40 were included in the study (7). A 30-min recovery period separated the VO_{2peak} and Wingate tests.

Familiarization Trial

Approximately 20–30 min after the Wingate tests, subjects performed a 3-min warm-up at 70% VO_{2peak} followed by three or four 60-s sprints at maximal effort with 3 min of active recovery (pedaling at a self-monitored pace with no resistance) between sprints. Subjects were specifically instructed not to pace themselves but to

Table 2   Sample Subject Schedule

Day	Trial
1	Pre-test (VO$_{2peak,}$ Wingate); familiarization trial
8	Baseline trial
13	Glycogen depletion ride–1
14	Diet manipulation–A
15	Performance trial–1
20	Glycogen depletion ride–2
21	Diet manipulation–B
22	Performance trial–2

exert maximal effort throughout the 60-s periods. They were allowed to stand up from the seat only during the first 3 s of the intervals.

The resistance used for the sprints would elicit 130% VO$_{2peak}$ if 90 rpm was maintained. This was selected since it was the level at which pilot subjects were able to perform approximately 15 sprints. We chose 90 rpm because pilot subjects often reached a plateau at 85–95 rpm when giving maximal effort for repeated 60-s sprints at this workload.

Baseline Trial

One week after the familiarization trial, subjects completed a baseline trial that mimicked the experimental protocol. The experimental protocol utilized was a modification of that previously used by Davis et al. (7) in which performance of repeated 60-s sprints at 120–130% VO$_{2max}$ was enhanced in untrained subjects by carbohydrate feeding during exercise. We modified this protocol by having our highly trained cyclists give maximal effort during each sprint rather than cycling consistently at 80 rpm at a fixed resistance, since our pilot data showed that trained cyclists could continue that protocol for well over 2 h.

For the baseline trial, subjects performed a 5-min warm-up at 70% VO$_{2peak}$ with maximal sprints in the final 10 s of min 3, 4, and 5 followed by 5 min of stretching. They then performed the same pattern of repeated sprints against the same workload as that used in the familiarization trial. Revolutions achieved during each sprint were recorded from a mechanical counter mounted to the back of the ergometer that was activated by the movement of the left pedal and counted to insure accuracy. Subjects continued the protocol until sprint rpm fell below 70% of the first sprint rpm for two consecutive sprints (30% fatigue).

Performance Trials

One to 2 weeks following the baseline trial, subjects completed two performance trials, HC and LC, separated by 7 days. Five subjects completed HC first, while 3

completed LC first. (One subject who had been originally assigned to HC first dropped out after his first trial.) Subjects fasted for 12 h prior to reporting to the lab for performance trials but were encouraged to consume water and non-caloric/caffeine-free fluids up until the trial.

Since the number of sprints performed during the baseline trial ranged from 3 to 20 (12 min to 80 min of exercise), the workload used for performance trials was adjusted based on baseline trial performance in an effort to induce homogeneous performance times and similar metabolic stress. If subjects performed 10 or fewer baseline sprints (2 subjects), 125% VO_{2peak} was used; if 15 or greater sprints were performed (2 subjects), 135% VO_{2peak} was used; and if 11 to 14 sprints were performed (4 subjects), workload was not changed from 130% VO_{2peak}. Subjects were verbally encouraged throughout trials by a researcher blind to subjects' treatment condition. Water was provided ad libitum during all trials, and consumption was encouraged during recovery periods. Subjects were urged to consume adequate fluids during and following glycogen depletion rides.

Glycogen Depletion Rides

On the evening 36 h prior to both performance trials, subjects completed a cycling bout designed to deplete leg muscles of glycogen. Subjects cycled at approximately 80 rpm at 70% VO_{2peak} for 80 min followed by four 60-s maximal effort sprints with 3 min recovery between sprints. Following the ride, subjects were given either a HC or LC snack of approximately 10% of their normal daily caloric intake and a corresponding HC or LC diet for consumption the next day.

Diet Manipulation

Diets consisted of the following: HC (80–85% CHO, 10–15% P, 5% F) or LC (5–10% CHO, 15–20% P, 65–75% F). Total energy provided was matched to a 24-h dietary record kept by subjects the day before the baseline trial [total energy, 830 ± 40 kJ (kcal 3474 ± 169), 62 ± 2% CHO, 15 ± 1% P, 25 ± 3% F]. Dietary records were analyzed with *Nutritionist V* computer software (First Data Bank, San Bruno, CA, USA). Foods provided for HC included pasta, tomato sauce, bananas, apple juice, bread, cereal, non-fat milk, and Gatorlode (25% CHO beverage, Gatorade, Barrington, IL). LC consisted of eggs, cheese, mayonnaise, tuna, turkey, margarine, peanut butter, and rice cakes. A non-caloric beverage (~1 L) was provided with both diets. In the case of vegetarian subjects (1 subject) or subjects with food allergies (1 subject), problem foods were omitted, and other list foods were increased proportionately. Subjects were instructed to consume all food given to them and to return any uneaten portions.

Blood and Muscle Collections

Three muscle biopsies and blood samples were collected with each performance trial, one at baseline (collected prior to the warm up), one during exercise (15% fatigue), and one immediately following exercise (30% fatigue). Each subject's left leg was used for first performance trial biopsies and right leg for second performance trial biopsies. For a baseline biopsy, lidocaine was injected into subjects' vastus lateralis muscle prior to their warm-up, and two 0.8-cm incisions were made

with a scalpel approximately 1.25 cm apart vertically. A 60–100-mg muscle sample was removed from the lower incision using the percutaneous needle biopsy technique with suction. Incisions were covered with a piece of gauze and medical tape, and subjects began warming up for the performance trial.

A second biopsy was taken from the lower incision (the needle was inserted at a different angle into the muscle than the baseline biopsy) after subjects' sprint rpm had declined 15% from the first sprint rpm (15% fatigue). A third biopsy was taken from the higher incision when rpm declined to 30% below first sprint rpm for two consecutive sprints (30% fatigue). Subjects were unaware of their performance throughout the trials and therefore did not know when biopsies were going to be taken. For biopsies taken during and after exercise, subjects were asked to immediately stop cycling and remove their legs from pedal clips as researchers removed gauze and tape. A sample was removed, and subjects were encouraged to continue cycling throughout the remainder of the recovery period.

Muscle samples were removed from the needle and cut into two sections with a scalpel. One portion was immediately frozen in liquid nitrogen for later analysis of glycogen, CP, Cr, and ATP. The amount of time required for samples to be removed and placed in liquid nitrogen averaged < 10 s (verified by videotaping trials of 4 subjects). Samples were later freeze dried, powdered, and dissected free of connective tissue, blood, and other non-muscle constituents. A portion of the muscle was extracted with acid, neutralized, and glucosyl units were analyzed enzymatically (16). Analysis of CP, Cr, and ATP was performed using a perchloric acid/$KHCO_3$ extraction and enzymatic spectrophotometric technique (16). Glycogen was analyzed in triplicate (9% variance among triplicates); CP, Cr, and ATP were analyzed in duplicate (6% variance among duplicates for CP and Cr and 4% for ATP). All muscle metabolites were adjusted for peak total creatine values within the same trial to account for potential contamination of muscle with connective tissue, blood, or fat (10, 16).

The second portion of muscle samples ($\sim$25 mg) was used for SR Ca^{2+}-uptake and release measurements. Samples were immediately put in an ice-cold homogenizing buffer containing sucrose, HEPES, NaN_3, and PMSF, and minced with dissecting scissors while kept on ice. Samples were then homogenized using a Pro 200 homogenizer with a 5-mm probe. Following centrifugation at 1600 g for 15 min (2 °C), the supernatant was removed and stored in a –80 °C freezer. Total protein concentration was analyzed as previously described (30).

Analysis of Ca^{2+}-uptake and release was performed using a technique described in detail by Williams et al. (30). Approximately 75–125 μg of homogenate protein was added to a buffer containing KCl, HEPES, Pyrophosphate, and $MgCl_2$. Free Ca^{2+} concentration was measured by a Jasco CAF-110 Intracellular Ion Analyzer in the presence of Fura (a flourescent Ca^{2+} indicator). This measurement was performed before and after addition of Ca^{2+} and ATP as a means of quantifying the rate of Ca^{2+}-uptake. Ca^{2+}-release was stimulated by addition of $AgNO_3$. We readily acknowledge that $AgNO_3$ is not a physiological releasing agent. However, it evokes SR Ca^{2+} release via sulfhydryl oxidation of the Ca^{2+} release channel, a process which some have argued is physiological. It is important to point out that in our study, we used $AgNO_3$ as a tool to activate the SR Ca^{2+} channel and examine the kinetics of Ca^{2+} release. We did not attempt to make inferences regarding the redox status or gating properties of the release channel.

Since there was not enough time to take blood samples during the rest period in which biopsies were collected, during exercise blood samples were collected in tubes without additives in the rest interval immediately following the 15% fatigue rest interval. The final sample was taken after the 30% fatigue biopsy, about 5 min after exercise had ceased. Following formation of the clot (~25–30 min), blood was centrifuged. The serum was frozen until later analysis of glucose (Sigma 16-UV) and lactate (13).

Statistical Analysis

Data are presented as averages with standard error of the mean. A one-tailed paired *t* test was performed to detect differences between trials in performance measurements. All other data were analyzed by repeated measures ANOVA to test for effect of Group, Time, and Group-by-Time interaction with Tukey as the post hoc test. Since statistical analysis showed that performance differences were not influenced by the order in which trials were performed, order was not included in subsequent analyses. Pearson's correlation analysis was performed to determine associations between dependent measures. Significance was defined at the $p < .05$ level.

Results

Compliance

All subjects maintained their body weight within 1.3 kg during the study (baseline = 73.5 ± 1.6 kg, HC = 73.5 ± 1.7 kg, and LC = 72.9 ± 1.6 kg). Subjects were verbally questioned regarding compliance to dietary and exercise instructions before performance trials. No deviance was reported at that time, with the exception of 1 subject who returned 287 kJ (1200 kcal) of food provided for his first performance trial. This subject was provided with a diet of the same energy content as the first trial for his second performance trial. No other subject returned more than 48 kJ (200 kcal) of unconsumed foods.

Performance

There was a superior performance in the HC trial (14.3 ± 2.5 sprints, 1311.9 ± 279.8 kJ total work) as compared to the LC trial (10.4 ± 0.9 sprints, 960.6 ± 118.0 kJ total work). Total exercise time was 37% longer in the HC trial (57.5 ± 10.0 min) than the LC trial (42.0 ± 3.6 min, $p = .04$). There was no difference between trials in the average amount of work performed per sprint (88.9 ± 3.8 and 90.0 ± 3.7 kJ for HC and LC, respectively). Six subjects performed more sprints in the HC trial, 1 performed more in the LC trial, and 1 performed the same in both trials.

Peak power was achieved by all subjects during both trials' first sprint. There was no difference in peak power between trials (HC, 641 ± 52.5 W vs. LC, 652 ± 57.0 W). The 15% fatigue point occurred, on average, at the same time in both trials (5.9 ± 1.5 intervals, 23.6 ± 6.0 min). Total work completed up to this point was not different in the HC and LC trials (558.4 ± 84.2 vs. 571.5 ± 87.2 kJ). Performance differences between trials appeared between 15 and 30% fatigue. Subjects cycled 87% longer following 15% fatigue in the HC condition than the LC condition (8.4 ± 2.5 sprints and 33.6 ± 10 min vs. 4.5 ± 0.9 sprints and 18.0 ± 3.6 min, respectively; $p <$

.05). Approximately 93% more work was completed between 15 and 30% fatigue in the HC condition (753.5 ± 231.5 kJ) than the LC condition (389.1 ± 69.2 kJ; $p < .05$).

Muscle Analyses

Exercise from baseline to 15% fatigue caused a 38% ($p < .001$) drop in muscle glycogen; the 6% decrease observed from 15 to 30% fatigue was not significant (Table 3). Diet influenced the absolute amount of muscle glycogen throughout exercise. Glycogen was 36% higher at baseline, 35% higher at 15% fatigue, and 42% higher at 30% fatigue for HC than LC (Table 3). There was no difference between trials in the rate of glycogen utilization over the entire exercise bout (HC, $5.7 ± 1.0$ vs. LC, $4.4 ± 1.4$ mmol · kg dw^{-1} · min^{-1}).

A significant reduction over time was found for CP, ATP, Ca^{2+}-uptake, and Ca^{2+}-release ($p < .01$), with no significant interaction of Groups over time. The decrease in ATP was significant from baseline to 15% fatigue and 15 to 30% fatigue ($p < .05$) with a tendency for a Diet by Time interaction ($p = .08$; Table 3). The overall rate of decrease in ATP was $0.1 ± 0.1$ mmol/kg dw^{-1} · min^{-1}. A significant decrease in CP and increase in Cr occurred in both trials from baseline to 15% fatigue ($p < .01$; Table 3). An overall decrease in Ca^{2+}-uptake was found from baseline to 15% fatigue (37%, $p < .001$) and 15 to 30% fatigue (8%, $p < .01$; Figure 1). Ca^{2+}-release decreased by 38% from baseline to 15% fatigue ($p < 0.001$) but remained unchanged from 15 to 30% fatigue (Figure 2). Analysis of rate of change in Ca^{2+}-uptake and Ca^{2+}-release from baseline to 15% fatigue, 15 to 30% fatigue, and baseline to 30% fatigue indicated that these rates were not different between groups when expressed per minute or per kilojoule of work performed (e.g., HC $0.03 ± 0.01$ and $0.02 ± 0.01$, LC $0.03 ± 0.02$ and $0.04 ± 0.01$ nmol/mg/min for rate of Ca^{2+}-uptake and Ca^{2+}-release from baseline to 30% fatigue, respectively). Neither the correlation between Ca^{2+}-release nor Ca^{2+}-uptake and whole muscle glycogen was significant ($r = 0.385$ and 0.435, respectively).

Blood Analyses

Both trials caused a similar increase in blood glucose at 15% fatigue that was maintained at 30% fatigue. The overall serum glucose for HC was higher than that for LC. None of the subjects demonstrated hypoglycemia; serum glucose was never less than 4.3 mmol during either trial for any subject. Serum lactate rose about eight fold in both trials by the 15% fatigue point, with a small reduction at 30% fatigue. (Table 3).

Discussion

The present experiment was designed to determine the effect of HC on performance of repeated 1-min sprints and to improve understanding of the mechanisms favoring a slower rate of fatigue with this diet manipulation. Our results indicate that consuming a HC diet for 36 hours after glycogen depletion improves performance of repeated sprints compared to a LC for the same time period. While fatigue was associated with reductions in CP and SR function, whole muscle glycogen was not linked to changes in either of these measures.
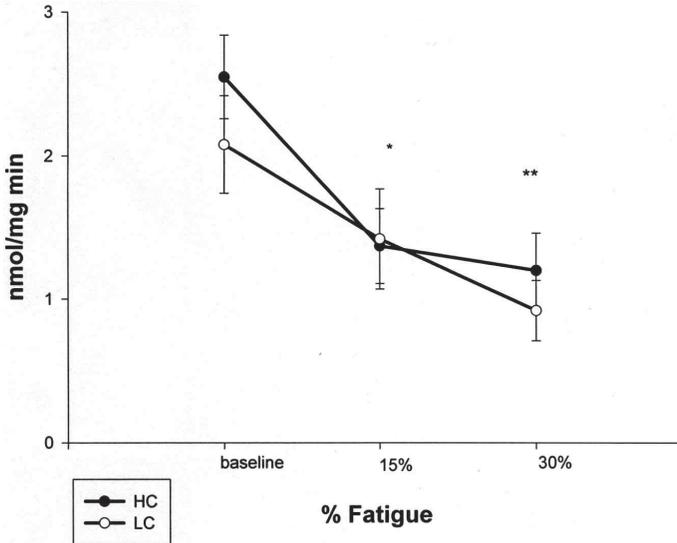
Figure 1 — Sarcoplasmic reticulum calcium-uptake at baseline, 15 and 30% fatigue; n = 6 for HC 15% fatigue, n = 7 for LC 30% fatigue, n = 8 for all others. Main effect of time ($p < .001$). *Significantly different from baseline for both HC and LC ($p < .001$); **significantly different from 15% fatigue for both HC and LC ($p < .01$).
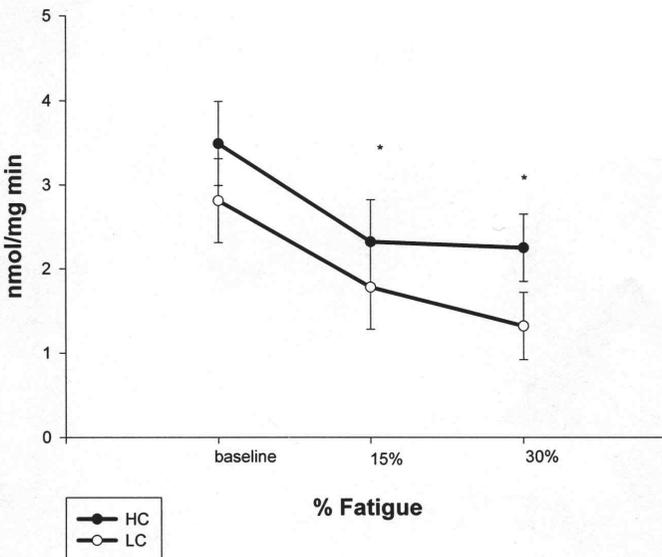
Figure 2 — Sarcoplasmic reticulum calcium-release at baseline, 15 and 30% fatigue; n = 6 for HC 15% fatigue, n = 7 for LC 15% fatigue, n = 8 for all others. Main effect of time ($p < .001$). *Significantly different from baseline for both HC and LC ($p < .001$).

Table 3 Muscle and Serum Metabolite Concentrations Before, During, and After Exercise in High and Low Carbohydrate Conditions

	HC			LC		
	Baseline	15% fatigue	30% fatigue	Baseline	15% fatigue	30% fatigue
Muscle						
Glycogen+,* (mmol/kg dw)	346 ± 19	214 ± 20	204 ± 16	222 ± 19	139 ± 15	118 ± 13
CP* (mmol/kg dw)	86 ± 5	59 ± 4	58 ± 6	86 ± 3	61 ± 5	53 ± 6
Free creatine* (mmol/kg dw)	47 ± 4	72 ± 4	74 ± 6	54 ± 3	80 ± 5	87 ± 6
ATP*,▼ (mmol/kg dw)	29 ± 1	27 ± 1	23 ± 1	26 ± 2	24 ± 1	23 ± 1
Serum						
Glucose* (mmol/L)	5.64 ± 0.15	9.75 ± 0.82	8.98 ± 1.19	5.08 ± 0.26	7.98 ± 0.91	7.20 ± 0.62
Lactate* (mmol/L)	1.89 ± 0.25	15.73 ± 0.62	13.13 ± 1.65	1.45 ± 0.28	16.30 ± 0.49	11.88 ± 1.82

Note. There were no Diet-by-Time interactions among variables. +Significant effect of diet; *significant difference between baseline and 15% value when groups combined; ▼significant difference between 15 and 30% value when groups combined.

The metabolic mechanism for the benefit of carbohydrate status on performance of our repeated sprint exercise protocol is unclear. Fatigue during high intensity exercise has been suggested to relate to reduction in fuels such as CP and glycogen, accumulation of metabolites (e.g., Pi, ADP, IMP) that may interfere with ATP production or the contractile process (e.g., SR function), metabolic acidosis, and reduction in central drive (for review, see 13). The muscle fuels, metabolites, and muscle calcium metabolism measured in our study did not vary by dietary treatment. Thus, these measures do not explain the reduced fatigue rate caused by a higher carbohydrate intake.

Initial muscle glycogen did not affect the change in CP and thus alterations in contribution of the phosphagen system to ATP generation do not appear to explain the performance benefit of HC. Others have observed more rapid utilization of CP in muscles with low glycogen. Larson et al. (20), for example, showed a sparing of CP during approximately 5 min of high intensity quadriceps exercise in HC compared to a LC. In a study by Tsintzas et al. (25), a carbohydrate solution consumed prior to submaximal running induced higher CP levels at exhaustion than a placebo solution, despite a longer time to exhaustion in the carbohydrate-fed group. Comparison of the results of these two studies and ours is complicated by the differing exercise protocols used. It is possible that low glycogen status may enhance CP utilization during some types and intensities of exercise but not others.

No difference in magnitude of glycogen reduction was observed in spite of beginning the exercise test with different initial total glycogen. This is similar to the findings of several other investigations that found no relationship between initial muscle glycogen content and rate of glycogen use during intense exercise (24, 27). As phosphorylase has a low K_m for glycogen, it was likely saturated with substrate even at the moderately reduced initial muscle glycogen levels in the LC trial observed in our study (24).

The difference in muscle glycogen between treatments remained throughout exercise such that different final glycogen values were achieved at the end of each trial despite the same level of muscle fatigue. Thus, the hypothesis that a drop in total muscle glycogen to a threshold value as the specific link to fatigue is not supported by our data. However, higher muscle glycogen in conjunction with elevated blood glucose throughout exercise in the HC condition may have delayed the conversion away from anaerobic glycolysis to oxidative metabolism and thus postponed fatigue. Exercise intensity declines when supported by oxidative metabolism, as this system cannot support as high of an exercise intensity as anaerobic glycolysis due to slower ATP generation rate.

Although total glycogen was not severely reduced for either group, it is possible that specific muscle fibers may have been more severely depleted of glycogen than others. Previous research has shown that glycogen reduction is higher in type II than type I fibers as a result of intense exercise (28). Future research could clarify whether a reduction in type II fiber muscle glycogen to some critical threshold predicts fatigue.

Because there was no difference between trials in absolute or in rate of change of Ca^{2+}-uptake or release and not a significant correlation between glycogen concentration and SR function, our data do not support a relationship between total muscle glycogen and SR function. More work was accomplished in HC compared to LC (i.e., LC subjects reached 30% fatigue faster than HC subjects), but the calculated rate of decline in SR Ca^{2+}-uptake and release was not different when

expressed per minute or per kilojoule of work performed. Although there was no statistically significant effect of dietary treatment on Ca^{2+}-uptake and release over the exercise bout, the numerical difference between treatments expanded as fatigue developed. It is possible that a significantly higher Ca^{2+}-uptake and/or release may have been maintained in HC if the exercise had been continued to a more severe fatigue level.

In contrast to evidence from animal studies for a relationship between muscle glycogen and SR function (6, 23), the only other published human study agreed with ours in not supporting a relationship between these factors (15). Hargreaves et al. (15) demonstrated that performance, muscle glycogen levels, and SR Ca^{2+}-uptake were depressed after two 30-s cycling sprints. A 90-min recovery period allowed Ca^{2+}-uptake and performance to rebound to baseline despite still-reduced muscle glycogen levels. Our study and that of Hargreaves et al. (15) were limited by only measuring total muscle glycogen and not glycogen specifically associated with the SR. Friden et al. (9) have reported human data that show selective and compartmentalized glycogen reduction in different cellular regions, including the SR membrane, that is significantly greater than glycogen degradation in the whole muscle. If glycogen was depleted from the SR more rapidly than from the whole muscle, changes in SR function would not be expected to reflect total muscle glycogen. Examination of this hypothesis would require measurement of changes in glycogen associated with SR following the exercise bout, a technique that has not been reported with human muscle samples. In addition, we acknowledge that this in vitro SR preparation may be different than SR function in situ, since conditions and substrates are optimal in our preparation. It remains possible that SR function may be differentially impaired by the dietary treatments under the in situ conditions within the muscle during exercise.

Summary

Performance of our repeated sprint protocol was enhanced 37% by a diet that resulted in elevated muscle glycogen levels throughout exercise relative to LC. The effect of diet was evident during the latter stage of exercise, from 15 to 30% fatigue, when exercise time was 87% longer after subjects had consumed HC. Initial muscle glycogen levels did not influence the rate of glycogen utilization, decrease in muscle CP, or impairment of SR function. Thus, other unmeasured factors must explain differential fatigue development when carbohydrate content of the diet is manipulated. The possibility that glycogen was selectively depleted during this intense exercise bout from specific muscle fibers or cellular compartments, including SR, deserves further research.

References

1. Balsom, P.D., G.C. Gaitanos, K. Soderlund and B. Ekblom. High-intensity exercise and muscle glycogen availability in humans. *Acta. Physiol. Scand.* 165:337-345, 1999.
2. Booth, J., M.J. McKenna, P.A. Ruell, T.H. Gwinn, G.M. Davis, M.W. Thompson, A.R. Harmer, S.K. Hunter, and J.R. Sutton. Impaired calcium pump function does not slow relaxation in human skeletal muscle after prolonged exercise. *J. Appl. Physiol.* 83:511-521, 1997.

3. Brautigan, D.L., W.G.L. Kerrick, and E.H. Fischer. Stimulation of sarcoplasmic reticulum by insulin and glucose-6-phosphate. *Fed. Proc.* 38:305-306, 1979.

4. Casey, A., A.H. Short, S. Curtis, and P.L. Greenhaff. The effect of glycogen availability on power output and the metabolic response to repeated bouts of maximal, isokinetic exercise in man. *Eur. J. Appl. Physiol.* 72:249-255, 1996.

5. Chin, E.R., and D.G. Allen. Effects of reduced muscle glycogen concentration on force, Ca^{2+} release and contractile protein function in intact mouse skeletal muscle. *J. Physiol.* 498:17-29, 1997.

6. Cuenda, A., M. Nogues, C. Gutierrez-Merino, and L. de Meis. Glycogen phosphorolysis can form a metabolic shuttle to support Ca^{2+} uptake by sarcoplasmic reticulum membranes in skeletal muscle. *Biochem. Biophys. Res. Commun.* 196:1127-1132, 1993.

7. Davis, J.M., D.A. Jackson, M.S. Broadwell, J.L. Queary, and C.L. Lambert. Carbohydrate drinks delay fatigue during intermittent, high-intensity cycling in active men and women. *Int. J. Sport Nutr.* 7: 261-273, 1997.

8. Febbraio, M.A., T.R. Flanagan, R.J. Snow, S. Zhao, and M.F. Carey. Effect of creatine supplementation on intramusclular TCr, metabolism and performance during intermittent, supramaximal exercise in humans. *Acta Physiol. Scand.* 155:387-395, 1995.

9. Friden, J., J. Seger, and B. Ekblom. Topographical localization of muscle glycogen: an ultrahistochemical study in the human vastus lateralis. *Acta Physiol. Scand.* 135:381-391, 1989.

10. Gaitanos, G.C., C. Williams, L.H. Boobis, and G. Brooks. Human muscle metabolism during intermittent maximal exercise. *J. Appl. Physiol.* 75:712-719, 1993.

11. Gollnick, P.D., P. Korge, J. Karpakka, and B. Saltin. Elongation of skeletal muscle relaxation during exercise is linked to reduced calcium uptake by the sarcoplasmic reticulum in man. *Acta Physiol. Scand.* 142:135-136, 1991.

12. Green, H.J. Mechanisms of muscle fatigue in intense exercise. *J. Sports Sci.* 15:247-256, 1997.

13. Green H.J., R.L. Hughson, G.W. Orr, and D.A. Ranney. Anaerobic threshold, blood lactate and muscle metabolites in progressive exercise. *J. Appl. Physiol.* 54:1032-1038, 1983.

14. Hargreaves, M., J.P. Finn, R.T. Withers, J.A. Halbert, G.C. Scroop, M. Mackay, R.J. Snow, and M.F. Carey. Effect of muscle glycogen availability on maximal exercise performance. *Eur. J. Appl. Physiol.* 75:188-192, 1997.

15. Hargreaves, M., M.J. McKenna, D.G. Jenkins, S.A. Warmingotn, J.L. Li, R.L. Snow, and M.A. Febbraio. Muscle metabolites and performance during high-intensity, intermittent exercise. *J. Appl. Physiol.* 84:1687-1691, 1998.

16. Harris R.C., E. Hultman, and L.L. Nordesjo. Glycogen, glycolytic intermediates and highenergy phosphates determined in biopsy samples of musculus quadriceps femoris of man at rest. *Scand. J. Clin. Invest.* 33:109-120, 1974.

17. Hill, C.A., M.W. Thompson, P.A. Ruell, J.M. Thom, and M.J. White. Sarcoplasmic reticulum function and muscle contractile character following fatiguing exercise in humans. *J. Physiol.* 531:871-878, 2001.

18. Jenkins, D.G., J. Palmer, and D. Spillman. The influence of dietary carbohydrate on performance of supramaximal intermittent exercise. *Eur. J. Appl. Physiol.* 67:309-314, 1993.

19. Langfort, J., R. Zarzenzny, W. Pilis, K. Nazar, and H. Kaciuba-Uscitko. The effect of a low-carbohydrate diet on performance, hormonal and metabolic responses to a 30-s bout of supramaximal exercise. *Eur. J. Appl. Phsiol.* 76:128-133, 1997.

20. Larson, D.E., R.L. Hesslink, M.I. Hrovat, R.S. Fishman, and D.M. Systrom. Dietary effects on exercising muscle metabolism and performance by P-MRS. *J. Appl. Physiol*. 77:1108-1115, 1994.

21. Maughan, R.J., P.L. Greenahff, J.B. Leiper, D. Ball, C.P. Lambert, and M. Gleeson. Diet composition and the performance of high-intensity exercise. *J. Sports Sci*. 15:265-275, 1997.

22. Pizza, F.X., M.G. Flynn, B. Duscha, J. Holden, and E.R. Kubitz. A carbohydrate loading regimen improves high intensity, short duration exercise performance. *Int. J. Sport Nutr*. 5:110-116, 1995.

23. Rossi, A.M., H.M. Eppenberger, P. Volpe, R. Cotrufo, and T. Wallinmann. Muscle-type MM creatine kinase is specifically bound to sarcoplasmic reticulum and can support Ca2+ uptake and regulate local ATP/ADP ratios. *J. Biol. Chem*. 265:5258-5266, 1990.

24. Spriet, L.L., L. Berardinucci, D.R. Marsh, C.B. Campbell, and T.E. Graham. Glycogen content has no effect on skeletal muscle glycogenoloysis during short-term titanic stimulation. *J. Appl. Physiol*. 68:1883-1888, 1990.

25. Tsintzas, O.K., C. Williams, L. Boobis, and P. Greenhaff. Carbohydrate ingestion and single fiber glycogen metabolism during prolonged running in men. *J. Appl. Physiol*. 81:801-809, 1996.

26. Tupling, R., H. Green, S. Grant, M. Burnett, and D. Ranney. Postcontractile force depression in humans is associated with an impairment in SR calcium pump function. *Am. J. Physiol. Regulatory Integrative Comp. Physiol*. 278:R87-R94, 2000.

27. Vandenberghe K., P. Hespel, B.V. Eynde, R. Lysens, and E.A. Richter. No effect of glycogen level on glycogen metabolism during high intensity exercise. *Med. Sci. Sports Exerc.* 27:1278-1283, 1995.

28. Vollestad, N.K., L. Tabata, and J.I. Medbo. Glycogen breakdown in different human muscle fibre types during exhaustive exercise of short duration. *Acta Physiol. Scand.* 144:135-141, 1992.

29. Williams, J.H., and G.A. Klug. Calcium exchange hypothesis of skeletal muscle fatigue: a brief review. *Muscle Nerve* 18:421-434, 1995.

30. Williams, J.H., C.W. Ward, E.E. Spangenburg, and R.M. Nelson. Functional aspects of skeletal muscle contractile apparatus and SR after fatigue. *J. Appl. Physiol*. 85:619-621, 1998.

31. Xu, K.Y., and L.C. Becker. Ultrastructual localization of glycolytic enzymes on sarcoplasmic reticulum vesicles. *J. Histochem. Cytochem*. 46:419-427, 1998.

Acknowledgments