Carbohydrate supplementation spares muscle glycogen during variable-intensity exercise

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YASPELKIS, B. B., III, J. G. PATTERSON, P. A. ANDERLA, Z. DING, AND J. L. IVY. *Carbohydrate supplementation spares muscle glycogen during variable-intensity exercise.* J. Appl. Physiol. 75(4): 1477–1485, 1993.—Effects of carbohydrate (CHO) supplementation on muscle glycogen utilization and endurance were evaluated in seven well-trained male cyclists during continuous cycling exercise that varied between low [45% maximal O_2 uptake ($\dot{V}O_{2\,max}$)] and moderate intensity (75% $\dot{V}O_{2\,max}$). During each exercise bout the subjects received either artificially flavored placebo (P), 10% liquid CHO supplement (L; 3 × 18 g CHO/h), or solid CHO supplement (S; 2 × 25 g CHO/h). Muscle biopsies were taken from vastus lateralis during P and L trials immediately before exercise and after first (124 min) and second set (190 min) of intervals. Subjects then rode to fatigue at 80% $\dot{V}O_{2\,max}$. Plasma glucose and insulin responses during L treatment reached levels of 6.7 ± 0.7 mM and 70.6 ± 17.2 μU/ml, respectively, and were significantly greater than those of P treatment (4.4 ± 0.1 mM and 17.7 ± 1.6 μU/ml) throughout the exercise bout. Plasma glucose and insulin responses of S treatment were intermediate to those of L and P treatments. Times to fatigue for S (223.9 ± 3.5 min) and L (233.4 ± 7.5 min) treatments did not differ but were significantly greater than that of P treatment (202.4 ± 9.8 min). After the first 190 min of exercise, muscle glycogen was significantly greater during L (79 ± 3.5 μmol/g wet wt) than during P treatment (58.5 ± 7.2 μmol/g wet wt). Furthermore, differences in muscle glycogen concentrations between L and P treatments after 190 min of exercise and in time to fatigue for these treatments were positively related ($r = 0.76$, $P < 0.05$). These results suggest that CHO supplementation can enhance prolonged continuous variable-intensity exercise by reducing dependency on muscle glycogen as a fuel source.

exercise performance; glucose; insulin; carbohydrate oxidation; maltodextrin; fructose

SUBSTANTIAL EVIDENCE EXISTS that indicates that the ingestion of carbohydrate (CHO) supplements during prolonged endurance activities can delay the onset of fatigue (7, 10, 11, 16, 19). It has been observed that CHO feedings delay the onset of fatigue during prolonged continuous moderate-intensity exercise [i.e, 70% maximal O_2 uptake ($\dot{V}O_{2\,max}$)] by preventing hypoglycemia and maintaining CHO oxidation rather than by reducing the rate of muscle glycogen utilization (10, 15). However, we have recently reported that the decline in muscle glycogen is reduced when CHO supplements are provided during 2 h of cycling exercise at 50% $\dot{V}O_{2\,max}$ (30). The differences in muscle glycogen responses elicited by a CHO supplement during moderate- and low-intensity steady-state exercise are possibly due to the differences in the plasma glucose and insulin responses. Ingestion of CHO supplements during low-intensity exercise (i.e., ≤50% $\dot{V}O_{2\,max}$) increases insulin and plasma glucose concentrations and maintains these variables at elevated levels throughout the exercise bout (1, 19, 30). In contrast, CHO supplementation during moderate-intensity exercise (i.e., 65–75% $\dot{V}O_{2\,max}$) only aids in the maintenance of plasma glucose and insulin concentrations (10).

It is not currently known whether the ingestion of a CHO supplement will alter the rate of decline in muscle glycogen during exercise of alternating intensity. However, on the basis of our previous research (30), we hypothesized that moderate-intensity exercise interspersed with low-intensity exercise would result in elevated plasma insulin and glucose levels, which in turn would increase reliance on blood glucose while reducing reliance on muscle glycogen. Therefore, we compared the effect of a 10% glucose polymer supplement and an artificially sweetened liquid placebo on muscle glycogen utilization and CHO metabolism during exercise that varied between low (45% $\dot{V}O_{2\,max}$) and moderate (75% $\dot{V}O_{2\,max}$) intensity.

It has previously been reported that blood glucose oxidation is unable to provide a sufficient amount of energy to support high-intensity work late in exercise (6). Coggan and Coyle (6) administered CHO supplements to subjects during cycling exercise that alternated between moderate (60% $\dot{V}O_{2\,max}$) and high (85% $\dot{V}O_{2\,max}$) intensity. They observed that the CHO supplements maintained blood glucose levels and CHO oxidation but were unable to support exercise at an intensity >75% $\dot{V}O_{2\,max}$ late in exercise. This finding suggested that adequate muscle glycogen stores must be available to support high-intensity exercise. Therefore, we further hypothesized that a reduction in muscle glycogen utilization resulting from the CHO feedings should translate into an extended time to fatigue during high-intensity work late in exercise. To test this hypothesis the exercise intensity was increased to 80% $\dot{V}O_{2\,max}$ after 200 min of the variable-intensity exercise, and time to fatigue was compared between the liquid CHO supplement and artificially sweetened liquid placebo treatments.

Lastly, it has been reported that a solid CHO supplement increases blood glucose and CHO oxidation and improves cycling sprint performance when compared with a water placebo (13, 16). However, we are not aware of any investigation that has directly assessed the meta-

bolic and ergogenic effects of a solid CHO supplement during a prolonged continuous exercise bout. Such an evaluation is warranted because many endurance athletes prefer a solid form of CHO as opposed to a liquid CHO supplement for reasons of satiety. Therefore, the secondary purpose of this study was to ascertain whether the consumption of a solid CHO supplement produces an ergogenic effect during prolonged continuous variable-intensity exercise.

METHODS

Subjects. The subjects were seven male competitive cyclists who were accustomed to cycling for prolonged periods (3–5 h). Their mean age was 23.0 ± 1.2 (SE) yr, and their mean body weight was 65.1 ± 1.7 kg. Before testing, all subjects were given a detailed explanation of the procedures to be used and the potential risks of the study, and all signed an informed consent form. The study was approved by the University's Institutional Review Board.

Experimental design. Each subject completed three randomly assigned treatments in which either an artificially flavored and colored water placebo (P), a 10.0% liquid CHO polymer (L; Performance, Shaklee US, San Francisco, CA), or a solid 25-g CHO (fructose-maltodextrin) source (S; Energy Bar, Shaklee US) was provided during exercise. Immediately before the S and L trials, the subjects received 1 g CHO/kg body wt of a 25% liquid CHO polymer (Performance, Shaklee US), whereas a similar volume of the liquid placebo was provided immediately before the P trial. The addition of a liquid CHO supplement before the ride was to maximize the glucose and insulin responses at the start of exercise and to ensure adequate CHO consumption (10). Preliminary testing indicated that the use of the solid CHO source in this manner caused gastrointestinal discomfort. During exercise, 180 ml of the P or L treatments were provided at 20 min of exercise and every 20 min thereafter. The S treatment was provided at 30 min of exercise and then every 30 min for the duration of the exercise bout. The subjects also ingested 270 ml of water with each bar during the S treatment to standardized the hourly fluid consumption among the experimental trials. In addition to the required fluid intake, the subjects were allowed ad libitum water consumption during the three experimental trials. The subjects performed each trial in a room maintained between 23 and 25°C at the same time of day and on the same day of the week over a 3-wk period.

Preliminary testing. Each subject reported to the laboratory before the start of the experiment for determination of their maximal aerobic power ($\dot{V}O_{2\,max}$) and lactate threshold. When the subjects reported to the laboratory, a catheter was inserted into an antecubital vein, fitted with a three-way stopcock, and taped in place. The subjects then mounted a Monark Ergomedic 829E bicycle ergometer equipped with clipless pedals (Look) and racing saddle and sat quietly for 5 min. The incremental test protocol consisted of 2-min stages that were set to elicit 40, 50, 60, 70, 80, 90, 100, and 110% of the subjects' estimated $\dot{V}O_{2\,max}$. Subjects breathed through a Daniel's valve, with expired gases directed to a mixing chamber

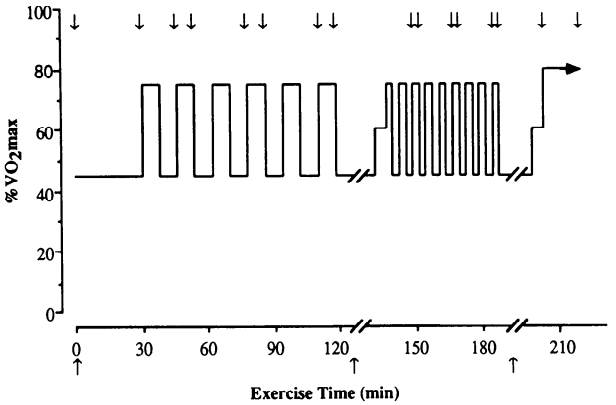

FIG. 1. Experimental protocol. ↓, Time of blood sample and measurement of O_2 consumption ($\dot{V}O_2$), respiratory exchange ratio, heart rate, and rate of perceived exertion (RPE) ($\dot{V}O_2$ and RPE not determined at rest, $\dot{V}O_2$ not determined at fatigue). ↑, Time of muscle biopsy. $\%\dot{V}O_{2\,max}$, percent maximal O_2 consumption.

for analysis of O_2 (Applied Electrochemistry S-3A/1, Ametek, Pittsburgh, PA) and CO_2 (Applied Electrochemistry CD-3A, Ametek). Inspired volumes were measured using a dry gas meter (Rayfield Equipment, Waitsfield, VT). Analog outputs from these instruments were directed to a laboratory computer for calculation of ventilation, O_2 consumption ($\dot{V}O_2$), CO_2 production, and respiratory exchange ratio (R) every 30 s. The criteria used to establish $\dot{V}O_{2\,max}$ were a plateau in $\dot{V}O_2$ with increasing exercise intensity and R > 1.10. The mean $\dot{V}O_{2\,max}$ for the group was 4.65 ± 0.1 l/min (71.4 ± 2.8 $ml \cdot kg^{-1} \cdot min^{-1}$).

Approximately 2 ml of venous blood were drawn while the subject was seated on the ergometer before the start of the incremental test and at the end of each 2-min stage. One-half milliliter of blood was deproteinized in 1 ml of cold 8% perchloric acid. The acid extract was recovered by centrifugation (1,000 *g* for 15 min) and subsequently used for the enzymatic analysis of lactate (17). Each subject's blood lactate threshold was determined by graphing the blood lactate concentration vs. $\dot{V}O_2$ and determining the percent $\dot{V}O_{2\,max}$ at which blood lactate started to accumulate (20). The mean lactate threshold for the group was 64.7 ± 3.2 $\%\dot{V}O_{2\,max}$.

One week after the $\dot{V}O_{2\,max}$ test the subjects reported to the laboratory to perform a practice ride to adjust and/or verify appropriate work rates for the experimental trials. No blood samples or tissue samples were collected during the practice ride. The cycling protocol is depicted in Fig. 1 and is a modification of the protocol used by Brouns (5) to simulate a stage of the Tour de France. The regimen consisted of cycling for 30 min at 45% $\dot{V}O_{2\,max}$, followed by six repeated 16-min periods composed of cycling for 8 min at 75% $\dot{V}O_{2\,max}$ and for 8 min at 45% $\dot{V}O_{2\,max}$. The subjects then dismounted the ergometer and sat quietly for 12 min. This period of time was determined to be of sufficient duration to perform a muscle biopsy and stop the bleeding before the subjects continued exercising. After the rest-biopsy period the subjects mounted the ergometer and cycled for 5 min at 45% $\dot{V}O_{2\,max}$ and then 5 min at 60% $\dot{V}O_{2\,max}$. A period of intervals then began that were composed of nine repeated 6-min bouts of cycling consisting of 3 min at 75% $\dot{V}O_{2\,max}$ and 3 min at 45% $\dot{V}O_{2\,max}$. After this sequence of intervals the subjects dis-

mounted the ergometer for the second 12-min rest-biopsy period. The subjects then mounted the ergometer and resumed cycling for 5 min at 45% $\dot{V}O_{2\,max}$, 5 min at 60% $\dot{V}O_{2\,max}$, and concluded at 80% $\dot{V}O_{2\,max}$ until fatigue. Fatigue was defined as the point when the subjects were unable to maintain a cycling cadence of 50 rpm. During the exercise bout, thermal stress was reduced by maintaining the laboratory temperature at 20–22°C and by directing two floor fans toward the subjects.

The subjects were instructed to maintain a training log and diet recall for 3 days before the practice session. These records were used to standardize the subjects' physical activity and diets on the days before each experimental trial. In addition, the subjects were instructed to maintain the same training schedule over the 3 wk of the experimental trials.

Experimental protocol. The subjects reported to the laboratory after a 12-h fast during which time they were allowed to consume only water. On reporting to the laboratory, body weight was obtained and a heart rate (HR) monitor (UNIQ Heartwatch model 8799, Computer Instruments, Hempstead, NY) was secured in place on the chest. A catheter was inserted into an antecubital vein, fitted with a three-way stopcock, and taped in place. The subjects mounted the ergometer and sat quietly for 10 min before the commencement of the test. The subjects then performed the above-described exercise protocol (Fig. 1). On conclusion of the exercise bout, body weight was recorded. Muscle biopsies were obtained from the vastus lateralis by the needle biopsy technique (2) for the P and L trials immediately before exercise, at 124 min of exercise, and at 190 min of exercise. The biopsies were obtained within the first 2 min of the subjects dismounting the cycle ergometer. Muscle biopsies were not obtained during the S trial.

The subjects were unaware as to whether they were on the P or L trial because the two treatments were similar in color, taste, and texture. However, it was not possible to administer the S treatment in a blinded manner. During each trial the subjects were not aware of their duration of riding or the time of day. Constant verbal encouragement to cycle to exhaustion was given to the subjects during the final exercise intensity (i.e., 80% $\dot{V}O_{2\,max}$). The results of the experiment were not discussed until all subjects had completed the three experimental trials.

Sample collection and analyses. Ventilation, $\dot{V}O_2$, CO_2 production, and R were recorded with the respiratory gas analysis system previously described. During the first 30 min of exercise, the 8-min interval stages, and the final exercise intensity (i.e., 80% $\dot{V}O_{2\,max}$), 5-min respiratory gas collection periods began at 5, 25, 41, 49, 73, 81, 105, 113, and 200 min of exercise. Respiratory gas collection periods were limited to 3 min during the 3-min interval stages. These 3-min collection periods began at 145, 148, 163, 166, 181, and 184 min of exercise.

HR was recorded immediately before the start of the exercise bout and throughout exercise at the time points indicated in Fig. 1. Subjective ratings of perceived exertion (3) were obtained during exercise as indicated in Fig. 1.

Five milliliters of venous blood were drawn while the subjects were seated on the ergometer immediately before the start of exercise and during exercise at the time points indicated in Fig. 1. Four milliliters of each blood sample were anticoagulated with 250 μl EDTA (24 mg/ml, pH 7.4), and the plasma was separated by centrifugation (1,000 *g*, 4°C). The plasma was split into equal portions and stored at −80°C for subsequent measurement of glucose, free fatty acids (FFA), and insulin. Plasma was analyzed for glucose concentration with a glucose analyzer (23A, Yellow Springs Instruments, Yellow Springs, OH) and for FFA according to Novack (25). The insulin concentration was measured by radioimmunoassay (Ref. 14; Radioassay System Laboratories, Carson, CA). One-half milliliter of blood was deproteinized in 1 ml of cold 8.0% perchloric acid and centrifuged (1,000 *g*, 4°C) for 15 min. The acid extract was then stored at −80°C for subsequent enzymatic analysis of lactate (17).

Percutaneous muscle biopsies were taken from the vastus lateralis and were divided into two pieces. One portion was quickly frozen in isopentane cooled in liquid N_2 and stored at −80°C until analyzed for glycogen. For glycogen determination, the biopsies were weighed and homogenized in a 50% glycerol-20 mM Na_2HPO_4 buffer (1:50 wt/vol, pH 7.4) that contained 0.5 mM EDTA, 0.02% bovine serum albumin, and 5 mM β-mercaptoethanol. Homogenization was performed over an ice-water slush. Two hundred microliters of the homogenate were added to 200 μl of 2 N HCl and incubated at 100°C for 120 min. The homogenate was cooled to room temperature and neutralized with 434 μl of 1 N NaOH. The muscle glycogen concentration was determined enzymatically (26).

The second piece of the biopsy sample was oriented in mounting media (OCT compound, Fisher Scientific, Pittsburgh, PA) and rapidly frozen in isopentane cooled to its freezing point in liquid N_2 and stored at −80°C. Serial sections (10 μm) were cut at −20°C in a cryostat and were stained for myosin adenosinetriphosphatase activity (pH 4.54) (4) and for glycogen via the periodic acid-Schiff (PAS) reaction (27). Sections from each biopsy sample were magnified, and the intensity of the PAS staining in the individual muscle fibers was rated visually on a scale of 1 (negative) to 5 (darkly stained). L and P biopsy sections from the same subject were always stained at the same time and in the same vessel. Each section contained an average of 226 ± 21 muscle fibers.

PAS stains were statistically analyzed by two procedures. First, the percentage of type I and II fibers stained a designated intensity (1 through 5) were compared between the P and L treatments. Second, a relative estimate of the glycogen concentration of the type I and II fibers for the P and L treatments was computed by multiplying the visual rating scale (i.e., 1 to 5) by the percentage of fibers that exhibited the corresponding staining intensity. For example, if 10% of the type I fibers for a particular muscle section exhibited a staining intensity of 5, then 10 was multiplied by 5 to obtain the weighted value of 50. This procedure was repeated for each of the staining intensities in the type I and II muscle fibers. The weighted values for the type I and II muscle fibers were then summed seperately for each subject. The sum of the weighted values was subsequently used to distingish differences between the P and L treatments in the type I and II muscle fibers.

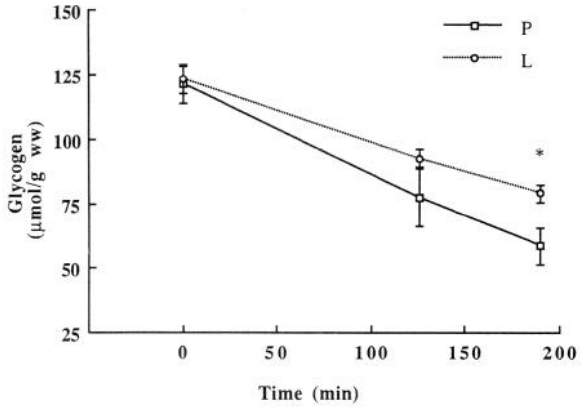

FIG. 2. Muscle glycogen concentration in vastus lateralis before exercise and at 126 and 190 min of exercise. L, 10% liquid carbohydrate (CHO) supplement; P, artificially sweetened liquid placebo. Values are means ± SE. ww, wet weight. * Significantly different from P ($P < 0.05$).

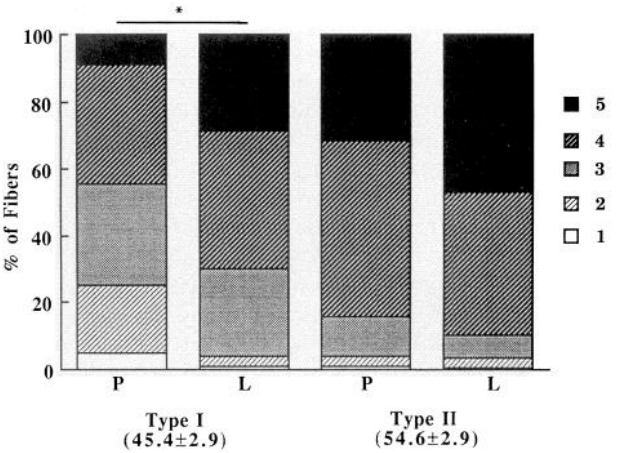

FIG. 3. Sections of muscle biopsies obtained from vastus lateralis at 190 min of exercise and stained for glycogen with periodic acid-Schiff reagent. Pattern of staining is displayed for both type I and II muscle fibers. Fiber type percentage is shown in parenthesis. Intensity of glycogen staining is rated on a scale of 1 (negative) to 5 (darkly stained). See METHODS for details. * Significantly different from corresponding treatment based on mean weighted values ($P < 0.01$).

Statistical analysis. A one-way analysis of variance was used to distinguish differences in time to fatigue among treatments. Two-way analysis of variance was used to statistically analyze all biochemical (treatment × time) and histochemical (treatment × staining intensity) variables. Significant differences between means were determined using Newman-Kuels post hoc test. Differences were considered significant if $P < 0.05$ was obtained.

RESULTS

Exercise performance. It was found that the S and L treatments enabled subjects to exercise for a significantly ($P < 0.05$) longer time than did the P treatment. Time to fatigue averaged 223.9 ± 3.5 and 233.4 ± 7.5 min for the S and L treatments, respectively. In contrast, the mean time to fatigue during the P treatment was 202.4 ± 9.8 min.

Glycogen concentration in the vastus lateralis muscle. Muscle glycogen did not differ between the P and L treatments before exercise and at 126 min of exercise (Fig. 2). However, at 190 min of exercise muscle glycogen levels were significantly greater during the L treatment than the P treatment (Fig. 2). Furthermore, it was observed that the difference in muscle glycogen levels between the P and L trials at 190 min of exercise was positively correlated ($r = 0.76$, $P < 0.05$) to the difference in time to fatigue between these trials.

As a result of the difference in muscle glycogen concentration between the P and L treatments at 190 min of exercise, PAS and myosin adenosinetriphosphatase stainings were performed to assess possible differences in response between type I and II muscle fibers (Fig. 3). On the basis of the weighted values for the PAS stains, it was found that the glycogen concentration of the type I fibers of the L treatment (393.3 ± 18.2 μmol/g) was significantly greater than that of the P treatment (314.5 ± 20.9 μmol/g) at 190 min of exercise ($P < 0.01$). However, no difference was observed between treatments for the type II muscle fibers (L: 431.6 ± 10.4 vs. 406.9 ± 10.1 μmol/g). The difference in muscle glycogen concentration in the type I muscle fibers was largely because of more fibers being stained lightly for glycogen (i.e., value of 2) during the P treatment (P: 24.7 ± 8.3% vs. L: 3.4 ± 1.0%; $P < 0.01$) and more fibers being stained darkly for glycogen (i.e., value of 5) during the L treatment (P: 7.4 ± 3.4% vs. L: 28.5 ± 10.2%; $P = 0.07$).

Plasma glucose, insulin, FFA, and blood lactate responses. As shown in Fig. 4, plasma glucose was similar among treatments before exercise, averaging 4.3 ± 0.1 mM. Over the first 30 min of exercise plasma glucose rose to 5.5 ± 0.3 mM during the S treatment and to 6.7 ± 0.7 mM during the L treatment but only slightly increased to 4.4 ± 0.1 mM during the P treatment. By 30 min of exercise the plasma glucose response during the L treatment was significantly greater than that which occurred during the P treatment. In addition, the L treatment maintained the plasma glucose concentration at a significantly greater level throughout exercise than the P treatment except at 151 and 200 min of exercise. An unanticipated finding was that even though a similar amount of CHO was administered during the S and L treatments the plasma glucose response of the L treat-

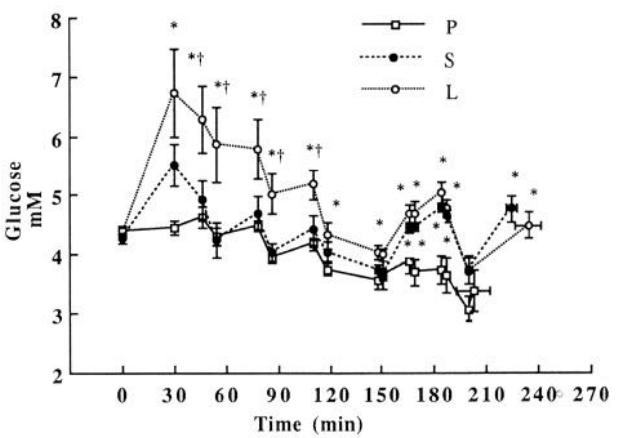

FIG. 4. Plasma glucose concentration during prolonged continuous variable-intensity exercise. S, solid CHO supplement. Values are means ± SE. * Significantly different from P ($P < 0.05$); † significantly different from S ($P < 0.05$).

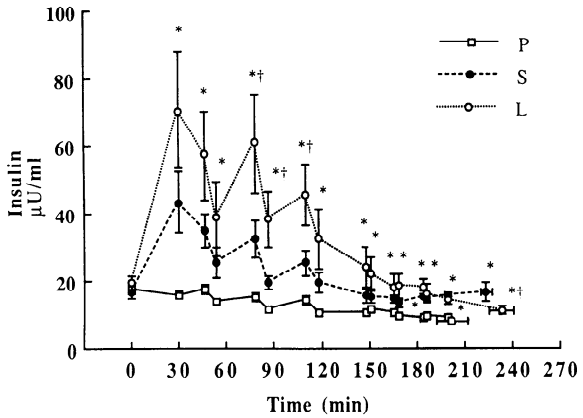

FIG. 5. Plasma insulin concentration during prolonged continuous variable-intensity exercise. Values are means ± SE. * Significantly different from P ($P < 0.05$); † significantly different from S ($P < 0.05$).

ment was significantly greater than that of the S treatment from 46 to 118 min of exercise. Furthermore, the plasma glucose response during the S treatment was similar to the P treatment except at 166, 169, 184, and 187 min of exercise and at fatigue.

In response to the increased plasma glucose during the L treatment, the plasma insulin concentration increased from 19.4 ± 2.3 μU/ml at rest to 70.6 ± 17.2 μU/ml after 30 min of exercise (Fig. 5). This plasma insulin concentration was significantly different from that of the P treatment (16.0 ± 1.3 μU/ml). During the remainder of the exercise bout, the plasma insulin response gradually declined but remained significantly greater than that of the P treatment at all time points. The plasma insulin response of the L treatment was also significantly greater than that of the S treatment at 78, 86, and 110 min of exercise. Plasma insulin levels did not differ between the P and S treatments except at 184 and 200 min of exercise and at fatigue.

Blood lactate was similar among treatments before exercise, averaging 0.8 ± 0.1 mM (Table 1). At 30 min of exercise blood lactate was ~1.1 mM for all treatments. Over the next 96 min of variable-intensity exercise the blood lactate concentration reflected the changing exercise intensity. During low-intensity exercise, blood lactate levels were near 1.6 mM, whereas moderate-intensity exercise produced a blood lactate concentration that was elevated to near 2.7 mM. During the second set of variable-intensity exercise (i.e., the next 64 min of exercise), the blood lactate concentration continued to reflect the changes in exercise intensity, but the magnitude of difference in blood lactate levels between the low and moderate exercise intensity was not as large compared with the previous 96 min of exercise. Most likely, this difference in blood lactate response was related to the shorter interval durations, which may not have provided sufficient time to allow blood lactate to fully accumulate in venous blood. No difference in blood lactate levels was found among treatments except at fatigue. At fatigue blood lactate was significantly elevated during the S (5.22 ± 0.68 mM) and L (5.05 ± 0.80 mM) treatments compared with the P treatment (3.00 ± 0.42 mM).

Plasma FFA concentrations were similar among treatments before exercise (Table 1). By 30 min of exercise the FFA levels declined during the S and L treatments, whereas the P treatment produced a slight rise in FFA concentration. During the P treatment, FFA levels increased throughout the exercise bout. However, the rise in FFA levels during the P treatment were influenced by exercise intensity. In general, during the P treatment, FFA levels rose during the low-intensity exercise intervals and declined slightly during the moderate-intensity intervals. In contrast, FFA levels remained relatively stable during exercise for the S and L treatments. Thus, the elevated FFA levels that occurred during the P treatment were significantly greater at all time points throughout exercise than those during S (except at 54 min of exercise) and L treatments.

$\dot{V}O_2$, R, and CHO oxidation. $\dot{V}O_2$ (Table 2) was similar among treatments during both the low- (~2.1 l/min) and moderate-intensity intervals (~3.4 l/min). Over time, $\dot{V}O_2$ did not increase more than 0.1 l/min during either the low or moderate exercise intensities. During the final high-intensity bout of exercise, $\dot{V}O_2$ was similar among treatments, averaging ~3.7 l/min.

R was not different among treatments at 10 min of exercise (Table 2). However, by 30 min of exercise R was significantly greater during the S (0.91 ± 0.01) and L (0.91 ± 0.01) treatments than during the P treatment (0.87 ± 0.01). R was found to be maintained at a significantly greater level throughout exercise during the L treatment than during the P treatment. In addition, the L treatment maintained R at a significantly greater level than did the S treatment at 151, 169, and 187 min of exercise. Over the first 126 min of exercise, R was significantly greater during the S treatment than during the P treatment but only during the moderate-intensity intervals. However, over the next 64 min of exercise R was greater during the S treatment than during the P treatment at all time points except at 187 min of exercise. During the final bout of high-intensity exercise, R was significantly greater during the S and L treatments than during the P treatment. $\dot{V}O_2$ and R were used to estimate the rate of CHO oxidation, which is presented in Table 2. Because $\dot{V}O_2$ was not different among treatments, the patterns of CHO oxidation generally paralleled the R responses.

Fluid consumption and weight loss during exercise. Fluid consumption did not differ among treatments. The average fluid consumption was 750.0 ± 47.5, 778.1 ± 47.0, and 809.4 ± 64.5 ml/h for the P, S, and L treatments, respectively. Similarly, the subjects' total and average hourly weight losses did not differ among treatments during exercise. Total body weight loss was 0.99 ± 0.16, 0.87 ± 0.15, and 0.99 ± 0.23 kg for the P, S, and L treatments, respectively. The average hourly weight loss was 0.30 ± 0.05, 0.23 ± 0.03, and 0.26 ± 0.06 kg/h for the P, S, and L treatments, respectively.

HR and perceived exertion. The exercise HR response was found to reflect the changes in exercise intensity. During the low-intensity exercise bout HR was near 130 beats/min, whereas during the moderate-intensity bout of exercise HR increased to ~160 beats/min. This pattern remained consistent throughout exercise, with no difference in the HR response being detected among treatments except at fatigue. At fatigue the HR response

TABLE 1. *Blood lactate and plasma FFA during prolonged continuous variable-intensity exercise*

	Time, min														
	Pre	30	46	54	78	86	110	118	148	151	166	169	184	187	200	End
								Lactate, mM								
P	0.82±0.14	0.89±0.12	1.35±0.15	2.86±0.36	1.42±0.20	2.80±0.39	1.31±0.14	2.37±0.20	1.47±0.12	2.07±0.12	1.58±0.10	2.20±0.25	1.43±0.13	2.07±0.21	1.28±0.07	3.00±0.42
S	0.77±0.13	1.21±0.12	1.66±0.28	3.20±0.51	1.73±0.26	2.87±0.34	1.63±0.28	2.34±0.22	1.27±0.10	1.59±0.13	1.36±0.13	1.71±0.21	1.35±0.12	1.80±0.14	1.04±0.09	5.22±0.68*
L	0.90±0.12	1.17±0.11	1.80±0.20	3.25±0.39	1.89±0.32	3.18±0.43	1.69±0.17	3.14±0.36	1.60±0.15	2.13±0.19	1.36±0.15	1.91±0.25	1.40±0.20	2.08±0.26	1.05±0.16	5.05±0.80*
								FFA, mM								
P	0.09±0.03	0.15±0.02	0.13±0.03	0.06±0.02	0.21±0.03	0.11±0.03	0.38±0.06	0.21±0.04	0.46±0.06	0.34±0.09	0.62±0.13	0.59±0.18	0.70±0.11	0.39±0.08	0.47±0.08	0.53±0.13
S	0.14±0.04	0.04±0.02*	0.04±0.02*	0.03±0.01	0.05±0.03*	0.03±0.01*	0.08±0.03*	0.05±0.02*	0.11±0.04*	0.05±0.02*	0.19±0.04*	0.11±0.04*	0.23±0.05*	0.16±0.04*	0.12±0.04*	0.09±0.03*
L	0.13±0.08	0.05±0.03*	0.02±0.01*†	0.01±0.01*	0.05±0.02*	0.04±0.02*	0.05±0.02*	0.02±0.01*	0.05±0.04*	0.03±0.02*	0.08±0.04*	0.04±0.03*	0.13±0.06*	0.06±0.04*	0.04±0.02*	0.06±0.04*

Values are means ± SE. FFA, free fatty acids; P, artificially sweetened liquid placebo; S, solid carbohydrate supplement; L, 10% liquid carbohydrate (CHO) supplement. * Significantly different from P ($P < 0.05$); † significantly different from S ($P < 0.05$).

TABLE 2. *$\dot{V}O_2$, R, and CHO oxidation during prolonged continuous variable-intensity exercise*

	Time, min														
	10	30	46	54	78	86	110	118	148	151	166	169	184	187	205
								$\dot{V}O_2$, l/min							
P	2.07±0.06	2.09±0.08	2.11±0.07	3.47±0.11	2.14±0.07	3.47±0.12	2.13±0.07	3.48±0.10	2.22±0.07	3.35±0.08	2.19±0.08	3.42±0.12	2.19±0.09	3.43±0.09	3.67±0.07
S	2.09±0.06	2.11±0.08	2.10±0.07	3.43±0.12	2.11±0.07	3.50±0.15	2.14±0.07	3.48±0.13	2.15±0.07	3.40±0.14	2.23±0.08	3.54±0.15	2.27±0.08	3.49±0.13	3.75±0.16
L	2.09±0.07	2.07±0.07	2.11±0.06	3.43±0.14	2.12±0.07	3.45±0.13	2.10±0.07	3.44±0.11	2.14±0.07	3.44±0.14	2.22±0.08	3.51±0.15	2.27±0.09	3.45±0.14	3.74±0.15
								R							
P	0.88±0.01	0.87±0.01	0.86±0.01	0.95±0.01	0.85±0.02	0.94±0.01	0.87±0.02	0.91±0.02	0.87±0.01	0.91±0.01	0.87±0.01	0.89±0.01	0.85±0.02	0.89±0.01	0.96±0.01
S	0.90±0.01	0.91±0.01*	0.89±0.01*	0.97±0.01*	0.88±0.01*	0.97±0.01*	0.88±0.01	0.96±0.01*	0.92±0.01*	0.94±0.01*	0.91±0.01*	0.92±0.01*	0.90±0.01*	0.90±0.01	0.99±0.01*
L	0.90±0.01	0.91±0.01*	0.90±0.01*	0.97±0.01*	0.90±0.01*	0.98±0.01*	0.90±0.01*	0.97±0.01*	0.94±0.01*	0.96±0.01*†	0.93±0.01*	0.94±0.01*†	0.92±0.01*	0.94±0.01*†	1.01±0.01*
								CHO oxidation, g/min							
P	1.50±0.04	1.39±0.06	1.28±0.07	3.42±0.11	1.25±0.11	3.36±0.17	1.46±0.16	2.92±0.24	1.46±0.09	2.74±0.06	1.44±0.08	2.58±0.16	1.28±0.16	2.58±0.21	3.78±0.11
S	1.70±0.08*	1.80±0.09*	1.60±0.10*	3.69±0.19	1.55±0.13	3.75±0.18	1.56±0.12	3.58±0.16*	1.87±0.08*	3.19±0.08*	1.84±0.06*	3.15±0.09*	1.81±0.07	2.85±0.19	4.44±0.23*
L	1.70±0.06*	1.74±0.07*	1.66±0.07*	3.74±0.21	1.66±0.07*	3.87±0.17*	1.66±0.10	3.79±0.16*	2.04±0.07*	3.58±0.11*†	2.07±0.12*	3.46±0.18	1.98±0.07*	3.30±0.17*	4.69±0.20*

Values are means ± SE. $\dot{V}O_2$, O_2 consumption; R, respiratory exchange ratio. * Significantly different from P ($P < 0.05$); † Significantly different from S ($P < 0.05$).

was significantly greater during the S (178 ± 2 beats/min) and L (181 ± 3 beats/min) treatments than during the P treatment (166 ± 5 beats/min).

The subjects did not perceive any difference in effort during exercise as a result of the treatments. However, the subjects' ratings of effort did reflect the alterations in exercise intensity. During low-intensity exercise, the subjects found the exercise to be relatively easy, although perceived exertion rose from ~9 units (very light) at 30 min of exercise to near 12 units (fairly light) by 184 min of exercise. On the other hand, the moderate-intensity exercise was perceived by the subjects to require an effort that elicited a rating of ~13 units (somewhat light) at 54 min of exercise, which then rose to near 15 units (hard) at 187 min of exercise. At fatigue the average rating of perceived exertion was just under 20 units (very very hard) for all treatments.

DISCUSSION

In the present investigation we observed that the L treatment reduced the rate of decline in muscle glycogen concentration during exercise compared with the P treatment. Muscle glycogen concentration during the L treatment was slightly greater than but not significantly different from the P treatment at 126 min of exercise. However, by 190 min of exercise the L treatment had maintained muscle glycogen at a concentration that was ~35% greater than that of the P treatment. We have previously reported that the ingestion of liquid CHO supplements during low-intensity exercise (50% $\dot{V}O_{2\,max}$) results in a sparing of muscle glycogen (30). These findings are in contrast to those of recent investigations that have demonstrated that CHO supplementation had no influence over muscle glycogen utilization during moderate-intensity exercise (70% $\dot{V}O_{2\,max}$) (10, 15).

These differences in muscle glycogen utilization in response to CHO supplementation during exercise are possibly due to differences in plasma glucose and insulin responses. During moderate-intensity exercise CHO supplementation prevents the decline in plasma glucose and insulin concentrations (10), whereas during low-intensity exercise plasma glucose and insulin levels become elevated (1, 19, 30). In the present investigation, we found that the L treatment rapidly increased plasma glucose and insulin levels by 30 min of exercise and continued to maintain these variables at a greater concentration throughout exercise than those of the P treatment. Most noteworthy, however, were the magnitudes of increase in the plasma glucose and insulin concentrations in response to the L treatment. The L treatment increased plasma glucose levels to 6.7 ± 0.7 mM and insulin concentrations to 70.6 ± 17.2 μU/ml by 30 min of exercise and prevented plasma glucose and insulin levels from falling below 5.0 mM and 30 μU/ml, respectively, over the following 80 min of exercise. These are the highest plasma insulin and glucose concentrations in response to CHO feedings during exercise that we are aware of having been reported.

In our present and previous investigations (30), we observed that plasma insulin and glucose levels became rapidly elevated in response to CHO feedings and contributed to reducing the decline in exercise muscle glycogen utilization. Our previous investigation utilized continuous low-intensity exercise, whereas our present investigation was composed of continuous variable low- to moderate-intensity exercise. It appears that these types of activities, as opposed to continuous moderate-intensity exercise, allow CHO feedings to generate hyperinsulemia and hyperglycemia of a sufficient magnitude to reduce exercise muscle glycogenolysis. Furthermore, elevations in both plasma glucose and insulin levels seem necessary to reduce exercise muscle glycogen utilization, since hyperglycemia alone does not appear to be a sufficient stimulus. Coyle et al. (12) reported that the maintenance of blood glucose levels at 10 mM by glucose infusion did not reduce exercise muscle glycogenolysis during 2 h of continuous moderate-intensity exercise (73% $\dot{V}O_{2\,max}$). These investigators observed that plasma insulin levels were similar during the glucose infusion and control trials until 60 min of exercise. In addition, peak insulin levels that reached only 24 μU/ml were not achieved until 80 min of exercise during the glucose infusion trial and then were observed to decline over the last 40 min of exercise.

The reduced decline in exercise muscle glycogen utilization that was observed during the L treatment appeared to result from muscle glycogen sparing. Histochemical analysis of the muscle biopsies obtained at 190 min of exercise indicated that the L treatment, compared with the P treatment, substantially reduced muscle glycogen utilization in the type I but not the type II muscle fibers. The low-intensity exercise utilized in the present investigation would be expected to recruit primarily type I muscle fibers, whereas the moderate-intensity exercise would be expected to recruit both type I and type II muscle fibers. It has been suggested that muscle glycogen synthesis can occur during low-intensity exercise if the muscle is glycogen depleted and if CHO is ingested (8, 22); however, this synthesis is restricted to nonactive muscle fibers (i.e., fast-twitch fibers) (22). Because the concentration of glycogen in the type II muscle fibers at 190 min of exercise did not differ between treatments, this would suggest that muscle glycogen synthesis did not account for the difference in muscle glycogen levels between treatments. Rather, it is likely that the greater concentration of muscle glycogen at 190 min of exercise during the L treatment was due primarily to muscle glycogen sparing, since the type I muscle fibers were probably active throughout the exercise bout. This hypothesis is consistent with our previous investigation (30) in which we reported that CHO feedings reduced the decline in muscle glycogen during low-intensity exercise by reducing the rate of muscle glycogen utilization in slow-twitch muscle fibers (type I muscle fibers).

The L treatment was found to significantly increase time to fatigue compared with the water placebo. This finding is in agreement with a number of previous investigations that have documented that the ingestion of CHO supplements during prolonged exercise can compensate for the reduction of endogenous CHO stores and delay the onset of fatigue (6, 7, 10, 11, 16, 19, 29). The manner by which CHO feedings delay the onset of fatigue

during prolonged continuous moderate-intensity exercise is by preventing hypoglycemia and maintaining CHO oxidation rather than by reducing the decline in muscle glycogen levels (10, 15, 28). However, Coggan and Coyle (6) have reported that blood glucose oxidation cannot fully support high-intensity work late in exercise when muscle glycogen stores are presumed to be depleted. We found that after 200 min of the variable-intensity exercise the subjects were only capable of performing ~2 min of exercise at a very high exercise intensity (80% $\dot{V}O_{2\,max}$) during the P treatment. In contrast, the L treatment allowed subjects to exercise for ~33 min at 80% $\dot{V}O_{2\,max}$. Because it has previously been reported that blood glucose oxidation cannot support exercise at an intensity >75% $\dot{V}O_{2\,max}$ late in exercise (6), it is unlikely that the L treatment would have been capable of significantly extending time to fatigue during exercise at 80% $\dot{V}O_{2\,max}$ without adequate muscle glycogen stores. Thus, the substantial increase in time to fatigue at a very intense work load late in exercise would further support our observation that muscle glycogen utilization was reduced during the first 190 min of the L treatment.

A second purpose of this investigation was to examine whether consuming a solid CHO supplement during prolonged continuous variable-intensity exercise would provide an ergogenic effect. Previous investigations have compared the effects of solid CHO feedings with a water placebo with respect to exercise metabolism and sprint performance (13, 16). However, we are not aware of any investigations that have examined the effect of solid CHO feedings on exercise performance or metabolism during prolonged continuous variable-intensity exercise.

We observed that the S treatment was capable of significantly improving time to fatigue during prolonged continuous variable-intensity exercise. Time to fatigue was increased by ~24 min during the S treatment compared with the P treatment. In addition, no statistical difference was found for time to fatigue between the S and L treatments. This is the first investigation to report that solid and liquid CHO supplements are similar in their ability to delay the onset of fatigue during prolonged continuous variable-intensity exercise compared with a water placebo.

During exercise we observed that the plasma glucose, plasma insulin, and total CHO oxidation responses during the S treatment were intermediate to those that occurred during the P and L treatments. It is possible that these intermediate responses were due to the S treatment being administered in a solid rather than a liquid form. However, it is more likely that the reduced plasma glucose and insulin responses that occurred during the S treatment compared with those occurring during the L treatment were due to the type of CHO in the S treatment being comprised predominantly of fructose as opposed to maltodextrin. This is in agreement with the findings of Murray et al. (24), who reported that ingestion of a 6% fructose solution produced lower plasma glucose and serum insulin levels and a reduced rate of CHO oxidation than did ingestion of a 6% glucose solution in humans during 115 min of intermittent cycling exercise. CHO type, caloric content, and solute osmolality have been reported to influence the rate of gastric emptying (9). Furthermore, it has been observed that the rate of intestinal absorption for glucose polymers is more rapid than that for glucose (21), whereas fructose is absorbed from the intestine at a much slower rate than is glucose (18). Thus, it is possible that the difference in plasma glucose and insulin responses between the L and S treatments was due to differences in the rate of intestinal absorption of the supplements.

In summary, we observed that the S and L treatments significantly delayed the onset of fatigue during prolonged continuous variable-intensity exercise compared with the P treatment. In response to the S and L treatments, plasma glucose, insulin, and CHO oxidation were elevated during exercise compared with the P treatment. However, the plasma glucose and insulin responses that occurred during the S treatment were intermediate to those of the P and L treatments. The difference in plasma glucose and insulin responses between the S and L treatments were likely related to the composition of the CHO supplements. Furthermore, it was observed that the L treatment significantly reduced the rate of decline in muscle glycogen utilization compared with that of the P treatment and could account for ~60% of the improved time to fatigue during the L treatment. The reduced rate of decline in muscle glycogen during the L treatment most likely resulted from the elevated plasma glucose and insulin levels that occurred during the variable low- to moderate-intensity exercise. These results suggest that CHO supplementation can enhance aerobic endurance during prolonged continuous exercise of varying low to moderate intensity in part by reducing dependency on muscle glycogen as a fuel source.

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