Muscle glycogen utilization during prolonged strenuous exercise when fed carbohydrate

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COYLE, EDWARD F., ANDREW R. COGGAN, MARI K. HEMMERT, AND JOHN L. IVY. *Muscle glycogen utilization during prolonged strenuous exercise when fed carbohydrate.* J. Appl. Physiol. 61(1): 165–172, 1986.—The purpose of this study was to determine whether the postponement of fatigue in subjects fed carbohydrate during prolonged strenuous exercise is associated with a slowing of muscle glycogen depletion. Seven endurance-trained cyclists exercised at 71 ± 1% of maximal O_2 consumption ($\dot{V}O_{2\ max}$), to fatigue, while ingesting a flavored water solution (i.e., placebo) during one trial and while ingesting a glucose polymer solution (i.e., 2.0 g/kg at 20 min and 0.4 g/kg every 20 min thereafter) during another trial. Fatigue during the placebo trial occurred after 3.02 ± 0.19 h of exercise and was preceded by a decline ($P < 0.01$) in plasma glucose to 2.5 ± 0.5 mM and by a decline in the respiratory exchange ratio (i.e., R; from 0.85 to 0.80; $P < 0.05$). Glycogen within the vastus lateralis muscle declined at an average rate of 51.5 ± 5.4 mmol glucosyl units (GU)·kg^{-1}·h^{-1} during the first 2 h of exercise and at a slower rate ($P < 0.01$) of 23.0 ± 14.3 mmol GU·kg^{-1}·h^{-1} during the third and final hour. When fed carbohydrate, which maintained plasma glucose concentration (4.2–5.2 mM), the subjects exercised for an additional hour before fatiguing (4.02 ± 0.33 h; $P < 0.01$) and maintained their initial R (i.e., 0.86) and rate of carbohydrate oxidation throughout exercise. The pattern of muscle glycogen utilization, however, was not different during the first 3 h of exercise with the placebo or the carbohydrate feedings. The additional hour of exercise performed when fed carbohydrate was accomplished with little reliance on muscle glycogen (i.e., 5 mmol GU·kg^{-1}·h^{-1}; NS) and without compromising carbohydrate oxidation. We conclude that when they are fed carbohydrate, highly trained endurance athletes are capable of oxidizing carbohydrate at relatively high rates from sources other than muscle glycogen during the latter stages of prolonged strenuous exercise and that this postpones fatigue.

endurance; physical performance; fatigue; glucose oxidation; athletes; diet

WE HAVE RECENTLY REPORTED that carbohydrate feedings during prolonged strenuous exercise (i.e., 74% of maximal O_2 uptake) can delay the development of fatigue (15). Although muscle glycogen data were not obtained in that study, we suggested, based upon the work of Bergström and Hultman (7) and Bagby et al. (5), that carbohydrate administration may result in increased utilization of blood glucose with a proportional slowing of muscle glycogen depletion.

The purpose of this study was to directly measure muscle glycogen utilization during strenuous exercise with and without carbohydrate feedings to determine whether muscle glycogen sparing can explain the postponement of fatigue. Our first approach in the present study was simply to quantify glycogen utilization during 105 min of cycling when subjects were fed carbohydrates and when they fasted. After observing no difference in glycogen utilization during these nonfatiguing bouts of exercise, we proceeded to quantify glycogen utilization and carbohydrate oxidation during the latter stages of prolonged exercise performed to fatigue when subjects fasted and were fed carbohydrates.

METHODS

Initial Study of Glycogen Utilization During 105 min of Exercise With and Without Carbohydrate Feedings

Our first study measured glycogen utilization following 105 min of exercise at 71.4 ± 0.8% of maximal O_2 uptake ($\dot{V}O_{2\ max}$) with and without carbohydrate feedings. These exercise trials employed a different group of five subjects, from the subsequent study described below, which exercised seven subjects to the point of fatigue. The specific methodology of this initial study is only briefly described at this point because it followed the same general procedures as employed during the first 105 min of exercise in the bouts performed to fatigue, which are described in detail below. The mean (±SE) age, weight, and $\dot{V}O_{2\ max}$ were 26 ± 2 yr, 71.9 ± 1.7 kg, and 4.67 ± 0.14 l/min, respectively, for this first group of five endurance-trained cyclists. The ordering of the carbohydrate and placebo trials was randomized in this first study and performed at 1-wk intervals.

Exercise Bouts Performed to Fatigue

Subjects. The subjects in these trials were seven endurance-trained male cyclists. Their mean (±SE) age, weight, and $\dot{V}O_{2\ max}$ were 28 ± 1 yr, 67.6 ± 1.5 kg, and 4.72 ± 0.18 l/min, respectively. These subjects were chosen because they were highly motivated and accustomed to exercising for prolonged periods (2–4 h). The nature and risks of the experimental procedures were explained in detail, and written informed consent was

obtained. This study was approved by the Human Studies Committee of the University of Texas.

Preliminary testing. All exercise tests were performed using an electrically braked Quinton model 845 cycle ergometer equipped with toe clips and straps. $\dot{V}O_{2\ max}$ was first determined using an incremental cycling protocol, and within 1 wk of this test, all subjects completed a practice trial (2–2.5 h of cycling at 70–75% $\dot{V}O_{2\ max}$) to familiarize themselves with the experimental procedures.

Experimental design. The work rate was adjusted to elicit 70–74% of $\dot{V}O_{2\ max}$, which was slightly below subjects' blood lactate threshold (14), and was kept constant for each subject across all trials. The laboratory temperature was maintained at 21°C, and subjects were cooled with a fan during exercise. During the first experimental trial, subjects ingested 4 ml/kg body wt of a cold aspartame-sweentened and lemon-flavored solution at 20-min intervals throughout the exercise period (i.e., placebo). After completing 2 h of exercise, an 8-min rest period was allowed and a muscle biopsy was performed. The subjects then continued exercising until they were fatigued, as defined by their inability to maintain the required work rate which is independent of pedaling frequency on this ergometer. At this time (~3 h) another muscle biopsy was performed within 5 min of the cessation of exercise.

The carbohydrate feeding trial was performed 1 wk later and followed the same exercise protocol as in the previous placebo trial. However, in this trial, the subjects ingested 2 g/kg body wt of a glucose polymer (Polycose, Ross Laboratories, Columbus, OH) in a 50% solution during the 20th min of exercise and 0.4 g/kg body wt in a 10% solution every 20 min thereafter. These solutions were also lemon flavored and sweetened with aspartame in an attempt to make them indistinguishable from the placebo solution. Muscle biopsies were performed prior to exercise, after 2 h of exercise, at the time that subjects fatigued during the placebo trial (i.e., ~3 h), and at the time of fatigue during the carbohydrate feeding trial (i.e., ~4 h). Subjects were kept naive to the experimental design and the exercise time beyond 2 h. Samples of blood and expired gases and ratings of perceived exertion (10) were collected every 20 min throughout exercise and at the times of fatigue.

A random sequencing of trials was not possible, since this study was designed to compare muscle glycogen utilization when subjects were fed carbohydrate after completing an exercise bout that resulted in fatigue during the previous placebo trial. Therefore, to examine a possible sequence effect on exercise time to fatigue, five of the seven subjects performed an additional placebo trial, either 1 wk before the experimental trials ($n = 3$) or 1 wk after the experimental trials. Identical procedures were employed as in the experimental placebo trial, with the exception that muscle biopsies were not performed.

The subjects were instructed to maintain a generally constant diet and training schedule throughout the experimental period, especially during the 2 days prior to each exercise bout. For all trials, subjects reported to the laboratory in the morning after a 16-h fast.

Tissue sampling and analysis. Blood samples were obtained every 20 min throughout exercise and just prior to fatigue from a Teflon catheter placed in an antecubital vein. The catheter was kept patent by frequent flushing with sterile saline. Three milliliters of each blood sample were placed in tubes containing 0.15 ml of ethylenediaminetetraacetic acid (EDTA) solution and stored on ice until the end of the exercise bout. This sample was then centrifuged at 4°C, and the separated plasma was used for measurement of glucose (Yellow Springs Instruments model 23A glucose analyzer) and free fatty acids (FFA) (28). Another milliliter of blood was deproteinized in cold 8% perchloric acid, and the supernatant fluid was used for the enzymatic determination of lactate (22) and glycerol (18). Insulin was determined on samples taken at rest, after 20 min, 1, 2, 3 h (when applicable) of exercise, and at fatigue. Three milliliters of blood were placed in tubes containing an aprotinin solution; plasma was separated by centrifugation at 4°C and stored at −80°C. The insulin concentration was subsequently measured by radioimmunoassay (20) (Radioassay System Laboratories, Carson, CA, no. 134).

Muscle biopsies were taken from the vastus lateralis using the needle-biopsy technique. One portion of the sample was quickly frozen in liquid N_2 and stored at −80°C. For glycogen determination, this frozen sample was divided into 2 to 3 pieces (10–20 mg each), weighed, hydrolyzed (2 h in 2 N HCl at 100°C) and neutralized with NaOH, and the glucose concentration of the hydrolysate determined enzymatically (25). Another portion of the biopsy sample was oriented in mounting media (Oct, Tissue Tech) and rapidly frozen in isopentane maintained at its freezing point with liquid N_2. Cryostat sections (10 μm) were cut at −20°C. Serial sections were stained for myosin adenosinetriphosphatase activity (pH 4.3) (11) and for glycogen via the periodic acid-Schiff (PAS) reaction (29). Sections from each biopsy sample were magnified (×90), and the intensity of the PAS staining in approximately 250 type I fibers and 150 type II fibers were rated visually on a scale of 0 (negative) to 4 (darkly stained). Capillaries were visualized by PAS staining following pretreatment with 1% amylase (4). Capillary density (per mm^2), capillaries per fiber, and the mean number of capillaries around each fiber were determined by projecting numerous artifact-free sections as previously described (16).

Measurement of gas exchange. $\dot{V}O_2$ and R were determined for a 3-min period beginning at the 8th min of exercise and then for 3 min at the beginning of every 20-min period thereafter. Subjects breathed through a Daniel's valve while inspired volumes were measured using a dry gas meter (Parkinson-Cowan CD4). Expired gases were continuously sampled from a mixing chamber and analyzed for O_2 (Applied Electrochemistry S3A) and CO_2 (Beckman LB-2). Outputs from these instruments were directed to a laboratory computer for calculation of $\dot{V}O_2$ and R. The gas analyzers were calibrated against gases analyzed by the micro-Scholander method, and the dry gas meter was calibrated against a Tissot spirometer.

Statistical analysis. Data were analyzed using a two-way (treatment by time) analysis of variance for repeated

measures. Significant differences were located using Tukey's post hoc analysis.

RESULTS

We first studied five subjects during 105 min of cycling at 71.4 ± 0.8% of $\dot{V}O_{2\,max}$, with and without the carbohydrate feedings. As shown in Table 1, muscle glycogen utilization was very similar during the two conditions. With this information, we proceeded to measure glycogen utilization during the latter stages of exercise performed to fatigue, in a separate group of seven subjects. The metabolic responses of the two groups were practically identical, and therefore only the results from the group who exercised to fatigue are reported.

Exercise Times to Fatigue

Mean exercise time to fatigue in subjects was 33% longer when fed carbohydrate compared with when they were fed the placebo solution (i.e., 3.02 ± 0.19 h vs. 4.02 ± 0.33 h; $P < 0.01$). All seven subjects showed improvements in performance which ranged from 21 to 149 min, when fed carbohydrates. In the five subjects who performed two placebo trials, the mean difference (± SE) in time to fatigue was only 7 ± 9 min. After fatiguing during the placebo trial, several subjects were also encouraged to continue exercising after resting 10 min, during which time the final muscle biopsy was obtained. Invariably, they were unable to continue exercising at their previous work rate for more than 5 min. Thus the 60 ± 16 min improvement ($P < 0.01$) in performance time can be confidently ascribed to the effects of the carbohydrate feedings rather than to sequencing of the trials or to the short rest period allowed by the third biopsy.

Plasma Glucose and Insulin Responses

As shown in Fig. 1*A*, plasma glucose began declining during the 2nd h of exercise when subjects received the placebo feedings, and it continued to decline from 3.4 to 2.5 mM during the 3rd and final hour of exercise. None of the subjects experienced subjective symptoms of hypoglycemia (i.e., overall weakness, lightheadedness, or nausea). When subjects received the carbohydrate feedings, plasma glucose concentration was maintained at 4.2–5.2 mM throughout the exercise period and was significantly elevated ($P < 0.01$) above placebo from 80 min to the end of exercise.

Preexercise plasma insulin concentration was low in these men following the overnight fast (Table 2). Plasma insulin concentrations were identical during the two trials immediately prior to the first feeding (i.e., at 20 min). Plasma insulin concentration was elevated only 1.4 μU/ml when fed compared with fasted ($P < 0.05$ for pooled data at 1, 2, and 3 h), which is a small physiological difference.

TABLE 1. *Initial study of muscle glycogen utilization after 105 min of cycling with and without carbohydrate feeding*

	Preexercise	Following 105 min of Cycling	Δ
Placebo	116.5±7.7	42.1±3.7	74.4±9.5
Carbohydrate	120.8±8.4	43.4±8.2	77.4±7.2

Values are means ± SE for 5 subjects. Exercise intensity averaged 71.4 ± 0.8% of maximal O_2 consumption. Values under Δ represent the difference between pre- and postexercise values.

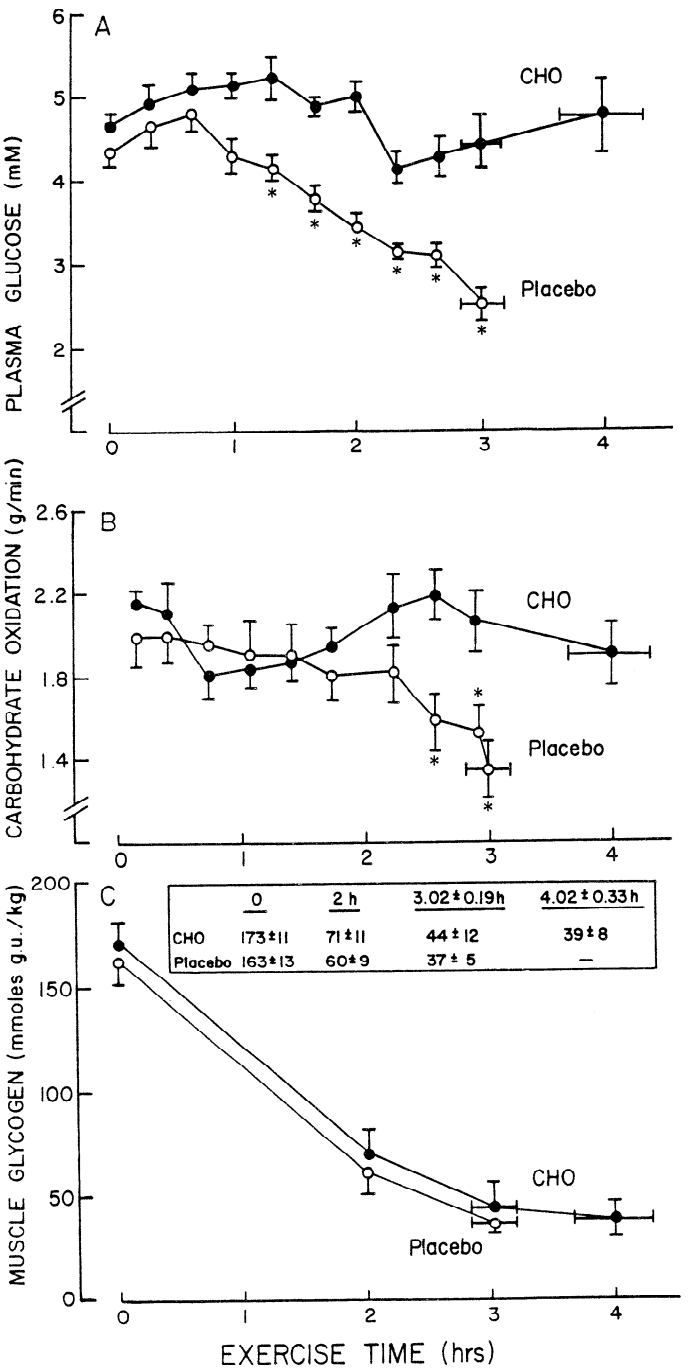

FIG. 1. Responses during exercise with the placebo solution and when carbohydrate was ingested every 20 min (CHO). *A*: plasma glucose responses. *B*: rate of carbohydrate oxidation estimated from O_2 uptake and respiratory exchange ratio. *C*: glycogen concentration, reported both graphically and numerically, measured in the vastus lateralis muscle. * Placebo significantly lower than carbohydrate; $P < 0.05$.

O_2 Uptake and Respiratory Exchange Ratio

Exercise elicited an average $\dot{V}O_2$ of 3.3 ± 0.2 l/min (i.e., 71.3 ± 1.3% of $\dot{V}O_{2\,max}$) after 10 min of exercise in

both trials and did not increase by more than 0.1 l/min throughout the remainder of the exercise. Respiratory exchange ratio (R) remained stable at 0.86–0.84 during the first 2 h of exercise with the placebo but began to decline ($P < 0.05$) during the 3rd h to a value of 0.80 ± 0.01 at fatigue (Table 2). In contrast, R was maintained at 0.84–0.87 throughout the entire 4-h period of exercise when subjects were receiving the carbohydrate feedings. $\dot{V}O_2$ and R were used to estimate the rate of carbohydrate oxidation, as presented in Fig. 1*B*. The patterns of carbohydrate oxidation are reflective of R, since $\dot{V}O_2$ was constant during the exercise tests.

Glycogen Concentrations in Vastus Lateralis Muscle

Muscle glycogen utilization with and without carbohydrate feedings is displayed in Fig. 1*C*. Muscle glycogen concentration was similar at the onset of exercise in the two trials, and the average rates of glycogen utilization during the first 2 h of exercise were nearly identical (i.e., 103 vs. 102 mmol glucosyl units (GU)·kg^{-1}·2 h^{-1} for placebo and carbohydrate, respectively). This agrees with our initial finding following 105 min of exercise. Muscle glycogen concentration continued to decline from 2 h to the time of fatigue during the placebo trial (i.e., 3.02 ± 0.19 h); however, the rate of decline was markedly slowed in both trials ($P < 0.01$; Fig. 1*C*). Muscle glycogen at fatigue during the placebo trial averaged 37 ± 5 mmol GU/kg. After performing the same amount of work while they were fed carbohydrate, the subjects experienced a muscle glycogen concentration not significantly different from placebo and averaged 44 ± 12 mmol GU/kg. The additional 60 ± 16 min of exercise performed before fatiguing when subjects were fed carbohydrates was accomplished without a significant reduction in glycogen within the vastus lateralis (i.e., 44 ± 12 to 39 ± 8 mmol GU/kg during the 3.02 ± 0.19 to 4.02 ± 0.33 h of exercise when subjects were fed carbohydrates; Fig. 1*C*).

The muscle sections subjected to PAS staining indicated that the pattern of glycogen utilization during the first 3 h of exercise was not appreciably different when comparing the two trials (Fig. 2). In agreement with the observation that glycogen concentration was similar in whole muscle biopsy samples obtained after 3 h of exercise during the placebo trial (i.e., fatigue) compared with those obtained after 3 and 4 h (i.e., fatigue) of exercise when fed (Fig. 1*C*), the mean distribution of PAS staining intensities for these three time points were also very similar (Fig. 2). At these times (i.e., 3- to 4-h periods), the majority of type I fibers and ~50% of all fibers appeared to contain little glycogen (i.e., negative or very light PAS stain). It can also be observed that few fibers stained dark for PAS, whereas all fibers from the preexercise samples were PAS dark (not shown). The slight (NS) increase in darkly stained fibers at fatigue when subjects were fed carbohydrates can be attributed to two subjects who also displayed a corresponding increase in whole muscle glycogen concentration. This suggests that, in these two subjects, the apparent glycogen synthesis that occurred during exercise when fed carbohydrate was a result of a larger increase in glycogen within some fibers and not a proportional increase in all fibers.

Plasma Free Fatty Acids and Blood Glycerol

As shown in Fig. 3, plasma FFA concentration began to increase rapidly during the 2nd h of the placebo trial, whereas the carbohydrate feedings resulted in a marked blunting of this response. FFA concentration was thus 35–70% lower ($P < 0.05$) from 80 min of exercise to fatigue when carbohydrates were ingested. The pattern of increase in blood glycerol and the difference between trials was generally similar to that described for plasma FFA (Fig. 3). The slight drop in plasma glucose (Fig. 1*A*), FFA, and blood glycerol (Fig. 3) observed after 2 h of exercise was probably related to the short exercise pause required for muscle sampling.

Perceived Exertion, Blood Lactate, and Heart Rate

Perceived exertion increased gradually and was not different during the first 2.5 h of exercise when subjects were fed carbohydrate or the placebo solution. As shown in Fig. 4, perceived exertion increased rapidly after 2.5 h of exercise in the placebo trial, and just prior to fatigue it was significantly elevated ($P < 0.05$) above that reported after 175 min of exercise when subjects were fed carbohydrate. The increase in perceived exertion from 2.5 h to fatigue during the carbohydrate trial occurred less rapidly.

As shown in Table 2, blood lactate tended to decline

TABLE 2. *Responses to exercise with placebo and carbohydrate feedings*

		0	20 min	1 h	2 h	3 h[a]	4 h[b]
Insulin, μU/ml	Placebo	7.7±1.3	6.7±1.0	6.5±0.8	5.9±0.9	5.6±0.8	
	Carbohydrate[c]	7.9±1.0	6.7±0.9	7.9±1.2	7.3±1.0	7.0±1.9	6.9±1.4
Blood lactate, mM	Placebo	0.7±0.1	1.5±0.1	1.2±0.1	1.2±0.1	2.0±0.2[d,e]	
	Carbohydrate	0.9±0.2	1.6±0.1	1.3±0.1	1.3±0.1	1.2±0.1	1.6±0.3[e]
Heart rate, beats/min	Placebo		146±3	150±3	152±3	157±3	
	Carbohydrate		147±4	150±4	153±3	159±3	164±3
R	Placebo		0.86±0.01	0.85±0.01	0.84±0.01	0.80±0.01[d,e]	
	Carbohydrate		0.87±0.01	0.85±0.01	0.86±0.01	0.86±0.01	0.85±0.01

Values are means ± for 7 subjects. R, respiratory exchange ratio; data were collected within 8 min of the times indicated. [a] These values were obtained at the time of fatigue during the placebo trial, which actually averaged 3.02±0.19 h. [b] These values were obtained at the time of fatigue during the carbohydrate trial, which actually averaged 4.02±0.33 h. [c] Pooled insulin values at 1, 2, and 3 h are significantly higher ($P < 0.05$) with carbohydrate compared with placebo. [d] Placebo significantly different from carbohydrate, $P < 0.05$. [e] Values when fatigued significantly different from previous values and different from the value when fatigued in the other trial, $P < 0.05$.

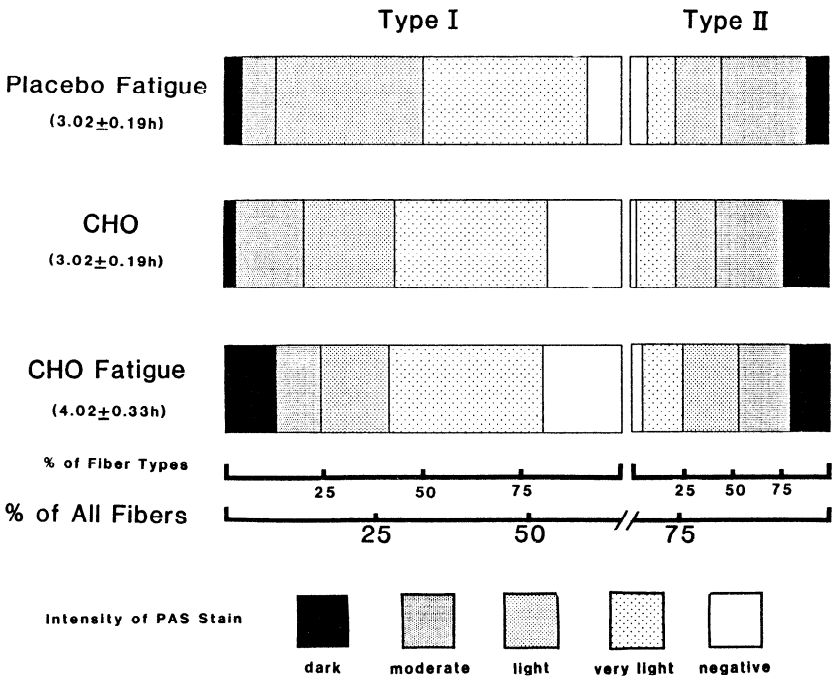

FIG. 2. Histochemical estimation of glycogen concentration in muscle sections stained with periodic acid-Schiff (PAS) reagent. Samples were obtained at fatigue during placebo, during exercise with carbohydrate feedings (CHO) after completing the same amount of exercise that resulted in fatigue during placebo trial, and at fatigue during carbohydrate trial. Pattern of staining is displayed in both type I and II muscle fibers.

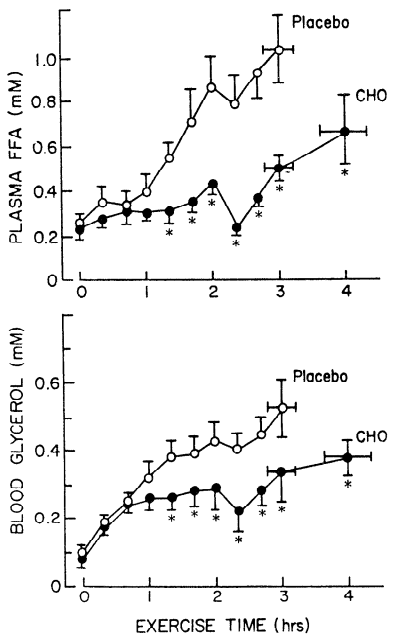

FIG. 3. Plasma free fatty acid and blood glycerol response to exercise with placebo solution and when carbohydrate was ingested every 20 min (CHO). Exercise was interrupted at 2 h and at 3.02 ± 0.19 h for muscle sampling. * Carbohydrate significantly lower than placebo; $P < 0.05$.

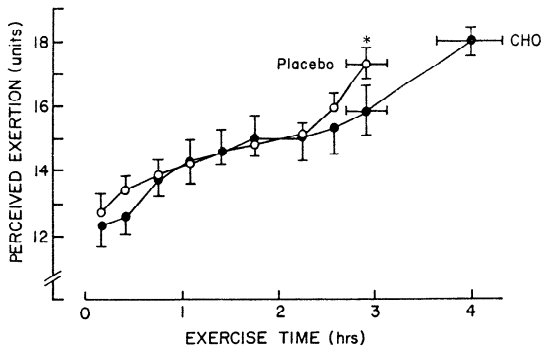

FIG. 4. Rating of perceived exertion during exercise with the placebo solution and when carbohydrate was ingested every 20 min (CHO). * Placebo significantly higher than carbohydrate; $P < 0.05$.

during the first 2 h of exercise before displaying a significant rise prior to fatigue in both trials. The blood lactate concentration at fatigue during intake of placebo was greater ($P < 0.05$) than when subjects were fed carbohydrates. These responses might reflect a catecholamine-induced stimulation of glycogenolysis in inactive muscle, as recently discussed by Ahlborg (1).

Muscle Fiber Type and Capillarization of Subjects

The vastus lateralis muscle of these endurance athletes possessed the following characteristics: 66 ± 4% slow-twitch muscle fibers; 2.71 ± 0.19 capillaries/fiber; 6.53 ± 0.33 capillaries around a fiber; and 441 ± 24 capillaries/mm^2.

DISCUSSION

As in our previous studies (15, 25), we have presently observed that carbohydrate feedings during prolonged exercise delay the development of fatigue, in this case by 1 h (i.e., from 3 to 4 h). Fatigue during exercise when subjects fasted was preceded by a decline in the rate of carbohydrate oxidation, whereas the postponement of fatigue when subjects were ingesting carbohydrates was associated with a maintained high rate of carbohydrate oxidation. A major finding of the present study is that carbohydrate feedings do not spare glycogen utilization in the vastus lateralis muscle during intense continuous cycling. This was demonstrated first in a group of subjects who exercised for 105 min (Table 1) and again in another group who exercised until fatigued. The maintenance of carbohydrate oxidation and work rate for an additional hour when subjects were fed compared with

when they fasted was accomplished with little reliance on muscle glycogen and presumably due to a sufficiently high rate of blood glucose oxidation.

Figure 5 summarizes our findings regarding the percentage of energy derived from the oxidation of fats and carbohydrates calculated from R. The percentage of energy derived from muscle glycogen was also calculated with the assumption that 10 kg of muscle were active and using glycogen at an average rate reflected by the decline in glycogen within the vastus lateralis (21). The difference between the rate of total carbohydrate oxidation and muscle glycogen utilization presumably reflects the oxidation of other carbohydrate sources, most notably blood glucose and to a lesser degree lactate (21). Since muscle glycogen utilization was similar during the two trials, the differences in total carbohydrate oxidation

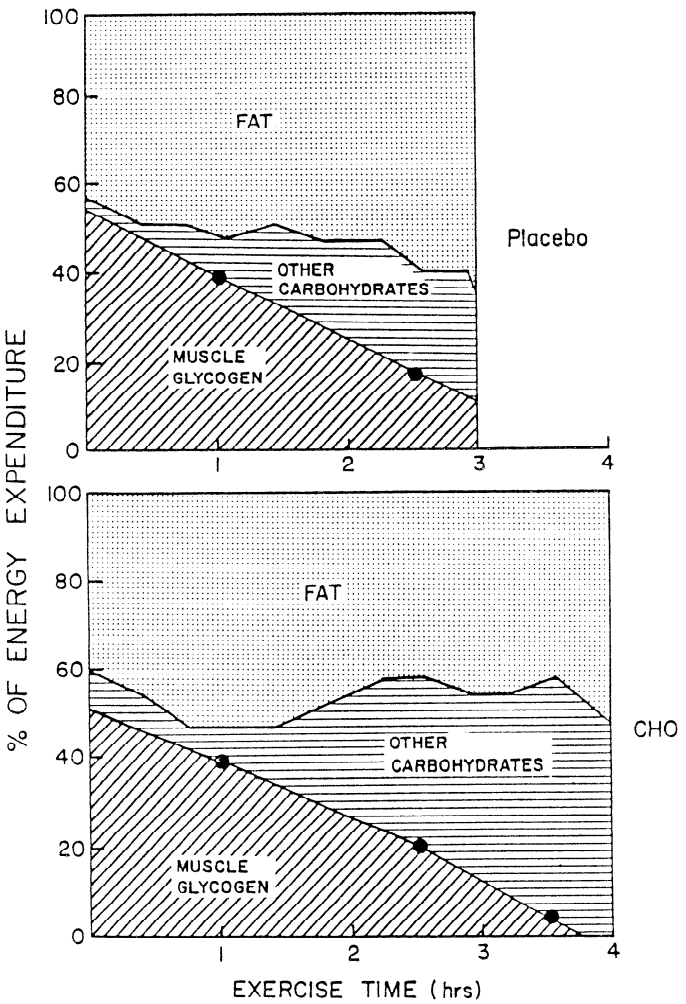

FIG. 5. Summary of estimated percent of energy expenditure derived from oxidation of muscle glycogen, carbohydrate sources other than muscle glycogen, and fats. Contribution of muscle glycogen was calculated from rate of decline in glycogen within vastus lateralis with assumption that a total of 10 kg of muscle were active and using glycogen at an average rate similar to that of vastus lateralis. Total carbohydrate and fat oxidation were calculated from O_2 uptake and respiratory exchange ratio, whereas percent of energy derived from carbohydrates other than muscle glycogen is displayed as difference between total carbohydrate oxidation and muscle glycogen oxidation. Rate of glycogen utilization averaged over 0- to 2-h, 2- to 3-h, and 3- to 4-h periods of exercise are plotted at midpoint of respective periods (i.e., 1, 2.5, and 3.5 h, respectively).

when subjects were fed, compared with when they fasted, reflect differences in oxidation of other carbohydrates. The rates of total carbohydrate oxidation were generally constant and similar during the first 2 h of exercise when subjects were fed and when they fasted (Figs. 1 and 5). Apparently the lowering of plasma glucose concentration during the 2nd h of exercise when fasted (i.e., 3.5 vs. 5.0 mM, placebo and carbohydrates, respectively; $P < 0.01$; Fig. 1*A*) did not reduce muscle glucose uptake enough to accelerate glycogen utilization or compromise carbohydrate oxidation. The oxidation of carbohydrates from sources other than muscle glycogen generally increased during the first 2 h of both exercise trials, and served to maintain total carbohydrate oxidation, as the contribution of muscle glycogen to energy expenditure declined progressively (Fig. 5).

Carbohydrate availability became limiting during the 3rd h of exercise when fasted as evidenced by declining carbohydrate oxidation and eventual fatigue. The progressive increase in carbohydrate oxidation from sources other than muscle glycogen was halted during the placebo trial at the time plasma glucose concentration declined to the 2.5–3.0 mM range (i.e., after 2.5 h; Figs. 1 and 5). This pattern parallels, and most likely reflects, leg glucose uptake, which increases during the first 2 h of moderate intensity cycling and then declines as blood glucose concentration declines from 3.5 to 2.8 mM (3).

The decline in total carbohydrate oxidation and eventual fatigue when subjects fasted occurred when the concentration of muscle glycogen and its contribution to energy expenditure were low and when plasma glucose declined to low levels, which likely compromised leg glucose uptake (3). We interpret these observations to indicate that lowering of blood glucose during the latter stages of prolonged strenuous exercise plays a major role in the development of fatigue by not allowing leg glucose uptake to increase sufficiently in order to offset reduced glycogen availability. Thus the rate of total carbohydrate oxidation was compromised, and intense exercise could not be maintained. Our present suggestion that hypoglycemia causes muscular fatigue when muscle glycogen is low is different from previous suggestions that hypoglycemia causes fatigue due to central nervous system dysfunction (13).

When plasma glucose was maintained at 4–5 mM, through carbohydrate ingestion, the rate of total carbohydrate oxidation was held constant and the subjects were able to exercise strenuously for an hour longer than when they fasted. This agrees with the well established concept that exercise eliciting 70% or more of $\dot{V}O_{2\,max}$ requires sufficient carbohydrate availability (6, 7, 9, 12, 24, 30). The concept that muscle glycogen is the obligatory substrate for exercise of this intensity has arisen from the fact that muscle glycogen is low at the point of fatigue and from the observations that endurance for such exercise can be increased by raising muscle glycogen and decreased by lowering muscle glycogen (6, 7, 24). However, we have presently demonstrated that by providing adequate blood glucose supplementation, exercise at 70% of $\dot{V}O_{2\,max}$ and the concomitant high rate of carbohydrate oxidation can be maintained during the

later stages of prolonged continuous exercise, with little reliance on muscle glycogen. This was dramatically demonstrated (Fig. 1) by the fact that the final hour of exercise when subjects were fed was accomplished without further reduction in muscle glycogen and with a continued high rate of carbohydrate oxidation. The histochemical pattern of glycogen depletion displayed in Fig. 2 also supports this point. Our present results therefore suggest that other carbohydrate sources (i.e., presumably blood glucose) can largely replace muscle glycogen in providing carbohydrate for oxidation during the latter stages of prolonged strenuous exercise.

Muscle glucose uptake increases with time during exercise when blood glucose is maintained (2, 21, 31). Additionally, Gollnick et al. (21) have found that glucose uptake by the exercising musculature increases in proportion to the number of glycogen-depleted fibers and largely compensates for the reduced glycogen availability. Along the same lines, Bonen et al. (9) reported that when glycogen-depleted subjects exercise intensely (i.e., 80% of $\dot{V}O_{2\ max}$), blood lactate levels and carbohydrate oxidation are 75–100% higher when glucose is ingested compared with when subjects fasted. These findings (9, 21) suggest that, in humans, a reduced muscle glycogen concentration may stimulate increases in leg glucose uptake.

Our present findings suggest that the majority of the 2 g/min rate of total carbohydrate oxidation was derived from blood glucose during the 3- to 4-h period of exercise when subjects were fed carbohydrates (Fig. 1*B*). This suggests a remarkably high rate of blood glucose uptake and oxidation compared with previous reports (2, 3, 31). It should be realized, however, that the present subjects were well-trained endurance athletes who possessed a high percentage of slow-twitch muscle fibers (i.e., 66 ± 4%) and a skeletal muscle capillarization that was 40–65% higher than in normally active men (16). They were also exercising at high steady-state rates of oxidative metabolism (i.e., 3.3 ± 0.2 l/min) and had relatively low muscle glycogen concentration following 3 h of exercise. These characteristics and the experimental conditions (including adequate carbohydrate supplementation) probably contributed greatly to their remarkable ability to rely on the ingested carbohydrate for their carbohydrate needs late in the exercise periods.

Animal studies provide some support for the idea that blood glucose can largely replace muscle glycogen as the source of carbohydrate for strenuous exercise when muscle glycogen is depleted (5, 17, 27). Nazar et al. (27), while studying dogs, found that exercise which results in hypoglycemia, fatigue, and muscle glycogen depletion after 2 h in the fasting control state can be continued for an additional 1–2 h, while glycogen stores remain depleted, when the animals were infused with glucose. Similarly, strenuous exercise in fasting rats normally results in carbohydrate depletion after 2–3 h. When infused with glucose, these animals are capable of exercising for almost 4 h, due largely to the fact that glycogen utilization in the running musculature is halted for a 2-h period (cf. Ref. 5; Fig. 2). Our present findings indicate that humans too, when muscle glycogen is low and when provided with adequate carbohydrate supplementation, are capable of cycling at 70% of $\dot{V}O_{2\ max}$ without significant reliance on glycogen from the active musculature. Galbo et al. (19), however, found nonglycogen-depleted men capable of running only an additional 16 min, at 70% of $\dot{V}O_{2\ max}$, after fatiguing and then receiving a continuous glucose infusion.

An interesting question regards the cause of fatigue during exercise with the carbohydrate feedings. There was no indication that the rate of carbohydrate oxidation was insufficient, and thus it might be expected that the subjects should be able to continue exercising for even longer than 4 h. It is clear from the perceived exertion ratings, however, that exercise became progressively harder for these athletes. The subjects commented that their legs felt glycogen depleted, yet they were quite surprised with their ability to maintain the work rate. As previously discussed, the low levels of muscle glycogen following 3 h of exercise may have contributed to their apparent ability to rely heavily on the feedings for energy during the 4th h of exercise.

Our present finding (that carbohydrate ingestion during continuous exercise at 70% of $\dot{V}O_{2\ max}$ does not alter the rate of muscle glycogen degradation) differs from previous studies that employed a different experimental design in men (7, 23). Bergström and Hultman (7) infused glucose into men at very high rates during high-intensity exercise that appears to have been performed intermittently. They found that glycogen was depleted after 60 min of exercise when subjects fasted, whereas it declined 25% less when carbohydrates were infused. Recently, Hargreaves et al. (23) had subjects perform intermittent exercise for 4 h with and without sucrose feedings and observed a greater rate of glycogen utilization during the first h when subjects were fed but then less net reduction in glycogen during the 1- to 4-h period when subjects were fed. It is possible that these two reports of muscle glycogen sparing with carbohydrate supplementation, and our presently reported lack of effect, can be reconciled by differing exercise protocols and methods of carbohydrate supplementation. Bjorkman et al. (8) employed an experimental design that is very similar to our present study regarding the exercise and feeding schedule. As in the present study, they observed that muscle glycogen when subjects fasted and fed was similar before exercise and declined to a similar absolute level at the time of fatigue during the two trials. As in the present study, they also found that carbohydrate feedings delay fatigue. They concluded, however, that carbohydrate feedings spare muscle glycogen utilization, since the glycogen utilization per minute of exercise appeared less when averaged over the longer exercise period. Our present observations, based on periodic determination of muscle glycogen, indicate no difference in the instantaneous rates of glycogen utilization and suggest that the apparent contradictory results of Bjorkman et al. (8) can be explained by mathematical artifact of the longer exercise duration allowed by carbohydrate feedings.

In summary, we observed that carbohydrate feedings during prolonged strenuous exercise resulted in the maintenance of a sufficiently high rate of carbohydrate oxidation and the postponement of fatigue. This was not a

result of a slowing of muscle glycogen depletion. Instead, it appeared that when blood glucose concentration was maintained, highly trained endurance athletes were capable of oxidizing carbohydrate sources other than muscle glycogen at high rates during the latter stages of prolonged strenuous exercise.

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REFERENCES

1. AHLBORG, G. Mechanism for glycogenolysis in nonexercising human muscle during and after exercise. *Am. J. Physiol.* 248 (*Endocrinol. Metab.* 11): E540–E543, 1985.
2. AHLBORG, G., AND P. FELIG. Influence of glucose ingestion on fuel-hormone response during prolonged exercise. *J. Appl. Physiol.* 41: 683–688, 1976.
3. AHLBORG, G., AND P. FELIG. Lactate and glucose exchange across the forearm, legs, and splanchnic bed during and after prolonged leg exercise. *J. Clin. Invest.* 69: 45–54, 1982.
4. ANDERSEN, P., AND J. HENRIKSSON. Capillary supply of the quadriceps femoris muscle of man: adaptive response to exercise. *J. Physiol. Lond.* 270: 677–690, 1977.
5. BAGBY, G. J., H. L. GREEN, S. KATSUTA, AND P. D. GOLLNICK. Glycogen depletion in exercising rats infused with glucose, lactate, or pyruvate. *J. Appl. Physiol.* 45: 425–429, 1978.
6. BERGSTRÖM, J., L. HERMANSEN, E. HULTMAN, AND B. SALTIN. Diet, muscle glycogen, and physical performance. *Acta Physiol. Scand.* 71: 140–150, 1967.
7. BERGSTRÖM, J., AND E. HULTMAN. A study of glycogen metabolism during exercise in man. *Scand. J. Clin. Lab. Invest.* 19: 218–228, 1967.
8. BJORKMAN, O., K. SAHLIN, L. HAGENFELDT, AND J. WAHREN. Influence of glucose and fructose ingestion on the capacity for long-term exercise in well-trained men. *Clin. Physiol. Oxf.* 4: 483–494, 1984.
9. BONEN, A., S. A. MALCOLM, R. D. KILGOUR, K. P. MACINTYRE, AND A. N. BELCASTRO. Glucose ingestion before and during intense exercise. *J. Appl. Physiol.* 50: 766–771, 1981.
10. BORG, G. A. Perceived exertion: a note on history and methods. *Med. Sci. Sports* 5: 90–93, 1973.
11. BROOKE, M. H., AND K. K. KAISER. Three "myosin ATPase" systems: the nature of their pH lability and sulfhydryl dependence. *J. Histochem. Cytochem* 18: 670–672, 1970.
12. CHRISTENSEN, E. H., AND O. HANSON. Arbeitsfahigkeit und Ermundung. *Skand. Arch. Physiol.* 81: 160–171, 1939.
13. CHRISTENSEN, E. H., AND O. HANSON. Hypoglykamie, Arbeitsfahigkeit und Ermundung. *Skand. Arch. Physiol.* 81: 172–179, 1939.
14. COYLE, E. F., W. H. MARTIN, A. A. EHSANI, J. M. HAGBERG, S. A. BLOOMFIELD, D. R. SINACORE, AND J. O. HOLLOSZY. Blood lactate threshold in some well-trained ischemic heart disease patients. *J. Appl. Physiol.* 54: 18–23, 1983.
15. COYLE, E. F., J. M. HAGBERG, B. F. HURLEY, W. H. MARTIN, A. A. EHSANI, AND J. O. HOLLOSZY. Carbohydrate feeding during prolonged strenuous exercise can delay fatigue. *J. Appl. Physiol.* 55: 230–235, 1983.
16. COYLE, E. F., W. H. MARTIN, D. R. SINACORE, M. J. JOYNER, J. M. HAGBERG, AND J. O. HOLLOSZY. Time course of loss of adaptations after stopping prolonged intense endurance training. *J. Appl. Physiol.* 57: 1857–1864, 1984.
17. DILL, D. B., H. T. EDWARDS, AND J. H. TALBOTT. Studies in muscular fatigue. VII. Factors limiting the capacity for work. *J. Physiol. Lond.* 77: 49–62, 1932.
18. EGGSTEIN, M., AND E. KUHLMANN. Triglycerides and glycerol. Determination after alkaline hydrolysis. In: *Methods of Enzymatic Analysis* (2nd ed.), edited by H. U. Bergmeyer. New York: Academic, 1974, p. 1825–1831.
19. GALBØ, H., J. J. HOLST, AND N. J. CHRISTENSEN. The effect of different diets and of insulin on the hormonal response to prolonged exercise. *Acta Physiol. Scand.* 107: 19–32, 1979.
20. GOETZ, F. C., AND B. Z. GREENBERG. A simple immunoassay for small amounts of insulin. *J. Lab. Clin. Med.* 58: 819–822, 1961.
21. GOLLNICK, P. D., B. PERNOW, B. ESSEN, E. JANSSON, AND B. SALTIN. Availability of glycogen and plasma FFA for substrate utilization in leg muscle of man during exercise. *Clin. Physiol. Oxf.* 1: 27–42, 1981.
22. GUTMAN, I., AND A. W. WAHLEFELD. L-(+)-Lactate. Determination with lactate dehydrogenase and NAD. In: *Methods of Enzymatic Analysis* (2nd ed.), edited by H. U. Bergmeyer. New York: Academic, 1974, p. 1464–1468.
23. HARGREAVES, M., D. L. COSTILL, A. COGGAN, W. J. FINK, AND I. NISHIBATA. Effect of carbohydrate feedings on muscle glycogen utilization and exercise performance. *Med. Sci. Sports Exercise* 16: 219–222, 1984.
24. HERMANSEN, L., E. HULTMAN, AND B. SALTIN. Muscle glycogen during prolonged severe exercise. *Acta Physiol. Scand.* 71: 129–139, 1967.
25. IVY, J. L., W. MILLER, V. DOVER, L. G. GOODYEAR, W. H. SHERMAN, S. FARRELL, AND H. WILLIAMS. Endurance improved by ingestion of a glucose polymer supplement. *Med. Sci. Sports Exercise.* 15: 466–471, 1983.
26. LOWRY, O. H., AND J. V. PASSONNEAU. *A Flexible System of Enzymatic Analysis.* New York: Academic, 1972.
27. NAZAR, K., Z. BRZEZINSKA, AND W. KOWALSKI. Mechanism of impaired capacity for prolonged muscular work following Beta-adrenergic blockade in dogs. *Pfluegers Arch.* 336: 72–78, 1972.
28. NOVAK, M. Colorimetric ultramicro method for determination of free fatty acids. *J. Lipid Res.* 6: 431–433, 1965.
29. PEARSE, A. G. E. *Histochemistry-Theoretical and Applied.* Boston, MA: Little, Brown, 1961, p. 832.
30. PERNOW, B., AND B. SALTIN. Availability of substrates and capacity for prolonged heavy exercise in man. *J. Appl. Physiol.* 31: 416–422, 1971.
31. WAHREN, J. Glucose turnover during exercise in man. *Ann. NY Acad. Sci.* 301: 45–55, 1977.