Muscle glycogen synthesis after exercise: effect of time of carbohydrate ingestion

J. L. IVY, A. L. KATZ, C. L. CUTLER, W. M. SHERMAN, AND E. F. COYLE
Exercise Physiology and Metabolism Laboratory, Department of Kinesiology and Health Education, University of Texas, Austin, Texas 78712

IVY, J. L., A. L. KATZ, C. L. CUTLER, W. M. SHERMAN, AND E. F. COYLE. Muscle glycogen synthesis after exercise: effect of time of carbohydrate ingestion. J. Appl. Physiol. 64(4): 1480–1485, 1988.—The time of ingestion of a carbohydrate supplement on muscle glycogen storage postexercise was examined. Twelve male cyclists exercised continuously for 70 min on a cycle ergometer at 68% VO\textsubscript{2}\text{max}, interrupted by six 2-min intervals at 88% VO\textsubscript{2}\text{max}, on two separate occasions. A 25% carbohydrate solution (2 g/kg body wt) was ingested immediately postexercise (P-EX) or 2 h postexercise (2P-EX). Muscle biopsies were taken from the vastus lateralis at 0, 2, and 4 h postexercise. Blood samples were obtained from an antecubital vein before and during exercise and at specific times after exercise. Muscle glycogen immediately postexercise was not significantly different for the P-EX and 2P-EX treatments. During the first 2 h postexercise, the rate of muscle glycogen storage was 7.7 μmol·g wet wt\textsuperscript{-1}·h\textsuperscript{-1} for the P-EX treatment, but only 2.5 μmol·g wet wt\textsuperscript{-1}·h\textsuperscript{-1} for the 2P-EX treatment. During the second 2 h of recovery, the rate of glycogen storage slowed to 4.3 μmol·g wet wt\textsuperscript{-1}·h\textsuperscript{-1} during treatment P-EX but increased to 4.1 μmol·g wet wt\textsuperscript{-1}·h\textsuperscript{-1} during treatment 2P-EX. This rate, however, was still 45% slower (P < 0.05) than that for the P-EX treatment during the first 2 h of recovery. This slower rate of glycogen storage occurred despite significantly elevated plasma glucose and insulin levels. The results suggest that delaying the ingestion of a carbohydrate supplement postexercise will result in a reduced rate of muscle glycogen storage.

SUFFICIENT STORES of muscle glycogen are essential for optimum performance during intense, prolonged exercise (1, 4, 15) and exercise of an anaerobic nature (22, 23). After the cessation of exercise, muscle glycogen is restored to normal preexercise concentrations within 24 h, provided sufficient carbohydrate is ingested (4, 5, 10). The amount of carbohydrate necessary to return the muscle glycogen stores to preexercise levels within a 24-h period was studied by Costill et al. (10). These investigators reported that consuming 150–600 g carbohydrate/day resulted in a proportionately greater muscle glycogen restoration during the 24-h period postexercise. However, consumption of >600 g carbohydrate/day was found to be of no additional benefit. It was also demonstrated by Costill et al. (9) that when the carbohydrate concentration of the diet was not adequate, successive days of intense prolonged exercise resulted in a marked reduction in the muscle glycogen stores and a deterioration in performance. Although it may be possible to restore muscle glycogen concentration to normal levels during successive days of prolonged exercise (36), there is little information on how to maximize glycogen synthesis during the hours immediately after exercise.

In the present study we investigated the effect the time of consumption of a carbohydrate supplement has on muscle glycogen restoration. After prolonged exhaustive exercise, there is a persistent elevation of insulin-antagonistic hormones and free fatty acids (8, 12, 13). We therefore tested the hypothesis that muscle glycogen restoration would be enhanced if the administration of the supplement was delayed several hours after exercise to allow the hormonal milieu and free fatty acid concentration to return to a more favorable condition. The results, however, indicated that providing the supplement 2 h postexercise resulted in a reduced glycogen storage rate when compared with providing the supplement immediately postexercise.

METHODS

Subjects. The subjects were 12 healthy males who cycled regularly. Their average (±SD) age, height, and weight was 26.1 ± 5.1 yr, 177.3 ± 11.1 cm, and 70.2 ± 9.9 kg, respectively. The average maximal O\textsubscript{2} uptake (VO\textsubscript{2}\text{max}) of the subjects was 4.19 ± 0.45 l/min. The protocol of this experiment and possible risks were fully explained to each subject before their signing an informed consent document. The research procedures were approved by the Institutional Review Board of the University of Texas.

Experimental protocol. Initially, the subjects trained for 2 wk in the laboratory. During this time they were acquainted with the laboratory setting and the exercise protocols. At the end of the second week of training their VO\textsubscript{2}\text{max} was determined on an electrically braked Monark cycle ergometer using a continuous exercise protocol (21). A respiratory exchange ratio of >1.0 and an increase in O\textsubscript{2} uptake (VO\textsubscript{2}) of <0.2 l/min over the previous work rate was the criteria used to ascertain that VO\textsubscript{2}\text{max} was achieved. A training log and diet recall was kept by the subjects during the training sessions. From these records, the daily physical activity patterns and diets of the subjects were established for use during the subsequent week of experimental testing.

To determine the effect of time of carbohydrate ingestion on the rate of muscle glycogen synthesis after exer-
cise, a carbohydrate supplement was administered to each subject on two separate occasions; immediately after 70 min of cycling (P-EX) or 2 h after 70 min of cycling (2P-EX). The 70 min of cycling to deplete the muscle glycogen stores consisted of six alternating work periods of 8 min at 68% \( \dot{V}O_2 \text{max} \) and 2 min at 88% \( \dot{V}O_2 \text{max} \). The subjects cycled at 68% \( \dot{V}O_2 \text{max} \), the last 10 min. \( \dot{V}O_2 \) was determined during the last 4 min of each 10 min of cycling. To prevent thermal stress during the rides, air was continuously circulated over the subjects and the laboratory temperature was maintained at 22°C. The subjects were also provided 125 ml of water after 20 and 40 min of cycling.

The two experimental treatments were separated by seven days, and the order of their administration was randomized. During the week between treatments, the subjects maintained a standard training schedule that was established during the 2-wk training period. Diets of the subjects were consistent for the 2 days before each treatment. This included a 14-h fast before the start of each glycogen depletion exercise bout.

The carbohydrate supplement consisted of a 25% commercially available glucose polymer solution (Excell, Ross Laboratories, Columbus, OH). The amount administered was based on the body weight of the subject (2 g/kg body wt). A placebo of similar taste, consistency, and volume to that of the carbohydrate supplement was administered 2 h after exercise during the P-EX treatments and immediately after the completion of exercise during the 2P-EX treatment.

**Tissue collection and analysis.** Blood samples (6 ml) were obtained before exercise, 55 min after the start of exercise, immediately postexercise, and 15, 45, 120, 135, 180, and 240 min postexercise from a catheter inserted in an antecubital vein. Five milliliters of blood were transferred to an ice-chilled test tube containing EDTA (24 mg/ml, pH 7.4) and an aprotinin solution (Trasylol, 10,000 KIU/ml). One milliliter of blood was transferred to a test tube containing 2 ml of 8% perchloric acid. Plasma and acid extracted samples were recovered by centrifugation (15 min at 1,000 g) and stored at −70°C. Plasma insulin concentration was determined by radioimmunoassay (14) (Endo Tech Laboratories, Carson, CA), and plasma glucose and free fatty acids were assayed as previously described (19). The acid extract samples were assayed for lactate according to the procedure of Hohorst (17).

Percutaneous muscle biopsies (3) were taken from the vastus lateralis immediately, 2 h, and 4 h postexercise. The muscle samples were immediately frozen in liquid N\(_2\) and stored at −70°C until assayed for glycogen and glycogen synthase. Muscle glycogen was assayed as described by Passonneau and Lauderdale (30). The muscle samples were weighed and placed in 2 N HCl and incubated at 100°C for 2 h. After neutralization, the liberated glucose units were assayed fluorometrically and glycogen content was expressed as micromoles glucosyl units per gram wet muscle weight. Glycogen synthase total activity was the same in the

**RESULTS**

The muscle glycogen concentrations were similar to the P-EX (35.8 ± 4.1 μmol/g wet wt) and 2P-EX (30.9 ± 3.4 μmol/g wet wt) treatments immediately postexercise. During the first 2 h of recovery, the muscle glycogen storage rate was threefold faster during the P-EX treatment than during the 2P-EX treatment (Fig. 1). During the second 2 h of recovery, the rate of glycogen storage slowed 44% during treatment P-EX but increased 67% during treatment 2P-EX. However, the rate of glycogen storage for the second 2 h of recovery during treatment 2P-EX was 45% slower than that which occurred in treatment P-EX during the first 2 h postexercise. At 4 h postexercise, the muscle glycogen concentration during the P-EX treatment (59.7 ± 6.2 μmol/g wet wt) was significantly higher than that during the 2P-EX treatment (44.3 ± 3.7 μmol/g wet wt).

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**FIG. 1.** Muscle glycogen storage during first 2 h and second 2 h of recovery for postexercise (P-EX) treatment (1) and the 2-h postexercise (2P-EX) treatment (11). *Significantly different from basal rate of synthesis which is represented by glycogen synthesis rate during first 2 h postexercise of treatment 2P-EX. **Significantly different from treatment 2P-EX during second 2 h of recovery (P < 0.05).**
P-EX and 2P-EX treatments immediately postexercise and at 2 and 4 h postexercise. There were no differences in the glycogen synthase activity ratio or fractional velocity immediately postexercise or 2 h postexercise between treatments (Fig. 2). The activity ratio was the same between treatments 4 h postexercise; however, the fractional velocity for treatment P-EX was significantly lower than that of treatment 2P-EX.

Plasma glucose before exercise was very similar in the two treatments (Fig. 3). After 55 min of cycling, the blood glucose was unchanged from the preexercise level, and immediately postexercise it was still similar to the preexercise level during both treatments. By 15 min postexercise, the plasma glucose level had increased ~60% during treatment P-EX. By 45 min postexercise, the plasma glucose had started to decline and had returned to the preexercise level by 120 min postexercise. It then remained low for the remainder of the recovery period. During treatment 2P-EX, plasma glucose remained at the preexercise level until 120 min postexercise, at which time the carbohydrate supplement was provided. By 135 min postexercise, 15 min after the carbohydrate supplement was ingested, the plasma glucose rose significantly. This increase was similar to that which occurred during the P-EX treatment. However, rather than the plasma glucose starting to decline during the next 45 min as it did during the P-EX treatment, it continued to rise. By 240 min postexercise, 120 min after the carbohydrate supplement, the 2P-EX plasma glucose level was still 28% above the preexercise level. It was also significantly elevated above the blood glucose level of the P-EX treatment at 120 postexercise, a time comparable in relation to carbohydrate ingestion.

Insulin levels were similar between treatments before, during, and immediately postexercise (Fig. 4). By 15 min postexercise, insulin in treatment P-EX had risen significantly above the preexercise level and remained significantly above the preexercise level until 180 min postexercise. During treatment 2P-EX, insulin remained similar to the preexercise level until 15 min after the carbohydrate supplement was administered, 135 min postexercise. It was also noted that during the 2P-EX treatment, the rise and decline in insulin after the carbohydrate supplement was slower than during treatment P-EX.

Plasma free fatty acids (FFA) were not significantly elevated above preexercise levels until 15 min postexercise (Fig. 5). At this time, the FFA level of treatment 2P-EX was significantly higher than that of the P-EX treatment. At 45 min postexercise the FFA level returned to the preexercise level in treatment P-EX. However, by 180 min postexercise it had started to rise once more,
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and by 240 min postexercise was again significantly elevated above the preexercise level. In contrast, the FFA level in treatment 2P-EX was still elevated above the preexercise level at 120 min postexercise. At 180 min postexercise the FFA level had returned to base line and remained there until the end of recovery, 240 min postexercise.

Blood lactate was not different between the two treatments at any time point. After 55 min of exercise lactate had risen from 1.0 ± 0.2 to 4.5 ± 0.4 mM. By the end of exercise it had decreased to 2.5 ± 0.3 mM. This decrease over the last 15 min was probably related to the work intensity before blood sampling. Blood drawn during exercise was preceded by 2 min of cycling at 88% V_{O2	ext{max}}. Blood drawn immediately after the completion of exercise was preceded by 10 min of cycling at 68% V_{O2	ext{max}}. By 45 min postexercise, lactate had returned to preexercise levels and remained stable for the remainder of the recovery period.

Discussion

The rate of glycogen storage when provided a carbohydrate supplement immediately after exercise has generally been reported to be between 5 and 8 μmol·g wet wt⁻¹·h⁻¹ (6, 26, 27, 31). Maehlum et al. (26) found that ingestion of 100 g (1.44 g/kg body wt) of glucose 15 min after an exhaustive bicycle exercise resulted in a 7.1 μmol·g wet wt⁻¹·h⁻¹ glycogen storage rate in the quadriceps during the subsequent 135 min. Maehlum et al. (27) also found a similar rate of glycogen storage after exercise when a carbohydrate-rich diet was consumed. Blom et al. (6) noted a glycogen storage rate of 5.3 μmol·g wet wt⁻¹·h⁻¹ during the 8 h immediately after exhaustive exercise when 0.7 g glucose/kg body wt was provided every 2 h. These results agree closely with those of the present study in which the rate of glycogen storage over the first 2 h of recovery during the P-EX treatment averaged 7.7 μmol·g wet wt⁻¹·h⁻¹.

The increase in glycogen storage above the basal rate during the first 2 h postexercise in the P-EX treatment appeared to be due in part to an increase in substrate availability, as evidenced by a rise in plasma glucose. This is supported by the finding that with the return of plasma glucose to the preexercise level 2 h postexercise, the rate of glycogen storage decreased from 7.7 to 4.3 μmol·g wet wt⁻¹·h⁻¹, and as the plasma glucose level increased in treatment 2P-EX after ingestion of the carbohydrate supplement, the rate of glycogen storage increased from 2.5 to 4.1 μmol·g wet wt⁻¹·h⁻¹. It was also observed that these shifts in the rate of glycogen storage occurred without a significant change in the activity ratio or fractional velocity of glycogen synthase. Furthermore, Roch-Norlund et al. (33) demonstrated that the rate of glycogen storage could be increased to 12.5 μmol·g wet wt⁻¹·h⁻¹ after exercise if glucose was infused at a rate of 1 g·kg body wt⁻¹·h⁻¹. The increase in plasma insulin in response to the rise in plasma glucose after ingestion of the carbohydrate supplement may have been of benefit as it is a powerful activator of glucose transport in muscle (28, 29). However, its stimulating effect on glycogen synthase was not evident (11).

Glycogen restoration after exercise has typically been reported to be slow when abstaining from carbohydrate consumption (4, 9, 10, 18, 26). In the present study, glycogen storage averaged 2.5 μmol·g wet wt⁻¹·h⁻¹ during the first 2 h of the 2P-EX treatment. This rate of storage was one-third as fast as that observed when the carbohydrate supplement was provided immediately postexercise. Maehlum et al. (26) observed that individuals who fasted for 15 h after depleting their muscle glycogen stores had an average glycogen storage rate of 1.6 μmol·g wet wt⁻¹·h⁻¹. An average rate of 0.3 μmol·g wet wt⁻¹·h⁻¹ was reported by Hultman et al. (18) for subjects glycogen depleted by exercise and fed a low-carbohydrate diet for 2 days. Hultman et al. (18) also found that it required 8–10 days for the muscle glycogen stores to return to normal if carbohydrate consumption was restricted. An exception to these studies is that reported by Hermansen and Vaage (16) in which subjects lowered their muscle glycogen levels by multiple 1-min maximal exercise bouts on a bicycle ergometer. During the first 30 min of recovery, the rate of muscle glycogen synthesis averaged 33.6 μmol·g wet wt⁻¹·h⁻¹. The increase in muscle glycogen was found to parallel the decline in muscle lactate, which had increased to 26.4 μmol/g wet wt after the last exercise bout. MacDougall et al. (25) also found a relatively rapid rate of storage after muscle glycogen depletion when subjects performed 1-min cycling sprints at 140% of V_{O2	ext{max}} to exhaustion. The difference in storage rates among the studies discussed can probably be explained by the type of exercise used to deplete the muscle glycogen stores. With multiple high-intensity sprints, glycogen depletion is accompanied by elevated blood glucose and blood and muscle lactate, which can be used immediately as substrate for glycogen synthesis (16). By contrast, prolonged sustained exercise severely reduces the endogenous precursors of carbohydrate.
muscle glycogen, therefore requiring an exogenous carbohydrate source for rapid muscle glycogen synthesis (4, 9, 10, 18, 26).

An important finding of the present study was that the rate of glycogen storage for the 2 h after the carbohydrate supplement during the 2P-EX treatment was 45% slower than that observed for the 2 h after the carbohydrate supplement during the P-EX treatment. This reduced rate of glycogen storage could not be attributed to a reduced blood glucose or insulin concentration, since their increase during the last 2 h of recovery was not compromised by the delayed administering of the supplement. The reduced rate of glycogen storage could not be accounted for by a lower glycogen synthase activity, since glycogen synthase activity either determined by its activity ratio or fractional velocity remained high throughout the 4 h of recovery during the 2P-EX treatment. It was noted that 2 h after the carbohydrate supplement during the 2P-EX treatment, plasma glucose and insulin were still significantly elevated above the preexercise values. In comparison, plasma glucose had returned to base line in the P-EX treatment by 2 h postsupplement and the plasma insulin concentration had declined substantially. These results suggest that the reduced rate of muscle glycogen storage during the 2P-EX treatment was due to a reduced muscle glucose uptake.

There are several explanations that could account for a reduced muscle glucose uptake during the 2P-EX treatment after the carbohydrate supplement. Glucose uptake by leg muscle is the product of leg blood flow and arteriovenous glucose difference. Leg blood flow is quite low at rest but can increase 20-fold during moderately intense exercise (2, 34). After exercise leg blood flow declines rapidly to a level slightly higher than resting and then decreases slowly over the next hour (2, 34). Although little is known about leg blood flow after the 1st hour postexercise, it is reasonable to assume that it decreases over time, reducing glucose delivery to the leg muscle.

Exercise also results in a non-insulin-dependent increase in muscle glucose transport, and this increase in transport decreases with time even in the absence of glycogen repletion (32). It is therefore possible that the reason for the greater muscle glycogen storage during the P-EX treatment as opposed to during the 2P-EX treatment was the result of a greater activation of muscle glucose transport at the time the P-EX carbohydrate supplement was provided.

An alternative explanation is that delaying the carbohydrate supplement results in a reduced insulin action. The reason for this reduced insulin action could be related to the elevated FFA during the 2P-EX treatment. It was reported by Schulch and Kipnis (35) that elevated blood FFA's impair glucose tolerance and reduce insulin sensitivity. The reduced insulin action might also be related to prolonged exposure to hormones antagonistic to insulin that increase during exercise and remain elevated for prolonged periods (8, 12, 13). Interestingly, insulin antagonistic hormones such as epinephrine, glucagon, and cortisol, as well as FFA, are suppressed in the presence of high blood glucose levels during and after exercise (12, 13) and therefore may explain why providing the carbohydrate supplement immediately after exercise results in an increased rate of glycogen synthesis.

If carbohydrate restriction after exercise results in a reduced insulin action, it is unlikely that it persists for an extended period of time. Bogardus et al. (7) found that insulin sensitivity, as determined by the rate of blood glucose clearance during an euglycemic clamp, was improved in subjects 15 h after exercise strenuous enough to significantly reduce their muscle glycogen stores. It has also been demonstrated that 16 h after prolonged strenuous exercise, the plasma insulin response to an oral glucose challenge is reduced without an increase in the plasma glucose response (20).

In summary, muscle glycogen storage after exercise was increased above the basal rate when a carbohydrate supplement was provided. However, the time of its administration was significant. A 2-h delay in administering the supplement resulted in a slower rate of glycogen storage than if the supplement was provided immediately postexercise. This reduced rate of glycogen storage occurred despite elevated plasma glucose and insulin levels and a comparable glycogen synthase activity. The results suggest that the reduced rate of muscle glycogen storage was due to a reduced rate of muscle glucose uptake.

This research was supported by a grant from Ross Laboratories. Present address of W. M. Sherman: 129 Larkins Hall, School HPER, 17th Ave., Ohio State Univ., Columbus, OH 43210. Address for reprint requests: J. L. Ivy, Dept. of Kinesiology and Health Education, Bellmont Hall 222, Univ. of Texas, Austin, TX 78712.

Received 27 May 1987; accepted in final form 5 November 1987.

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