Early postexercise muscle glycogen recovery is enhanced with a carbohydrate-protein supplement

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Ivy, John L., Harold W. Goforth, Jr., Bruce M. Damon, Thomas R. McCauley, Edward C. Parsons, and Thomas B. Price. Early postexercise muscle glycogen recovery is enhanced with a carbohydrate-protein supplement. JAppl Physiol 93: 1337-1344, 2002. First published July 12, 2002; 10.1152/japplphysiol.00394.2002.-In the present study, we tested the hypothesis that a carbohydrate-protein (CHO-Pro) supplement would be more effective in the replenishment of muscle glycogen after exercise compared with a carbohydrate supplement of equal carbohydrate content (LCHO) or caloric equivalency (HCHO). After 2.5 \pm 0.1 h of intense cycling to deplete the muscle glycogen stores, subjects (n = 7) received, using a rank-ordered design, a CHO-Pro (80 g CHO, 28 g Pro, 6 g fat), LCHO (80 g CHO, 6 g fat), or HCHO (108 g CHO, 6 g fat) supplement immediately after exercise (10 min) and 2 h postexercise. Before exercise and during 4 h of recovery, muscle glycogen of the vastus lateralis was determined periodically by nuclear magnetic resonance spectroscopy. Exercise significantly reduced the muscle glycogen stores (final concentrations: $40.9 \pm 5.9 \text{ mmol/l CHO}$ -Pro, 41.9 \pm 5.7 mmol/l HCHO, 40.7 \pm 5.0 mmol/l LCHO). After 240 min of recovery, muscle glycogen was significantly greater for the CHO-Pro treatment (88.8 \pm 4.4 mmol/l) when compared with the LCHO (70.0 \pm 4.0 mmol/l; P = 0.004) and HCHO (75.5 \pm 2.8 mmol/l; P = 0.013) treatments. Glycogen storage did not differ significantly between the LCHO and HCHO treatments. There were no significant differences in the plasma insulin responses among treatments, although plasma glucose was significantly lower during the CHO-Pro treatment. These results suggest that a CHO-Pro supplement is more effective for the rapid replenishment of muscle glycogen after exercise than a CHO supplement of equal CHO or caloric content.

catecholamines; glucose; lactate; insulin; nuclear magnetic resonance spectroscopy

MUSCLE GLYCOGEN IS an essential fuel source for moderate- to high-intensity exercise. Once depleted, the capacity to perform at these exercise intensities is lost or severely limited (1, 2, 9, 11). Therefore, the faster the muscle glycogen stores can be replenished after exercise the faster the recovery process and theoretically

the greater the return of performance capacity. In recent years, different methods of rapidly increasing the muscle glycogen stores have been extensively investigated. Research studies addressing this question have focused on the timing (13, 18), frequency (10), amount of supplementation (3, 12, 14, 15), as well as type of supplement to ingest (6, 21, 24, 30. 32). With regard to the type of supplement, Zawadzki et al. (32) found that the combination of carbohydrate and protein was more effective than carbohydrate alone in the replenishment of muscle glycogen during the 4 h immediately after exercise. It was suggested that this greater rate of muscle glycogen storage was the result of a greater plasma insulin response when the carbohydrate-protein (CHO-Pro) supplement was provided. However, more recent studies have taken issue with the benefits of adding protein to a carbohydrate supplement (7, 15, 27, 29, 30). Results from these studies suggested that the enhancement in muscle glycogen storage found in the study by Zawadzki et al. was simply due to a greater amount of calories provided during the CHO-Pro treatment compared with the carbohydrate treatment. Moreover, it was suggested that if adequate carbohydrates were provided, the addition of protein would have no beneficial effect on muscle glycogen recovery (15). It should be noted, however, that the carbohydrate supplement used by Zawadzki et al. had previously been shown to maximize muscle glycogen storage during recovery when provided immediately postexercise and at 2 h intervals thereafter (3, 12, 14). Thus it is possible that significant differences in experimental designs and supplement compositions could account for the lack of agreement among studies.

In the present study, we reevaluated the potential of a CHO-Pro supplement to enhance muscle glycogen storage after vigorous exercise. We employed natural abundance ¹³C-nuclear magnetic resonance (NMR) spectroscopy to measure glycogen concentrations primarily in vastus lateralis, with some small contribu-

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tion from vastus medialis and rectus femoris. ¹³C-NMR provides noninvasive and continuous assessment of muscle glycogen concentrations and their dependence on diet and exercise.

With muscle biopsies, the number and frequency of measurements and the sampling of only a small volume in a nonhomogeneous tissue have been a limitation. Because of the noninvasive nature of the NMR technique (22), we were able to measure glycogen with better time resolution (frequency), repeatability, and precision during recovery than previously employed in biopsy studies (22). We hypothesized that 1) this increase in time resolution would enable us to detect subtleties in postexercise glycogen recovery that would not otherwise be available and 2) a CHO-Pro supplement would increase the rate of muscle glycogen storage compared with a carbohydrate supplement of equal carbohydrate content (LCHO) or equal caloric content (HCHO).

METHODS

Subjects

Seven trained male cyclists were studied. Subjects were 23 ± 1 yr of age (range of 19–26 yr), 181 ± 1 cm in height (range of 178–183 cm), and weighed 74 \pm 2 kg (range of 70-82 kg). Subjects were screened by an interview for medical history and had no family history of diabetes, hypertension, or metabolic disorder. Maximum oxygen uptakes (VO_{2 max}) and maximum heart rates were measured by using an expired-gas analyzer (SensorMedics Vmax 29, Yorba Linda, CA) and Polar heart monitor (Polar Electro, Oy, Finland) at least 72 h before the beginning of the study. $\mathrm{\dot{Vo}_{2\,max}}$ and maximum heart rate were $61.1 \pm 2.1 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ $(50.6-66.4 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1})$ and 190 ± 4 beats/min $(178-201 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1})$ beats/min), respectively. Resting heart rates were 44 \pm 2 beats/min (40-50 beats/min). Resting quadriceps glycogen concentrations were $147.0 \pm 6.6 \text{ mmol/l} (84.6-210.1 \text{ mmol/l})$. Subjects gave informed consent to participate in this study according to a protocol approved by the Human Investigation Committee of the Yale University School of Medicine.

Experimental Protocol

Subjects participated in the study on three separate occasions spaced at least 1 mo apart. Subjects were rank ordered according to Vo_{2 max} and assigned treatments according to a predetermined counter-balanced design to reduce any sequence effects. Subjects arrived at the Yale University School of Medicine General Clinical Research Center (GCRC) at 6:00 PM on the evening before each study. Subjects were given a mixed meal (50% carbohydrate, 30% fat, 20% protein) 12 h before beginning the exercise protocol. During the 12 h immediately preceding exercise, subjects remained in the GCRC where they were monitored and consumed only water. At 6:00 AM on the morning of the study, after the overnight fast and at least 30 min before any samples were collected, a Teflon catheter was inserted in an antecubital vein to obtain blood samples. To establish resting glycogen levels in the vastus lateralis, a natural abundance ¹³C-NMR scan was performed at the midthigh by using the methods described below. Baseline blood samples were also obtained during this period. Subjects then performed the exercise protocol. A blood sample and a single ¹³C-NMR scan were obtained immediately on cessation of exercise. After the NMR scan, 10 min into the recovery period, subjects were given the first of two, 472-ml (16 oz) postexercise nutritional supplements. During the remainder of the first hour of recovery, ¹³C-NMR scans were performed at 20, 40, and 60 min, and blood samples were collected at 30 and 60 min. At 120 min into the recovery period, subjects were given the second 472-ml dose of the nutritional supplement. Additional ¹³C-NMR scans were performed at 120, 180, and 240 min of recovery. A total of seven ¹³C-NMR measurements of quadriceps glycogen were obtained during the 240-min initial recovery period. Blood samples were collected at 120, 150, 180, 210, and 240 min of recovery. After 240 min of recovery, subjects were given a mixed meal and were released from the GCRC.

Exercise Protocol

After baseline NMR and blood measurements, subjects exercised on their own bicycles equipped with stationary adapters. Each subject performed 2 h of cycling at 65-75% of his Vo_{2 max}. Oxygen uptake was measured every 30 min, and workload was adjusted accordingly. After 2 h of cycling, subjects performed a series of 1-min sprints at maximum effort. Each sprint was separated by 1 min of rest during which the subject cycled at a self-selected leisurely rate. This sprint phase of the exercise protocol was maintained until the subject's plasma glucose level had dropped below 3.89 mmol/l. This was to ensure that liver glycogen stores were depleted to the same degree during each trial, thus reducing variability in carbohydrate availability during recovery. There were no significant differences in the total number of sprints completed between the three occasions that each subject performed the exercise protocol. The mean number of sprints completed was 15 ± 2 .

Nutritional Supplementation

Subjects, performing identical exercise protocols each time, were studied on three separate occasions. In each study, subjects were given one of three different nutritional supplements immediately after exercise (within 10 min of cessation) and again 2 h after cessation of exercise. The three different nutritional supplements were carbohydrate + protein (CHO-Pro), a commercial product (Systems GO International) containing 378 kcal [240 kcal (80 g) of carbohydrate + 84 kcal (28 g) of protein + 54 kcal (6 g) of fat]; HCHO containing 378 kcal [324 kcal (108 g) of carbohydrate + 54 kcal (6 g) of fat]; and LCHO containing 294 kcal [240 kcal (80 g) of carbohydrate + 54 kcal (6 g) of fat]. Nutritional supplements were delivered in liquid form (472 ml) at the two separate time points for a total of 944 ml (32 fluid oz.) over the 4 h recovery period. Total caloric intake for the CHO-Pro and HCHO supplements was 756 kcal, and for LCHO supplements caloric intake was 588 kcal. Because the CHO-Pro supplement included a small amount of fat (3 g/236 ml) for flavor, subjects assigned the HCHO and LCHO treatments consumed 3 g of clarified warm butterfat, followed immediately by the aqueous CHO portion of the treatment. The ratio of simple to complex CHO of all treatments was identical. This was achieved by making flavored aqueous solutions of HCHO and LCHO by using the same formula of sucrose and maltodextrin as the commercial CHO-Pro supplement. We opted to use an available CHO-Pro supplement because its percentage of carbohydrate to protein was similar to what was employed in the Zawadzki et al. (32) study and because it was thought to contain a sufficient amount of protein for our purpose.

NMR Spectroscopy

Natural abundance ¹³C-NMR spectroscopy was performed at 2.1 T on a Bruker Biospec spectrometer with a 100-cm-diameter magnet bore. During the measurements, subjects remained supine within the magnet with a surface coil radio-frequency (RF) probe resting midthigh, directly above the vastus lateralis. Power deposition profiles indicate that the majority of NMR signal was received from the vastus lateralis, with some small contribution (<10%) from the vastus medialis and the rectus femoris. During the data acquisition period, RF power was pulsed through the surface coil at a frequency of 22.5 MHz (^{13}C resonance frequency). A 9-cm-diameter circular ¹³C surface coil RF probe was used for spectral acquisitions. Shimming, imaging, and $^1\mathrm{H}$ decoupling at 89.5 MHz was performed with a 12 imes12-cm series butterfly coil. Proton line widths are typically shimmed to <70 Hz. A microsphere containing a ¹³C-labeled formate was fixed at the center of the RF coil for calibration of RF pulse widths. Subjects were positioned by an image-guided localization routine that employs a T1-weighted gradient-echo image (repetition time = 82 ms, echo time = 21 ms). Subjects' thighs were positioned so that the isocenter of the magnetic field was ~ 2 cm into the vastus lateralis muscle. By determining the 180° flip angles at the center of the observation coil from the microsphere standard, RF pulse widths were set so that the 90° pulse was sent to the center of the muscle. This technique maximizes suppression of the lipid signal that arises from the subcutaneous fat layer and optimizes signal from the muscle. The ¹H decoupled ¹³C RF pulse sequence has been designed so that 5,472 ¹³C transients are obtained. The repetition time for ¹³C acquisition was 87 ms, and ¹H continuous wave decoupling was truncated to 25 ms at the beginning of each ¹³C acquisition to prevent excessive RF power deposition in the muscle. Power deposition, assessed by magnetic vector potential specific absorption rate (5), was calculated at <4 W/kg. The total scan time for each spectrum was 8 min.

Blood Sampling

Venous blood samples were assayed for glucose, lactate, insulin, catecholamines, and free fatty acids (FFA). Plasma glucose was measured by the glucose oxidase method (Glucose Analyzer II, Beckman Instruments, Fullerton, CA) (17). Lactate was also assayed by an enzymatic method (8). Plasma insulin (26) and catecholamines (23) were assayed by a double-antibody radioimmunoassay technique. FFA were assayed by a microfluorometric method (19).

Statistical Analysis

Statistical analyses were performed by using SPSS software (SPSS, Chicago, IL). Values are reported as means \pm SE. For muscle glycogen analysis, a two-way ANOVA (treatment \times time) with repeated measures was employed to identify significant major effects. Significant differences among means were determined by pairwise comparisons using estimated marginal means. Blood and plasma samples were analyzed in the same manner as muscle glycogen except that the samples drawn during exercise were analyzed separately from those drawn during recovery. Statistical significance was set at $P \leq 0.05$.

RESULTS

Muscle Glycogen

There were no significant differences in muscle glycogen concentration among the three treatments before (141.8 \pm 15.4 mmol/l CHO-Pro, 150.8 \pm 9.5 mmol/l

after exercise $(40.9 \pm 5.9 \text{ mmol/l CHO-Pro}, 41.9 \text{ mmol/l}$ HCHO, 40.7 mmol/l LCHO mmol/l). Figure 1 illustrates the pattern of glycogen storage for each treatment during the 4-h recovery period. Total glycogen storage at 4 h was significantly greater during the CHO-Pro treatment compared with the HCHO and LCHO treatments (Fig. 2). There was no difference in glycogen storage between the HCHO and LCHO treatments. In the CHO-Pro treatment, the difference in total glycogen storage resulted from a greater rate of glycogen storage during the first 40 min of recovery and a more sustained rate of glycogen storage during the final 2 h of recovery (Figs. 1 and 3). Of the total glycogen utilized during exercise, the amount recovered in the first 40 min was 22% for the CHO-Pro treatment but only 11.5 and 5.5% for the HCHO and LCHO treatments, respectively. Between 40 and 120 min of recovery, the rate of glycogen restoration declined during the CHO-Pro and HCHO treatments but accelerated during the LCHO treatment. After 120 min of recovery, glycogen restoration was 30.4% during the CHO-Pro treatment, 23.9% during the HCHO treatment, and 23.0% for the LCHO treatment. After the 2-h supplement, the rate of glycogen storage increased again with the CHO-Pro treatment. This secondary increase in glycogen storage did not occur during the HCHO or LCHO treatments. After 4 h of recovery, 46.8% of the glycogen utilized during exercise had been replenished with the CHO-Pro treatment, 31.1% with the HCHO treatment, and 28.0% with the LCHO treatment.

HCHO, 148.5 \pm 9.8 mmol/l LCHO) or immediately

Plasma Metabolites and Hormones

Lactate. Plasma lactate rose significantly during exercise but was not different among the three treatments (Fig. 4A). During recovery, however, plasma



Fig. 1. Patterns of muscle glycogen storage during recovery as determined by nuclear magnetic resonance spectroscopy for the carbo-hydrate-protein (CHO-Pro; **D**), isocarbohydrate (LCHO; \triangle), and isocaloric carbohydrate (HCHO; **O**) supplements. Supplements were provided immediately after and 2 h after exercise. *CHO-Pro significantly different from LCHO and HCHO (P < 0.05). †CHO-Pro significantly different from LCHO (P < 0.05). ¶HCHO significantly different from LCHO (P < 0.05).

lactate was significantly lower during the CHO-Pro treatment compared with the HCHO and LCHO treatments at 30, 60, 180, 210, and 240 min. There were no differences in plasma lactate between HCHO and LCHO treatments at any time point during recovery.

Insulin. Plasma insulin levels did not differ at any time among treatments (Fig. 4B). After ingestion of the first supplement, insulin levels rose significantly and then reached a plateau until the second supplement was ingested. Insulin levels then increased during the next hour and declined steadily thereafter.

Glucose. Plasma glucose declined during exercise below 3.89 mmol/l for each treatment (Fig. 4*C*). Within 30 min after ingestion of the first supplement, blood glucose increased significantly regardless of the treatment ingested. However, the increase was significantly greater after the HCHO and LCHO treatments than after the CHO-Pro treatment. Blood glucose during the HCHO treatment remained significantly elevated above the CHO-Pro treatment through the first 3 h of recovery. Similar results were found when comparing the CHO-Pro and LCHO treatments, except that blood glucose was not different between these treatments at 120 min of recovery.

FFA. No differences in plasma FFA occurred during exercise or recovery for the three treatments (Fig. 5*A*). Plasma FFA increased significantly during exercise. They declined precipitously during the first 60 min of recovery and then continued declining at a slower rate, reaching baseline values by 180 min of recovery.

Epinephrine and norepinephrine. Catecholamine responses during exercise and recovery were similar for each treatment. However, the response between epinephrine (Fig. 5C) and norepinephrine (Fig. 5C) differed. Plasma levels of both epinephrine and norepinephrine increased during the first 70 min of exercise. Epinephrine continued to rise during the final 60 min,



Fig. 2. Total muscle glycogen storage in the vastus lateralis during 4 h of recovery from intense cycling. Treatments were with CHO-Pro, LCHO, and HCHO supplements provided immediately after and 2 h after exercise. *Significantly different from HCHO and LCHO (P < 0.05).



Fig. 3. Percent recovery of depleted glycogen stores of the vastus lateralis from 0 to 40, 40 to 120, and 120 to 240 min of recovery. Initial and postexercise muscle glycogen stores were not different among treatments.

but during this time the plasma concentration of norepinephrine declined. During recovery, there was a rapid decline in epinephrine back to baseline values within 30 min, whereas norepinephrine approached baseline more slowly.

DISCUSSION

In the present study, the addition of a CHO-Pro supplement yielded significantly greater muscle glycogen storage in the 4 h immediately after heavy exercise compared with LCHO or HCHO supplements. There was no significant difference in the muscle glycogen storage between HCHO and LCHO supplements. The percentage of glycogen restored during the 4-h recovery period was 46.8, 31.1, and 28.0% for the CHO-Pro, HCHO, and LCHO treatments, respectively.

The finding that CHO-Pro supplement enhanced the storage of muscle glycogen compared with LCHO supplement is in agreement with the early research of Zawadzki et al. (32) and the more recent research of van Loon et al. (30). Furthermore, the present results reveal that CHO-Pro may be more effective for the restoration of muscle glycogen than HCHO. Because of the noninvasive nature of the ¹³C-NMR method for glycogen determination, we were also able to dramatically increase the time resolution of the glycogen measurements. The enhanced time resolution revealed subtle effects of different nutritional supplements on muscle glycogen recovery that have not been previously observed. From these additional data, we found that ingestion of a CHO-Pro supplement promptly after severe exercise enhances glycogen synthesis during the initial minutes (minutes (0-40)) of recovery and that muscle glycogen recovery is further enhanced when a second CHO-Pro supplement is consumed 120 min into the recovery period. This enhancement was not observed with either the HCHO or LCHO supplements.

Zawadzki et al. (32) found that muscle glycogen storage during the 4 h immediately after exercise was



Fig. 4. Plasma lactate (A), insulin (B), and glucose (C) during exercise and 4 h of recovery. Treatments were with CHO-Pro (\blacksquare), LCHO (\triangle), and HCHO (\bullet) supplements provided immediately after and 2 h after exercise. *Significantly different from HCHO and LCHO (P < 0.05).

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increased by 38% if protein was added to a LCHO supplement. The supplements were provided immediately and 2 h after exercise with the CHO-Pro supplement consisting of \sim 1.6 g carbohydrate and 0.6 g pro-

tein/kg body wt and the carbohydrate supplement consisting of 1.6 g carbohydrate/kg body wt. However, van Hall et al. (28, 29) could find no difference in postexercise muscle glycogen storage when comparing





supplements similar to those used by Zawadzki et al. (32). Moreover, van Hall et al. (29) reported that leg glucose uptake was similar during recovery for the carbohydrate and CHO-Pro supplements.

Recently, van Loon et al. (30) compared the effects of a CHO-Pro supplement with carbohydrate supplements containing either an equal weight of carbohydrate or caloric equivalency. They reported that with the addition of a protein-amino acid mixture to a supplement of 0.8 g carbohydrate·kg body wt⁻¹·h⁻¹ increased the rate of glycogen storage by >100% above that produced by an equivalent carbohydrate supplement. However, they also reported that glycogen storage was similar when comparing a carbohydrate plus protein-amino acids HCHO supplement. Tarnopolsky et al. (27) and Carrithers et al. (7) also found no difference in muscle glycogen storage during the initial hours of recovery when LCHO or HCHO supplements were compared.

Considering the research that has been presented, it appears that the addition of protein to a carbohydrate supplement will increase the rate of muscle glycogen storage during the hours immediately after exercise if the supplement contains a low to moderate amount of carbohydrate. What is less evident is whether the advantage of a CHO-Pro supplement relative to muscle glycogen storage is maintained when compared with a HCHO supplement. There are several possible explanations accounting for the contrasting results among studies. For example, Tarnopolsky et al. (27) and Carrithers et al. (7) supplemented with lower protein concentrations than used in the present study. There were also differences in the frequency of supplement administration among studies. In the present study, supplements were provided immediately after and 2 h after exercise. In the studies that found no difference in glycogen storage among isocaloric supplements, supplements were provided every 15 or 30 min (7, 15, 27, 30). Large doses of carbohydrate provided at frequent intervals such as every 15 min have been reported to promote glycogen storage rates considerably higher than those seen when supplementing at 2-h intervals (10, 21). Thus supplementing with smaller doses, but more frequently, could alter the rate of absorption of carbohydrate and protein and possibly limit the advantage of the protein.

The time allotted for glycogen to recover may also yield different results. In the present study, differences in muscle glycogen between the CHO-Pro treatment and HCHO treatment occurred only after 40 min and 4 h of recovery. Therefore, detection of a treatment effect may also depend on experimental design differences in the recovery protocol. This possibility is particularly relevant when considering the negative finding of van Hall et al. (28). These investigators reported that glycogen storage rates during 3 h of recovery, although not statistically significant, were ${\sim}20\%$ higher after supplementation with either a carbohydrate and whey or wheat protein hydrolysate supplement compared with supplementing with a comparable carbohydrate supplement. Supplements were provided at 1-h intervals. The results are similar to those in the present study and raise the possibility that a significant difference in glycogen storage might have been detected if the recovery period had been extended an additional hour.

The extent to which the addition of protein to a large carbohydrate supplement could increase the rate of postexercise glycogen storage was recently addressed by Jentjens et al. (15). They provided 1.2 g of carbohydrate \cdot kg body wt⁻¹ \cdot h⁻¹ with and without a protein-amino acid mixture. Supplements were provided immediately after exercise and every 30 min thereafter. No difference in the rate of glycogen storage was found between treatments. Jentiens et al. (15) concluded that when the total carbohydrate content in a supplement is very high, the addition of a proteinamino acid mixture does not further increase the rate of muscle glycogen storage; i.e., the rate of glycogen storage can be maximized if sufficient carbohydrate is provided. Although the present study was not designed to address this question directly, it should be noted that glycogen storage was similar for the HCHO and LCHO treatments and that this finding is in agreement with earlier research (3, 12, 14). Our results, therefore, suggest that the addition of protein to a carbohydrate supplement can in fact raise the rate of muscle glycogen storage beyond the maximal rate produced by carbohydrate alone. The ability of protein to maximize the carbohydrate response, however, may be restricted to conditions when supplements are provided at intervals of 2 h or greater. As mentioned previously, carbohydrate supplements provided at more frequent intervals appear to increase their effectiveness and thus render the addition of protein to the supplement less effective (10, 21).

Results from several studies have suggested that the rate of muscle glycogen storage after carbohydrate supplementation is related in part to the plasma insulin response (24, 30, 32). Thus the rationale for adding protein to a carbohydrate supplement has been to increase the effectiveness of the supplement toward raising the plasma insulin concentration (30, 32). In the present study, the increased glycogen storage during the CHO-Pro treatment could not be attributed to a greater plasma insulin response, nor could it be attributed to differences in plasma catecholamines or circulating FFA levels, both of which can antagonize the action of insulin (4, 16, 20, 25). It therefore appears that the mechanism by which CHO-Pro supplementation increases the rate of muscle glycogen storage is unrelated to an enhanced plasma insulin response as previously theorized (30, 32). It was observed, however, that during recovery plasma glucose and lactate were lower during the CHO-Pro treatment than during the HCHO or LCHO treatments. This observation might indicate an increase in plasma glucose uptake and a redistribution of intracellular glucose disposal by the addition of protein to a carbohydrate supplement. In support of this possibility is the finding of Yaspelkis and Ivy (31) that the addition of arginine to a carbohydrate supplement lowered glucose oxidation during recovery while it tended to increase the rate of muscle glycogen storage.

An important observation in the present study was the rapid increase in muscle glycogen storage during the first 40 min of recovery as a result of the CHO-Pro treatment. Glycogen storage rates with the CHO-Pro treatment were 2–4 times faster than with the HCHO and LCHO treatments and accounted for \sim 50% of the glycogen stored during the 4-h CHO-Pro recovery period. Glycogen storage for the HCHO and LCHO treatments did not reach the level obtained during the first 40 min of the CHO-Pro treatment for 2 h. These results have practical implications and suggest that a CHO-Pro supplement would be very advantageous when recovery time is extremely limited.

In conclusion, the present results suggest that a distinct advantage in muscle glycogen storage can be achieved after exercise with the addition of protein to a carbohydrate supplement. When supplementation occurs immediately postexercise and 2 h postexercise, this advantage appears to be maintained even when compared with a HCHO supplement. The increased rate of muscle glycogen storage after CHO-Pro supplementation does not appear to be due to an enhanced plasma insulin response and is most evident during the first 40 min of recovery. This later finding would suggest that a CHO-Pro supplement might be most beneficial during short recovery periods. CHO-Pro supplementation for exercise recovery might also be advantageous if minimizing carbohydrate consumption is necessary or a personal preference, such as during a weight-management program.

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REFERENCES

- 1. Ahlborg B, Bergström J, Ekelund LG, and Hultman E. Muscle glycogen and muscle electrolytes during prolonged physical exercise. *Acta Physiol Scand* 70: 129–142, 1967.
- Bergström J, Hermansen L, Hultman E, and Saltin B. Diet, muscle glycogen and physical performance. Acta Physiol Scand 71: 140–150, 1967.
- Blom PCS, Høstmark AT, Vaage O, Kardel KR, and Mæhlum S. Effect of different post-exercise sugar diets on the rate of muscle glycogen synthesis. *Med Sci Sports Exerc* 19: 491-496, 1987.
- Boden G, Jadali F, White J, Liang Y, Mozzoli M, Chen X, Coleman E, and Smith C. Effects of fat on insulin-stimulated carbohydrate metabolism in normal men. J Clin Invest 88: 960– 966, 1991.
- Bottomly PA, Hardy CJ, Roemer PB, and Mueller OM. Proton-decoupled, Overhauser-enhanced, spatially-localized carbon-13 spectroscopy in humans. *Magn Reson Med* 12: 348–363, 1989.
- Burke LM, Collier GR, and Hargreaves M. Muscle glycogen storage after prolonged exercise: effect of the glycemic index of carbohydrate feedings. J Appl Physiol 75: 1019–1023, 1993.
- Carrithers JA, Williamson DL, Gallagher PM, Godard MP, Schulze KE, and Trappe SW. Effects of postexercise carbohydrate-protein feedings on muscle glycogen restoration. J Appl Physiol 88: 1976–1982, 2000.
- 8. Clark LC Jr, Noyes LK, Grooms TA, and Moore MS. Rapid micromeasurement of lactate in whole blood. *Crit Care Med* 12: 461–464, 1984.
- 9. Coyle EF, Coggan AR, Hemmert MK, and Ivy JL. Muscle glycogen utilization during prolonged strenuous exercise when fed carbohydrate. *J Appl Physiol* 61: 165–172, 1986.
- Doyle JA, Sherman WM, and Strauss RL. Effects of eccentric and concentric exercise on muscle glycogen replenishment. *J Appl Physiol* 74: 1848–1855, 1993.
- Hermansen L, Hultman E, and Saltin B. Muscle glycogen during prolonged severe exercise. Acta Physiol Scand 71: 334– 346, 1965.

- Ivy JL. Glycogen resynthesis after exercise: effect of carbohydrate intake. Int J Sports Med 19, Suppl: 142–146, 1998.
- Ivy JL, Katz AL, Cutler CL, Sherman WM, and Coyle EF. Muscle glycogen synthesis after exercise: effect of time of carbohydrate ingestion. J Appl Physiol 64: 1480-1485, 1988.
- Ivy JL, Lee MC, Brozinick JT, and Reed MJ. Muscle glycogen storage after different amounts of carbohydrate ingestion. *J Appl Physiol* 65: 2018–2023, 1988.
- 15. Jentjens RLPG, van Loon LJC, Mann CH, Wagenmakers AJM, and Jeukendrup AE. Addition of protein and amino acids to carbohydrates does not enhance postexercise muscle glycogen synthesis. J Appl Physiol 91: 839–846, 2001.
- Juhlin-Dannfelt A. Metabolic effects of β-adrenoceptor blockade on skeletal muscle at rest and during exercise. Acta Med Scand Suppl 665: 113–115, 1982.
- Kadish AH and Sternberg JC. Determination of urine glucose by measurement of rate of oxygen consumption. *Diabetes* 18: 467-470, 1969.
- Levenhagen DK, Gresham JD, Carlson MG, Maron DJ, Borel MJ, and Fakoll PJ. Postexercise nutrient intake timing in humans is critical to recovery of leg glucose and protein homeostasis. Am J Physiol Endocrinol Metab 280: E982–E993, 2001.
- Miles J, Glasscock R, Aikens J, Gerich J, and Haymond M. A microfluorometric method for the determination of free fatty acids in plasma. J Lipid Res 24: 96–99, 1983.
- Piatti PM, Monti LD, Pacchioni M, Pontiroli AE, and Pozza G. Forearm insulin and non-insulin-mediated glucose uptake and muscle metabolism in man: role of free fatty acids and blood glucose levels. *Metabolism* 40: 926–933, 1991.
- 21. Piehl AK, Soderlund K, and Hultman E. Muscle glycogen resynthesis rate in humans after supplementation of drinks containing carbohydrates with low and high molecular masses. *Eur J Appl Physiol* 81: 346–351, 2000.
- Price TB, Rothman DL, and Shulman RG. NMR of glycogen in exercise. Proc Nutr Soc 58: 1–9, 1999.
- Raum WJ. Methods of plasma catecholamine measurement including radioimmunoassay. Am J Physiol Endocrinol Metab 247: E4-E12, 1984.
- Reed MJ, Brozinick JT, Lee MC, and Ivy JL. Muscle glycogen storage postexercise: effect of mode of carbohydrate administration. J Appl Physiol 66: 720-726, 1989.
- Rizza RA, Cryer PE, Haymond MW, and Gerich JE. Adrenergic mechanisms for the effects of epinephrine on glucose production and clearance in man. J Clin Invest 65: 682-689, 1980.
- Starr JI and Rubenstein AH. Insulin, proinsulin, and C peptide. In: *Methods of Hormone Radioimmunoassay*. New York: Academic, 1974, p. 289–315.
- 27. Tarnopolsky MA, Bosman M, MacDonald JR, Vandeputte D, Martin J, and Roy BD. Postexercise protein-carbohydrate and carbohydrate supplements increase muscle glycogen in men and women. J Appl Physiol 83: 1877–1883, 1997.
- Van Hall Saris GWH, van de Schoor PA, and Wagenmakers AJ. The effect of free glutamine and peptide ingestion on the rate of muscle glycogen resynthesis in man. *Int J Sports Med* 21: 25–30, 2000.
- Van Hall G, Shirreffs SM, and Calbet JAL. Muscle glycogen resynthesis during recovery from cycle exercise: no effect of additional protein ingestion. J Appl Physiol 88: 1631-1636, 2000.
- Van Loon LJC, Saris WHS, Kruijshoop M, and Wagenmakers AJM. Maximizing postexercise muscle glycogen synthesis: carbohydrate supplementation and the application of amino acid and protein hydrolysate mixtures. *Am J Clin Nutr* 72: 106–111, 2000.
- Yaspelkis BB III and Ivy JL. The effect of a carbohydratearginine supplement on post-exercise carbohydrate metabolism. Int J Sport Nutr 9: 241-250, 1999.
- Zawadzki KM, Yaspelkis BB III, and Ivy JL. Carbohydrateprotein complex increases the rate of muscle glycogen storage after exercise. J Appl Physiol 72: 1854–1859, 1992.