Carbohydrate-protein complex increases the rate of muscle glycogen storage after exercise

K. M. ZAWADZKI, B. B. YASPELKIS III, AND J. L. IVY
Exercise Physiology and Metabolism Laboratory, Department of Kinesiology,
The University of Texas at Austin, Austin, Texas 78712

ZAWADZKI, K. M., B. B. YASPELKIS III, AND J. L. IVY. Carbohydrate-protein complex increases the rate of muscle glycogen storage after exercise. J. Appl. Physiol. 72(5): 1854-1859, 1992.—Carbohydrate, protein, and carbohydrate-protein supplements were compared to determine their effects on muscle glycogen storage during recovery from prolonged exhaustive exercise. Nine male subjects cycled for 2 h on three separate occasions to deplete their muscle glycogen stores. Immediately and 2 h after each exercise bout, they ingested 112.0 g carbohydrate (CHO), 40.7 g protein (PRO), or 112.0 g carbohydrate and 40.7 g protein (CHO-PRO). Blood samples were drawn before exercise, immediately after exercise, and throughout recovery. Muscle biopsies were taken from the vastus lateralis immediately and 4 h after exercise. During recovery the plasma glucose response of the CHO treatment was significantly greater than that of the CHO-PRO treatment, but the plasma insulin response of the CI20-PRO treatment was significantly greater than that of the CHO treatment. Both the CHO and CHO-PRO treatments produced plasma glucose and insulin responses that were greater than those produced by the PRO treatment (P < 0.05). The rate of muscle glycogen storage during the CHO-PRO treatment [35.5 ± 3.3 (SE) μmol·g protein⁻¹·h⁻¹] was significantly faster than during the CHO treatment (25.6 ± 2.3 μmol·g protein⁻¹·h⁻¹), which was significantly faster than during the PRO treatment (7.6 ± 1.4 μmol·g protein⁻¹·h⁻¹). The results suggest that postexercise muscle glycogen storage can be enhanced with a carbohydrate-protein supplement as a result of the interaction of carbohydrate and protein on insulin secretion.

AFTER PROLONGED endurance exercise, restoration of muscle glycogen is very slow unless a carbohydrate supplement is provided (3, 15, 16). When an adequate amount of carbohydrate is consumed immediately after exercise and at 2-h intervals thereafter, the rate of muscle glycogen storage is rapidly increased and maintained up to 6 h after exercise (4, 6, 16, 21, 26). However, efforts to further increase the rate of storage by increasing the amount and frequency of carbohydrate consumption or by changing the type and form of the carbohydrate used in the supplement have been unsuccessful (6, 16, 18, 26).

Insulin is a strong activator of muscle glycogen synthesis (9). This activation can occur potentially by two mechanisms. First, insulin increases the rate of muscle glucose transport, thus providing substrate for glycogen synthesis (2, 14, 17). Second, it activates glycogen synthase, the rate-limiting enzyme in the glycogen synthesis pathway (14, 17). Although glucose is the primary regulator of pancreatic insulin secretion, protein will also stimulate its release (22, 23, 25). Furthermore, when protein and carbohydrate are consumed together, the insulin response is greater than that which would be predicted from the addition of their individual responses (22, 23, 25, 28). Therefore the purpose of this study was to determine whether ingestion of a carbohydrate-protein supplement after prolonged endurance exercise would have a synergistic effect on the plasma insulin response and would enhance the rate of muscle glycogen storage above that produced with a carbohydrate supplement.

METHODS

Nine male cyclists were recruited to participate in this study. The average weight and maximum O₂ consumption (VO₂ max) of the subjects were 73.1 ± 3.1 (SE) kg and 66.6 ± 2.9 ml·kg⁻¹·min⁻¹, respectively. The protocol and the potential benefits and risks associated with participation were fully explained to each subject before they signed an informed consent document. The study was approved by the Institutional Review Board of The University of Texas at Austin.

Preliminary testing. The subjects were initially trained in the laboratory for 1–2 wk to familiarize them with the equipment, the laboratory setting, and the experimental protocol. During this time VO₂ max was determined by use of a continuous exercise test performed on a manually braked Monark 818 E cycle ergometer (26). A respiratory exchange ratio (R) > 1.10 and an increase in O₂ consumption (VO₂) of <0.21/min over the previous work rate were the criteria to determine whether VO₂ max was achieved. VO₂ was monitored continuously throughout the test by a computer-based open-circuit gas analysis system. Subjects breathed through a two-way Daniels valve while inspired volumes were measured with a Parkinson-Cowan CD-4 dry gas meter. Exhaled gases were continuously sampled from a mixing chamber and analyzed with O₂ (Applied Electrochemistry S-3A, Pittsburgh, PA) and CO₂ (Applied Electrochemistry SD-3A) analyzers. Analog outputs from the analyzers were directed to a laboratory computer for calculation of pulmonary ventilation, VO₂, CO₂ production, and R every 30 s.

During the preliminary testing a 2-h practice ride was conducted to adjust and/or verify appropriate work rates for the experimental trials. The regimen consisted of cycling for 15 min at 60–65% VO₂ max and 15 min at 70–75% VO₂ max. This was repeated three times during the first 90 min of the ride. During the last 30 min, subjects cycled at

maltodextrins; glucose; insulin
60–65% of their \( \text{VO}_{2\text{max}} \) for 10 min, 70–75% \( \text{VO}_{2\text{max}} \) for 10 min, 50% \( \text{VO}_{2\text{max}} \) for 5 min, and 80–85% \( \text{VO}_{2\text{max}} \) for the final 5 min. During the practice ride, no blood or tissue sampling was conducted and the recovery period was neglected. A training log and diet recall were kept by the subjects for 3 days before the training sessions. These records were used to standardize the subjects physical activity and diets on the days before each experimental trail.

**Experimental protocol.** After preliminary testing, each subject underwent three randomized experimental treatments separated by ~7 days. The subjects rode on a cycle ergometer for 2 h at the work rates established during the practice ride to deplete muscle glycogen stores and to lower blood glucose. Glycogen depletion was facilitated by having the subjects fast 12 h before reporting to the laboratory. To verify that the subjects were working at the proper intensity, \( \text{VO}_{2} \) was determined during the last 4 min of each 15-min interval for the 1st h of the ride. To minimize thermal stress, we circulated air past the subjects with two standing floor fans, and 2 ml of water per kg body weight were provided every 15 min during exercise.

Immediately after exercise and again 2 h after exercise, the subjects received a supplement containing 1) 112.0 g carbohydrate (CHO; dextrose-maltodextrin mixture, 21.0% wt/vol), 2) 40.7 g protein (PRO; milk and whey protein isolate mixture, 7.6% wt/vol), or 3) 112.0 g carbohydrate and 40.7 g protein (CHO-PRO; 21.0% wt/vol carbohydrate and 7.6% wt/vol protein mixture). The amount of carbohydrate provided was selected because it has been reported to maximize muscle glycogen storage after exercise (15). The amount of protein provided was selected because it has been reported to significantly increase insulin secretion in the absence and presence of carbohydrate (22, 28). All supplements were donated by Shaklee US (San Francisco, CA).

**Tissue collection and analysis.** Muscle biopsies were taken immediately after exercise and after 4 h of recovery from the vastus lateralis according to Bergström (2). The biopsies were frozen in isopentane cooled in liquid \( \text{N}_2 \) and stored at \(-80^\circ\text{C}\) for subsequent determination of glycogen.

Blood samples (5 ml) were drawn from a catheter inserted into an antecubital vein before and during the last 5 min of exercise and 15, 30, 60, 90, 120, 150, 180, and 240 min after ingestion of the first supplement after exercise. Four milliliters of blood were transferred to a chilled test tube containing EDTA (24 mg/ml, pH 7.4) and an apro tinin solution (Trasylol; 10,000 kallikrein-inactivating units/ml). From this tube 0.5 ml of blood was transferred to a tube containing 1 ml of 8% perchloric acid. Plasma and acid extract samples were recovered by centrifugation (15 min at 1,000 g) and were stored at \(-80^\circ\text{C}\).

During the recovery period, \( \text{VO}_{2} \) and \( \text{CO}_{2} \) were determined during a 10-min interval starting at 20, 50, 110, 170, and 230 min after ingestion of the first supplement. Carbohydrate oxidation was then calculated from these data with the tables of Lusk (20), assuming a nonprotein R.

For glycogen determination, the biopsies were weighed and homogenized in a 50% glycerol, 20 mM \( \text{Na}_2\text{HPO}_4 \) buffer (50:1 wt/vol, pH 7.4) that contained 0.5 mM EDTA, 0.02% bovine serum albumin, and 5 mM \( \beta \)-mercaptoethanol. Homogenization was performed in a dry ice acetone bath. Two hundred microliters of the homogenate were added to 200 \( \mu\text{l} \) of 2 N HCl and were incubated at 100°C for 120 min. The homogenate was cooled to room temperature and was neutralized with 1 N NaOH. The muscle glycogen concentration was determined enzymatically (24) and made relative to the protein concentration of the muscle (\( \mu\text{mol/g protein} \)). Muscle protein concentration was measured colorimetrically as described by Bradford (7). Plasma samples were analyzed for glucose with the use of a YSI 23A glucose analyzer (Yellow Springs Instrument, Yellow Springs, OH). Plasma insulin concentration was determined by radioimmunoassay with the use of a double antibody procedure (ICN Biomedical/Diagnostic Division, Costa Mesa, CA). Blood lactate concentrations were determined on the acid extracts according to the enzymatic procedure of Hohorst (13).

**Statistical analysis.** The data were analyzed using a one-way or two-way analysis of variance where appropriate. Post-hoc analyses were performed using Fisher's protected least significant differences. Differences were considered significant if \( P < 0.05 \) were obtained.

**RESULTS**

**Plasma glucose and insulin and blood lactate responses.** Plasma glucose concentrations during the three treatments declined from an average of 4.27 ± 0.05 mM before exercise to 3.67 ± 0.11 mM immediately after exercise (Fig. 1). Thirty minutes after ingestion of the first supplement, glucose levels increased 47% above preexercise concentrations in the CHO-PRO treatment and remained near 6.0 mM throughout the first 3 h of recovery. During the CHO treatment, plasma glucose concentrations rose 61% in the first 30 min of recovery and ranged between 6.15 and 7.06 mM throughout the first 3 h of recovery. Plasma glucose concentrations during the CHO treatment were significantly elevated above those
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CHO-PRO treatments produced significantly faster treatment and remained significantly different throughout the 4-h recovery period. Values are means ± SE. *Significantly different from PRO; †Significantly different from CHO.

Fig. 2. Plasma insulin concentrations before and at the end of 2 h of exercise and during 4-h recovery period for subjects receiving CHO-PRO, CHO, or PRO supplement. Supplements were provided immediately and 2 h after exercise. Values are means ± SE. *Significantly different from PRO.

Muscle glycogen storage. Muscle glycogen concentrations were similar for all treatments immediately after exercise (Table 1). After the 4-h recovery period, the muscle glycogen concentrations did not differ between the CHO and CHO-PRO treatments. However, the rate of storage during the CHO-PRO treatment was 38% faster than that which occurred during the CHO treatment ($P < 0.5$; Table 1 and Fig. 4). Both the CHO and CHO-PRO treatments produced significantly faster storage. Muscle glycogen and protein concentrations for muscle biopsy samples taken from vastus lateralis immediately after exercise and after 4-h recovery period

<table>
<thead>
<tr>
<th>Glycogen, mg/g wet wt</th>
<th>CHO-PRO</th>
<th>CHO</th>
<th>PRO</th>
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<tbody>
<tr>
<td>After Exercise</td>
<td>217.1±33.6</td>
<td>233.0±29.3</td>
<td>188.5±36.3</td>
</tr>
<tr>
<td>4 h After Exercise</td>
<td>359.0±33.7</td>
<td>335.9±31.5</td>
<td>218.8±34.8</td>
</tr>
<tr>
<td>Δ</td>
<td>141.9±13.6</td>
<td>102.7±9.2</td>
<td>30.3±5.6</td>
</tr>
</tbody>
</table>

Values are means ± SE. Δ, difference between after exercise and 4 h after exercise recovery glycogen concentrations. CHO-PRO, carbohydrate plus protein; CHO, carbohydrate; PRO, protein. *Significantly different from PRO; †significantly different from CHO ($P < 0.06$).

DISCUSSION

Rapid storage of muscle glycogen occurs in response to a carbohydrate feeding after exercise that significantly lowers the muscle glycogen stores (4, 6, 16, 21, 26). If the feeding is continued at 2-h intervals, a rapid rate of stor-
age can be maintained for 6–8 h after exercise (6, 16, 26). However, previous efforts to further enhance the rate of muscle glycogen storage after exercise by increasing the amount of carbohydrate or by changing the type and form of carbohydrate consumed has proven unsuccessful (6, 16, 18, 26). This inability of carbohydrate-supplement modification to further stimulate muscle glycogen storage does not appear to be limited by gastric emptying but is limited at the level of glucose transport or the glycogen synthesis pathway within muscle (5, 26).

Insulin is a strong activator of muscle glycogen synthesis because of its stimulating effect on glucose transport (1, 14, 17) and glycogen synthase (9), the rate-limiting enzyme in the glycogen synthesis pathway. Pancreatic insulin secretion is primarily regulated by the blood glucose concentration. However, protein as well as some amino acids will also stimulate insulin secretion (11, 22, 23, 25). Furthermore, when protein and carbohydrate are consumed together, the insulin response is greater than that which would be predicted from the addition of their individual responses (22, 23, 25, 28).

In the present study the addition of protein to a carbohydrate supplement resulted in a synergistic insulin response. In conjunction with the greater insulin response was a significantly lower plasma glucose response and a 38% faster rate of muscle glycogen storage compared with the CHO treatment alone. It was also noted that carbohydrate oxidation rates and blood lactate concentrations for the CHO-PRO and CHO treatments were similar. These results suggest that the increased rate of muscle glycogen storage during the CHO-PRO treatment was the result of an increased clearance of glucose by the muscle due to the increased plasma insulin response. This is supported by the recent finding of Kiens et al. (19) that the carbohydrates that have a high glycemic index are also the most effective in restoring muscle glycogen during the initial hours after exhaustive exercise.

One could also assume that the higher rate of glycogen storage during the CHO-PRO treatment was due to an increased availability of gluconeogenic precursors contributed by the protein. This is suggested by the finding that the sum of glycogen storage during the CHO and PRO treatments was approximately equal to that stored during the CHO-PRO treatment. However, there are several reasons why this possibility is not feasible. First, the rate of storage during the PRO treatment was very slow and not different from what has been previously observed when no supplement was provided after exercise (15, 16). This suggests that protein alone will not enhance the normally slow muscle glycogen storage rate that occurs immediately after prolonged intense exercise. Second, if the increase in glycogen storage during the CHO-PRO treatment was due to an increased rate of gluconeogenesis, an equal or higher glucose response during this treatment compared with the CHO treatment would have been expected. This of course did not occur. Third, the synergistic insulin response produced by the combining of the carbohydrate and protein supplements should have inhibited rather than enhanced gluconeogenesis.

It should also be mentioned that during the CHO-PRO treatment the rate of glycogen storage was not exceptionally fast. Similar rates have been seen after standard carbohydrate supplementation (6, 15, 21). It is therefore possible that if additional CHO supplement or a different carbohydrate supplement was used, there would have been no difference in the rates of storage between the CHO-PRO and CHO treatments. That is, the addition of protein to a carbohydrate supplement may be beneficial only when an inadequate CHO source is used.

Table 2. Carbohydrate oxidation estimated from the nonprotein R and the resting \( \text{VO}_2 \) during postexercise recovery

<table>
<thead>
<tr>
<th></th>
<th>30 min</th>
<th>60 min</th>
<th>120 min</th>
<th>180 min</th>
<th>240 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHO-PRO</td>
<td>0.62±0.03</td>
<td>0.74±0.02</td>
<td>0.77±0.00*</td>
<td>0.81±0.01*</td>
<td>0.84±0.01*</td>
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<tr>
<td>CHO</td>
<td>0.66±0.02*</td>
<td>0.74±0.03</td>
<td>0.78±0.01*</td>
<td>0.82±0.01*</td>
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</tr>
<tr>
<td>PRO</td>
<td>0.38±0.02</td>
<td>0.68±0.02</td>
<td>0.70±0.01</td>
<td>0.71±0.02</td>
<td>0.74±0.01</td>
</tr>
<tr>
<td>( \text{VO}_2 ), l/min</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHO-PRO</td>
<td>0.41±0.02</td>
<td>0.40±0.02</td>
<td>0.38±0.02*</td>
<td>0.40±0.02</td>
<td>0.39±0.01</td>
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<tr>
<td>CHO</td>
<td>0.37±0.01</td>
<td>0.36±0.02</td>
<td>0.33±0.01*</td>
<td>0.34±0.02*</td>
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</tr>
<tr>
<td>PRO</td>
<td>0.40±0.01</td>
<td>0.40±0.02</td>
<td>0.37±0.01</td>
<td>0.41±0.03</td>
<td>0.37±0.02</td>
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Carbohydrate oxidation, g/min

<table>
<thead>
<tr>
<th></th>
<th>30 min</th>
<th>60 min</th>
<th>120 min</th>
<th>180 min</th>
<th>240 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHO-PRO</td>
<td>0.01±0.01</td>
<td>0.07±0.02</td>
<td>0.10±0.02*</td>
<td>0.16±0.02*</td>
<td>0.22±0.03*</td>
</tr>
<tr>
<td>CHO</td>
<td>0.01±0.01</td>
<td>0.07±0.03*</td>
<td>0.10±0.02*</td>
<td>0.16±0.01*</td>
<td>0.23±0.02*</td>
</tr>
<tr>
<td>PRO</td>
<td>0.02±0.01</td>
<td>0.02±0.01</td>
<td>0.02±0.01</td>
<td>0.04±0.02</td>
<td>0.06±0.02</td>
</tr>
</tbody>
</table>

Values are means ± SE. \( \text{O}_2 \) consumption (\( \text{VO}_2 \)) measurements were made for 10-min intervals. R, respiratory exchange ratio. * Significantly different from PRO; † significantly different from CHO (P < 0.05).
The observation of a higher plasma insulin response and reduced plasma glucose response when subjects were fed carbohydrate and protein compared with carbohydrate alone is in agreement with previous research (22, 23, 25, 28). Rabinowitz et al. (25) and Pallotta and Kennedy (23) compared the plasma insulin and glucose responses after ingestion of a carbohydrate meal, a protein meal, and a combination carbohydrate and protein meal. The peak insulin concentration after the carbohydrate-protein meal was approximately twice that of the carbohydrate meal and about four times that after ingestion of the protein meal. Nuttal et al. (22) found that ingestion of 50 g protein in combination with 50 g carbohydrate compared with 50 g of carbohydrate alone resulted in a higher plasma insulin response but a lower plasma glucose response. Spiller et al. (28) also demonstrated an increased plasma insulin response and decreased plasma glucose response with the addition of protein to a carbohydrate supplement. Moreover, Spiller et al. found that the insulin response was directly proportional and the glucose response inversely proportional to the protein content of the carbohydrate-protein supplement. The reason for a synergistic plasma insulin response after a carbohydrate-protein supplement is not known, but it has been suggested to be due to an increased release of insulin secretagogues from the gastrointestinal tract (23).

There are several possible reasons for the lower plasma glucose response during the CHO-PRO treatment in addition to an increased rate of glucose clearance by the muscle. One explanation is that hepatic glucose production was suppressed to a greater extent during the carbohydrate-protein supplementation. Nuttal et al. (22) found that ingestion of 50 g protein in combination with 50 g carbohydrate compared with 50 g of carbohydrate alone resulted in a higher plasma insulin response but a lower plasma glucose response. Spiller et al. (28) also demonstrated an increased plasma insulin response and decreased plasma glucose response with the addition of protein to a carbohydrate supplement. Moreover, Spiller et al. found that the insulin response was directly proportional and the glucose response inversely proportional to the protein content of the carbohydrate-protein supplement. The reason for a synergistic plasma insulin response after a carbohydrate-protein supplement is not known, but it has been suggested to be due to an increased release of insulin secretagogues from the gastrointestinal tract (23).

In summary, it was found that a carbohydrate-protein supplement was more effective than a carbohydrate supplement for the resynthesis of muscle glycogen during the initial hours of recovery from prolonged intense exercise. The increased rate of muscle glycogen storage during the carbohydrate-protein supplementation appeared to be due to an increased plasma insulin response, although other possibilities exist. Protein supplementation alone was found to have little influence on muscle glycogen storage after exercise.

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Address for reprint requests: J. L. Ivy, Dept. of Kinesiology, Bellmont Hall 222, The University of Texas at Austin, Austin, TX 78712.

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