Effects of postexercise carbohydrate-protein feedings on muscle glycogen restoration

JOHN A. CARRITHERS, DAVID L. WILLIAMSON, PHILIP M. GALLAGHER, MICHAEL P. GODARD, KIMBERLEY E. SCHULZE, AND SCOTT W. TRAPPE

Human Performance Laboratory, Ball State University, Muncie, Indiana 47306

Carrithers, John A., David L. Williamson, Philip M. Gallagher, Michael P. Godard, Kimberley E. Schulze, and Scott W. Trappe. Effects of postexercise carbohydrate-protein feedings on muscle glycogen restoration. J Appl Physiol 88:1976–1982, 2000.—The purpose of this investigation was to determine the effects of postexercise eucaloric carbohydrate-protein feedings on muscle glycogen restoration after an exhaustive cycle ergometer exercise bout. Seven male collegiate cyclists [age = 25.6 ± 1.3 yr, height = 180.9 ± 3.2 cm, wt = 75.4 ± 4.0 kg, peak oxygen uptake (V̇O₂peak) = 4.20 ± 0.2 l/min] performed three trials, each separated by 1 wk: 1) 100% α-d-glucose (carbohydrate [CHO]), 2) 70% carbohydrate-20% protein (PRO)-10% fat, and 3) 86% carbohydrate-14% amino acid (AA). All feedings were eucaloric, based on 1.0 g·kg body wt⁻¹·h⁻¹ of CHO, and administered every 30 min during a 4-h muscle glycogen restoration period in an 18% wt/vol solution. Muscle biopsies were obtained immediately after exercise and every 0.5 h for 4 h during the restoration period. Increases in muscle glycogen concentrations for the three feedings (CHO, CHO-PRO, CHO-AA) were 118 mmol/kg dry wt; however, no differences among the feedings were apparent. The serum glucose and insulin responses did not differ throughout the restoration period among the three feedings. These results suggest that muscle glycogen restoration does not appear to be enhanced with the addition of proteins or amino acids to an eucaloric CHO feeding after exhaustive cycle exercise.

Methods

Subjects

Eight male collegiate cyclists (nonsmokers with no metabolic or cardiovascular disorders) were recruited for this investigation. In addition, subjects were required to be glucose tolerant, having a fasted serum glucose concentration of <115 mg/dl, a peak value of <200 mg/dl, and a 2-h concentration of <140 mg/dl (16). One subject’s peak serum glucose concentration was 214 mg/dl and the 2-h concentration was 156 mg/dl; therefore, this subject was dismissed from the investigation, and all data presented are based on seven subjects (Table 1). Subjects were informed of all experimental procedures and risks associated with the investigation and gave their written, informed consent in accordance with the University Institutional Review Board.

Subjects performed three different muscle glycogen depletion/restoration trials, each separated by 1 wk. One trial investigated the effect of a CHO-only feeding on muscle glycogen restoration, whereas the other two trials consisted of a 70% CHO-20% PRO-10% fat feeding and an 86% CHO-14% amino acid (AA) feeding (see Experimental Feedings). All trials were performed in random order.

Preliminary Testing

Aerobic capacity test. One week before the initial trial, subjects performed a graded exercise test on an electronically braked cycle ergometer (Lode, Groningen, Holland) to determine aerobic capacity or peak oxygen uptake (V̇O₂peak). A

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one-way, nonrebreathing respiratory valve and a nose clip were utilized to collect expired oxygen and carbon dioxide. A personal computer was interfaced with a Parkinson-Cowan dry-gas meter, an oxygen analyzer (Applied Electrochemistry S-3A), and a carbon dioxide analyzer (Sensormedics LB-2) to obtain the respiratory exchange ratio and oxygen consumption ($V\dot{O}_2$) values every 30 s. $V\dot{O}_2$peak was determined when subjects attained a respiratory exchange ratio value of 1.10 and/or a $V\dot{O}_2$ increase from the previous exercise intensity of $0.2 \text{ l/min}$ (14, 24).

70% Check ride. Two days after the $V\dot{O}_2$peak test, subjects underwent a 20–25 min $V\dot{O}_2$ check ride on a cycle ergometer (Cybex, Ronkonkama, NY). Due to the differences in cycle ergometers (Cybex vs. Lode) used for the $V\dot{O}_2$peak test and the muscle glycogen depletion rides, the check ride established the appropriate exercise intensity necessary to achieve 70% of the subject's predetermined $V\dot{O}_2$peak for the muscle glycogen depletion rides. This test also established the appropriate workload necessary to elicit 125% of the subject's $V\dot{O}_2$ for the sprints. During the check ride, the subject's $V\dot{O}_2$ was analyzed in a process identical to that used in the $V\dot{O}_2$peak test.

Experimental Trials

Each of the three muscle glycogen depletion/restoration trials was composed of a 4-day period. On days 1 and 2 of the trial, subjects consumed their "normal" diet. On day 3, subjects performed a fasted OGTT, consumed a controlled diet, and cycled in the evening for 45 min. On day 4, subjects cycled for 75 min and underwent six, 1-min sprints, followed by a 4-h muscle glycogen restoration period. This cycle ergometer protocol was modified from previous studies that demonstrated it to be an effective mechanism for decreasing muscle glycogen concentrations (15). An overview of the 4-day muscle glycogen depletion/restoration protocol is presented in Fig. 1.

Dietary Control (Days 1, 2, and 3)

On days 1 and 2 of the trial, subjects consumed their normal dietary intake over the 48-h period. Diets were analyzed for total caloric intake and for amounts (g) of CHO, PRO, and fat by a dietary analysis program (ESHA Food Processor, version 7.11). A copy of this diet was given to the subjects to repeat for the ensuing two trials. This was done to assist in the standardization of muscle glycogen concentration before each trial. In addition, no exercise was permitted during this 48-h period.

On day 3 of each trial, subjects were fed a controlled diet (breakfast, lunch, and dinner) consisting of 55% CHO, 20% PRO, and 25% fat. The total caloric intake for each subject was based on the Harris-Benedict (12) equation for men: total daily expenditure = $[66 + (13.7 \times \text{weight}) + (5 \times \text{height}) - (6.8 \times \text{age})] \times \text{activity factor} \times \text{injury factor}$. All calories for the controlled diet on day 3 were consumed after the OGTT and before the 45-min ride that evening.

OGTT (Day 3: Morning)

On day 3 of the trial, after a 12-h overnight fast, subjects performed an OGTT. A 75-g (300 calories) load of $\alpha$-D-glucose was dissolved in 400 ml of water and administered for the OGTT of the CHO trial (16). The OGTT for the CHO-PRO and CHO-AA trials were based on the 75-g load administered in the CHO trial. The feeding consumed for the OGTT was identical to the one used during the muscle glycogen restoration phase on day 4 of the trial. Before the OGTT, a fasted blood sample was collected from an anticubital vein, via Teflon catheter kept patent with 0.9% saline. After the fasted blood sample, subjects consumed the feeding, and a blood sample was then taken every 30 min for 2 h (16).

Muscle Glycogen Depletion Protocol

Day 3: evening The evening of day 3, subjects reported to the laboratory and exercised on a cycle ergometer (Cybex) for 45 min at 70% of their $V\dot{O}_2$peak. At 15 and 45 min, the subject's heart rate was measured via telemetry (Polar Vantage XL, Polar Electro, Port Washington, NY), and $V\dot{O}_2$ was measured by collection of expired air into Douglas bags for 60 s. Douglas

### Table 1. Subject demographics

| Age, yr | 25.6 ± 1.3 |
| Height, cm | 180.9 ± 3.2 |
| Weight, kg | 75.4 ± 4.0 |
| Body fat, % | 9.5 ± 1.2 |
| $V\dot{O}_2$peak, l/min | 4.20 ± 0.2 |
| Fiber type I, % | 54.3 ± 2.7 |
| Fiber type II, % | 45.7 ± 2.7 |

Values are means ± SE. $V\dot{O}_2$peak, peak oxygen consumption.
bags were analyzed for oxygen and carbon dioxide using gas analyzers to determine V\textsubscript{O\textsubscript{2}}. After the 45-min ride, subjects were instructed to consume no additional food and, to ensure adequate hydration, were given 1 liter of water to consume that evening.

Day 4. On day 4 of the trial, subjects reported to the laboratory in the morning and exercised for 75 min at 70% of their V\textsubscript{O\textsubscript{2}}\textsubscript{peak} on a cycle ergometer (Cybex). This was followed by six, 1-min sprints at 125% of their V\textsubscript{O\textsubscript{2}}\textsubscript{peak} with a 1-min rest period between sprints. Additional sprints were performed if subjects were not completely exhausted. The subject’s heart rate and V\textsubscript{O\textsubscript{2}} were measured at 15, 45, and 75 min, via telemetry and Douglas bag collection, respectively. Throughout the 45- and 75-min rides, subjects were allowed to consume water ad libitum for the first trial. For the ensuing two trials, individual water consumption was limited to the amount consumed during the initial trial.

Muscle Biopsy

At the conclusion of the final sprint, a muscle biopsy was obtained with aid of suction (1, 9) from the vastus lateralis muscle. The time from cessation of exercise to the muscle biopsy was ~2 min. At the conclusion of the 4-h restoration period, another needle biopsy of the vastus lateralis muscle was obtained, ~3 cm proximal to the previous sample (8). The muscle samples were dissected of any visible connective tissue and immediately frozen in liquid nitrogen until assayed for total muscle glycogen.

Blood Sampling

Before the 75-min muscle glycogen depletion ride was started, a fasted blood sample was obtained from an antecubital vein, via a vacutainer vial containing SST gel and clot activator (Vacutainer, Franklin Lakes, NJ). Blood samples were obtained immediately after the depletion ride and every 30 min thereafter for 4 h. All samples were centrifuged at 3,000 rpm for 15 min, and the serum was decanted from each sample and frozen at −10°C until analyzed for glucose and insulin.

Experimental Feedings

Consumption of the first feeding occurred immediately after the initial biopsy and blood sample. For the remainder of the restoration period, subjects consumed seven additional feedings, each separated by 30 min. The last feeding was consumed at the 210-min point, for a total of eight feedings. CHO, carbohydrate feeding; CHO-PRO, carbohydrate-protein feeding. CHO-PRO 1,206 (150) 214.5 (26.8) 61.3 (7.7) 1.5 (1.4)

Table 2. Example muscle glycogen restoration feeding for a 75-kg man

<table>
<thead>
<tr>
<th>Feeding</th>
<th>Energy, kcal</th>
<th>CHO, g</th>
<th>PRO, g</th>
<th>Fat, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHO</td>
<td>1,206 (150)</td>
<td>301.6</td>
<td>37.7</td>
<td>0</td>
</tr>
<tr>
<td>CHO-PRO</td>
<td>1,206 (150)</td>
<td>214.5</td>
<td>26.8</td>
<td>61.3</td>
</tr>
<tr>
<td>CHO-AA</td>
<td>1,206 (150)</td>
<td>258.5</td>
<td>32.3</td>
<td>43.1</td>
</tr>
</tbody>
</table>

Numbers in parentheses represent amounts consumed during feeding. CHO, carbohydrate feeding; CHO-PRO, carbohydrate-protein feeding; CHO-AA, carbohydrate-amino acid feeding.

The proteins within the CHO-PRO feeding have previously demonstrated (17, 22, 25) to elicit an elevated insulin response both with and without carbohydrates. The CHO-AA feeding contained sucrose, fructose, dextrose, and L-lysine, L-leucine, L-valine, L-phenylalanine, L-threonine, L-histidine, L-isoleucine, and L-methionine. It was hypothesized that, because exercise stimulates the release of nonessential amino acids (5, 10), the CHO-AA feeding containing only essential amino acids would produce an insulin response greater than that demonstrated by the CHO-only feeding.

Muscle Glycogen Analysis

Muscle samples were sectioned into three pieces and subsequently freeze-dried (FTS Systems, Stone Ridge, NY) at −35°C for 7 days. Once the muscle sample was freeze-dried, each piece was weighed on a microbalance (CAHN C-35 ATI Orion, Boston, MA) and placed in hydrochloric acid for the hydrolysis of glycogen to glucose residue. After hydrolysis, the sample was neutralized with sodium hydroxide, and a reagent cocktail was added. The total muscle glycogen was determined fluorometrically (Ratio-2 system fluorometer, Optical Technology Devices, Elmsford, NY) by measuring the appearance of NADPH.

Blood Analysis

Serum glucose was determined by an enzymatic hexokinase/glucose-6-phosphate dehydrogenase reaction (Sigma Chemical no. 16-UV, St. Louis, MO). The absorbance of NADPH was measured on a spectrometer (spectrometer 501, Milton Roy) at 340 nm. A RIA kit (Coat-A-Count, Diagnostic Products, Los Angeles, CA) was used to analyze the serum insulin concentration of each sample in duplicate. Serum insulin samples, after a 24-h incubation, were analyzed via a gamma counter (1470 Wizard gamma counter, Wallac, Gaithersburg, MD). Each subject’s glucose and insulin samples were assayed simultaneously to account for interassay variances.

Statistical Analysis

Comparison of means between trials was conducted using a two-way repeated-measures ANOVA (treatment x time). Significance level was set at P < 0.05. When significance occurred between means, a Tukey’s post hoc test was used to determine where significance occurred. All data are presented as means ± SE.

RESULTS

OGTT

Glucose. The mean serum glucose concentration for the three trials increased 32% from baseline to 30 min (peak concentration), and, at the conclusion of the OGTT, the response returned to baseline measurements (Fig. 2). The glucose concentrations at 0, 30, 90, and 120 min were similar among the three trials. However, at 60 min, the serum glucose concentration for the CHO trial was greater (P < 0.05) than that of the CHO-PRO and CHO-AA trials. No statistical differences were observed for the area under the curve (AUC) in the CHO (150.1 ± 18.6 mM), CHO-PRO (96.1 ± 12.3 mM), and CHO-AA (97.0 ± 18.6 mM) trials.

Insulin. During the 2-h OGTT, no differences were observed at any point for the serum insulin concentrations (Fig. 2). The average insulin concentration for the three trials peaked at 30 min, and, at the conclusion of
the OGTT, the insulin response returned to the baseline measurements. Additionally, no differences were observed for the AUC for CHO (2,671.2 ± 6429.1 µU/ml), CHO-PRO (2,413.1 ± 316.7 µU/ml), and CHO-AA (1,964.3 ± 338.3 µU/ml) trials.

Glucose and Insulin Response During the 4-h Restoration Period

Glucose. Serum glucose concentrations increased, on average, from 4.38 ± 0.08 mM before exhaustive exercise to 5.28 ± 0.28 mM immediately postexercise for the three trials (Fig. 3). With serial feedings administered throughout the restoration period, the mean serum glucose concentration peaked at 60 min, with an increase of 33% from the immediate postexercise glucose concentration. There was a 12% decrease in the serum glucose concentration from 60 to 120 min. From 120 min to the conclusion of the 4-h restoration period, the mean serum glucose concentration ranged from 5.00–6.11 mM for all three trials. However, the CHO trial at 90 min had a greater (P < 0.05) glucose concentration than either the CHO-PRO and CHO-AA trials. In addition, the AUC did not differ for the three trials (CHO = 287.9 ± 35.0, CHO-PRO = 277.8 ± 14.4, and CHO-AA = 211.6 ± 37.6 mM).

Insulin. Throughout the restoration period, no differences were observed in the serum insulin concentrations at any time (Fig. 3). The insulin response of the three trials averaged an increase of 87% from immediately postexercise to the 120-min mark of the restoration period and remained elevated until the conclusion of the restoration period. At no time during the restoration period did the insulin concentrations approach the baseline concentrations found immediately postexercise; this can be attributed to the serial feedings administered every 0.5 h. The AUC during the restoration period did not differ for the three trials (CHO = 5,095.7 ± 1,231.9, CHO-PRO = 4,567.3 ± 843.7, and CHO-AA = 3,216.0 ± 630.9 µU/ml).

Muscle Glycogen Restoration

The muscle glycogen concentrations immediately after exercise were similar among the three trials (Fig. 4) (CHO = 107 ± 27, CHO-PRO = 118 ± 35, and CHO-AA = 87 ± 36 mmol/kg dry wt). As expected, there was an increase (P < 0.05) in muscle glycogen concentrations at the conclusion of the 4-h restoration period (CHO = 231 ± 37, CHO-PRO = 230 ± 29, and CHO-AA = 205 ± 40 mmol/kg dry wt); however, no differences were apparent among the three trials. Over the 4-h restoration period, the three feedings caused an average increase in muscle glycogen concentration of 118 mmol/kg dry wt.

Fig. 2. Glucose (A) and insulin (B) concentrations during 2-h oral glucose tolerance test. Values are means ± SE. *P < 0.05 for carbohydrate (CHO)-only feeding compared with carbohydrate-protein (CHO-PRO) and carbohydrate-amino acid (CHO-AA) feedings.

Fig. 3. Glucose (A) and insulin (B) concentrations before exhaustive exercise and during 4-h muscle glycogen restoration period. Values are means ± SE. *P < 0.05 for CHO trial compared with CHO-PRO and CHO-AA trials.

Fig. 4. Muscle glycogen concentrations immediately after exhaustive exercise and during 4-h muscle glycogen restoration period.
The number of sprints (CHO) were similar within and among the three trials. These results contradict the findings of Zawadzki et al. (25), in which subjects were administered three feedings: 1) 112 g of CHO, 2) 40.7 g of PRO, and 3) 152.7 g of a CHO-PRO mixture. They demonstrated that, during a 4-h restoration period, the CHO-PRO feeding elicited a 38% greater muscle glycogen restoration than the CHO-only feeding, and both were greater than the PRO-only feeding.

Two previous investigations (21, 23) similar to the present study examined the effects of isoenergetic CHO and CHO-PRO feedings (based on 1.0 g/kg body wt of CHO) on muscle glycogen restoration after an exhaustive exercise bout. These results demonstrated that both feedings had similar effects on muscle glycogen restoration. The results from the present investigations, along with those of Roy and Tarnopolsky (21) and Tarnopolsky et al. (23), demonstrate that the caloric content of the feedings may impact muscle glycogen restoration. The additional caloric content of the CHO-PRO feeding in the investigation by Zawadzki et al. (25) may have been a critical determinate for the enhanced muscle glycogen storage of the CHO-PRO feeding compared with the CHO- and PRO-only feedings.

The muscle glycogen concentration immediately after the exhaustive exercise bout in the present investigation averaged 104 mmol/kg dry wt for the three trials. These findings are similar to the results reported by Tarnopolsky et al. (23), who also used endurance trained cyclists. In contrast, the investigation by Roy and Tarnopolsky (21) reported that the immediate postexercise muscle glycogen concentrations for the CHO and CHO-PRO trials were ~228 mmol/kg dry wt. The most likely cause for the differences in muscle glycogen concentrations between the studies is the different exhaustive exercise protocols, i.e., resistance training in the investigation by Roy and Tarnopolsky (21) vs. a cycle ergometer protocol in the present investigation and that of Tarnopolsky et al. (23). At the conclusion of the 4-h restoration period, a greater absolute increase in muscle glycogen concentration (118 vs. 84 mmol/kg dry wt) was evident in the present investigation compared with the increase found by Roy and Tarnopolsky (21), which could result in an elevated glycogen synthase activity (13, 19, 24).

Previous investigations by Blom et al. (3) and Ivy et al. (14) demonstrated that CHO ingestion between 0.70 and 1.5 g·kg body wt⁻¹·h⁻¹ after exhaustive exercise provided adequate stimulus for maximal muscle glycogen restoration over a 4-h period. However, additional CHO ingestion (>0.7 g·kg body wt⁻¹·h⁻¹) did not appear to provide an enhanced benefit in the restoration of muscle glycogen (3, 14). The CHO content of the

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**Table 3. Dietary intake of calories, carbohydrates, protein, and fat during the first 3 days of each trial**

<table>
<thead>
<tr>
<th>Day</th>
<th>Energy, kcal</th>
<th>CHO, g</th>
<th>PRO, g</th>
<th>Fat, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>3,224 ± 509</td>
<td>425 ± 71 (53)</td>
<td>116 ± 17 (14)</td>
<td>119 ± 43 (33)</td>
</tr>
<tr>
<td>Day 2</td>
<td>2,955 ± 419</td>
<td>400 ± 72 (54)</td>
<td>95 ± 25 (13)</td>
<td>106 ± 32 (32)</td>
</tr>
<tr>
<td>Day 3</td>
<td>3,220 ± 546</td>
<td>474 ± 78 (57)</td>
<td>156 ± 25 (19)</td>
<td>91 ± 15 (25)</td>
</tr>
</tbody>
</table>

Values are means ± SE. Numbers in parentheses represent percentage of daily diet.
three feedings in the present investigation differed (CHO = 1.0, CHO-PRO = 0.71, and CHO-AA = 0.86 g·kg body wt\(^{-1}·h^{-1}\)), but muscle glycogen restoration among the three trials was similar, a finding that supports the results of Blom et al. (3) and Ivy et al. (14). Therefore, muscle glycogen restoration does not appear to be altered with a CHO-PRO feeding, provided that adequate carbohydrates are consumed.

The addition of protein to a CHO feeding has been shown to have a synergistic effect on the serum insulin response compared with a CHO-only feeding (17, 18, 22, 25). Zawadzki et al. (25) theorized that the elevated plasma insulin response in the CHO-PRO trial was responsible for the decrease in the serum glucose concentrations and for the greater muscle glycogen restoration compared with the CHO and PRO feedings. The addition of protein or amino acids to the CHO feeding in the present study and in previous investigations (21, 23) did not elicit a synergistic insulin response. Furthermore, in the present study, during the resting OGTT and restoration periods, no significant differences were found in the insulin and glucose responses for the three feedings. Likewise, the AUC for the glucose and insulin responses demonstrated similar responses for the three trials during both the resting OGTT and the 4-h restoration period. Neither the investigations by Roy and Tarnopolsky (21) and Tarnopolsky et al. (23) nor the present study demonstrated greater muscle glycogen restoration with CHO-PRO or CHO-AA feedings compared with CHO-only feeding, thus indicating that the caloric intake of the feedings does not appear to be identical among the three trials was similar immediately postexercise and in previous investigations (21, 23) did not elicit a synergistic insulin response. Furthermore, in the present study, during the resting OGTT and restoration periods, no significant differences were found in the insulin and glucose concentrations for the feedings does not appear to be altered with a CHO-PRO feeding, provided that adequate carbohydrates are consumed.

The serum glucose responses at 60 min of the resting OGTT and at 90 min of the 4-h restoration period in the present study were greater in the CHO trial than in either the CHO-PRO or the CHO-AA trial. The elevated glucose response may be due to the greater CHO load in the CHO trial compared with the CHO-PRO and CHO-AA trials (1.0 vs. 0.70 and 0.86 g·kg body wt\(^{-1}·h^{-1}\), respectively). Additionally, after the 60- and 90-min time points of the resting OGTT and 4-h restoration period, respectively, the serum insulin response demonstrated a trend to be greater during the CHO trial than in the CHO-PRO and CHO-AA trials, thereby compensating for the elevated glucose response elicited at the 60- and 90-min time points.

Carbohydrate metabolism may be altered by the simultaneous ingestion of fats, as each substrate competes as an oxidative fuel source (20). Therefore, the addition of fat in the CHO-PRO feeding may have decreased glucose utilization, and a concomitant increase in the serum glucose concentration would be expected. Previous investigations (4, 21, 23) have demonstrated that the addition of minimal amounts of fats (11 and 31%) to a CHO-only feeding did not affect the glucose response or muscle glycogen restoration. The CHO-PRO feeding demonstrated a serum glucose response similar to that for CHO-AA and CHO feedings in the present study, supporting the findings of previous investigations (4, 21, 23).

In summary, muscle glycogen concentrations for the three trials were similar immediately postexercise and again 4 h after the cessation of exercise. In addition, the serum insulin and glucose responses among the three eucaloric feedings displayed no differences at any time throughout the 4-h restoration period. Therefore, it appears that the addition of protein or amino acids to an eucaloric postexercise CHO feeding does not enhance the restoration of muscle glycogen compared with a CHO-only feeding. Thus, provided that the caloric content is similar and adequate amounts of CHO are consumed (>)0.70 g·kg body wt\(^{-1}·h^{-1}\) after exhaustive exercise, the addition of protein or amino acids to a CHO feeding does not appear to enhance muscle glycogen restoration.

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