Postexercise Muscle Glycogen Recovery Enhanced with a Carbohydrate–Protein Supplement

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ABSTRACT

BERARDI J. M., T. B. PRICE, E. E. NOREEN, and P. W. LEMON. Postexercise Muscle Glycogen Recovery Enhanced with a Carbohydrate–Protein Supplement. *Med. Sci. Sports Exerc*., Vol. 38, No. 6, pp. 1106–1113, 2006. **Purpose:** This study assessed whether liquid carbohydrate–protein (C + P) supplements, ingested early during recovery, enhance muscle glycogen resynthesis versus isoenergetic liquid carbohydrate (CHO) supplements, given early or an isoenergetic solid meal given later during recovery (PLB). **Methods:** Two hours after breakfast (7.0 kcal·kg^{-1}; 0.3 g·kg^{-1} P, 1.2 g·kg^{-1} C, 0.1 g·kg^{-1} F), six male cyclists performed a 60-min time trial (AM_{ex}). Pre- and postexercise, vastus lateralis glycogen concentrations were determined using nMRS. Immediately, 1 h, and 2 h postexercise, participants ingested C + P (4.8 kcal·kg^{-1}; 0.8 g·kg^{-1} C, 0.4 g·kg^{-1} P), CHO (4.8 kcal·kg^{-1}; 1.2 g·kg^{-1} C), or PLB (no energy). Four hours postexercise, a solid meal was ingested. At that time, C + P and CHO received a meal identical to breakfast, whereas PLB received 21 kcal·kg^{-1} (1 g·kg^{-1} P, 3.6 g·kg^{-1} C, 0.3 g·kg^{-1} F); energy intake during 6 h of recovery was identical among treatments. After 6 h of recovery, measurement and cycling protocols (PM_{ex}) were repeated. **Results:** Absolute muscle glycogen utilization was 18% greater ($P \leq 0.05$) during AM_{ex} (C + P: −42.75 ± 5.24 mmol·L^{-1}; CHO: −37.08 ± 7.59 mmol·L^{-1}; PLB: −53.78 ± 11.59 mmol·L^{-1}; $P = 0.302$) relative to PM_{ex} (C + P: −38.40 ± 4.37 mmol·L^{-1}; CHO: −31.16 ± 3.78 mmol·L^{-1}; PLB: −40.33 ± 1.47 mmol·L^{-1}; $P = 0.292$), but there were no differences between groups. During 6 h of recovery, muscle glycogen resynthesis was greater in C + P (+ 28.62 ± 2.10 mmol·L^{-1}) versus CHO (+ 22.20 ± 1.19 mmol·L^{-1}, $P \leq 0.05$) or PLB (+ 18.50 ± 7.67 mmol·L^{-1}, $P \leq 0.05$). Cycling performance was similiar ($P = 0.282$) among treatments during both AM_{ex} (C + P: 37.61 ± 0.63 km; CHO: 37.03 ± 0.60 km; PLB: 37.24 ± 0.34 km) and PM_{ex} (C + P: 36.31 ± 0.83 km; CHO: 36.38 ± 0.80 km; PLB: 35.34 ± 0.45 km). **Conclusions:** C + P supplements, given early after exercise, enhance glycogen resynthesis relative to CHO and PLB. However, this does not influence performance in this type of exercise bout. **Key Words:** ENDURANCE ATHLETES, CYCLING TIME TRIAL, POSTEXERCISE NUTRITION, MAGNETIC RESONANCE SPECTROSCOPY

Prolonged moderate-intensity exercise and high-intensity intermittent exercise cause significant muscle glycogen depletion (5,10,16). Because skeletal muscle glycogen is an important fuel for exercise performance (9,10,21,25), researchers and athletes alike have sought nutritional strategies to both maximize muscle glycogen storage prior to exercise and glycogen resynthesis following exercise. Historically, these strategies have focused on dietary carbohydrate (CHO) manipulation and include various preexercise glycogen supercompensation protocols (9,25). In addition, postexercise CHO ingestion timing (12,17), type (2), amount (13), and frequency (3) have also been studied to determine the most effective way to accelerate muscle glycogen recovery. Many of these investigations have demonstrated that higher preexercise muscle glycogen concentration can delay fatigue (9,25), and some also indicate that for repeated exercise, glycogen resynthesis between bouts is a critical performance determinant, especially when recovery time is short (6,26).

A recent nutritional manipulation proposed to improve muscle glycogen resynthesis involves replacing some of the CHO consumed during recovery with protein (4,11,15, 19,20,23,26,27,29). Apparently, when consumed alone, ingested amino acids and protein promote a rapid and large insulin response (7). Furthermore, when consumed along with CHO, some amino acids promote a synergistic hyperinsulinemia (22,24). Because insulin plays a major role in skeletal muscle CHO uptake and glycogen storage, especially when glycogen concentrations are greater than 30–35 mmol·L^{-1} (known as the insulin-dependent phase of glycogen resynthesis), it has been suggested that the rate of muscle glycogen resynthesis should be elevated further when a CHO–protein or CHO–amino acid beverage is consumed (11,14).

To this end, Zawadzki et al. (29) demonstrated that CHO plus protein (C + P) supplementation improved muscle glycogen resynthesis during 4 h of recovery following

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exercise relative to CHO-only supplementation. Whereas the investigators speculated that this greater rate of muscle glycogen resynthesis was due to a greater plasma insulin response with C + P, the treatments were not isoenergetic; the C + P condition received more total energy. It is therefore conceivable that this effect was due, at least in part, to the greater energy provision in the C + P group versus the CHO-only group (29). More recent research with isoenergetic supplementation is equivocal, with some studies showing no effect of C + P ingestion relative to isoenergetic CHO (4,15,19,23), others indicating a tendency toward higher glycogen resynthesis with C + P or C + arginine (20,27), and one study demonstrating greater glycogen resynthesis with C + P relative to isoenergetic CHO (11). Further complicating this issue are potentially important methodological differences among the aforementioned studies.

In this investigation, the effect of supplementation type (CHO vs C + P) as well as timing (early vs 4 h following strenuous exercise) on glycogen resynthesis and repeat exercise performance was studied. It was hypothesized that liquid C + P supplements ingested early during recovery would enhance muscle glycogen resynthesis and subsequent exercise performance versus either early isoenergetic liquid CHO feedings or an isoenergetic solid meal given 4 h into recovery (placebo, PLB). It was also hypothesized that the liquid CHO supplements would be superior to the isoenergetic solid meal on both measures. ^{13}C-nuclear magnetic resonance (NMR) was chosen to assess muscle glycogen not only because it is a noninvasive, highly sensitive technique, but also because it samples from a much larger percentage of the thigh mass versus the needle biopsy, thereby providing a more representative measure of thigh glycogen content.

METHODS

Participants. The same six competitive male cyclists were studied with both C + P and CHO; however, due to scheduling conflicts, only four of these six subjects were evaluated under the PLB condition. All subjects were members of the Yale University cycling team and were screened by interview for medical history. None of the subjects had known neuromuscular, metabolic, or musculoskeletal disorders. In addition, anyone using dietary supplements other than multivitamin tablets, glucose–electrolyte solutions (liquid carbohydrate supplements), or protein supplements, was excluded from the study. Prior to collecting any measures, participants provided written informed consent according to a protocol approved by the human investigation committee at the Yale University School of Medicine.

Participants in the C + P and CHO conditions (N = 6) were 23.8 ± 3.5 yr (mean ± SD) of age (range 20–27 yr) and weighed 80.2 ± 5.4 kg (range 72–85 kg). They exercised 10.7 ± 4.1 h·wk^{-1} (range 7–20 h), and, of this exercise, 8.7 ± 3.7 h·wk^{-1} (range 7–20 h) was spent cycling. Participants in the PLB condition (N = 4) were 24.0 ± 3.6 yr of age (range 20–27 yr), weighed 80.7 ± 6.4 kg (range 72–85 kg), exercised 11.8 ± 4.7 h·wk^{-1} (range 7–20 h), and cycled 9.3 ± 4.2 h·wk^{-1} (range 7–20 h).

Experimental protocol. Each condition was separated by at least 1 wk. Subjects were assigned to treatments according to a predetermined counterbalanced design to eliminate potential order effects. On the day prior to their first cycling time trial, each subject kept a weighed food record detailing dietary intake (both type and amount). These subjects then ingested an identical diet on the day prior to each subsequent experimental day (verified again via food records). In addition, participants refrained from exercising on the day prior to each experiment. On the study day, a standardized breakfast consisting of cereal, energy bars, and skim milk (see the Nutritional supplementation section below for details) was ingested 120 min prior to measurement of body mass (calibrated weigh scale). Resting vastus lateralis glycogen concentration was assessed via a natural abundance ^{13}C-NMR scan at the midthigh (as described in the NMR spectroscopy section below). Following ingestion of the meal, subjects consumed only water until the postexercise drinks were given. After the muscle scan (8-min duration), participants walked to an adjacent room and, following a 5-min warm-up, began a 60-min best-effort cycling bout (AM_{ex}). Immediately after the AM_{ex}, another ^{13}C-NMR scan was performed to establish postexercise muscle glycogen concentration, and subjects ingested 1 L of liquid supplement (see below for details) at 10, 60, and 120 min postexercise. At 240 min postexercise (120 min before a second 60-min best-effort cycling bout (PM_{ex}), participants in all groups consumed a solid meal. At this time, C + P and CHO received a meal identical to their breakfast meal, whereas PLB received a larger meal containing 200% more energy than the breakfast meal. This manipulation ensured that energy intake was consistent (21 kcal·kg^{-1}) across all treatments by the end of the 6-h recovery period. During this 6-h recovery period, participants consumed only the foods and beverages provided. Six hours following the AM_{ex}, the exercise and measurement protocols were repeated (all measures were retaken, the PM_{ex} was completed, and a postexercise muscle glycogen measurement was made).

Exercise protocol. Subjects exercised on their own bicycles with a Blackburn wind trainer providing rear-wheel resistance. As mentioned, during the first study day, participants completed a 5-min self-paced warm-up. This warm-up was recorded, and the work output and duration were duplicated during each subsequent exercise bout. At the conclusion of the warm-up, each cycled as far as possible in 60 min, treating the exercise bout as a time trial. During the exercise bouts, heart rate (HR; Polar heart rate monitor) as well as average speed and distance traveled (Cateye Micro bicycle computer mounted on and calibrated to the rear-wheel circumference) were measured. Distance-traveled feedback was provided to the subjects every 15 min (although no information was given relative to prior trials). During the first study day, participants consumed water *ad libitum* (mean intake = 700 ± 150 mL) while exercising,

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and this amount of fluid ingestion was duplicated during each subsequent exercise bout. In addition, subjects made a music selection on day 1, and this music was replayed during all subsequent exercise bouts. Prior to the ride, subjects were asked to ride as far as possible in 1 h, but no verbal encouragement was given at any time.

Nutritional supplementation. The standardized breakfast meal (consumed 120 min prior to AM_{ex}) consisted of cereal (Vector meal replacement), cereal bars (Vector), and skim milk (7 kcal·kg^{-1}; 1.2 g·kg^{-1} C, 0.3 g·kg^{-1} P, 0.1 g·kg^{-1} F). The liquid supplements ingested 10, 60, and 120 min postexercise were CHO + protein (C + P; 33% maltodextrin, 33% glucose, and 33% whey protein hydrolysates), CHO (100% maltodextrin), and PLB (artificially sweetened, colored, flavored water). Each supplement plus 3 g of Crystal Light (to provide color and flavor consistency between drinks) was dissolved in 1 L of water, ensuring an 8–12% (by mass) solution. Drink energy content was 4.8 kcal·kg^{-1} body mass; C + P (0.8 g·kg^{-1} C and 0.4 g·kg^{-1} P) and CHO (1.2 g·kg^{-1} C) or 0 kcal·kg^{-1} (PLB). The solid meal ingested 4 h post-AM_{ex} was identical to the standardized breakfast for both C + P and CHO (7 kcal·kg^{-1}), whereas PLB ingested a larger meal consisting of identical foods types containing 21 kcal·kg^{-1} (3.6 g·kg^{-1} C, 1.0 g·kg^{-1} P, 0.3 g·kg^{-1} F). For the 6-h recovery period, mean energy intake was 1680 kcal for all three groups (C + P: 288 g C, 120 g P, 8 g F; CHO: 384 g C, 24 g P, and 8 g F; PLB: 288 g C, 80 g P, and 24 g F). It is important to note that all three conditions ingested a mixture of carbohydrates, proteins, and fats during the recovery period. Because we estimated total energy expenditure to be between 2200 and 2500 kcal (exercise expenditure plus resting metabolic rate) for the entire experimental period, the mean energy intake of 2240 kcal (breakfast plus recovery nutrition) was predicted to be adequate to achieve energy balance during the study period. As total energy intake during recovery was similar between groups, it was the breakdown of the individual macronutrients that varied by condition, with each group ingesting differing amounts of carbohydrates, proteins, and fats.

NMR spectroscopy. Natural abundance ^{13}C-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 over the vastus lateralis to ensure that the majority of the NMR signal was received from the vastus lateralis. A microsphere containing a ^{13}C-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). Thighs were positioned so the isocenter of the magnetic field was approximately 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 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 the signal from the muscle. The ^{1}H-decoupled ^{13}C RF pulse sequence has been designed so that 5472 ^{13}C transients are obtained. The repetition time for ^{13}C acquisition was 87 ms, and ^{1}H continuous wave decoupling was truncated to 25 ms at the beginning of each ^{13}C acquisition to prevent excessive RF power deposition in the muscle. Power deposition, assessed by magnetic vector potential specific absorption rate, was calculated at < 4 W·kg^{-1}. 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 ^{13}C surface coil RF probe was used for spectral acquisitions. Shimming, imaging, and ^{1}H decoupling at 89.5 MHz was performed with a 12 × 12-cm series butterfly coil. Proton line widths are typically shimmed to < 70 Hz. The total scan time for each spectrum was 8 min.

Statistical analysis. Statistical analyses were performed using SPSS software (SPSS Version 10, Chicago, IL). Values are reported as means ± SEM. Group × time ANOVA with repeated measures were used to determine differences between muscle glycogen concentration, heart rate, exercise performance, and body mass. In addition, a one-way ANOVA was performed to determine differences in nutritional intake between groups on the day prior to each study period. Furthermore, planned comparisons were conducted between pre-AM_{ex} and post-AM_{ex} (3 × 2 ANOVA) to examine group differences in glycogen depletion during AM_{ex}; between pre-PM_{ex} and post-PM_{ex} (3 × 2 ANOVA) to examine group differences in glycogen depletion during PM_{ex}; between post-AM_{ex} and pre-PM_{ex} (3 × 2 ANOVA) to examine group differences in glycogen resynthesis during recovery; between calculated glycogen utilization during AM_{ex} and PM_{ex} (3 × 2 ANOVA), and on the calculated percent depletion during AM_{ex} and PM_{ex} (3 × 2 ANOVA). In all analyses, when main effects were found, significant differences among means were determined using Tukey's honestly significant difference calculation. Statistical significance was set at $P \leq 0.05$.

RESULTS

Muscle glycogen. Despite attempts to control food intake and exercise prior to the study, pre-AM_{ex} muscle glycogen concentration appeared to vary among groups (PLB + 19% vs CHO and + 17% vs C + P), although these differences were not statistically different. As hypothesized, there was a significant time effect ($P \leq 0.05$) indicating muscle glycogen concentration either increased or decreased with the experimental protocol. Although there was no significant group by time interaction using the 3 (group) × 4 (time) ANOVA, this interaction approached

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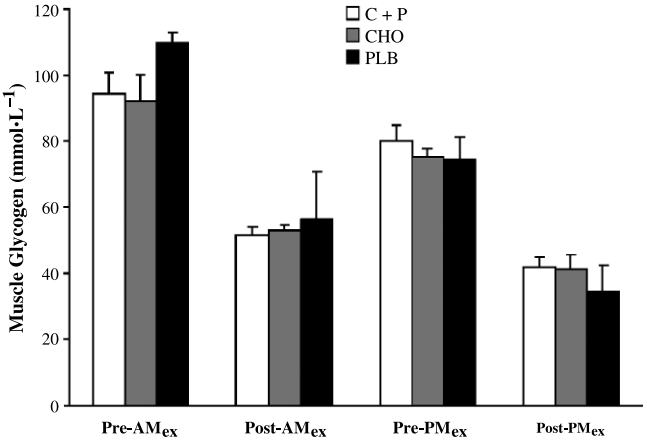

FIGURE 1—Muscle glycogen concentrations in the vastus lateralis before and after each 60-min time trial. During the 6-h recovery period between post-AM_{ex} and pre-PM_{ex}, isoenergetic nutritional interventions included carbohydrate + protein supplements (C + P) and a solid meal, carbohydrate-only supplements (CHO) and a solid meal, and placebo supplements (PLB) and a solid meal. There were no main effects of time, group, or interaction; however, the interaction effect approached significance ($P = 0.069$). For planned comparisons, see Figure 2.

significance ($P = 0.069$; Fig. 1). Planned comparisons demonstrated that between AM and PM_{ex} muscle glycogen resynthesis was greater ($P \leq 0.05$) with C + P versus both CHO and PLB, with no significant differences between CHO and PLB ($P = 0.348$; Fig. 2). The total amount of glycogen resynthesis during the 6-h recovery represented 67% recovery of the glycogen utilized in the C + P group, 57% recovery of the glycogen utilized in the CHO group, and 34% recovery of the glycogen utilized in the PLB group. Consequently, the muscle glycogen concentration prior to PM_{ex} represented 85, 81, and 68% of AM baseline values in the C + P, CHO, and PLB conditions, respectively. Absolute glycogen utilization during AM_{ex} was greater ($P \leq 0.05$) than that observed during PM_{ex}. This amounted to 18% less absolute glycogen utilization (7.57 $mmol \cdot L^{-1}$) during the PM_{ex} (36.5 ± 3.95 $mmol \cdot L^{-1}$) versus the AM_{ex} (44.5 ± 3.87 $mmol \cdot L^{-1}$) (Fig. 3). However, when expressed relative to preexercise glycogen concentration, the utilization was similar: 45% (AM_{ex}) and 48% (PM_{ex}) ($P = 0.158$).

Exercise performance. There was no significant group or group by time interaction with respect to total distance traveled during AM_{ex} or PM_{ex} ($P = 0.282$; Fig. 4); however, there was a main effect of time, indicating a 3% reduction (1.2 km) in performance from AM_{ex} to PM_{ex} ($P \leq 0.05$).

Body mass. There were no significant group, time, or group by time interactions for body mass (pre-AM_{ex}: C + P: 80.7 ± 2.2, CHO: 81.2 ± 2.5, PLB: 81.8 ± 3.3 kg; pre-PM_{ex}: C + P: 80.5 ± 2.2, CHO: 81.2 ± 2.5, PLB: 81.5 ± 3.4 kg), indicating subjects remained at stable weights throughout the experimental day.

Heart rate. There were no significant group or group by time interactions for mean heart rate during the 1-h exercise trials (AM_{ex}: C + P: 161.0 ± 5.0, CHO: 161.0 ± 3.0, PLB: 163.8 ± 2.0 bpm; PM_{ex}: C + P: 159.0 ± 4.0, CHO: 158.4 ± 2.7, PLB: 155.9 ± 2.9 bpm). There was a main effect of time, however, indicating a 2% (5 bpm) reduction in heart rate during PM_{ex} relative to AM_{ex} ($P \times 0.05$).

DISCUSSION

The main finding of the present study is that liquid C + P supplements given early during a 6-h recovery period yielded significantly greater muscle glycogen resynthesis versus isoenergetic liquid CHO supplements or an isoenergetic solid meal consumed 4 h into recovery (keeping in mind that all three conditions did receive a mixed nutrient intake during the isoenergetic recovery period). The absolute differences of 6.42 $mmol \cdot L^{-1}$ (C + P vs CHO) and 10.12 $mmol \cdot L^{-1}$ (C + P vs PLB) represent 22 and 34% more glycogen accumulation with C + P, respectively ($P \leq 0.05$). Further, the absolute amount of glycogen resynthesized during the 6-h recovery period appeared to be greater with CHO versus PLB (16.7%; 3.70 $mmol \cdot L^{-1}$), but this difference did not attain statistical significance ($P = 0.348$).

These data demonstrating the superiority of C + P supplements relative to isoenergetic CHO in muscle glycogen resynthesis may be of considerable importance practically and are consistent with the recent work of Ivy et al. (11). In their study, C + P supplements containing approximately 7 $kcal \cdot kg^{-1}$ (1.1 $g \cdot kg^{-1}$ C, 0.4 $g \cdot kg^{-1}$ P, 0.1 $g \cdot kg^{-1}$ F) were given immediately and 2 h postexercise, whereas isoenergetic (~7 $kcal \cdot kg^{-1}$) CHO-only supplements (1.5 $g \cdot kg^{-1}$ C) were given at the same time intervals. They (11) reported approximately 40% more glycogen resynthesis (~14 $mmol \cdot L^{-1}$) with C + P versus CHO during 4 h of recovery. Because Ivy et al. (11) did not measure subsequent performance in their study, the impact of this approximately 40% increase in muscle glycogen on exercise performance was not investigated. Regardless, our work, along with the work of Ivy et al. (11), suggests that macronutrient composition is an important factor influencing the rate of muscle glycogen resynthesis during short-term recovery from endurance exercise.

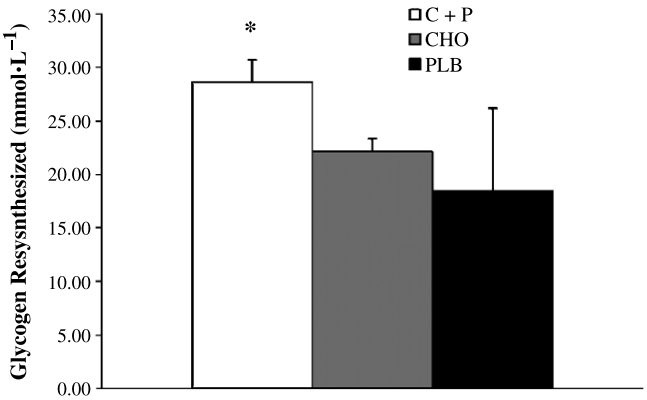

FIGURE 2—Muscle glycogen resynthesis in the vastus lateralis during 6 h of recovery following a 60-min time trial. During this time, isoenergetic nutritional interventions included carbohydrate + protein supplements (C + P) and a solid meal, carbohydrate-only supplements (CHO) and a solid meal, and placebo supplements (PLB) and a solid meal. * Significantly greater than CHO ($P \leq 0.05$) and PLB ($P \leq 0.05$).

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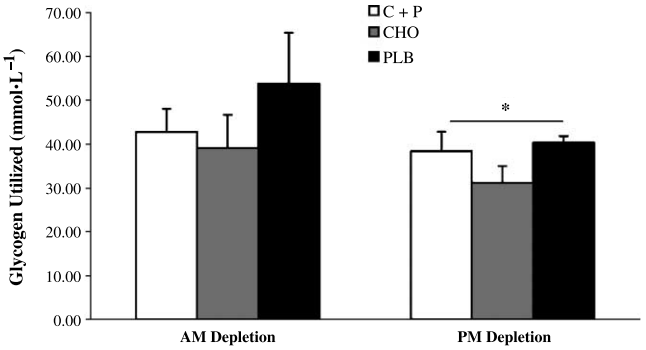

FIGURE 3—Muscle glycogen utilization in the vastus lateralis before and after each 60-min time trial. During the 6-h recovery period between post-AM_{ex} and PM_{ex}, isoenergetic nutritional interventions included carbohydrate + protein supplements (C + P) and a solid meal, carbohydrate-only supplements (CHO) and a solid meal, and placebo supplements (PLB) and a solid meals. * There was a main effect of time ($P \leq 0.05$); however, there were no group effects of group by time interactions.

In contrast, several other studies have shown no enhanced postexercise glycogen resynthesis with C + P supplements versus CHO alone (4,15,19,23). However, important methodological differences may exist between our work and the work of Carrithers et al. (4), Jentjens et al. (15), Tarnopolsky et al. (19), and van Loon eat al. (23). First, each of these studies used a more severe muscle glycogen depletion protocol than used in our study. In these studies, postexercise muscle glycogen concentrations were in the approximately 100-mmol·kg^{-1}·dm^{-1} range (~25 mmol·L^{-1}), whereas subjects in our current study saw much less depletion, with postexercise muscle glycogen concentrations in the approximately 50-mmol·L^{-1} range (~200 mmol·kg^{-1}·dm^{-1}). Likewise, depletion in the Ivy et al. study (11) referenced above led to postexercise muscle glycogen concentrations in the 40- to 42-mmol·L^{-1} range (~160 mmol·kg^{-1}·dm^{-1}). These could be very important differences because muscle glycogen resynthetic rates are known to be highest following severe depletion (1,8,25,28). During such a glycogen-depleted state (below 30–35 mmol·L^{-1}), Price et al. (18) have demonstrated an insulin independence to glycogen resynthesis and a high physiological drive to resynthesize utilized muscle glycogen. Under these conditions, CHO feedings alone may be sufficient to stimulate maximal resynthesis rates. However, as the addition of protein and/or amino acids to carbohydrate may stimulate a synergistic hyperinsulinemia (7,11,15,22,24), when glycogen is depleted to only a moderate extent and postexercise concentrations are greater than 30–35 mmol·L^{-1}, the addition of protein to carbohydrate may be necessary to maximize muscle glycogen resynthesis. In support of this contention, in the four studies mentioned above, studies in which the rates of muscle glycogen resynthesis were not different between C + P and CHO conditions, severe depletion stimulated greater rates of glycogen resynthesis in shorter time periods. In our study, subjects resynthesized 30–56% in 6 h, whereas subjects in the other four studies resynthesized 95–135% in 4 h (Carrithers et al. (4)), 45–112% in 3 h (Jentjens et al. (15)), 73–91% in 4 h (Tarnopolksy et al. (19)), and 110–170% in 5 h (van Loon et al. (23)).

Another potentially important difference between our study and the aforementioned studies (4,15,19,23) is the use of ^{13}C-NMR versus needle biopsy to measure muscle glycogen concentration. Certainly, the needle biopsy technique is a recognized method of glycogen determination, but recent work has demonstrated that ^{13}C-NMR glycogen determinations may offer a greater degree of repeatability and precision (18). The most likely explanation for this is the more representative area of muscle sampled with the ^{13}C-NMR procedure. Further, with increased error in the needle biopsy technique, decreases in statistical power may be seen. Finally, because repeated needle biopsies may cause muscle damage, perhaps impacting glycogen resynthesis rate, ^{13}C-NMR could offer advantages when repeated glycogen determinations are desired.

Also, differences in the timing of ingestion as well as the amount of energy and protein provided during recovery could be responsible for the different findings seen in the literature. In our work and the work of Ivy et al. (11), supplements were provided 1–2 h apart, wherea in the work of Carrithers et al. (4), Jentjens et al. (15), and van Loon et al. (23), supplements were provided every 30 min. Further, the amount of protein provided to subjects during the recovery period was greater in our work and in the Ivy et al. (11) study when compared with Carrithers et al. (4) and Tarnopolsky et al. (19). Finally, the amount of energy provided during recovery differed between studies; we provided 3.5 kcal·h^{-1}, Ivy et al. (11) provided 3.5 kcal·h^{1}, Carrithers et al. (4) provided 4.25 kcal·h^{-1}, Jentjens et al. (15) provided 13 kcal·h^{-1}, Tarnopolsky et al. (19) provided 2 kcal·h^{-1}, and van Loon et al. (23) provided 4.8 kcal·h^{-1}. How each of these factors may have impacted glycogen resynthesis independently is not completely understood,

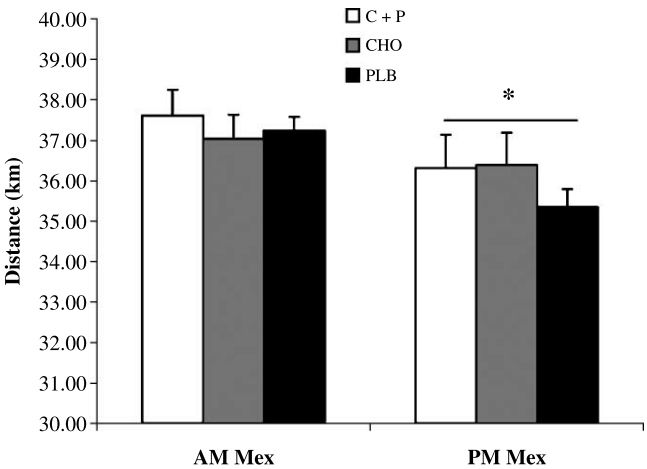

FIGURE 4—Exercise performance during the first (AM_{ex}) and second (PM_{ex}) cycling bout of each study day. During this 6-h recovery period between bouts, isoenergetic nutritional interventions included carbohydrate + protein supplements (C + P) and a solid meal, carbohydrate-only supplements (CHO) and a solid meal, and placebo supplements (PLB) and a solid meal. * There was a main effect of time ($P \leq 0.05$); however, there were no group or group by time interactions.

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and any explanation for the differences between studies may be multifactorial.

In addition to addressing the C + P versus CHO question, the present study is among the first investigations examining the effects of isoenergetic postexercise nutrient ingestion timing during short-term recovery. It was hypothesized that CHO supplementation at an early postexercise stage (and a standardized meal 4 h postexercise) would lead to greater glycogen resynthesis than PLB (a standardized meal given 4 h postexercise), but, surprisingly, the observed increase with CHO (+ 16.7% vs PLB) did not attain statistical significance (Fig. 2). Although a clear explanation for this finding is not available, it is possible that 1) the larger amount of protein ingested (and the potentially increased insulin response) in the PLB group (1.0 $g \cdot kg^{-1}$ protein vs 0.3 $g \cdot kg^{-1}$ protein in CHO) negated any deficit in muscle glycogen resynthesis induced by the delayed nutrient ingestion; 2) carbon compounds mobilized during exercise were converted into muscle glycogen during the immediate postexercise period when no energy was received with PLB; and 3) sufficient blood glucose may have been available for muscle glycogen resynthesis, especially early in recovery, as shown in other studies (1,8), because our exercise protocol was unlikely severe enough to deplete liver glycogen. Consequently, endogenous substrate recycling into muscle glycogen during the first 4 h of recovery, in combination with enhanced glycogen resynthesis during the last 2 h of recovery (due to a large insulin-dependent increase in resynthesis rate), could explain, in part, the lack of difference between glycogen resynthesis in the CHO and PLB conditions.

Parkin et al. (17) have demonstrated previously that after glycogen-depleting exercise, delaying postexercise nutrient ingestion from immediately postexercise to 2 h postexercise had no effect on muscle glycogen resynthesis measured at 8 and 24 h postexercise. Our findings measured at 6 h are consistent with this in that there were no differences in muscle glycogen resynthesis between the CHO (given early in recovery) and PLB (given 4 h later) conditions. Our data also expand their observations by suggesting that early postexercise ingestion of C + P can enhance glycogen resynthesis, at least when the resynthesis rate is submaximal. However, any conclusions from these studies must be made carefully because several methodological differences exist. For example, the Parkin et al. study (17) used identical meals, whereas in our study, the groups were isoenergetic yet differences in the macronutrient mix existed (C + P: 3.6 $g \cdot kg^{-1}$ C, 1.5 $g \cdot kg^{-1}$ P, 0.1 $g \cdot kg^{-1}$ F; CHO: 4.8 $g \cdot kg^{-1}$ C, 0.3 $g \cdot kg^{-1}$ P, 0.1 $g \cdot kg^{-1}$ F; PLB: 3.6 $g \cdot kg^{-1}$ C, 1 $g \cdot kg^{-1}$ P, 0.3 $g \cdot kg^{-1}$ F). Moreover, the form of supplementation differed across groups in our study (C + P and CHO: two thirds of the total supplementation was given early postexercise and in liquid form, whereas one third was ingested as solid food at 4 h; PLB: all of the energy as solid food was consumed at 4 h). Clearly, more experimentation is necessary to adequately sort out these possibilities.

Mean cycling performance during the PM bout was reduced significantly in all groups relative to the AM bout by approximately 3% (−3.4% C + P, −1.7% CHO, and −5.6% PLB), and these performance decrements were matched by absolute reductions in mean glycogen utilization in all groups (−18%) when comparing PM with AM (−10% C + P, −16% CHO, and −25% PLB). Surprisingly, despite the differences in muscle glycogen resynthesis among conditions, there were no group differences (P = 0.282) in exercise performance between the AM and PM cycling bouts (Fig. 4).

Although coaches suggest routinely that enhanced glycogen resynthesis during short-term recovery can improve subsequent performance, few studies have investigated this question directly. In a study by Niles et al. (available online at www.asep.org/jeponline/issue/Jan2001JEPonline/php), subjects ingesting C + P supplements during recovery from an earlier exercise bout were able to run for 21% longer versus isoenergetic CHO supplements in a time to exhaustion trial performed at 10% above the individual anaerobic threshold (IAT). However, as the performance trials lasted between 7 and 9 min, it is unlikely that the performance benefit seen in the C + P trial was due to increased muscle glycogen concentrations. In another study examining the relationship between short-term glycogen resynthesis and exercise performance, subjects were able to cycle 55% longer at 85% $\dot{V}O_{2max}$ when ingesting C + P (106 g C and 28 g P) during recovery from an earlier exercise bout versus a sports drink (42 g C) (25). Subjects in the C + P condition had 26% more muscle glycogen (and 100% more functional glycogen, noted as the difference between glycogen concentration at fatigue and glycogen concentration after recovery) at the start of the performance trial versus subjects in the sports drink condition.

Direct comparison of these studies and our data is difficult because the depletion and subsequent performance trials were quite different. Although our participants were instructed to ride as far as they could in the 60-min cycling bout to simulate a time trial, it appears that they did not cycle at intensities where glycogen was limiting. Whether time trial performances are sensitive to small differences in muscle glycogen concentrations is an important question that requires further research.

Whereas results from the present study suggest that small differences in muscle glycogen do not impact time trial performance, this may be a limitation of the exercise testing device used in our study rather than a true effect. Although the wind trainer device used recorded total distance traveled, the cyclists did not receive continuous feedback about heart rate, watt production, speed, or distance traveled relative to prior bouts, and as a result they may not have been able to gauge their effort accurately from one bout to the next, limiting their motivation and resulting in a lower than optimal exercise intensity. In support of this argument, data from our laboratory with 22 cyclists and the identical supplementation protocol indicate that when real-time feedback

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about distance traveled, speed, watt output, and heart rate are provided (via a Computrainer device interfaced to a laboratory computer), exercise intensity over a 1-h exercise bout is greater (~76–80 vs ~70–73% $\dot{V}O_{2max}$ in the present study). Further, under these conditions, C + P early in recovery results in a statistically significant performance enhancement during the PM_{ex} versus early CHO or later isoenergetic feedings. Therefore, subjects in the present study may not have cycled intensely enough during the time trial for the supplement intervention to demonstrate benefit. In fact, with elite cyclists, typical time trial bouts are performed at greater intensities than 70–73% $\dot{V}O_{2max}$. Future investigations need to consider carefully the exercise intensity used when investigating the impact of macronutrient composition on simulated race performance.

In conclusion, the results of this study suggest that liquid C + P supplements (ingested early during recovery from a 1-h cycling bout) enhance muscle glycogen resynthesis when compared with either CHO or isoenergetic PLB feedings (given later in recovery). However, the increase in muscle glycogen concentration seen in the C + P condition does not impact performance with the self-selected exercise intensities (~70% $\dot{V}O_{2max}$) chosen in this study. Perhaps during exercise bouts of higher intensities (~80% $\dot{V}O_{2max}$) that are more reliant on muscle glycogen, a performance benefit might be uncovered.

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