Addition of protein and amino acids to carbohydrates does not enhance postexercise muscle glycogen synthesis

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Jentjens, Roy L. P. G., Luc J. C. van Loon, Christopher H. Mann, Anton J. M. Wagenmakers, and Asker E. Jeukendrup. Addition of protein and amino acids to carbohydrates does not enhance postexercise muscle glycogen synthesis. J Appl Physiol 91: 839–846, 2001.—Ingestion of a protein-amino acid mixture (Pro; wheat protein hydrolysate, leucine, and phenylalanine) in combination with carbohydrate (CHO; 0.8 g·kg–1·h–1) has been shown to increase muscle glycogen synthesis after exercise compared with the same amount of CHO without Pro. The aim of this study was to investigate whether coingestion of Pro also increases muscle glycogen synthesis when 1.2 g CHO·kg–1·h–1 is ingested. Eight male cyclists performed two experimental trials separated by 1 wk. After glycogen-depleting exercise, subjects received either CHO (1.2 g·kg–1·h–1) or CHO+Pro (1.2 g CHO·kg–1·h–1 + 0.4 g Pro·kg–1·h–1) during a 3-h recovery period. Muscle biopsies were obtained immediately, 1 h, and 3 h after exercise. Blood samples were collected immediately after the exercise bout and every 30 min thereafter. Plasma insulin was significantly higher in the CHO+Pro trial compared with the CHO trial (P < 0.05). No difference was found in plasma glucose or in rate of muscle glycogen synthesis between the CHO and the CHO+Pro trials. Although coingestion of a protein amino acid mixture in combination with a large CHO intake (1.2 g·kg–1·h–1) increases insulin levels, this does not result in increased muscle glycogen synthesis.

wheat hydrolysate; carbohydrate-protein drinks; insulin; recovery

FATIGUE DURING PROLONGED EXERCISE is often associated with muscle glycogen depletion (2, 15); therefore, high preexercise muscle glycogen concentrations are believed to be essential for optimal performance (5, 9, 17). Because endurance athletes often train twice daily for several days and may compete on consecutive days, rapid restoration of muscle glycogen is of crucial importance to optimize recovery.

The complete restoration of muscle glycogen after prolonged exercise can occur within 24 h, depending on the degree of glycogen depletion and provided that sufficient carbohydrates (CHO) are ingested (23, 24). It has been suggested that muscle glycogen synthesis after glycogen-depleting exercise occurs in two phases (31). Initially, there is a period of rapid synthesis of muscle glycogen that does not require the presence of insulin and lasts ~30–60 min. This early postexercise recovery period is marked by an exercise-induced permeability of the muscle cell membrane to glucose (18). GLUT-4 translocation occurs during exercise, and the increase in the density of GLUT-4 transporters in the muscle membrane seems to persist for some time after exercise (27, 36). Together with the continued activation of glycogen synthase (46), this seems to lead to the initial rapid period of insulin-independent synthesis of muscle glycogen. The second phase is dependent on insulin, and glycogen synthesis occurs at a rate that is 10–30% lower than in the first rapid phase (31). When a CHO supplement is consumed after exercise, blood glucose and insulin concentrations will rise, and it has been suggested that this is the mechanism by which the combined ingestion of CHO and protein can enhance glycogen synthesis (41, 47). Insulin stimulates both muscle glucose uptake and the activation of glycogen synthase (20), the rate-limiting enzyme in glycogen synthesis. The ability of insulin to stimulate glucose uptake and glycogen synthase activity is even more pronounced in the first hours after exercise (7, 20). Several studies have attempted to increase insulin concentrations in the postexercise recovery period to optimize the rate of muscle glycogen storage (37, 38, 41, 45, 47). Although the pancreatic insulin secretion is primarily regulated by the blood glucose concentration, certain amino acids (37, 42) and proteins (41, 47) have a synergistic effect on the insulin release when administered in combination with a CHO load (38, 39, 41, 47). Zawadzki et al. (47) observed that the addition of whey protein to a CHO supplement resulted in an enhanced rate of glycogen storage during a 4-h recovery period (47). The authors attributed this effect to a larger insulin response caused by the ingestion of whey protein. Recently, van Loon et al. (40, 42) investigated which type, combination, and quantity of free amino acids...
Acids or protein sources would maximize the insulin response when coingested with CHO. It was shown that a mixture of whey protein hydrolysate, free leucine, and free phenylalanine, when added to a CHO drink (0.8 g CHO·kg⁻¹·h⁻¹), resulted in higher insulin concentrations and an increased glycogen synthesis rates compared with a CHO-only drink. In this study (41), drinks were ingested at regular intervals (30 min), in contrast to the study of Zawadzki et al. (47), in which drinks were given as two large boluses (120-min intervals).

van Loon et al. (41) showed that, when the rate of CHO intake was increased to 1.2 g·kg⁻¹·h⁻¹, this also resulted in significantly higher muscle glycogen synthesis rates compared with the ingestion of 0.8 g·kg⁻¹·h⁻¹. These findings suggest that maximal glycogen synthesis rates were not reached when 0.8 g CHO·kg⁻¹·h⁻¹ was ingested.

It is not known whether the addition of a protein-amino acid mixture to a larger amount of CHO would further increase glycogen synthesis after exercise. Therefore, the purpose of the present study was to investigate whether coingestion of an insulinotropic amino acid mixture and a high rate of CHO intake (1.2 g·kg⁻¹·h⁻¹) provided at 30-min intervals would increase the rate of muscle glycogen resynthesis in the postexercise recovery period compared with a high-CHO intake only.

METHODS

Subjects. Eight healthy, trained male cyclists participated in this study. Subjects trained at least three times per week for >2 h/day and had been involved in endurance training for at least 3–5 yr. 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 South Birmingham Local Research Ethics Committee. Subject characteristics are listed in Table 1.

Preliminary testing. At least 1 wk before the experimental trials, the individual maximum power output (Wmax) and maximal oxygen consumption (V˙O₂ max) were determined by using an incremental exercise test to exhaustion. Wmax values were used to determine the power output settings employed in the glycogen depletion protocol. The exercise test was performed on an electromagnetically braked cycle ergometer (Lode Excalibur Sport, Groningen, The Netherlands), modified to the configuration of a racing bicycle with adjustable saddle height and handlebar position. Subjects started with a 3-min warm-up at 95 W, followed by increment mental steps of 35 W every 3 min until exhaustion. Wmax was determined by the following formula [adapted from Kuipers et al. (25)]

\[
W_{\text{max}} = W_{\text{out}} + (t/180) \times 35
\]

where \(W_{\text{out}}\) is the last completed stage and \(t\) is the time in the final stage. Heart rate (HR) was recorded continuously by a radiotelemetry HR monitor (Polar Vantage NV, Kempele, Finland). Breath-by-breath measurements were performed throughout exercise using an Oxycron Alpha automated gas analysis system (Jaeger, Wuerzberg, Germany). Average inspired and expired volume, O₂ consumption (V˙O₂), and CO₂ production were averaged over eight breaths. V˙O₂ was considered maximal (V˙O₂ max) when at least two of the following three criteria were met: 1) a leveling off of V˙O₂ with increasing workload (increase of no more than 2 ml·kg⁻¹·min⁻¹), 2) a HR within 10 beats/min of predicted maximum (HR of 220 beats/min – age), and 3) a respiratory exchange ratio > 1.05. V˙O₂ max was calculated as the average V˙O₂ over the last 60 s of the test.

General design. All subjects performed two randomized “glycogen depletion-restoration” experiments with at least 7 days in between. To minimize differences in resting muscle glycogen concentration, subjects completed an activity and diet recall log in which they recorded diet and activity patterns 48 h before the first trial. Subjects were instructed to follow the same patterns before the second trial. Furthermore, they were asked to avoid vigorous exercise 1 day in advance of both trials and to fast 10 h before the start of each experimental exercise trial. To deplete muscle glycogen stores, subjects exercised on a cycle ergometer with alternating exercise intensities. After cessation of the exercise protocol, a muscle biopsy from the m. vastus lateralis was taken, and subjects received, in random order, either a CHO plus wheat hydrolysate amino acid drink (CHO + Pro) or a CHO-only drink (CHO). All drinks were lemon flavored to make the taste comparable in the two trials. One and three hours after ingestion of the first drink, a second and third biopsy were taken. Blood samples were collected at registered time intervals. Throughout the entire 3-h recovery period, subjects remained seated during which time they could watch television or read.

Experimental protocol. Subjects reported to the Human Performance Laboratory at the University of Birmingham after an overnight fast (≥10 h). Muscle glycogen depletion was induced as follows: subjects performed a graded exercise test to exhaustion as described before (Wmax protocol); they then rested for 10 min and subsequently performed an intermittent exercise protocol as described by Kuipers et al. (25). In short, after a 10-min warm-up period at 50% Wmax, subjects were instructed to cycle 2-min block periods at alternating workloads of 90 and 50% Wmax. When the subject was unable to complete a 2-min block at 90% Wmax (i.e., subjects were unable to maintain a cadence of 60 rpm), despite encouragement from the experiment leader, the workload was lowered to 80% Wmax. Again subjects continued cycling until they were unable to complete a 2-min block at 80% Wmax, after which the high-intensity block was reduced to 70% Wmax. The exercise was stopped when the 2-min block at 70% Wmax could not be completed. This protocol has been shown to result in very low muscle glycogen concentrations (26). All tests were performed under normal and standard environmental conditions (22–25°C dry bulb temperature and 50–60% relative humidity). During the exercise tests, subjects were cooled with standing floor fans to minimize thermal stress, and water was available ad libitum.

Table 1. Physical and physiological characteristics of the subjects

| Age, yr | 27.1 ± 2.6 |
| Height, cm | 176.2 ± 1.5 |
| Body mass, kg | 69.6 ± 2.9 |
| V˙O₂ max, ml·kg⁻¹·min⁻¹ | 63.3 ± 1.5 |
| Wmax, W | 353.6 ± 13.7 |
| Wmax per kilogram, W/kg | 5.1 ± 0.2 |
| Heart rate maximum, beats/min | 191 ± 2 |

Values are mean ± SE; n = 8 subjects. V˙O₂ max, maximum O₂ consumption; Wmax, maximum power output.
After cessation of the depletion exercise protocol, subjects were allowed to take a shower, and, within 20 min postexercise, a 50- to 100-mg muscle sample was then obtained from the vastus lateralis using the percutaneous needle biopsy technique modified to include suction (1). After the skin was cleaned and 5-ml local anesthesia (2% lignocaine) was administered, a 4- to 6-mm incision in the skin and muscle fascia was made 12–20 cm above the patella on the lateral side of the vastus lateralis. The obtained muscle samples were immediately frozen in liquid nitrogen and stored at −70°C for later analysis of muscle glycogen content.

A 21-gauge Teflon catheter (Baxter, Norfolk, UK) was then inserted in an antecubital vein for blood sampling. A resting blood sample (10 ml) was collected, stored on ice, and later centrifuged. The catheter was kept patent by flushing with 0.5 ml of isotonic saline (0.9%; Baxter). Immediately after the resting blood sample was taken (t = 0; Fig. 1), subjects received the first bolus of test drink. Blood samples were taken at 30-min intervals for determination of plasma glucose and insulin until t = 180 min. A second and third muscle biopsy sample were taken at t = 60 min using the same incision and at t = 180 min from the contralateral leg. The second biopsy was obtained by angling the biopsy needle at least 3 cm distal to the initial incision. To reduce local tissue inflammation and/or membrane disruption after repeated muscle biopsy sampling from one leg, the third muscle sample was taken from the contralateral leg (10).

**Beverages.** At t = 0, 30, 60, 90, 120, and 150 min, subjects received a beverage volume of 3.5 ml/kg to ensure a given dose of 1.2 g·kg⁻¹·h⁻¹ of CHO (50% glucose and 50% maltodextrin) in the CHO trial and 1.2 g·kg⁻¹·h⁻¹ of CHO (50% glucose and 50% maltodextrin) and 0.4 g·kg⁻¹·h⁻¹ of a protein hydrolysate and amino acid mixture in the CHO + Pro trial. The protein hydrolysate and amino acid mixture consisted of a wheat protein hydrolysate (50% mass) and two free amino acids: leucine (25% mass) and phenylalanine (25% mass). This protein hydrolysate and amino acid mixture has previously been reported to result in high-insulin responses (40, 42) and increased glycogen synthesis rates when co-ingested with moderate amounts of CHO (41). The compositions of the test drinks are presented in Table 2.

Glucose (dextrose monohydrate) was obtained from Cerestar (Manchester, UK). A correction factor was applied to correct for the difference in molecular weight between glucose and dextrose monohydrate by multiplying the amount of glucose by 1.1 (5/180/180). Maltodextrin was obtained from Nutriforce (Sittard, The Netherlands), amino acids were obtained from Sigma Aldrich (Gillingham, UK), and protein hydrolysate (Hyprol DEV 4107) was prepared by Quest (Naarden, The Netherlands). The protein hydrolysate is a polypeptide derived from wheat protein with a medium chain length of 11 amino acids. The exact amino acid profile of the wheat hydrolysate has been described previously (42). The maltodextrins used had a medium chain length of 10–16 glycosyl units. To make the taste comparable in both trials, 0.8 g sodium-saccharinate solution (25% wt/wt) and 3.6 g citric acid solution (50% wt/wt) was added for each liter of drink.

**Analyses.** Blood samples were collected into prechilled tubes containing 200 µl of 0.2 M EDTA and centrifuged at 1,500 g for 5 min at 4°C. Aliquots of plasma were stored at −70°C until further analysis for glucose and insulin. Glucose (Glucose K kit, 07260102: Sigma-Aldrich, Costa Mesa, CA) was analyzed on a COBAS BIO semiautomatic analyzer (La Roche, Basel, Switzerland). Plasma insulin was determined by radioimmunoassay by using a commercially available kit (insulin 125I-RIA 100 kit, ICN Pharmaceuticals, Costa Mesa, CA). The intra-assay coefficient of variation for glucose and insulin was <2%.

Muscle biopsy samples (~50 mg wet wt) were freeze dried for 2 days at approximately −40°C and for 0.5 days at room temperature. Then the muscle samples were dissected free of connective tissue, visible fat, and blood using a light microscope. Each muscle sample was minced with a scalpel, and ~3 mg were weighed (Mettler, PM 6400, accuracy of 0.001 mg) for determination of muscle glycogen concentration. Muscle samples were hydrolyzed in 1 M HCl at 100°C for 3 h. After cooling down to room temperature, 175 µl Tris-KOH neutralization mixture (1.44 g Tris and 12 g KOH per 100 ml distilled water) was added. Samples were centrifuged at 9,000 rpm for 10 min at 4°C, and muscle glycogen concentrations were determined as glucose residues on the Cobas Fara semiautomatic analyser (La Roche).

**Calculations.** Muscle glycogen synthesis rate was calculated from the following equation: muscle glycogen synthesis rate = (Gₐ₋₆₀ − Gₐ₋₀)/Δt, where Gₐ₋₆₀ and Gₐ₋₀ are the muscle glycogen concentrations at A and B hours postexercise (i.e., Gₐ₋₆₀ − Gₐ₋₀, Gₐ₋₆₀ = Gₐ₋₄, and Gₐ₋₀ = G₀, respectively), and Δt is the time between the two biopsies. Muscle glycogen synthesis rates were expressed as millimoles of glycosyl units per kilogram of dry weight (dm) per hour (mmol·kg⁻¹·dw⁻¹·h⁻¹).

**Questionnaires.** Subjects were asked to fill out a questionnaire immediately after the exercise test (before the first beverage was received) and every hour thereafter. This questionnaire contained questions regarding the presence of gastrointestinal (GI) problems at that moment and addressed the following complaints: stomach problems, GI cramping, bloated feeling, diarrhea, nausea, dizziness, headache, belching, urge to urinate and/or defecate. The items were scored on
Statistics. All data are expressed as means ± SE. Two-way (time × treatment) ANOVA for repeated measurements was used to compare differences in glycogen, plasma insulin, and glucose concentration. When these analyses revealed significant differences, a Tukey’s post hoc test was used to locate the difference. Plasma glucose and insulin responses were calculated as areas under the curve. Statistical analysis of these data were calculated by using a paired Student’s t-test. Differences were considered to be significant at \( P < 0.05 \).

RESULTS

Exercise trials. After subjects had performed a graded exercise to exhaustion, they cycled on average the following number of 2-min blocks: 11.3 ± 1.7, 7.8 ± 1.3, 8.4 ± 1.5, and 27.8 ± 3.4 at 90, 80, 70, and 50% \( \text{W}_{\text{max}} \), respectively. The average work rate corresponding to 90, 80, 70, and 50% \( \text{W}_{\text{max}} \) was 318 ± 12, 283 ± 11, 248 ± 10, and 177 ± 7 W, respectively. The total exercise duration and the average workload in the CHO and CHO+Pro trials were not significantly different (143 ± 10 vs. 128 ± 11 min and 229 ± 10 vs. 232 ± 10 W, respectively). Over the entire exercise period, the average HR was 158 ± 1.5 beats/min, which corresponded to 83 ± 1% maximum heart rate.

Insulin and glucose. The changes in plasma glucose and insulin concentrations during the recovery period are shown in Fig. 2, A and B, respectively. Plasma insulin concentration immediately after exercise was 9.1 ± 1.8 \( \mu \text{U/ml} \) in the CHO trial and 9.7 ± 0.9 \( \mu \text{U/ml} \) in the CHO+Pro trial. In both trials, plasma insulin levels increased during the first 120 min and remained relatively constant during the final 60 min of recovery. During the final 60 min of recovery, plasma insulin concentration was significantly higher in the CHO+Pro trial compared with the CHO trial.

The addition of the protein hydrolysate and amino acid mixture resulted in a significantly higher insulin response (expressed as area under the curve) between 60–180 min and 0–180 min of recovery compared with the CHO trial (Fig. 3B) (12.9 ± 2.5 vs. 6.1 ± 0.8 \( \mu \text{U/ml} \) at 120 min and 14.7 ± 2.9 vs. 7.3 ± 0.9 \( \mu \text{U/ml} \) at 180 min, respectively; \( P < 0.05 \)). However, during the first 60 min postexercise, the area under the curve for insulin was not significantly different between the two trials \( [1.1 ± 0.2 (\text{CHO}) vs. 1.8 ± 0.4 \mu \text{U/ml} (\text{CHO+Pro})] \) at 60 min.

There was no significant difference between the two trials in plasma glucose concentration at any time point during the recovery period. Plasma glucose concentration was low immediately after exercise (3.3–3.5 mmol/l) but increased rapidly during the first hour (6.5–7.2 mmol/l), after which glucose levels remained stable in the second hour and finally declined to values of 5.1 and 5.6 mmol/l at 180 min postexercise for the CHO and CHO+Pro trial, respectively. The area under the curve for glucose calculated for each time interval (0–60, 60–180, and 0–180 min) did not differ between the two trials (Fig. 3A).

Muscle glycogen. Muscle glycogen concentrations were obtained from only seven subjects because of technical problems. The muscle glycogen concentration immediately after exercise was similar between the two trials [106 ± 19 (CHO) vs. 176 ± 31 mmol/kg dw (CHO+Pro)]. Over the entire 3-h recovery period, muscle glycogen concentration increased to 225 ± 22 mmol/kg dw in the CHO trial and to 252 ± 48 mmol/kg dw in the CHO+Pro trial (Table 3). There were no significant differences in muscle glycogen synthesis rates between the CHO and CHO+Pro trials for either the first 60 min or between 60 and 180 min postexercise. No differences in glycogen synthesis rates were found between the first 60 min and the final 120 min of recovery [35 ± 22 (0–60 min) vs. 31 ± 10 mmol·kg dw\(^{-1}\)·h\(^{-1}\) (60–180 min)].

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GI complaints. In general, subjects reported nonsevere GI symptoms (a score < 5). However, two subjects reported a high score regarding nausea, belching, and bloated feeling after consuming the CHO + Pro beverages (Table 4).

DISCUSSION

Previous studies have shown that the addition of certain amino acids and/or proteins to a CHO supplement can increase glycogen synthesis rates as a result of an enhanced insulin response (41, 47). The major finding of the present study was that the ingestion of an insulinotropic protein hydrolysate and amino acid mixture, in combination with a large CHO intake (1.2 g·kg\(^{-1}\)·h\(^{-1}\)), provided at 30-min intervals, did not affect the rate of muscle glycogen synthesis during the early postexercise recovery period. Although the CHO+Pro supplement resulted in significantly higher insulin concentrations, a difference in the glucose response or in the rate of muscle glycogen storage between the two trials was not observed. Furthermore, the higher prevalence of GI discomfort after ingestion of CHO+Pro drinks may limit their usefulness as recovery drinks, at least in some subjects.

Zawadzki et al. (47) found that the insulin rise and glycogen repletion rate was 39% greater with a combined CHO-protein supplement compared with a CHO supplement only. This study has been criticized by several investigators because it did not include a control trial (isoenergetic amount of CHO intake) (6, 34, 35). Previous studies (6, 34, 35) found similar muscle glycogen resynthesis rates for both CHO and isoenergetic CHO + Pro (+fat) supplements. They suggested that the total amount of energy consumed in the postexercise period is an important factor for muscle glycogen synthesis. The present study did not examine the effects of isoenergetic CHO and CHO + Pro feedings. However, it is also possible that the increased glycogen synthesis rate was indeed the result of elevated insulin levels caused by the addition of protein rather than a difference in energy intake between the two trials (41, 42, 47). Recently, van Loon et al. (41) showed that the addition of an insulinotropic protein hydrolysate and amino acid mixture to a CHO beverage increased mus-

Table 3. Mean muscle glycogen contents and synthesis rates postexercise

<table>
<thead>
<tr>
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<th>Glycogen Content, mmol/kg dw</th>
<th>Synthesis Rate, mmol·kg dw(^{-1})·h(^{-1})</th>
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<tr>
<td></td>
<td>0 h</td>
<td>1 h</td>
</tr>
<tr>
<td>CHO</td>
<td>106 ± 19</td>
<td>141 ± 25</td>
</tr>
<tr>
<td>CHO + Pro</td>
<td>176 ± 31</td>
<td>211 ± 41</td>
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Values are mean ± SE; n = 7 subjects. dw, Dry weight.
cle glycogen synthesis rates by more than twofold. The increased rate of muscle glycogen synthesis after exercise was attributed to an increased clearance of glucose by the muscle as a result of higher insulin levels (41, 46). In the present study, we have used the same protein hydrolysate and amino acid supplement as was used by van Loon et al. (40, 41) but a higher amount of CHO. Ingestion of 1.2 g CHO·kg⁻¹·h⁻¹ in this study resulted in a similar insulin response as in the study by van Loon et al. (40). Addition of the protein hydrolysate and amino acids resulted in an even larger insulin response (Fig. 3B). However, despite this increase in plasma insulin levels, we did not find an effect on glycogen synthesis, which seems to be in contrast to earlier findings by Zawadzki et al. (47). There may be two reasons for this apparent discrepancy. In this study, a larger amount of CHO (1.2 g CHO·kg⁻¹·h⁻¹) was ingested compared with that in the study of Zawadzki et al. (47) (0.77 g·kg⁻¹·h⁻¹), and feeding was more frequent (every 30 min compared with 120 min).

The present data, together with those of van Loon et al. (41), suggest that insulin is not the rate-limiting factor for muscle glycogen synthesis when total CHO intake is high and provided at 30-min intervals. Recent work from van Hall et al. (38) has shown that, despite higher insulin levels with CHO-protein ingestion compared with CHO ingestion alone, leg glucose uptake was not increased in the CHO-protein trial. Similar to the results of the present study, van Hall et al. (38) found no beneficial effect of the increased insulin levels on muscle glycogen synthesis when CHO was ingested at rates of 1.0 g·kg⁻¹·h⁻¹. The authors suggested that a relatively low insulin level may already elicit maximal achievable GLUT-4 migration to the cell membrane and glycogen synthesis rates when CHO intake is ample. Together, the present study and other recent studies (38, 41) suggest that the availability of substrate (i.e., glucose) is the main limiting factor for glycogen synthesis.

The availability of glucose is dependent on the rate of gastric emptying and intestinal absorption of the ingested glucose, glucose output by the liver, and glucose entry into the muscle. Studies that have examined gastric emptying in relation to exogenous CHO oxidation have shown that the rate of gastric emptying is not the limiting step in the oxidation of the oral ingested glucose (29, 33). It is, therefore, unlikely that the rate of gastric emptying will limit the rate of glycogen synthesis when only CHO is ingested. However, it is well known that the rate of gastric emptying is affected by the energy density of the food consumed (43, 44). The higher energy density in the CHO+Pro trial may have confounded the results in the present study by inhibiting gastric emptying. This may have slowed down the rapid delivery of glucose to the muscle and amino acids to simulate the pancreas for insulin secretion. If gastric emptying would hinder the delivery of glucose to the muscle, it is possible that less glycogen synthesis would have occurred in the first phase of glycogen synthesis when contraction-induced GLUT-4 migration was still present. In this study, however, we did not measure gastric emptying, and, therefore, we could not investigate the effects of gastric emptying on muscle glycogen synthesis. Adding a smaller amount of protein might have reduced a potential negative effect on the rate of gastric emptying and could have increased the rate of appearance of glucose into the circulation.

There is also convincing evidence that this limitation is not located at the muscular level (14). Hansen et al. (14) reported an initial glycogen synthesis rate of 185 mmol·kg⁻¹·h⁻¹ after a prolonged infusion of supraphysiological concentrations of glucose and insulin after exercise. Although this study was based on only two subjects, the rate of muscle glycogen storage was far above the maximal glycogen synthesis rate of 40–50 mmol·kg⁻¹·h⁻¹ often found in studies where glucose was ingested orally after glycogen-depleting exercise (4, 8, 11, 30, 41). Therefore, more likely the rate of muscle glycogen synthesis is limited by the rate of digestion and absorption of CHO by the intestine and subsequent transport of glucose into the blood stream regulated by the liver. It has been suggested that the upper limit for glucose absorption in human is ~1 g/min (22, 32), which may be slightly higher in the postexercise state (13). Assuming an active muscle mass of 10 kg during cycling (12), the maximal rate of muscle glycogen storage after oral glucose consumption should be in the range of 0.3–0.4 g/min. Thus the maximal glycogen synthesis rate in the leg seems to be 60–65% lower than the maximal absorption rate by the gut, which indicates that part of the absorbed glucose must be extracted by other tissues (i.e., other muscle groups, fat tissue, or the liver). Therefore, the transport capacity of the intestine for glucose cannot be the only factor that determines the (maximal) rate of muscle glycogen synthesis.

Although the amount of CHO intake seems to be a very important factor determining the rate of glycogen synthesis, there is still no conclusive answer as to how much CHO needs to be consumed in the postexercise recovery phase to maximize the rate of glycogen synthesis. Initially, Blom et al. (3) demonstrated that increasing CHO intake from 0.35 to 0.7 g·kg⁻¹·h⁻¹ did not result in an increased muscle glycogen storage rate. Ivy et al. (21) found similar glycogen storage rates during the first 4 h of recovery when a CHO supplement of either 0.75 or 1.5 g·kg⁻¹·h⁻¹ was provided (19.6 vs. 22.0 mmol·kg dw⁻¹·h⁻¹). However, in both studies, CHO drinks were supplemented at 2-h intervals. It was suggested that this feeding protocol may not have adequately increased and maintained blood glucose and insulin levels for 2 h (16), which could explain the discrepancy between these results and those of others (8, 11, 30, 41). Several studies have reported higher glycogen synthesis rates (between 40 and 45 mmol·kg dw⁻¹·h⁻¹) when 1.0–1.85 g·kg⁻¹·h⁻¹ of CHO were consumed more frequently (15- to 60-min intervals) during a 3- to 5-h recovery period (8, 11, 30, 41). The glycogen synthesis rates found in the present study are consistent with previously reported results of several other studies in which almost similar amounts of CHO
were ingested (6, 19, 28, 35, 38, 41). The glycolgen concentrations found immediately after exercise were in good agreement with the results reported by van Loon et al. (41), where subjects performed a similar amount of work (exercise duration >90 min at workloads varying between 50 and 90% Wmax).

**Summary.** The results of the present study do suggest that a further increase in the insulin concentration by additional supplementation of protein and amino acids does not increase the rate of glycogen synthesis when CHO intake is sufficient and supplemented at regular intervals of ≤30 min. Insulin can, therefore, be excluded as the limiting factor for glycogen synthesis. The total amount of glucose intake post-exercise, on the other hand, seems to play a more important role when maximal rates of muscle glycogen synthesis are required. An intake of 1.2 g·kg\(^{-1}\)·h\(^{-1}\) or more seems to be required to achieve the maximal glycogen resynthesis rate.

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