

# Maximizing postexercise muscle glycogen synthesis: carbohydrate supplementation and the application of amino acid or protein hydrolysate mixtures<sup>1-3</sup>

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## ABSTRACT

**Background:** Postexercise muscle glycogen synthesis is an important factor in determining the time needed to recover from prolonged exercise.

**Objective:** This study investigated whether an increase in carbohydrate intake, ingestion of a mixture of protein hydrolysate and amino acids in combination with carbohydrate, or both results in higher postexercise muscle glycogen synthesis rates than does ingestion of  $0.8 \text{ g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$  carbohydrate, provided at 30-min intervals.

**Design:** Eight trained cyclists visited the laboratory 3 times, during which a control beverage and 2 other beverages were tested. After the subjects participated in a strict glycogen-depletion protocol, muscle biopsy samples were collected. The subjects received a beverage every 30 min to ensure ingestion of  $0.8 \text{ g} \text{ carbohydrate} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$  (Carb trial),  $0.8 \text{ g} \text{ carbohydrate} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$  plus  $0.4 \text{ g}$  wheat protein hydrolysate plus free leucine and phenylalanine  $\cdot \text{kg}^{-1} \cdot \text{h}^{-1}$  (proven to be highly insulinotropic; Carb + Pro trial), or  $1.2 \text{ g} \text{ carbohydrate} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$  (Carb + Carb trial). After 5 h, a second biopsy was taken.

**Results:** Plasma insulin responses in the Carb + Pro and Carb + Carb trials were higher than those in the Carb trial ( $88 \pm 17\%$  and  $46 \pm 18\%$ ;  $P < 0.05$ ). Muscle glycogen synthesis was higher in both trials than in the Carb trial ( $35.4 \pm 5.1$  and  $44.8 \pm 6.8$  compared with  $16.6 \pm 7.8 \mu\text{mol glycosol units} \cdot \text{g dry wt}^{-1} \cdot \text{h}^{-1}$ , respectively;  $P < 0.05$ ).

**Conclusions:** Addition of a mixture of protein hydrolysate and amino acids to a carbohydrate-containing solution (at an intake of  $0.8 \text{ g} \text{ carbohydrate} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ ) can stimulate glycogen synthesis. However, glycogen synthesis can also be accelerated by increasing carbohydrate intake ( $0.4 \text{ g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ ) when supplements are provided at 30-min intervals. *Am J Clin Nutr* 2000;72:106–11.

**KEY WORDS** Insulin, leucine, phenylalanine, glycogen synthase, muscle glycogen synthesis, glycogen depletion, healthy men, exercise

## INTRODUCTION

Muscle glycogen has been recognized as an important fuel during prolonged exercise since the early studies by Bergström et al (1–3). The reliance on muscle glycogen increases with

increasing exercise intensity and a direct relation between fatigue and depletion of muscle glycogen stores has been described (1–3). Therefore, the postexercise glycogen synthesis rate is an important factor in determining the time needed to recover. Glycogen synthesis is affected not only by the extent of glycogen depletion but also in a more direct manner by the type, duration, and intensity of the preceding exercise because these will differentially influence the acute enzymatic changes as well as recovery from the acute changes that are induced by strenuous exercise (4–6).

To optimize glycogen synthesis rates, adequate amounts of carbohydrate should be ingested (1, 7, 8). Blom et al (9) suggested initially that a carbohydrate intake of  $0.35 \text{ g} \cdot \text{kg body wt}^{-1} \cdot \text{h}^{-1}$ , provided at 2-h intervals, maximized muscle glycogen synthesis. Ivy et al (7) observed no differences in glycogen storage rates after subjects ingested  $0.75$  or  $1.5 \text{ g} \text{ carbohydrate} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$  provided at 2-h intervals. In a follow-up study, Ivy (10) reported that an intake of  $>0.5 \text{ g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$  is necessary to maximize postexercise glycogen synthesis if supplements are administered at 2-h intervals. Higher glycogen synthesis rates have been reported in studies in which carbohydrates were ingested more frequently and at higher ingestion rates than in Ivy's study (4, 11). Other efforts to increase glycogen synthesis rates by changing the form of administration (ie, as a solution, as a solid, or intravenously) have been unsuccessful (8, 12).

Zawadzki et al (13) reported that addition of an intact protein to a carbohydrate-containing solution resulted in higher glycogen synthesis rates in subjects after exercise than did ingestion of carbohydrate only at a rate of  $0.8 \text{ g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ . This was explained by the observed additional increase in plasma insulin concentrations after ingestion of the carbohydrate-protein mixture. Elevated insulin concentrations may lead to increased glucose uptake (14) and to an increase in glycogen synthase activity (15, 16), which

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forms the major factor in determining the rate of glycogen synthesis when the substrate supply is adequate (13, 17). The stimulating effect of the combined ingestion of carbohydrate and protein on plasma insulin concentrations was investigated previously (18–21) and is described in detail in an accompanying article (22). We showed that ingestion of a mixture of a wheat hydrolysate, free leucine, and free phenylalanine in combination with a carbohydrate drink leads to a substantial increase in plasma insulin in healthy subjects after an overnight fast (22).

The first aim of this study was to investigate whether, and to what extent, the ingestion of this highly insulinotropic protein hydrolysate and amino acid mixture in combination with carbohydrate ( $0.8 \text{ g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ ) can accelerate postexercise muscle glycogen synthesis. The second aim was to determine whether glycogen synthesis rates can also be elevated by increasing carbohydrate intake. The ingestion rates chosen were based on those used by Zawadzki et al (13). However, we decided to provide the supplements at 30-min intervals because more frequent carbohydrate ingestion could result in higher glycogen synthesis rates (4, 11). To realize these aims, 8 highly trained cyclists performed a glycogen-depletion test on 3 occasions, after which beverages containing carbohydrate ( $0.8 \text{ g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ ), carbohydrate and an amino acid and protein hydrolysate mixture ( $0.8$  and  $0.4 \text{ g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ , respectively), or an isoenergetic amount of carbohydrate ( $1.2 \text{ g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ ) were ingested during a 5-h period. Plasma insulin and glucose concentrations were measured and muscle biopsies were taken immediately postexercise and 5 h later to determine glycogen synthase activity and muscle glycogen content.

## SUBJECTS AND METHODS

### Subjects

Eight well-trained male cyclists or triathletes [ $\bar{x} \pm \text{SEM}$ : age,  $24.0 \pm 0.6$  y; body mass,  $70.0 \pm 1.0$  kg; body mass index (in  $\text{kg}/\text{m}^2$ ),  $21.4 \pm 0.6$ ; maximum workload ( $W_{\text{max}}$ ),  $390 \pm 8$  W; maximum heart rate,  $191 \pm 3$  beats/min] participated in this study. Subjects trained  $\geq 3$  times/wk for  $\geq 2$  h and had a training history of  $>5$  y. All subjects were informed about the nature and risks of the experimental procedures before their informed consent was obtained. This study was approved by the Ethical Committee of the Academic Hospital Maastricht.

### Pretesting

$W_{\text{max}}$  was measured on an electronically braked cycle ergometer (Lode Excalibur, Groningen, Netherlands) during an incremental exhaustive exercise test (23) 1 wk before the first experimental trial. The results were used to determine the workload used in the glycogen-depletion protocol.

### Experimental trials

The subjects performed 3 randomized tests, each separated by  $\geq 1$  wk. During those trials the subjects were subjected to a glycogen-depletion protocol. Thereafter, the subjects were studied for 5 h while ingesting only carbohydrate (Carb trial), carbohydrate together with an amino acid and protein hydrolysate mixture (Carb + Pro trial), or an isoenergetic amount of carbohydrate (Carb + Carb trial). During the 5-h postexercise period, the subjects remained seated. Beverages were provided in a randomized, double-blind manner. All drinks were vanilla flavored

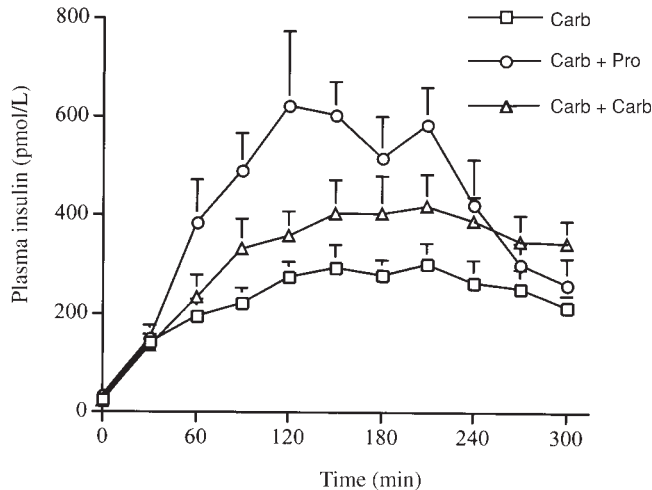
to make the taste comparable in all trials (*see* below). The subjects were instructed to refrain from any sort of heavy physical labor and to eat the same meals the day before each of the trials.

### Protocol

During each of the trials, the subjects reported to the laboratory at 0830 after an overnight fast. Muscle glycogen depletion was established by having the subjects perform an intense cycle ergometer test (24). This muscle glycogen depletion protocol started with a 10-min warm-up period at a workload of 50%  $W_{\text{max}}$ . Thereafter, the subjects were instructed to cycle 2-min block periods at alternating workloads of 90% and 50% of  $W_{\text{max}}$ , respectively. This was continued until the subjects were no longer able to complete the 2 min at 90%  $W_{\text{max}}$ . That moment was defined as the time at which the subject was unable to maintain cycling speed at 60 revolutions/min. At that moment the high-intensity block was reduced to 80%  $W_{\text{max}}$ . Again, the subjects had to cycle until they were unable to complete a 2-min block at 80%  $W_{\text{max}}$ , after which the high-intensity block was reduced to 70%  $W_{\text{max}}$ . The subjects were allowed to stop when pedaling speed could not be maintained at 70%  $W_{\text{max}}$ . Water was provided ad libitum during the exercise protocol. After cessation of the depletion exercise protocol, the subjects were allowed to take a quick shower, and within 15 min postexercise a muscle biopsy (25) was taken from the vastus lateralis muscle. Thereafter, a polytetrafluoroethylene sampling catheter (Baxter BV, Utrecht, Netherlands) was inserted in the antecubital vein, a resting blood sample (0 min) was taken, and the subjects immediately received the first bolus of test drink ( $3.5 \text{ mL}/\text{kg}$ ). The subjects were observed for the next 5 h, during which they received a beverage volume of  $3.5 \text{ mL}/\text{kg}$  every 30 min until 270 min. Blood samples were taken at 30-min intervals for measurement of plasma glucose and insulin until 300 min. Immediately after the final blood sample was acquired, a second biopsy was taken from the same leg. Muscle biopsies were analyzed for glycogen content and glycogen synthase activity.

### Beverages

At 0, 30, 60, 90, 120, 150, 180, 210, 240, and 270 min, the subjects received a beverage volume of  $3.5 \text{ mL}/\text{kg}$  to ensure a given dose of  $0.8 \text{ g carbohydrate} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$  (50% glucose and 50% maltodextrin) in the Carb (control) trial,  $0.8 \text{ g carbohydrate} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$  and  $0.4 \text{ g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$  of an amino acid and protein hydrolysate mixture in the Carb + Pro trial, and an isoenergetic amount of  $1.2 \text{ g carbohydrate} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$  (50% glucose and 50% maltodextrin) in the Carb + Carb trial. The amino acid and protein hydrolysate mixture consisted of 2 free amino acids, leucine (25 mass percentage), and phenylalanine (25 mass percentage) and a wheat-gluten protein hydrolysate (50 mass percentage). The compositions of all test drinks are listed in an accompanying article (22). Glucose and maltodextrin were obtained from AVEBE (Veendam, Netherlands), crystalline amino acids were obtained from BUFA (Uitgeest, Netherlands), and the protein hydrolysate (Hyprol) was prepared by Quest International (Naarden, Netherlands). The protein hydrolysate is prepared from wheat protein by enzymatic digestion and has a medium chain length of 11 amino acids. The amino acid profile of the wheat hydrolysate is listed in the accompanying article (22). The maltodextrins used had a medium chain length of 14–16 glycosyl units. To make the taste comparable in all trials,  $0.8 \text{ g}$  sodium saccharinate solution (25% by wt),  $3.6 \text{ g}$  citric acid



**FIGURE 1.** Mean ( $\pm$ SEM) plasma insulin concentrations after ingestion of the control drink (carbohydrate only; Carb) and drinks containing carbohydrate plus wheat-protein hydrolysate plus free leucine and phenylalanine (Carb + Pro) or an isoenergetic amount of carbohydrate (Carb + Carb).  $n = 8$ . To convert from pmol/L to mU/L, divide by 7.25.

solution (50% by wt), and 5.0 g cream vanilla flavor (Quest International) were added for each liter of drink. In the accompanying article (22) and in pilot experiments, we showed repeatedly the large insulinotropic effect of this carbohydrate and protein drink compared with various mixtures of carbohydrate and protein and amino acids.

### Analysis

Blood was collected in EDTA-containing tubes and centrifuged at  $1000 \times g$  and  $4^\circ\text{C}$  for 5 min. Aliquots of plasma were frozen immediately in liquid nitrogen and stored at  $-40^\circ\text{C}$ . Glucose (Uni Kit III, 07367204; Roche, Basel, Switzerland) was analyzed with the Cobas Fara semiautomatic analyzer (Roche, Basel, Switzerland). Insulin was analyzed by radioimmunoassay (Insulin RIA 100 Kit; Pharmacia, Uppsala, Sweden).

The amino acid composition of the wheat protein hydrolysate was analyzed on an automated dedicated amino acid analyzer (Pharmacia LKB Biotechnology, Roosendaal, Netherlands). The various amino acids were separated with ion-exchange chromatography with use of lithium citrate buffers. After postcolumn derivatization with ninhydrine, the derivatives were detected at 570 and 440 nm.

Muscle biopsies were immediately frozen in liquid nitrogen and stored at  $-80^\circ\text{C}$ . For glycogen determination,  $\approx 50$  mg muscle (wet wt) was freeze-dried. Collagen, blood, and other non-muscle fiber material was removed from the muscle fibers under a microscope. The isolated muscle fiber mass (2–3 mg) was weighed and 500  $\mu\text{L}$  of 1 mol hydrochloric acid/L was added. After heating for 3 h at  $100^\circ\text{C}$  to hydrolyze the glycogen to glycosyl units and cooling down to room temperature, the solution was neutralized by adding 267 mL tris/KOH. Thereafter, 150  $\mu\text{L}$  was analyzed for glucose (Uni Kit III, 07367204; La Roche

with the Cobas Fara semiautomatic analyzer. Glycogen synthase activity was measured by the method of Danforth (26). Approximately 30 mg muscle tissue was homogenized in SET buffer, 250 mmol sucrose/L, 2 mmol EDTA/L, and 10 mmol tris/KOH with a pH of 7.4. The enzyme activity was assayed at pH 7.4 and  $30^\circ\text{C}$  in a reaction mixture containing 60 mmol tris/L, 1.2 mmol EDTA/L, 3 mmol mercaptoethanol/L, 1.2 mmol NaF/L, 7.5 mmol uridine 5'-diphosphate (UDP)-glucose/L, and 1.2% glycogen. The assay was carried out in the presence and absence of 12 mmol glucose-6-P/L to measure total glycogen synthase activity and the percentage of active enzyme. The reaction was terminated by heating for 2 min in a boiling water bath. The denatured protein was removed by centrifugation and the supernatant solution was assayed enzymatically for UDP. UDP was measured by allowing UDP to react with phosphoenolpyruvate in the presence of pyruvate kinase. The pyruvate formed was measured spectrophotometrically with lactate dehydrogenase. Activity is expressed as  $\mu\text{mol}$  incorporated as UDP-glucose into glycogen  $\cdot \text{min}^{-1} \cdot \text{g}^{-1}$  wet muscle at  $30^\circ\text{C}$ .

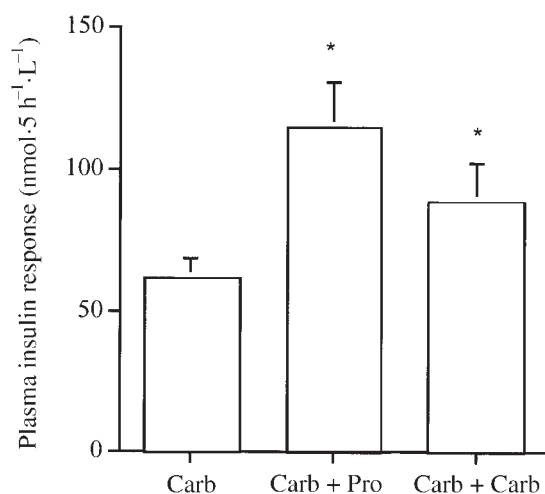
### Statistics

All data are expressed as means  $\pm$  SEMs. Plasma glucose and insulin responses were calculated as areas under the curve minus baseline values. The effect of each treatment on the glucose and insulin responses, glycogen synthesis rate, and glycogen synthase activity was compared with that of the control trial by using paired  $t$  tests with Bonferroni correction. Significance was set at  $P < 0.05$ . Data were analyzed by using SYSTAT (version 5.2; Evanston, IL).

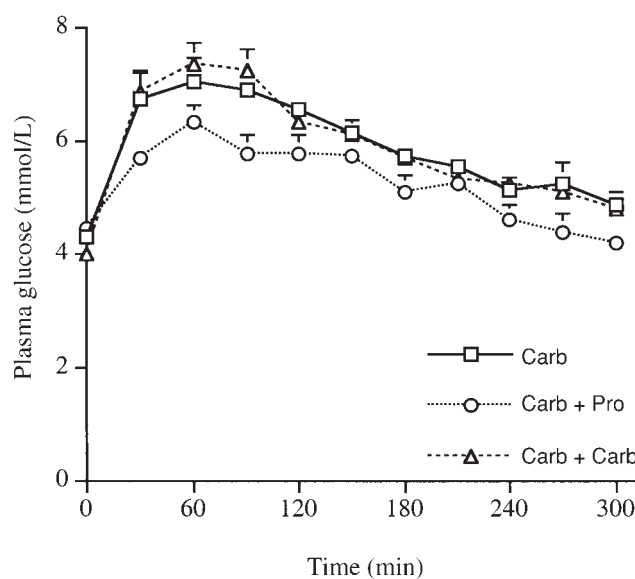
### RESULTS

Mean  $W_{\text{max}}$  measured during pretesting was  $390 \pm 8$  W ( $5.6 \pm 0.1$  W/kg); therefore, average workload settings in the depletion protocol were  $195 \pm 4$ ,  $351 \pm 7$ ,  $312 \pm 6$ , and  $273 \pm 6$  W at 50%, 70%, 80%, and 90%  $W_{\text{max}}$ , respectively. On average, the subjects cycled a total of  $45.6 \pm 2.8$  high-intensity blocks (total cycling time:  $>90$  min). This vigorous exercise protocol resulted in an average postexercise glycogen content of  $190 \pm 29$ ,  $174 \pm 36$ , and  $138 \pm 30$   $\mu\text{mol}$  glycosyl units/g dry muscle wt in the Carb, Carb + Pro, and Carb + Carb trials, respectively. Both values did not differ significantly from those of the Carb trial. Postexercise glycogen synthase activity (active form) in the Carb + Pro and Carb + Carb trials were not significantly different from those in the control trial and averaged  $2.9 \pm 0.8$ ,  $3.0 \pm 0.5$ , and  $3.8 \pm 0.7$   $\mu\text{mol} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$  ( $\mu\text{mol}$  incorporated as UDP-glucose into glycogen  $\cdot \text{min}^{-1} \cdot \text{g}^{-1}$  wet muscle at  $30^\circ\text{C}$ ) in the Carb, Carb + Pro, and Carb + Carb trials, respectively.

In all trials, plasma insulin concentrations increased during the first 2 h postexercise, after which insulin concentrations plateaued in the Carb and Carb + Carb trials. However, in the Carb + Pro trial, insulin concentrations decreased during the last hour (Figure 1). Plasma insulin responses, expressed as area under the curve minus baseline values (Figure 2), were significantly higher in both the Carb + Pro and Carb + Carb trials than in the control trial ( $15.9 \pm 2.2$  and  $12.3 \pm 1.8$  compared with  $8.6 \pm 0.9$   $\text{U} \cdot 5 \text{ h} \cdot \text{L}^{-1}$ , respectively). The addition of the amino acid and protein hydrolysate mixture resulted in an insulin response that was  $87.5 \pm 17\%$  higher than in the control trial. The addition of an isoenergetic amount of carbohydrate resulted in an insulin response that was  $45.8 \pm 18\%$  higher than in the control



**FIGURE 2.** Mean ( $\pm$ SEM) plasma insulin responses after ingestion of the control drink (carbohydrate only; Carb) and drinks containing carbohydrate plus wheat-protein hydrolysate plus free leucine and phenylalanine (Carb + Pro) or an isoenergetic amount of carbohydrate (Carb + Carb).  $n = 8$ . \*Significantly higher than Carb,  $P < 0.05$  (paired  $t$  tests with Bonferroni correction).



**FIGURE 3.** Mean ( $\pm$ SEM) plasma glucose concentrations after ingestion of the control drink (carbohydrate only; Carb) and drinks containing carbohydrate plus wheat-protein hydrolysate plus free leucine and phenylalanine (Carb + Pro) or an isoenergetic amount of carbohydrate (Carb + Carb).  $n = 8$ .

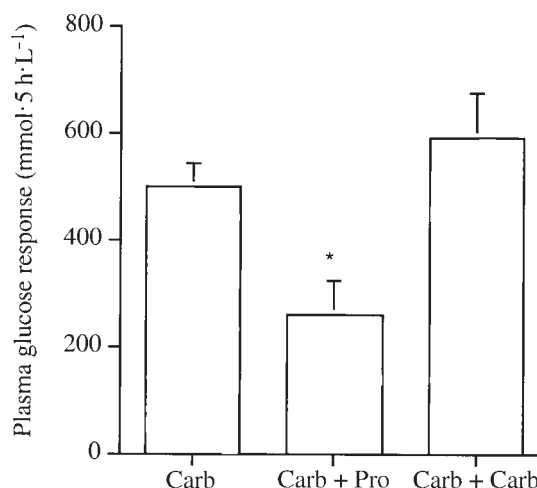
trial. Plasma glucose concentrations increased during the first 30–60 min in all trials, after which concentrations slowly decreased over time (Figure 3). After glucose response was expressed as area under the curve above baseline values (Figure 4), glucose responses were significantly lower in the Carb + Pro trial than in the control trial ( $258 \pm 66$ ,  $500 \pm 46$ , and  $592 \pm 83$   $\text{mmol} \cdot 5 \text{ h}^{-1} \cdot \text{L}^{-1}$ , respectively).

Five hours postexercise, muscle glycogen content averaged  $272 \pm 54$ ,  $351 \pm 39$ , and  $362 \pm 46$   $\mu\text{mol}$  glycosol units/g dry muscle  $\text{wt}^{-1}$  in the Carb, Carb + Pro, and Carb + Carb trials, respectively. Muscle glycogen synthesis was significantly higher in both the Carb + Pro and Carb + Carb trials than in the control trial ( $35.4 \pm 5.1$  and  $44.8 \pm 6.8$  compared with  $16.6 \pm 7.8$   $\mu\text{mol}$  glycosol units  $\cdot$  g dry muscle  $\text{wt}^{-1} \cdot \text{h}^{-1}$ ) (Figure 5). Glycogen synthase activity (active form) measured at 300 min averaged  $1.9 \pm 0.2$ ,  $2.2 \pm 0.4$ , and  $1.8 \pm 0.3$   $\mu\text{mol} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$  in the Carb, Carb + Pro, and Carb + Carb trials, respectively. No significant increase was observed in active form, total activity, or the percentage of active glycogen synthase enzyme between the Carb + Pro or Carb + Carb trial and the control trial 5 h postexercise.

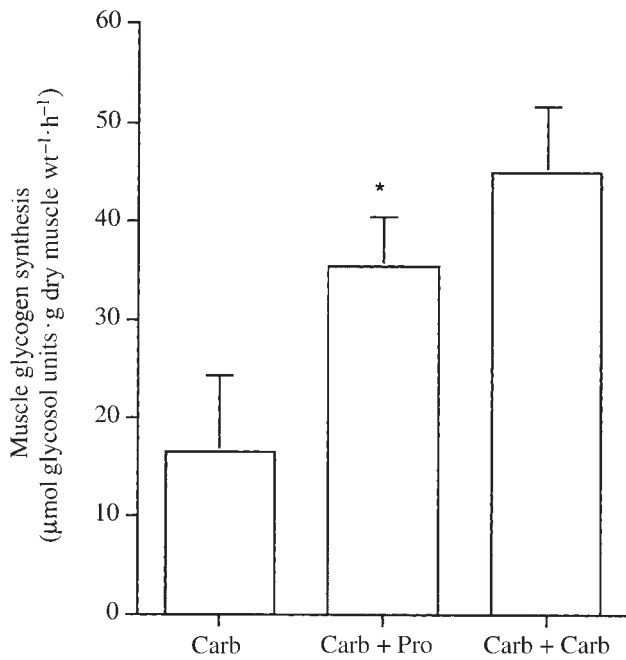
## DISCUSSION

In an accompanying study we observed that the ingestion of a mixture of wheat protein hydrolysate, leucine, and phenylalanine added to a carbohydrate-containing solution resulted in a larger insulin response than did any other mixture of carbohydrate and protein (hydrolysate) or amino acids (22). In the present study, ingestion of a carbohydrate solution ( $0.8 \text{ g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ ) in combination with this amino acid and protein hydrolysate mixture ( $0.4 \text{ g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ ) resulted in an insulin response, measured over a 5-h period, that was 88% higher than the response observed during the ingestion of carbohydrate only ( $P < 0.05$ ; Figures 1 and 2). The plasma glucose response was accordingly significantly lower than in the Carb

trial ( $P < 0.05$ ; Figures 3 and 4). Glycogen synthesis rates during this 5-h postexercise period were  $>113\%$  higher than the synthesis rates observed in the control trial. These results agree with the findings by Zawadzki et al (13), who found that glycogen synthesis rates were 38% higher over a 4-h period after carbohydrate and protein ingestion ( $0.77$  and  $0.28 \text{ g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ , respectively) than after ingestion of carbohydrate only. This increase in glycogen synthesis rate was explained by the addi-



**FIGURE 4.** Mean ( $\pm$ SEM) plasma glucose responses after ingestion of the control drink (carbohydrate only; Carb) and drinks containing carbohydrate plus wheat-protein hydrolysate plus free leucine and phenylalanine (Carb + Pro) or an isoenergetic amount of carbohydrate (Carb + Carb).  $n = 8$ . \*Significantly lower than Carb,  $P < 0.05$  (paired  $t$  tests with Bonferroni correction).



**FIGURE 5.** Mean ( $\pm$ SEM) muscle glycogen synthesis rates after ingestion of the control drink (carbohydrate only; Carb) and drinks containing carbohydrate plus wheat-protein hydrolysate plus free leucine and phenylalanine (Carb + Pro) or an isoenergetic amount of carbohydrate (Carb + Carb). \*Significantly higher than Carb,  $P < 0.05$  (paired  $t$  tests with Bonferroni correction).

tional increase in insulin concentrations after the combined ingestion of carbohydrates and protein.

Both glucose availability and insulin concentrations determine the rate of glucose uptake in skeletal muscle. Insulin stimulates glucose utilization by muscle cells through activation of glucose transport (GLUT4 translocation) and stimulation of intracellular enzymes regulating pathways for oxidative and nonoxidative glucose metabolism (10, 14). Kruszynska et al (16) concluded that in rats, increases in blood glucose concentrations were attended by an increase in glycogen synthase activity in the liver whereas in skeletal muscle, increases in plasma insulin concentrations seem to be of primary importance. Bak et al (15) confirmed the latter finding in a study in humans, in whom they found that hyperinsulinemia for 2.5 h increased glycogen synthase activity, independently of plasma glucose concentrations. At the same time it was shown that during short-term hyperinsulinemia or hyperglycemia, insulin receptor binding and kinase function were not modified. Glycogen synthase catalyzes the incorporation of glycosyl units from UDP-glucose into an amylose chain. This is considered to be the rate-limiting step in the glycogen synthetic pathway (26, 27). As such, hyperinsulinemia could accelerate glycogen synthesis by increasing glucose uptake and glycogen synthase activity when substrate supply is adequate (13, 17). In the present study we observed no significant differences in the percentage of glycogen synthase in the active form at 0 and 300 min; nor were differences seen between the trials. This may be explained by the timing of muscle biopsy sampling in this study, because at 300 min, plasma insulin concentrations had returned to similar values in the Carb + Pro trial as in the control trial (Figure 1).

It has been suggested that the accelerated glycogen synthesis rates observed in the carbohydrate and protein trials can also be attributed to an increased gluconeogenic flux after the ingestion of protein or amino acids. However, this is unlikely because the high insulin concentrations observed in the Carb + Pro trial would have inhibited rather than stimulated gluconeogenesis. In addition, several studies on glycogen synthesis rates included only protein as a control and found very low rates, not different from those in trials without any nutrient intake (7, 13, 28). Burke et al (29) did not observe a positive difference in glycogen synthesis rates over a 24-h period after ingestion of carbohydrate plus protein plus fat ( $7.0$ ,  $1.2$ , and  $1.6$   $\text{g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ , respectively) compared with the intake of carbohydrate only ( $7.0$   $\text{g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ ) and a matched energy diet containing an additional  $4.8$   $\text{g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  carbohydrates ( $11.8$   $\text{g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ ). These findings can be explained by the feedback inhibition that increasing glycogen stores have on glycogen synthase activity (26, 27). This negative feedback process is likely to reduce glycogen synthesis rates over time, resulting in similar glycogen storage in the long term. However, fast replenishment of glycogen stores is crucial in sports practice, especially in multiple-day sports events like the Tour de France, because in such cases athletes simply do not have 24 h to restore muscle glycogen concentrations.

This study also clearly showed that in highly trained cyclists, higher glycogen synthesis rates are attained at a carbohydrate intake of  $1.2$   $\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$  than at an intake of  $0.8$   $\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$  over a 5-h period if supplements are provided at 30-min intervals ( $P < 0.05$ ; Figure 5). Ivy et al (7) observed no differences in glycogen storage rates after providing subjects with  $0.75$  or  $1.5$   $\text{g}$  carbohydrate  $\cdot \text{kg}^{-1} \cdot \text{h}^{-1}$  ingested at 2-h intervals. In a follow-up study, Ivy (10) suggested that those muscle glycogen synthesis rates could be submaximal because of the inability of a carbohydrate supplement to adequately increase and maintain blood glucose and insulin concentrations for 2 h and that more frequent provision could be beneficial in the optimization of glycogen synthesis. This idea is supported by the relatively high muscle glycogen synthesis rates observed in studies in which carbohydrates were provided more frequently (4, 11). The results of the present study confirm this hypothesis because we clearly observed an increase in muscle glycogen synthesis rates when carbohydrate ingestion was increased from  $0.8$  to  $1.2$   $\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$  provided at 30-min intervals ( $P < 0.05$ ). Because a carbohydrate ingestion rate of  $0.8$   $\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ , provided at 30-min intervals, limits postexercise muscle glycogen synthesis rates, further research will be necessary to investigate whether the addition of this insulinotropic amino acid and protein hydrolysate mixture can further increase glycogen synthesis rates when an adequate amount of carbohydrate is provided at frequent intervals.

The main conclusion to be derived from this study is that ingestion of a mixture of wheat hydrolysate and free leucine and phenylalanine in combination with carbohydrate can result in an 88% higher postexercise insulin response than can the intake of carbohydrate only. At a carbohydrate intake of  $0.8$   $\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ , provided at 30-min intervals, addition of this insulinotropic mixture resulted in a significant increase in glycogen synthesis rates (113%) during the 5-h postexercise period. An increase in the carbohydrate ingestion rate up to  $1.2$   $\text{g}$  carbohydrate  $\cdot \text{kg}^{-1} \cdot \text{h}^{-1}$  provided at 30-min intervals also resulted in faster muscle glycogen synthesis (170%) than did an intake of  $0.8$   $\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ . 🌱

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