

## Effect of carbohydrate intake on net muscle protein synthesis during recovery from resistance exercise

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**Børsheim, Elisabet, Melanie G. Cree, Kevin D. Tipton, Tabatha A. Elliott, Asle Aarsland, and Robert R. Wolfe.** Effect of carbohydrate intake on net muscle protein synthesis during recovery from resistance exercise. *J Appl Physiol* 96: 674–678, 2004. First published October 31, 2003; 10.1152/jappphysiol.00333.2003.—The purpose of this study was to determine the effect of ingestion of 100 g of carbohydrates on net muscle protein balance (protein synthesis minus protein breakdown) after resistance exercise. Two groups of eight subjects performed a resistance exercise bout (10 sets of 8 repetitions of leg presses at 80% of 1-repetition maximum) before they rested in bed for 4 h. One group (CHO) received a drink consisting of 100 g of carbohydrates 1 h postexercise. The other group (Pla) received a noncaloric placebo drink. Leg amino acid metabolism was determined by infusion of  $^2\text{H}_5$ - or  $^{13}\text{C}_6$ -labeled phenylalanine, sampling from femoral artery and vein, and muscle biopsies from vastus lateralis. Drink intake did not affect arterial insulin concentration in Pla, whereas insulin increased several times after the drink in CHO ( $P < 0.05$  vs. Pla). Arterial phenylalanine concentration fell slightly after the drink in CHO. Net muscle protein balance between synthesis and breakdown did not change in Pla, whereas it improved in CHO from  $-17 \pm 3 \text{ nmol}\cdot\text{ml}^{-1}\cdot 100 \text{ ml leg}^{-1}$  before drink to an average of  $-4 \pm 4$  and  $0 \pm 3 \text{ nmol}\cdot\text{ml}^{-1}\cdot 100 \text{ ml leg}^{-1}$  during the second and third hour after the drink, respectively ( $P < 0.05$  vs. Pla during last hour). The improved net balance in CHO was due primarily to a progressive decrease in muscle protein breakdown. We conclude that ingestion of carbohydrates improved net leg protein balance after resistance exercise. However, the effect was minor and delayed compared with the previously reported effect of ingestion of amino acids.

resistance exercise; muscle protein metabolism; carbohydrate ingestion; stable isotopes

NET MUSCLE PROTEIN BALANCE (i.e., the difference between muscle protein synthesis and protein breakdown) generally remains negative in the recovery period after resistance exercise in the absence of nutrient intake (4, 14–16), i.e., the muscle is in a catabolic state. We have previously shown that either infusion (5) or ingestion (20) of amino acids after exercise stimulates muscle protein synthesis. Furthermore, as little as 6 g of essential amino acids (EAA) alone effectively stimulated net protein synthesis after a strenuous resistance exercise bout (7). The response to 6 g of EAA did not appear to differ from the response when 6 g of EAA was given together with 35 g of carbohydrates (17). However, the independent effects of carbohydrates on muscle protein metabolism after resistance exercise are not clear. Roy et al. (19) found only a trend toward higher fractional muscle protein synthetic rate (FSR) in exercised vs. resting leg when carbohydrates (1 g/kg) were given at

0 and 1 h after unilateral knee extension. FSR was measured over 10 h postexercise. Simultaneously, urinary 3-methylhistidine excretion (marker of protein breakdown) was decreased after the carbohydrate intake compared with a placebo condition. However, muscle protein breakdown was not directly measured, and the time course of changes in net protein balance is not known. Furthermore, it is unclear how a possible change of net protein balance between synthesis and breakdown relates to changes in plasma insulin concentration after carbohydrate intake.

Interpretation of the response of muscle protein to insulin is complicated by the fact that a systemic increase in insulin concentration causes a fall in plasma amino acid concentrations, and this reduced amino acid availability could potentially counteract a direct effect of insulin on synthesis (25). For this reason, we have previously assessed the response to local hyperinsulinemia induced by infusion into the femoral artery. This approach raises insulin concentration in muscle without affecting amino acid concentrations. Using local insulin infusion, we found that hyperinsulinemia stimulated muscle protein synthesis at rest (2). However, after resistance exercise, local hyperinsulinemia caused no further stimulation of muscle protein synthesis (6). The normal postexercise increase in muscle protein breakdown was ameliorated by insulin, thus improving net muscle protein balance. However, whereas local infusion of insulin may effectively isolate the effect of insulin per se, the response may differ from when insulin release is stimulated by ingestion of carbohydrates.

Hence, the purpose of the present study was to investigate the independent effect of carbohydrate intake on muscle protein net balance during recovery from resistance exercise.

### MATERIALS AND METHODS

**Subjects.** Sixteen healthy subjects took part in the study. Subjects were recreationally active. They were fully informed of the purpose and procedures of the study before written consent was obtained. Before participation in the experiments, each subject had a complete medical screening, including vital signs, blood tests, urine tests, and a 12-lead electrocardiogram, for determination of health status at the General Clinical Research Center (GCRC) of the University of Texas Medical Branch (UTMB) at Galveston, Texas. The protocol was approved by the Institutional Review Board of the UTMB.

**Preexperimental procedures.** At least 1 wk before an experiment, subjects were familiarized with the exercise protocol, and their one repetition maximum (1 RM; the maximum weight that can be lifted for one repetition) for leg extension was determined by the procedure

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described by Mayhew et al. (10). The leg volume of each subject was estimated from anthropometric measures of leg circumference and height at multiple points down the length of the leg (Table 1).

**Experimental protocol.** The subjects were assigned to one of two groups: carbohydrate group (CHO;  $n = 8$ , Table 1) or placebo group (Pla;  $n = 8$ , Table 1). Subjects were instructed not to exercise for at least 48 h before an experiment, not to use tobacco or alcohol during the 24 h before an experiment, and not to make any changes in their dietary habits. The subjects reported to the GCRC in the evening before an experiment for an overnight stay and were fasted from 10:00 PM.

The experimental protocol is shown schematically in Fig. 1. At ~6:00 AM, an 18-gauge polyethylene catheter (Cook, Bloomington, IN) was inserted into an antecubital arm vein for the primed continuous infusion of stable isotopes of amino acids. After a blood sample for measurement of background amino acid enrichment was obtained, a primed, constant infusion of labeled phenylalanine was started at -120 min (~06:30 AM). In Pla, L-[ $^{13}\text{C}_6$ ]phenylalanine was used (priming dose: 2  $\mu\text{mol}/\text{kg}$ ; infusion rate: 0.07  $\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ), whereas L-ring[ $^2\text{H}_5$ ]phenylalanine (priming dose: 2  $\mu\text{mol}/\text{kg}$ ; infusion rate: 0.1  $\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ) was used in CHO. Isotopes were purchased from Cambridge Isotopes (Andover, MA). They were dissolved in 0.9% saline and were filtered through a 2- $\mu\text{m}$  filter before infusion.

At ~7:00 AM, a 3-Fr, 8-cm polyethylene catheter (Cook, Bloomington, IN) was inserted into both the femoral vein and femoral artery under local anesthesia. Both femoral catheters were used for blood sampling, and the femoral arterial catheter was also used for indocyanine green dye infusion for determination of leg blood flow (3). A constant infusion of indocyanine green dye (0.5 mg/min) was given at intervals during the experiment (Fig. 1). The infusion ran for at least 10 min before peripheral, and femoral venous blood samples were drawn for measurement of blood flow. The peripheral venous blood samples were drawn from an 18-gauge polyethylene catheter inserted into an antecubital vein of the opposite arm into which the amino acids were infused. Patency of catheters was maintained by saline infusion.

Subjects rested in bed until the exercise started at -20 min (8:45 AM). Subjects performed 10 sets of 8 repetitions of leg extensions at 80% of the 1 RM. Each set was completed in ~30 s with a 2-min rest between sets, and the entire bout was completed in ~20 min. Subjects then returned to bed and rested for 4 h.

In CHO, subjects were given a drink consisting of 100 g of carbohydrates (maltodextrin) in double distilled water at 1 h postexercise. In Pla, a placebo drink with artificial sweetener was ingested instead.

To measure the isotopic enrichment of free and bound amino acid tracers in the muscle, biopsies were sampled at 5 min before start of exercise and at 55, 120, and 240 min after exercise (Fig. 1). The muscle biopsies were taken under local anesthesia from the lateral portion of the vastus lateralis ~10–15 cm above the knee. A 5-mm Bergstrom biopsy needle (Depuy, Warsaw, IN) was used to sample ~30–50 mg of mixed muscle tissue. The sample was quickly rinsed,

Table 1. Physical characteristics and exercise data for the subjects in the CHO and Pla groups

|                     | CHO ( $n = 8$ )  | Pla ( $n = 8$ )  |
|---------------------|------------------|------------------|
| Gender              | 3 F, 5 M         | 3 F, 5 M         |
| Age, yr             | 29 $\pm$ 7       | 24 $\pm$ 3       |
| Height, cm          | 171 $\pm$ 11     | 173 $\pm$ 6      |
| Weight, kg          | 74.4 $\pm$ 13.1  | 75.4 $\pm$ 8.5   |
| Leg volume, liters  | 10.3 $\pm$ 1.7   | 10.0 $\pm$ 1.2   |
| Leg press, 1 RM, kg | 119.9 $\pm$ 40.2 | 142.8 $\pm$ 37.4 |

Values are mean  $\pm$  SD. F, females; M, males; 1 RM, 1-repetition maximum; CHO, carbohydrate group; Pla, placebo group.

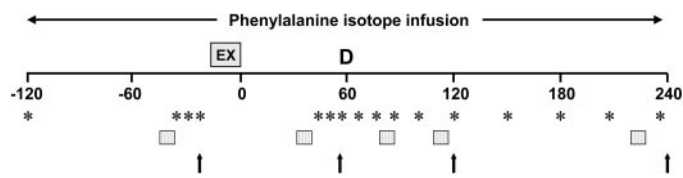


Fig. 1. Infusion protocol. D, drink; \*, blood sample; squares, blood flow measurements; arrows, biopsy.

blotted, and immediately frozen in liquid nitrogen and stored at  $-80^{\circ}\text{C}$  for later analysis.

Blood samples were drawn from the femoral artery and venous catheters at 15, 10, and 5 min before start of exercise and at 45, 50, 55, 70, 80, 90, 105, 120, 150, 180, 210, 230, and 240 min after the end of exercise (Fig. 1). The samples were analyzed for phenylalanine enrichments and concentrations, and most of the samples were also analyzed for glucose and insulin concentrations. More frequent samples were drawn for glucose and insulin concentrations in CHO than in Pla. Less frequent samples were drawn from the antecubital vein and femoral vein for determination of blood flow in five different periods (Fig. 1).

**Sample analyses.** Blood samples for determination of amino acid enrichment and concentrations were immediately precipitated in pre-weighed tubes containing 15% sulfosalicylic acid and a weighed amount of an appropriate internal standard, consisting of phenylalanine labeled differently than the infused phenylalanine, was added (3, 4, 14). The supernatant was passed over a cation exchange column (Dovex AG 50W-8X, 100–200 mesh  $\text{H}^+$  form; Bio-Rad, Richmond, CA) and dried under vacuum with a Speed Vac (Savant Instruments, Farmingdale, NY). Enrichments of intracellular free phenylalanine were then determined on the tertiary-butyl dimethylsilyl derivatives by using gas chromatography mass spectrometry (Hewlett-Packard 5973, Palo Alto, CA) and selected ion monitoring (23). Enrichments were expressed as tracer-to-tracee ratio. Appropriate corrections were made for overlapping spectra (23).

To determine muscle intracellular enrichment of infused tracers, muscle tissue was weighed, and the protein was precipitated with perchloroacetic acid. The tissue was then homogenized and centrifuged, and the supernatant was collected. The procedure was then repeated, and the pooled supernatant was processed in the same way as the supernatant from the blood samples.

Indocyanine green dye concentration in serum for the determination of leg blood flow was measured spectrophotometrically at wavelength ( $\lambda$ ) = 805 nm (9, 22). Plasma glucose concentration was determined enzymatically (YSI 1500, Yellow Springs Instruments, Yellow Springs, OH). Plasma insulin concentration was determined by a radioimmunoassay method (Diagnostic Products, Los Angeles, CA).

**Calculations.** Net muscle phenylalanine balance, which was considered as the primary endpoint, was calculated as follows: (phenylalanine arterial concentration - venous concentration)  $\times$  blood flow. Because phenylalanine is neither produced nor metabolized in muscle, net phenylalanine balance reflects net muscle protein synthesis, provided there is no significant increase in the free intracellular pool of phenylalanine. Area under the curve of net phenylalanine uptake was determined for the 3-h period after drink ingestion, with net average uptake at time = 45–55 min postexercise used as the zero point.

The rate of incorporation of phenylalanine from blood into muscle protein [rate of disappearance ( $R_d$ ) of phenylalanine from blood] was calculated as  $R_d = [(Ea \cdot Ca) - (Ev \cdot Cv)] \cdot BF \cdot Ea^{-1}$ , where Ea and Ev are the arterial and venous phenylalanine enrichments (expressed as mole percent excess), respectively, Ca and Cv are the total (tracer + tracee) arterial and venous phenylalanine concentrations, respectively, and BF is blood flow (23). The rate of release of phenylalanine from protein breakdown into blood [rate of appearance ( $R_a$ ) of phenylalanine in blood] was calculated as  $R_a = R_d - [(Ca - Cv) \cdot BF]$  (23).

FSR was determined for the time periods between muscle biopsies after drink intake and for the entire postdrink period. FSR (%/h) was calculated as described previously (14). Briefly, mixed muscle protein FSR was determined by using the free intracellular phenylalanine enrichment as the precursor pool, which appears to be the optimal surrogate for the true precursor enrichment (1). The mixed muscle FSR was calculated as the mean of the incorporation of labeled phenylalanine into mixed muscle protein over time divided by the precursor enrichment.

**Statistical methods.** Overall significance of differences in response to drink between the two groups was analyzed by using a two-factor (treatment and time) repeated-measures analysis of variance, followed by Dunnett's test (SigmaStat 2.03, SPSS, Chicago, IL). Results were considered significant if  $P < 0.05$ . The results are presented as means  $\pm$  SE, unless otherwise noted.

## RESULTS

**Plasma glucose concentration.** Arterial glucose concentration was not different between the two groups in the morning before exercise (Pla:  $89 \pm 4$  mg/dl; CHO:  $85 \pm 1$  mg/dl) or before the drink after exercise (Pla:  $89 \pm 6$  mg/dl; CHO:  $85 \pm 1$  mg/dl; Fig. 2A). No change in concentration was seen during the recovery period in Pla, whereas the concentration increased rapidly after the drink in CHO, reaching a peak after 30 min ( $175 \pm 8$  mg/dl). Arterial glucose was significantly increased in CHO vs. Pla until 210 min after intake of drink.

**Plasma insulin concentration.** Plasma concentration of insulin reflected the changes in glucose concentration. Before exercise, arterial insulin concentration was  $5.3 \pm 1.2$   $\mu$ IU/ml

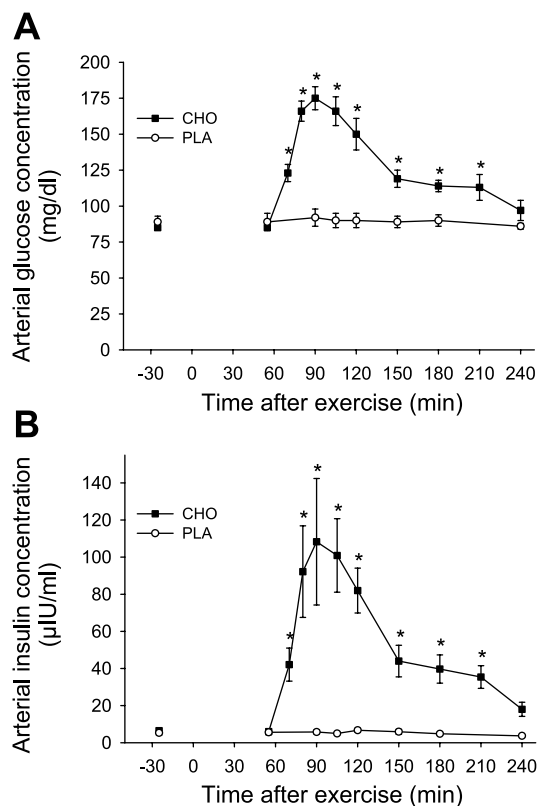


Fig. 2. Time course of arterial glucose concentration [A; carbohydrate group (CHO):  $n = 8$ ; placebo group (Pla):  $n = 7$ ] and serum insulin concentration (B;  $n = 8$  in each group). Drink was consumed at 60 min after exercise. Values are means  $\pm$  SE. \* $P < 0.05$  vs. Pla.

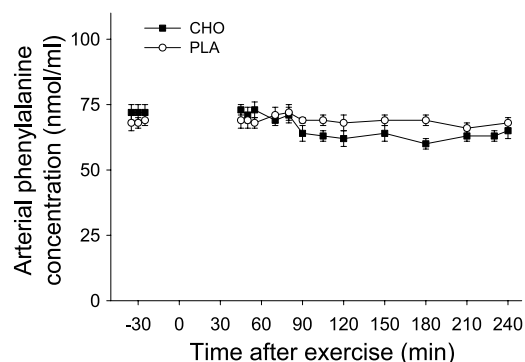


Fig. 3. Time course of arterial phenylalanine concentration. Drink was consumed at 60 min after exercise. Values are means  $\pm$  SE;  $n = 8$  in each group.

in Pla vs.  $6.5 \pm 0.9$   $\mu$ IU/ml in CHO [ $P =$  not significant (NS); Fig. 2B]. Drink intake did not affect arterial insulin concentration in Pla, whereas arterial insulin increased by several times after the drink in CHO ( $P < 0.05$  vs. Pla).

**Phenylalanine concentration.** Arterial phenylalanine concentration was  $\sim 70$  nmol/ml in both groups before exercise and also before the drink after exercise ( $P =$  NS; Fig. 3). Phenylalanine concentration did not change after intake of drink in Pla, but in CHO the concentration decreased and stabilized at a level  $\sim 10$  nmol/ml lower than predrink level from 30 min post-drink until the end of the experiment [time effect:  $P < 0.001$ ; treatment (drink)  $\times$  time effect:  $P < 0.001$ ].

**Phenylalanine kinetics.** Net balance of phenylalanine did not differ in the two groups before intake of drink (Fig. 4). Similarly, no differences were seen during the first hour after exercise, but during the second hour after drink, net balance started to increase in CHO. There was a significant interaction effect between treatment (drink) and time on the average net balance for each hour after drink ( $P = 0.02$ ). The difference between groups reached statistical significance during the third hour after intake of drink.

Net phenylalanine balance is determined by the disappearance of phenylalanine from the blood into the muscle (i.e., protein synthesis from plasma phenylalanine) and the rate of appearance of phenylalanine into the blood from the muscle (estimate of protein breakdown). There were no differences in

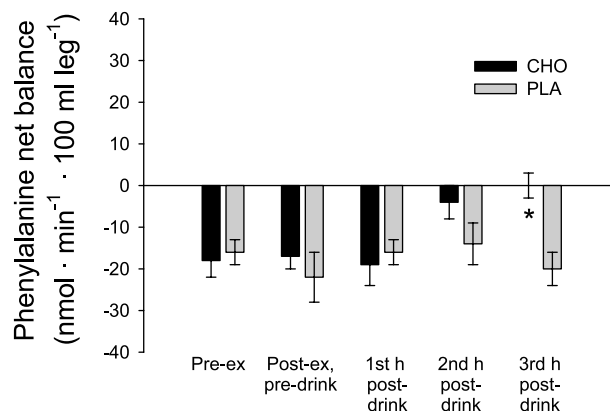


Fig. 4. Average net balance of phenylalanine before exercise (Pre-ex), after exercise but before drink (Post-ex, pre-drink), and during the first, second, and third hour after intake of drink (post-drink). Values are means  $\pm$  SE;  $n = 8$  in each group. \* $P < 0.05$  vs. Pla.

protein synthesis between the groups either before or after intake of drink (Fig. 5). Protein breakdown was not different between groups before the drink (Fig. 6), but a time effect ( $P = 0.048$ ) was seen after intake of drink. This was not significant within Pla, but in CHO there was a gradual drop in protein breakdown after intake of drink ( $P < 0.05$ ).

There was no difference in muscle intracellular phenylalanine concentration between the groups in the morning (Pla:  $63 \pm 5$  nmol/ml; CHO:  $74 \pm 5$  nmol/ml) or after exercise before drink (Pla:  $72 \pm 5$  nmol/ml; CHO:  $72 \pm 8$  nmol/ml). There was no effect of drink intake, but an overall effect of time was found ( $P < 0.001$ ), with a lower concentration in the two biopsies taken after drink vs. the predrink biopsy (last biopsy Pla:  $63 \pm 6$  nmol/ml; CHO:  $52 \pm 4$  nmol/ml;  $P = NS$  between groups).

Total area under the curve for phenylalanine uptake into muscle above predrink value over the 3 h after drink was  $32.0 \pm 10.3$  mg/leg in CHO vs.  $23.7 \pm 21.3$  mg/leg in Pla ( $P = NS$ ). Similarly, no differences between groups in phenylalanine FSR were found over the first hour after drink (Pla:  $0.0843 \pm 0.0065\%/h$ ; CHO:  $0.0851 \pm 0.0120\%/h$ ), between 1 and 3 h after drink (Pla:  $0.0682 \pm 0.0099\%/h$ ; CHO:  $0.0876 \pm 0.0112\%/h$ ), or when the entire postdrink period was compared (Pla:  $0.0735 \pm 0.0077\%/h$ ; CHO:  $0.0869 \pm 0.0087\%/h$ ).

## DISCUSSION

This study is the first to compare net muscle protein balance (protein synthesis minus breakdown) after carbohydrate ingestion with control after exercise. The principal finding was that intake of 100 g of carbohydrates after resistance exercise improved muscle net protein balance. However, this improvement was of questionable physiological significance because the net balance did not reach positive values and the improvement was minor compared with the reported effect of intake of amino acids (7, 20).

The present findings show that intake of carbohydrates alone can improve net protein balance between synthesis and breakdown. Previous studies have provided some evidence that carbohydrate ingestion improves protein metabolism when taken alone. Rennie et al. (18) showed improved leucine balance during carbohydrate-supplemented endurance exer-

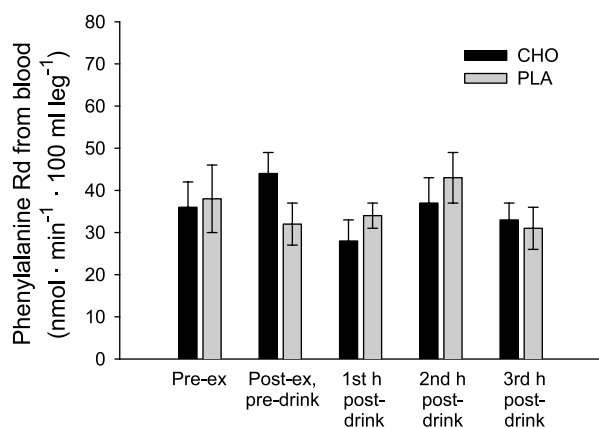


Fig. 5. Average rate of disappearance ( $R_d$ ) of phenylalanine out of the blood into the muscle (estimate of protein synthesis) Pre-ex; Post-ex, pre-drink; and during the first, second, and third hour post-drink. Values are means  $\pm$  SE;  $n = 8$  in each group.

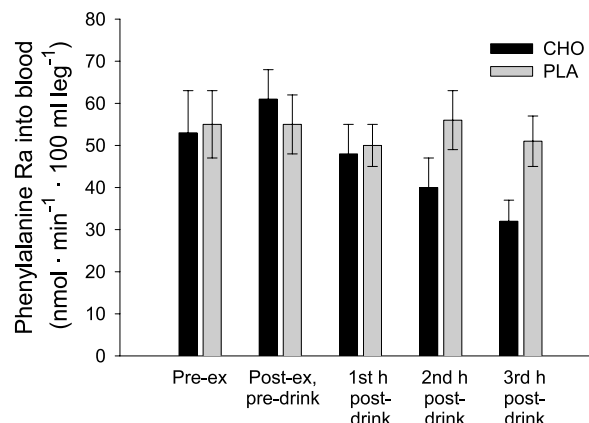


Fig. 6. Average rate of appearance ( $R_a$ ) of phenylalanine into the blood from muscle (estimate of protein breakdown) Pre-ex; Post-ex, pre-drink; and during the first, second, and third hour post-drink. Values are means  $\pm$  SE;  $n = 8$  in each group.

cise, and Roy et al. (19) found a trend toward higher FSR in response to carbohydrate ingestion after unilateral knee extension. They also observed a decreased excretion of urinary 3-methylhistidine, suggesting a suppression of muscle protein breakdown. However, there was no determination of net muscle protein balance.

In our study, the gradual improvement in net muscle protein balance after carbohydrate intake was due principally to a progressive reduction in breakdown. However, the improvement was small compared with previous findings after intake of amino acids or amino acids + carbohydrates (7, 11, 17, 20, 21). In fact, the balance between synthesis and breakdown did not reach positive values during the 3 h after drink (Fig. 4). The modest effect of carbohydrates alone on net balance supports the findings that intake of 6 g of EAA appeared to stimulate net protein balance as effectively as when 6 g of EAA was given together with 35 g of carbohydrates (7, 17). The response to amino acids is not only greater than the response to carbohydrates, but the onset is also more rapid. The delayed onset of response to carbohydrate intake occurs despite the rapid increases in both arterial plasma glucose and insulin concentrations after intake of the carbohydrates (Fig. 2). Thus it may be that insulin causes a delayed effect on protein metabolism.

The apparently delayed action of insulin on net muscle protein anabolism, as opposed to the rapid effect of amino acids, suggests that delayed intake of amino acids relative to carbohydrates could amplify the potential interactive effect. With this approach, amino acids would be taken up at the peak of insulin action on muscle. With such a staggered approach, a physiologically significant effect of carbohydrate intake after exercise might be evident. However, when given alone or at the same time as free EAA, carbohydrates seem to have little effect on net muscle protein synthesis.

There was no effect of the ingested carbohydrates on protein synthesis in the present study. Similarly, Miller et al. (11) did not find any effect of a smaller dose of carbohydrates ( $2 \times 35$  g) postexercise, generating a lower increase in plasma glucose and insulin concentrations compared with the present study, on the disappearance of phenylalanine from plasma into leg muscle. Whereas there is little doubt that insulin stimulates muscle protein synthesis in vitro (24), some controversy exists

regarding the effect of insulin on muscle protein synthesis *in vivo*. Several experiments in animals have supported the notion that insulin stimulates protein synthesis (8, 12, 13). A review of experiments in humans (25) shows that the effect of insulin on muscle protein synthesis is closely related to the concurrent amino acid delivery. When amino acid delivery to muscle is maintained or increased by either infusion or ingestion of amino acids, insulin has been shown to have a positive effect on protein synthesis, whereas little or no effect of insulin on synthesis has been found when there is a simultaneous decrease in amino acid delivery. In the present study, a modest drop in amino acid concentration was observed after carbohydrate intake (Fig. 3), but there was no difference between the two groups in the inflow of amino acids (delivery) to the leg, because blood flow was slightly (but statistically nonsignificantly) higher in CHO. However, muscle intracellular amino acid concentration was lower after drink, thereby limiting the potential response of protein synthesis to the increase in insulin concentration (24).

In conclusion, the principal finding of this study was that intake of 100 g of carbohydrates after resistance exercise improved muscle net protein balance, but the improvement was only minor compared with the reported effect of intake of amino acids. We conclude that intake of carbohydrates alone after resistance exercise will modestly improve the anabolic effect of exercise. However, amino acid intake is necessary for a maximal response.

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