

# Independent and Combined Effects of Amino Acids and Glucose after Resistance Exercise

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## ABSTRACT

MILLER, S. L., K. D. TIPTON, D. L. CHINKES, S. E. WOLF, and R. R. WOLFE. Independent and Combined Effects of Amino Acids and Glucose after Resistance Exercise. *Med. Sci. Sports Exerc.*, Vol. 35, No. 3, pp. 449–455, 2003. **Purpose:** This study was designed to assess the independent and combined effects of a dose of amino acids (~6 g) and/or carbohydrate (~35 g) consumed at 1 and 2 h after resistance exercise on muscle protein metabolism. **Methods:** Following initiation of a primed constant infusion of <sup>2</sup>H<sub>5</sub>-phenylalanine and <sup>15</sup>N-urea, volunteers performed leg resistance exercise and then ingested one of three drinks (amino acids (AA), carbohydrate (CHO), or AA and CHO (MIX)) at 1- and 2-h postexercise. **Results:** Total net uptake of phenylalanine across the leg over 3 h was greatest in response to MIX and least in CHO. The individual values for CHO, MIX, and AA were 53 ± 6, 114 ± 38, and 71 ± 13 mg·leg<sup>-1</sup>·3 h<sup>-1</sup>. Stimulation of net uptake in MIX was due to increased muscle protein synthesis. **Conclusions:** These findings indicate that the combined effect on net muscle protein synthesis of carbohydrate and amino acids given together after resistance exercise is roughly equivalent to the sum of the independent effects of either given alone. The individual effects of carbohydrate and amino acids are likely dependent on the amount of each that is ingested. Further, prior intake of amino acids and carbohydrate does not diminish the metabolic response to a second comparable dose ingested 1 h later. **Key Words:** STABLE ISOTOPES, UREA PRODUCTION, PHENYLALANINE, LEG BALANCE, HUMAN SUBJECTS

Resistance exercise stimulates muscle protein synthesis in both an absolute sense and in relation to the rate of muscle protein breakdown (3,11). However, in the absence of nutritional intake, the balance between synthesis and breakdown (i.e., net muscle protein synthesis) remains negative after resistance exercise (3,4). Therefore, the increase in muscle size (anabolism) that results from resistance training must reflect an interaction between exercise and nutritional intake. Although it is known that exogenous amino acids alone (4,16) or amino acids plus glucose (12) can stimulate muscle protein synthesis after exercise, the optimal composition of a nutritional supplement to promote this response is unknown.

Because amino acids are known to stimulate muscle protein synthesis (13), the ingestion of amino acids or protein can be predicted to be an essential component of a supplement designed to promote anabolism. However, the importance of energy intake in conjunction with amino acids/protein is less clear. On the one hand, there is little evidence to support the importance of fat as a source of energy to promote efficient utilization of ingested amino acids. On the other hand, there is reason to expect an interactive effect

between amino acid and carbohydrate intake stemming from the resultant insulin response to the carbohydrate (20).

Insulin is an important anabolic hormone, but its mechanism of action on muscle is controversial (5,7). Evaluation of the effect of insulin on muscle is complicated by the fact that systemic insulin infusion causes a pronounced hypoaminoacidemia. This decrease in amino acid levels tends to counteract any direct action of insulin to stimulate muscle protein synthesis *in vivo* (20). Infusion of insulin directly into the femoral artery to create local hyperinsulinemia in the absence of any change in amino acid concentrations stimulated muscle protein synthesis at rest (2). However, following resistance exercise local hyperinsulinemia caused no further stimulation of muscle protein synthesis, although the postexercise elevation in the rate of protein breakdown was attenuated (5). It is possible that the failure of insulin alone to further stimulate synthesis after exercise is due to a relative insufficient supply of amino acids. If true, then supplying exogenous amino acids after exercise should allow expression of the stimulatory effect of insulin. This response would be manifest as an interactive effect of insulin and amino acids, such that the combined effects of hyperinsulinemia and ingested amino acids would be greater than the sum of their independent effects. It was therefore a principal goal of this project to assess the individual and interactive effects of ingestion of amino acids and/or carbohydrate (to stimulate insulin release) on muscle protein synthesis after exercise.

A second aspect of this study was designed to assess the time course of the response of muscle protein turnover to an oral supplement after exercise. We have previously shown that ingestion of essential amino acids (EAA) plus carbo-

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hydrate after exercise significantly increased muscle protein anabolism by increasing muscle protein synthesis without altering muscle protein breakdown (12). Although the increase in net muscle protein synthesis was statistically significant, it was relatively short-lived and persisted for only 1 h following ingestion of the drink. A second drink could potentially amplify both duration and magnitude of the response given the relatively transient response to a single drink. Alternatively, it is possible that a second bolus might elicit a lesser response. This outcome is considered because we recently found that during a 6-h intravenous infusion of amino acids, protein synthesis was only stimulated for the first 2 h, and by 6 h, the rate had returned to the basal level despite persistent elevations in amino acid concentrations (6). Further, in our previous study in which a bolus of amino acids and glucose were ingested (12) arterial amino acid and insulin concentrations were still significantly elevated at the time when the rate of net muscle protein synthesis returned to the basal value. Therefore, a second goal of this study was to determine if the response to a second bolus of amino acids and/or carbohydrate was affected by the prior ingestion of the same dose 1 h earlier.

## METHODS

Ten volunteers (six male, four female) participated in the study, which was approved by the Institutional Review Board at the University of Texas Medical Branch (UTMB). All subjects provided written informed consent and underwent a preliminary health screening to ensure that they were healthy and free of metabolic disease. At this time, subjects were also strength tested to determine their one-repetition maximum (1 RM) on leg press and knee extension exercises. Leg volume was estimated from anthropometric measures of leg circumference and height at multiple points down the length of the leg (10).

On the day before a scheduled study, subjects were instructed to avoid exercise, alcohol, and deviation from their normal diet. Volunteers reported to the General Clinical Research Center (GCRC) at UTMB and fasted overnight. The following morning, peripheral venous catheters were placed for primed continuous infusion of a stable isotope tracer. Femoral arterial and venous catheters were placed for sampling arterial and venous blood and for arterial infusion of indocyanine green dye (ICG) to assess leg blood flow (9). Femoral catheters were inserted using lidocaine, and patency of all catheters was maintained by saline infusion.

The general infusion protocol is shown schematically in Figure 1. After obtaining a blood sample for measurement of background amino acid enrichment and ICG concentration, a primed-continuous infusion of L-[<sup>2</sup>H5]-phenylalanine (infusion rate =  $0.10 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ , priming dose =  $2 \mu\text{mol}\cdot\text{kg}^{-1}$ ) and <sup>15</sup>N-urea (infusion rate =  $0.227 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ , priming dose =  $113 \mu\text{mol}\cdot\text{kg}^{-1}$ ) was started. Subjects then rested comfortably in bed for approximately 1 h, after which they prepared to perform the resistance exercise routine. Exercise was completed in approximately 40 min and consisted of leg press (10 sets of 10

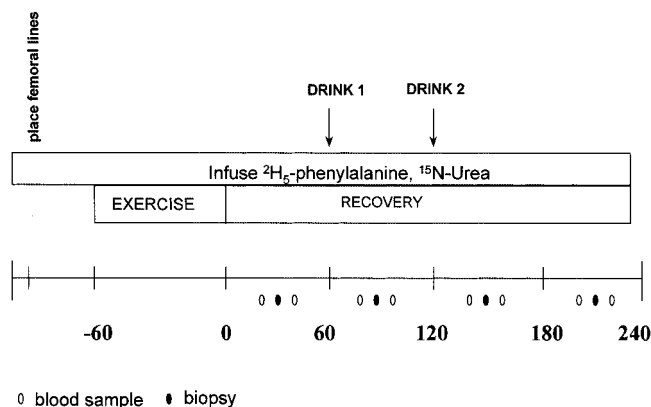


FIGURE 1—Study protocol.

repetitions) and knee extensions (8 sets of 8 repetitions), both at ~75% of the subject's 1 RM. Each set was completed in approximately 30 s with a 2-min rest between sets. Subjects returned to bed following completion of the exercise and the first samples were taken at 30 min following completion of the last set of exercise. At 1 h after exercise, subjects ingested a drink containing either carbohydrate alone (CHO), amino acids alone (AA), or both carbohydrate and amino acids together (MIX). The amount of carbohydrate (glucose) and amino acids was adjusted according to body weight, based on 35 g of carbohydrate and 6 g of amino acids per 70 kg subject. The details of the drinks, including the mixture of amino acids, is shown in Table 1. The amino acid mixture was approximately 50% EAA (2.8 g/6 g total). Five study subjects (three male, two female) completed all three trials while an additional one finished two trials and four others participated in one trial each. An equivalent number of trials,  $N = 7$ , was completed for each treatment.

Approximately 10 min before each sampling period, a primed-continuous infusion of ICG was started into the femoral artery and maintained throughout the sampling period. Femoral and peripheral venous blood samples for blood flow measurement were taken, followed by femoral arterial and venous blood sampling. The dye infusion was stopped briefly to allow sampling from the femoral artery and then quickly resumed. Blood samples were taken at 30-min postexercise, at 10-min intervals following ingestion

TABLE 1. Supplement composition.

	CHO	MIX	AA
Energy (kcal)	140	164	24
Carbohydrate (g)	$0.5 \text{ g}\cdot\text{kg}^{-1}$	$0.5 \text{ g}\cdot\text{kg}^{-1}$	0
Amino acids (g)	0	$0.087 \text{ g}\cdot\text{kg}^{-1}$	$0.087 \text{ g}\cdot\text{kg}^{-1}$
Amino acid Composition			
Essential	2.8 g/6 g	Nonessential	3.2 g/6 g
Histidine	0.298	Alanine	0.358
Isoleucine	0.275	Arginine	0.433
Leucine	0.544	Aspartate	0.597
Lysine	0.426	Cystine	0
Methionine	0.087	Glutamine	1.075
Phenylalanine	0.453	Glycine	0.234
Threonine	0.403	Proline	0.234
Tryptophan	0	Serine	0.164
Valine	0.321	Tyrosine	0.119

of each drink at 1-h and 2-h postexercise, and at four time points during the third and final hour of the recovery period. Blood samples were used for determination of arterial-venous differences of substrate across the leg muscle and for measurement of urea and phenylalanine (Phe) enrichment. Enrichments and Phe concentration were determined by gas chromatography mass spectrometry, as described previously (19). Glucose concentrations were determined enzymatically using an autoanalyzer (YSI 2300 STAT, Yellow Springs, OH).

Muscle biopsies were taken to quantify the intracellular Phe concentration. The first muscle biopsy was taken 30-min postexercise from the lateral portion of the right vastus lateralis muscle, approximately 20 cm above the knee, using a 5-mm Bergstrom biopsy needle (Stille, Stockholm, Sweden) (1). Approximately 30–50 mg of muscle tissue was obtained with each biopsy. This procedure yields a sample of mixed skeletal muscle (1). Blood and visible fat and connective tissue were removed from the remainder of the specimen, and the tissue was immediately frozen in liquid nitrogen and stored at  $-80^{\circ}\text{C}$  for later analysis. Biopsies were taken at 30 min following exercise, ~30 min following ingestion of the first and second drink, and toward the end of the third hour of recovery.

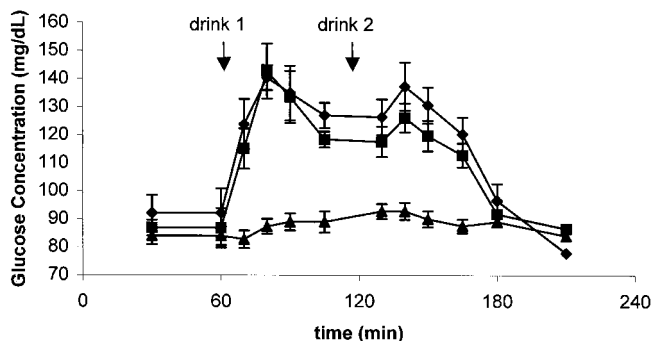
Phe net uptake into the muscle was calculated by the product of blood flow (milliliters per minute per 100 mL leg volume) and the difference in arterial and venous concentrations. Rates of appearance and disappearance of Phe from the blood were calculated using a model based only on blood measurements. Rate of disappearance (Rd) of Phe across muscle was calculated using the formula:

$$\text{Rd} = [\text{EA} \cdot \text{CA} - \text{EV} \cdot \text{CV}] \cdot \text{BF} / \text{EA}$$

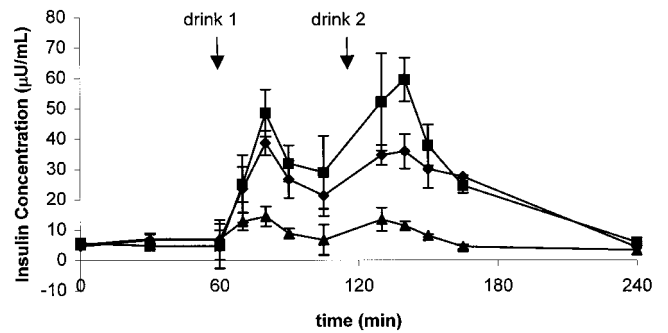
where EA and EV are the arterial and venous Phe enrichments, respectively, CA and CV are the arterial and venous Phe concentrations, respectively, and BF is the rate of blood flow across the leg. The rate of appearance (Ra) of Phe across muscle is then calculated by the formula:

$$\text{Ra} = \text{Rd} - (\text{CA} - \text{CV}) \cdot \text{BF}$$

where Ra represents the amino acids released from muscle protein breakdown that appear in the plasma, and Rd represents



**FIGURE 2—Arterial glucose concentration.** O represents the end of exercise. Drink 1 was 60-min postexercise. Drink 2 was 120-min postexercise.  $\diamond$  carbohydrate alone (CHO);  $\blacktriangle$  amino acids alone (AA);  $\blacksquare$  carbohydrate + amino acids (MIX).



**FIGURE 3—Plasma insulin concentration.**  $\diamond$  carbohydrate alone (CHO);  $\blacktriangle$  amino acids alone (AA);  $\blacksquare$  carbohydrate + amino acids (MIX).

resents irreversible loss of amino acids from plasma. Since Phe cannot be metabolized within muscle, Rd represents muscle protein synthesis from plasma-bound Phe.

Area under the curve (AUC) of Phe uptake for the 3-h recovery period was determined using the trapezoid method.

Urea production was calculated from enrichment and tracer infusion rates as described in (21).

**Statistical analysis.** Average values were calculated for all measured outcomes and are expressed as mean  $\pm$  SEM. Differences between treatment groups were determined by two-way ANOVA where one factor is subject and the other factor is group. If a significant difference was indicated ( $P < 0.05$ ), Tukey *post hoc* analysis was used to determine the treatments between which differences were significant.

## RESULTS

**Glucose.** Plasma glucose concentrations rose significantly in response to carbohydrate containing drinks, CHO and MIX ( $P < 0.05$ ) (Fig. 2). Glucose uptake also increased significantly more so in these trials compared with uptake during amino acid supplemented recovery ( $P < 0.01$ ).

**Insulin.** Plasma insulin levels followed the glucose increase and peaked at ~20 min following ingestion of CHO or MIX (Fig. 3). Insulin AUC was significantly greater in response to MIX ( $P < 0.001$ ), and CHO also increased insulin AUC more than AA alone ( $P < 0.001$ ). However, AA did tend to increase insulin concentrations above the baseline and postexercise concentrations ( $P = 0.08$ ). By the end of the third hour of recovery, insulin concentrations returned to postexercise levels in all three treatments.

**The concentration and uptake.** Arterial Phe concentrations increased significantly within 10 min after ingestion of AA ( $P < 0.05$ ) or MIX ( $P < 0.05$ ) but remained relatively constant in response to CHO (Fig. 4). Values in amino acid drink trials started to decline before ingestion of the second drink but were still increased significantly above baseline (MIX  $P < 0.05$ , AA  $P < 0.001$ ). Response to the second drink was comparable to that of the first amino acid containing drink.

**Blood flow.** Blood flow was highest in 30-min postexercise in all groups, averaging  $6.69 \pm 0.6$ ,  $6.63 \pm 1.2$ , and

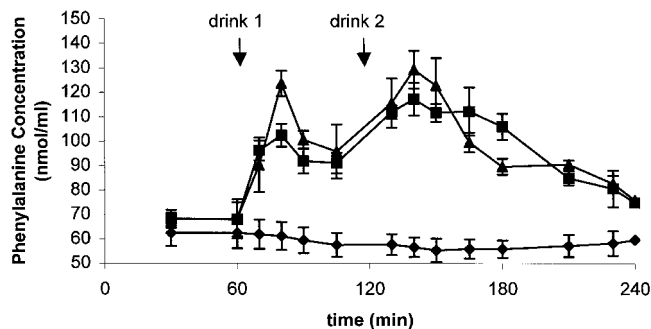


FIGURE 4—Arterial phenylalanine concentration.  $\diamond$  carbohydrate alone (CHO);  $\blacktriangle$  amino acids alone (AA);  $\blacksquare$  carbohydrate + amino acids (MIX).

$5.32 \pm 0.5 \text{ mL}\cdot\text{min}^{-1}\cdot 100 \text{ mL}^{-1}\cdot\text{leg}^{-1}$  in the CHO, AA, and MIX groups, respectively. The values fell to  $5.13 \pm 0.9$ ,  $4.70 \pm 0.6$ , and  $4.64 \pm 0.6$  in the same groups 3-h postexercise. There were no significant differences between groups, and the reductions over time, while consistent, were similarly not significantly different.

The net uptake into the muscle followed the same general temporal pattern as blood concentration changes in all three trials. However, in both the AA and MIX group, the net Phe uptake had returned to the predrink value by 1 h, even though the blood concentration of Phe was still elevated. Similarly, the stimulated net uptake after the second drink of AA or MIX returned to baseline by 120 min after exercise, despite persistent elevations in amino acid concentrations. AUC for the total 3 h was significantly greater for MIX than CHO ( $P < 0.05$ ) but not significantly different between AA and CHO or between AA and MIX. The individual values for CHO, MIX, and AA were  $53.0 \pm 6.0$ ,  $114.4 \pm 38.3$ , and  $71.1 \pm 13.3 \text{ (mg}\cdot\text{leg}^{-1}\cdot 3 \text{ h}^{-1})$  (Fig. 5), respectively. All three groups showed a significant elevation above the basal value (30-min time point). Previous reports of Phe kinetics during unsupplemented recovery from this exercise protocol indicate that values over this time period do not change (12). Estimations of AUC for each hour of the recovery indicate a significantly greater response in the first and second hours to MIX and AA relative to CHO (Fig. 6). The response during the third hour did not differ significantly between groups, although the value was the highest for the CHO group and lowest for the AA group.

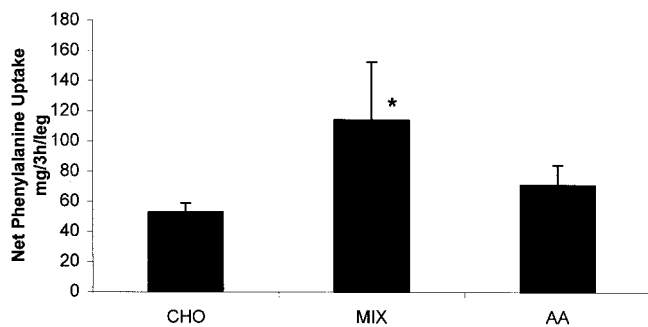


FIGURE 5—Area under the curve for net uptake of phenylalanine over 3 h after ingestion of first drink. \*Significantly greater than CHO,  $P < 0.05$ .

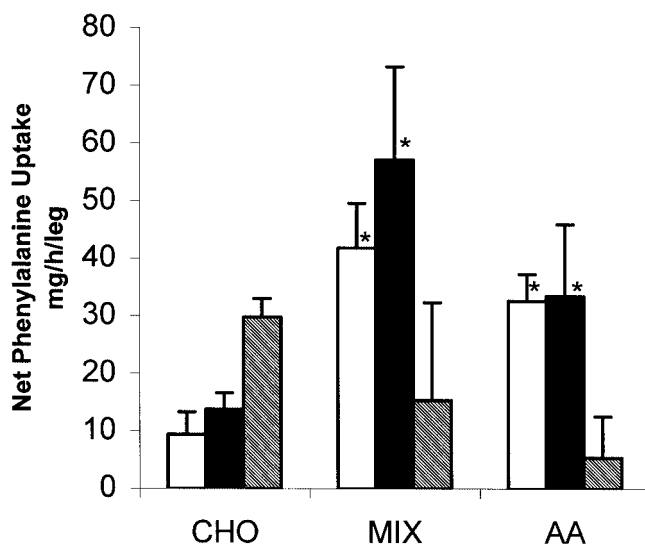


FIGURE 6—Area under the curve for net uptake of phenylalanine, expressed on hourly basis after ingestion of first drink. \*Significantly different from CHO group,  $P < 0.05$ .  $\square$  = 1 h;  $\blacksquare$  = 2 h;  $\square$  (hatched) = 3 h.

Changes in intramuscular Phe concentration between treatments could not explain differences in Rd. Although intramuscular Phe concentration dropped in the CHO group, the value was constant over the last 90 min of the experiment, so changes in the pool size would not explain the response of Rd over that time. There was no significant change in intramuscular Phe concentrations in the two groups receiving exogenous amino acids.

When Ra values over each of the first 3 h after ingestion of the first drink are expressed as an average, there was a progressive fall in the CHO group, such that the value over the third hour was significantly reduced below the value over the first hour (Fig. 7). There were no other significant changes in Ra. Rd did not change significantly in the CHO

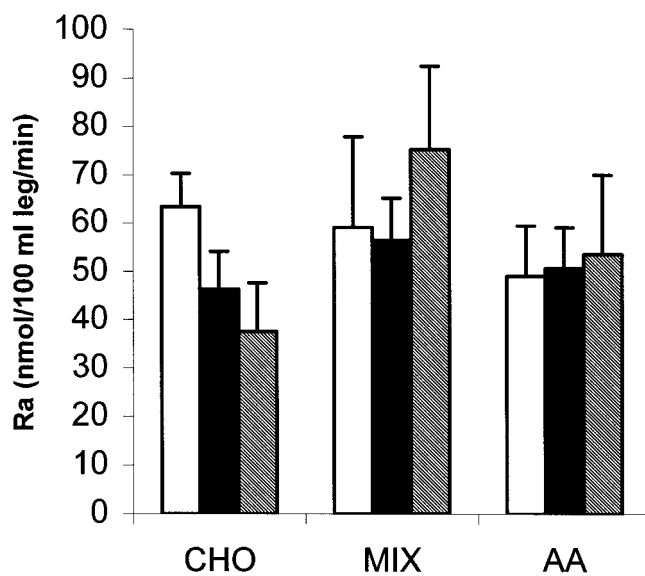


FIGURE 7—Rate of appearance (Ra) of phenylalanine from muscle into blood. The value over the third hour after CHO was significantly reduced below the value over the first hour after the drink,  $P < 0.05$ . There were no other significant differences.  $\square$  = 1 h;  $\blacksquare$  = 2 h;  $\square$  (hatched) = 3 h.

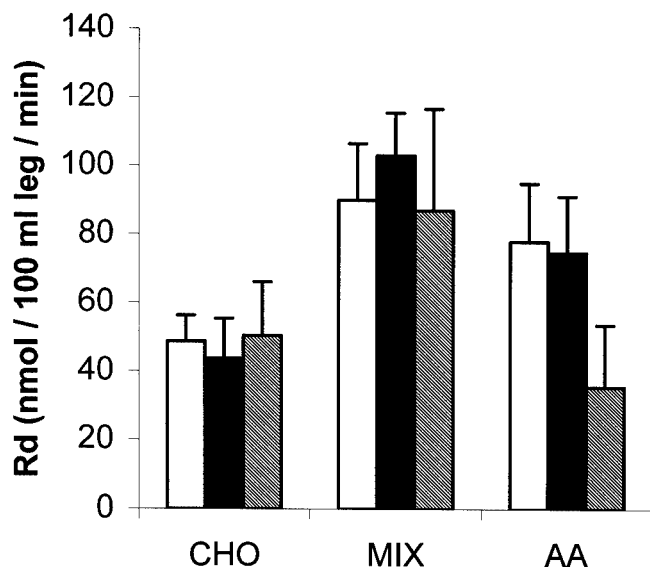


FIGURE 8—Rate of disappearance (Rd), or irreversible loss, of phenylalanine from blood into muscle. All values for MIX were greater than the corresponding values for CHO. The values over the first 2 h for AA were significantly greater than the corresponding values for CHO ( $P < 0.05$ ). □ = 1 h; ■ = 2 h; ▨ = 3 h.

group over time. Similarly, the increase in Rd in MIX was significantly greater than the corresponding values for CHO over each of the 3 h. Rd was elevated for the first 2 h in the AA group, but in the third hour Rd returned to the basal level (Fig. 8).

**Urea enrichment and production.** Urea enrichment gradually increased over time in CHO but was constant in MIX and AA. Calculated rates of urea production thus indicated a significant fall during the third hour after CHO but no changes in the other trials. Urea production coincided with Ra of Phe, i.e., both were significantly reduced during the third hour after CHO but did not change over time in the other trials (Fig. 9).

## DISCUSSION

Results from this study expand upon previous findings from this laboratory, which showed an increase in net muscle protein synthesis when a large dose of amino acids was either infused (4) or ingested (12,16) after exercise. A mixture of 6 g EAA plus 35 g glucose also increased net muscle protein synthesis when given either immediately before or at one of three times after exercise, although the anabolic effect was greatest when given before exercise (17). These previous studies did not address the relative importance of components of the EAA/glucose mixture. The results from the current study show that when ~35 g CHO was combined with ~6 g of a balanced amino acid mixture (i.e., EAA and nonessential AA (NEAA)) the effect on net muscle protein synthesis was roughly equivalent to the sum of the independent effect of either given alone (Fig. 5). During the 2-h period after ingestion of CHO plus AA (MIX) and amino acids alone (AA), net muscle protein synthesis was significantly greater than CHO alone, but there was no difference between MIX and AA (Fig. 6).

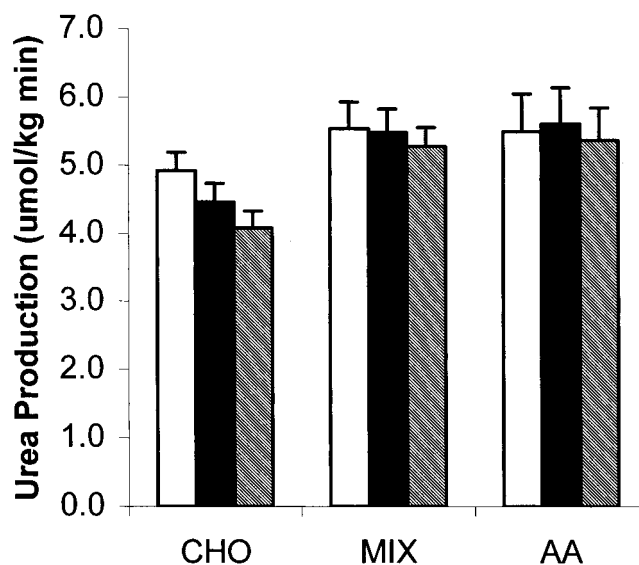


FIGURE 9—Rate of production of urea. Value over third hour for CHO was significantly ( $P < 0.05$ ) lower than the value over the first hour. □ = 1 h; ■ = 2 h; ▨ = 3 h.

Previous studies have provided some evidence that carbohydrate ingestion alone improves protein metabolism. Rennie et al. showed improved leucine balance during carbohydrate supplemented endurance exercise (13), whereas Roy et al. reported diminished myofibrillar proteolysis when carbohydrate was ingested following resistance exercise (14). Our results are consistent with this latter observation, as both urea production and Phe Ra were significantly lower in the CHO group relative to the predrink value at 30 min, and net balance was greater than zero throughout the 3 h after ingestion (Fig. 6). Implicit in this interpretation of our results is the assumption that in the absence of any intervention the net balance of Phe would have remained constant throughout the time during which the response to the drink was measured. The basis for this assumption is the result from our previous study in which a group of subjects received no intervention until 4 h after exercise (12). In that study there were no changes in net balance over the first 3 h after exercise (12).

Although the net effect of CHO alone on net muscle protein synthesis indicated moderate anabolism (i.e., the rate of synthesis exceeding the rate of breakdown), there was no effect of CHO on Rd, indicating no change in the rate of muscle protein synthesis. This is consistent with our previous observation that local hyperinsulinemia failed to stimulate muscle protein synthesis after exercise (5). When studies assessing the response of muscle protein synthesis to insulin are taken together, it is clear that insulin only stimulates muscle protein synthesis in the setting of adequate amino acid availability (20). Thus, it is possible that after exercise insulin increases the potential for accelerated muscle protein synthesis, but this can only be reflected in an increased production of protein if amino acid availability is increased contemporaneously. Thus, when an increase in insulin was coupled with an increase in amino acid concentration (MIX), there was an interactive effect in which the

normal response of muscle protein synthesis to amino acids was amplified by the elevation of insulin, even though an isolated increase in insulin was ineffective.

Our experimental design did not control for caloric intake. Thus, the MIX group, which had the most positive net balance response, received more total calories (164) than the AA (24) or the CHO (140) group. However, it is unlikely that the observed responses can be explained by the caloric imbalances. Thus, when amino acids (24 cal) were added to carbohydrate (140 cal) a significant improvement in net balance resulted (MIX vs CHO). In contrast, when carbohydrate (140 cal) was added to amino acids (24 cal), no significant improvement in net balance resulted over the response to amino acids alone (MIX vs AA).

In contrast to the situation with CHO ingestion, there is little doubt that amino acids alone can stimulate net muscle protein synthesis. This response is elicited by either the infusion or ingestion of amino acids, whether at rest or after exercise (4,15,17). In the current study, the anabolic action of amino acids in both the AA and MIX groups was principally due to a stimulation of muscle protein synthesis, as reflected by Rd (Fig. 8). We previously showed that 40 g of a balanced mixture of amino acids stimulated muscle protein synthesis after exercise (18), but in the current study we gave a total of only two doses of 6 g each (3 g of EAAs and 3 g of NEAAs per dose). When combined with CHO ingestion, this resulted in a total net Phe uptake of  $43 \pm 8$   $\text{mg}\cdot\text{h}^{-1}\cdot\text{leg}^{-1}$  over the first hour after ingestion of the first drink. This uptake is approximately half the value for the response of net Phe uptake we previously obtained ( $107$   $\text{mg}\cdot\text{h}^{-1}\cdot\text{leg}^{-1}$ ) when we gave 6 g of EAAs + 35 g of glucose as a single dose 1 h after exercise (12). These results are consistent with our notion that only the EAAs are effective in stimulating muscle protein synthesis (18).

We can estimate that approximately 50% of ingested Phe was incorporated into whole body muscle if we assume that the Phe incorporated into one leg represented 25% of the total. If another 20% of ingested Phe was incorporated into protein in other tissues, then approximately 30% was oxidized. Comparable percentages for the other EAA in the mixture would be anticipated, and a greater percentage of NEAA intake would be expected to be oxidized. The nitrogen from the oxidized amino acids would be expected to eventually contribute to urea production, yet urea production did not increase in either group receiving amino acids. However, the lack of change in urea production in the groups receiving amino acids has to be interpreted in the context of the reduction in urea production in the group receiving only glucose (Fig. 9) (8). Also, our ability to detect the magnitude of expected change in urea production on the basis of amino acid ingested was marginal, considering the relatively high efficiency of incorporation of ingested amino acids into protein.

The response to ingestion of the second dose of each drink at 2 h after exercise was essentially the same as the response to the first dose (Fig. 6). Whereas the second drink sustained increased concentrations of glucose, Phe, or insulin (depending on the drink) throughout the 3 h following

exercise, essentially all measured variables related to muscle protein metabolism returned to baseline by the end of the second hour. The rapid, transient response of amino acid concentrations to a free amino acid bolus drink observed in this study were in contrast to a previous study in which we tested the response to a primed-constant infusion of amino acids that elicited a 1.7-fold increase in amino acid concentrations that was essentially constant for the duration of the 6-h experiment (6). Muscle protein synthesis, however was only increased for the first 2 h of the elevation in amino acid concentrations (6). This diminished response over time implies that during a steady-state increase in amino acid concentrations muscle protein synthesis becomes refractory to the stimulatory effect of the amino acids. One might therefore have anticipated that there would have been a diminished response to the second drink after 1 h because the Phe concentration was still double the basal value, yet net muscle protein synthesis had dropped back to the basal value at that time. However, ingestion of the second drink 1 h after ingestion of the first drink stimulated a similar net uptake of amino acids as seen in response to the first drink (Fig. 6). The practical implication of this finding is that a supplement or beverage containing amino acids taken after exercise would appear not to diminish the stimulation of muscle protein synthesis that would result, for example, from a meal consumed at some point following exercise. From a mechanistic perspective, it may be that muscle protein synthesis responds to a change in blood or intracellular amino acid concentrations, rather than to their absolute values.

Another aspect of timing of the response suggested by our data is that the maximal insulin effect may not coincide with its peak concentration. Both Ra (reflecting muscle protein breakdown) and urea production were only significantly reduced in the third hour after the initial dose of CHO alone (Figs. 7 and 9). This reduction explains the positive net balance in this group during the third hour (Fig. 6). Furthermore, the MIX group had an almost identical response of net Phe uptake to the AA group over the first hour, and it was only in the second and particularly the third hour that the response was more positive than the AA group alone (Fig. 6). If, in fact, there is a delay in insulin action, then the peak amino acid effect may not have coincided in time with the peak insulin effect. If this is the case, amino acids ingested in a more slowly absorbed form (i.e., intact protein) may elicit a greater interactive effect between the amino acids and carbohydrate, so that the peak in amino acid concentration would coincide with the peak action of insulin.

In conclusion, our findings confirm the previously described anabolic effect on muscle protein of amino acids and glucose given after resistance exercise. Further, the results indicate that the combined effects of carbohydrate and amino acid ingestion approximately reflects the sum of their individual effects. When the results of the current study are combined with our previous results (12), it appears that ingestion of only the EAAs are required to stimulate muscle

protein synthesis after exercise. Finally, the response to the second drink shows that prior ingestion of a modest amount of amino acids or carbohydrate does not diminish the metabolic response to a second comparable dose ingested 1 h later.

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