Effect of an Amino Acid, Protein, and Carbohydrate Mixture on Net Muscle Protein Balance After Resistance Exercise

Elisabet Børsheim, Asle Aarsland, and Robert R. Wolfe

This study tests the hypotheses that (a) a mixture of whey protein, amino acids (AA), and carbohydrates (CHO) stimulates net muscle protein synthesis to a greater extent than isoenergetic CHO alone after resistance exercise; and (b) that the stimulatory effect of a protein, AA, and CHO mixture will last beyond the 1st hour after intake. Eight subjects participated in 2 trials. In one (PAAC), they ingested 77.4 g CHO, 17.5 g whey protein, and 4.9 g AA 1 h after resistance exercise. In the other (CON), 100 g CHO was ingested instead. They received a primed constant infusion of L-[\textsuperscript{2}H\textsubscript{5}] phenylalanine, and samples from femoral artery and vein, and biopsies from vastus lateralis were obtained. The area under the curve for net uptake of phenylalanine into muscle above pre-drink value was $128 \pm 42 \, \text{mg} \cdot \text{leg}^{-1}$ (PAAC) versus $32 \pm 10 \, \text{mg} \cdot \text{leg}^{-1}$ (CON) for the 3 h after the drink ($p = .04$). The net protein balance response to the mixture consisted of two components, one rapid immediate response, and a smaller delayed response about 90 min after drink, whereas in CON only a small delayed response was seen. We conclude that after resistance exercise, a mixture of whey protein, AA, and CHO stimulated muscle protein synthesis to a greater extent than isoenergetic CHO alone. Further, compared to previously reported findings, the addition of protein to an AA + CHO mixture seems to extend the anabolic effect.

Key Words: resistance exercise, protein and carbohydrate supplement, stable isotopes

Introduction

Net muscle protein balance between protein synthesis and breakdown is generally improved in the recovery period after resistance exercise (2, 7). However, in the absence of nutrient intake, net muscle protein balance remains negative—that is, the muscle is in a catabolic state. The optimal composition of a nutrient designed to promote muscle anabolism after exercise is not known. It can be predicted that AA or protein should be an essential component, because amino acids (AA) are known...
to stimulate muscle protein synthesis (17). Thus, we have previously shown that both infusion (3) or ingestion of either free AA (20) or combinations of AA and carbohydrates (CHO; 16, 21) after exercise stimulates muscle protein synthesis. Further, as little as 6 g of essential AA (EAA) alone stimulated net muscle protein synthesis after resistance exercise (5).

It is likely that energy intake affects muscle protein metabolism. Calloway and Spector (6) showed that regardless of the amount of nitrogen intake, nitrogen balance improved as energy intake increased. These findings are also reported in exercising subjects who were given varying energy and protein intakes (22). The results indicate that energy balance may be equally or more important than nitrogen intake as a determinant of nitrogen balance. However, the mechanism by which energy affects nitrogen retention, and perhaps muscle protein metabolism, is not clear. There is little evidence to support a direct effect of either glucose or FFA availability on muscle protein metabolism, because there is adequate substrate availability in the basal state to produce the ATP needed for protein synthesis (24). It is more likely that an effect of CHO is mediated by the resulting insulin response. Not only does insulin have an important anabolic effect on net muscle protein balance, an interactive effect between insulin and increase in AA availability would be anticipated. Whereas insulin stimulates the capacity of muscle protein synthesis (9), a sufficient availability of AA is required for that to be reflected in an increase in the actual rate of synthesis. Thus, we propose that a mixture of AA, protein, and CHO would have a significantly greater anabolic effect on muscle than an isoenergetic amount of CHO alone.

A large rapid response in net muscle protein balance was observed after intake of a bolus of AA or AA + CHO (5, 13, 16), but net balance returned to basal level within 60 min after intake of drink. It may be that a combination of protein and AA may result in a more prolonged response because of the combination of the rapid initial response to the free AA and the delayed absorption of AA from protein. Prolonging the appearance of AA may be particularly beneficial when given with CHO, because it appears there may be a time lag of as much as 2 h before the insulin effect on muscle protein peaks after CHO ingestion (13). Thus, if only free AA + CHO are given, the response may be limited by the fact that the insulin effect may occur at a time when there is no longer an increased availability of AA. We therefore have quantified the time course of response to a protein + AA + CHO mixture. We proposed that there would be at least two phases to the response: an initial response to the free AA and a later response to insulin and AA from the protein. The second aim of the study was therefore to investigate if addition of whey protein to free AA and CHO extends the response of net muscle protein balance beyond the 1st hour after intake.

**Materials and Methods**

**Subjects**

Eight healthy subjects (5 men and 3 women) participated in the study (Table 1). Subjects were recreationally active. They were fully informed about the purpose and procedures of the study before written consent was obtained. Prior to participation in the experiment, each subject had a complete medical screening, including vital signs, blood tests, urine tests, and a 12-lead electrocardiogram for determina-
tion 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.

**Pre-experimental Procedures**

At least 1 wk before an experiment, the subject was familiarized with the exercise protocol, and his/her one repetition maximum (1RM; the maximum weight that can be lifted for one repetition) was determined by the procedure described by Mayhew et al. (12; Table 1). 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**

Each subject participated in two double-blinded trials in randomized order. The subjects were instructed not to exercise for 48 h before an experiment, not to use tobacco or alcohol during the 24 h preceding an experiment, and not to make any changes in their dietary habits. Each subject kept a food diary for the last 24 h before each experiment. They followed the same regimen also before the second study. 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 Figure 1. At ~6:00 AM, an 18-G polyethylene catheter (Cook, Inc., Bloomington, IN, USA) was inserted into an antecubital arm vein for the primed continuous infusion of stable isotopes of AA. After obtaining a blood sample for measurement of background AA enrichment, a primed, constant infusion of L-[ring-H$_5$]-phenylalanine was started at –120

**Table 1 Physical Characteristics and Exercise Data for All Subjects**

<table>
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<tr>
<th>Subject #</th>
<th>Gender</th>
<th>Age (yr)</th>
<th>Height (m)</th>
<th>Weight (kg)</th>
<th>Mean volume of legs (L)</th>
<th>1RM (kg)</th>
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</tr>
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min (~06:30 AM). The priming dose was 2 µmol · kg⁻¹, and the infusion rate was 0.10 µmol · kg⁻¹ · min⁻¹. Isotopes were purchased from Cambridge Isotopes (Andover, MA, USA). They were dissolved in 0.9% saline and filtered through a 2 µm filter before infusion.

At ~7:00 AM, a 3 Fr, 8-cm polyethylene catheter (Cook, Inc., Bloomington, IN, USA) 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 (ICG) infusion for determination of leg blood flow (1). A constant infusion of ICG (0.5 mg · min⁻¹) was given at intervals during the experiment (Figure 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-G polyethylene catheter inserted into an antecubital vein of the opposite arm into which the AA 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 1RM. Each set was completed in approximately 30 s with a 2-min rest between sets, and the entire bout was completed in about 20 min. Subjects then returned to bed and rested for 4 h.

In one of the trials (PAAC), the subjects were given a drink consisting of 77.4 g CHO, 17.5 g whey protein, and 4.9 g AA (Pro Performance Distance®, General Nutrition Corporation) 1 h post exercise. In the other trial (CON), a drink of 100 g CHO was ingested instead. The compositions of the drinks are given in Table 2. Each supplement solution was composed of 590 ml of double-distilled water and the appropriate mixture. L-[ring-²H₅]-phenylalanine was added to the PAAC drink in amounts to equal 10% enrichment to allow maintenance of isotopic equilibrium during ingestion of drink. The drinks were administered in a double-blind and randomized order.

To measure the glycogen concentration and the isotopic enrichment of free and bound amino acid tracers in the muscle, muscle biopsies were sampled at 5 min before the start of exercise, and at 55, 120, and 240 min after exercise (Figure 1). The biopsies were taken under local anesthesia, from the lateral portion of the vastus lateralis approximately 10–15 cm above the knee. A 5-mm Bergstrom biopsy needle (Depuy, Warsaw, IN, USA) was used to sample approximately 30 to 50 mg of mixed muscle tissue. The sample was quickly rinsed, blotted, and immediately frozen in
liquid nitrogen and stored at –80 °C for later analysis. Two incisions approximately 4 cm apart were made during each experiment, and two biopsies were sampled from each incision, with the needle angled differently for each sample.

Blood samples were drawn for determination of net muscle protein and CHO balance 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 (Figure 1). The samples were analyzed for phenylalanine enrichments and concentrations, and most of them also for glucose and insulin concentrations. Less frequent samples were drawn from the antecubital vein and femoral vein for determination of blood flow in five different periods (Figure 1).

Sample Analyses

Blood samples for determination of amino acid enrichment and concentrations were immediately precipitated in pre-weighed tubes containing 15% sulfosalisyl acid (SSA), and a weighed amount of an appropriate internal standard, consisting of phenylalanine labeled differently than the infused phenylalanine, was added (1, 2, 15). The supernatant was passed over a cation exchange column (Dovex AG 50W-8X, 100-200 mesh H⁺ form; Bio-Rad Laboratories, Richmond, CA, USA) and dried under vacuum with a Speed Vac (Savant Instruments, Farmingdale, NY, USA). Enrichments of intracellular free phenylalanine were then determined on the ter-

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Repletion drink (g)</th>
<th>Control drink (g)</th>
</tr>
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<tbody>
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<tr>
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<tr>
<td>Fructose</td>
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<tr>
<td>Maltodextrin</td>
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<tr>
<td>Whey protein concentrate</td>
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<td></td>
</tr>
<tr>
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<tr>
<td>L-isoleucine</td>
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<td>L-lysine</td>
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</tr>
<tr>
<td>L-valine</td>
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<tr>
<td>L-methionine</td>
<td>0.84</td>
<td></td>
</tr>
<tr>
<td>L-phenylalanine</td>
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<td></td>
</tr>
<tr>
<td>L-arginine</td>
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<td></td>
</tr>
<tr>
<td>L-aspartate</td>
<td>0.21</td>
<td></td>
</tr>
<tr>
<td>L-glutamine</td>
<td>0.53</td>
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</tr>
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</table>
tiary-butyl dimethylsilyl (t-BDMS) derivatives, using gas chromatography mass spectrometry (GCMS; Hewlett-Packard 5973, Palo Alto, CA, USA) and selected ion monitoring (25). Enrichments were expressed as a tracer-to-tracee ratio. Appropriate corrections were made for overlapping spectra (25).

To determine muscle intracellular enrichment of infused tracers, muscle tissue was weighed and the protein 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.

Muscle tissue was also analyzed for glycogen concentration. Muscle tissue was weighed and then boiled in 2N HCl for 2 h. After cooling, the solution was titrated to neutral pH with 2N NaOH. Thereafter, it was kept in room temperature until concentration of glycosyl units was determined enzymatically by an automated system (YSI 1500, Yellowspring Instruments, Yellowspring, OH, USA). Glycogen concentrations were found by correcting for added fluid.

ICG concentration in serum was measured spectrophotometrically at $\lambda = 805$ nm for the determination of leg blood flow (10, 23). Plasma glucose concentration was determined enzymatically (YSI 1500, Yellowspring Instruments, Yellowspring, OH, USA). Plasma insulin concentration was determined by a radioimmunoassay method (Diagnostic Products Corporation, Los Angeles, CA, USA).

**Calculations**

Net muscle phenylalanine balance, which was considered as the primary endpoint, was calculated as follows: (phenylalanine arterial concentration – venous concentration) × blood flow. Since phenylalanine is neither produced nor metabolized in muscle, net phenylalanine balance reflects net muscle protein synthesis, provided there are no significant changes in the free intracellular pool of phenylalanine. Area under the curve (AUC) of net phenylalanine uptake was determined for each individual hour following drink ingestion, with net average uptake at $t = 45–55$ min post exercise used as the zero point for each hour of the recovery period. This approach assumes a constant net balance after the basal sample if nutrient is not given. The basis for this assumption is the relatively constant net balance for 3 h after exercise in the absence of nutrient intake we have previously observed (16).

The rate of incorporation of phenylalanine from blood into muscle protein (Rd; rate of disappearance of phenylalanine from blood) was calculated as $\text{Rd} = \frac{(\text{Ea} \cdot \text{Ca}) – (\text{Ev} \cdot \text{Cv})}{\text{BF} \cdot \text{Ea}^{-1}}$, 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; and BF is blood flow (25). The rate of release of phenylalanine from protein breakdown into blood (Ra; rate of appearance of phenylalanine in blood) was calculated as $\text{Ra} = \text{Rd} – [(\text{Ca} – \text{Cv}) \cdot \text{BF}]$ (25). Calculation of Ra and Rd requires an isotopic, but not physiological, steady state. By adding an appropriate amount of tracer to the ingested AA, we were able to maintain a relatively stable isotopic steady state, despite changing concentrations of plasma AA (see below).

AUC of net glucose uptake was also determined for each individual hour following drink ingestion, with net uptake at $t = 55$ min post exercise, used as the zero point for each hour of the recovery period.
**Statistical Methods**

Overall significance of differences between the two trials was analyzed using a two-factor (treatment and time) repeated measures analysis of variance, followed by Bonferroni’s test (SigmaStat 2.03, SPSS Inc., Chicago, IL, USA). Results were considered significant if $p < .05$. The results are presented as means ± SE unless otherwise noted.

**Results**

**Phenylalanine Concentration and Balance**

At rest before exercise the arterial blood phenylalanine concentration was approximately 70 nmol · ml$^{-1}$ in both trials (Figure 2; NS between trials) and was a similar value at 45–55 min after exercise. In PAAC, arterial phenylalanine concentration increased rapidly after intake of drink and remained on an elevated plateau of about 115 nmol · ml$^{-1}$ until 90 min after intake of drink. Thereafter, the concentration declined, but it increased above the pre-drink level until 210 min after the end of exercise ($p < .001$). The concentration in PAAC was significantly higher than in CON until 230 min after exercise ($p = .002$). Muscle intracellular phenylalanine concentration was 69 ± 6 nmol · ml$^{-1}$ before exercise in PAAC versus 74 ± 5 nmol · ml$^{-1}$ in CON (NS vs. PAAC). There was no main effect of Time or Treatment on the intracellular phenylalanine concentration, but an interaction effect of Treatment × Time was found, with a significant difference between the two trials for the last biopsy (PAAC: 79 ± 8 nmol · ml$^{-1}$ vs. CON: 51 ± 4 nmol · ml$^{-1}$, $p = .003$).

There was a significant difference between the effects of the drinks on net muscle phenylalanine balance ($p = .042$; Figure 3). Net balance increased rapidly
from negative to positive values after intake of drink in PAAC, whereas no changes
could be seen in response to drink in CON. After about 20 min, net balance in PAAC
fell rapidly back towards the basal level and, at 45 min after drink, net balance was
no longer significantly increased versus pre-drink level, despite a persistent elevation
in blood phenylalanine concentration. Thereafter, a new small but significant
increase in net balance was seen around 90 min after drink ($p < .05$ vs. pre-drink).
Also in CON, there was a small increase at the same time; thus, the difference
between the two trials lasted only for 45 min after intake of drink.

Total area under the curve (AUC) for phenylalanine uptake into muscle above
pre-drink value was $128 \pm 42$ mg · leg$^{-1}$ (PAAC) versus $32 \pm 10$ mg · leg$^{-1}$ (CON) for
the 3 h after the drink ($p = .04$). The difference between groups was mainly over the
1st hour after intake of drink (Figure 4).

**Phenylalanine Kinetics**

Enrichment of phenylalanine in blood was relatively constant throughout the experi-
ment (tracer/tracee ratio of approximately 0.11), despite changes in concentra-
tion. No statistical changes in enrichment were observed during the different calcu-
lation periods. This was accomplished by adding tracer to the drink that contained
AA.

There was no difference between drinks on the rate of appearance of phenylal-
anine in blood, which is a reflection of muscle protein breakdown (Figure 5), but an
overall Time effect was found, with the periods after drinks being lower than the pre-
drink level ($p < .001$).
The rate of muscle protein synthesis from plasma phenylalanine was higher in PAAC versus CON after drink (Treatment × Period: \( p = .001 \); Figure 6). This was mostly due to the difference over the 1st hour after drink; 75 ± 13 nmol phenylalanine \( \cdot \) min\(^{-1} \cdot \) 100 ml leg\(^{-1} \) (PAAC) versus 28 ± 5 nmol phenylalanine \( \cdot \) min\(^{-1} \cdot \) 100 ml leg\(^{-1} \) (CON), respectively \( p < .001 \).
**Plasma Glucose Concentration and Uptake**

Arterial glucose concentration during rest before exercise was $89 \pm 2.5 \text{ mg} \cdot \text{dl}^{-1}$ in PAAC and $85 \pm 0.7 \text{ mg} \cdot \text{dl}^{-1}$ in CON (NS vs. PAAC; Figure 7). In the sample 55 min

![Figure 6 — Average rate of disappearance of phenylalanine out of the blood into the muscle (estimate of protein synthesis) before exercise, after exercise (but before drink), and during the 1st, 2nd, and 3rd hours after intake of drink (mean $\pm \text{SE}, n = 8$). *$p < .05$, PAAC vs. CON; #$p < .05$, value vs. pre-drink value.](image)

![Figure 7 — Time course of arterial glucose concentration (mean $\pm \text{SE}, n = 8$). Drink was consumed at 60 min after exercise. *$p < .05$, PAAC vs. CON; #$p < .05$, value vs. pre-drink value.](image)
after exercise (immediately before drink), the arterial glucose concentration was not different from the pre-exercise concentration. After drink, the glucose concentration increased in both trials but to a slightly higher level in CON. In both studies, the concentration peaked 30 min after intake of drink, before it decreased again. In PAAC, the concentration was increased versus the pre-drink level for 90 min after drink, whereas in CON, glucose concentration was increased until 150 min after drink. There was a significant overall difference between the two trials after drink ($p = .015$).

No significant Treatment effect could be observed for the time course of glucose AV-difference ($p = .054$) or glucose uptake ($p = .061$) but, expressed as area under the curve (AUC) for total glucose uptake over each hour after intake of drink, a significant overall difference was found between the two trials ($p = .01$). Total AUC for net uptake of glucose was $7.7 \pm 1.0$ g · leg$^{-1}$ (PAAC) vs. $13.3 \pm 1.6$ g · leg$^{-1}$ (CON) for the 3 h after drink ($p = .01$). Total muscle uptake of CHO in the two legs corresponded to $19.9 \pm 2.5\%$ (PAAC) and $26.6 \pm 3.1\%$ (CON) of the amount of CHO in each drink, respectively (NS between trials).

**Muscle Glycogen Concentration**

Muscle glycogen was $116 \pm 12$ µmol · g wet mass$^{-1}$ in PAAC versus $126 \pm 22$ µmol · g wet mass$^{-1}$ in CON (NS vs. PAAC) during rest before exercise. The values were slightly lower after exercise (overall $p = .10$) and did not change in the post exercise period. No difference in effect of the drink on muscle glycogen concentration was found.

**Plasma Insulin Concentrations**

Arterial insulin concentration was not different between the studies before exercise and, after exercise, before drink (Figure 8). In response to the drink, insulin increased...
several-fold in both studies. There was no main effect of Treatment on insulin concentration. One subject had an exaggerated response in both studies compared to the other subjects.

**Discussion**

The principal finding of this study was that ingestion of a mixture of AA, whey protein, and CHO stimulated net muscle protein synthesis to a greater extent than isoenergetic CHO alone. Even though addition of protein resulted in a sustained increase in plasma AA concentration, the initial increase in phenylalanine net balance peaked 20 min after intake of drink and thereafter fell rapidly. A new small but significant increase in phenylalanine net balance was seen around 90 min after drink. This was likely due to an insulin effect and the sustained elevation in AA. Thus, the addition of protein to an AA + CHO mixture prolongs the anabolic response as compared to previously reported response to AA + CHO mixtures (13, 16).

**Energy Effect on Net Protein Balance**

The effect of provision of energy on net muscle protein balance is not clear. Because AA stimulate muscle protein synthesis (17), it is evident that AA or protein should be an essential component of a nutrient designed to promote muscle anabolism after exercise. Thus, both mixed AA (13, 20) or EAA alone (5, 20) have been shown to stimulate net muscle protein synthesis after resistance exercise. No studies of post-exercise supplementation with intact protein alone have been performed.

There is little evidence that fat is an important source of energy to promote efficient utilization of AA, but an effect of CHO is more likely because of its insulin effect. Insulin stimulates protein synthesis, and thus net balance, when adequate AA are available (26). When AA and CHO were given together after resistance exercise, an increase in net protein balance was observed (13, 16, 21). Miller et al. (13) tested the independent effect of each and found that the response to a drink consisting of 6 g mixed AA + 35 g CHO was roughly equivalent to the sum of the independent effect of either given alone. Because the drinks were not isoenergetic, it is possible that differences in energy provision partially explained the results. This was unlikely, however, because the response to AA alone was larger than the response to CHO alone, even though the CHO drink was of greater caloric value than the AA drink. The results of the present study support the notion that addition of AA and/or protein to CHO has an effect on muscle AA metabolism beyond the caloric value. Total AUC for phenylalanine uptake into muscle above pre-drink value was approximately four times higher in PAAC versus CON (Figure 4). When water content of muscle (about 73%) is taken into account, this would represent a net gain of 24 versus 6 g of muscle synthesized in total in the two legs over the 3 h after drink. Thus, about 18 g more muscle was synthesized in response to the protein + AA + CHO drink versus isoenergetic CHO alone.

The effect of the mixture of whey protein, AA, and CHO on net balance can largely be explained by the effect on protein synthesis. In accordance with this, Levenhagen et al. (11) found that leg protein synthesis (average over 180 min) was four times higher after intake of a protein + CHO + lipid supplement immediately after 60 min cycling at 60% of VO$_{2\text{max}}$ as compared to when protein was excluded in the drink (i.e., not isoenergetic), whereas leg protein breakdown was not different.
between treatments. Consistent with this observation, free AA or AA + CHO intake primarily stimulate protein synthesis, whereas protein breakdown is less/not affected (5, 13, 16, 21).

A recent outcome study indicated an anabolic effect of ingestion of a protein + CHO + fat supplement in conjunction with resistance training in elderly men (8). However, the supplement was found to be effective in increasing muscle cross-sectional area only when ingested immediately after exercise as opposed to 2 h after. Our own studies have found the precise timing of ingestion of AA + CHO after exercise to be less important in terms of improving net muscle balance (16). One would predict from the results of the current study that the subjects receiving the protein + AA + CHO would gain more muscle mass over a training period than the CHO alone. However, it would require many weeks of comparable response for a difference in muscle mass to become detectable, during which time all variables such as activity and other nutritional intake would have to be strictly controlled. If our young subjects were to undergo a training program using exercises similar to the one used in the current experiment, a muscle gain of 864 g wet mass can be predicted over 12 weeks, as compared to 216 g wet mass with the CHO drink, on the basis of our results.

The net balance response to CHO alone was much smaller and delayed as compared to the effect of protein + AA + CHO (Figure 3). The insulin effect on muscle is complicated by the fact that it stimulates AA uptake from plasma throughout the body, thereby lowering the plasma concentrations. Thus, a decrease in AA availability may counteract any direct action of insulin to stimulate muscle protein synthesis in vivo (27).

The slight increase in phenylalanine net balance approximately 90 min after intake of drink in PAAC was likely a response to insulin because an increase was seen also in CON (Figure 3). Thus, it is clear that the main effect on net balance in PAAC comes from the protein/AA in the drink and that the principal role of CHO in a post-exercise drink for protein recovery is to improve palatability and stimulate a small increase in protein net balance probably as a response to insulin, but the latter will only be effective if it occurs rapidly enough to coincide with increased availability of AA.

**Duration of Response**

The increase in net amino acid balance after a bolus of free AA (5) or AA + CHO (13, 16) is transient. Typically it peaks after 20–30 min before rapidly decreasing again, and no increase is observed beyond the 1st hour after drink. The results of the present study show that addition of protein to free AA resulted in a sustained elevation in plasma AA (phenylalanine) concentration, with no decrease from the peak until after 90 min post-drink (Figure 2), probably because protein takes longer to digest. Nonetheless, the initial increase in phenylalanine net balance peaked only 20 min after the drink and thereafter fell rapidly again. After the first rapid decrease in phenylalanine net balance, it changed direction again and increased to a new peak 90 min after drink, which was significantly elevated versus the pre-drink level and which is longer after ingestion than what was observed when EAA was given alone (5). The net balance response to the protein + AA + CHO supplement thus consisted of at least two phases: an initial stimulatory response, which probably was due to the free AA in the mixture, and a later response, which probably was caused by insulin,
since a parallel peak was seen in the CHO trial at the same time. Thus, the maximal effect of insulin seems not to coincide with its peak concentration. It may thus be hypothesized that CHO intake should precede free AA intake to achieve maximal anabolic effect of insulin. In the present study, we tried to achieve a greater interaction between AA and insulin by adding protein to the mixture so that AA would be provided over a longer time period.

The return of protein synthesis to resting values despite prolonged elevations of plasma AA is in agreement with Bohe et al. (4) who found that when blood AA concentrations were elevated to a steady-state level, about twice the basal values for 6 h, muscle protein synthesis was stimulated over the first 2 h but thereafter returned to the resting level, despite persistent elevations in blood AA concentrations. These observations were probably not a result of the muscle becoming refractory to a persistent elevation in AA concentrations, since we have shown that the response of net muscle protein synthesis to a dose of EAA was not affected by elevated AA concentrations resulting from a previous dose 1 h earlier (5). Rather, the response in net protein balance to the second dose was comparable to the first dose. Thus, it may be speculated that muscle protein synthesis is being regulated by changes in arterial, and presumably interstitial, AA concentrations, rather than by the absolute AA concentration.

**Effect of Essential Amino Acids (EAA)**

We have previously shown a dose-dependent effect of EAA ingestion on muscle protein synthesis (5, 13, 16). In the present study, the protein + AA + CHO mixture contained 3.9 g free EAA, in addition to approximately 8.49 g EAA in the whey protein. Thus, the mixture contained in total ~12.39 g EAA. The total AUC over the 3 h after intake of the mixture (128 ± 42 mg · leg⁻¹) corresponds well to what was found when a bolus of 6 g EAA (0.087 g · kg body mass⁻¹) was given 1 and 2 h after resistance exercise—that is, a total of 12 g EAA (5). In that study, total AUC was 139 ± 42 mg · leg⁻¹ over 3 h after intake of the first bolus. (AUC was back to pre-drink values during the 3rd hour.) This was estimated to represent a net gain of ~26 g of muscle tissue synthesized in response to the drinks versus ~24 g in the present study. So even though the initial net phenylalanine balance response was lower in this study, the response was more prolonged and, over a 3-h period, is comparable to the response to 12 g of EAA. Similarly, when 6 g EAA + 35 g CHO was given 1 h after exercise, 1h-AUC was 64 ± 9 mg · leg⁻¹ (16), which is half of the AUC in the present study. Thus, it appears that it is the EAA that is of principal importance for the stimulation of protein synthesis. In agreement with this notion, it has been shown that ingestion of non-EAA is not necessary for stimulation of protein synthesis (5, 20).

The practical implication of this comparison is that even though a smaller amount of EAA has been shown to stimulate net muscle protein synthesis to the same extent as the mixture tested in the current study (5), it appears that the same effect can be achieved by intake of a cheaper and more palatable form of nutrient. The response to protein (±CHO) alone has not been quantified, but the absence of a rapid initial response may limit the overall effectiveness of a supplement without free AA.
Muscle Glucose Uptake

Glucose uptake was higher in the CHO trial versus PAAC. The total AUC for glucose uptake was $7.7 \pm 1.0$ g · leg$^{-1}$ in PAAC and $13.3 \pm 1.6$ g · leg$^{-1}$ in CON, but when the uptake was related to the percentage of CHO taken in, there was no difference between the trials. Hence, simultaneous intake of AA or protein does not affect CHO uptake.

Resistance exercise has been shown to result in a decrease in muscle glycogen (18), and CHO supplementation in the recovery period after resistance exercise stimulates glycogen resynthesis (14). Whereas some authors have observed greater rates of muscle glycogen resynthesis and higher plasma insulin levels when protein is added to CHO after resistance exercise (28), others have found similar rates of glycogen resynthesis after isoenergetic mixtures of CHO versus CHO + protein + fat (19). In this study, we observed only a slight overall decrease in muscle glycogen concentration as a result of the exercise, and we found no difference in the effect of the drinks on muscle glycogen concentration. Similarly, there was no difference in serum insulin time course between the two trials, even though less CHO was given in the mixed drink compared to the control drink (77 vs. 100 g, respectively), and some of the CHO was given as fructose in that drink. This may be because the proteins in the mixed drink also stimulate insulin.

Methodological Considerations

Drinks normally are ingested as boluses; however, quantifying the response to a bolus ingestion of unlabeled AA introduces potential methodological problems because of rapid dilution of the tracer. We therefore added tracer to the ingested AA so that a relatively constant enrichment in the blood was maintained, even during absorption of the bolus. Nonetheless, we used net protein balance, which is not dependent on the measurement of isotopic enrichment, as our primary endpoint. This is because in terms of gain or loss of muscle protein, the net balance (i.e., synthesis minus breakdown) is the most relevant parameter. Furthermore, it can be determined in non–steady state conditions.

Because previous studies have shown that net muscle protein balance remains slightly negative for several hours after resistance exercise in the absence of nutrient intake (2, 7), a placebo group was not included in this study. Further, in a previous study from our lab, Rasmussen et al. (16) gave a placebo drink at 60 min after a similar resistance exercise bout. They found no significant change in net balance over the first 3 h after exercise. Hence, the significant positive net balance observed after drinks in the present study can be ascribed to the intake of the AA rather than to changes that would have occurred anyway.

Conclusion

We conclude that a combination of whey protein, AA, and CHO resulted in a greater response of muscle protein net balance after resistance exercise than when CHO were given alone. Further, addition of protein to a mixture of free AA + CHO resulted in a response lasting beyond the 1st hour after intake.
References


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