

# Stimulation of net muscle protein synthesis by whey protein ingestion before and after exercise

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**Tipton KD, Elliott TA, Cree MG, Aarsland AA, Sanford AP, Wolfe RR.** Stimulation of net muscle protein synthesis by whey protein ingestion before and after exercise. *Am J Physiol Endocrinol Metab* 292: E71–E76, 2007. First published August 8, 2006; doi:10.1152/ajpendo.00166.2006.—Timing of nutrient ingestion has been demonstrated to influence the anabolic response of muscle following exercise. Previously, we demonstrated that net amino acid uptake was greater when free essential amino acids plus carbohydrates were ingested before resistance exercise rather than following exercise. However, it is unclear if ingestion of whole proteins before exercise would stimulate a superior response compared with following exercise. This study was designed to examine the response of muscle protein balance to ingestion of whey proteins both before and following resistance exercise. Healthy volunteers were randomly assigned to one of two groups. A solution of whey proteins was consumed either immediately before exercise (PRE;  $n = 8$ ) or immediately following exercise (POST;  $n = 9$ ). Each subject performed 10 sets of 8 repetitions of leg extension exercise. Phenylalanine concentrations were measured in femoral arteriovenous samples to determine balance across the leg. Arterial amino acid concentrations were elevated by ~50%, and net amino acid balance switched from negative to positive following ingestion of proteins at either time. Amino acid uptake was not significantly different between PRE and POST when calculated from the beginning of exercise ( $67 \pm 22$  and  $27 \pm 10$  for PRE and POST, respectively) or from the ingestion of each drink ( $60 \pm 17$  and  $63 \pm 15$  for PRE and POST, respectively). Thus the response of net muscle protein balance to timing of intact protein ingestion does not respond as does that of the combination of free amino acids and carbohydrate.

muscle protein synthesis; arteriovenous balance; net muscle protein balance; muscle biopsies

THE STUDY OF NUTRITIONAL INFLUENCES on muscle during recovery from exercise has received increasing attention in recent years. Despite the increased attention, many aspects of the response of muscle protein metabolism to nutritional interventions need to be delineated.

It is clear that provision of amino acids, whether in free form (3, 6, 8, 10) or as intact proteins (9), in association with resistance exercise increases muscle protein synthesis and results in net muscle protein synthesis, i.e., positive net muscle protein balance. Recently, the timing of ingestion of proteins and amino acids in relation to resistance exercise has received some attention. Previously, we demonstrated that there was a similar response to an essential amino acid (EAA)/carbohy-

drate solution when given at 1 compared with 3 h postexercise (7). However, when EAA was ingested before exercise, the response of muscle anabolism was greater than immediately (11) or 1 or 3 h after exercise (7). The most obvious explanation for the superior response when EAA were ingested before exercise was increased delivery of amino acids to the muscle (11).

It is not clear that the response of muscle to ingestion of intact proteins would be similar to that of free amino acids. In elderly subjects, muscle fiber cross-sectional area was increased more when protein was ingested immediately following exercise, rather than 2 h post during a period of training (5). Whereas no measures of muscle protein synthesis and net muscle protein balance were made during this study, the results suggest that the response may be different than for EAA. The response of net muscle protein balance to ingestion of intact proteins before and following exercise has not previously been compared. Thus the aim of the present study was to determine if the response of amino acid uptake, representative of net muscle protein balance, to resistance exercise is affected by the timing of ingestion.

## METHODS

### Subjects

Subjects were healthy, young males and females who had not participated in regular resistance training for at least five years before participation in this study. Subjects were randomly assigned to one of two groups receiving a 300-ml solution containing 20 g of whey proteins either immediately before (PRE;  $n = 8$ ) or immediately following (POST;  $n = 9$ ) a heavy leg resistance exercise bout (Table 1).

### Pretesting

**Medical screening.** The study design, purpose, and possible risks were explained to the subjects, and written consent was obtained. The Institutional Review Board and the General Clinical Research Center (GCRC) of The University of Texas Medical Branch at Galveston approved the study. Before participation in the experiments, each subject had a complete series of medical screening tests for the purpose of disclosing any preexisting medical or physical conditions that would preclude participation in the study.

**Exercise test.** At least 5 days before the research protocol, a one-repetition maximum (1RM) for leg extension exercise was determined for each subject. The 1RM was defined as the maximum weight that could be lifted and held for a 1-s count.

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Table 1. Subject characteristics for the two groups studied following resistance exercise

	n	Age, yrs	Wt, kg	Height, meters	BMI, kg/m <sup>2</sup>	1RM, kg	Leg Volume, liters
PRE	8	26.0±2.7	72.3±4.5	1.68±0.02	25.3±1.0	89.1±12.4	10.0±0.6
POST	9	27.8±2.0	74.8±2.8	1.73±0.03	25.3±0.8	116.4±11.7	9.1±0.5

Values are means ± SE; n, no. of subjects. BMI, body mass index; 1RM, one repetition maximum, i.e., the maximum resistance that can be lifted one time, for leg extension exercise; PRE, ingested whey protein before exercise; POST, ingested whey proteins following exercise.

### Experimental Protocol

**General.** This experimental protocol was designed to quantify the response of net muscle protein balance, as represented by phenylalanine balance across the leg in two groups of subjects following ingestion of a bolus of whey proteins. For each group, sampling began before protein ingestion and continued for 4 h following ingestion of the protein bolus. Thus amino acid uptake is quantified for ~4 h from the protein ingestion for each group. In addition, to examine a more practical interpretation of the question, amino acid uptake is quantified from the beginning of exercise and for the next 5 h for each group.

PRE ingested whey proteins immediately before a leg resistance exercise bout. POST consumed the whey protein bolus 1 h following the resistance exercise bout. One hour following exercise was chosen because, in previous studies, the mean uptake of amino acids was greater following bolus ingestion of EAA and carbohydrates at 1 h vs. immediately following resistance exercise (7, 11). We previously demonstrated that there is no change in net muscle protein balance when a placebo is ingested immediately before or following resistance exercise (9, 11). Therefore, no placebo groups were included in this study.

**Study protocol.** Subjects were admitted to the GCRC the night before each study, given a standard dinner, and then allowed only water until the start of the study the following morning. The general protocol for each group is depicted in Fig. 1. At ~0545 on each study day, an 18-gauge, polyethylene catheter was inserted in a vein on the forearm for blood sampling. Additionally, a 3-Fr, 8-cm, polyethylene catheter (Cook, Bloomington, IN) was inserted in the femoral vein and femoral artery under local anesthesia. Both femoral catheters were used for blood sampling, while the femoral arterial catheter also was used for indocyanine green (ICG) infusion for determination of leg blood flow. Systemic concentration of ICG was measured from a

peripheral vein. Patency of all catheters was maintained by saline infusion.

Muscle biopsies from the vastus lateralis were taken to measure intracellular amino acid concentrations. The muscle biopsies were taken immediately before the beginning of exercise for the resting period for PRE. Postexercise period biopsies were taken at ~55 min (i.e., ~90 min following protein ingestion for PRE and immediately before consumption of the protein for POST) and at the end of the study. Each biopsy was taken under local anesthetic from the lateral portion of the vastus lateralis ~10–15 cm above the knee. A 5-mm Bergstrom biopsy needle was used to sample ~50 mg of mixed muscle tissue. The sample was quickly rinsed, blotted, and divided into two to three pieces, frozen in liquid nitrogen, and stored at -80°C for future processing for intracellular amino acid concentration analysis. Biopsies were taken at least 1 cm apart in an attempt to minimize the impact of local inflammation from previous biopsy samples.

Blood flow was measured in periods chosen in an attempt to best characterize the leg blood flow during and following exercise and during protein ingestion (Fig. 1; see Refs. 8 and 9). A continuous ICG (infusion rate = 0.5 mg/min) infusion was initiated 10 min before each blood flow measurement period. If it was necessary to interrupt the ICG infusion for arterial sampling, ICG was allowed to flow uninterrupted for at least 5–6 min before subsequent sampling for blood flow. Three blood flow samples were drawn for each period. Each blood flow value was based on the mean of triplicate samples during that time period. The mean coefficient of variation (CV) for the triplicates was 12%.

At ~0600 (time = -120 min) background blood samples for insulin concentration and ICG concentration were taken. For both PRE and POST, three arteriovenous samples were taken immediately after cessation of the ICG infusion and 35, 30, and 25 min before exercise for the resting period, 45, 50, and 55 following exercise for the postexercise period, and 70, 80, 90, 105, 120, 150, 180, 210, 240, 270, and 300 min following exercise to determine the amino acid concentrations for determination of net muscle protein balance following ingestion of each protein solution. For PRE, additional samples were taken during exercise, following sets 6 and 10, and at 15 and 30 min following exercise. Because previous studies showed that there was no response of net muscle protein balance during or following exercise when no nutrients were ingested (4, 11), no samples were taken during or immediately following exercise for POST. Arterial blood samples for insulin analysis also were collected periodically throughout the protocol, 120, 150, 180, 240, and 300 min following exercise. Blood samples were analyzed for phenylalanine and leucine concentrations using the internal standard method and gas chromatography-mass spectrometry (GCMS) analysis (1, 2, 10).

For each trial, the exercise routine was begun immediately after the resting samples and lasted ~25 min. It consisted of 10 sets of 8 repetitions of leg extensions at 80% of 1RM. Each set was completed in ~30 s with a 2-min rest between sets. We have previously utilized this routine to increase blood flow and muscle protein metabolism (8, 9).

### Analysis of Samples

**Blood.** Concentrations of free amino acids were determined by GCMS (model 5973; Hewlett Packard, Palo Alto, CA) using an

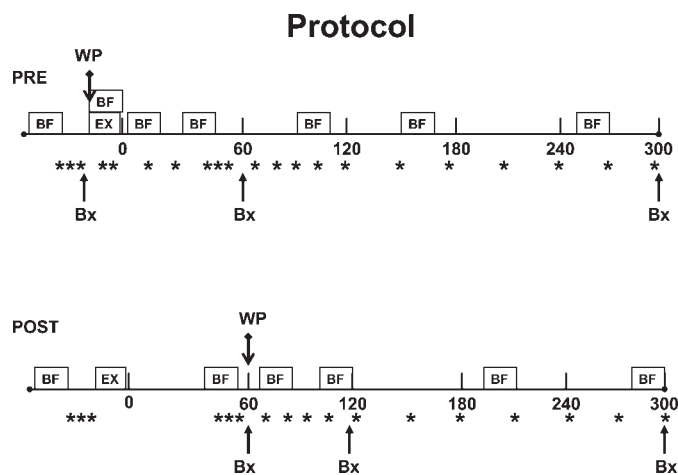


Fig. 1. Schematic diagram for infusion protocol designed to measure net muscle protein balance across the leg during ingestion of 20 g of whey protein immediately preceding exercise (PRE) or following exercise (POST). EX, resistance exercise bout; BF, measurement of leg blood flow using indocyanine green; WP, ingestion of 20 g of whey proteins; Bx, muscle biopsy from the vastus lateralis. \*Arteriovenous blood sampling.

internal standard solution, as previously described (1, 2, 10). The internal standard used was U- $^{13}\text{C}_9$ - $^{15}\text{N}$ ]phenylalanine (50  $\mu\text{mol/l}$ ) added in a ratio of  $\sim 100$   $\mu\text{l/ml}$  of blood. Because the tube weight and the amount of blood was known, the blood amino acid concentration could also be determined from the internal standard enrichments measured by GCMS based on the amount of blood and internal standard added. Leg blood flow was determined by spectrophotometrically measuring the ICG concentration in serum from the femoral vein and the peripheral vein, as described previously (1, 2). Leg plasma flow was calculated from steady-state values of dye concentration and converted to blood flow using the hematocrit (1, 2). Serum insulin levels were determined by RIA (Diagnostic Products, Los Angeles, CA). Intra-assay CV was  $<10\%$ .

**Muscle.** Muscle biopsy tissue samples were analyzed for free intracellular amino acid concentrations as previously described (1, 2). Tissue biopsies ( $\sim 50$  mg) of the vastus lateralis were immediately blotted and frozen in liquid nitrogen. Samples were then stored at  $-80^\circ\text{C}$  until processed. Upon thawing,  $\sim 20$ – $25$  mg of tissue were weighed, and protein was precipitated with 0.5 ml of 10% perchloroacetic acid. Tissue was then homogenized and centrifuged, and the supernatant was collected. This procedure was repeated two more times, and the pooled supernatant ( $\sim 1.3$  ml) was processed as were the blood samples described under *Blood*. Muscle free amino acid concentration was measured with the internal standard method, with corrections for the contribution of extracellular fluid as described in *Blood* and in previous studies (1, 2).

### Calculations

**Phenylalanine delivery to the leg and net muscle protein balance.** Phenylalanine delivery to the leg was calculated as the arterial phenylalanine concentration  $\times$  leg blood flow. Phenylalanine delivery was calculated for rest and for each hour following exercise by averaging the samples taken during that hour. For PRE, delivery was calculated during exercise. Because no exercise samples were taken during POST, no delivery values were calculated. Previous studies showed that delivery would be expected to increase  $\sim 2.5$ – $3.5$  times during exercise without exogenous amino acids from protein or free amino acid ingestion, i.e., entirely due to increased blood flow during exercise (Elliot TA, Tipton KD, Aarsland AA, Wolfe RR, unpublished data and Refs. 4 and 11).

Net muscle protein balance (NB) was represented by

$$NB = (C_a - C_v) \times BF \quad (1)$$

where  $C_a$  is arterial amino acid concentration,  $C_v$  is venous amino acid concentration, and BF is leg blood flow.

**Amino acid exchange across the leg.** Total amino acid exchange (mg) was calculated for phenylalanine from the area under the curve (AUC) of the net balance over the time following ingestion of the drinks (8, 9, 11). Positive values represent net uptake (anabolism) and negative values represent net release (catabolism).

For the present study, AUC was calculated in two ways. First, AUC was calculated for 5 h from the beginning of exercise for both groups. The baseline was the preexercise, resting samples for both PRE and POST, and AUC was calculated for the entire sampling time, i.e., during exercise plus 300 min of recovery from exercise. Previously, we used this method to compare free amino acids and carbohydrates consumed pre and post exercise (11). Thus the value determined was for the entire study period and can be considered the more practical examination of the data as this value represents, assuming that the complete response is represented in that time period and further sampling would not have provided more information, the entire response. Because our previous studies indicated that there was no change in net balance during and immediately following exercise when a placebo was ingested (9, 11), we chose not to sample during exercise for POST. Thus AUC for POST assumes that there was no change during and immediately following exercise.

The second way was to calculate AUC for an equal time following ingestion of the protein. The baseline was the mean of the preexercise, resting samples for PRE and the mean postexercise values from samples taken at 45, 50, and 55 min following exercise for POST. Because the total time following protein ingestion was shorter for POST, AUC during PRE was calculated from drink ingestion only to 210 min following exercise to equalize the time for AUC calculation. Thus uptake values represented the AUC for  $\sim 4$  h for both groups, i.e., beginning of exercise to 210 min following exercise for PRE and 60–300 min following exercise for POST. This method may be interpreted as more mechanistic, since it reflects how the muscle responds to the same perturbation when protein is ingested at different times. Because exchange of phenylalanine was essentially zero (exchange nonsignificantly different from zero,  $P = 0.80$  for phenylalanine) for placebo groups during previous studies (9, 11), the reported exchange for PRE and POST represents the value due to the ingestion of the protein.

**Insulin.** AUC was calculated for the insulin values with the baseline value from the sample immediately before drink ingestion. Calculation of AUC for insulin was stopped when the insulin value returned to baseline ( $\sim 120$  min following exercise). Because insulin did not change over this time period for the placebo group in previous studies (9, 11), the AUC represents the change due to protein ingestion.

### Data Presentation and Statistical Analysis

Data are presented as means  $\pm$  SE. Phenylalanine arterial concentrations and net balances, as well as insulin concentrations, are presented across time. Phenylalanine exchange, i.e., AUC for net balance, across the leg is presented for PRE and POST as individual values for the entire sampling period, including exercise and recovery, as well as for the 4 h following ingestion of the whey protein for each group. Muscle intracellular concentrations are presented for each biopsy sample for each group.

For the primary endpoint, phenylalanine AUC, as well as insulin AUC, PRE and POST were compared by two-tailed Student's *t*-test assuming unequal variances. Significance was set at  $P = 0.05$ .

For the secondary endpoints, muscle intracellular concentration, arterial phenylalanine concentration, and phenylalanine net balance over time were analyzed with one-way, repeated-measures ANOVA separately for each group. Because the sampling patterns were not identical for PRE and POST, statistical analyses were restricted to determining when the values for each changed from baseline. For muscle intracellular concentrations, wherever ANOVA revealed a significant difference, Tukey's post hoc procedure was used to locate the pairwise differences. For arterial phenylalanine concentration and phenylalanine net balance, Dunnett's post hoc procedure was used to detect differences between each time point and the resting value.

## RESULTS

### Serum Insulin AUC

There was no difference in insulin AUC between PRE ( $1,483 \pm 303$   $\mu\text{U} \cdot 120 \text{ min}^{-1} \cdot \text{ml}^{-1}$ ) and POST ( $1,168 \pm 180$   $\mu\text{U} \cdot 120 \text{ min}^{-1} \cdot \text{ml}^{-1}$ ).

### Leg Blood Flow

Leg blood flow for each group is presented in Fig. 2. For PRE, blood flow was significantly greater than rest during and immediately following exercise. By 45 min following exercise and throughout the remainder of the sampling protocol, there were no differences from rest. For POST, blood flow was significantly greater than rest at 45 min following exercise and during the final measurement at 295 min following exercise.

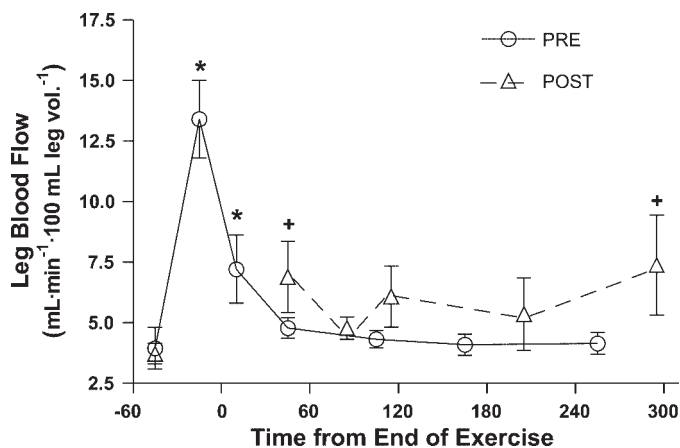


Fig. 2. Leg blood flow measured by indocyanine green infusion at rest, during, and following exercise during ingestion of 20 g of whey protein immediately preceding exercise or following exercise. All values are means  $\pm$  SE. \*Significantly different from resting values for PRE. +Significantly different from resting values for POST.

#### Blood Amino Acid Concentrations and Net Balance over Time

Arterial phenylalanine concentrations for PRE and POST are shown in Fig. 3. Phenylalanine concentration increased immediately in response to ingestion of whey proteins at both times. Arterial phenylalanine was significantly ( $P < 0.05$ ) increased over baseline until 120 min following exercise for PRE and 150 min following exercise for POST.

Net phenylalanine balance over time is summarized in Fig. 4. Phenylalanine balance was significantly ( $P < 0.05$ ) increased following ingestion during exercise for PRE but declined to baseline levels near the end of exercise. Net balance increased a second time following exercise and declined back to baseline at 30 min postexercise. For POST, phenylalanine balance was significantly increased over baseline at 80 and 90 min following exercise (20 and 30 min following drink ingestion) and thereafter returned to values no different from baseline.

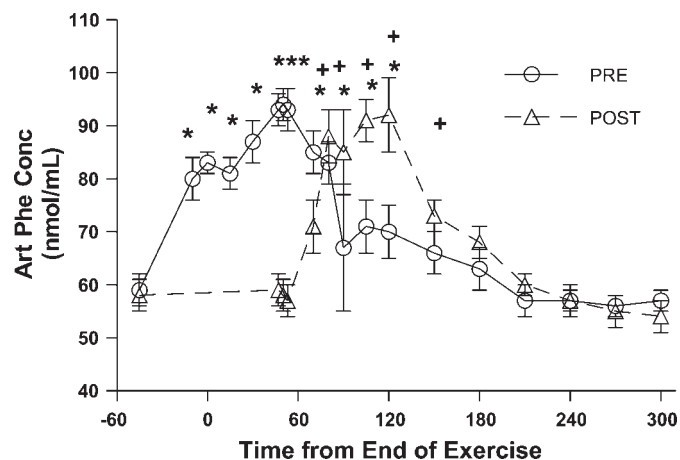


Fig. 3. Arterial phenylalanine concentration at rest and for 5 h following resistance exercise during ingestion of 20 g of whey protein immediately preceding exercise or following exercise. All values are means  $\pm$  SE. \*Significantly different from resting values for PRE. +Significantly different from resting values for POST.

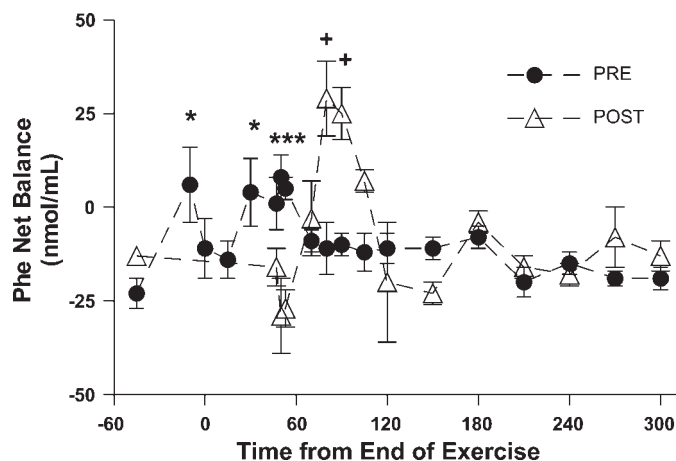


Fig. 4. Phenylalanine net balance across the leg at rest and for 5 h following resistance exercise during ingestion of 20 g of whey protein immediately preceding exercise or following exercise. All values are means  $\pm$  SE. \*Significantly different from resting values for PRE. +Significantly different from resting values for POST.

#### Amino Acid Delivery to the Leg

Delivery of phenylalanine to the leg (arterial phenylalanine concentration  $\times$  BF) was increased during exercise for PRE by  $\sim 4.4$  times and returned to values no different from rest in the 3rd h following exercise (Table 2). Phenylalanine delivery was increased above rest for the entire postexercise period during POST. In our previous studies (4, 11), phenylalanine delivery during exercise was  $\sim 2.5$ – $3$  times resting values during exercise due to a  $\sim 2.5$ – $3$  times increase in blood flow and with no change in arterial amino acid concentrations. Furthermore, using an identical exercise protocol, phenylalanine delivery during exercise was  $\sim 3.5$  times resting values with no change in phenylalanine concentration (Elliot TA, Tipton KD, Aarsland AA, Wolfe RR, unpublished data). Thus we could expect that delivery of phenylalanine increased approximately three times in the present study as well.

#### Muscle Intracellular Amino Acid Concentration

Muscle intracellular phenylalanine concentration did not change over time for PRE or POST. Before exercise, phenylalanine was  $77 \pm 4$  nmol/ml of intracellular fluid for PRE. Following exercise, phenylalanine was  $89 \pm 5$  and  $67 \pm 4$  nmol/intracellular fluid at 55 min and 300 min, respectively. Following exercise for POST, phenylalanine was  $70 \pm 6$ ,  $70 \pm 8$ , and  $54 \pm 4$  nmol/intracellular fluid before protein ingestion at 55 min postexercise, 1 h following protein ingestion at 120 min postexercise, and at the end of the sampling 300 min postexercise, respectively.

#### Amino Acid Exchange (AUC)

Phenylalanine exchange was not significantly different between PRE and POST calculated over the 4 h following ingestion of the whey proteins (Fig. 5A). When calculated for 5 h, phenylalanine uptake was not statistically greater for PRE than POST ( $P = 0.16$ ) despite a large difference between means. The difference between means is largely due to one individual value that is much greater than all other values, in fact almost two times the next largest uptake value (Fig. 5B).

Table 2. Phenylalanine delivery to the leg before, during, and each hour after exercise in two groups of subjects

	Rest	EX	PE1	PE2	PE3	PE4	PE5
PRE	249 ± 71	1,099 ± 137*	493 ± 32*	347 ± 19*	264 ± 25	233 ± 17	232 ± 17
POST	206 ± 20	NA	372 ± 54*	436 ± 23*	374 ± 46*	312 ± 41*	357 ± 43*

Values are means ± SE in nmol phenylalanine/ml of arterial blood. Rest, resting value; EX, value during exercise for PRE, no samples were taken during exercise for POST; PE1, 1st h following exercise; PE2, 2nd h following exercise; PE3, 3rd h following exercise; PE4, 4th h following exercise; PE5, 5th h following exercise; NA, no measurements taken during exercise for POST. \*Significantly different from rest ( $P < 0.05$ ).

DISCUSSION

The present study was designed to determine if the anabolic response of muscle to whey protein ingestion would be different depending on the timing of ingestion in relation to resistance exercise. There was an anabolic response to ingestion of 20 g of whey proteins whether ingested before or 1 h following resistance exercise, and no differences were detected between time points. The anabolic response to the protein ingestion was calculated in two ways. AUC of the net balance was determined from the beginning and for 5 h following the exercise bout. This method of calculating the response to timing of ingestion was similar to the approach we used in our previous

study examining the response to timing of EAA and carbohydrate ingestion (11). However, it could be argued that this approach biases the analysis in favor of the PRE trial because PRE had amino acids available for ~1.5 h more than POST. Even so, there was no significant difference between the two trials, although there was large variability in the response and some subjects did exhibit large uptake of amino acids by the leg when whey proteins were ingested before exercise. The response also was calculated for 4 h following ingestion of the whey proteins. This method provides an illustration of the basic response of the muscle to whey proteins before and after exercise. Unlike the response to EAA in our previous study (11), the anabolic response to whey protein ingestion was similar whether ingested before or following exercise. Thus it seems that muscle protein accretion is stimulated by ingestion of whey proteins, and the timing of the ingestion in relation to the exercise is not as important for this response as it was for EAA.

Previously, we demonstrated that ingestion of EAA before exercise resulted in a greater anabolic response than following exercise (11). It is not immediately apparent why free amino acids plus carbohydrate would engender a different response to timing of ingestion than whey proteins. The most likely reason that EAA ingested before exercise lead to superior amino acid uptake vs. following exercise was increased delivery of amino acids to the leg. In the present study, phenylalanine delivery was not different between PRE and POST. The AUC for phenylalanine delivery was  $45 \pm 16 \mu\text{M} \times 5 \text{ h/ml}$  for PRE and  $49 \pm 13 \mu\text{M} \times 5 \text{ h/ml}$  for POST. If delivery of amino acids to the leg is a key component for the anabolic response, then these data suggest that it is delivery that explains the difference between the present study and our previous study (11). Our data do not allow a clear explanation of why amino acid delivery was greater for EAA ingested before than following exercise but not different for whey proteins. It is tempting to speculate that digestion of the protein may limit the amount of amino acids in the blood during exercise when blood flow is high. Support for this speculation comes from examination of the amino acid concentration and delivery data during exercise. The increase in arterial amino acid concentrations during exercise was ~100% for EAA (11) but only ~30% for whey proteins when ingested immediately before exercise (Fig. 3). Thus the delivery of phenylalanine during exercise was increased by ~7.5 times for EAA, but only ~4.4 times for whey proteins. Therefore, this evidence suggests that amino acid delivery during exercise is greater when EAA is ingested than when intact whey proteins are ingested immediately before exercise. It is possible that consuming proteins at other time points before exercise, e.g., 30, 45, or 60 min, may have resulted in greater amino acid levels and increased delivery.

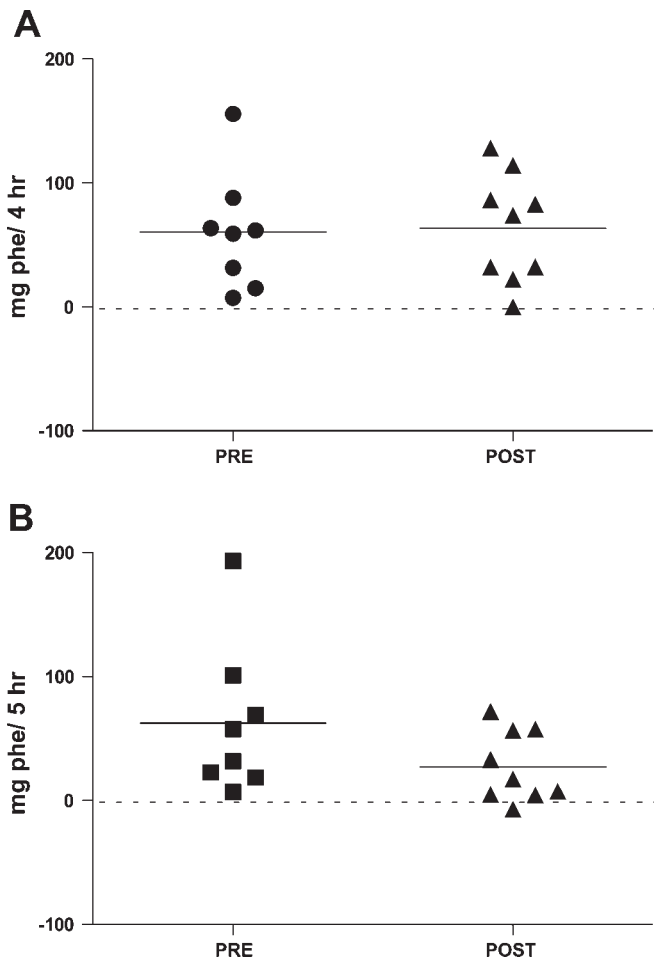


Fig. 5. Individual and mean values for phenylalanine uptake (area under the curve for net balance) across the leg for 4 h following ingestion of 20 g of whey protein immediately preceding exercise or following exercise (A) and during and for 5 h following resistance exercise with ingestion of 20 g of whey protein immediately preceding exercise or following exercise (B). All values are means ± SE.

Future studies should examine the response to ingestion of intact proteins at other time points before exercise.

There are several differences between the present study and our previous examination of the effect of the timing of ingestion of EAA (11) that may contribute to the different responses. Aside from the free amino acids vs. intact proteins, there was no carbohydrate added to the whey protein. It is possible that the insulin response to the carbohydrates influenced the response of muscle to timing of amino acid ingestion. However, the insulin response to the whey proteins in the present study was similar to that to the EAA in our previous study (11). Thus it is not likely that the differences in insulin response would explain the different responses of amino acid uptake following ingestion of EAA or protein. In our previous study (11), we compared the response to ingestion of EAA immediately before exercise to immediately after exercise. In the present study, we compared whey proteins ingestion immediately before exercise to 1 h following exercise. We chose 1 h because the response to ingestion of EAA at 1 h following exercise (7) was slightly greater than immediately following exercise (11). However, we cannot rule out the possibility that amino acid uptake from whey proteins would not be the same as EAA ingested at different times following exercise.

The difference in means between PRE and POST without statistical significance for the 5-h AUC suggests the possibility that our results are due to limitations in power, and we have failed to detect a real difference between PRE and POST. Support for this notion comes from the fact that the EAA ingestion in our previous study (11) did result in a large, significant difference between PRE and POST. Thus we expected the amino acid uptake for PRE to be superior to POST due to ingestion of whey proteins. Power calculations revealed that a statistically significant difference could be detected with two times the number of subjects, given the variability and difference between means. It is apparent that the large variability in the response of our subjects contributes to the lack of clarity. Careful examination of Fig. 5 reveals that much of the difference between means is due to a very large response from one subject. If we consider this value to be a statistical outlier ( $>2$  SD above the mean) and remove the value, the difference between means is reduced by  $>50\%$ , and it is less clear that our conclusions would be affected by a type II error. Without this value, we would need  $>50$  subjects to detect a difference. However, we cannot dismiss the possibility that we failed to detect a true difference between means for the 5-h AUC due to a small sample size.

Another interpretation of the data is that certain individuals are more responsive than others. Thus the practical conclusion that ingestion of whey proteins before exercise will translate into significantly more accretion of muscle proteins may be true for some individuals. Whereas our study design does not allow a direct comparison for any one individual, it is interesting that four of the eight subjects in PRE had greater uptake over 5 h than any of the subjects in POST.

Other studies have examined changes in muscle mass with variable timing of ingestion of proteins in relation to exercise. Esmarck et al. (5) demonstrated that protein intake immediately following exercise resulted in greater muscle fiber hyper-

trophy than 2 h following exercise in elderly subjects. These data suggest that timing of protein intake may be crucial, at least in elderly subjects, but the data are not directly comparable to the present results since there was no pretraining protein ingested in the aforementioned study (5). To our knowledge, no comparison of protein ingestion before and following exercise has been made.

Thus our two studies suggest that EAA and whey proteins may not engender the same response before and following exercise. Although it is clear that the response of muscle protein balance to EAA ingestion is superior when ingested before exercise, it is not clear that this is the case for whey proteins.

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