

Ingestion of Casein and Whey Proteins Result in Muscle Anabolism after Resistance Exercise

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ABSTRACT

TIPTON, K. D., T. A. ELLIOTT, M. G. CREE, S. E. WOLF, A. P. SANFORD, and R. R. WOLFE. Ingestion of Casein and Whey Proteins Result in Muscle Anabolism after Resistance Exercise. *Med. Sci. Sports Exerc.*, Vol. 36, No. 12, pp. 2073–2081, 2004. **Purpose:** Determination of the anabolic response to exercise and nutrition is important for individuals who may benefit from increased muscle mass. Intake of free amino acids after resistance exercise stimulates net muscle protein synthesis. The response of muscle protein balance to intact protein ingestion after exercise has not been studied. This study was designed to examine the acute response of muscle protein balance to ingestion of two different intact proteins after resistance exercise. **Methods:** Healthy volunteers were randomly assigned to one of three groups. Each group consumed one of three drinks: placebo (PL; $N = 7$), 20 g of casein (CS; $N = 7$), or whey proteins (WH; $N = 9$). Volunteers consumed the drink 1 h after the conclusion of a leg extension exercise bout. Leucine and phenylalanine concentrations were measured in femoral arteriovenous samples to determine balance across the leg. **Results:** Arterial amino acid concentrations were elevated by protein ingestion, but the pattern of appearance was different for CS and WH. Net amino acid balance switched from negative to positive after ingestion of both proteins. Peak leucine net balance over time was greater for WH ($347 \pm 50 \text{ nmol}\cdot\text{min}^{-1}\cdot 100 \text{ mL}^{-1} \text{ leg}$) than CS ($133 \pm 45 \text{ nmol}\cdot\text{min}^{-1}\cdot 100 \text{ mL}^{-1} \text{ leg}$), but peak phenylalanine balance was similar for CS and WH. Ingestion of both CS and WH stimulated a significantly larger net phenylalanine uptake after resistance exercise, compared with the PL (PL $-5 \pm 15 \text{ mg}$, CS $84 \pm 10 \text{ mg}$, WH $62 \pm 18 \text{ mg}$). Amino acid uptake relative to amount ingested was similar for both CS and WH ($\sim 10\text{--}15\%$). **Conclusions:** Acute ingestion of both WH and CS after exercise resulted in similar increases in muscle protein net balance, resulting in net muscle protein synthesis despite different patterns of blood amino acid responses. **Key Words:** MUSCLE PROTEIN SYNTHESIS, ARTERIOVENOUS BALANCE, NET MUSCLE PROTEIN BALANCE, MUSCLE BIOPSIES

Determination of the anabolic response to exercise and nutrition is important for individuals who might benefit from increased muscle mass. The metabolic basis for skeletal muscle growth lies in the relationship of muscle protein synthesis to muscle protein breakdown. Muscle hypertrophy occurs only from net protein synthesis; that is, when muscle protein synthesis exceeds breakdown. It is clear that exercise, especially resistance exercise, has a profound effect on muscle protein metabolism, often resulting in muscle growth. Acutely, resistance exercise may result in improved muscle protein balance, but, in the absence of food intake, the balance remains negative (i.e., catabolic) (3,13,14). Thus, the impact of exercise on muscle growth and function must be considered in light of the

interactive effects of exercise and nutrient intake on muscle protein metabolism.

Amino acid availability is critical to the control of muscle protein metabolism (5,19). Thus, a meal or a supplement containing protein or amino acids will influence muscle protein. Recently, we examined the response of muscle protein metabolism in human volunteers during ingestion of amino acids after resistance exercise. Whereas net muscle protein balance was negative during placebo ingestion, net muscle protein balance was positive during ingestion of mixed amino acids (16), as well as essential amino acids only (7,16). The increase in net balance was primarily a result of increased muscle protein synthesis. These data demonstrate that amino acid availability can be increased as effectively via oral intake as with intravenous infusion. It would also follow that any essential amino acid source, including whole proteins, ingested after exercise should result in muscle anabolism. However, to date, no study has examined the response of net muscle protein balance to ingestion of whole proteins after resistance exercise. Thus, the primary purpose of the present study was to examine the acute response of net muscle protein balance to ingestion of intact proteins after resistance exercise.

A further goal of the project was to compare the response of net muscle protein synthesis after exercise of two whole

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Submitted for publication March 2004.

Accepted for publication July 2004.

0195-9131/04/3612-2073

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DOI: 10.1249/01.MSS.0000147582.99810.C5

TABLE 1. Subject characteristics for the three groups studied after resistance exercise.

	<i>N</i>	Age (yr)	Wt (kg)	Ht (m)	BMI (kg·m ⁻²)	1RM (kg)	Leg Volume (L)
Placebo	7	23.7 ± 1.1	73.4 ± 3.0	1.73 ± 0.03	24.7 ± 1.0	142.6 ± 15.2	9.7 ± 0.4
Casein	7	24.0 ± 2.7	78.2 ± 5.9	1.77 ± 0.03	24.9 ± 1.4	134.5 ± 14.3	9.8 ± 0.7
Whey	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

BMI, body mass index; 1RM, one repetition maximum, that is, the maximum resistance that can be lifted one time, for leg extension exercise.

proteins with different digestive properties. Casein is emptied from the stomach more slowly than whey proteins, so casein is considered a “slow” protein, and whey proteins “fast” proteins. Amino acids from slow proteins appear in the blood more slowly, and peak at a lesser magnitude, but the response lasts longer than with fast proteins. Recent studies indicated that whole-body leucine balance was greater after ingestion of casein than of whey proteins, because of differences in their digestive properties (6,8). It is unknown whether the differences in digestive properties between casein and whey proteins would impact the response of net muscle protein balance after resistance exercise. We examined the response of net muscle protein balance to ingestion of 20 g of casein, 20 g of whey proteins, and a placebo in healthy volunteers after exercise. Arterio-venous balances of phenylalanine and leucine across the leg were measured to determine the muscle anabolic response.

METHODS

Subjects

Subjects were healthy young males and females who had not participated in regular resistance training for at least 5 yr before participating in this study. Subjects were randomly assigned to one of three groups receiving a 300-mL solution containing one of two whole proteins or a placebo, 1 h after a heavy leg resistance exercise bout. The three groups were: placebo (PL), *N* = 7; casein (CS), *N* = 7; and whey proteins (WH), *N* = 9. Subject characteristics are presented in Table 1. There were no significant differences among groups for any of the subject characteristics.

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. These screening tests included: vital signs, blood tests (chemistries, auto chem. panel, lipids, iron, hematology, coagulation, hepatitis B surface antigen and antibody, hepatitis B core Ab, hepatitis A antibody IgM, hepatitis A antibody total, and HIV AB), urine tests (drug screen I: abuse, marijuana, pregnancy test), and a 12-lead electrocardiogram to exclude any individuals displaying heart irregularities.

Exercise test. At least 5 d before the research protocol, a one-repetition maximum (1RM) utilizing 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.

Experimental Protocol

General. The general approach to addressing the hypotheses was to determine net muscle protein balance at rest and after resistance exercise during ingestion of placebo and each of two whole proteins. Groups consisted of resistance exercise plus ingestion of: 1) placebo (artificially flavored, colored water); 2) 20 g of casein; and 3) 20 g of whey protein. The casein solution contained 1.7 g of leucine and 0.9 g of phenylalanine, and whey proteins contained 2.3 g of leucine and 0.6 g of phenylalanine.

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 is depicted in Figure 1. At approximately 0545 on each study day, an 18-gauge polyethylene catheter was inserted into a vein on the forearm for blood sampling. Additionally, a 3-Fr, 8-cm, polyethylene catheter (Cook, Inc., Bloomington, IN) was inserted into 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.

This experimental protocol was designed to quantify the response of net muscle protein balance, as represented by amino acid balance across the leg. Two sampling periods were utilized during each trial, one resting period and the other from 0 to 300 min after the end of resistance exercise. In each trial, the protein or placebo was ingested in 300 mL of solution 60 min after resistance exercise.

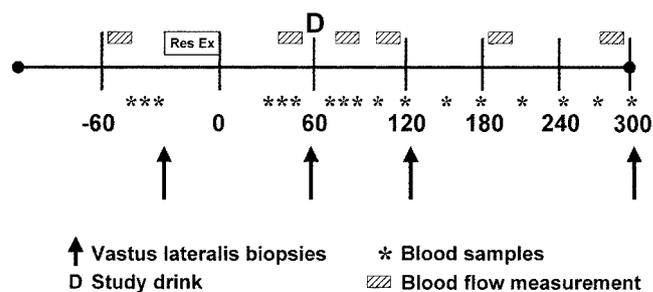


FIGURE 1—Schematic diagram of the study protocol.

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. Postexercise period biopsies were taken at ~55 min (immediately before consumption of the study drink), 120 min, and 300 min after exercise. Each biopsy was taken under local anesthetic from the lateral portion of the vastus lateralis approximately 10–15 cm above the knee. A 5-mm Bergstrom biopsy needle (Stille, Stockholm, Sweden) was used to sample ~50 mg of mixed muscle tissue. The sample was quickly rinsed, blotted, and divided into 2–3 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.

A continuous indocyanine green (ICG; infusion rate = $0.5\text{ mg}\cdot\text{min}^{-1}$) 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. Samples to calculate leg blood flow at rest were taken from 45 min before exercise until 35 min before exercise. Blood flow samples were taken five times (from 40–45 min, 80–90 min, 115–120 min, 200–210 min, and 290–300 min after exercise) during the postexercise period. These five periods were chosen in an attempt to best characterize the leg blood flow after exercise and during protein ingestion. Sampling time varied somewhat among periods, depending on concurrent arteriovenous sampling for amino acid concentrations. Each blood flow value was based on the mean of triplicate samples during that time period, regardless of the length of time over which the samples were taken. The mean CV for the triplicates was 12%.

Blood sampling was identical for all experiments. At ~0600 (time = -120 min), background blood samples for insulin concentration and ICG concentration were taken (3,7,12,15–17). Additionally, 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 after exercise for the postexercise period; and 70, 80, 90, 105, 120, 150, 180, 210, 240, 270, and 300 min after exercise to determine the amino acid concentrations for determination of net muscle protein balance after ingestion of each protein solution or placebo. Arterial blood samples for insulin analysis also were collected throughout the time after exercise at -5 , 53, 90, 105, 120, 150, 180, 240, and 300 min after exercise. Blood samples were analyzed for phenylalanine and leucine concentrations using the internal standard method and gas chromatography-mass spectrometry (GCMS) analysis (2,3).

For each trial, the exercise routine was begun immediately after the first muscle biopsy was completed, and lasted ~25 min. It consisted of 10 sets of 8 repetitions of leg extensions at 80% of 1RM. Each set was completed in approximately 30 s, with a 2-min rest between sets. We have

previously utilized this routine to increase blood flow and muscle protein metabolism (13,15).

Analysis of Samples

Blood. Concentrations of free amino acids were determined by GCMS (Hewlett Packard 5973, Palo Alto, CA) using an internal standard solution as previously described (2,3). The internal standards used were U- $^{13}\text{C}_9$ - (15N)phenylalanine ($50\text{ }\mu\text{mol}\cdot\text{L}^{-1}$) and L- $^{13}\text{C}_6$ leucine ($115\text{ }\mu\text{mol}\cdot\text{L}^{-1}$), added in a ratio of $\sim 100\text{ }\mu\text{L}\cdot\text{mL}^{-1}$ of blood. Because the tube weight and the amount of blood were 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 (2,3). Leg plasma flow was calculated from steady-state values of dye concentration, and converted to blood flow using the hematocrit (2,3). Serum insulin levels were determined by radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA). Intraassay coefficient of variation (CV) was $<10\%$.

Muscle. Muscle biopsy tissue samples were analyzed for free intracellular amino acid concentrations as previously described (2,3,15). Tissue biopsies (~50 mg) of the vastus lateralis were immediately blotted and frozen in liquid nitrogen. Samples were then stored at -80°C until processed. Upon thawing, ~20–25 mg of tissue was weighed and protein precipitated with 0.5 mL of 10% perchloroacetic acid. Tissue was then homogenized and centrifuged, and the supernatant collected. This procedure was repeated two more times, and the pooled supernatant (~1.3 mL) was processed, as were the blood samples described above in blood. Muscle free amino acid concentration was measured with the internal standard method, with corrections for the contribution of extracellular fluid as described in the section “Blood” and in previous studies (2,3). No replicates were available to determine an intraassay CV, but the intersubject CV for phenylalanine and leucine for PL were 19 and 25%, respectively, and for protein ingestion were 27 and 28%, respectively.

Calculations

Net muscle protein balance. Net muscle protein balance (NB) was represented by:

$$\text{NB} = (\text{Ca} - \text{Cv}) \cdot \text{BF}$$

where:

Ca = arterial amino acid concentration, Cv = venous amino acid concentration, and BF = leg blood flow.

Amino acid exchange across the leg. Total amino acid exchange (mg) was calculated for phenylalanine and leucine from the area under the curve of the net balance over the time after ingestion of the drinks for each amino acid (14). Positive values represent net uptake (anabolism) and negative values represent net release (catabolism). Area

under the curve was calculated from all samples after the drink ingestion (i.e., 70–300 min after exercise). The baseline was the mean postexercise values from samples taken at 45, 50, and 55 min after exercise. Because exchange of both phenylalanine and leucine was essentially zero (exchange nonsignificantly different from zero, $P = 0.80$ and 0.85 for phenylalanine and leucine, respectively) for PL (i.e., the control), the reported exchange for CS and WH represents the value due to the ingestion of the respective protein.

Insulin. Area under the curve was calculated for the insulin values with the baseline value from the sample immediately before drink ingestion. Calculation of area under the curve for insulin was stopped when the insulin value returned to baseline (~90–120 min after drink ingestion). Because insulin did not change over this time period for the placebo group, the area under the curve for CS and WH represents the change due to protein ingestion.

Data Presentation and Statistical Analysis

Data are presented as means \pm SE. Phenylalanine and leucine arterial concentrations and net balances, as well as insulin concentrations, are presented across time. Phenylalanine and leucine exchange (i.e., area under the curve for net balance) across the leg are presented for each group. Muscle intracellular concentrations are presented for each biopsy sample for each group.

For the primary endpoints, phenylalanine and leucine exchange, ANOVA was used to determine differences between groups. Significance was set at $P = 0.05$. If the overall P value was significant, then Tukey's *post hoc* test was used to determine pairwise differences. Insulin area under the curve for CS and WH were compared by two-tailed Student's *t*-test assuming unequal variances.

Two-way ANOVA with repeated measures was used to analyze leg blood flow and muscle intracellular concentration with time as the within factor and group (PL, CS, WH) as the between factor. Two-way ANOVA also was used to detect differences in phenylalanine and leucine arterial and venous concentrations and net balance across the leg over time. Time was the within factor and group (PL, CS, WH) the between factor. Wherever ANOVA revealed a significant difference, Tukey's *post hoc* procedure was used to locate the pairwise differences.

RESULTS

Leg blood flow. Leg blood flow for each group is presented in Figure 2. No significant interaction between time and drink ingested was detected. There was no effect of protein ingestion on leg blood flow. However, an overall effect of time was significant at $P = 0.001$. Blood flow peaked in the first hour after exercise and was significantly greater than rest, 80–90, and 200–210 measurement periods at that time. Blood flow was significantly decreased by 80–90 min after exercise. There was a slight elevation in blood flow during the final measurement period from 290 to 300 min after exercise ($P = 0.004$ vs rest).

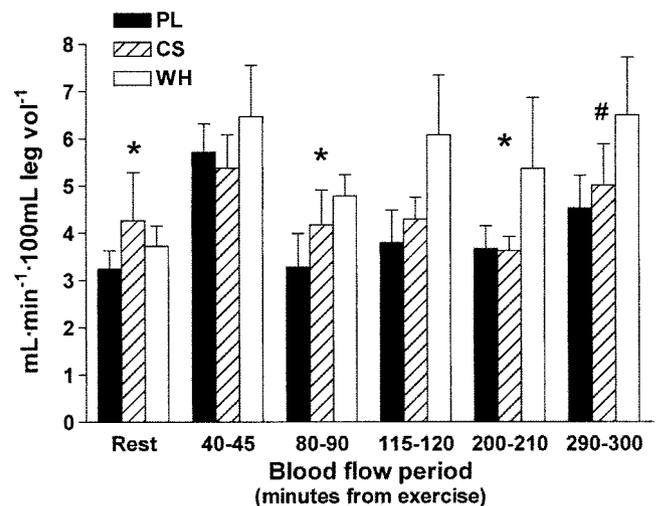


FIGURE 2—Leg blood flow at rest and at five time periods after resistance exercise. At 60 min postexercise (i.e., between the second and third blood flow measurements), a drink containing flavored water (PL), 20 g of casein (CS), or 20 g of whey proteins (WH) was consumed. ANOVA revealed no significant interactions, but there was a significant overall effect of time; * significantly different from 45 to 55; # significantly different from rest. All values are mean \pm SE.

Blood amino acid concentrations and net balance over time.

Concentrations of leucine and phenylalanine in the femoral artery and vein are summarized in Figures 3 and 4. There was no significant change over time in either leucine or phenylalanine concentrations during PL. There was a significant interaction of time and drink ingested for leucine concentration in the artery and vein ($P < 0.001$). Arterial leucine concentration (Fig. 3A) was significantly increased by 20 min after ingestion of CS (80 min from exercise), and remained elevated until 180 min after drink ingestion (240 min from exercise). WH ingestion significantly elevated leucine at 20 min after drink ingestion, and remained elevated until 150 min after drink ingestion (210 min after exercise), except for the value at 90 min after drink ingestion. Leucine concentration for CS and WH were significantly greater than for PL from 10 to 150 min after drink ingestion (70–210 min after exercise). Furthermore, at 180 min after drink ingestion (240 min after exercise), arterial leucine for CS, but not WH, was significantly greater than PL. WH leucine was significantly greater than CS from 20 to 90 min after drink ingestion (80–150 min after exercise). Venous leucine concentrations followed similar patterns for both CS and WH (Fig. 3B).

There was a significant interaction of time and drink ingested for phenylalanine concentration in the artery and vein ($P < 0.001$). Phenylalanine concentration in the artery (Fig. 4A) was significantly elevated by 10 min after ingestion of both CS and WH. During CS, phenylalanine concentration remained elevated until 180 min after drink ingestion (240 min after exercise); however, it returned to levels no different from baseline much more quickly during WH. Phenylalanine concentration was significantly different from baseline at 90 min after drink ingestion (150 min after exercise), but had returned to levels no different from baseline by 120 min after drink (180 min after exercise).

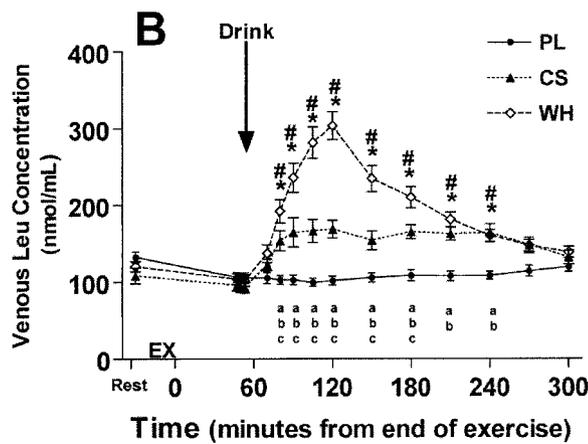
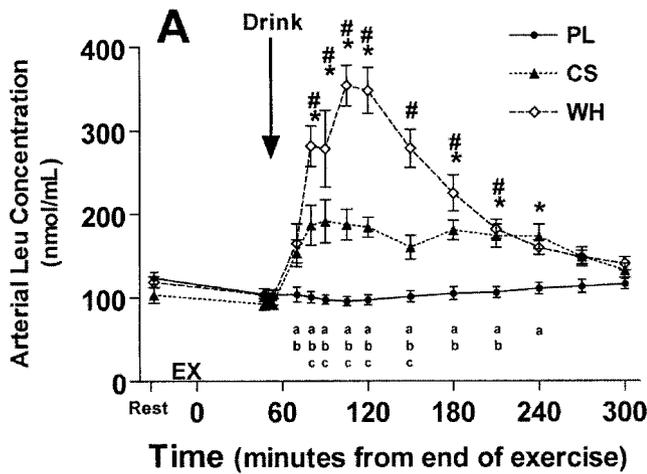


FIGURE 3—Arterial (A) and venous (B) leucine concentration at rest and for 5 h after resistance exercise and ingestion of a placebo (PL), 20 g of casein (CS), or 20 g of whey protein (WH) 1 h after exercise; a, CS significantly different from PL, $P < 0.05$; b, WH significantly different from PL, $P < 0.05$; c, CS significantly different from WH, $P < 0.05$; * CS value significantly different from rest, $P < 0.05$; # WH value significantly different from rest. All values are mean \pm SE.

Arterial phenylalanine was greater for both CS and WH than for PL from 10 to 60 min after drink ingestion (70–120 min postexercise). During CS phenylalanine concentration in the artery was greater than PL at 120 min after drink ingestion (180 min postexercise). At 180 min after drink ingestion (240 min after exercise), CS phenylalanine was greater than WH. The pattern of venous phenylalanine concentrations was similar to the arterial concentrations (Fig. 4B).

Muscle intracellular amino acid concentration.

Muscle intracellular leucine and phenylalanine concentrations are presented in Figure 5. There was a significant interaction between group (drink) and time (biopsy) for leucine concentration ($P < 0.001$) (Fig. 5A). Leucine concentration during CS was higher at 120 min after exercise (60 min after drink ingestion) than before drink ingestion (55 min after exercise; $P < 0.001$). During WH, leucine concentration at 120 min after exercise (60 min after drink ingestion) was significantly greater than at all other sampling times ($P < 0.001$). Furthermore, leucine concentration during WH was significantly greater than for CS and PL at

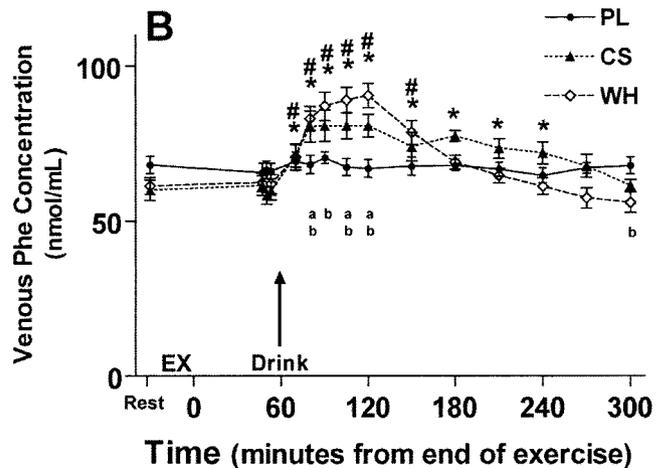
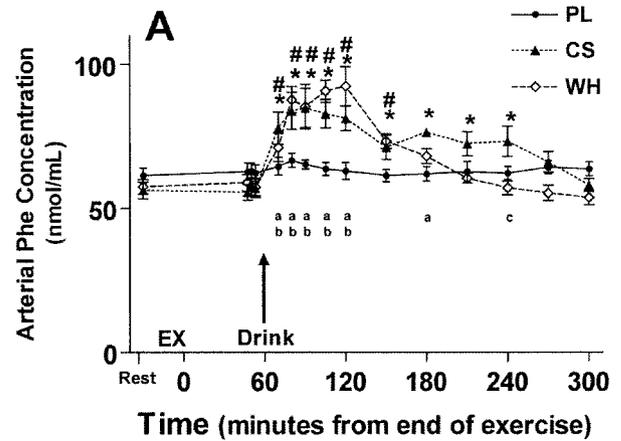


FIGURE 4—Arterial (A) and venous (B) phenylalanine concentration at rest and for 5 h after resistance exercise and ingestion of a placebo (PL), 20 g of casein (CS), or 20 g of whey protein (WH) 1 h after exercise; a, CS significantly different from PL, $P < 0.05$; b, WH significantly different from PL, $P < 0.05$; c, CS significantly different from WH, $P < 0.05$; * CS value significantly different from rest, $P < 0.05$; # WH value significantly different from rest. All values are mean \pm SE.

120 min after exercise (60 min after drink ingestion; $P < 0.001$).

Differences in mean muscle intracellular phenylalanine concentrations did not reach statistical significance (Fig. 5B); however, the interaction between group and drink approached statistical significance ($P = 0.06$).

Serum insulin concentration. There was no change in insulin concentration from rest to postexercise or at any time during PL (Fig. 6). Insulin concentrations increased more rapidly and to a greater magnitude for WH than for CS. The magnitude of the insulin response was not great for ingestion of either protein reaching a peak of only $\sim 20 \mu\text{U}\cdot\text{mL}^{-1}$ at 30 min after WH ingestion and $\sim 11 \mu\text{U}\cdot\text{mL}^{-1}$ at 45 min after CS ingestion. Area under the curve for insulin was greater for WH than for CS (752 ± 130 and $372 \pm 69 \mu\text{U}\cdot\text{mL}^{-1}\cdot\text{min}^{-1}$, respectively; $P = 0.02$).

Amino acid exchange. Amino acid balance across the leg over time is presented in Figure 7, and the amino acid exchange (i.e., area under the curve for net balance) is presented in Figure 8. Leucine net balance changed from

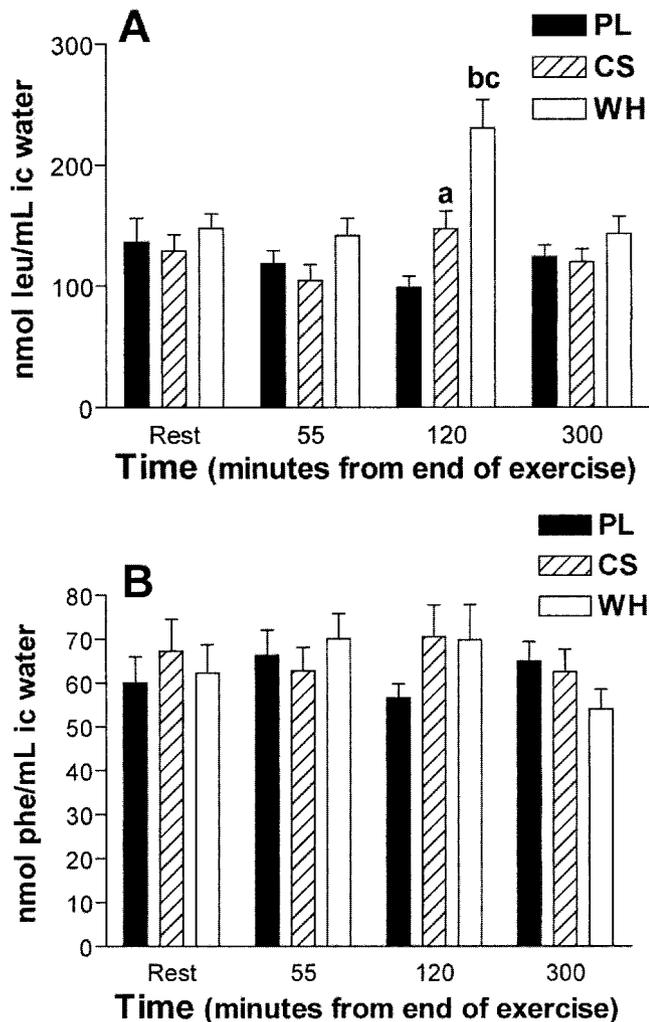


FIGURE 5—Muscle intracellular leucine (A) and phenylalanine (B) concentration at rest and in biopsy samples taken 55 min after exercise (immediately before drink ingestion; 55), 1 h after drink ingestion (120), and 4 h after drink ingestion (300) from the vastus lateralis. All samples were after resistance exercise during ingestion of a placebo (PL), 20 g of casein (CS), or 20 g of whey protein (WH); a, significantly different ($P < 0.001$) from CS biopsy 2; b, significantly different ($P < 0.001$) from WH biopsies 1, 2, and 4; c, significantly different ($P < 0.001$) from PL and CS for biopsy 3. All values are mean \pm SE.

negative to positive after ingestion of both CS and WH. Net leucine balance was significantly greater than baseline at 10 and 20 min after drink ingestion (70 and 80 min after exercise) during CS, and from 10 to 60 min after drink ingestion for WH. Both WH and CS leucine balances were greater than PL from 10 to 90 min after drink ingestion (70–150 min after exercise). WH leucine balance was greater than PL at 60 and 90 min after drink ingestion (120 and 150 min postexercise). WH leucine balance was greater than CS at 20–60 min after drink ingestion (80–120 min after exercise). The mean leucine exchange was not significantly different from zero during PL (Fig. 8A).

Phenylalanine net balance remained negative throughout PL (Fig. 7B), indicating a release of phenylalanine; however, phenylalanine exchange after the drink ingestion was not significantly different from zero (Fig. 8B). Net phenylalanine balance changed from negative to positive in re-

sponse to ingestion of CS and WH (Fig. 7B). Phenylalanine balance was significantly increased over baseline at 20 and 30 min after ingestion of CS (80 and 90 min after exercise) and at 10 min after ingestion of WH (70 min after exercise). Phenylalanine balance during CS was greater than during PL at 10 and 30 min after drink ingestion (70 and 90 min after exercise) and greater than WH at 10 min after drink ingestion (70 min postexercise). Phenylalanine balance was greater during WH than PL at 20–45 min after drink ingestion (80–105 min after exercise).

Both CS ($P < 0.01$) and WH ($P < 0.001$) resulted in much greater uptake of leucine than PL (Fig. 8A). WH leucine uptake was $\sim 30\%$ greater than CS leucine uptake ($P < 0.05$). The proportion of ingested leucine taken up by the leg was similar for CS and WH ($\sim 11.6 \pm 1.6$ and $14.7 \pm 2.2\%$, respectively).

Both CS and WH improved net balance to positive resulting in a net uptake of phenylalanine after drink ingestion (Fig. 8B). Phenylalanine uptake was $\sim 35\%$ greater for CS than WH, but the variability was large, and there was no significant difference between CS and WH for uptake of phenylalanine. The proportion of ingested phenylalanine taken up by the leg was similar for CS and WH ($\sim 9.4 \pm 0.9$ and $10.4 \pm 2.4\%$, respectively). Amino acid uptake relative to the amount ingested varied. Whereas the amount of amino acid taken up by the leg relative to that ingested was similar for leucine and phenylalanine during CS, it was significantly greater for leucine than phenylalanine during WH.

DISCUSSION

The principle result of this study was that both casein and whey protein ingestion after resistance exercise resulted in positive amino acid balance, indicative of net muscle protein synthesis. A secondary goal of the present investigation was to determine whether ingestion of proteins with differing digestive properties engendered differing anabolic responses in the muscle. There was no significant difference in net phenylalanine balance in response to ingestion of

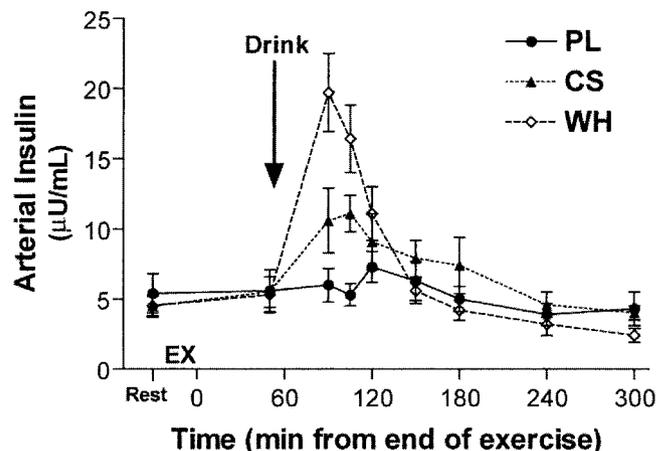


FIGURE 6—Serum insulin concentration at rest and for 5 h after resistance exercise during ingestion of a placebo (PL), 20 g of casein (CS), or 20 g of whey protein (WH). All values are mean \pm SE.

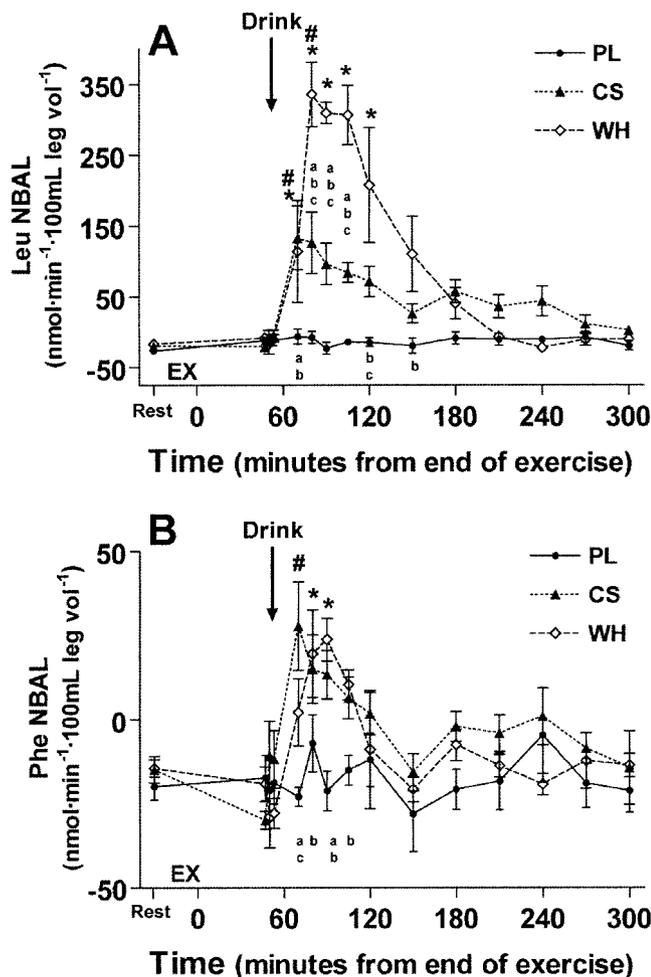


FIGURE 7—Leucine (A) and phenylalanine (B) net balance across the leg at rest and for 5 h after resistance exercise during ingestion of a placebo (PL), 20 g of casein (CS), or 20 g of whey protein (WH). All values are mean \pm SE.

casein and whey proteins, suggesting that any difference in the response of muscle to ingestion of the two proteins was not large.

Previously, we have demonstrated that net muscle protein synthesis results from infusion (4) and ingestion (7,12,16) of free amino acids after resistance exercise. Moreover, non-essential amino acids are unnecessary to engender an anabolic response by the muscle (7,12,16). The present study is the first to demonstrate that single proteins stimulate net muscle protein synthesis after resistance exercise. Taken together, these results suggest that proteins stimulate muscle protein accretion by supplying the essential amino acids necessary for muscle anabolism after exercise. Although not measured in this study, several studies have demonstrated that the stimulation of net muscle protein synthesis by amino acids after exercise is primarily due to increased muscle protein synthesis, rather than decreased muscle protein breakdown (7,12,15–17). It could be that a similar mechanism is responsible for changes in muscle protein synthesis caused by changes in blood amino acid concentrations.

The pattern of amino acid appearance in the blood was markedly different for casein than for whey proteins (Fig. 3

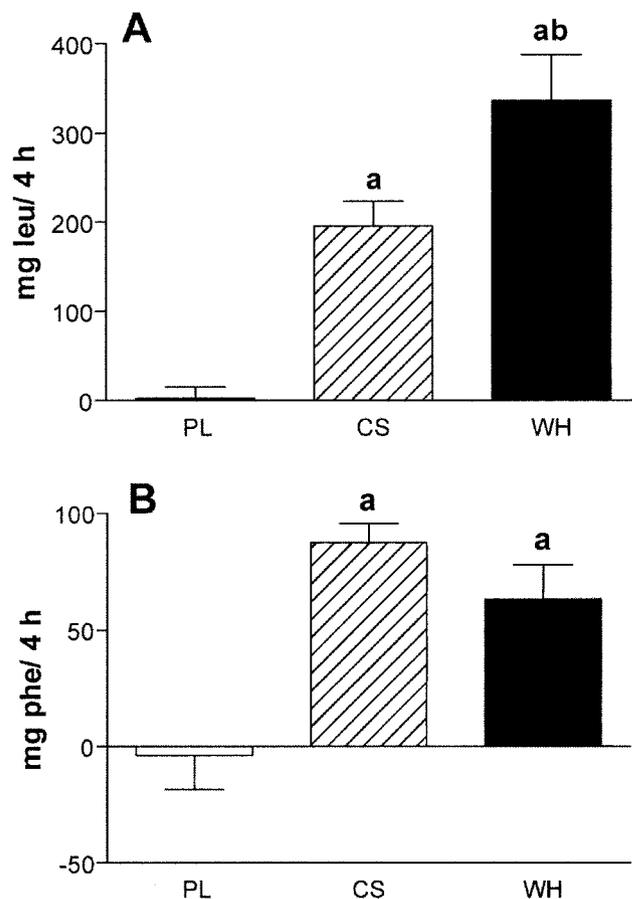


FIGURE 8—Leucine (A) and phenylalanine (B) exchange (area under the curve for net balance) across the leg for 5 h after resistance exercise during ingestion of a placebo (PL), 20 g of casein (CS), or 20 g of whey protein (WH); a, significantly ($P < 0.01$) different from PL; b, significantly ($P < 0.05$) different from CS. All values are mean \pm SE.

and 4). Arterial leucine concentrations peaked earlier and at a greater magnitude during WH than CS. Boirie et al. (6) reported a similar pattern of leucine concentrations in response to whey and casein ingestion at rest. The differences in leucine appearance in the blood were attributed to differences in digestive properties (6,8,9). Whey proteins remain soluble in the stomach, and thus are emptied rapidly, whereas casein is converted into a solid clot, and emptied more slowly from the stomach (10,11). These differences in digestive properties likely contribute to the different pattern of amino acid concentrations noted after ingestion of whey proteins and casein. Thus, casein has been termed a “slow” protein, and whey protein has been termed a “fast” protein (6). In this study, although the pattern of leucine in the blood of the leg after protein ingestion was as would be expected from previous studies, phenylalanine concentrations did not seem to follow the previously reported patterns as clearly. Arterial phenylalanine concentrations initially were similar for the proteins; the arterial concentrations remained elevated for a longer period of time after casein ingestion. It is possible that the large amount of leucine transported into the blood from the gut overloads the transport proteins, competitively inhibiting the amount of phenylalanine that can be transported at the same time. Phenylalanine and leucine

share the L amino acid transport system, and this system can be competitively inhibited, thus blunting the peak phenylalanine concentrations. Although in this study it was not possible to measure inward amino acid transport, this notion is supported by the muscle intracellular concentration data shown in Figure 5. One hour after ingestion of the whey protein solution, intracellular leucine concentration was significantly elevated by ~110%. However, there was no significant change in intracellular phenylalanine concentration, despite a 60% increase in blood concentrations. If the phenylalanine transport into the muscle was being inhibited by excess leucine, then the lower uptake of phenylalanine during WH may have been due to the increased leucine resulting in decreased protein accretion.

Differences in digestive properties of the proteins after exercise also may lead to differences in the anabolic response to each protein. Boirie and coworkers (6,8,9) concluded that whole-body leucine balance was improved to a greater extent by casein than by whey proteins in resting subjects. Subsequent data suggested that it was the pattern of appearance that was crucial to this difference, rather than the amino acid composition of the proteins (6,8,9). Our results suggest that this conclusion is not applicable to the muscle after exercise. Postexercise net leucine balance across the leg was greater after ingestion of whey proteins than of casein (Fig. 8). On the other hand, there was no significant difference in phenylalanine balance, although the mean value was ~35% less for whey than for casein. Perhaps more importantly, further evidence for a lack of difference in the anabolic response between the two proteins is provided when the uptake of the amino acids is examined relative to that ingested. In the whole-body studies, the amount of protein ingested was adjusted to ensure that the amount of leucine was the same (6,8,9). However, in our study, the amount of leucine and phenylalanine consumed varied according to the protein ingested. There is no difference in the percent of amino acid taken up by the leg that was ingested between the ingested proteins (~10% for phenylalanine and ~11–15% for leucine), suggesting that there is no difference in the anabolic response. It is not clear why our results differ from those of the previous studies. It is possible that tissues other than muscle account for the superior whole-body response to casein noted in previous studies (6,8,9). Alternatively or additionally, the stimulation of muscle protein metabolism by resistance exercise (3,13,14) may alter the response to ingestion of these proteins.

Determination of the response of muscle protein balance to protein ingestion is based on the uptake of individual amino acids. It is assumed that amino acid uptake represents net muscle protein synthesis and the potential for muscle protein accretion. The discrepancy in the response of leucine and phenylalanine uptake to the two proteins indicates that reliance on one amino acid to represent muscle protein balance may not be advisable. These results suggest that in an acutely changing situation, such as what occurs after bolus ingestion of proteins, the metabolism of amino acids may differ. In this case, the uptake of phenylalanine can be

considered indicative of the true response of muscle protein balance. Phenylalanine is not oxidized in the muscle (18), so the only metabolic fate for phenylalanine taken up by the muscle is for protein synthesis. The muscle intracellular pool of phenylalanine at the end of sampling was the same as at baseline (Fig. 5B), and net phenylalanine balance had returned to baseline levels by the end of sampling (Fig. 7B), indicating that sampling accounted for the entire response to the ingested protein. Thus, it can be assumed that all the phenylalanine taken up by the muscle was utilized for muscle protein synthesis. Leucine taken up across the leg may be utilized for muscle protein synthesis, as well as oxidation by the muscle. Thus, the uptake values for leucine do not truly represent the potential for muscle protein accretion.

The much greater blood leucine concentration in response to WH than CS was likely responsible for the greater insulin response, as well as increased leucine oxidation. It has been shown previously that rates of leucine oxidation on a whole-body level are primarily a function of leucine availability (20). Hence, it is likely that the faster digestion of whey proteins resulting in much greater leucine levels in the blood and delivery to the muscle resulted in much greater leucine oxidation in the muscle. A greater percentage of the ingested leucine than phenylalanine was taken up by the WH group, but not the CS group, consistent with excess oxidation of the leucine. If we assume that the phenylalanine uptake value represents the true value of net muscle protein synthesis, and that ratio of leucine to phenylalanine in muscle protein is 9/4, we can then estimate the amount of leucine oxidized by the muscle after ingestion of each protein. These calculations indicate that casein results in negligible oxidation of leucine, whereas whey protein ingestion resulted in oxidation of ~57% of the leucine taken up by the muscle. It is clear that the intracellular leucine concentration was elevated at 1 h after ingestion of both CS and WH, but that WH was greater than CS (Fig. 5A).

There is some suggestion that leucine alone stimulates muscle protein synthesis after exercise, thus suggesting that the elevated leucine was responsible for stimulation of muscle protein synthesis and net muscle protein synthesis after protein ingestion. Previously, it was demonstrated that ingestion of leucine alone stimulates muscle protein synthesis after prolonged treadmill exercise in rats (1). However, the relevance of those results in rats running on a treadmill to the situation in the present study is unclear. In that study, a maximal dose of leucine (equivalent to the amount normally eaten in a 24-h period) was given to rats after treadmill running, resulting in recovery of muscle protein synthesis to baseline resting levels (1). In our study, a relatively smaller amount of leucine was ingested by the subjects after a resistance exercise bout. To our knowledge, no study has demonstrated that levels of leucine ingested by these volunteers' results in increased muscle protein anabolism in humans after resistance exercise.

Because the mean value of phenylalanine uptake for CS is 35% greater than for WH, we cannot dismiss the possibility that there is a difference between CS and WH, but it is not significantly different, because of insufficient sample

size and a Type II error. However, power calculations reveal that the addition of 16 more subjects (7 in CS and 9 in WH) responding with identical values to the present subjects would give us only a 50% chance of statistical significance with a one-tailed *t*-test. Furthermore, the phenylalanine uptake relative to ingested (i.e., the ratio of intake/ingested phenylalanine) is virtually identical for the two proteins, lending support to the notion that there is no difference in the anabolic response to the two proteins. The variability in the response to WH was much greater than for CS. The variability in the individual response to WH ingestion may be an important physiological factor. It is conceivable that the response of net muscle protein synthesis to whey protein is mediated by several factors, all stemming from the rapidity of release from the gut, thus complicating the response and increasing individual variation. Regardless, it is clear that both proteins stimulate an anabolic response in muscle after exercise, but it is less clear whether one protein results in greater stimulation than the other.

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In summary, ingestion of casein and whey proteins after resistance exercise stimulated net muscle protein synthesis by providing essential amino acids. Thus, consumption of whole proteins after exercise may be an effective strategy to increase muscle size and strength. The blood amino acid pattern of appearance differs for casein and whey proteins, likely resulting in greater oxidation of leucine when whey protein was ingested. Our results do not clearly demonstrate a difference in the response of net muscle protein synthesis between casein and whey proteins.

We would like to thank the nurses and staff of the General Clinical Research Center at the University of Texas Medical Branch-Galveston. We also thank Dr. David Chinkes for statistical assistance, and the volunteers who participated in the studies for their time and effort.

This work was supported in part by the Gatorade Sports Science Institute and by grants 8940 and 15489 from the Shriners Hospitals for Children. Studies were conducted on the General Clinical Research Center at the University of Texas Medical Branch at Galveston, funded by grant M01 RR 00073 from the National Center for Research Resources, NIH, USPHS.