Consumption of fluid skim milk promotes greater muscle protein accretion after resistance exercise than does consumption of an isonitrogenous and isoenergetic soy-protein beverage[1–3]

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ABSTRACT

Background: Resistance exercise leads to net muscle protein accretion through a synergistic interaction of exercise and feeding. Proteins from different sources may differ in their ability to support muscle protein accretion because of different patterns of postprandial hyperaminoacidemia.

Objective: We examined the effect of consuming isonitrogenous, isoenergetic, and macronutrient-matched soy or milk beverages (18 g protein, 750 kJ) on protein kinetics and net muscle protein balance after resistance exercise in healthy young men. Our hypothesis was that soy ingestion would result in larger but transient hyperaminoacidemia compared with milk and that milk would promote a greater net balance because of lower but prolonged hyperaminoacidemia.

Design: Arterial-venous amino acid balance and muscle fractional synthesis rates were measured in young men who consumed fluid milk or a soy-protein beverage in a crossover design after a bout of resistance exercise.

Results: Ingestion of both soy and milk resulted in a positive net protein balance. Analysis of area under the net balance curves indicated an overall greater net balance after milk ingestion ($P < 0.05$). The fractional synthesis rate in muscle was also greater after milk consumption (0.10 ± 0.01%/h) than after soy consumption (0.07 ± 0.01%/h; $P = 0.05$).

Conclusions: Milk-based proteins promote muscle protein accretion to a greater extent than do soy-based proteins when consumed after resistance exercise. The consumption of either milk or soy protein with resistance training promotes muscle mass maintenance and gains, but chronic consumption of milk proteins after resistance exercise likely supports a more rapid lean mass accrual. *Am J Clin Nutr* 2007;85:1031–40.

KEY WORDS Skeletal muscle, protein synthesis, dietary protein, feeding, hypertrophy

INTRODUCTION

Both hyperaminoacidemia (1–3) and resistance exercise (4–8) independently stimulate muscle protein synthesis. Furthermore, there is an additive effect of combining resistance exercise with feeding (3, 9–12), which leads to an enhanced anabolic environment. The gain in muscle protein mass induced by resistance training is due to the summation of the series of acute responses of muscle protein synthesis and breakdown caused by the combined stimulus of exercise and feeding (13, 14).

It is currently unclear whether proteins from different sources induce a greater anabolic response after resistance exercise. Different milk proteins result in a different time course of hyperaminoacidemia (15, 16). Proteins, such as soy and whey, which are digested rapidly, lead to a large but transient rise in aminoacidemia, stimulate protein synthesis, and are referred to as "fast" proteins. By contrast, casein protein is considered a "slow" protein because it promotes a slower, more moderate, and longer lasting rise in plasma amino acids and does not stimulate protein synthesis, at least at the whole body level, but suppresses proteolysis (15). Our hypothesis was that, to promote an anabolic environment for muscle protein synthesis after resistive exercise, a supply of both fast dietary proteins, which stimulate protein synthesis, and slow dietary proteins, which suppress muscle protein breakdown, are advantageous (15, 16). Such a combination of fast and slow proteins is available in fluid bovine milk, which contains ≈80% casein and ≈20% whey protein by mass. Whole-body protein turnover data support the hypothesis that milk provides a combination of whey to stimulate synthesis and casein to inhibit breakdown (16). Using a modeling approach, Fouillet et al (17) estimated that ingestion of soy protein resulted in a lower whole-body retention of dietary nitrogen than did milk protein. Furthermore, soy protein induced a more rapid digestion, transit time, and absorption of nitrogen from the intestine, which was more readily retained by the splanchnic bed. This sequestering of amino acids by the splanchnic bed caused a subsequent reduction in amino acid uptake by peripheral tissues, including skeletal muscle (17). Data from previous studies suggest that the digestibility of a protein source differentially affects whole-body protein turnover at rest;

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however, it has yet to be fully elucidated what effect the protein source has on whole-body and muscle protein turnover after resistance exercise.

Given our knowledge of the effect of dietary protein ingestion at rest on whole-body protein turnover, we aimed to investigate the effect of oral ingestion of either fluid nonfat milk or an isonitrogenous and isoenergetic macronutrient-matched soy-protein beverage on whole-body and muscle protein turnover after an acute bout of resistance exercise in trained men. We hypothesized that the ingestion of milk protein would stimulate muscle anabolism to a greater degree than would the ingestion of soy protein, because of the differences in postprandial aminoacidemia.

SUBJECTS AND METHODS

Subjects

Eight healthy men with a mean (±SE) age of 21.6 ± 0.3 y, body mass of 81.7 ± 5.9 kg, and height of 177.6 ± 4.1 cm who regularly engaged in resistance training (≥4 d/wk) were recruited for the study. Each participant was advised of the purposes of the study and its associated risks. The participants were required to complete a health questionnaire and were deemed healthy on the basis of the responses. All subjects were nonsmokers, did not use any medication chronically, and gave their written informed consent before participation. The Hamilton Health Sciences Research Ethics Board approved the project, which complies with all standards set by the Declaration of Helsinki.

Experimental protocol

The subjects performed 2 trials in random order separated by ≥1 wk. On each trial day, the participants received either a soy or milk beverage after a unilateral resistance exercise bout. A unilateral bout was used to isolate the effect of protein ingestion, after resistance exercise, to a single muscle mass with ample postexercise hyperemia and amino acid supply. On each trial day, the samples were taken only from the exercised leg. The drink order and leg that was tested, in terms of dominance based on strength, were randomized in a counterbalanced manner.

Each subject's single repetition maximum (1 RM, ie, the maximal amount of weight lifted at one time) for each leg was tested on 2 separate occasions ≥2 wk before the trials began ($\bar{x}$ ± SE: seated leg press, 122 ± 7 kg; prone hamstring curl, 51 ± 3 kg; and seated leg extension, 69 ± 4 kg). The mean (±SE) leg volume was 12.7 ± 0.7 L, which was determined by using an anthropometric approach (18).

The participants were asked to refrain from participating in strenuous exercise and from consuming alcohol for 2 d before each trial day. On each trial day, the subjects consumed a beverage with a defined formula (2170 kJ, 67% of energy as carbohydrate, 17% of energy as protein, and 16% of energy as fat; Boost, Novartis Nutrition Corporation, Fremont, MI) in the morning (0600) after an overnight fast (no food after 2000 the previous night). After 2.5 h (postabsorptive), the subjects reported to the exercise metabolism laboratory at McMaster University. A baseline breath sample was collected into a 100-L Douglas bag before being injected into a 10-mL evacuated tube for subsequent analysis of baseline $^{13}CO_2/^{12}CO_2$. Breath enrichment was analyzed by using an automated $^{13}CO_2$ isotope ratio mass spectrometry breath-analysis system (BreathMat plus; Thermo Finnigan, San Jose, CA) per previously described methods (19). Breath-by-breath carbon dioxide production was measured for 5 min. with an online gas collection system (Moxus; AEI Technologies, Pittsburg, PA).

A polyethylene catheter was then inserted into a forearm vein, from which a baseline blood sample was taken to determine background amino acid enrichment. After the baseline blood sample was drawn, the bicarbonate pool was primed with $Na^{13}CO_3$ (3.5 μmol/kg), and primed constant infusions of L-[1-^{13}C]leucine (prime: 7.6 μmol/kg; infusion rate: 7.6 $\mu mol \cdot kg^{-1} \cdot h^{-1}$) and L-[*ring*-$^2H_5$]phenylalanine (prime: 2 μmol/kg; infusion rate: 2.4 $\mu mol \cdot kg^{-1} \cdot h^{-1}$) were initiated (**Figure 1**). All isotopes were purchased from Cambridge Isotopes (Andover, MA), dissolved in 0.9% saline, filtered through a 0.2-μm filter, and infused with the use of a calibrated syringe pump (KD Scientific, Holliston, MA). The infusion protocol was designed so that steady state was achieved within 1.5 h in both the intramuscular and plasma pools. After baseline sampling, the subjects rested for 1.5 h, during which time a 20-gauge polyethylene catheter was inserted into the radial artery for blood sampling (Figure 1). The catheter was kept patent by using periodic flushes of 0.9% saline containing 1 IU heparin/mL, which was maintained at a pressure above systolic pressure. At ≈1–2 cm distal to the inguinal crease, a 3 French 10-cm polyethylene catheter was inserted into the femoral vein in an anterograde orientation.

After 1.5 h, blood samples were taken from the radial artery and femoral vein. Femoral artery blood flow was determined by using pulsed-wave Doppler ultrasonography, and a percutaneous muscle biopsy sample was obtained. The subjects then performed a standardized leg workout, ie, leg press, hamstring curl, and knee extension with a single leg. The subjects performed 4 sets of each exercise, with 10 repetitions per set for the first 3 sets, and the last set to exhaustion. Exercise intensity was set at 80% of 1 RM with an interset rest period of 2 min. After the resistance exercise protocol was completed, blood samples and muscle biopsy samples were obtained. The subjects then ingested (in a randomized single-blinded fashion) a 500-mL drink that contained either fluid nonfat milk or an isonitrogenous, isoenergetic, and macronutrient-matched soy-protein beverage (745 kJ, 18.2 g protein, 1.5 g fat, and 23 g carbohydrate as lactose for milk and as maltodextrin for the soy beverage). The drinks were made from commercially available isolated soy protein (GeniSoy, Fairfield, CA) or skim milk powder. After drink consumption, femoral artery blood flow, breath samples, arterial and venous blood samples, and muscle biopsy samples were obtained every hour thereafter for 3 h (Figure 1). The biopsy samples were taken only from the exercised leg within a given experimental condition. On a second day, ≥1 wk after the initial trial, the subjects followed the same protocol, except that the contralateral leg was tested and they received the alternative beverage after exercise.

Analytic methods

Blood flow

Femoral artery mean blood velocity (MBV) was measured by using pulsed-Doppler ultrasonography (model system 5; GE Medical Systems, Horten, Norway). Data were acquired continuously with a 10-MHz probe, corrected for insonation angle, placed on the skin surface 2–3 cm proximal to bifurcation of the femoral artery into the superficial and profundus segments. The

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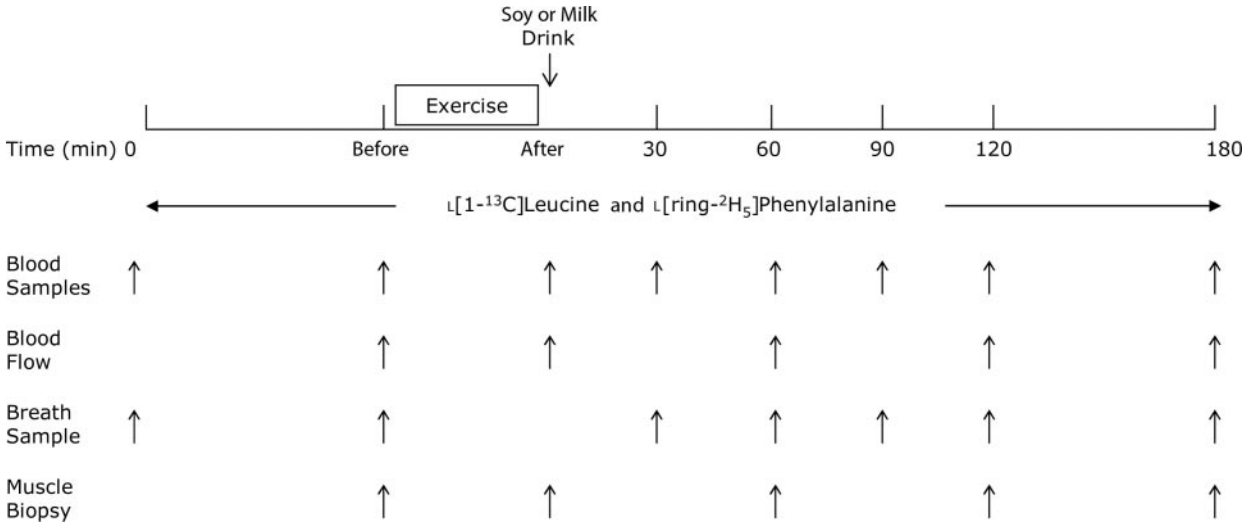


FIGURE 1. Experimental protocol.

ultrasound gate was maintained at full width to ensure complete insonation of the entire vessel cross-section with constant intensity (20). MBV data were recorded at 200 Hz and stored on a computer for subsequent analysis. Average MBV was calculated by integrating the total area under the MBV profile for 15 subsequent heart cycles at each time point. Femoral artery diameter was measured simultaneously by using 2-dimensional echo-Doppler ultrasound (10-MHz probe) and stored to videotape for subsequent analysis. Arterial diameter was determined in triplicate before and immediately after exercise and 1, 2, and 3 h after drink ingestion. At each time point, 3 measures of systolic and diastolic diameters were used to determine mean diameter.

$$\text{Mean leg blood flow (mL/min)} = \text{MBV(cm/s)} \times r^2 \times 60 \text{ s/min} \quad (1)$$

Blood samples

Blood samples were collected into heparinized evacuated containers. Whole blood (100 μL) was added to ice-cold perchloric acid (PCA; 0.6 mol/L, 500 μL); the solution was mixed and allowed to sit on ice for 10 min to precipitate all proteins. This mixture was then centrifuged at 4000 $\times$ *g* (15 000 rpm) for 2 min at 4 °C. The PCA was neutralized with 250 μL of 1.25 mol $KHCO_3$/L, and the reaction was allowed to proceed on ice for 10 min. The samples were then centrifuged at 4000 $\times$ *g* (15 000 rpm) for 2 min at 4 °C. The supernatant fluid was stored at −50 °C until analyzed further (blood amino acid concentrations and blood phenylalanine enrichment). Blood plasma was obtained by centrifuging the evacuated tube at 4 °C for 10 min at 4000 $\times$ *g* (4500 rpm). The plasma was stored at −50 °C for the measurement of plasma insulin and glucose concentrations and plasma α-ketoisocaproic acid enrichment as described below.

Muscle biopsy samples

Needle biopsy samples from the vastus lateralis were obtained under local anesthesia (1% xylocaine). A 5-mm Bergström biopsy needle modified for manual suction was used to obtain $\approx$100 mg of muscle tissue from each biopsy. Biopsies were obtained from separate incisions from the same leg during each trial and from the contralateral leg during the following trial. The muscle was dissected free of any visible fat and connective tissue and was immediately frozen in liquid nitrogen and stored at −80 °C before analysis.

Blood analysis

Plasma was assayed for insulin by using a commercially available radioimmunoassay kit from Diagnostic Products Corporation (Los Angeles, CA). Neutralized blood PCA extract was assayed for glucose by using a standard enzymatic method (21). Plasma α-ketoisocaproic acid enrichment was determined by using methods described previously (22, 23).

Muscle sample analysis

Muscle samples were lyophilized to dryness while being incubated on dry ice (Savant, Rockville, MD). Samples were manually powdered and weighed. To determine intracellular amino acid concentration and phenylalanine enrichments, a portion of the muscle sample was extracted with 0.5 mol PCA/L and neutralized with 2.2 mol $KHCO_3$/L. The PCA extract was removed and stored at −50 °C until analyzed further. Subsequently, to determine protein-bound phenylalanine enrichment, the remaining muscle pellet was washed with distilled water, dried, and then hydrolyzed in 6 mol HCl/L at 100 °C for 24 h. The protein hydrolysate was neutralized and passed over a PepClean C_{18} Spin Column (Pierce, Rockford, IL) for purification. Desorption of amino acids from the column was accomplished with a 70% acetonitrile solution, and the eluate was collected and dried under nitrogen gas.

HPLC amino acid analysis

To determine whole blood and muscle intracellular amino acid concentrations, the whole-blood and muscle PCA extract was derivatized by using a Waters AccQ·Fluor reagent kit (Milford, MA) by heating for 30 min at 55 °C to form the 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate derivative of all physiologic amino acids. Samples and standards (Sigma, St Louis, MO) were run on an HPLC (HPLC: Waters model 2695; column: Waters Nova-Pak C_{18}, 4 μm; detector: Waters 474 scanning fluorescence detector). The amino acids were detected by using a scanning fluorescence detector with excitation and emission wavelengths of 250 and 395 nm, respectively. Amino acid peak areas

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were integrated and compared with known standards and analyzed by using a Waters Millenium[32] software package (Milford, MA). This method achieved separation of 19 of the 20 physiologic amino acids, with the exception of tryptophan, which was not included in the analysis.

Protein amino acid content analysis

To determine the amino acid content of the milk and soy proteins ingested by the participants, 5 aliquots of each protein were hydrolyzed in 6N HCl for 24 h at 100 °C. The samples were then neutralized with 6N NaOH and filtered through a 0.2-μm filter. A small portion of the sample was then derivatized in the same manner as were the blood and muscle samples and run on the HPLC to determine the percentage of each individual amino acid (mg amino acid/mg protein). The milk protein was composed of 43% essential and 23% branched-chain amino acids (7.6% Lys, 2.6% Met, 4.3% Phe, 5.5% Thr, 5.6% Ile, 10.5% Leu, and 7.0% Val). Analysis of the soy-protein amino acid content showed that it was made up of 41% and 21% essential and branched-chain amino acids, respectively (7.0% Lys, 1.4% Met, 5.0% Phe, 5.7% Thr, 5.4% Ile, 9.6% Leu, and 6.4% Val).

Phenylalanine enrichment

To determine the enrichment of phenylalanine in blood and muscle, a *tert*-butyl dimethylsilyl (*t*-BMDS) derivative was prepared. The blood and intracellular muscle PCA extracts were transferred into threaded borosilicate tubes and lyophilized in a SpeedVac rotary evaporator (Savant Instruments, Farmingdale, NY). To derivatize the dried eluent from the column clean-up for the bound sample and dried PCA extract, 50 μL HPLC grade acetonitrile and 50 μL *N*-methyl-*N*-(*tert*-butyldimethylsilyl) trifluoro-acetaminde + 1% *tert*-butyldimethylchlorosilane (MTBSTFA and 1% TBDMCS; Regis, Morton Grove, IL) were added to the sample. Phenylalanine enrichment was analyzed by electron-impact ionization capillary gas chromatography–mass spectrometry (GC Hewlett-Packard 6890: Palo Alto, CA; MSD Agilent 5973: Palo Alto, CA) in electron ionization mode (23). The enrichment of phenylalanine in the PCA blood and muscle intracellular extracts was analyzed at mass-to-charge (*m/z*) ratios of 234 ($m + 0$ – baseline) and 239 ($m + 5$). For the protein-bound phenylalanine enrichment, a standard curve was used and *m/z* ratios of 234, 237, and 239 were used (24, 25).

Calculations

The fractional synthetic rate (FSR) of muscle proteins was calculated as the rate of tracer incorporation into mixed muscle proteins by using the enrichment of intracellular free phenylalanine as the precursor, according to a previously published equation (6).

Chemical phenylalanine and total amino acid (TAA) net balance (NB) across the leg was calculated, as described elsewhere (9–12), from the difference between arterial and venous concentrations multiplied by femoral artery blood flow:

$$\mathrm{NB} = (C_a - C_v) \times \mathrm{BF} \quad (2)$$

where C_a is the arterial amino acid concentration, C_v is the venous amino acid concentration, and BF is femoral artery blood flow. Because phenylalanine is not metabolized in muscle, a positive net balance signifies net uptake and muscle protein anabolism and a negative net value indicates net release of amino acids and muscle protein catabolism. Nitrogen NB was calculated by multiplying the concentration of each amino acid by nitrogen content per amino acid.

Area under the NB curve was calculated by using the PRISM software package (GraphPad Software Inc, San Diego, CA). A baseline of 0 was used to determine the total positive area under the curve for the time points after drink consumption (30, 60, 90, 120, and 180 min).

In the 2-pool model, muscle protein synthesis and breakdown is estimated by using the rate of appearance (R_a) and disappearance (R_d), respectively, of L-[*ring*^{2}H$_5$]phenylalanine in the blood (23):

$$R_a = (E_a/E_v - 1) \times C_a \times \mathrm{BF} \quad (3)$$

$$R_d = \mathrm{NB} + R_a \quad (4)$$

where E_a is the arterial enrichment of L-[*ring*^{2}H$_5$] phenylalanine, E_v is the venous enrichment of L-[*ring*^{2}H$_5$] phenylalanine, and C_a is the arterial amino acid concentration.

Leucine flux (Q), oxidation, and nonoxidative leucine disposal (NOLD) were calculated by using previously published equations (26). Exercise and feeding is known to effect the retention of carbon dioxide in the body (23); therefore, values of 0.81 (26) and 0.83 (22) were used for calculations before exercise and during the recovery period, respectively. NOLD was used as an index of whole-body protein synthesis, Q was used as an index of whole-body protein breakdown, and oxidation was used as an index of whole-body protein oxidation.

Statistics

Sample size estimates were based on the ability to detect a 25% difference between groups in mixed muscle fractional synthetic rate using an α value of 0.05 and a β value of 0.2, with an estimated population variance in the measure based on past studies from our lab and from literature values. To protect power, we added 2 subjects to the final calculated sample size estimate. Data were analyzed by using STATISTICA (version 6.0; Statsoft, Tulsa, OK) with a repeated-measures analysis of variance. Area under the curve measures were analyzed by using paired *t* tests. When a significant *F* ratio was observed, a post hoc analysis with Tukey's honestly significant difference test was used to determine differences. Significance was set at $P < 0.05$. Data are presented as means ± SEMs.

RESULTS

All subjects completed the exercise protocols. The number of repetitions and sets were evenly matched so that the exercise stimulus was similar in each trial. Plasma insulin and glucose concentration increased above concentrations before exercise 60 min after drink consumption, with no differences observed between the drinks (**Table 1**). By 120 min after drink consumption, blood glucose and insulin concentrations were no different from those observed before exercise.

Femoral artery blood flow was significantly elevated immediately after the resistance exercise bout and returned to concentrations not different from those before exercise by 60 min after drink consumption (**Table 2**).

The sum of TAA concentration showed a time-by-beverage interaction such that concentration was elevated after both soy- and milk-protein consumption (**Figure 2**) 30, 60, and 90 min

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TABLE 1
Effect of milk- and soy-protein consumption on plasma glucose and insulin concentration[1]

	Before exercise	After exercise	60 min	120 min	180 min
Glucose (mmol/L)					
Milk					
Artery	4.5 ± 0.2	4.7 ± 0.2	5.1 ± 0.3[2]	4.9 ± 0.1	4.7 ± 0.1
Vein	4.0 ± 0.1	4.5 ± 0.2	4.7 ± 0.3[2]	4.4 ± 0.2	4.4 ± 0.1
Soy					
Artery	4.5 ± 0.1	4.8 ± 0.2	5.4 ± 0.2[2]	4.7 ± 0.1	4.6 ± 0.1
Vein	4.0 ± 0.1	4.5 ± 0.2	4.9 ± 0.3[2]	4.4 ± 0.1	4.4 ± 0.1
Insulin (μIU/mL)					
Milk	3.0 ± 0.2	4.1 ± 0.5	14.0 ± 3.2[2]	5.0 ± 0.8	3.6 ± 0.4
Soy	3.4 ± 0.3	4.3 ± 0.5	16.5 ± 4.9[2]	4.1 ± 0.6	3.3 ± 0.4

[1] All values are $\bar{x}$ ± SEM; $n = 8$. A 3-factor ANOVA was performed to test for main effects of time, beverage, and site (artery or vein) on the glucose data. No 3-factor interaction was significant. A 2-factor ANOVA was performed to test for main effects of time and beverage. There was a significant time-by-beverage interaction ($P < 0.05$). A 2-factor ANOVA was performed on the insulin data to test for main effects of time and beverage. A main effect of time was observed. When a significant F ratio was observed, post hoc analysis with Tukey's honestly significant difference test was used to determine differences.
[2] Significantly different from before exercise with both beverage groups combined, $P < 0.05$.

after each drink; however, by 120 min after drink consumption, AA concentrations were no different from those observed before or immediately after exercise. The sum of TAA was significantly greater in the soy trial than in the milk trial 30 min after drink consumption ($P = 0.05$).

Intramuscular lysine and phenylalanine concentrations were significantly elevated above concentrations before exercise by 60 min after both drinks were consumed, but returned to concentrations no different from those before exercise by 120 min after drink consumption (**Table 3**). The intramuscular concentrations of Ile, Leu, Lys, Phe, and Val and the sum of essential amino acids were all significantly reduced at 180 min after the drink was consumed compared with concentrations observed 60 min after the proteins were consumed (Table 3).

Leucine oxidation did not change significantly over the entire protocol (**Table 4**). NOLD, a measure of whole-body protein synthesis, was significantly elevated 1 h after both milk- and soy-protein consumption compared with values before beverage consumption (Table 4). In observations made 120 and 180 min after drink consumption, NOLD was not significantly different from values observed before exercise. Leucine flux, an indication of whole-body protein breakdown, was significantly elevated 60 min after and protein-drink consumption compared with values observed before exercise (Table 4). However, by 120 min after exercise, leucine flux was not significantly different from that before exercise. There were no differences between soy and milk in any variables measured by whole-body protein oxidation or turnover.

FSR showed a significant time-by-beverage interaction such that FSR was significantly greater during the 3 h of recovery from exercise after both soy and milk drink consumption than after the time period when resistance exercise was performed (**Figure 3**). There was no difference in muscle FSR between the soy and milk trials during the exercise time period; however, muscle FSR observed after milk consumption was 34% greater than that after soy consumption ($P < 0.05$).

There was no effect of either protein or time on the R_a of phenylalanine (**Figure 4**A). The R_d of phenylalanine showed a main effect of time and was elevated 30 min after protein consumption, when both beverages were combined (Figure 4B). Net phenylalanine balance showed a significant time-by-beverage interaction such that values were negative before exercise (Figure 4C); however, 30 and 60 min after both soy- and milk-protein consumption, NB became positive and remained significantly elevated above concentrations seen before consumption. In the soy trial, NB was again negative by 120 min after drink consumption, whereas NB remained positive in the milk condition and different from that in the soy condition at the 90- and 120-min time points. By 180 min after drink consumption, NB was negative in both the soy and milk trial. Positive area under the milk NB curve was significantly greater than that in the soy trial

TABLE 2
Effect of milk- and soy-protein consumption on femoral artery blood flow[1]

	Before exercise	After exercise	60 min	120 min	180 min
Blood flow (mL · min^{-1} · 100 mL leg^{-1})					
Milk	1.46 ± 0.19	6.44 ± 1.02[2]	1.88 ± 0.30	1.55 ± 0.29	1.42 ± 0.24
Soy	1.51 ± 0.23	6.88 ± 0.98[2]	1.80 ± 0.27	1.49 ± 0.23	1.45 ± 0.25
Blood flow (L/min)					
Milk	0.19 ± 0.03	0.82 ± 0.13[2]	0.25 ± 0.05	0.20 ± 0.04	0.19 ± 0.04
Soy	0.19 ± 0.03	0.84 ± 0.12[2]	0.23 ± 0.04	0.19 ± 0.03	0.18 ± 0.03

[1] All values are $\bar{x}$ ± SEM; $n = 8$. A 2-factor ANOVA was performed to test for main effects of time and beverage. No time-by-beverage interaction was observed. A main effect of time was observed, and post hoc analysis with Tukey's honestly significant difference test was used to determine differences.
[2] Significantly different from before exercise with both beverage groups combined, $P < 0.05$.

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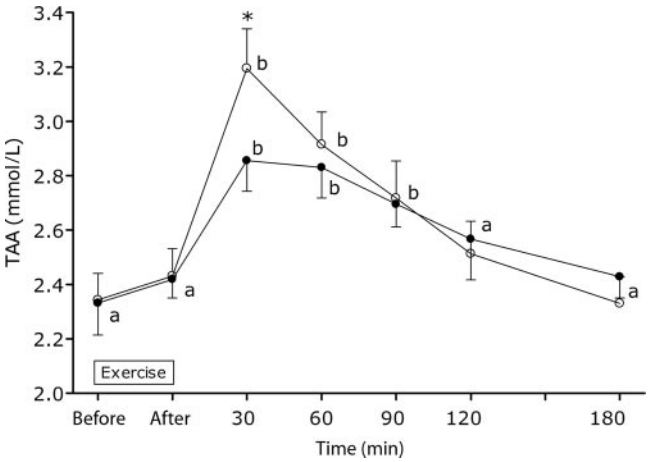


FIGURE 2. Mean (±SEM) whole-blood total amino acid (TAA) concentrations after the consumption of a nonfat milk-protein beverage (●) or an isonitrogenous, isoenergetic, macronutrient-matched (750 kJ, 18.2 g protein, 1.5 g fat, and 23 g carbohydrate) soy-protein beverage (○). A 2-factor ANOVA was performed to test for main effects of time and beverage. A main effect of time and a time-by-beverage interaction was observed, and a post hoc analysis was conducted with Tukey's honestly significant difference test to determine differences. *Significantly different from the milk group at the same time point, $P < 0.05$. Main effect of time: values with different lowercase letters are significantly different, $P < 0.05$. $n = 8$.

(Figure 4D), which indicated a more sustained net positive balance over the course of the trial.

The TAA arterial-venous NB showed a significant time-by-beverage interaction. The TAA balance was negative before exercise and protein consumption (**Figure 5**). After protein consumption, the balance became positive and remained significantly elevated above concentrations before consumption at 30 and 60 min after both soy- and milk-protein consumption. The TAA NB was still positive at 90 and 120 min after drink consumption and was significantly greater than the values at the same times in the soy trial. At 180 min after beverage consumption, the values for TAA balance for both milk and soy were not different from those after consumption. When the area under the TAA NB curve (Figure 5) after drink consumption was analyzed, milk was significantly greater than soy ($P < 0.05$). Nitrogen balance across the leg followed the same pattern as did TAA NB (data not shown). The area under the curve analysis indicated that milk consumption provided a more positive nitrogen balance over the 3 h after milk-protein consumption (53 887 ± 16 524 mmol N/100 mL leg) than after soy-protein consumption (19 485 ± 4820 mmol N/100 mL leg) ($P < 0.01$).

DISCUSSION

The primary finding of the current study was that intact dietary proteins can support an anabolic environment for muscle protein accretion. We observed a significantly greater uptake of amino acids across the leg and a greater rate of muscle protein synthesis in the 3 h after exercise and milk-protein consumption than after soy-protein ingestion. There were no differences in blood flow or in insulin and blood glucose concentrations in response to the drinks. Additionally, the measured essential amino acid content of both proteins was not significantly different.

TABLE 3
Effect of milk- and soy-protein consumption on muscle intracellular indispensable amino acid concentrations[1]

	Before exercise	After exercise	60 min	120 min	180 min
			mmol/kg dry wt		
Ile					
Milk	0.51 ± 0.08	0.51 ± 0.08	0.64 ± 0.15[2]	0.47 ± 0.08	0.45 ± 0.06
Soy	0.43 ± 0.08	0.49 ± 0.09	0.51 ± 0.09[2]	0.45 ± 0.08	0.46 ± 0.11
Leu					
Milk	0.56 ± 0.04	0.57 ± 0.05	0.69 ± 0.06[2]	0.55 ± 0.03	0.54 ± 0.04
Soy	0.50 ± 0.03	0.54 ± 0.03	0.59 ± 0.04[2]	0.54 ± 0.04	0.44 ± 0.02
Lys					
Milk	1.38 ± 0.11	1.41 ± 0.15	1.64 ± 0.09[2,3]	1.39 ± 0.14	1.38 ± 0.13
Soy	1.19 ± 0.10	1.28 ± 0.16	1.53 ± 0.12[2,3]	1.40 ± 0.08	1.29 ± 0.08
Met					
Milk	0.21 ± 0.03	0.20 ± 0.02	0.23 ± 0.03	0.19 ± 0.01	0.18 ± 0.02
Soy	0.19 ± 0.01	0.21 ± 0.01	0.20 ± 0.01	0.19 ± 0.01	0.17 ± 0.02
Phe					
Milk	0.24 ± 0.02	0.25 ± 0.03	0.30 ± 0.03[2,3]	0.24 ± 0.02	0.24 ± 0.02
Soy	0.23 ± 0.01	0.26 ± 0.02	0.28 ± 0.01[2,3]	0.27 ± 0.01	0.23 ± 0.02
Thr					
Milk	3.97 ± 0.35	3.87 ± 0.48	4.16 ± 0.28	3.65 ± 0.44	3.50 ± 0.45
Soy	3.78 ± 0.53	4.06 ± 0.42	3.64 ± 0.43	3.68 ± 0.43	3.30 ± 0.49
Val					
Milk	0.93 ± 0.10	0.98 ± 0.10	1.05 ± 0.07[2]	0.87 ± 0.06	0.85 ± 0.06
Soy	0.80 ± 0.03	0.85 ± 0.05	0.85 ± 0.04[2]	0.83 ± 0.05	0.72 ± 0.02
ΣEAA					
Milk	7.80 ± 0.62	7.80 ± 0.80	8.72 ± 0.49[2]	7.37 ± 0.67	7.15 ± 0.67
Soy	7.13 ± 0.59	7.68 ± 0.60	7.77 ± 0.60[2]	7.36 ± 0.48	6.60 ± 0.50

[1] All values are $\bar{x}$ ± SEM; $n = 8$. EAA, essential amino acids. A 2-factor ANOVA was performed to test for main effects of time and beverage. No time-by-beverage interaction was observed. A main effect of time was observed, and post hoc analysis with Tukey's honestly significant difference test was conducted to determine differences.

[2] Significantly different from 180 min with both beverages combined, $P < 0.05$.

[3] Significantly different from before exercise with both beverage groups combined, $P < 0.05$.

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TABLE 4
Effect of milk- and soy-protein consumption on leucine oxidation, nonoxidative leucine disposal (NOLD), and leucine flux[1]

	Before exercise	60 min	120 min	180 min
Leucine oxidation (μmol · kg^{-1} · h^{-1})				
Milk	23 ± 2	25 ± 1	28 ± 1	23 ± 2
Soy	25 ± 2	26 ± 2	26 ± 2	22 ± 2
NOLD (μmol · kg^{-1} · h^{-1})				
Milk	108 ± 2	139 ± 10[2]	119 ± 6	104 ± 4
Soy	109 ± 4	133 ± 5[2]	120 ± 4	101 ± 4
Leucine flux (μmol · kg^{-1} · h^{-1})				
Milk	132 ± 4	163 ± 10[2]	147 ± 6	127 ± 5
Soy	139 ± 5	164 ± 7[2]	152 ± 5	126 ± 6

[1] All values are $\bar{x}$ ± SEM; $n = 8$. A 2-factor ANOVA was performed to test for main effects of time and beverage. No time-by-beverage interaction was observed. A main effect of time was observed, and post hoc analysis with Tukey's honestly significant difference test was used to determine differences.
[2] Significantly different from before exercise with both beverage groups combined, $P < 0.05$.

To date, 2 studies have shown that the ingestion of whole proteins after resistance exercise can support positive muscle protein balance (27, 28). Both studies examined the effect of fluid milk (27) or its constituent protein fractions, whey and casein (28), on muscle protein balance. Ours, however, is the first study to show that the source of intact dietary protein (ie, milk compared with soy) is important for determining the degree of postexercise anabolism. We found, using arterial-venous balance, that milk protein promoted a more sustained net positive protein balance after resistance exercise than did soy protein. On the basis of our analysis of the amino acid content of the proteins, which showed that milk and soy proteins provide equal amounts of essential amino acids, it is unlikely that the differences in muscle protein synthesis and net protein balance seen in the present study are related to the amino acid content of the respective proteins. Alternatively, because of differences in digestion rates, milk proteins may provide a slower pattern of amino acid delivery to the muscle than soy protein. Therefore, we propose that a difference in the digestion rate of milk and soy protein affects the pattern of amino acid appearance, which ultimately leads to differences in the net amino acid uptake and muscle protein synthesis after resistance exercise.

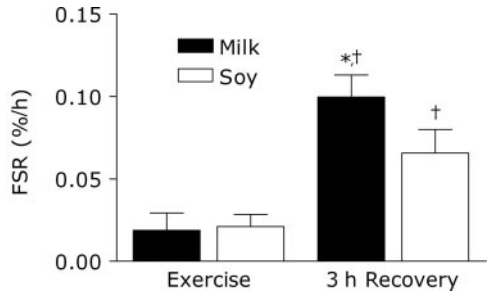


FIGURE 3. Mean (±SEM) fractional synthetic rate (FSR) of muscle proteins during the resistance exercise time period (Exercise) and 3 h after exercise and the consumption of a nonfat milk-protein beverage or an isonitrogenous, isoenergetic, macronutrient-matched (750 kJ, 18.2 g protein, 1.5 g fat, and 23 g carbohydrate) soy-protein beverage (3 h Recovery). A 2-factor ANOVA was performed to test for main effects of time and beverage. A main effect of time and a time-by-beverage interaction was observed, and a post hoc analysis with Tukey's honestly significant difference test was conducted to determine differences. *Significantly different from the soy group at the same time point, $P < 0.05$. †Significantly different from Exercise, $P < 0.05$. $n = 8$.

Hyperaminoacidemia resulting from the ingestion of protein or amino acids after resistance exercise provides a potent stimulus for muscle protein accretion. In particular, essential amino acids appear crucial, and are perhaps all that are necessary, for this process (29). Both soy and milk are high-quality proteins (30). Analysis of the proteins yielded an essential amino acid composition of the milk and soy proteins of 43% and 41% of TAAs, respectively. Analysis of the individual amino acid content of the milk and soy showed that ingestion of 18.2 g protein provided ≥70% of the Recommended Dietary Allowance for all of the individual essential amino acids, except methionine (31). The content of methionine in the soy protein (1.4%) was lower than that in milk protein (2.6%); hence, 18.2 g protein provided 30% and 50% of the Recommended Dietary Allowance for methionine with consumption of soy and milk, respectively. In a series of nitrogen balance studies, Young (30) confirmed that the quality of soy protein is comparable with that of good-quality animal-protein sources, such as milk, and that methionine supplementation was not needed to maintain nitrogen balance. In agreement, our data suggest that the essential amino acid content is likely not the underlying reason why there were no differences between the milk and soy proteins, because no differences in the intramuscular concentration of any of the essential amino acids were detected. This suggests that the availability of essential amino acids, and thus the availability of the amino acids to charge transfer RNA in the muscle for protein synthesis, was not different between the trials. We propose that the rapid digestion of soy protein, and therefore the faster and greater increase in delivery of amino acids from the gut to the liver, may have resulted in an increased utilization of these amino acids for the synthesis of serum proteins and urea, as seen by Bos et al (32), rather than for muscle protein synthesis.

Ingestion of soy protein results in a rapid rise and fall in blood TAA concentrations, whereas milk protein ingestion produces a more moderate rise and a sustained elevation in blood amino acid concentrations (32). In support, our data show that the postexercise consumption of soy protein resulted in a rate of increase in blood TAA concentrations, between the time of ingestion and the first 30 min after exercise, of 25 μmol · L^{-1} · min^{-1} that was followed by a rate of decline of 9 μmol · L^{-1} · min^{-1} in the following 30 min. In contrast, with postexercise milk consumption, we saw a more modest rise in TAA concentration of 14 μmol · L^{-1} · min^{-1} that was followed by a much less rapid

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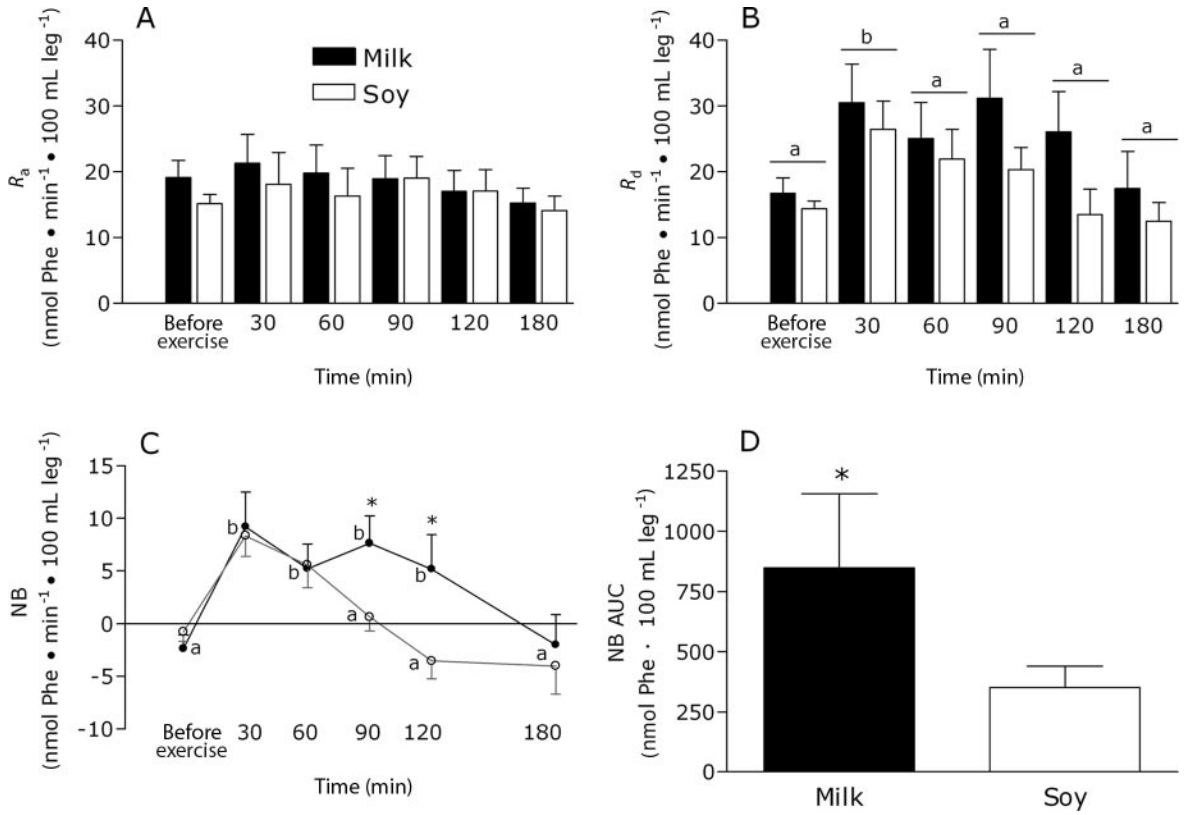


FIGURE 4. Two-pool model–derived mean (±SEM) values for rate of appearance (R_a; A) and rate of disappearance (R_d; B) of phenylalanine, the chemical net balance (NB) of phenylalanine across the leg (C: ●, milk; ○, soy), and the positive area under the curve (AUC) for chemical NB of phenylalanine across the leg after consumption of a nonfat milk-protein beverage or an isonitrogenous, isoenergetic, macronutrient-matched (750 kJ, 18.2 g protein, 1.5 g fat, and 23 g carbohydrate) soy-protein beverage (D). A 2-factor ANOVA was performed on the R_a, R_d, and NB data to test for main effects of time and beverage. Main effects were analyzed with Tukey's honestly significant difference test to determine differences. The NB AUC was analyzed by using a paired *t* test. A significant time-by-beverage interaction was found for the chemical NB of phenylalanine ($P < 0.05$). *Significantly different from the soy group, $P < 0.05$. Significant differences across time are represented by lowercase letters; means with different lowercase letters are significantly different, $P < 0.05$. The data in panel B were analyzed with both beverage groups combined. $n = 8$.

decline of 0.8 μmol · L^{-1} · min^{-1}. The only statistically significant difference in TAA concentration between the soy and milk periods was that at 30 min after consumption. The peak in amino acid concentration that we observed occurred earlier than that observed by Bos et al (32), who found that the amino acid concentration peaked between 1 and 2 h after protein consumption. The test meals consumed by participants in this study (32) had 30% of total energy from fat, which would likely have slowed digestion and, thus, the rate appearance of amino acids into general circulation. We propose that the digestion rate and, therefore, the ensuing hyperaminoacidemia that differed between the milk and soy groups after exercise is what affected the net uptake of amino acids in the exercised leg. Bohé et al (1) have reported that extracellular, not intracellular, amino acid concentrations are regulators of the rise in muscle protein synthesis. Miller et al (10) showed that 2 drinks containing ≈6 g crystalline amino acids given 1 h after resistance exercise transiently and independently stimulated amino acid uptake. These data also showed, despite sustained elevations in amino acid concentration in the hour after the second drink, that amino acid uptake fell sharply. In light of previous data (1, 10), one possibility is that a rise in the extracellular amino acid concentration is a stimulus for muscle protein synthesis; decreases in extracellular amino acid concentration may actually shut off protein synthesis; this scheme is similar in nature to that proposed by Bohé et al (1). This may explain why the sharp rise then fall in aminoacidemia in the soy condition resulted in a lower uptake and net synthesis than in the milk condition.

Previous studies that examined the effect of ingestion of similar quantities of crystalline amino acids on muscle protein turnover have shown that increases in net protein balance with the ingestion of 40 g crystalline indispensable amino acids (8.3 g leucine; 12) were similar in magnitude to that seen with the ingestion of only 6 g crystalline amino acids (2.2 g leucine; 9). These data suggest that, when large quantities of amino acids are ingested, amino acids are likely being directed to deamination and oxidation. In the current experiment, we observed no change in whole-body protein oxidation during the entire study protocol, which indicated that the dose of protein (≈7.5 g indispensable amino acids) did not stimulate amino acid oxidation.

The combined stimulus of resistance exercise and protein or amino acid consumption results in a net protein balance greater than that from either stimulus alone (33, 34). Although the exercise- and feeding-induced response to a single exercise bout is small, muscle protein accumulates and fiber hypertrophy occurs over time with resistance exercise training (34). Muscle fiber hypertrophy occurs when there is a sustained positive balance between muscle protein synthesis and breakdown. Therefore, consumption of milk after resistance exercise, which sustains a more positive net protein balance acutely, should theoretically result in

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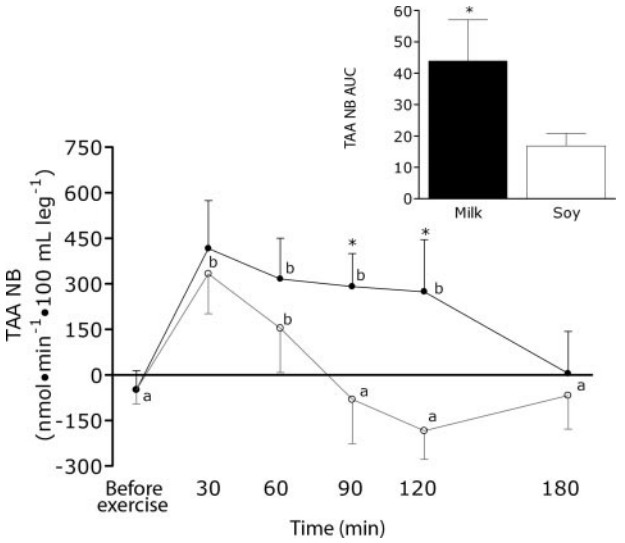


FIGURE 5. Mean (±SEM) total amino acid (TAA) chemical net balance (NB) after consumption of a nonfat milk-protein beverage (○) or an isonitrogenous, isoenergetic, macronutrient-matched (750 kJ, 18.2 g protein, 1.5 g fat, and 23 g carbohydrate) soy-protein beverage (●). Inset: positive area under the curve (AUC) for TAA NB after consumption of the milk or soy beverage. A 2-factor ANOVA was performed on the TAA NB data to test for main effects of time and beverage. Main effects were analyzed by using Tukey's honestly significant difference test to determine differences. The TAA NB AUC was analyzed by using a paired *t* test. A significant time-by-beverage interaction was found for TAA NB ($P < 0.01$). *Significantly different from the soy group, $P < 0.05$. Significant differences across time are represented by lowercase letters; means with different lowercase letters are significantly different, $P < 0.05$. $n = 8$.

greater muscle hypertrophy than consumption of soy protein after exercise. This ability of milk-protein consumption to enhance anabolism after resistance exercise might be particularly valuable to persons with compromised muscle function.

Our value for blood flow of ≈0.21 L/min at rest, excluding the immediate postexercise hyperemic response, compares relatively well with other resting flow values obtained by using Doppler ultrasound measurements (for a review, *see* reference 35). However, because our subjects were all strength-trained men, their average leg volume was 12.7 ± 0.7 L; thus, our reported resting flow (≈ 1.56 mL^{-1} · 100 mL leg volume · min^{-1}) is lower than values reported in other studies (3, 8, 28, 33). The result is that our estimates of R_a, and R_d are lower than those previously reported (3, 8, 28, 33); the differences between our studies and others appear to be due, for the most part, to a lower blood flow. However, we did not observe any between-treatment effects on blood flow (Table 2), which is not surprising given that the same exercise bouts (volume and relative intensity) were performed, and similar insulin responses were observed between trials (Table 1). Hence, we acknowledge that our flow values, collected by an experienced investigator using established procedures that have been shown to be valid in a variety of situations (36–38), might be lower than what others have observed but believe it is unlikely that the between-trial differences were influenced by our measurements of flow.

In conclusion, we found that the consumption of intact dietary proteins resulted in a positive net protein balance and an increased rate of muscle protein synthesis after resistance exercise. Further analysis of area under the NB curves indicated a greater net amino acid balance across the leg, and the measures of muscle FSR indicated greater rates of muscle protein synthesis after milk ingestion than after soy ingestion. These increases in anabolic processes were seen without any concurrent increases in whole-body protein oxidation. It appears unlikely that our results were due to differences in amino acid composition between the proteins, which were minimal. Instead, we favor the hypothesis that differences in the delivery of and patterns of change in amino acids are responsible for the observed differences in net amino acid balance and rates of muscle protein synthesis.

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The authors' responsibilities were as follows—all authors: study conduct, data analysis, and writing and editing of the manuscript. None of the authors had a conflict of interest to declare.

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