

Milk Ingestion Stimulates Net Muscle Protein Synthesis following Resistance Exercise

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ABSTRACT

ELLIOT, T. A., M. G. CREE, A. P. SANFORD, R. R. WOLFE, and K. D. TIPTON. Milk Ingestion Stimulates Net Muscle Protein Synthesis following Resistance Exercise. *Med. Sci. Sports Exerc.*, Vol. 38, No. 4, pp. 667–674, 2006. **Purpose:** Previous studies have examined the response of muscle protein to resistance exercise and nutrient ingestion. Net muscle protein synthesis results from the combination of resistance exercise and amino acid intake. No study has examined the response of muscle protein to ingestion of protein in the context of a food. This study was designed to determine the response of net muscle protein balance following resistance exercise to ingestion of nutrients as components of milk. **Method:** Three groups of volunteers ingested one of three milk drinks each: 237 g of fat-free milk (FM), 237 g of whole milk (WM), and 393 g of fat-free milk isocaloric with the WM (IM). Milk was ingested 1 h following a leg resistance exercise routine. Net muscle protein balance was determined by measuring amino acid balance across the leg. **Results:** Arterial concentrations of representative amino acids increased in response to milk ingestion. Threonine balance and phenylalanine balance were both > 0 following milk ingestion. Net amino acid uptake for threonine was 2.8-fold greater ($P < 0.05$) for WM than for FM. Mean uptake of phenylalanine was 80 and 85% greater for WM and IM, respectively, than for FM, but not statistically different. Threonine uptake relative to ingested was significantly ($P < 0.05$) higher for WM ($21 \pm 6\%$) than FM ($11 \pm 5\%$), but not IM ($12 \pm 3\%$). Mean phenylalanine uptake/ingested also was greatest for WM, but not significantly. **Conclusions:** Ingestion of milk following resistance exercise results in phenylalanine and threonine uptake, representative of net muscle protein synthesis. These results suggest that whole milk may have increased utilization of available amino acids for protein synthesis. **Key Words:** AMINO ACID UPTAKE, MUSCLE BIOSIES

Recent research has focused on the role of various nutrients for increasing muscle mass. Muscle protein synthesis can be stimulated during recovery from resistance exercise, but in the absence of nutrient intake, net muscle protein balance—the difference between protein synthesis and breakdown—remains negative (2,3,16,22). Acute investigations of the response of muscle protein to nutrient ingestion following resistance exercise have provided information that may be used to formulate the optimal nutritional strategy for muscle growth. To date, the work has focused on individual nutrients alone and in combination with others. Amino acid ingestion, either in free form (6,14,22) or the form of isolated milk proteins (21), stimulate muscle protein synthesis, resulting in net muscle protein synthesis. Increased insulin concentrations resulting from carbohydrate ingestion increase net muscle protein balance, but in the absence of an amino acid source, balance will remain negative (4). When amino acids and carbohydrates are ingested simultaneously, the response of net muscle protein balance following exercise

is greater than either alone (5,14). Thus, it seems that the combination of an amino acid source and carbohydrates to stimulate insulin secretion may be an optimal nutrient combination to stimulate net muscle protein synthesis and thus muscle growth.

The response of muscle protein metabolism to the ingestion of amino acids and carbohydrates has not been examined in the context of whole foods. Because milk is a potent insulin secretagogue (10) and provides protein as a source of amino acids, milk ingestion would seem to be an ideal postexercise strategy to provide stimulation of muscle anabolism. However, no study has systematically examined the response of net muscle protein balance to ingestion of a food, including all three macronutrients.

The interaction of the response of protein metabolism to various nutrients has been examined. Whole body leucine balance was higher with the addition of carbohydrates and fat in a meal than with protein alone in resting subjects (9). A series of studies in resting humans using whole body measurements and a multicompartiment model determined that the concurrent ingestion of fat and sucrose with milk proteins modified the uptake of amino acids into peripheral tissues (8,9,13). Addition of sucrose to milk proteins increased whole body N retention, but primarily in splanchnic tissues, whereas addition of fat to milk proteins resulted in greater dietary N retention in peripheral tissues (9,13). However, in resting muscle there is no evidence that provision of exogenous fat stimulates muscle protein synthesis or net muscle protein synthesis (19). There is no information on the response of net muscle protein synthesis

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TABLE 1. Subject characteristics for three groups studied following resistance exercise during ingestion of milk.

	N (M/F)	Age (yr)	Wt (kg)	Ht (m)	BMI (kg·m ⁻²)	1RM (kg)	Leg Volume (L)
FM	8 (3/5)	26.0 ± 2.0	59.7 ± 4.9	1.62 ± 0.03	22.60 ± 1.31	75.7 ± 11.1	7.7 ± 0.8
WM	8 (6/2)	27.8 ± 2.5	81.2 ± 7.6	1.74 ± 0.04*	26.49 ± 1.78	116.7 ± 13.7	9.4 ± 0.7
IM	8 (7/1)	23.8 ± 0.7	80.5 ± 5.4	1.80 ± 0.02†	24.73 ± 1.49	121.5 ± 9.0	9.9 ± 0.5*

Expressed as mean ± SE. M/F, number of males and females in each group; FM, fat-free milk group; WM, whole milk group; IM, isocaloric fat-free milk group; BMI, body mass index; 1RM, one-repetition maximum (i.e., the maximum resistance that can be lifted one time) for leg extension exercise.

* Significantly different from FM, *P* < 0.05.

† Significantly different from FM, *P* < 0.01.

following resistance exercise with ingestion of a naturally occurring food, including all three macronutrients, as the source of amino acids.

The primary aim of this investigation was to determine the response of net muscle protein balance following resistance exercise to ingestion of amino acids as a component of a real food, milk. Our hypothesis was that ingestion of milk following resistance exercise would result in net muscle protein synthesis, as represented by uptake of the amino acids phenylalanine and threonine. A secondary aim of this study was to investigate the influence of the components in the milk on the uptake of amino acids from the milk proteins. By manipulating the type and quantity of the ingested milk, the influence of energy, fat, and carbohydrates on the response was also investigated.

METHODS

Subjects were healthy young volunteers who had not participated in regular resistance training for at least 5 yr prior to participation in this study. Subjects were randomly assigned to one of three groups receiving one of three milk drinks following 10 sets of 8 repetitions of knee extension exercise. The three groups were fat-free milk (FM), *N* = 8; whole milk (WM), *N* = 8; and isocaloric fat-free milk (IM), *N* = 8. Subject characteristics are presented in Table 1. ANOVA revealed no differences between groups for age, weight, body mass index (BMI), or strength (one-repetition maximum (1RM)).

Pretesting

Medical screening. Prior to participation in the experiments, 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. All

subjects participated in a health screen to eliminate those with health risks that may preclude participation in the protocol. 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 antibody, hepatitis A antibody IgM, hepatitis A antibody total, and human immunodeficiency virus antibody), urine tests (drug screen I: abuse, marijuana, pregnancy test) and a 12-lead ECG to exclude any individuals displaying heart irregularities.

Exercise test. At least 5 d prior to the first trial, 1RM for knee extension was determined for each subject. The 1RM was defined as the maximum weight that could be lifted and held for a 1-s count. The mean leg extension values are presented in Table 1.

Experimental Protocol

Study protocol. The sampling protocol was identical to that used in a previous study (21) and is presented in Figure 1. Subjects were admitted to the GCRC the night before each trial, given a standard dinner, and then allowed only water after 10 p.m. until the start of the study the following morning. At approximately 5:30 a.m., an 18-gauge polyethylene catheter was inserted into a vein in the forearm for blood sampling. Additionally, a 3-French, 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. The experimental protocol was designed to quantify the response of net muscle protein balance, as represented by amino acid balance across the leg. Milk was consumed at 60 min following resistance exercise. FM consumed 237 g of fat-free milk, WM 237 g of whole milk, and IM 393 g of fat-free milk. FM and WM were isonitrogenous and WM and IM were isocaloric. The nutritional breakdown for each is given in Table 2. In a previous study with an identical sampling protocol, we demonstrated that there was no change in amino acid concentrations or net muscle protein balance following an identical resistance exercise bout (21), resulting in zero uptake of amino acids when a placebo was ingested. Thus, we did not include a placebo in this protocol and any changes in net muscle protein balance in this study could be attributed to milk ingestion. Muscle biopsy specimens from the vastus lateralis were taken to measure intracellular amino

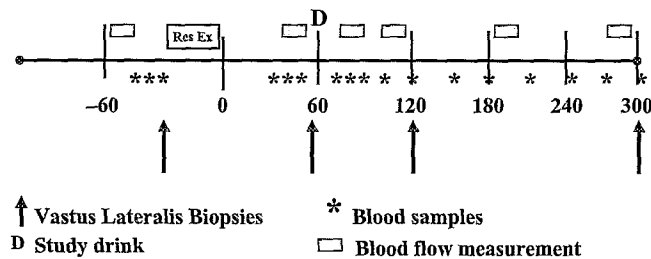


FIGURE 1—Schematic of study protocol.

TABLE 2. Nutrient content of the milk consumed by each group following resistance exercise.

	Energy (kJ)	CHO (g)	Fat (g)	Protein (g)	Phenylalanine (mg)	Threonine (mg)
FM	377	12.3	0.6	8.8	420	390
WM	627	11.4	8.2	8.0	390	360
IM	626	20.4	1.0	14.5	696	647

FM, 237 g of fat-free milk; WM, 237 g of whole milk; IM, 393 g of fat-free milk; CHO, carbohydrate.

acid concentrations. A biopsy was taken immediately prior to beginning exercise for the resting period measurement. Postexercise biopsy samples were taken at approximately 55 min (immediately prior to consumption of the study drink) and at 120 and 300 min following the resistance exercise bout or -5, 60, and 240 min following ingestion of the milk. Each biopsy was performed 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 approximately 50 mg of mixed muscle tissue. The sample was quickly rinsed, blotted, and divided into two to three pieces, frozen in liquid N, and stored at -80°C for future processing for intracellular amino acid concentration analysis. Biopsies were performed at sites at least 1 cm apart in an attempt to minimize the impact of local inflammation from previous biopsies.

A continuous ICG (infusion rate = 0.5 mg·min⁻¹) infusion was initiated 10 min prior to 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 prior to 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 prior to exercise until 35 min prior to exercise. Blood flow samples were taken five times (from 40 to 45, 80 to 90, 115 to 120, 200 to 210, and 290 to 300 min following exercise) during the postexercise period. These five periods were chosen in an attempt to best characterize the leg blood flow following exercise and milk ingestion. Each blood flow value was based on the mean of triplicate samples during that time period.

Blood sampling was identical for all experiments. At approximately 6:00 a.m. (time = -120 min), background blood samples for insulin concentration and ICG concentration were taken. Additionally, three arteriovenous samples were taken immediately after cessation of the ICG infusion and 35, 30, and 25 min prior to 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 milk. Arterial blood samples for glucose and insulin analysis also were collected at -7, 30, 45, 60, 90, 120, 120, and 240 min following milk ingestion.

For each trial, the exercise routine was begun immediately after the first muscle biopsy was completed and lasted approximately 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 (17,20,21).

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 (1,2,22). The internal standards used were U-[¹³C₉-¹⁵N] phenylalanine (50 μmol·L⁻¹) and [¹⁵N]threonine (120 μmol·L⁻¹) added in a ratio of approximately 100 μL·mL⁻¹ of blood. Threonine concentration was determined for only 6, 6, and 7 subjects for FM, IM, and WM, respectively. 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. The mean cv for steady-state samples taken at rest using this method was 3 ± 2%. Leg blood flow was determined from spectrophotometric measurements of the ICG concentration in serum from the femoral vein and the peripheral vein as described previously (1,2,22). Serum insulin concentrations were determined by radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA). Intraassay coefficient of variation was < 10%. Glucose concentrations were determined enzymatically using an autoanalyzer (YSI 2300 STAT, Yellow Springs, OH).

Muscle. Muscle tissue samples were analyzed for free intracellular amino acid concentrations as previously described (1,2,20,22). Upon thawing, approximately 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 was collected. This procedure was repeated two more times, and the pooled supernatant (~1.3 mL) was processed as were the blood samples described above for blood. Muscle free amino acid concentration (phenylalanine only) was measured with the internal standard method, with corrections for the contribution of extracellular fluid as described in the previous

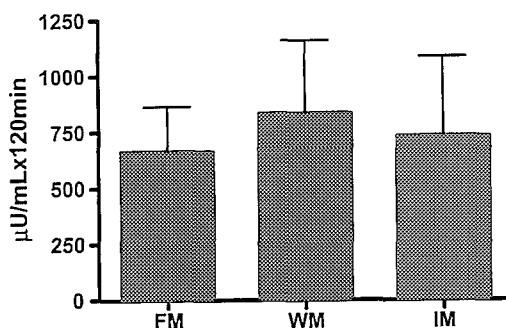


FIGURE 2—Area under the curve of the serum insulin response for 4 h after milk ingestion during recovery from resistance exercise. Each subject consumed one of three milk drinks at 60 min following exercise. Area under the curve was calculated from the baseline pre-milk ingestion values. FM, subjects consumed 237 g of fat-free milk; WM, subjects consumed 237 g of whole milk; IM, subjects consumed 393 g of fat-free milk isocaloric to the WM.

TABLE 3. Muscle intracellular phenylalanine concentration measured at rest and following resistance exercise during ingestion of one of three milk drinks.

	Time			
	Rest	55 min	120 min	300 min
FM	67 ± 7	63 ± 8	65 ± 7	56 ± 6
WM	66 ± 5	68 ± 7	66 ± 22	61 ± 5
IM	52 ± 6	55 ± 5	60 ± 5	60 ± 7

Mean ± SE (nmol·mL⁻¹ intracellular water).

Time, time following exercise that the biopsy was taken; FM, fat-free milk group; WM, whole milk group; IM, isocaloric fat-free milk group.

section and in previous studies (1,2,20,22). There are no muscle threonine concentration data available.

Calculations

Net muscle protein balance. Net muscle protein balance (NB) was represented by arteriovenous balance of amino acids across the leg using the formula $NB = (Ca - Cv) \cdot BF$, where Ca = arterial amino acid concentration, Cv = venous amino acid concentration, and BF = leg blood flow.

Amino acid exchange across the leg. The primary endpoint of the study is the comparison of the amino acid (phenylalanine or threonine) uptake or release (i.e., exchange). Because phenylalanine and threonine are not metabolized in muscle, the uptake of these amino acids was considered to represent net muscle protein synthesis, the primary endpoint of the study (6,20–22). Total amino acid exchange (mg) was calculated for phenylalanine and threonine from the area under the curve of the net balance over the time from all samples following the drink ingestion (i.e., 70–300 min following exercise for each amino acid). Positive values represent net uptake (anabolism) and negative values represent net release (catabolism). The baseline was the mean postexercise values from samples taken at 45, 50, and 55 min following exercise.

Insulin. The area under the curve of insulin concentration was calculated following milk ingestion. Baseline was the insulin concentration immediately prior to milk ingestion.

Data Presentation and Statistical Analysis

Data are presented as means ± SE. Phenylalanine and threonine arterial concentrations and net balance following drink ingestion are presented across time. Phenylalanine and threonine exchange (i.e., area under the curve for net balance across the leg, are presented for each group). Due to missing samples, threonine exchange is presented for 6, 6, and 7 subjects for FM, IM, and WM, respectively. The uptake of each amino acid relative to the amount of that amino acid ingested is presented for each of the three milk groups. Muscle intracellular concentrations of phenylalanine, but not threonine (data not available), are presented for each biopsy sample for each group. The insulin response to the drinks is presented as the area under the insulin curve for 4 h following drink ingestion.

For the primary endpoints, phenylalanine and threonine exchange and the amino acid uptake/ingested, one-way ANOVA was used to determine differences between groups. Whenever overall significance ($P < 0.05$) was indicated,

TABLE 4. Leg blood flow at rest and following resistance exercise during ingestion of one of three milk drinks.

	Time					
	Rest	40–45 min	80–90 min	110–120 min	200–210 min	290–300 min
FM	3.60 ± 0.55	3.90 ± 0.51*	3.72 ± 0.44	4.02 ± 0.53	3.98 ± 0.61	4.53 ± 0.57
WM	3.28 ± 0.28	6.77 ± 0.96	5.03 ± 0.85	4.41 ± 0.64	3.71 ± 0.41	4.09 ± 0.54
IM	2.96 ± 0.27	5.03 ± 0.94	4.58 ± 0.81	3.97 ± 0.50	3.50 ± 0.61	4.16 ± 0.49

Mean ± SE (mL·min⁻¹·100 mL⁻¹ leg volume). Time is time following exercise. Groups as in Table 3.

Tukey's test was used to determine pairwise differences. Furthermore, to determine whether the response was different from zero balance, exchange of each group was compared to zero (indicative of a positive response by the muscle). One-way ANOVA was also used to determine differences between the areas under the insulin curves.

Two-way ANOVA with repeated measures was used to analyze muscle intracellular concentration with time as the within factor and group (FM, IM, and WM) as the between factor. Two-way ANOVA also was used to detect differences in glucose, phenylalanine, and threonine arterial concentrations and the net balance of each amino acid across the leg over time. Time was the within factor and group (FM, IM, and WM) the between factor. Wherever two-way ANOVA revealed a significant interaction ($P < 0.05$), Bonferroni's test was used to locate the pair wise differences. When the

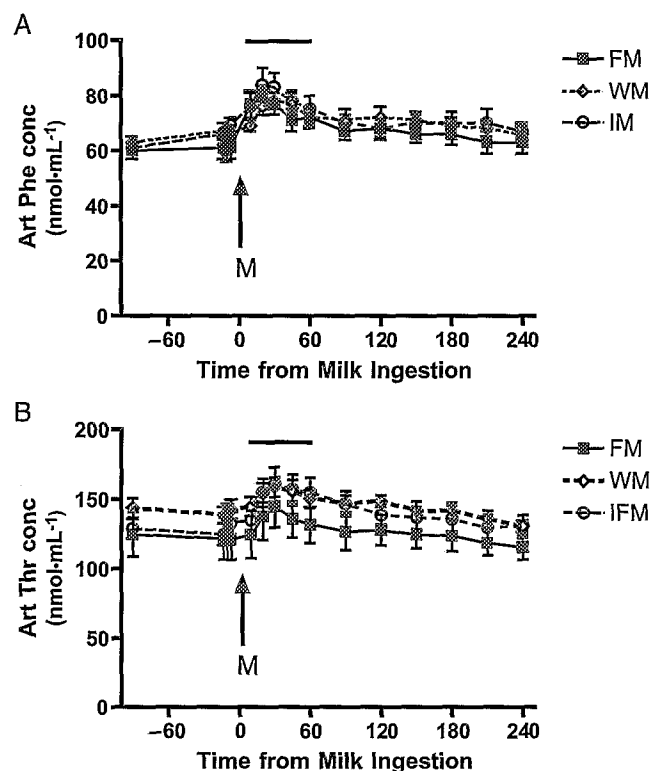


FIGURE 3—Arterial phenylalanine (A) and threonine (B) concentrations following ingestion of milk during recovery from resistance exercise. Lines at the top indicate the time points at which there is an overall (all groups together) significant difference from baseline (postexercise prior to ingestion of milk). Groups as in Figure 1.

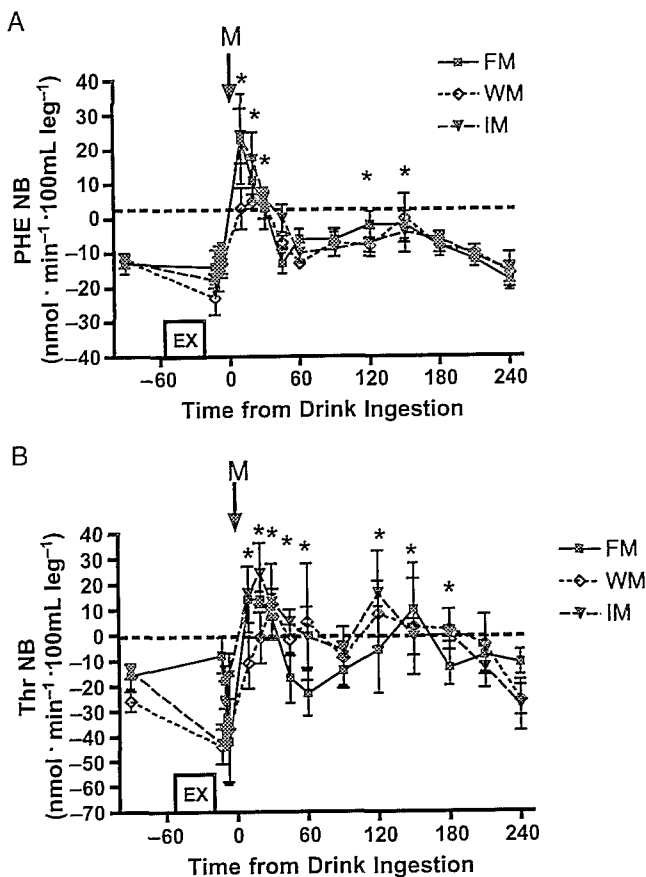


FIGURE 4—Net balance of phenylalanine (A) and threonine (B) following ingestion of milk or during recovery from resistance exercise. *Overall (all groups together) significant difference from baseline (postexercise prior to ingestion of milk). Groups as in Figure 1.

two-way ANOVA revealed significant overall effects of time or milk group, a one-way ANOVA was performed to determine the differences over time or between groups.

RESULTS

Glucose and insulin. Glucose increased following milk ingestion, but there were no differences between the three groups (data not shown). For all three milk groups pooled, arterial glucose was increased from $84.8 \pm 4.1 \text{ mg} \cdot \text{dL}^{-1}$ prior to milk ingestion and peaked at $98.1 \pm 4.6 \text{ mg} \cdot \text{dL}^{-1}$ ($P = 0.001$) 30 min following milk ingestion. Glucose concentration was back to baseline values by 60 min following drink ingestion. Milk ingestion resulted in increased insulin concentrations, but there were no significant differences between groups in the area under the insulin curves (Fig. 2).

Muscle amino acid concentration. Ingestion of milk did not affect muscle intracellular amino acid concentrations. There were no significant differences in muscle intracellular phenylalanine concentrations among the three treatment groups or across time (Table 3).

Leg blood flow. Leg blood flow is presented in Table 4. There was a group \times time interaction ($P = 0.0019$) and a

significant overall effect of time ($P < 0.0001$). The increase in leg blood flow following exercise was less for FM than for IM and WM. Overall, blood flow peaked at 45 min following exercise and was significantly greater than all other time periods (66% greater than at rest). Blood flow was significantly decreased back to values no different from rest by 120 min following exercise.

Blood amino acid concentration. Figure 3 illustrates the arterial amino acid concentrations following ingestion of milk during recovery from resistance exercise. There was an overall effect of time for arterial phenylalanine ($P < 0.0001$). Phenylalanine peaked at 20–30 min following drink ingestion and returned to baseline by 90 min. There were no significant differences between milk groups. Our previous study showed that blood amino acid concentrations do not change following an identical resistance exercise routine when a placebo is ingested (21). Threonine concentration in the femoral artery increased from 10 to 30 min following drink ingestion, then declined back to predrink values by 90 min. Threonine concentration did not vary due to type of milk ingested.

Net balance over time and exchange of phenylalanine and threonine. Figure 4 illustrates the net balance of phenylalanine and threonine during recovery from resistance exercise. Net balance for both amino acids changed from negative to positive following ingestion of all three milk drinks. Previously, we showed that net amino acid balance is unchanged and remains negative following an identical-resistance exercise bout when subjects ingested a placebo (21). Net phenylalanine balance was significantly increased over baseline and peaked at 10–20 min for all three milk drinks, but subsequently declined to concentrations no different from baseline by 45 min following drink ingestion ($P < 0.001$) and for the remainder of the blood collection period except for differences at 120 and 150 min following milk ingestion. Threonine balance changed over time for all groups combined ($P = 0.001$). Net threonine balance across the leg peaked at 20–30 min following drink ingestion and was also significantly different from baseline from 120 to 180 following milk ingestion.

Phenylalanine and threonine exchange (area under the curve of net balance) for 4 h following drink ingestion during recovery from resistance exercise is presented in Table 5. Phenylalanine exchange was significantly greater than zero ($P < 0.05$) (i.e., phenylalanine uptake indicating an anabolic response in the muscle to drinking milk following resistance exercise) for WM and IM, but not FM. Mean uptake was approximately 80 and 85% more for WM and IM, respectively, than for FM, but these differ-

TABLE 5. Amino acid exchange (area under the curve of net balance across the leg) for 4 h following ingestion of one of three milk drinks during recovery from resistance exercise.

	Phenylalanine (mg)	Threonine (mg)
FM	22.8 ± 9.6	26.7 ± 10.4
WM	$40.5 \pm 11.1^*$	$101.5 \pm 24.6^{\dagger}$
IM	$42.4 \pm 10.6^*$	$95.2 \pm 23.2^*$

* Significantly greater than 0.

\dagger Significantly different from FM

FM, 237 g of fat-free milk; WM, 237 g of whole milk; IM, 393 g of fat-free milk; CHO, carbohydrate.

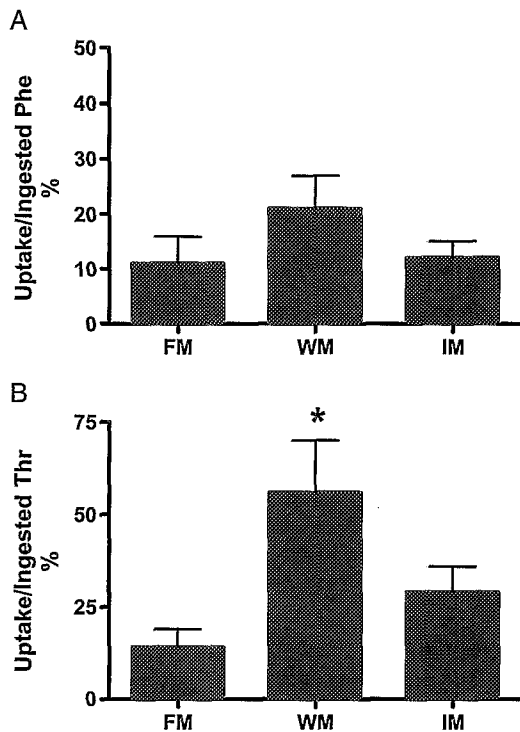


FIGURE 5—Ratio of the amino acid uptake relative to the amount ingested for phenylalanine (A) and threonine (B) following ingestion of milk during recovery from resistance exercise. Groups as in Figure 1.

ences were not statistically significant. Threonine uptake for WM and IM was significantly greater than zero ($P < 0.01$), but FM was not. Threonine uptake for WM was approximately 2.8-fold greater ($P < 0.05$), and IM was approximately 2.5-fold greater than FM, but the latter failed to reach statistical significance.

Phenylalanine and threonine uptake for both legs relative to the amount ingested for each milk drink is summarized in Figure 5. Mean phenylalanine uptake/ingested during WM was 90 and 70% greater than FM and IM, respectively. However, these differences failed to reach statistical significance. Threonine uptake relative to that ingested was 312% greater for WM than FM ($P < 0.05$). Mean WM threonine uptake relative to ingested was 91% greater than IM, but this difference failed to reach statistical significance.

DISCUSSION

The principal result of the present study is that milk ingestion stimulated net uptake of phenylalanine and threonine representing net muscle protein synthesis following resistance exercise. Net muscle protein balance changed from negative to positive, indicating muscle anabolism, following milk ingestion. Previous data demonstrated that there was no change in net muscle protein balance following an identical resistance exercise bout with placebo ingestion (21), indicating that net muscle protein synthesis in this study primarily was due to the milk. The impact of individual nutrients, especially amino acids (6,

14,22), proteins (21), and carbohydrates (5,14), and the combination of these nutrients (5,14) on the response of net muscle protein balance following resistance exercise has been examined previously. To our knowledge, this study is the first to determine the response of net muscle protein balance during recovery from resistance exercise to ingestion of protein in the form of food.

Net muscle protein balance may be increased by increasing muscle protein synthesis or decreasing protein breakdown. The methodology used in this study did not allow us to discern the metabolic mechanism for this increase in net balance, but it is likely that both an increase in synthesis and a decrease in breakdown contributed. Presumably, increased muscle protein synthesis due to provision of essential amino acids was responsible for the majority of the response (3,6,14,20,22). The increase in insulin in response to milk ingestion also may have contributed to the increase in net muscle protein synthesis by decreasing muscle protein breakdown (4). Thus, it is not difficult to accept that net muscle protein synthesis resulting from ingestion of milk following resistance exercise stems from both increased muscle protein synthesis and decreased muscle protein breakdown.

In this and previous studies (3,6,12,14,20–23), it is assumed that net uptake of particular amino acids by the leg is representative of net muscle protein balance and that the acute measurement of net muscle protein balance represents the potential for accretion of muscle protein over longer time periods. Whereas it is true that amino acid uptake across the leg would include synthesis of skin and bone as well as muscle protein, at rest it has been determined that the contribution of skin and bone to leg muscle protein synthesis is less than 15% (1). If anything, it does not seem likely that the total contribution of skin and bone to the total amino acid uptake following exercise would increase. Thus, the majority of the leg amino acid uptake following resistance exercise likely is due to muscle. Because phenylalanine and threonine are not oxidized in muscle (25), the uptake of these amino acids by the leg can result only from their use for protein synthesis. Further, there was no expansion of the muscle intracellular phenylalanine pool (Table 3), indicating that all phenylalanine taken up over the 4 h of sampling was utilized for muscle protein synthesis. In previous studies, intracellular phenylalanine concentration was representative of the response of other amino acids at the end of the sampling period (6,23).

The relevance of the acute response to nutrient ingestion and resistance exercise for muscle protein accretion is supported by data from two previous studies (15,20). The 24-h response of net muscle protein balance was shown to be represented by the acute response during amino acid ingestion and resistance exercise (20). Furthermore, the acute measurement of net phenylalanine balance provided a close estimate of the change in muscle mass during 28 d of bed rest as measured by dual-energy x-ray absorptiometry (DXA) (15). Thus, the measurement of the uptake of essential amino acids that cannot be oxidized in muscle (i.e., threonine and phenylalanine) over an acute time

period following resistance exercise demonstrating that milk ingestion stimulates net muscle protein balance seems to be a reasonable estimate of the impact on muscle protein accretion over a longer time period. One study has examined milk ingestion following resistance exercise during a longer period of training. Rankin et al. (18) demonstrated that milk consumption following each training session during 10 wk of training resulted in significant gains in fat-free mass measured by DXA. These gains were compared to those in subjects given an isocaloric carbohydrate solution. Milk consumption resulted in a trend toward greater gains than carbohydrate consumption.

Uptake of threonine was greater for WM than for FM. Although it failed to reach statistical significance, the trend was similar for phenylalanine. Furthermore, the uptake of threonine relative to the amount ingested was greatest for WM. A similar trend was noted for phenylalanine. These data suggest that some property of WM enhanced the amount of threonine, and possibly phenylalanine, utilized for muscle protein synthesis. If true, it is not clear which property of WM was responsible for increased utilization. WM contained more energy, primarily in the form of fat than FM. The extra energy provided may be especially important following exercise. It is clear that increased energy is important to N balance over longer time periods when exercise is performed (24). Acutely in rats, muscle protein synthesis was depressed immediately after exercise and was associated with declines in energy compounds, adenosine triphosphate, creatine phosphate, and adenosine triphosphate/adenosine diphosphate ratio (7). On the other hand, other data suggest that energy per se may not be the critical factor for optimal uptake of dietary amino acids. Muscle protein synthesis was not stimulated in rats subjected to intense endurance exercise when carbohydrates were consumed during recovery, only when protein was consumed, although the total energy intake was equivalent (11). During recovery from resistance exercise in humans, ingestion of isocaloric (5), and even greater (14), amounts of carbohydrate resulted in less amino acid uptake than when protein/amino acids were included.

Other nutrients ingested with protein will influence the N utilization of the ingested protein. In this study, there were no differences in the insulin response to milk ingestion between groups. Therefore, it is unlikely that insulin was a factor in the uptake across the leg in our study. On a whole body level in resting subjects, incorporation of dietary N into peripheral proteins was highest when fat was ingested concurrently (9). However, there is little direct evidence that fat affects amino acid uptake in muscle (19). Thus, future work should examine whether extra energy and fat ingested concurrently with an amino acid source will increase amino acid utilization for muscle protein synthesis.

It is notable that the blood flow values for FM were lower immediately following the exercise bout. Similarly, because blood flow was greater following exercise for WM, the values used to calculate the baseline for the net amino acid uptake were slightly, but not statistically, more negative than for FM. There is no obvious reason why the baseline

FM blood flow would be lower. There were more females randomized to FM than the other two groups, and thus the mean size of the subjects was somewhat smaller and the mean strength was less, albeit not significantly so. However, it is uncertain how these differences would affect the blood flow following exercise. All subjects performed the same exercise bout at the same relative intensity (80%) of their 1RM. Presumably, the blood flow response to the exercise bout is relative to each individual's maximum strength rather than the absolute amount of weight lifted. Thus, we expected the response of blood flow to be similar for all groups regardless of the maximum resistance utilized during the exercise bout. However, to our knowledge, this notion has never been tested.

Because blood flow is different among groups, the baseline values would be different and could thus be a confounding factor. If so, then it is possible that the differences between groups are artifactual due to differences in baseline values. However, there is evidence to suggest that our results are not an artifact of the differential postexercise blood flow for the different groups. A closer examination of the individual values indicates that the decreased blood flow is primarily due to two subjects in FM. In one of these subjects, the blood flow does not change following exercise, and in the other, the blood flow is actually reduced. However, removing the data from either or both of these subjects does not change the results and thus does not change the conclusion. Furthermore, the change from baseline for net balance is not different for any of the groups (Fig. 4). Thus, starting at a different baseline did not affect the data. Moreover, the change from baseline to immediately (10–20 min) following milk ingestion was not different between groups (Fig. 4). Finally, the total amino acid uptake was similar for WM and IM, yet WM seemed to have greater utilization of amino acids for net muscle protein synthesis despite there being little differences in their baseline values. Regardless of the differences between groups, net amino acid balance changed from negative to positive in response to all three milk drinks; thus, the primary finding that there was a stimulation of net muscle protein synthesis by food ingestion following exercise is not affected by blood flow differences.

It also is worth noting the variability in the net uptake values. Clearly, this variability affects the conclusions in that statistical significance is not obtained in some cases despite large differences between means. Furthermore, whereas the pattern of phenylalanine and threonine uptake in the three groups was similar, statistical significance was reached only for the threonine data. It is likely that the variability inherent in the uptake of amino acids contributed to this discrepancy. There seems to be a great deal of variability among individuals in their response to nutrient ingestion following exercise. Examination of our previous studies shows that variability of this range is common to these studies when net balance is measured following a bolus ingestion of amino acids (5,6,14, 20,21,23). The variability among individuals in response to amino acid infusions or repeated ingestions does not seem to be as large (3,12,22). So it seems that the response to bolus ingestion is more variable among individuals, perhaps due to

splanchnic removal, and this variability may be natural. Thus, we have chosen not to remove individuals with seemingly, or at least possibly, anomalous net uptake values. The variability clearly affects the statistical power for this study. It is possible that an increased number of subjects may have increased the power and thus statistical significance.

These data support our primary hypothesis that milk ingestion results in uptake of phenylalanine and threonine, representative of net muscle protein synthesis. These data are the first to quantify the response of muscle amino acid balance to ingestion of a food source rather than free amino acids or individual proteins, following resistance exercise. From a practical standpoint, these results suggest that milk

would be suitable for ingestion during recovery from exercise and provides an alternative to supplements.

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