Consumption of fat-free fluid milk after resistance exercise promotes greater lean mass accretion than does consumption of soy or carbohydrate in young, novice, male weightlifters[1–3]

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ABSTRACT

Background: Acute consumption of fat-free fluid milk after resistance exercise promotes a greater positive protein balance than does soy protein.

Objective: We aimed to determine the long-term consequences of milk or soy protein or equivalent energy consumption on training-induced lean mass accretion.

Design: We recruited 56 healthy young men who trained 5 d/wk for 12 wk on a rotating split-body resistance exercise program in a parallel 3-group longitudinal design. Subjects were randomly assigned to consume drinks immediately and again 1 h after exercise: fat-free milk (Milk; $n = 18$); fat-free soy protein (Soy; $n = 19$) that was isoenergetic, isonitrogenous, and macronutrient ratio matched to Milk; or maltodextrin that was isoenergetic with Milk and Soy (control group; $n = 19$).

Results: Muscle fiber size, maximal strength, and body composition by dual-energy X-ray absorptiometry (DXA) were measured before and after training. No between-group differences were seen in strength. Type II muscle fiber area increased in all groups with training, but with greater increases in the Milk group than in both the Soy and control groups ($P < 0.05$). Type I muscle fiber area increased after training only in the Milk and Soy groups, with the increase in the Milk group being greater than that in the control group ($P < 0.05$). DXA-measured fat- and bone-free mass increased in all groups, with a greater increase in the Milk group than in both the Soy and control groups ($P < 0.05$).

Conclusion: We conclude that chronic postexercise consumption of milk promotes greater hypertrophy during the early stages of resistance training in novice weightlifters when compared with isoenergetic soy or carbohydrate consumption.   *Am J Clin Nutr* 2007; 86:373–81.

KEY WORDS   Hypertrophy, strength, resistance exercise, protein

INTRODUCTION

The accretion of muscle protein as a result of resistance exercise occurs because of successive periods of positive muscle protein balance (1–3). Periods of positive protein balance are due to a synergistic interaction of an exercise and feeding-induced stimulation of muscle protein synthesis (MPS) (4–7). Protein ingestion provides essential amino acids for protein synthesis (8,

9), which also act, in the case of leucine, to stimulate the translational machinery [for review see Kimball and Jefferson (10)]. Protein ingestion also increases systemic insulin, which has a mild stimulatory effect on MPS (11, 12). Rather than a dose-dependent stimulation, a minimal threshold of insulin is required to allow MPS to proceed unabated; however, further stimulation of MPS is not seen at higher doses [for reviews see Kimball et al (13, 14)].

Boirie et al (15) showed that consumption of whey protein induced a rapid aminoacidemia with a greater amplitude than did casein protein. Whey protein consumption resulted in a rapid stimulation of whole-body protein synthesis and also oxidation, whereas casein resulted in a suppression of whole-body proteolysis. The result was that casein ingestion induced a more positive whole-body leucine balance than did whey (15). Recent findings showed that milk proteins appear superior to, or at least equivalent to, either isolated whey or casein alone in terms of supporting postprandial dietary nitrogen utilization (16). In addition, ingestion of intrinsically labeled soy and milk proteins resulted in greater incorporation of nitrogen into serum proteins and urea when soy was ingested (17). The suggestion from these results (17), and from a modeling approach (18), was that proteins from soy are directed toward splanchnic metabolism, whereas milk proteins are directed to peripheral sites.

We have recently shown that when milk and soy proteins are ingested after resistance exercise, milk protein resulted in a more positive net amino acid balance and a greater postexercise stimulation of protein synthesis (19). The acute findings we observed should result, over the longer term, in a greater increase in muscle protein accrual with regular milk than with soy protein consumption (19). We tested this hypothesis by having subjects consume
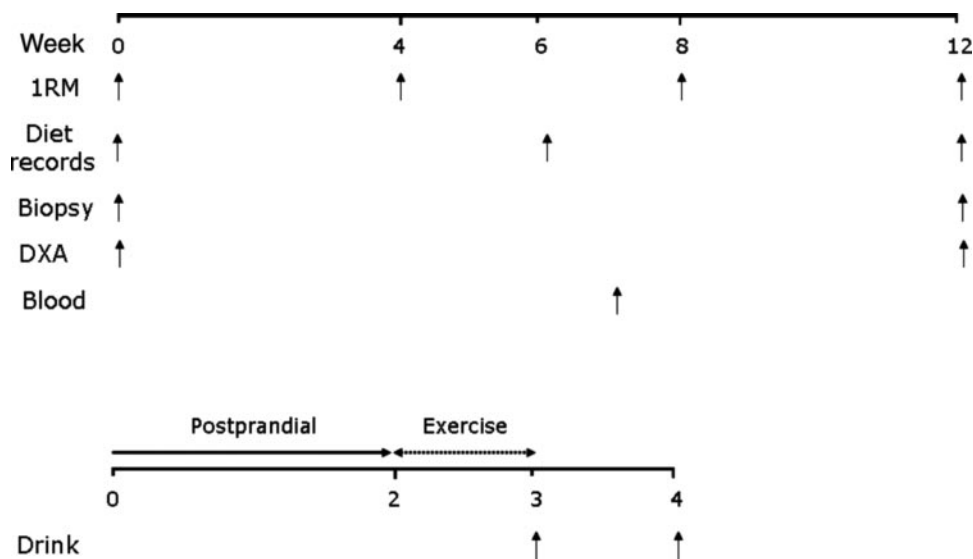
FIGURE 1. Schematic representations of the study protocol, including variables examined and timing of collection and the acute protocol before and after exercise followed daily by subjects when training. 1RM, single repetition maximal strength; DXA, dual-energy X-ray absorptiometry.

either fluid milk or soy protein, with inclusion of an energy-matched (carbohydrate only) control group, immediately after exercise to promote muscle hypertrophy. Subjects also consumed a second drink 1 h after having worked out because 2 separate periods of hyperaminoacidemia only 1 h apart can still promote a full anabolic response (20). Our hypotheses were that both protein-consuming groups would have greater protein accretion with resistance training than an energy-matched carbohydrate-consuming group, but that the milk-consuming group would have greater muscle protein accretion than both the soy- and carbohydrate-consuming groups.

SUBJECTS AND METHODS

Participants

Participants were all young men between 18 and 30 y of age and recruited locally from the McMaster University campus or local Hamilton area by posters and advertisements. Subjects were screened before participation by questionnaire against standard criteria to ensure they were free from any medical condition that would preclude their participation in the study. On the basis of the subjects' responses to the questionnaire, subjects were deemed healthy. Subjects could not be actively participating in any weightlifting activities ≤8 mo before the study and were only permitted to enter the study if the only recreational activity the subjects performed was the weightlifting study itself. Hence, subjects were not completely sedentary but participated in <2–3 h/wk of structured physically active or leisure-time activities. Subjects who reported being lactose intolerant ($n = 2$ declined entry into the study) or having any history of milk protein allergy were not allowed to participate (no subjects reported). Subjects could also not be consuming, or have consumed in the past 6-8 mo, any dietary supplements (including vitamins) or protein supplements. Before participating, all subjects were informed of the risks associated with the study and gave their written informed consent. The protocol was approved by the local ethics committee of McMaster University and Hamilton Health Sciences Research Ethics Board. The study protocol conformed to

all local and international standards, including the revised 1983 Declaration of Helsinki, on research with human subjects. We initially recruited 60 participants but had 4 subjects drop out of the study for reasons unrelated to the study protocol itself.

Protocol

The study protocol consisted of 12 wk, 5 d/wk, of resistance exercise training on a rotating whole-body split routine (see below). Subjects were required to refrain from consuming any food or macronutrient-containing beverage for 2 h before exercise. Subjects were randomly assigned to 1 of 3 groups that defined their postexercise nutritional intake: *1*) Milk: 500 mL fluid (fat-free) milk (735 kJ; ≈17.5 g protein, ≈25.7 g carbohydrate, ≈0.4 g fat) ($n = 18$); *2*) Soy: 500 mL fluid (fat-free) soy protein drink (GeniSoy, Tulsa, OK) that was isoenergetic, isonitrogenous, and macronutrient ratio-matched (carbohydrate:protein:fat) to the milk and was also certified to be isoflavone free by the manufacturer and did not contain any dietary fiber ($n = 19$); or *3*) control: 500 mL flavored fluid containing carbohydrate (maltodextrin as a 9% solution) that was isoenergetic with the soy and milk drinks ($n = 19$). Under supervision, the subjects consumed a drink immediately after exercise and again 1 h after exercise. All drinks were flavored with vanilla to taste roughly equivalent and were served to subjects in opaque containers to prevent subjects from discerning any subtle differences in color of the drinks. Although minor taste differences existed, subjects were asked not to discuss the drinks with other participants or the investigators. All participants consumed the drinks, and none reported any adverse side effects as a result. The study design, variables examined, and day-to-day dietary control are shown in **Figure 1**.

Training

Three workout slots (0600–0900, 1200–1500, and 1800–2100) were designated to accommodate subjects' schedules. Subjects chose one workout slot and completed ≥90% of their workouts in that time slot, which also meant that their dietary controls before exercise could be monitored and were similar for

the duration of the study. There were no significant differences between or within groups with respect to the number of subjects that trained in each time slot. Subjects were monitored during each training session either individually or with one supervisor per 2 subjects to ensure compliance with good lifting form, with rest intervals as specified, and to maintain the subjects' motivation. Training consisted of 3 groups of exercises: pushing exercises (military press, bench press, seated chest fly, and seated triceps extension), pulling exercises (seated lateral pull down, seated wide grip row, seated reverse fly, seated biceps curl, and a series of abdominal exercises without weights); and leg exercises [incline (45 °) leg press, 2-leg knee extension, 2-leg hamstring curl, and seated calf raise]. In any given week subjects would complete pushing and pulling exercises, followed by leg exercises, and then complete 2 more days of pushing and pulling exercises. In the ensuing week they would begin with leg exercises, followed by pushing and pulling exercises and a second leg and pushing exercise workout. Thus, by the end of the study subjects would have completed 60 weightlifting sessions consisting of 20 pushing, 20 pulling, and 20 leg-resistance exercise workouts. All exercises were performed on guided motion machines (Nautilus, Tulsa, OK) at 80% of the subject's voluntary single repetition maximal strength (1RM). Subjects began the protocol, weeks 1–2, by completing 2 sets of each exercise for 10–12 repetitions with a 2-min rest between sets. From weeks 3–12 all exercises were performed to voluntary failure in the last set. In weeks 3–5 subjects completed 3 sets of each exercise for 10–12 repetitions. In weeks 6 and 7 subjects completed 4 sets for 8–10 repetitions. Weeks 8–10 consisted of 4 sets at 6–8 repetitions per set. In the final stages of the training, weeks 11 and 12, subjects completed 4 sets at 4–6 repetitions per set. Compliance with the prescribed training protocol was $86 \pm 2\%$ ($\bar{x} \pm$ SEM) overall with all subjects achieving $\geq 75\%$ compliance and 13 of 54 subjects had 100% compliance (ie, attendance at and completion of every training session).

In general the protocol was well tolerated by the participants. During the protocol, 4 subjects developed tendonitis of their shoulders (2 from the Milk group and 2 from each of the Soy and control groups) for which they required a rest period (ie, omission of exercises that aggravated their tendonitis) for a 1–2-wk duration. Two subjects also developed patellar tendonitis, which required omission of leg extensions and hamstring curls for 2–3 wk. However, no subjects had an acute, or developed a chronic, injury that did not resolve with rest, application of ice, or both or with a short course of nonprescription analgesics. All of the aforementioned subjects completed the protocol, however, and showed strength and lean mass gains that were not below average or out of the normal range (within 95% CI limits) of values; hence, their data were included in all analyses.

Strength testing

The 1RM testing was conducted over a 3-d period, training days 1–3 at the beginning of each week, for each of the exercises before training (Pre), every 4 wk during, and 3 d after (Post) the last training bout for each exercise group. Leg exercise 1RMs were always tested on the first day followed by pushing exercises and pulling exercises. Subjects initially performed a warm-up set for each exercise at an estimated 40–50% of their 1RM for 12–15 repetitions. A successful 1RM was when the subject was able to move the machine lever arm through a full range of motion as judged by 1 of only 2 researchers who performed all of the

strength testing. If the subject was not successful on the first attempt or was successful then the weight was increased or decreased accordingly, the subjects were allowed a full 3-min rest before attempting a second lift. In this manner a true 1RM was established. This 1RM value was subsequently checked by asking the subject to report back to the testing center and perform only their 1RM. Any subject who could maximally lift weight that was 10% greater than their previously determined 1RM had a subsequent new 1RM determined.

Dietary analysis

Before and during the 6th and 12th weeks of the study, participants were required to complete 3-d weighed diet records to determine their daily macronutrient intake. Records were scrutinized and questions asked of any participants about entries that were not understood by the chief dietary analyst. All dietary data were analyzed with the use of NUTRITIONIST V software (First Data Bank, San Bruno, CA).

Muscle biopsies

Percutaneous needle muscle biopsies were taken before and after the 12 wk of training from the vastus lateralis muscle under local anesthesia (2% xylocaine) with the use of a 5-mm Bergstrom needle modified for manual suction as described previously (21, 22). Muscle samples (100–150 mg) were immediately dissected free of any visible fat or connective tissue and were oriented under low power magnification ($\times 20$) so that the muscle could be embedded in optimal cutting temperature medium and sectioned for histochemical analyses of fiber type as previously described (21–23). With the use of these methods we have been able to clearly identify 3 muscle fiber subtypes: I, IIa, and IIx as previously described (21, 22); however, for this study to establish a clear relation between fiber size and the intervention we chose only to examine type I and type II fibers, which meant that on average we included between 130 and 250 fibers of each type in all analyses.

Dual-energy X-ray absorptiometry

Subjects also underwent a whole-body dual-energy X-ray absorptiometry (DXA) scan (QDR-4500A; Hologic Inc, Waltham, MA) both Pre and Post to establish changes in body composition. Participants were scanned at the same time of day before and after training and were asked to maintain the same dietary conditions, which were recorded for accuracy, at both times. All scans were performed by the same investigator, and body positions were recorded with the use of bony landmarks and scan table references to ensure that body position was maintained as constant as possible. All participants were scanned with their hands by their sides in a suppinated position with their feet ≈ 30 cm apart. During each test the standard bar was placed to the right of the subject, and a phantom spine was scanned each day to account for any significant variations in day-to-day variability. In a reproducibility experiment with the same machine it was found that the CVs in 6 subjects for 3 scans during the course of 18 wk were between 1.5% and 1.1%, and 1.8% and 1.3% for fat- and bone-free mass (FBFM), and fat mass, respectively.

Blood profile

At week 7 of the training all subjects had a one-time blood profile drawn after a representative leg workout in which they

TABLE 1
Subjects' anthropometric and body-composition characteristics by group, before (Pre) and after (Post;12 wk) training[1]

	Control			Soy			Milk		
	Pre	Post	Change	Pre	Post	Change	Pre	Post	Change
			%			%			%
BW (kg)	80.5 ± 3.8^2	82.4 ± 3.8	2.3	83.3 ± 4.1	85.9 ± 4.1	3.1	78.8 ± 2.5	81.9 ± 2.3	3.9
FM (kg)	14.6 ± 2.0	14.1 ± 1.7^3	−3.4	16.4 ± 1.8	16.2 ± 1.8	−1.5	13.5 ± 1.1	$12.7 \pm 0.9^{3-5}$	−5.5
FBFM (kg)	63.0 ± 2.1	65.4 ± 2.2^3	3.7	64.0 ± 2.5	66.8 ± 2.5^3	4.4	62.4 ± 1.7	$66.3 \pm 1.6^{3,4}$	6.2
BMC (kg)	2.90 ± 1.1	2.92 ± 1.2	0.6	2.92 ± 1.2	2.94 ± 1.3	0.8	2.95 ± 0.8	3.00 ± 0.8	1.7

[1] Control group (carbohydrate beverage; $n = 19$); Soy, soy drink–consuming beverage group ($n = 19$); Milk, milk-consuming beverage group ($n = 18$); BW, body weight; FM, fat mass; FBFM, fat- and bone-free mass; BMC, bone mineral content. Statistical analysis was by 2-factor ANOVA with within (Pre, middle, and Post) and between (control, Soy, or Milk) variables. BW showed a main effect of time ($P < 0.05$). FM and FBFM showed a significant time-by-group interaction ($P < 0.05$).

[2] $\bar{x} \pm$ SEM (all such values).

[3] Significantly different from Pre, $P < 0.05$.

[4] Significantly different from control, $P < 0.05$.

[5] Significantly different from Soy, $P < 0.05$. No statistical analysis was performed on the percentage change.

also consumed their assigned drinks immediately and 1 h after exercise. We used this blood sampling to characterize what might be considered an aggregate systemic circulating concentration of metabolites and hormones relevant to the investigation. Subjects reported to the laboratory in their usual dietary state, but ≥2 h postprandially. A 20-gauge catheter was inserted into a prominent forearm vein for blood sampling. A 3-way stopcock closed off the catheter which was kept patent by periodic flushing with 0.9% saline. Subjects had a blood sample drawn at rest, immediately after exercise, and at 30, 60, 90, and 120 min after exercise. Blood was drawn into heparinized evacuated tubes, and portions were divided into aliquots to isolate deproteinized whole blood by perchloric acid precipitation of all proteins and of plasma, as described previously (24). Whole blood was treated and measured for amino acid concentrations according to previously described procedures (24). Whole-blood glucose concentrations were measured with the use of fluorimetry and standard enzymatic methods as described (24). Plasma insulin was measured with the use of a commercially available radioimmunoassay (Diagnostic Products, Los Angeles, CA). To minimize variability equivalent numbers of samples from subjects and times from each group were analyzed within each run. CVs for amino acids are generally <4–6% and <4–5% for glucose and insulin, respectively.

Statistics

Data were analyzed (SIGMASTAT, version 3.1; Systat Software Inc, San Jose, CA) with the use of orthogonal analyses in a repeated measures 2-factor analysis of variance (ANOVA) with time (weeks in the case of longitudinal variables and minutes in the case of blood variables) as a within factor and group as a between factor. Training-induced changes in lean mass and lifting volume were analyzed with the use of a 1-factor ANOVA. Significant interactions by ANOVA were further examined and isolated with the use of Tukey's honestly significantly difference post hoc test, with adjustments for multiple comparisons. Correlation analysis was performed with the use of Pearson's product coefficient. Statistically significant differences were considered to be when $P < 0.05$. Values are presented as means ± SEMs.

RESULTS

Initial mean subject heights were not different between the groups (control: 1.81 ± 0.05 m; Soy: 1.79 ± 0.06 m; Milk: 1.77 ± 0.04 m; $P = 0.45$). Initial mean subject body mass index (in kg/m^2) was also not significantly different between the groups (control: 24.6 ± 1.2; Soy: 26.0 ± 1.4; Milk: 25.1 ± 1.2; $P = 0.45$). The subjects' weight changed significantly over time (main effect) with gains in weight from Pre to Post of $2.3 \pm 1.1\%$, $3.1 \pm 1.7\%$, and $3.9 \pm 1.2\%$, for the control, Soy, and Milk groups, respectively. Fat loss from Pre to Post showed a time-by-group interaction with subjects in the Milk and control groups losing body fat, with the change being greater in the Milk group than in both the control and Soy groups (**Table 1**). Resistance training and postexercise drink consumption showed an interaction with both the control and Soy groups showing significant increases in FBFM; however, a greater increase in FBFM was observed in the Milk group than in both the control and Soy groups (Table 1). Although the comparison of means is relevant, the data in **Figure 2** show individual responses and group means (±SEM) in terms of training-induced lean mass changes, by group, with a division showing the overall median
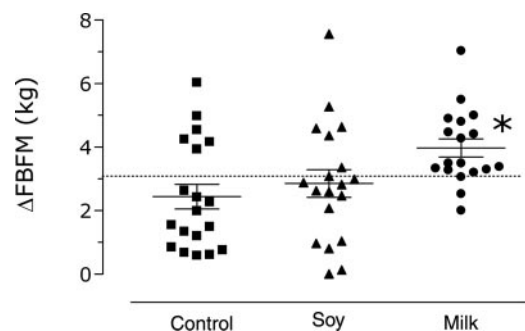
FIGURE 2. Individual and mean (±SEM) changes (Δ) in fat- and bone-free mass (FBFM) measured by dual-energy X-ray absorptiometry, plotted as a function of group: milk-consuming group (Milk; $n = 18$), soy-consuming group (Soy; $n = 19$), and isoenergetic carbohydrate-consuming group (control; $n = 19$). Analysis of means was by one-factor ANOVA with Tukey's post hoc test. *Significantly different from the Soy and control groups ($P < 0.01$). The dashed line represents the overall median change in FBFM.

TABLE 2
Subjects' estimated dietary intakes by group, before (Pre) at the midpoint (Mid; 6 wk) of, and after (Post; 12 wk) exercise[1]

	Control (n = 19)			Soy (n = 19)			Milk (n = 18)		
	Pre	Mid	Post	Pre	Mid	Post	Pre	Mid	Post
Energy intake (MJ/d)	12.7 ± 0.8	14.1 ± 0.9	13.3 ± 1.0	13.0 ± 0.8	12.2 ± 0.8	12.7 ± 0.7	12.6 ± 0.9	13.2 ± 1.0	13.5 ± 1.2
Energy intake (kJ · kg^{-1} · d^{-1})	159 ± 13	175 ± 15	164 ± 18	157 ± 12	148 ± 13	150 ± 10	159 ± 14	164 ± 17	164 ± 20
Protein intake (g/d)	117 ± 7	138 ± 9	131 ± 7	105 ± 5	145 ± 9	139 ± 8	111 ± 8	146 ± 6	149 ± 5
Protein intake (g · kg^{-1} · d^{-1})	1.4 ± 0.1	1.7 ± 0.1	1.6 ± 0.2	1.2 ± 0.1	1.7 ± 0.1	1.6 ± 0.1	1.4 ± 0.1	1.8 ± 0.2	1.8 ± 0.2
CHO intake (g/d)	403 ± 33	449 ± 32[2,3]	434 ± 45	376 ± 22	329 ± 18	388 ± 26	401 ± 46	358 ± 32	429 ± 37
CHO intake (g · kg^{-1} · d^{-1})	5.0 ± 0.4	5.5 ± 0.4	5.1 ± 0.5	4.5 ± 0.3	3.9 ± 0.3[4]	4.5 ± 0.3	5.1 ± 0.5	4.5 ± 0.4[3]	5.2 ± 0.5
Fat intake (g/d)	103 ± 7	111 ± 10	99 ± 13	99 ± 10	105 ± 8	94 ± 9	103 ± 7	104 ± 9	104 ± 18
Fat intake (g · kg^{-1} · d^{-1})	1.3 ± 0.1	1.4 ± 0.1	1.2 ± 0.3	1.2 ± 0.2	1.3 ± 0.2	1.1 ± 0.2	1.3 ± 0.1	1.3 ± 0.2	1.2 ± 0.3

[1] All values are $\bar{x}$ ± SEM. CHO, carbohydrate. Statistical analysis was by 2-factor ANOVA with within (Pre, Mid, and Post) and between (control, Soy, or Milk) variables with Tukey's post hoc test. Main effect of time for protein intake for all groups was pooled: Mid = Post > Pre ($P < 0.05$). There was a significant group-by-time interaction ($P < 0.05$) for total CHO intake and for CHO intake per kilogram of body weight.

[2] Significantly different from Soy and Milk at the same time point, $P < 0.05$.

[3] Significantly different from Pre and Post within the same group, $P < 0.05$.

[4] Significantly different from control and Milk at the same time point, $P < 0.05$.

gain in muscle mass which shows, perhaps more clearly than a comparison of means, the greater gain in FBFM in the Milk group than in both the Soy and control groups. The comparison of changes in lean mass showed a significant treatment effect ($P < 0.01$) in that the change in FBFM in the Milk group was greater than that observed in the Soy and control groups.

Only one interaction was seen in the dietary intake data (that included the postexercise beverage consumption) such that a significant between-group difference in the control group had significantly greater dietary carbohydrate intake at the 6-wk midpoint (Mid) compared with both the Soy and Milk groups (**Table 2**). At the Mid, the daily carbohydrate intake was greater than at Pre in the control group (Table 2). All groups had higher protein intakes (main effect) at the Mid and Post times compared with before training, with no differences between the groups (Table 2).

Changes in voluntary 1RM for the exercises for the leg exercises are shown in **Table 3**. The changes in strength, although recorded continuously throughout the study, are only reported for changes from Pre to Post. In addition, 1RM values were only recorded for exercises for which values could reliably be determined; hence, we have not reported 1RM values for seated calf raises or abdominal exercises. All groups (main effect for time; Post > Pre; $P < 0.001$) experienced strength gains. A trend toward greater strength gains was observed for the leg press in the Milk and Soy groups than in the control group ($P = 0.075$). A trend for greater strength gains was also observed in the Milk group than in both the Soy and control groups for knee extension ($P = 0.077$) and hamstring curls ($P = 0.082$). Strength gains were not statistically significantly different in the following exercises: military press (mean overall increase: 37 ± 3%), bench press (mean overall increase: 52 ± 3%), triceps pushdown (mean overall increase: 44 ± 7%), front pectoral fly (mean overall increase: 56 ± 6%), lateral pull down (mean overall increase: 34 ± 4%), wide grip seated row (mean overall increase: 42 ± 3%), standing biceps curl (mean overall increase: 52 ± 7%), and rear deltoid fly (mean overall increase: 50 ± 3%).

Type II muscle fiber size showed a time-by-group interaction ($P < 0.05$) such that all groups showed increases in fiber cross-sectional area (CSA) as a result of training ($P < 0.01$). After

training, the CSA of type II fibers was greater in the Milk group than in both the Soy and control groups ($P < 0.05$; **Figure 3**).

Type I muscle fiber CSA also showed a time-by-group interaction ($P < 0.05$) such that increases from Pre to Post were seen in the Soy and Milk groups but not in the control group. After training, type I muscle fiber area was not significantly different between the Soy and Milk groups but was greater in the Milk group than in the control group ($P < 0.05$; **Figure 4**).

We also calculated lifting volume (repetitions × load lifted) to see whether changes in FBFM (Table 1) and fiber type (Figures 3 and 4) were in any way a result of differences in load lifted. Although there were significant interindividual differences, comparison across groups showed no statistically significant differences ($P = 0.46$; data not shown). Moreover, when lifting volume was normalized to body weight, the differences between groups were also not significant ($P = 0.84$; data not shown).

Acutely, neither blood glucose nor insulin were affected by exercise; hence, the preexercise values for both are not presented and the 0 time is from the blood sample taken immediately after exercise (see "Subjects and Methods"). Blood glucose showed a group-by-time interaction such that it remained constant after consumption of both the soy and milk drinks but increased after consumption of the control drink at 30 min after exercise. Blood glucose at 30 min after exercise was higher in the control group than in both Soy and Milk groups (**Figure 5**). Insulin was elevated at 30 min after exercise in all groups with the rise being greatest in the control group (Figure 5). Insulin returned to preexercise concentrations at all other times in the Soy and Milk groups but remained elevated with a secondary rise at 90 min in the control group compared with the Soy and Milk groups (Figure 5).

Acutely, differences in the arterialized concentrations of no summed (or individual) amino acids were observed between the preexercise and immediately postexercise samples (neither were there any between group or interaction effects); hence, the preexercise values are not presented and the 0 time point (**Figure 6**) is from the blood sample taken immediately after exercise (see Subjects and Methods). Blood amino acid responses showed group-by time interactions ($P < 0.001$) for leucine, the sum of all

TABLE 3
Subjects' single repetition maximal leg strength (1RM) by group, before (Pre) and after (Post; 12 wk) exercise[1]

|	Control (n = 19)			Soy (n = 19)			Milk (n = 18)		
	Pre	Post[2]	Change	Pre	Post[2]	Change	Pre	Post[2]	Change
	kg	kg	%	kg	kg	%	kg	kg	%
Incline leg press	210 ± 19[3]	394 ± 27	87	213 ± 15	423 ± 32	98	186 ± 11	377 ± 18	102
Knee extension	122 ± 12	178 ± 13	46	131 ± 13	209 ± 13	60	128 ± 13	214 ± 13	67
Hamstring curl	71 ± 2	107 ± 5	51	78 ± 5	112 ± 5	42	71 ± 3	115 ± 4	62

[1] No statistical analysis was performed on the percentage change.

[2] Main effect of time for all treatments ($P < 0.001$).

[3] $\bar{x} \pm$ SEM (all such values).

branched-chain amino acids (BCAAs; leucine, valine, and isoleucine), and the sum of all essential amino acids and total amino acids (Figure 6). Amino acid profiles were not statistically significantly different between the Soy and Milk groups with respect to time-dependent or between-group differences (Figure 6). The concentration of amino acids declined in the control group with concentrations for leucine, and all summed amino acids were lower at 90 and 12 min than at 0 and 30 min (differences not indicated on graph; Figure 6).

DISCUSSION

Our data show that the chronic consumption of fluid skim milk (500 mL) immediately and 1 h after resistance exercise promoted greater gains in FBFM (ie, lean mass) than consumption of either an isonitrogenous, isoenergetic, and macronutrient ratio-matched soy protein–containing beverage or an isoenergetic carbohydrate drink. We also observed a greater reduction in body fat mass associated with chronic postexercise milk consumption. Previously, we reported acute data that showed fluid milk consumption resulted in greater net amino acid uptake and fractional protein synthesis than did the same soy protein-containing drink used in the present study (19). In addition, consumption of whole or fat-free milk has also been shown to give rise to a positive muscle protein balance (25). We hypothesized that our acute findings (19) would translate into greater lean mass gains with chronic milk consumption than with soy protein consumption. As proof-of-principle, the present study confirms that our acute findings were at least qualitatively predictive of a greater lean mass gain with long-term training. However, calculations based on our previous data (19) indicate that the Milk group would have
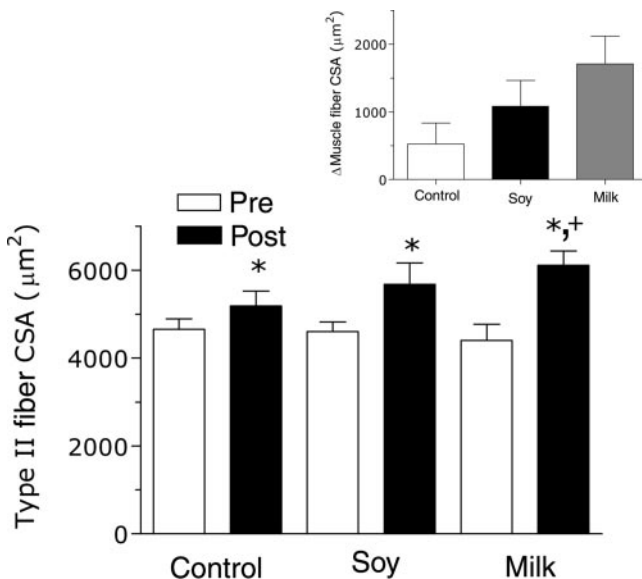
FIGURE 3. Mean (±SEM) type II muscle fiber cross-sectional area (CSA) before (Pre) and after (Post) 12 wk of resistance exercise training and the change (Δ) in muscle fiber CSA (not analyzed statistically) in all groups: milk-consuming group (Milk; n = 18), soy-consuming group (Soy: n = 19), and isoenergetic carbohydrate-consuming group (control; n = 19). A 2-factor ANOVA with Tukey's post hoc test was used for the statistical analysis. Time-by-group interaction: P = 0.006. *Significantly different from Pre, within the same group (P < 0.01). +Significantly different from Post in the Soy and control groups (P < 0.05).
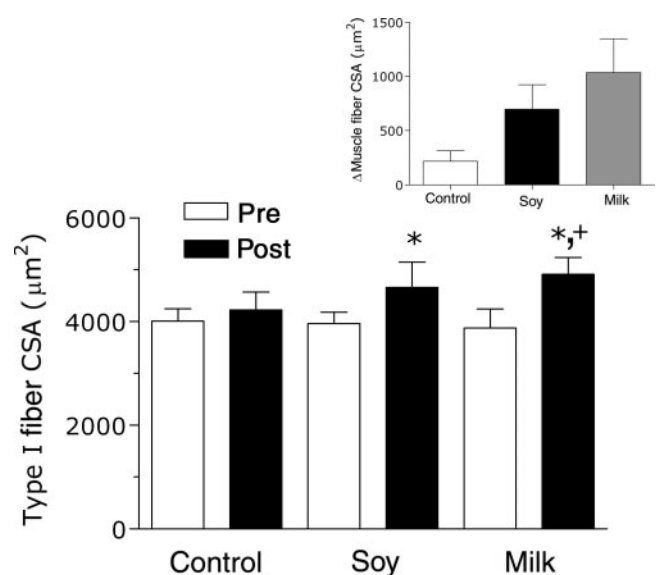
FIGURE 4. Mean (±SEM) type I muscle fiber cross-sectional area (CSA) before (Pre) and after (Post) 12 wk of resistance exercise training and the change (Δ) in muscle fiber CSA (not analyzed statistically) in all groups: milk-consuming group (Milk; n = 18), soy-consuming group (Soy; n = 19), and isoenergetic carbohydrate-consuming group (control; n = 19). A 2-factor ANOVA with Tukey's post hoc test was used for the statistical analysis. Time-by-group interaction: P = 0.04. *Significantly different from Pre within the same group (P < 0.05). +Significantly different from Post in the control group only (P < 0.05).
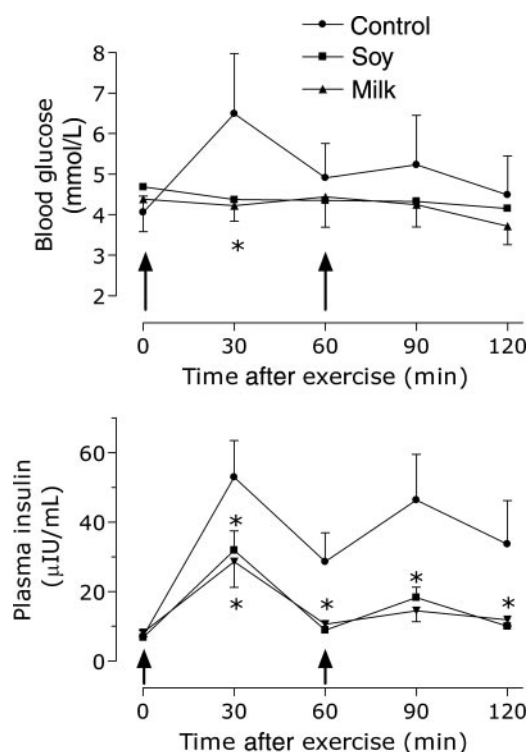
FIGURE 5. Mean (±SEM) acute blood glucose and plasma insulin responses to leg exercise in all groups: milk-consuming group (Milk; $n = 18$), soy-consuming group (Soy; $n = 19$), and isoenergetic carbohydrate-consuming group (control; $n = 8$ of 19). The arrows indicate the time of drink consumption (carbohydrate, soy, or milk). A 2-factor ANOVA with Tukey's post hoc test was used for the statistical analysis. Time-by-group interaction: $P = 0.01$ for both insulin and glucose. *Significantly different from the Milk and Soy groups at the same time point ($P < 0.01$). Time-dependent changes were omitted for clarity.

accrued ≈5.4 ± 1.2 g muscle protein (≈21 g hydrated muscle tissue) in a single exercised leg, whereas the Soy group would have accrued only 2.6 ± 0.8 g muscle protein (≈10 g hydrated muscle tissue). On the basis of these results, extrapolations would predict that someone would gain twice as much lean mass with milk consumption as opposed to soy consumption, if only leg exercises were performed during a training program. Thus, it would appear that our acute findings overestimated the magnitude of the effect in terms of differences in muscle protein accretion seen with soy and milk ingestion after exercise. However, several factors could account for this, including nonlinear changes in protein accrual (ie, a plateau in muscle mass gains at the later stages of training), lack of control over total daily dietary intake, changes in training intensity and duration, and that our original data were a within-subject design as opposed to a between-subject design.

Milk has been given to subjects as a postexercise supplement previously and was shown to be no more effective in promoting fat-free mass gains than the postexercise provision of carbohydrate alone (26). In this latter study, which was 10 wk in duration, the training intensity was much lower than what we had used here, only with the use of 80% of 1RM by week 7 of 10 and was conducted only 3 d/wk. Consequently, the gains in FBFM were only 0.8 kg in the carbohydrate-supplemented group ($n = 9$) and 1.6 kg in the milk-supplemented group ($n = 10$) (26). Hence, it is possible that the study design used by Rankin et al (26) was too

short in duration, was likely underpowered to detect differences between interventions, and may have lacked the training intensity to stimulate differential gains in muscle mass brought about by consumption of milk as opposed to carbohydrate.

As a representative systemic response to a single strenuous (leg) workout session, including postexercise drinks as specified, to capture how blood substrates might be affecting changes in muscle protein accrual, we sampled blood at a single time (7 wk) during training. We chose only one time point because it is unlikely that training would have changed how the respective postexercise drinks affected the substrates we measured. On the basis of our data of venous amino acid profiles, insulin, and glucose, we cannot readily discern why milk proteins provide a greater anabolic stimulus than soy proteins. As with our acute data (19), which was from arterial amino acid concentrations, we did not observe marked differences in blood total amino acid, essential amino acid, BCAA, or leucine concentrations (Figure 6). Previously, we attributed the greater net amino acid uptake and greater stimulation of muscle fractional protein synthetic rate with milk consumption to subtle differences in the pattern of aminoacidemia and not to the amino acid composition of the proteins themselves, which were not statistically significantly different for any amino acid.

Work comparing milk proteins with isolated whey and casein proteins indicates that postprandial nitrogen utilization with milk proteins may support greater anabolism than either isolated protein alone (16). The reason for this difference was not readily apparent, but in light of other data showing the superiority of milk proteins, including isolated casein (27), compared with soy to support peripheral protein anabolism (17, 18, 28), we view the recent data of Lacroix et al (16) as supportive of the concept that milk proteins appear to have an inherently greater capacity to support resistance exercise-stimulated muscle protein accretion than does soy protein. Moreover, we do not see this finding being solely a function of the protein amino acid content (19). However, supplementation of soy protein with BCAAs can alter interorgan amino acid fluxes to favor muscle protein anabolism in aged and patient populations (29), whether this would be true in young persons under conditions of exercise-induced anabolism is not known.

When compared with postexercise provision of energy as carbohydrate, which increases postexercise net protein balance to a value that is not significantly different from zero (30), milk proteins were more effective in promoting lean mass gains. Our subjects consuming only carbohydrate gained muscle mass and had gains in FBFM and type II fiber hypertrophy, findings that are in contrast to those of another chronic study in which postexercise nutrition was manipulated (31). Andersen et al (31) reported that after 14 wk of training a group who received 25 g protein (a mixture containing predominantly whey, but also casein and egg protein) showed hypertrophy, whereas the carbohydrate-only group did not. The findings of Andersen et al (31) are interesting, given that the effect of resistance exercise alone appears to be to increase postexercise protein synthesis for ≥48 h (32). In this 48-h window one would expect that eating protein at any time would have resulted in an additive exercise-induced increase in muscle protein synthesis and promote protein accrual (1), which, although it may not have been as great as when protein is consumed immediately after exercise, is presumably what happened in our control group.
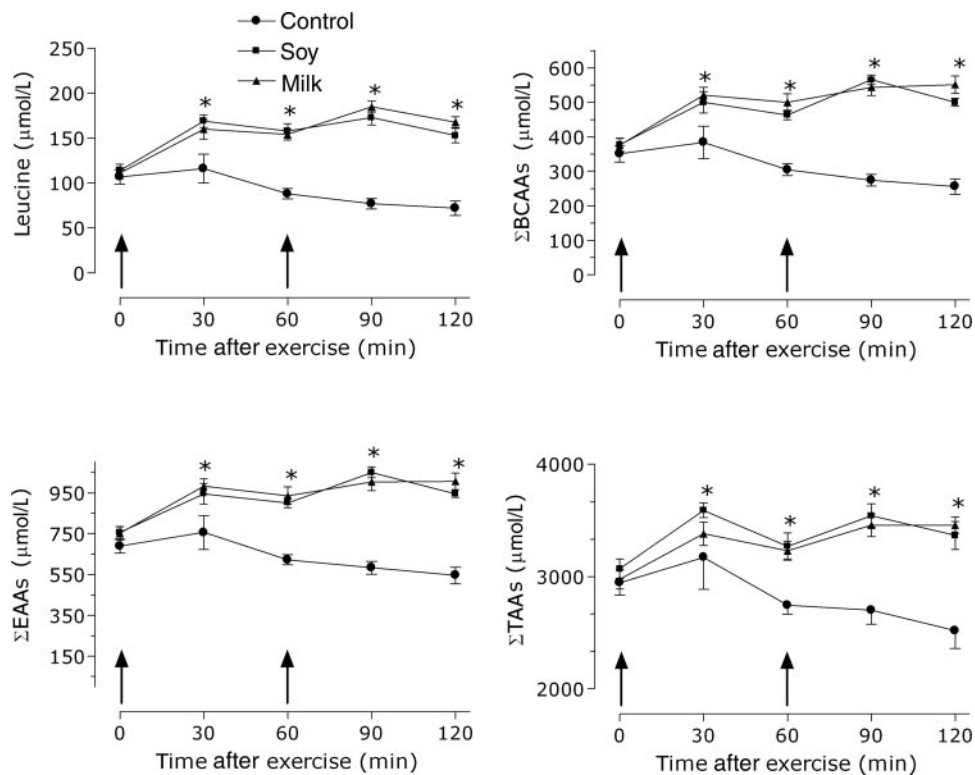
FIGURE 6. Mean (±SEM) acute venous blood amino acid concentrations [leucine, summed branched-chain amino acids (ΣBCAAs), summed essential amino acids (ΣEAAs),and summed total amino acids (ΣTAAs)] after exercise in all groups: milk-consuming group (Milk; $n = 18$), soy-consuming group (Soy; $n = 19$), and isoenergetic carbohydrate-consuming group (control; $n = 8$ of 19). The arrows indicate the time of drink consumption (carbohydrate, soy, or milk). A 2-factor ANOVA with Tukey's post hoc test was used for the statistical analysis. Time-by-group interaction: $P = 0.01$ for all variables. *Significantly different from the Milk and Soy groups at the same time point ($P < 0.01$). Time-dependent changes were omitted for clarity.

Other studies, in which postexercise protein intake was manipulated (either in terms of timing or supplement content), appear to agree with a general statement that timing of protein consumption after exercise may be a determinant of muscle mass and strength gains. Esmarck et al (33) reported that delaying the postexercise delivery of a protein-containing supplement to elderly men by 2 h prevented exercise-induced hypertrophy and slowed strength gains. In addition, strength and muscle mass gain in patients who had just undergone knee surgery were promoted to a greater degree by protein and carbohydrate consumption than simply carbohydrate or a placebo (34). Hence, the data presented here, combined with previous data from chronic studies manipulating postexercise protein consumption (31, 33, 34), support the general thesis that immediate consumption of protein, particularly milk protein, after resistance exercise serves to maximize exercise-induced increases in muscle mass.

We observed a significantly greater reduction in fat mass in the Milk group than in both the Soy and control groups (Table 1). The mechanism for this result is not clear but may be due, in part, to a greater daily calcium intake of the Milk group (35), which, from analysis of the dietary records, was ≈700 mg/d greater ($P < 0.05$) in the Milk group (1696 ± 265 mg/d) than in the Soy (945 ± 188 mg/d) and control (1023 ± 213 mg/d) groups. In addition to a greater dietary calcium intake in the Milk group, it was proposed that an inherent property of dairy proteins themselves accelerate loss of adipose tissue mass during energy restriction (35, 36). Hence, the increased ingestion of calcium and dairy-based proteins may be playing a role in the greater fat loss

in the Milk group despite being in positive energy balance, which would be necessary for muscular hypertrophy to occur.

In conclusion, immediate and 1-h postexercise milk consumption, as opposed to soy or isoenergetic carbohydrate, resulted in greater gains in FBFM and type II muscle fiber area. Increases in type I muscle fiber area were greater in the Milk and the Soy groups than in the control group. All groups showed increased strength as a result of the training program; however, there were no between-group effects. A greater fat mass loss was seen in subjects who consumed the postexercise milk supplement than in both the Soy and control groups, which may be related to dietary calcium intake or an endogenous property of the milk proteins themselves.

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