No Difference between Spray Dried Milk and Native Whey Supplementation with Strength Training

HÅVARD HAMARSLAND[1], VILDE HANDEGARD[1], MAURITZ KÅSHAGEN[1], HAAKON B. BENESTAD[2], and TRULS RAASTAD[1]

[1]Department of Physical Performance, Norwegian School of Sport Sciences, Oslo, NORWAY, and [2]Section of Anatomy, Institute of Basis Medical Sciences, University of Oslo, Oslo, NORWAY

ABSTRACT

HAMARSLAND, H., V. HANDEGARD, M. KÅSHAGEN, H. B. BENESTAD, and T. RAASTAD. No Difference between Spray Dried Milk and Native Whey Supplementation with Strength Training. *Med. Sci. Sports Exerc.*, Vol. 51, No. 1, pp. 75–83, 2019. **Background**: A rapid digestibility and high leucine content are considered important for maximal stimulation of muscle protein synthesis. Consequently, with these properties, native whey may hold greater anabolic potential than milk, when supplemented in combination with strength training. Our aim was to compare the effects of supplementation with milk or native whey, during a 12-wk strength training period, on gains in muscle mass and strength in young adults. **Methods**: In this double-blinded, randomized, controlled study a total of 40 untrained young men and women received two daily servings of either milk or native whey containing 20 g of protein, during a 12-wk strength training intervention. Muscle strength, lean mass, thigh muscle cross-sectional area, *m. vastus lateralis* thickness and muscle fiber cross-sectional area were assessed before and after the training period. In addition, the acute phosphorylation of the anabolic kinases p70S6K, 4E-BP1 and eEF-2 in response to a standardized workout and supplementation was investigated before and after the 12-wk training period. **Results**: Muscle mass and strength increased, by all measures applied (5%–16%, $P < 0.001$), with no differences between groups ($P > 0.25$). p70S6K phosphorylation increased (~1000%, $P < 0.02$) 2 h after exercise in the untrained and trained state, but no differences in anabolic signaling were observed between supplements ($P > 0.40$). No correlation between these acute measures and changes in muscle mass or strength were observed. **Conclusion**: Supplementation with milk or native whey during a 12-wk strength training period did not differentially affect muscle mass and strength in young untrained individuals. **Key Words**: PROTEIN SUPPLEMENTATION, AMINO ACIDS, PROTEIN QUALITY, RESISTANCE TRAINING

Resistance exercise and protein intake are strong stimuli for a transient increase in net muscle protein balance (1). If repeated over time, the summation of periods with positive net balance results in adaptation, such as muscle hypertrophy and gains in strength (2). Studies on the acute muscle protein synthesis (MPS) response have

indicated blood concentrations of amino acids (3,4), depending on amino acid composition (5) and leucine content (6), to be an important factor determining the anabolic effect of a protein source. Whey protein is generally considered an especially potent stimulator of MPS (7), even when compared to other high-quality proteins like casein (3). However, not all studies find this (8,9). Long-term studies investigating differences between whey and casein supplementation are few and equivocal (10,11). We have previously shown native whey supplementation to result in greater blood leucine concentrations, p70S6K phosphorylation and mixed muscle FSR than milk during a 5-h postresistance exercise period (12). Native whey is produced by filtration of unprocessed raw milk, leaving proteins intact. In addition, the leucine content of native whey is about 15% and 25% higher than in regular whey and milk, respectively (13). The aim of the current study was to investigate whether the previously measured short-term differences between native whey and milk (12) might lead to long-term adaptations in muscle mass and strength when supplemented two times daily during a 12-wk strength training regime in young participants. Moreover, little is known about the changes in

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TABLE 1. Changes in regional muscle mass, muscle CSA, muscle fiber area and myonuclei in young men and women receiving milk or native whey supplementation for 12 wk combined with strength training.

	Milk, n = 18 (10♂/8♀)			Native Whey, n = 18 (10♂/8♀)			ES between Groups; 95% CI	P Values for Group Difference (% Change)
	Pre Mean ± SD	Post Mean ± SD	% Change ± SD; 95% CI	Pre Mean ± SD	Post Mean ± SD	% Change ± SD; 95% CI		
Body mass (kg)	77.8 ± 16.0	80.0 ± 15.3*	3.1 ± 3.4; 1.7[0]	77.9 ± 11.7	81.3 ± 12.1*	4.4 ± 3; 1.5[1]	0.39 to 0.51	0.21
Fat mass (kg)	22.0 ± 7.1	21.7 ± 6.4	−0.9 ± 6.9; 3.5[0]	20.9 ± 7.8	21.4 ± 8.3	3.26 ± 9.3; 4.6[0]	0.11 to 0.14	0.15
Lean mass (kg)	53.4 ± 11.0	56.2 ± 11.6*	5.2 ± 2.9; 1.6[1]	54.2 ± 8.0	57.2 ± 8.2*	5.6 ± 2.7; 1.3[1]	0.13 to 0.36	0.32
Leg lean mass (kg)	18.8 ± 4.3	19.9 ± 4.5*	5.5 ± 3.6; 1.9[1]	19.0 ± 3.0	20.2 ± 3.1*	6.4 ± 4.5; 2.3[1]	0.04 to 0.13	0.73
Arm lean mass (kg)	6.31 ± 1.8	6.77 ± 2.0*	7.5 ± 6.2; 3.2[1]	6.38 ± 1.45	6.89 ± 1.6*	8.23 ± 3.78; 1.9[1]	0.01 to 0.05	0.66
Trunk lean mass (kg)	24.8 ± 4.4	25.9 ± 4.6*	4.2 ± 3.7; 1.9[1]	25.5 ± 3.6	26.9 ± 3.7*	5.8 ± 3.7; 1.8[1]	0.11 to 0.16	0.25
Quadriceps CSA (cm²)	74.8 ± 17.8	82.9 ± 19.2*	11.3 ± 8.0; 4.5[2]	76.2 ± 12.7	85.0 ± 15.5*	11.6 ± 7.8; 4.0[2]	0.13 to 0.56	0.63
Pectoralis + triceps CSA (cm²)	65.4 ± 21.6	75.1 ± 23.7*	15.8 ± 7.0; 5.2[1]	71.2 ± 18.5	81.2 ± 19.0*	15.1 ± 10.1; 3.2[1]	0.03 to 0.40	0.84
Vastus lateralis thikness (cm)	2.47 ± 0.39	2.74 ± 0.39*	12 ± 6.0; 3.2[2]	2.64 ± 0.46	2.91 ± 0.49*	11 ± 7; 3.3[2]	−0.11 to 0.71	0.75
MFA type I (μm²)	4299 ± 799	5071 ± 691*	20.6 ± 22.3; 12.3[3]	4605 ± 1037	4987 ± 1092	10.9 ± 25.4; 13.6[1]	0.40 to 0.74	0.27
MFA type II (μm²)	4761 ± 1187	6145 ± 1320*	31.7 ± 24.6; 13.6[3]	4841 ± 1378	6116 ± 1684 *	28.4 ± 27.4; 14.6[3]	−0.13 to 0.74	0.71
Type I nuclei (per fiber)	1.56 ± 0.34	1.80 ± 0.41*	20.1 ± 37.4; 21.1[2]	1.57 ± 0.27	1.76 ± 0.29*	14.7 ± 25.2; 13.0[2]	−0.17 to 0.71	0.63
Type II nuclei (per fiber)	1.69 ± 0.29	2.01 ± 0.36*	22.4 ± 29.4; 16.8[3]	1.74 ± 0.40	2.03 ± 0.40*	23.4 ± 42.0; 21.6[3]	0.03 to 0.70	0.94
1RM leg press (kg)	269 ± 83	349 ± 74*	32.6 ± 14.6; 8.8[3]	269 ± 77	344 ± 83*	30.1 ± 11.8; 5.9[3]	−0.19 to 0.76	0.61
1RM bench press (kg)	62 ± 22	79 ± 28*	27.3 ± 7.2; 3.9[2]	64 ± 23	80 ± 26*	26.9 ± 11.2; 5.6[2]	−0.04 to 0.71	0.90

Prevalues and postvalues are means ± SD. Changes are percent ± SD; 95% confidence intervals, * indicates difference between pre- and postvalues ($P < 0.05$), upper case letters indicates effect sizes ([0]0.0–0.20, [1]0.20–0.49, [2]0.50–0.80, and [3]≥0.8. Differences between groups are effect size; 95% confidence interval and P value ($P < 0.05$). MFA; muscle fiber area.

anabolic signaling in response to prolonged resistance training. We therefore included an acute study before and after the training intervention to investigate signaling responses in the untrained and trained state, in the same study group.

METHODS

Participants and ethical approval. A total of 40 young (29 ± 6 yr; Table 1) men and women were included in the study. Four participants withdrew from the study after inclusion. One withdrew after the first acute study, after which this participant experienced headaches. The other three withdrawals were not related to the study. Before entering the study all participants underwent a medical screening. To take part participants had to be healthy and without any injuries to the musculoskeletal system that would interfere with the execution of training. If using supplements participants had to stop at least 2 wk before the study. Participants were untrained in the sense that they had not done resistance exercise on a regular basis (less than once a week) for the past six months. The study was approved by the Regional Ethics Committee for Medical and Health Research of South-East Norway and performed in accordance with the *Declaration of Helsinki*. All participants signed a written informed consent form before entering the study. The trial was registered at clinicaltrials.gov as NCT03033953.

Study design. This study was a double-blinded, randomized, controlled trial. The design involved one bout of strength training in the untrained state and one in the trained state, separated by 12 wk of strength training amounting to a total of 36 workouts. Thus, muscle biopsies were collected in four different states: (1) untrained rested, (2) untrained acutely exercised, (3) trained rested, (4) trained acutely exercised. The acute measures were performed in a subgroup of the participants (n = 22). Participants were randomized to one of two groups stratified by sex and lean mass, receiving either native whey or milk.

Supplements. Native whey was bought from Lactalis® (Laval, Mayenne, France) and the milk was produced by Tine ASA (Oslo, Norway). In order to match the two supplements concerning macronutrients, cream (Tine, Norway), lactose (Arla food ingredients, Denmark) and water was added to the native whey protein (Table 2). Both the milk and native whey were dried to powder to be dissolved in 400 mL of water before ingestion. The reason for giving the milk as a powder was for supplements to be matched for appearance and flavor. Each serving (69 g for milk and 73 g for native whey) contained 19.3 g and 20.0 g of protein in the milk and native whey, respectively. Supplements were consumed two times per day, in the morning and in the evening. On training days one of the servings was ingested immediately after exercise. Participants were instructed to not consume other food for 1 h after the training session. Compliance to the supplementation was self-reported and noted at each training day by the instructors.

TABLE 2. Amino acid and macronutrient content of supplements.

Amino Acids (g per Serving)	Milk	Native Whey
Alanine	0.6	1.0
Arginine	0.6	0.6
Aspartic acid	1.5	2.2
Cysteine	0.2	0.5
Phenylalanine	0.9	0.9
Glutamic acid	4.1	3.9
Glycine	0.4	0.4
Histidine	0.5	0.5
Isoleucine	1.0	1.1
Leucine	1.9	2.5
Lysine	1.6	2.1
Methionine	0.5	0.5
Proline	1.9	1.3
Serine	1.1	1.0
Threonine	0.8	1.0
Tyrosine	0.8	0.7
Valine	1.2	1.2
Tryptophan	0.2	0.4
Total protein	19.1	20.0
Fat	7.5	6.9
Carbohydrate	6.9	7.5

Daily food intake. Participants were encouraged to maintain their habitual diet during the intervention. At the start, midway and at the end of the training intervention participants participated in two 24-h dietary recall interviews. A trained dietitian conducted the interviews and analyzed dietary nutrient content using Mat på Data 5.1 (Mattilsynet, Oslo, Norway, 2009). Five participants with a protein intake lower than 1.0 g·(kg body weight)$^{-1}$ at the first recording were recommended to increase their protein intake and given advice on how to achieve this.

Training program. Participants followed a traditional whole body strength-training program with three sessions per week. The program consisted of three lower body exercises (Squat, leg press and knee extensions) and four upper body exercises (Bench press, seated row, close grip pull-down and shoulder press). Training on Mondays and Fridays were maximal in terms of intensity and load, whereas Wednesdays were submaximal with 90% of the Monday load for the same amount of reps (two repetitions less during weeks 1–3). During the program Mondays progressed from one to two sets of 12 RM the first 3 wk to two sets of 10 and three sets 8 RM in weeks 4 to 9 and weeks 10 to 12, respectively. Fridays progressed from one to two sets of 8 RM in weeks 1 to 6 to two to three sets of 6 RM in weeks 7 to 12. Interset rest periods lasted 2 to 3 min. Qualified instructors supervised participants during all training sessions. If a participant missed an exercise session another session would be added to their program in order for all participants to reach the goal of 36 sessions.

Dual-energy X-ray absorptiometry. Body composition was assessed by dual energy X-ray absorptiometry (Lunar iDXA GE Healtcare, Madison, WI. Using the enCORE Software, version 14.10.022) before and after the intervention. Participants were scanned from head to toe in a supine position, providing values for lean tissue, fat mass and bone mineral content. The coefficient of variation (CV) for the assessment was less than 1% for lean mass.

MRI. Transverse section images were captured of the thighs (15 images), arms and chest (10 images; GE Signa 1.5 Tesla Echospeed; GE Medical Systems, Madison, WI) before and after the training intervention. Joint-gaps on the right side were used as reference points and all measures were done on the right thigh, arm and chest. The distance between images was individualized based on the length of femur and humerus. The images [Digital Imaging and Communications in Medicine (DICOM)] were analyzed using OsiriX 3.9.3 (Pixmeo, Bernex, Switzerland), giving the cross-sectional area (CSA) of individual muscles. The CV of these assessments was less than 2%.

Maximal strength. Familiarization to performance tests were done approximately 1 wk before testing. Maximal strength in bilateral leg press and bench press was assessed by one repetition maximum (1RM) before and after the training intervention. After a 10-min treadmill warm-up, a specific warm-up was performed with 10, 6, 3, and 1 repetitions at 50%, 70%, 80%, and 90% of expected 1RM, respectively. Two to 3 min of rest was given between attempts. The expected 1RM before the training intervention was based on the 1RM familiarization test. After the training intervention estimation of 1 RM was based on the training load of the participant. Two to five attempts were used to find 1RM. Range of motion was strictly controlled. Knee flexion during leg press was set to 90° and grip width in bench press was standardized. The load could be adjusted with increments of 5 kg in leg press and 1 kg in bench press. The CV for these measurements were <5%.

Unilateral maximal knee extension strength was assessed by isometric maximal voluntary contraction (MVC) in a custom-made knee extension apparatus (Gym2000, Geithus, Norway). Participants were seated in a chair with a four-point belt fixing the chest and hips, with 90° in the hip and knee joints. Three attempts of 5 s with 1-min rest between were given to reach MVC. Force was measured with a force transducer (HMB U2AC2, Darmstadt, Germany). Maximal voluntary contraction was tested after 5 min warm-up on a cycle ergometer, except for when performed immediately after the workout. The CV for these measurements was <5%.

Blood analyses. Serum was analyzed for glucose, insulin, urea and creatine kinase at Fürst Medical Laboratory (Oslo, Norway). Plasma amino acid concentration was measured as described earlier (13) with an EZfaast amino acid analysis kit (Phenomenex®, Torrance, CA) and gas chromatography/mass spectrometry (Shimadzu QP-2010 Ultra GCMS; Shimadzu Scientific Instruments, Columbia, MD).

Biopsy collection and preanalytical processing. Muscle biopsies were collected from the mid portion of *m. vastus lateralis* with a modified Bergström technique with suction. Muscle specimens were used to make a homogenate of soluble proteins and mounted for immunohistochemistry. Preanalytical processing of muscle tissue was performed as described by Paulsen and colleagues (14).

Western blot. Samples for Western blot were treated as previously described (14), quantified with ChemiDoc MP (BioRad Laboratories, Hercules, CA) and analyzed with Image Lab (v5.1, BioRad Laboratories). Primary antibodies against p70S6K and phosphor-p70S6K Thr^{389} (1:1000 for both, cat. no. 8209), eEF-2 (1:5000, cat. no. 2332), phosphor-eEF-2 Thr^{56} (1:5000, cat. no. 2331), 4EBP-1 (1;1000, cat. no. 9452), phosphor-4EBP-1 Thr$^{37/46}$ (1:1000, cat. no. 9455) and secondary antibody against antirabbit (1:3000, cat. no. 7074) were bought from Cell Signaling (Beverly, MA), diluted in a 1% fat-free skimmed milk and 0.05% TBS-t solution. All samples were run in duplicates.

Immunohistochemistry. Eight-micrometer-thick cross-sections were blocked for 30 min with 1% bovine serum albumin; (Sigma Life Science, St Louis, MO) in a 0.05% PBS-t solution (Calbiochem, EMD Biosciences, Darmstadt, Germany) before incubation for 2 h at room temperature with antibodies against myosin heavy chain II (SC71; hybriodomabank, DSHB, IA) and dystrofin (Abcam, Cambridge, UK), dissolved in the blocking solution. This was followed by incubation with appropriate secondary antibodies (A11005 or A11001; Life Technologies, Invitrogen, Eugene,

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OR) for 30 min at room temperature, before covered with a coverslip and mounted with ProLong Gold Antifade Reagent with DAPI (Invitrogen Molecular Probes). Muscle sections were then left to dry overnight at room temperature. Between stages, the sections were washed three timesfor 5 min in a 0.05% PBS-t solution. For each section, 50 type I fibers and 50 type II fibers were analyzed.

Acute strength training experiment. During the first and last bout of the strength training intervention, 22 participants took part in an extension of the project to investigate the acute effects of the milk and native whey supplements. Participants were asked to write down and repeat their dietary intake on the days before the acute experiments. After an overnight fast, these participants received a standardized breakfast consisting of oats (0.85 g·(kg body mass)$^{-1}$), water, rapeseed oil (0.2 g·(kg body mass)$^{-1}$), and 5 g sugar (30 kJ, 0.14 g protein, 0.36 g fat, and 0.84 g carbohydrates·kg^{-1} body mass). Participants followed an individual diet plan, based on body mass for length of this acute experiment, providing participants with 40 kcal·(kg·body mass)$^{-1}$ and 1.5 g protein·kg^{-1}·d^{-1}. One serving of milk or native whey was to be consumed within 5 min after the exercise session. The session was standardized, but training load was increased for the workout at the end of the training program in order to have the appropriate RM-load. After a 10-min warm-up on a treadmill, the participants completed three sets of 10 repetitions in hammer squat, leg press, knee extension, bench press seated row, one set of close grip pull down, and two sets of shoulder press. The load was 10RM for all exercises and a new set was started every third minute. Specific warm-up was done with one submaximal set (60% of working set load) in hammer squat, bench press, and seated rowing. Muscle biopsies and blood samples were collected, and MVC was tested as outlined in Figure 1.

Statistics. Non-normally distributed data (D'Agostino and Pearson omnibus normality test) were log-transformed prior to statistical analysis. All data are illustrated in original form. A two-way ANOVA with repeated measures (time × group) was applied to test group differences before and after the 12-wk training period and relative changes from before to after, and between the acute experiments. Sidak and Tukey's test was used as *post hoc* tests to specify significant differences between selected groups or time points and all comparisons, respectively. Dunnet's test was used as a *post hoc*

test for comparisons within groups for blood amino acid concentrations, glucose, insulin, urea and creatine kinase (CK) as comparisons were only made against prevalues. Comparisons of relative changes (%) between groups from before to after the training period were tested with an unpaired Student's *t* test. A sample size calculation was conducted with a power of 80% based on lean muscle mass results from an earlier study comparing whey and soy protein supplementation in young men ((15); StatMate, Graphpad Software, San Diego, CA). Based on the power calculation, our goal was to include 20 subjects in each group to obtain a 5% significance for a 1.5 percentage points difference between groups. Statistical analyses were made using Prism Software (Graphpad 6, San Diego, CA). All results are expressed as means ± SD. Statistical significance level was set at $P \leq 0.05$. For changes in muscle size and strength, we have also included 95% confidence intervals and effect sizes in order for readers to have a more nuanced interpretation of the data (Table 1).

RESULTS

Participant characteristics and compliance. There were no significant differences in participant characteristics between the milk and native whey groups at the beginning of the study (Table 1). Participants attended an average of 35.8 ± 0.5 and 35.4 ± 1.2 sessions in the native whey and milk group, respectively. The total load (repetitions × sets × kg × sessions) lifted during the 12-wk intervention was 390,000 ± 110,000 kg and 400,000 ± 95,000 kg in the milk and native whey group, respectively ($P = 0.72$). The self-reported compliance to the supplementation was 94.8% ± 2.5% and 96.4% ± 1.9% in the milk and native whey group, respectively. The total energy intake and protein [g·(kg body mass)$^{-1}$·d^{-1}] increased in during the intervention, with no differences between groups (Table 3). The milk group had a shift toward a greater energy percent (E%) from carbohydrates during the intervention and had a significantly higher E% from carbohydrates and lower E% from fat than the native whey groups.

Muscle hypertrophy and strength. All measures of total body and whole muscle growth increased by 5% to 12% in both groups, with no differences between groups (Table 1). Type II fiber CSA increased in both groups (milk, $P = 0.024$; native whey, $P = 0.022$; Table 1). Whereas, type I fiber area
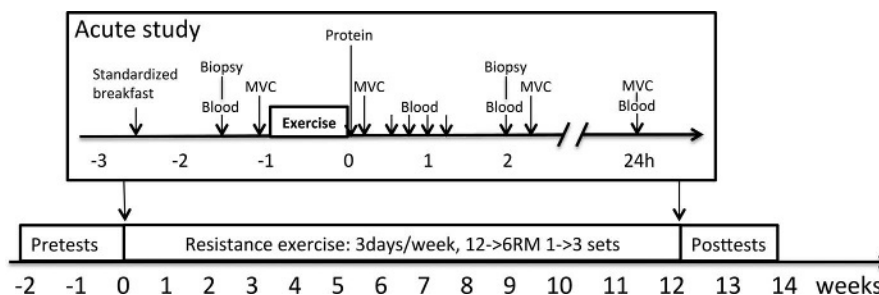

FIGURE 1—Timeline of the study.

TABLE 3. Daily energy and macronutrient intake before and during the intervention.

	Milk		Native Whey	
	Baseline	Intervention	Baseline	Intervention
Energy (kJ)	9100 ± 2700	12,600 ± 3000*	10,100 ± 3400	12,400 ± 2500*
Protein g·(kg body mass)$^{-1}$	1.5	2.0 ± 0.6*	1.4	2.0 ± 0.5*
Protein (E%)	19 ± 4	20 ± 3	19 ± 4	21 ± 3
Carbohydrate (E%)	40 ± 6	47 ± 7*	41 ± 9	39 ± 8**
Fat (E%)	41 ± 7	33 ± 6*	40 ± 8	40 ± 7**

Values are means ± SD. Values are average of two 24-h recall interviews.
*Difference between pre and post.
**Difference between milk and native whey ($P = 0.05$).

increased only in the milk group, but there were no significant differences between groups for any of the measures of muscle growth. The number of nuclei per muscle fiber also increased to a similar extent in both groups (Table 1). During the 12-wk training period, both the milk and native whey group increased 1RM in leg press and bench press (Table 1). The magnitude of strength gain did not differ between groups.

Acute experiment. Both in the untrained and in the trained state the acute exercise session resulted in muscular fatigue, demonstrated by a reduced force-generating capacity 15 min after the session in both groups (native whey, $-21.1\% \pm 10.1\%$ vs $-16.5\% \pm 2.6\%$, and milk, $-20.2\% \pm 3.0\%$, vs $-18.3\% \pm 5.3\%$, in the untrained and trained state, respectively, $P < 0.001$ for all, data not shown). Force-generating capacity was significantly reduced in the native whey group at 24 h in the untrained state ($-7.8\% \pm 12.7\%$; $P = 0.003$), but not in the trained state ($-2.3\% \pm 6.5\%$; $P = 0.740$). In the milk group, force-generating capacity was also significantly reduced at 24 h both in the untrained ($-6.8\% \pm 4.1\%$; $P < 0.007$) and trained state ($-6.9\% \pm 6.7\%$; $P = 0.005$). There were no differences between groups.

Blood measures in the acute experiment. Fasting serum concentrations of glucose did not change significantly from before to after the training intervention ($P > 0.87$). Baseline levels of serum insulin increased during the intervention (milk, 47–64 pmol·L^{-1}; $P = 0.02$; native whey, 42–78 pmol·L^{-1}; $P = 0.004$). Acutely after exercise glucose and insulin increased to about 6 mg·dL^{-1} and 400 to 600 mIU·L^{-1}, respectively, and returned toward baseline during the first hour. Urea remained stable at all sampling time points. Creatine kinase increased to between 200 and 450 U·L^{-1} at 24 h. No differences were observed between groups for glucose ($P = 0.65–0.99$), insulin ($P = 0.11–0.99$, urea ($P = 0.59–0.99$), or CK ($P = 0.93–0.99$).

Amino acid concentration in blood in the acute experiment. Fasting blood concentrations of BCAA (except isoleucine in the milk group), EAA (except histidine in both groups and phenylalanine and tryptophan in the native whey group), and total amino acids increased during the intervention period ($P < 0.05$). Blood concentrations of leucine ($P < 0.001$), BCAA ($P < 0.004$), and EAA ($P < 0.015$) were higher in the native whey than in the milk group, both in the untrained and trained state (Fig. 2). The area under the curve was greater for leucine ($P < 0.001$), BCAA ($P < 0.002$), EAA ($P = 0.001$), and total amino acids

($P < 0.033$) with native whey, compared with milk, before and after the intervention.

Signaling. Resting levels of total p70S6K protein did not change appreciably from before to after the training intervention ($P > 0.49$ for both groups; Fig. 3A). Resting phosphorylation levels of p70S6K decreased with native whey (-13%, $P = 0.041$), milk displayed a similar but not significant decrease (-10%, $P = 0.234$). Phosphorylation of p70S6K increased 2 h after exercise (milk, $370\% \pm 400\%$, $P < 0.001$; native whey, $380\% \pm 180\%$; $P < 0.001$) in the untrained state and in the trained state (milk, $390\% \pm 380\%$; $P < 0.001$; native whey, $300\% \pm 180\%$; $P = 0.016$), with both supplements. There were no significant differences between the supplements. Postexercise phosphorylation of p70S6K was significantly lower in the trained state than in the untrained state for the native whey group ($P < 0.001$).

Total resting levels of 4E-BP1 remained stable during the training period with native whey ($+3\%$, $P = 0.54$) and tended to increase with milk ($+18\%$, $P = 0.066$). Resting phosphorylation of 4E-BP1 did not change appreciably. Values of 4E-BP1 phosphorylation did not increase acutely after exercise (Fig. 3B). There were no differences between groups for any measure of 4E-BP1.

Both total and baseline phosphorylations of eEF-2 were similar before and after the training intervention in both groups. The phosphorylation of eEF-2 did not change acutely in response to exercise and protein supplementation (Fig. 3C). There were no differences between groups for any measure of eEF-2.

DISCUSSION

In this study, we tested the hypothesis that supplementation with a leucine-rich native whey protein would result in greater increases in muscle mass and strength than milk supplementation, during a 12-wk resistance training program. In an attempt to clarify potential mechanisms beyond the possible long-term differences between supplements, a more detailed acute study was conducted at the beginning and the end of the training program, on a subgroup of participants. There were three primary findings. 1) 12 wk of heavy resistance training increased muscle mass and strength, with no differences observed between supplements. 2) No significant differences in phosphorylation of p70S6K, 4E-BP1, and eEF-2 were observed between supplements. 3) Phosphorylation of p70S6K
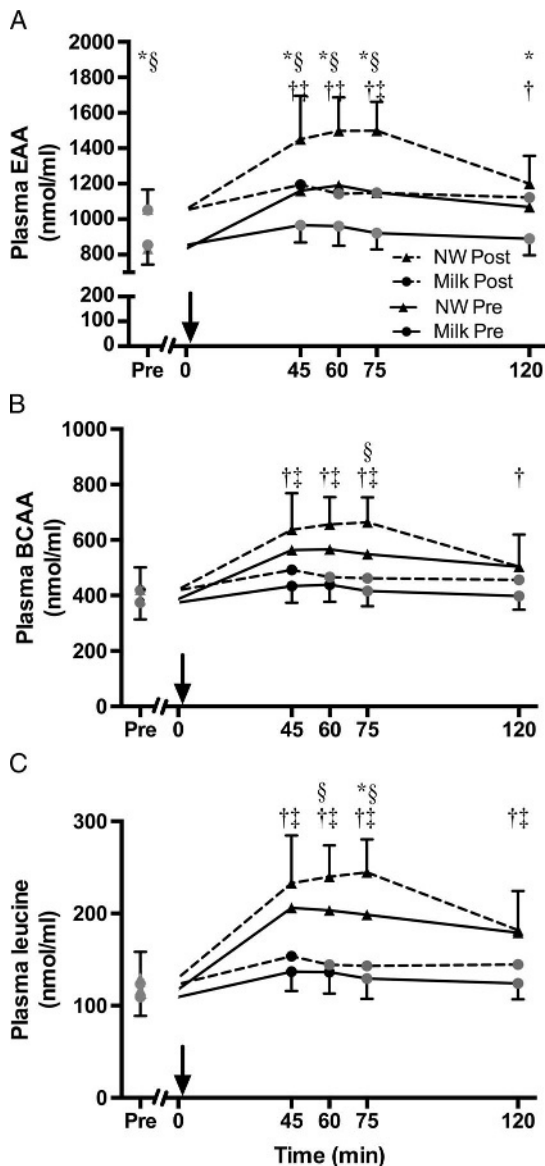
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were not able to show any meaningful correlations between fasting blood concentrations of amino acids and changes in lean body mass. The observed increase was small compared with what is needed to acutely stimulate MPS, and it is unlikely to have a direct stimulatory effect on MPS. Acutely, blood amino acid profiles were similar to previous studies, reporting a greater potential of native whey to increase blood concentrations of leucine, BCAA, and EAA compared with milk (12,13).

FIGURE 2—Blood concentrations of essential amino acids (A), branched chain amino acids (B) and leucine (C) following intake of 20 g of milk protein, or native whey immediately after a bout of resistance exercise. Arrow indicates time point of protein supplement ingestion. Values are mean ± SD (only shown for highest and lowest values). n = 12 and 10 in the milk group and native whey group, respectively. Data were analyzed with a two-way repeated measures ANOVA (time–supplement). Multiple comparisons tests were used as *post hoc* tests to specify the significant differences between groups (Tukey) and within groups (Dunnett). Black data points indicate difference form resting values, gray data points indicate no significant difference from resting levels. *Milk difference between pre and post, §native whey difference between pre and post, †milk and native whey different at pre, ‡milk and native whey different at post, $P < 0.05$.

in response to resistance exercise and protein supplementation was reduced in the trained state with native whey, compared to the untrained state.

Amino acid concentrations in blood. Both groups increased their fasting levels of most EAAs (between 10% and 30%) and total amino acids during the supplementation period. However, in contrast to a previous study (15), we
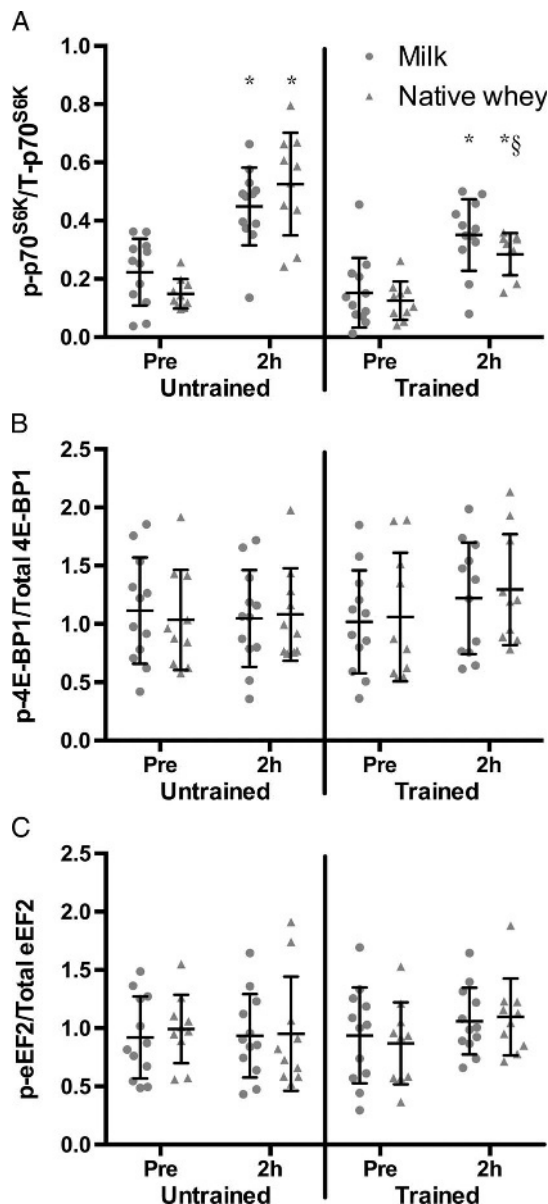
FIGURE 3—Phospho/total ratio of P70S6K (A), 4E-BP1 (B) and eEF-2 (C) after intake of milk or native whey immediately after a bout of resistance exercise. Values are mean ± SD. n = 12 and 10 in the milk group and native whey group, respectively. Data were analyzed with a two-way repeated-measures ANOVA (time–supplement). Multiple-comparisons tests were used as *post hoc* tests to specify the significant differences between groups (Tukey) and within groups (Sidak). *Different from pre within group, §Relative difference from pre, difference between untrained and trained state ($P < 0.05$).

Intracellular signaling. We observed a robust increase in phosphorylation of p70S6K after resistance exercise and protein supplementation in the untrained and trained state, for both groups. In line with previous studies, we observed no differences in intracellular signaling between whey and milk (8,16). However, these results do not align with our previous findings (12). This may result from differences in supplementation regime and timing of biopsies. Previously we supplemented with 20 g of protein immediately after and 2 h, and collected biopsies at 1 and 3 h after resistance exercise. Moreover, a change of native whey manufacturer leading to a smaller difference in leucine content per serving between milk and native whey in the current study (0.59 g vs 0.76 g), may have affected the results.

After the training intervention, the measured p70S6K response was reduced in both groups, only reaching significance with native whey. A similar diminished response to intake of whey for p70S6K, mTOR and rps6 phosphorylation, has previously been reported in elderly, but not in young, after a 12-wk strength training intervention with protein supplementation (17). Based on this and previous studies, we suggest that p70S6K is less responsive to anabolic stimuli in resistance-trained individuals than in untrained individuals (17,18). This reduced responsiveness of key signaling molecules may be a mechanism explaining a part of the reduced rate of muscle protein synthesis response to exercise observed after prolonged strength training in young adults (19,20). However, caution is warranted when interpreting these results as alterations in the anabolic response, as the p70S6K response to anabolic stimuli is not necessarily a good predictor of the magnitude of the MPS response (21,22).

Changes in the resting levels of total and phosphorylated p70S6K did not seem to explain the reduced p70S6K response to native whey in the trained state.

An alternative explanation to the seemingly reduced p70S6K response to native whey in the trained state may be temporal changes in the signaling events after exercise and protein ingestion. A more transient p70S6K (18) and MPS (20) response after resistance exercise and protein intake has previously been reported in resistance-trained individuals, when compared with nonresistance-trained individuals. Accelerated signaling events could shift the peak phosphorylation of p70S6K away from the 2-h biopsy in the trained state. A slower digestion and absorption of amino acids from milk may lead to a more prolonged phosphorylation of p70S6K, thus explaining why no significant difference in p70S6K phosphorylation 2 h after exercise and protein ingestion was observed in this group. Because we only had one postexercise biopsy, the potential changes in the temporal pattern of signaling events remain speculations.

In line with previous studies (12,23,24), there were no changes in the phosphorylation state of 4E-BP1 or eEF-2 in the untrained or trained state. 4E-BP1 is down-regulated during resistance exercise (25), supposedly to suppress the costly ATP turnover of MPS. Some studies, potentially due to a large volume of exercise (comparable to the current study), show a delayed increase in phosphorylation of 4E-BP1 after exercise (8,26,27). As 4E-BP1 has been shown to respond to protein intake (28), the phosphorylation of 4E-BP1 may already have been elevated in our resting biopsy, disguising a potential increase in response to the resistance exercise and supplements.

The anabolic signaling was similar between supplements, which agrees with the long-term outcome measures of muscle mass and strength in our study. The lack of correlation between acute signaling measures and long-term outcomes is not surprising, because investigating only a few of all kinases involved in hypertrophy is likely to give an incomplete picture. Furthermore, a potential measurable relationship between a 1-s "snap-shot" and long-term outcomes may easily be missed.

Recovery of force-generating capacity. Participants experienced a 10% to 40% reduction in muscle force-generating capacity 10 min after performing the resistance exercise, both in the untrained and the trained state. Together with a small increase in CK, this indicates mild to moderate muscular stress (29). Or results are in line with previous results from our group reporting no difference in recovery of force-generating capacity between native whey and milk groups after a "normal" bout of resistance exercise (12,13).

Effect of protein type on muscle mass and strength. Different muscle anabolic responses caused by different protein types are evident in acute settings, but have proven difficult to relate directly to hypertrophy over time (23,30). Our long-term results are in line with several acute studies comparing whey protein to milk protein or casein (8,9,16), but not all (3,12). Despite considerable gains in muscle mass, we were not able to show any differences in terms of muscle hypertrophy between milk and native whey supplementation. The supplement servings contained 1.9 g and 2.5 g of leucine for milk and native whey, respectively. This is equal to or above the estimate needed to maximally stimulate MPS in young individuals after leg exercise (1.8–2.0 g) (30). Based on earlier studies, it seems that effects of differing protein quality, on changes in muscle mass, are only evident when suboptimal doses of protein are given (15,31), and disappear as protein dose increases (11,32–34). However, a study by Cribb and colleagues (10) does not follow this trend, reporting unusually large and small effects for whey and casein, respectively. Furthermore, a recent study suggests a greater postexercise muscle protein synthesis response with 40 g of whey protein compared to 20 g after high-volume whole body resistance exercise (35). These results would suggest at least an acute advantage with native whey supplementation compared with milk in the current study. Unfortunately, MPS was not measured, but no signaling differences were observed. The importance of protein quality may be greater in populations with lower protein intake, or with a reduced anabolic response to protein ingestion, such as in the elderly (36,37).

In the case of a greater acute stimulation of MPS, it is still unclear whether this would lead to different long-term

adaptations. Supplementation increased protein intake by $0.6 \ \mathrm{g \cdot kg^{-1} \cdot d^{-1}}$ to $2.0 \ \mathrm{g \cdot kg^{-1} \cdot d^{-1}}$ in both groups. Although an insufficient protein intake hampers muscular adaptations to resistance exercise (38), the supplementation of protein on top of an already sufficient diet is generally considered to have less effect. A meta-analysis by Cermak and colleagues (39) showed greater increases in lean mass (0.81 kg) and 1RM in leg press (14.4 kg) with protein supplementation compared with placebo, in young individuals, consuming more ($1.2 \ \mathrm{g \cdot kg^{-1} \cdot d^{-1}}$) than the recommended daily protein intake. However, a later meta-analysis by Morton and colleagues (40) found no effect of protein supplementation for individuals initially consuming above $1.6 \ \mathrm{g \cdot kg^{-1} \cdot d^{-1}}$, which is close to the initial intake in our participants. Thus, any potential differences between supplements may have been masked by a relatively high-protein intake in our participants.

As untrained individuals generally are very responsive to exercise stimuli it is possible the results would be different for a trained population. The large exercise stimuli may have been enough to maximally stimulate the adaptations in our untrained participants making the anabolic stimuli of the supplements redundant. Trained individuals may need greater stimuli and are perhaps in greater need for the additional stimuli of protein ingestion, wherein the quality of protein may play a role. These speculations receive some support from meta-analyses suggesting greater effects of protein supplementation in trained individuals (39,40).

A difference between protein supplements is expected to be less than that between protein and placebo. Previous studies comparing whey and casein in combination with strength training suffer from methodological challenges, such as a suboptimal number of participants and limited control of diet, and results are equivocal (10,11). Whey and milk are considered high-quality proteins, and have both been shown to be superior to soy protein for increasing lean mass (15,31). Thus, small differences between groups should be expected when comparing these supplements. To obtain a significant difference between our groups for lean mass, using the results of the current study, a sample size of about 600 participants would be needed. However, a lean mass difference of 0.19 kg gained after 12 wk of training is small and not clinically relevant. The fact that we observed minor or no differences between groups by using several methods quantifying muscle mass (DXA, MRI, ultrasound, immunohistochemistry) strengthens our main finding in this study that very small differences occurred between the supplements. In accordance with the observed changes in muscle mass, 1RM strength also increased substantially, and to a similar extent in both groups. The increase in 1RM leg press and bench press was within the expected range based on previous comparable studies (15,33,41).

Limitations. The large variability in response to the training intervention and the modest size of the study groups makes it difficult to confidently exclude a type II statistical error. However, the differences observed were small, substantiated by the measurements of a number of variables, and increasing the statistical power is not likely to add any clinical relevance to the outcomes.

It is important to note that our results may have been different in other populations with a lower protein intake or in populations with a greater need for anabolic stimuli to maximize adaptation, such as trained individuals, elderly and intensive care patients. The large protein intake during the intervention may have masked potential differences between supplements in our participants.

Because participants ingested supplements immediately after exercise, the postexercise intake had a compliance of 100%. For all other supplement intakes, we relied on the participants to report their own adherence to the intervention. Although participants were highly motivated and reported supplement intake three times per week, we cannot exclude an overestimation of adherence to the supplementation scheme.

CONCLUSIONS

Whole-body heavy resistance exercise for $3 \ \mathrm{d \cdot wk^{-1}}$, over 12 wk, effectively increased muscle mass and strength in young participants. Our results suggests no relevant differences in changes in muscle mass or strength when supplementing 2×20 g milk or native whey daily in combination with strength training. Acute phosphorylation of p70S6K, 4E-BP1, and eEF-2 did not differ between supplements.

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