

Applied nutritional investigation

# Impact of differing protein sources and a creatine containing nutritional formula after 12 weeks of resistance training

Chad M. Kerksick, Ph.D.<sup>a,\*</sup>, Chris Rasmussen, M.S.<sup>b</sup>, Stacy Lancaster, M.S.<sup>b</sup>,  
Michael Starks, Ph.D.<sup>b</sup>, Patty Smith, M.S.<sup>b</sup>, Charlie Melton, M.S.<sup>b</sup>,  
Mike Greenwood, Ph.D.<sup>b</sup>, Anthony Almada, M.S.<sup>c</sup>, and Richard Kreider, Ph.D.<sup>b</sup>

<sup>a</sup> Applied Biochemistry and Molecular Physiology Laboratory, Health and Exercise Science Department, University of Oklahoma, Norman, Oklahoma, USA

<sup>b</sup> Exercise and Sport Nutrition Laboratory, Center for Exercise, Nutrition and Preventive Health Research, Department of Health, Human Performance and Recreation, Baylor University, Waco, Texas, USA

<sup>c</sup> IMAGINutrition, Inc., Laguna Niguel, California, USA

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## Abstract

**Objective:** We evaluated whether colostrum (Col) or an isocaloric and isonitrogenous blend of whey and casein in addition to creatine (Cr) affects body composition, muscular strength and endurance, and anaerobic performance during resistance training.

**Methods:** Forty-nine resistance-trained subjects participated in a standardized 12-wk total body resistance training program. In a double-blind and randomized manner, subjects supplemented their diet with a protein control (Pro), Pro/Col, Pro/Cr, or Col/Cr. Supplements were isocaloric and isonitrogenous and provided 60 g/d of casein/whey (Pro) or Col as the protein source. At 0, 8, and 12 wk of supplementation, subjects were weighed, had body composition determined using dual-energy X-ray absorptiometry (DXA), performed one-repetition maximum (1RM) and 80% of 1RM tests on the bench press and leg press, and 30-s anaerobic sprint capacity tests. Data (mean  $\pm$  SD) were analyzed by repeated measures analysis of variance and reported as raw data in all tables and as changes from baseline for all figures for the Pro, Pro/Col, Pro/Cr, and Col/Cr groups, respectively.

**Results:** Resistance training increased 1RM strength, muscular endurance, and anaerobic sprint capacity equally in all groups. Significant main and interaction effects ( $P < 0.05$ ) were found for body mass, DXA total scanned mass, and fat-free mass (FFM; lean plus bone), whereas no changes ( $P > 0.05$ ) were noted for fat mass, percent fat, or bone content. Post hoc analysis showed that, compared with Pro, subjects ingesting Pro/Col, Pro/Cr, and Col/Cr showed greater gains in body mass and DXA total scanned mass. Subjects ingesting Pro/Cr and Col/Cr had greater increases in FFM during training in comparison with Pro/Col.

**Conclusion:** In conjunction with 12 wk of resistance training, ingestion of Col or a blend of whey and casein protein with a vitamin/mineral supplement containing Cr resulted in greater improvements in FFM in comparison with Pro and Pro/Col. © 2007 Elsevier Inc. All rights reserved.

**Keywords:** Colostrum; Casein; Whey; Protein; Creatine; Nutritional supplementation

## Introduction

Athletes involved in intense training are thought to have greater dietary protein needs than individuals who do not

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\* Corresponding author. Tel.: +405-325-9021; fax: +405-325-0594.

E-mail address: chad\_kerksick@ou.edu (C. M. Kerksick).

train [1,2]. Collectively, the milk proteins (e.g., whey, casein, and colostrum) are the most popular forms of protein supplements [3]. Although all three types of proteins are complete proteins with an abundance of essential amino acids and other peptide components (e.g., lactoferrin,  $\beta$ -lactalbumin, etc.), each has some unique characteristics [3,4]. For example, whey protein has been considered “fast” protein because its constituent amino acids are released into the gut at a faster rate when compared with casein. Whey is

hypothesized to have an effect on promoting protein synthesis but having very little role in protein breakdown [4]. Conversely, casein protein is considered a “slow” protein and has been shown to release its amino acids from the gut at a much slower rate in comparison with whey protein [4]. Consequently, casein works to decrease the rate of protein breakdown but has little influence on protein synthesis [4].

Colostrum is an additional protein source, which has become more popular in recent years [5–7]. Bovine colostrums are the “early” milk produced by cows during the first few days after giving birth [6]. This milk has a much different nutrient profile and immunologic composition compared with regular milk [6,8]. The macronutrient profile is similar to other forms of milk, but bovine colostrums have a much higher concentration of immunoglobulins, growth factors, and antimicrobial constituents [6,8]. Insulin-like growth factor-1 (IGF-1) is a growth factor that is mechanistically linked to skeletal muscle hypertrophy [9]. It is purported that supplementation with colostrum can increase serum levels of IGF-1 [10], although others have not supported this relation [11]. These characteristics have led many investigators to suggest that colostrum supplementation may enhance training adaptations and subsequent physical performance [12–15]. In addition to the growth factors, concentrations of various antimicrobial agents such as lactoperoxidase, lactoferrin, lysozymes, and immunoglobulins (A, G, and M) have been hypothesized to provide colostrum with increased immune support and function [5,16]. Studies have shown that bovine colostrum has ~100-fold higher concentrations of immunoglobulins A, G, and M compared with normal milk [10].

Many nutritional supplements have been developed in an attempt to provide an ergogenic benefit in addition to promoting accretion of lean tissue while resistance training. Many of these products have been marketed as anabolic and/or ergogenic agents. In this regard, creatine monohydrate has become extremely popular for its efficacy to increase short-term, explosive activity performance, promoting lean tissue accretion and mRNA expression of myogenic regulators [17–19]. The overall safety and efficacy of creatine monohydrate has also been well documented [20–22].

Some recent studies have shown that combinations of proteins (e.g., whey/casein) in addition to creatine and free form amino acids during resistance training may not provide any advantage over a carbohydrate control [23,24], whereas other studies have reported improvements of 3 to 5 kg of fat-free mass (FFM) during resistance training [3,17]. Fry et al. [25] investigated the impact a creatine-containing formulation with different combinations of protein (e.g., whey, casein, and colostrum) had on resistance training adaptations. This study, completed in collaboration with other colleagues in our research group, reports data from a subset of 19 participants of the entire cohort who provided vastus lateralis muscle biopsies before and after the 12-wk supplementation period. This initial publication suggested no greater effect on changes in body composition, force

production and cellular adaptations, fiber type percentages, fiber cross-sectional area, relative fiber area, and relative major histocompatibility complex expression in comparison with a protein control [25]. The purpose of this study was to present data on the entire cohort of participants and determine the impact various forms of protein (e.g., whey, casein, colostrum) supplementation with or without a creatine-containing nutrition formulation may have had on body mass, body water, body composition, muscular strength, muscular endurance, and anaerobic capacity.

## Materials and methods

### Experimental design

This study was conducted as a double-blind, placebo-controlled, randomized clinical trial with subjects matched according to age and FFM before the study. All subjects were tested at 0, 8, and 12 wk to determine any changes in criterion variables. Several a priori hypothesis was made: 1) all groups would improve their strength and body composition; 2) colostrum supplementation (Pro/Col) would promote greater improvements in strength and body composition over the protein control (Pro) group; and 3) the addition of creatine to colostrum (Col/Cr) and protein control (Pro/Cr) would further promote improvements in strength and body composition over non-creatine groups (Pro/Col).

### Subjects

Forty-nine apparently healthy male ( $n = 36$ ) and female ( $n = 13$ ) subjects, 18 to 45 y of age, volunteered to participate. Subjects were informed about the experimental procedures and signed informed consent statements and medical history forms in adherence with the human subjects' guidelines of the University of Memphis and the American College of Sports Medicine before any data collection. Subjects' descriptive characteristics are presented in Table 1.

### Entrance criteria

To participate in this study, subjects had to 1) sign statements indicating they had no history of anabolic steroid use; 2) be experienced with resistance training (>1 y of training) and currently training >3 h/wk with a program that included the bench press and leg press/squat exercises; 3) refrain from participating in any non-leisure endurance training for >20 min at a time (e.g., running, cycling, swimming, etc.) for the entire study; 4) have not ingested creatine,  $\beta$ -hydroxy- $\beta$ -methylbutyrate, or thermogenics before the study and to not take any nutritional supplements or non-prescription drugs during the study; 5) agree to follow a predetermined workout program; 6) not have any existing medical conditions that would compromise participation in the study; and 7) avoid any

Table 1  
Descriptive characteristics of subjects

Variable	Gender*	Mean	SD
Body weight (kg)	Male	84.1	11.9
	Female	65.0	14.4
	Total	79.0	15.1
Height (cm)	Male	178.4	6.9
	Female	164.8	5.8
	Total	174.8	9.0
Age (y)	Male	27.3	6.5
	Female	27.1	5.3
	Total	27.2	6.2
DXA percent body fat	Male	16.5	4.5
	Female	26.9	7.7
	Total	19.3	7.2

DXA, dual-energy X-ray absorptiometry

\*  $n = 36$  males,  $n = 13$  females,  $n = 49$  total subjects.

regular nutritional practices that might confound the results of the study (i.e., vegetarianism, caloric restriction, food allergies, etc.).

#### Familiarization and testing sessions

Subjects participated in one familiarization session and three identical testing sessions at 0, 8, and 12 wk. During the familiarization session, informed consent statements were signed and medical and exercise history forms were completed. A general physical examination (e.g., heart rate, blood pressure, breath sounds, reviewing medical history form, etc.) was completed and participants were risk-stratified according to American College of Sports Medicine criteria. Subjects completed practice trials of all strength testing and anaerobic capacity equipment before being provided specific instructions on exercise technique and recording of training data. Approximately 1 wk separated the familiarization session from the baseline testing session (T0) to allow time for subjects to complete dietary recalls. Presupplementation assessments included 1) a 4-d dietary record, 2) measurement of body mass and total body water by bioelectrical impedance analysis, 3) body composition assessment using dual-energy X-ray absorptiometry (DXA), and 4) one-repetition maximum (1RM) strength tests (normalized per unit of body mass) on the bench press and leg presses. After each respective 1RM test, subjects completed a maximal repetitions to fatigue test with 80% of their 1RM as a measurement of muscular endurance; and 5) peak power, total work, and fatigue rate using a computerized 30-s Wingate testing system on a cycle ergometer.

Subjects were matched according to FFM and age by stratifying participants into clusters. Participants from all clusters started supplementation and training at the same time. In a double-blinded and randomized manner, subjects were assigned to one of four isocaloric and isonitrogenous supplement groups (Table 2).

#### Procedures

Subjects were instructed to report to a research nurse at the end of each week of training to report the frequency and/or severity of any possible side effects (i.e., bloating,

Table 2  
Nutrition information for supplements\*

Nutrient	Pro	Pro/Col	Pro/Cr	Col/Cr
<b>Protein (g)</b>				
Casein	43.5	7.5	43.5	7.5
Whey	31.5	7.5	31.5	7.5
Colostrum		60		60
<b>Carbohydrates (g)</b>				
Lactose	1.525	1.525	1.525	1.525
Saccharose	15.1	15.1	15.1	15.1
Organic acids	0.9	0.9	0.9	0.9
Others	0.2	0.2	0.2	0.2
<b>Fat (g)</b>				
Saturated	1.3	1.3	1.3	1.3
Monounsaturated	4.4	4.4	4.4	4.4
Linoleic acid	1.73	1.73	1.73	1.73
$\alpha$ -Linolenic acid	0.37	0.37	0.37	0.37
<b>Minerals (mg)</b>				
Sodium			108	108
Potassium			750	750
Chlorine			203	203
Calcium			315	315
Phosphorus			183	183
Magnesium			20	20
<b>Trace elements</b>				
Iron (mg)			1	1
Zinc (mg)			15	15
Copper (mg)			1.5	1.5
Manganese (mg)			3.0	3.0
Fluorine (mg)			1.0	1.0
Molybdenum ( $\mu$ g)			50	50
Selenium ( $\mu$ g)			50	50
Chromium ( $\mu$ g)			33	33
Iodine ( $\mu$ g)			100	100
<b>Vitamins</b>				
Vitamin A ( $\mu$ g RE)			668	668
Vitamin D ( $\mu$ g)			5	5
Vitamin E (mg $\alpha$ -TE)			538	538
Thiamin (mg)			25	25
Riboflavin (mg)			3.0	3.0
Niacin (mg NE)			20	20
Pantothenate (mg)			4.0	4.0
Vitamin B6 (mg)			6.0	6.0
Folate ( $\mu$ g)			600	600
Vitamin B12 ( $\mu$ g)			3.0	3.0
Biotin ( $\mu$ g)			100	100
Vitamin C (mg)			250	250
<b>Other nutrients</b>				
Carnitine (g)			3	3
Choline (mg)			100	100
Creatine (g)			3	3
Taurine (g)			3	3
Coenzyme Q10 (mg)			50	50

$\alpha$ -TE,  $\alpha$ -tocopherol equivalents; Col/Cr, colostrum + creatine formulation; NE, niacin equivalents; Pro, protein control; Pro/Col, protein control + colostrum; Pro/Cr, protein control + creatine formulation; RE, retinol equivalents.

\* Values represent approximately daily intake.

cramps, diarrhea, etc.) and their compliance to the training and supplementation protocols. If participants failed to contact the nurse, the nurse initiated this follow-up.

Supplements were prepared in powder form with similar smell, texture, taste, and appearance and independently packaged/labeled in single-serving foil packets for double-blinded administration. All supplements were verified for macronutrient content and for creatine and vitamin/mineral contents by Covance Laboratories, (Madison, WI, USA). Subjects were instructed to mix the supplement with water, juice, or milk and ingest the solution as soon as possible, but ideally within 1 h [26], after their workouts on training days and in the morning (~0900 h) of non-training days. Supplement ingestion date/time was recorded daily and empty supplement packets were collected and counted as a percentage of those consumed to verify subject compliance with supplementation. Subjects were instructed to maintain their normal diet and record all food and fluid consumed over a 4-d period (3 weekdays, 1 weekend day) at designated times to ensure dietary habits did not change. All dietary records were analyzed by the same individual who had several years of experience entering dietary records using ESHA Food Processor 7.8 (ESHA Research, Salem, OR, USA). Unavailable foods were entered into the database from the manufacturer's label. Four-day averages of caloric and macronutrient intake were calculated from each dietary record and expressed per unit of body weight per day.

For initial and follow-up testing, subjects reported to the laboratory between 0800 and 1000 h to control for diurnal variation. Due to the work schedules of some participants, their testing sessions were completed during similar times in the afternoon for the three sessions. Body weight was obtained using a calibrated Healthometer digital strain gauge electronic scale (Bridgeview, IL, USA) with a precision of  $\pm 0.02$  kg. Total body water was estimated using a Valhalla Bioelectrical Impedance Analyzer (Valhalla Scientific, San Diego, CA, USA) [27].

Whole-body (excluding cranium) composition was estimated according to previous procedures [28,29] by certified investigators using a Hologic QDR-4500W DXA using Hologic 9.80C (Waltham, MA, USA). This DXA device scans and measures bone, fat, lean (bone-free) mass, and FFM (lean plus bone) in the right and left arms and legs and the torso. Hologic software uses these values to calculate whole-body (excluding cranial) bone, fat, and lean masses. Percent body fat was determined by dividing the amount of fat mass by the total scanned mass. DXA is a highly reliable method of determining soft tissue body composition and percent body fat for specific body regions and for the whole body [28–31]. Manufacturer and state-certified investigators performed all DXA analyses. Quality control calibration procedures were performed on a spine phantom (Hologic X-CALIBER Model DPA/QDR-1 anthropometric spine phantom) before each testing session according to standard procedures [32]. Mean coefficients of varia-

tion in bone mineral content and bone mineral density measurements on the spine phantom ranged from 0.41% to 0.55% throughout the life of the unit. Subjects were positioned on the DXA table using standardized methods for each test. Test-retest reliability studies performed on male athletes with this DXA machine yielded a mean deviation for total bone mineral content and total fat free/soft tissue mass of 0.31% with a mean intraclass correlation of 0.985 [32].

After body composition analysis, subjects performed 1RMs and maximal repetitions to fatigue tests using 80% of their predetermined 1RM with the bench press and leg press. All data were normalized per unit of body mass and the maximal load (repetitions completed times weight used) was calculated from the maximal repetitions test as a measurement of muscular endurance. A warmup of two sets of 10 repetitions at ~50% 1RM was typically followed by three to five progressive 1RM attempts with 2-min rest between attempts using a standard 20-kg barbell and bench press (AMF, Jefferson, IA, USA). Grip width was recorded and all weight plates used were numbered and similar across all testing sessions. Subjects were required to maintain good lifting form (i.e., feet in contact with the floor, buttocks remaining in contact with the bench, no bouncing of the bar off of the chest) during all lifts. Once bench press 1RM was determined, subjects were allowed a 5-min rest and completed a maximal repetitions to fatigue test with 80% of their bench press 1RM. Subjects were given 5 min of rest, and leg press 1RM was determined on an AMF hip sled. Subjects were positioned flat on their back in a back/shoulder support, which was adjusted so that the subject was positioned with thighs approximately 1 to 2 inches from their torso and their knees at an angle approximately equal to 90 degrees. Back/shoulder support, foot placement, and weight plates used were numbered and recorded for subsequent testing sessions. Subjects were required to maintain good lifting form (hands/forearms at their sides with the lower back flat on the back pad) [33]. Subjects typically used four to six attempts to achieve their leg press 1RM while appropriately adjusting the weight with a 2-min rest between attempts. Subjects were given a 5-min rest and completed a maximal repetitions to fatigue test with 80% of their leg press 1RM. During all testing sessions, subjects were equally encouraged by the investigators. Test-retest reliability of performing these strength tests in our laboratory on resistance-trained subjects have yielded low mean coefficients of variation and high reliability for the bench press (1.9%, intraclass  $r = 0.94$ ) and hip sled/leg press (0.7%, intraclass  $r = 0.91$ ) [17].

Subjects completed a 30-s Wingate anaerobic capacity sprint test on a cycle ergometer. The sprint tests were performed at a standardized resistance of 0.70 N on a computerized  $\text{CardiO}_2$  cycle ergometer (ErgometR<sub>x</sub> Corp., St. Paul, MN, USA) equipped with toe clips. Seat position and height were recorded and standardized be-

Table 3  
Resistance training program

Exercise order	
Monday, Thursday*†	Tuesday, Friday*†
Bench press	Leg press
Chest flies	Leg extensions
Lateral pull	Dead lift
Seated row	Lunges
Shoulder press	Lying leg curls
Shoulder shrugs	Heel raises
Biceps curls	
Triceps extensions	
Weeks	Sets × repetitions
1–4	3 × 10
5–8	3 × 8
9–12	3 × 6
Abdominal crunches remained at 3 × 25	

\* One-minute rest between sets.

† Two-minute rest between exercises.

tween trials. The ergometer was connected with a RS232 parallel interface to a Dell 466/Le Optiplex computer (Dell Computer Corp., Austin, TX, USA) using ErgometR<sub>x</sub> Cardioscribe and Exerscribe software (ErgometR<sub>x</sub> Corp.). Crank frequency was measured using a crystal referenced optic encoder with a precision range of 0–200 rpm and an accuracy of ±1 rpm. Power production was determined by a calibrated strain gauge with a range of 0–2000 W and an accuracy of ±1.0%. Data were collected and downloaded into the computer at 0.5-s intervals. Test-retest reliability in our laboratory for Wingate sprints tests is  $r = 0.96$  [34].

The training program consisted of four workouts per week (two for upper body and two for lower body), which primarily used multijoint exercises that targeted the major muscle groups (Table 3). All subjects were required to perform each exercise to the point of reaching muscular failure at the last repetition of each set [33,35]. Subjects were instructed to rest for approximately 1 min between sets and 2 min between each exercise. Workouts were completed at each participant's own training facility and were verified by a training partner, fitness instructor, or personal trainer.

### Statistical analysis

A priori power analysis revealed values of 0.16, 0.78, and 0.98 for small (0.25), moderate (0.75), and large (1.25) effect sizes, respectively for the sample size used in this study. All criterion-dependent variables were analyzed by separate  $4 \times 3$  (group × test) univariate analysis of variance with repeated measures on test. Dietary intake (calorie, carbohydrate, protein, and fat intakes) were evaluated by one-way analysis of variance using SPSS 11.5 for Windows (SPSS, Inc., Chicago, IL, USA). Group means were considered significantly different when the probability of type I error was ≤0.05. Post hoc procedures were conducted when necessary using

Tukey's  $t$  test. Delta scores for selected variables were calculated as post-test less pretest values and are presented graphically. Remaining raw data are listed in tables and presented as mean ± standard deviation.

## Results

### Monitoring of side effects

The training and supplementation protocols were well tolerated by most participants. As expected, mild side effects (i.e., bloating, cramps, diarrhea) were reported by a small number ( $n < 10$ ) of participants, but none of these side effects compromised compliance to the training or supplementation regimen. One woman terminated her participation due to an inability to consume the assigned supplement.

### Nutritional data

There were no statistically significant differences ( $P > 0.05$ ) between groups for total calorie, carbohydrate, protein, and fat intakes (Table 4).

### Body composition

There were no significant changes for total body water, DXA fat mass, DXA percent body fat, and bone mineral content for all four groups over time (Table 5). Significant increases across time for all four groups were seen for body mass, DXA total scanned mass, and DXA FFM. Significant interactions and subsequent post hoc analysis of the body

Table 4  
Dietary intake (normalized per unit body weight) for the Pro, Pro/Col, Pro/Cr, and Col/Cr groups

Variable	Group*	Mean ± SD	Significance
Energy intake (kcal · kg <sup>-1</sup> · d <sup>-1</sup> )	Pro	38.9 ± 14.4	0.558
	Pro/Col	38.9 ± 11.3	
	Pro/Cr	34.1 ± 5.0	
	Col/Cr	39.2 ± 9.9	
Carbohydrate intake (g · kg <sup>-1</sup> · d <sup>-1</sup> )	Pro	4.5 ± 1.6	0.480
	Pro/Col	4.6 ± 1.2	
	Pro/Cr	3.9 ± 0.7	
	Col/Cr	4.3 ± 1.0	
Protein intake (g · kg <sup>-1</sup> · d <sup>-1</sup> )	Pro	2.2 ± 0.9	0.488
	Pro/Col	1.9 ± 0.4	
	Pro/Cr	2.0 ± 0.4	
	Col/Cr	2.3 ± 0.9	
Fat intake (g · kg <sup>-1</sup> · d <sup>-1</sup> )	Pro	1.3 ± 0.6	0.539
	Pro/Col	1.3 ± 0.7	
	Pro/Cr	1.1 ± 0.2	
	Col/Cr	1.3 ± 0.5	

Col/Cr, colostrum + creatine formulation; Pro, protein control; Pro/Col, protein control + colostrum; Pro/Cr, protein control + creatine formulation

\* Pro group,  $n = 12$ ; Pro/Col group,  $n = 13$ ; Pro/Cr group,  $n = 13$ ; Col/Cr group,  $n = 11$ .

Table 5  
Body water and body composition changes for the Pro, Pro/Col, Pro/Cr, and Col/Cr groups

Variable	Group*	Week 0	Week 8	Week 12	Significance	
Body water (L)	Pro	48.9 ± 11.0	48.8 ± 10.3	48.9 ± 10.4	Group	0.751
	Pro/Col	48.4 ± 7.7	48.3 ± 5.2	50.1 ± 7.7	Time	0.519
	Pro/Cr	49.4 ± 15.4	48.0 ± 10.8	50.0 ± 10.4	Group × time	0.892
	Col/Cr	52.1 ± 15.7	54.4 ± 10.3	52.1 ± 11.9		
Body water (%)	Pro	61.8 ± 3.1	61.4 ± 3.2	61.6 ± 3.0	Group	0.414
	Pro/Col	61.9 ± 5.9	61.2 ± 4.5	61.4 ± 6.0	Time	0.950
	Pro/Cr	59.6 ± 4.7	61.4 ± 6.5	60.5 ± 5.7	Group × time	0.374
	Col/Cr	63.1 ± 4.3	64.5 ± 4.4	63.0 ± 4.7		
DXA total scanned mass (kg)	Pro	71.4 ± 14.9	72.1 ± 14.6	72.0 ± 15.2 <sup>†</sup>	Group	0.953
	Pro/Col	73.5 ± 11.0	74.9 ± 11.3	75.6 ± 11.0 <sup>‡</sup>	Time	<0.001 <sup>†</sup>
	Pro/Cr	72.1 ± 16.2	74.3 ± 16.8	73.7 ± 16.7 <sup>‡</sup>	Group × time	0.021 <sup>‡</sup>
	Col/Cr	73.2 ± 17.0	75.7 ± 17.0	76.4 ± 17.2 <sup>‡</sup>		
DXA fat mass (kg)	Pro	12.2 ± 3.6	11.9 ± 3.6	12.0 ± 3.4	Group	0.506
	Pro/Col	14.2 ± 6.9	14.4 ± 7.2	15.0 ± 6.9	Time	0.093
	Pro/Cr	15.8 ± 7.5	16.0 ± 7.1	15.8 ± 7.1	Group × time	0.274
	Col/Cr	13.6 ± 9.8	13.9 ± 6.7	14.2 ± 6.8		
DXA body fat (%)	Pro	17.3 ± 5.0	16.6 ± 4.5	16.9 ± 4.2	Group	0.444
	Pro/Col	19.3 ± 7.3	19.1 ± 7.5	19.8 ± 7.2	Time	0.743
	Pro/Cr	21.7 ± 7.7	21.4 ± 7.0	21.2 ± 7.1	Group × time	0.401
	Col/Cr	18.6 ± 8.4	18.5 ± 8.1	18.7 ± 8.2		
DXA fat-free mass (kg)	Pro	59.2 ± 13.5	60.2 ± 13.0	60.0 ± 13.5	Group	<0.911
	Pro/Col	59.2 ± 9.8	60.5 ± 9.9	60.5 ± 9.8	Time	<0.001 <sup>†</sup>
	Pro/Cr	56.3 ± 13.6	58.3 ± 13.9	57.9 ± 13.6	Group × time	<0.035 <sup>‡</sup>
	Col/Cr	59.6 ± 14.1	61.7 ± 14.5	62.2 ± 14.6		
DXA bone content (kg)	Pro	2.26 ± 0.5	2.25 ± 0.5	2.28 ± 0.5	Group	0.955
	Pro/Col	2.18 ± 0.4	2.21 ± 0.4	2.20 ± 0.4	Time	0.062
	Pro/Cr	2.15 ± 0.5	2.17 ± 0.5	2.13 ± 0.5	Group × time	0.930
	Col/Cr	2.21 ± 0.5	2.22 ± 0.5	2.24 ± 0.5		

Col/Cr, colostrum + creatine formulation; DXA, dual-energy x-ray absorptiometry; Pro, protein control; Pro/Col, protein control + colostrum; Pro/Cr, protein control + creatine formulation

\* Pro group,  $n = 12$ ; Pro/Col group,  $n = 13$ ; Pro/Cr group,  $n = 13$ ; Col/Cr group,  $n = 11$ . Raw data are presented as mean ± SD.

<sup>†</sup> Significant main effect for time ( $P < 0.05$ ).

<sup>‡</sup> Pro/Cr and Col/Cr greater than Pro ( $P < 0.05$ ).

<sup>§</sup> Pro/Col, Pro/Cr, and Col/Cr greater than Pro ( $P < 0.05$ ).

composition data revealed that the Pro/Col, Pro/Cr, and Col/Cr groups had significantly greater increases in comparison with the Pro group for body mass ( $P = 0.02$ ) and DXA total scanned mass ( $P = 0.02$ ). Participants who ingested Pro/Cr and Col/Cr had greater ( $P < 0.05$ ) gains in DXA FFM than those who ingested Pro ( $P = 0.04$ ). Delta values were graphed to highlight the changes made over time (Figs. 1 and 2).

#### Muscular strength and endurance

There were significant increases across time ( $P < 0.05$ ) for bench press 1RM and load and for leg press 1RM and load. No significant group × time interactions were found for the normalized bench press 1RM, bench press load, leg press 1RM, and leg press load (Table 6).

#### Anaerobic capacity

For the 30-s Wingate test, there was a significant increase over time for peak power across groups; however, there were no changes for total work and fatigue

index (Table 6). There were no significant group × time interactions for peak power, total work, and fatigue index.

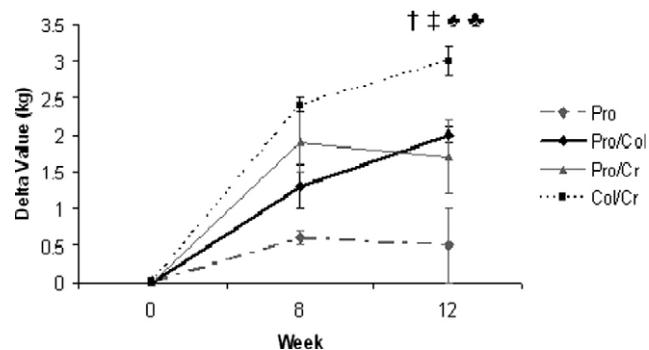


Fig. 1. Delta value change in body mass (kilograms) at 0, 8, and 12 wk. Data are mean ± SD. <sup>†</sup>All groups showed a significant increase from time 0. <sup>‡</sup>Pro/Col significantly greater than Pro. <sup>§</sup>Col/Cr significantly greater than Pro. <sup>¶</sup>Pro/Cr significantly greater than Pro. Col/Cr, colostrum + creatine formulation ( $n = 11$ ); Pro, protein control (whey protein + casein protein;  $n = 12$ ); Pro/Col, protein control + colostrum ( $n = 13$ ); Pro/Cr, protein control + creatine formulation ( $n = 13$ ).

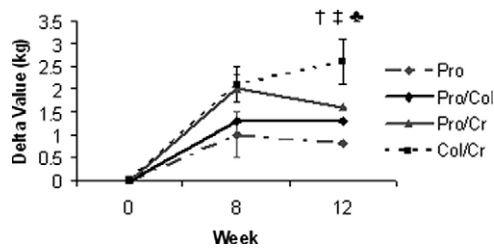


Fig. 2. Delta value change in dual-energy X-ray absorptiometric fat-free mass (kilograms) at 0, 8, and 12 wk. Data are mean  $\pm$  SD.  $\dagger$ All groups showed a significant increase from time 0.  $\ast$ Col/Cr significantly greater than Pro.  $\ddagger$ Pro/Cr significantly greater than Pro. Col/Cr, colostrum + creatine formulation ( $n = 11$ ); Pro, protein control (whey protein + casein protein;  $n = 12$ ); Pro/Col, protein control + colostrum ( $n = 13$ ); Pro/Cr, protein control + creatine formulation ( $n = 13$ ).

## Discussion

The present study is one of the few studies that have examined changes in body composition, including body water, and muscular performance after 12 wk of rigorous resistance training while supplementing with equivalent amounts of protein but from different sources. The primary findings of the

present study suggest that combined supplementation of colostrum protein plus creatine or a whey/casein protein blend plus creatine (Table 2) promotes the greatest increases in FFM mass while completing 12 wk of rigorous resistance training when compared with isocaloric, isonitrogenous controls. All groups demonstrated a significant increase over time ( $P < 0.05$ ) for body mass, DXA total scanned mass, and FFM; however, the Pro/Cr and Col/Cr groups showed greater increases in FFM compared with the Pro group.

Protein supplementation has been suggested to increase resistance training adaptations and has increased in popularity over the past decade [36,37]. Milk proteins such as casein and whey are the predominant sources of protein for these products [3,24,38]. Scientific evidence supports the use of these proteins to effectively deliver key nutrients and enhance performance [1,4,26,38]. Regarding colostrum supplementation, one study used lower doses (20 g/d) during resistance training [7], whereas others have looked at other modes of exercise (e.g., treadmill running, high-intensity rowing, repeated sprinting performance, etc.) while implementing dosing regimens similar to that in the present study [11–13,39].

Table 6

Maximal strength (normalized per unit body weight), lifting load (repetitions  $\times$  weight), and Wingate anaerobic capacity changes for the Pro, Pro/Col, Pro/Cr, and Col/Cr groups

Variable	Group*	Week 0	Week 8	Week 12	Significance	
Relative bench press 1RM (kg/kg BW)	Pro	0.48 $\pm$ 0.17	0.51 $\pm$ 0.15	0.51 $\pm$ 0.15	Group	0.573
	Pro/Col	0.48 $\pm$ 0.14	0.51 $\pm$ 0.13	0.49 $\pm$ 0.12	Time	0.001 $\ddagger$
	Pro/Cr	0.41 $\pm$ 0.15	0.45 $\pm$ 0.15	0.44 $\pm$ 0.14	Group $\times$ time	0.436
	Col/Cr	0.48 $\pm$ 0.13	0.51 $\pm$ 0.13	0.51 $\pm$ 0.13		
Bench press load (repetitions/kg)	Pro	692 $\pm$ 276	735 $\pm$ 357	728 $\pm$ 352	Group	0.727
	Pro/Col	681 $\pm$ 276	818 $\pm$ 370	716 $\pm$ 310	Time	0.019 $\ddagger$
	Pro/Cr	629 $\pm$ 351	605 $\pm$ 305	715 $\pm$ 342	Group $\times$ time	0.553
	Col/Cr	730 $\pm$ 274	757 $\pm$ 353	879 $\pm$ 318		
Relative leg press 1RM (kg/kg BW)	Pro	0.78 $\pm$ 0.27	0.86 $\pm$ 0.25	0.85 $\pm$ 0.22	Group	0.655
	Pro/Col	0.78 $\pm$ 0.23	0.86 $\pm$ 0.19	0.87 $\pm$ 0.21	Time	<0.001 $\ddagger$
	Pro/Cr	0.66 $\pm$ 0.30	0.76 $\pm$ 0.28	0.77 $\pm$ 0.26	Group $\times$ time	0.299
	Col/Cr	0.73 $\pm$ 0.26	0.84 $\pm$ 0.25	0.87 $\pm$ 0.24		
Leg press load (repetitions/kg)	Pro	2048 $\pm$ 1268	2840 $\pm$ 1947	2816 $\pm$ 2236	Group	0.499
	Pro/Col	1937 $\pm$ 1148	2584 $\pm$ 917	2551 $\pm$ 1156	Time	<0.001 $\ddagger$
	Pro/Cr	1117 $\pm$ 828	2156 $\pm$ 1147	2341 $\pm$ 1169	Group $\times$ time	0.481
	Col/Cr	1369 $\pm$ 895	2295 $\pm$ 1292	2457 $\pm$ 1410		
Peak power (W)	Pro	836 $\pm$ 248	868 $\pm$ 217	856 $\pm$ 217	Group	0.673
	Pro/Col	864 $\pm$ 235	922 $\pm$ 222	925 $\pm$ 193	Time	0.002 $\ddagger$
	Pro/Cr	748 $\pm$ 228	830 $\pm$ 261	834 $\pm$ 248	Group $\times$ time	0.832
	Col/Cr	863 $\pm$ 236	926 $\pm$ 246	892 $\pm$ 214		
Total work (J)	Pro	216.4 $\pm$ 66.6	214.3 $\pm$ 64.4	205.6 $\pm$ 61.2	Group	0.866
	Pro/Col	214.4 $\pm$ 44.4	231.6 $\pm$ 49.1	221.4 $\pm$ 50.4	Time	0.566
	Pro/Cr	213.1 $\pm$ 58.0	220.0 $\pm$ 57.2	204.5 $\pm$ 43.9	Group $\times$ time	0.821
	Col/Cr	199.4 $\pm$ 60.6	218.9 $\pm$ 53.8	194.9 $\pm$ 83.8		
Fatigue index (%)	Pro	64.2 $\pm$ 12.3	65.1 $\pm$ 8.6	59.7 $\pm$ 8.9	Group	0.501
	Pro/Col	67.8 $\pm$ 12.3	69.4 $\pm$ 13.4	69.7 $\pm$ 11.5	Time	0.369
	Pro/Cr	66.2 $\pm$ 10.9	68.3 $\pm$ 12.0	64.8 $\pm$ 12.7	Group $\times$ time	0.579
	Col/Cr	67.1 $\pm$ 13.4	64.4 $\pm$ 12.3	65.3 $\pm$ 11.0		

1RM, one-repetition maximum; BW, body weight; Col/Cr, colostrum + creatine formulation; DXA, dual-energy x-ray absorptiometry; Pro, protein control; Pro/Col, protein control + colostrum; Pro/Cr, protein control + creatine formulation

\* Pro group,  $n = 12$ ; Pro/Col group,  $n = 13$ ; Pro/Cr group,  $n = 13$ ; Col/Cr group,  $n = 11$ . Raw data are presented as mean  $\pm$  SD.

$\ddagger$  Significant main effect for time ( $P < 0.05$ ).

Colostrum is the initial (~3 d) lacteal secretion of cows upon giving birth. Colostrum has a greater concentration of various growth factors (e.g., epidermal growth factors, IGF-1, transforming growth factors, tumor necrosis factor, etc.) and increased concentration of immunoglobulins [6,10]. Colostrum supplementation may stimulate anabolism and/or be ergogenic; however, this effect has not been elucidated. The wide array of growth factors and constituents found in colostrum may provide an additional non-nutritive factor that is anabolic in skeletal muscle. A potential anabolic factor may be IGF-1, which is a factor that regulates muscle protein turnover and increases in response in resistance training [40]. Mero et al. [10] found that colostrum supplementation increased circulating levels of IGF-1 and immunoglobulin A in male sprinters. In addition, Mero et al. [5] reported that 2 wk of colostrum supplementation (20 g/d) increased serum concentrations of essential amino acids and promoted a positive net balance of protein [26]. In contrast, Buckley and others reported that colostrum supplementation has no role at increasing circulating levels of IGF-1 in physically active males who were completing run training or resistance training [11,14,39] but may promote increases in run performance during subsequent maximal exercise bouts [14].

Fry et al. [25] reported on a subset ( $n = 19$ ) of participants from the present study who provided muscle biopsies before and after supplementation and resistance training to investigate changes in cellular responses (e.g., fiber types, fiber cross-sectional area, relative fiber area, and relative major histocompatibility complex expression) in addition to selected performance and body composition changes. The investigators reported no significant changes for any of the criterion variables, which included DXA lean mass and body mass. Upon including the addition of 30 study participants who did not provide a biopsy to the previously reported data, significant increases in FFM were shown in the Col/Cr and Pro/Cr groups in comparison with the Pro group. The initial publication presented tissue-related changes, whereas the present study is presenting the physiologic adaptations using the entire cohort.

In the present study, increases in FFM were greater in the Pro/Cr group than in the Pro group. The protein composition in the Pro and Pro/Cr groups was a blend of whey and casein protein. This combination has been previously reported to promote greater increases in lean tissue during resistance training in comparison with isocaloric and isonitrogenous controls [3]. Previous research by Boirie et al. [4] has suggested that whey and casein may possess different kinetic patterns. Whey protein releases its amino acids at a fast rate, stimulating protein synthesis, whereas digestion of casein results in a slower release of amino acids, which serves to prevent the breakdown of protein. Although not directly investigated in this study, the combination of whey and casein may have been responsible for the increases seen in FFM in these groups [3].

In the present study, the inclusion of creatine monohydrate may be the likely candidate for explaining the greater increase in FFM in the Pro/Cr and Col/Cr groups over the Pro group. Creatine monohydrate is an extremely popular nutritional supplement, which has been widely reported to have ergogenic and anabolic effects in individuals undergoing rigorous resistance training [17–19,38]. The dosage of creatine used in the present study was similar to dosages provided in other studies, which increased intramuscular creatine levels, increased FFM, and improved strength and anaerobic performance [18,41,42]. Considering the topic of responders and non-responders to creatine supplementation [44], most subjects in the Pro/Cr and Col/Cr groups were likely “responders” because these groups had greater gains in FFM compared with the Pro group. Measurement of muscle creatine concentrations could be used to confirm whether subjects were creatine responsive, but that was not done in the present study [43]. Nonetheless, these findings are consistent with previous studies, which used other combinations of protein and creatine [24,44] and reported no change in muscular strength and endurance. Fat mass was unchanged in the present study, which is consistent with similar studies [7]. In addition, percent body fat, bone mineral content, and total body water were unchanged throughout the present study.

Many studies have investigated possible ergogenic effects of colostrums with various modes of exercise. These results are equivocal with previous reports suggesting no ergogenic effect [12,13,15] and others reporting an ergogenic effect [11,14,16]. Other investigators have also suggested that colostrum improves recovery from prolonged, intense exercise [14,15]. A plausible explanation for the increase in FFM in the Col/Cr and Pro/Cr groups without any performance changes relates to the improvement of recovery within and between each workout. This effect has been reported for creatine supplementation [45]; however, problems with the recording of training volume (e.g., participants not turning in their workout cards, properly recording them) throughout the study made an accurate calculation of training volume impossible. Nonetheless, the results of the present study do not provide additional support for any possible role colostrum may have as an ergogenic agent. Similar gains were made by all groups for muscular strength, muscular endurance, peak power, total work, and fatigue index. These findings, however, do provide efficacy for the resistance training program used to promote muscle hypertrophy.

Our data demonstrate no significant differences in total caloric intake and in carbohydrate, protein, and fat intakes throughout our 12-wk study. The changes observed for DXA FFM and body mass are unlikely to be due to differences in macronutrient intake. These findings should be interpreted with caution because previous research has indicated that people typically under-report their nutritional intake [46].



## Conclusion

Protein supplementation from whey, casein, and colostrum sources during resistance training promotes increases in body mass and FFM in addition to increases in strength. The combination of whey and casein protein plus creatine or colostrum plus creatine promoted greater increases in FFM compared with protein alone or protein plus colostrum. However, these changes may have resulted solely from the inclusion of creatine. These data are important for any clinical population, dietitian, athlete, or coach who may use protein supplementation to support energy and macronutrient requirements to optimize training adaptations, mitigate muscle loss, and prevent muscle atrophy.

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