# The Effect of Whey Protein Supplementation With and Without Creatine Monohydrate Combined With Resistance Training on Lean Tissue Mass and Muscle Strength

Darren G. Burke, Philip D. Chilibeck, K. Shawn Davison, Darren G. Candow, Jon Farthing, and Truis Smith-Palmer

Our purpose was to assess muscular adaptations during 6 weeks of resistance training in 36 males randomly assigned to supplementation with whey protein (W; 1.2 g/kg/day), whey protein and creatine monohydrate (WC; 0.1 g/kg/day), or placebo (P; 1.2 g/kg/day maltodextrin). Measures included lean tissue mass by dual energy x-ray absorptiometry, bench press and squat strength (1-repetition maximum), and knee extension/flexion peak torque. Lean tissue mass increased to a greater extent with training in WC compared to the other groups, and in the W compared to the P group (p < .05). Bench press strength increased to a greater extent for WC compared to W and P (p < .05). Knee extension peak torque increased with training for WC and W (p < .05), but not for P. All other measures increased to a similar extent across groups. Continued training without supplementation for an additional 6 weeks resulted in maintenance of strength and lean tissue mass in all groups. Males that supplemented with whey protein while resistance training demonstrated greater improvement in knee extension peak torque and lean tissue mass than males engaged in training alone. Males that supplemented with a combination of whey protein and creatine had greater increases in lean tissue mass and bench press than those who supplemented with only whey protein or placebo. However, not all strength measures were improved with supplementation, since subjects who supplemented with creatine and/or whey protein had similar increases in squat strength and knee flexion peak torque compared to subjects who received placebo.

Key Words: strength, ergogenic aids, exercise, dual energy x-ray absorptiometry

D.G. Burke is with the Department of Human Kinetics at St. Francis Xavier University, Antigonish, Nova Scotia, B2G 2W5, Canada. P.D. Chilibeck, K.S. Davison, D.G. Candow, and J. Farthing are with the College of Kinesiology at the University of Saskatchewan, Saskatoon, Saskatchewan, S7N 4L3, Canada. T. Smith-Palmer is with the Department of Chemistry at St. Francis Xavier University, Antigonish, Nova Scotia, B2G 2W5, Canada

#### Introduction

Protein needs of individuals engaged in strength training have been shown to be up to two times that of sedentary individuals (21, 28). Protein turnover is elevated substantially following resistance-training exercise (6, 22), and the rate of protein synthesis following exercise is enhanced with oral consumption of amino acids (30). Given these findings, one would expect that protein supplementation during resistance training would enhance muscle hypertrophy and strength. The anabolic hormone response to resistance training is enhanced with protein supplementation (5, 17), but studies that have assessed muscular changes have been equivocal. Some have demonstrated an enhanced adaptation in muscle mass (10, 23) and strength (10) with supplementation, and others have shown no beneficial effects (3, 21, 28, 29).

Whey protein is a supplement that has recently gained popularity among strength-trained athletes. Numerous studies have demonstrated the positive immune system benefits and antioxidant potential of whey protein supplementation (1, 16, 20, 24, 35). Compared to other protein sources, whey has been found to possess a greater compliment of essential amino acids and branched chain amino acids, and to result in greater biological value in mice, rats, and humans (1, 20, 24). Whey protein may therefore be of potential benefit during exercise training.

Protein requirements of individuals engaged in strength training are related to the intensity and volume of training. Intensity and volume of acute bouts of exercise are augmented with creatine monohydrate supplementation (4, 34). Protein supplementation may therefore prove to be more beneficial if combined with creatine supplementation.

The purpose of this study was to assess the effect of whey protein supplementation by itself, and when combined with creatine monohydrate, on adaptations to resistance training. It was hypothesized that whey protein supplementation would enhance adaptations (lean tissue mass and muscular strength and peak torque) during resistance training. It was also hypothesized that whey protein supplementation, when combined with creatine monohydrate, would result in a greater increase in lean tissue mass and muscular strength than supplementation with whey protein alone. This hypothesis is based on the fact that creatine monohydrate has the potential to increase training intensity and volume due to enhanced energy system effects, and that protein needs are most likely proportional to training intensity and volume.

#### Methods

# Subjects

Forty-two males between 18–31 years of age were recruited from the university population. No subject reported a history of anabolic steroid use, and no subject had supplemented with protein or creatine within the previous 6 weeks. This is greater than the time (30 days) required for creatine levels within skeletal muscle to return to baseline levels following cessation of supplementation (9). All subjects were familiar with weight training and had a minimum of 3 years experience (Table 1). Each subject signed an informed consent and was free to withdraw from the study at any time. This study was approved by the university's ethics review committee for research involving human experimentation.

Table 1 Subjects Resistance Training Experience Prior to the Study (Mean  $\pm$  SE)

Group	Experience (y)	Training Frequency (d/wk)	Duration(h/wk)
WC	$5.6 \pm 0.6$	5 ± 0.3	$8.3 \pm 0.6$
$\mathbf{W}$	$4.3 \pm 0.3$	$4 \pm 0.3$	$6.7 \pm 0.6$
P	$4.2 \pm 0.3$	$4\pm0.3$	$7.1 \pm 0.6$

<sup>\*</sup>There were no significant differences in prior training experience among groups.

Table 2 Ingredients in Whey Protein Blend Supplement (g/kg/d)

Whey Protein	$1.2  imes 10^{ m o}$	
Creatine	$1.0 \times 10^{-1}$	
Guar Gum	$2.0 \times 10^{-2}$	
Inositol	$1.0 \times 10^{-2}$	
Glucomannan	$5.0 \times 10^{-3}$	
Arginine	$5.0 \times 10^{-3}$	
Potassium Phosphate	$5.0  imes 10^{-3}$	
American Ginseng	$2.0 \times 10^{-3}$	
Folic Acid	$2.0 \times 10^{-5}$	
N-acetyl-cysteine	$3.0 \times 10^{-4}$	
Pyridoxine Hydrochloride	$1.0 \times 10^{-3}$	
α-tocopherol	$2.0 \times 10^{-5}$	

A double-blind protocol was used, where an individual who did not participate in any other aspect of the study randomly assigned subjects to one of three groups: whey protein (W; 1.2 g/kg body mass/day), whey protein (1.2 g/kg body mass/day) combined with creatine monohydrate (0.1 g/kg body mass/day; WC), or placebo (P; 1.2 g/kg body mass/day of maltodextrin) groups. The number of creatine-naïve subjects who finished all aspects of the first 6 weeks of training in each group (subjects who had never before taken creatine) was 7/13 in WC, 6/12 in W, and 6/11 in P. Each serving from all three supplements was isocaloric. However, the WC supplement was slightly more nitrogenous than W because it contained creatine, inositol, arginine, and N-acetyl-cysteine. The WC supplement also uniquely contained American ginseng, but all other ingredients were the same among supplements. Substances found in the whey protein combined with creatine blend supplement are listed in Table 2 as amount per kilogram of body mass. Supplement or placebo was distributed in powder form in unmarked containers containing approximately a 1-week supply. An individual who was not associated with any other

aspect of the experiment filled the unmarked containers. This ensured blinding of subjects and investigators. Subjects were instructed to consume their supplement dosage in four equal servings across the day by mixing their powder with a non-caffeinated beverage. These specific instructions are based on findings that caffeine counteracts muscle creatine loading (31). Subjects returned to the lab once a week with empty containers to pick up refills. At these times, subjects were asked about compliance to the protocol. All subjects indicated that all supplements were consumed and when asked what they thought they were taking only guessed correctly about 30% of the time. Six subjects (1 W, 1 WC, and 4 P) dropped out of the study because of lack of time (n=4) or due to minor injuries (n=2).

# **Exercise Program**

All subjects followed the same high volume, heavy load, free-weight resistancetraining program for 12 weeks. Weight training started on the first day of supplementation and consisted of a 4-day split routine involving whole body musculature. Day 1 involved chest and triceps musculature and included the following exercises in order: bench press, incline bench press, flat bench dumbbell flys, incline dumbbell flys, cable triceps extensions, rope reverse triceps extensions, and French curls. Day 2 involved back and biceps musculature and included the following exercises in order: chin-ups, low row, "lat" pull-downs, alternate dumbbell row, standing EZcurls, preacher curls, and alternate dumbbell curls. Day 3 involved leg, shoulder, and abdominal musculature and included the following exercises in order: squats, leg extensions, hamstring curls, standing calve raises, military dumbbell press, upright row, shrugs, deltoid flys, and abdominal crunches. Day 4 was a rest day. This 4-day cycle was repeated continuously throughout the duration of the study. The training program was broken into 5 blocks of 2 cycles for 8 days, for a total of 40 days. Block 1 consisted of 4 sets of 10-12 repetitions, with 1-min rest between sets. Block 2 (days 9-16) consisted of four sets of 8-10 repetitions, with 1.5-min rest between sets. Block 3 (days 17-24) consisted of five sets of 6-8 repetitions, with 2min rest between sets. Block 4 (days 25-32) consisted of four sets of 8-10 repetitions, with 2-min rest between sets. Block 5 (days 33-40) consisted of four sets of 10-12 repetitions, with 1.5-min rest between sets. Following this initial 6 weeks of training, 23 subjects (8 WC, 10 W, 5 P) continued training for 6 weeks, repeating the training schedule, but without W, WC, or P supplementation. Subjects trained in a supervised fitness facility with at least one partner and adjusted their exercise load each day to permit completion of the desired repetitions. Training logs detailing the weight used and numbers of sets and repetitions performed for each exercise were completed for every workout. Weekly calculations of training load were used to compare progress among groups.

# **Body Composition**

Lean tissue mass was assessed by dual energy x-ray absorptiometry (DEXA) at the beginning of the study, following 6 weeks of supplementation and 6 weeks after cessation of supplementation. Whole-body DEXA scans were performed on a Hologic QDR-2000 in array mode and analyzed using system software version 7.01. The same technician conducted all DEXA scans and regional body analysis. Reproducibility of DEXA in our lab was determined by measuring 10 subjects on two separate

occasions. The coefficients of variation (CVs) were 0.54% and 2.95% for whole-body lean tissue and fat mass, respectively. According to power calculations using these CVs (7), this level of reproducibility is sufficient to detect expected changes in lean tissue mass with supplementation (2, 18, 19, 27) at an alpha level of .05. Body weight was measured on a Toledo scale, accurate to the nearest 0.1 kg.

# Strength and Peak Torque Measures

Muscular strength and isokinetic knee extension and flexion peak torques were measured at the beginning of the study, following 6 weeks of supplementation and 6 weeks after cessation of supplementation. Strength was assessed by one repetition maximum (1-RM) for bench press and squat (18). To measure the 1-RM squat, a squat rack and an Olympic barbell was used. Each subject positioned his feet approximately shoulder width apart inside the squat rack and in front of a full body mirror. Subjects were instructed to lower the squat bar until there was an internal angle at the knees of 90°, which was approximated by the investigator administrating the test, before returning to the upright position. A warm-up consisted of the modified hurdler's stretch held twice on each leg for 20 s, followed by 10 squat repetitions using a weight determined by each subject as an appropriate warm-up weight.

For bench press, subjects were positioned on the bench with both feet flat on the floor. Subjects were not allowed to lift their buttocks off the bench or arch their backs during a lift. A complete repetition went from the top straight-arm position down until the bar touched the chest, then ended with the bar returning to the top straight-arm position. A warm-up consisted of 20 push-ups; 2 static stretches of the chest musculature against a wall, held for 8 s each; and 10 repetitions with a comfortable starting weight as determined by each subject.

Following warm-up, for both squat and bench press testing, subjects selected a weight with which they felt they could complete three repetitions. At this weight, they only performed one repetition. Subjects then selected a weight they felt would be their 1-RM and attempted one repetition with this weight. Following successful attempts, weight was increased by 2–5 kg for subsequent 1-RM attempts. The 1-RM was usually reached in less than 6 sets, including the warm-up set. There was 3 min of rest between attempts, and two assistants changed the weight on the bar between attempts. Reproducibility of the bench press and squat 1-RM was determined on 2 separate days in 12 subjects. The CVs for bench press and squat 1-RM were 1.89% and 5.97%, respectively.

Peak torque was measured in the right knee extensors and flexors, using an isokinetic dynamometer (Biodex System 3, Biodex Medical Systems Inc., Shirley NY). The dynamometer was set in the concentric mode for knee extension/flexion at an angular velocity of 60° per second. Range of motion consisted of movement from 90° to 170° of knee flexion (internal angle). Sitting against a back support, subjects produced an angle of 85° of hip flexion. Stabilizing belts were placed over the lap, across the chest, and across the distal one third of the tested leg thigh. The rotational axis of the dynamometer was positioned to be coaxial with the knee axis (lateral condyle) during testing. Subjects were given two practice trials where they were told to perform knee extension and flexion as fast and hard as possible. Following the practice trials, subjects performed test trials consisting of one repetition of knee extension, followed by knee flexion at maximal effort, repeated three times with 1 min of rest between sets. The highest peak torque obtained during the three repetitions

was recorded for extension and flexion. Peak torque measures were corrected for the effects of gravity on the lower leg and the dynamometer's resistance pad. The torque output on the dynamometer was checked with a calibration weight on a weekly basis throughout the study duration. Reproducibility of the peak torque measurements was determined on 2 separate days in 12 subjects. The CVs for knee extension and flexion peak torque were 2.43% and 6.16%, respectively.

The order of tests was the same each time: squat, bench press, and isokinetic knee extension/flexion, with at least 10 min of rest between tests.

### Dietary Intake

Subjects kept 3-day dietary records three times during the study: at the start, following the 6-week supplementation period, and at the end of the 12th week. Dietary intake was recorded over 2 weekdays and 1 weekend day each time. Subjects were given detailed instruction on filling out the dietary records, which included information and examples on sample sizes. Caloric, protein, fat, and carbohydrate consumption was determined using Fuel 2.1a Pro Nutrition Software.

# Statistical Analysis

A  $3 \times 2$  repeated measures analysis of variance was used to assess changes in body composition, strength, and peak torque for the three groups (WC vs. W vs. P) across time (baseline to 6 weeks of training). Tukey's post hoc tests were used to determine differences amongst individual means. Significance was set at an alpha level of .05.

#### Results

#### Baseline to End of Week 6

There were no differences among groups in any of the baseline measurements. There was a significant Group  $\times$  Time interaction for lean tissue mass (p < .05). Both the WC (+4.0 kg or 6.5%) and W (+2.3 kg or 3.8%) groups had significant gains in lean tissue mass with training (post hoc; p < .05), while the P group had a nonsignificant change (+0.9 kg or 1.5%; Figure 1). The gain was greatest in the WC group, as lean tissue mass after 6 weeks of training was greater compared to the W and P groups (post hoc; p < .05). There were no significant changes in fat mass with training. The nonsignificant changes for fat mass were -0.4 kg or 3% for WC, -0.6 kg or 4% for W, and -0.2 kg or 2% for P. There was a significant Group  $\times$  Time interaction for total body mass. Post hoc analysis indicated that body mass significantly increased for all groups by 3.7 kg, 1.5 kg, and 1.0 kg for WC, W, and P, respectively (p < .05). Body mass after 6 weeks of training was greater in WC and W groups compared to P (p < .05).

There was a significant Group  $\times$  Time interaction for bench press 1-RM (p < .05). Post hoc analysis indicated that bench press 1-RM significantly increased for all three groups with training (p < .05; Figure 2). After 6 weeks of training, bench press 1-RM was significantly greater in the WC (+15.2 kg or 17%) compared to the W (+6.3 kg or 7%) and P (+7.2 kg or 8%) groups (p < .05) and similar between W and P groups. For squat 1-RM, there was a significant time main effect (p < .05), with similar increases with training across groups (Figure 3). The pre to post increase in 1-RM squat was +25.7 kg or 20% for WC, +22.5 kg or 17% for W, and +21.5 kg or 15% for P.

#### **Lean Tissue Mass**

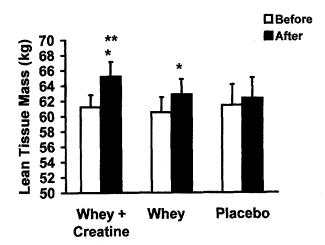


Figure 1 — Lean tissue mass before and after training with supplementation of whey protein and creatine, whey protein alone, and placebo. Values are means  $\pm SD$ . \*Significantly different from before training (p < .05). \*\*Significantly different than whey protein-supplemented and placebo groups after training (p < .05).

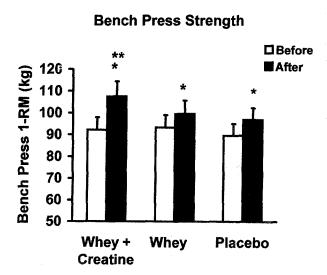


Figure 2 — Bench press strength before and after training with supplementation of whey protein and creatine, whey protein alone, and placebo. Values are means  $\pm SD$ . \*Significantly different from before training (p < .05). \*\*Significantly different than whey protein-supplemented and placebo groups after training (p < .05).

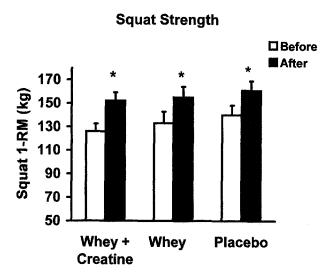


Figure 3 — Squat strength before and after training with supplementation of whey protein and creatine, whey protein alone, and placebo. Values are means  $\pm SD$ . \*Significantly different from before training (p < .05).

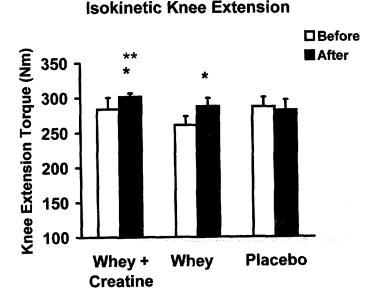


Figure 4 — Knee extension peak torque before and after training with supplementation of whey protein and creatine, whey protein alone, and placebo. Values are means  $\pm SD$ . \*Significantly different from before training (p < .05). \*\*Significantly different than placebo group after training (p < .05).

There was a significant Group  $\times$  Time interaction for isokinetic knee extension peak torque. Post hoc analysis indicated that the WC (+26.2 Nm or 10%) and W (+16.5 Nm or 7%) groups experienced significant increases in knee extension peak torque after 6 weeks of training (p < .05), while the P(+11.6 Nm or 5%) group did not (Figure 4). The WC group had a greater peak torque at 6 weeks compared to the P group (p < .05) but not the W group. The peak torque at 6 weeks was similar between W and P groups. There was no effect of training on knee flexion peak torque, but the Group  $\times$  Time interaction approached significance (p = .066), with the WC group increasing by 6.8%, the W group by 1.5%, and the P group decreasing by 2.6% (Figure 5).

From analysis of 3-day dietary intakes, there were no differences between groups or across time (baseline to 6 weeks) for caloric intake, dietary protein, fat, or carbohydrate (Table 3).

## Isokinetic Knee Flexion

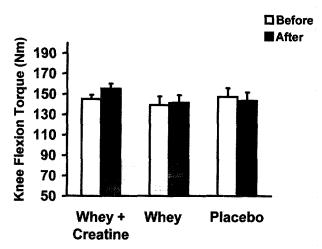


Figure 5 — Knee flexion peak torque before and after training with supplementation of whey protein and creatine, whey protein alone, and placebo. Values are means  $\pm$  SD. There were no significant changes in knee flexion peak torque with training.

Table 3 Group Carbohydrate (CHO), Protein (PRO), and Fat (FAT) Intake Expressed in g/kg Body Weight/Day Averaged Across 12 Weeks (Mean ± SE)

Group	СНО	PRO	FAT
			<u> </u>
WC	$4.2 \pm 0.3$	$1.0 \pm 0.1$	$1.3 \pm 0.1$
W	$4.1 \pm 0.3$	$2.1 \pm 0.3$	$1.5 \pm 0.1$
P	$3.9 \pm 0.3$	$1.2\pm0.2$	$1.7 \pm 0.1$
			1.3

<sup>\*</sup>All values are based on 3-day food records and do not include the addition of the supplement or placebo.

#### Week 6 to the End of Week 12

Twenty-three subjects continued training without supplementation for an additional 6 weeks and were tested on the same measures at the end of week 12. There were no significant changes across any of the groups during this time period for bench press and squat strength, and for lean tissue, fat, or body mass (Table 4). There was a time main effect for knee extension and flexion peak torque (p < .05), with all three groups experiencing a reduction in these measures. There were no dietary differences between groups during this time period (Table 3).

## Discussion

To our knowledge, this is the first study to investigate the effects of whey protein supplementation during resistance training. We demonstrated that males who received whey protein supplementation in combination with resistance training had slightly greater increases in lean tissue mass compared to males who trained and received placebo. One of four muscular strength measurements, knee extension peak torque, was also increased to a greater extent in males that supplemented with whey protein compared to those that received a placebo. Other strength measurements, such as squat and bench press strength and knee flexion peak torque, were unaffected. Males who supplemented with both creatine and whey protein had greater increases in lean tissue mass and strength (bench press) than males that supplemented with only whey protein or placebo. Again, squat strength and knee flexion peak torque was unaffected.

The observation that knee extension peak torque, but not squat strength, was enhanced with whey protein and creatine supplementation could be due to the differences in complexity of these two exercises. It has previously been demonstrated that early gains in strength for complex movements (such as leg exercises involving movement at a multiple number of joints) are not due to muscle hypertrophy but to neural adaptations or a "learning effect" (8). Early gains in strength during less complex exercises, such as those involving movement at a single joint (i.e., knee extension) are due mainly to muscle hypertrophy (8). Strength during a less complex task, such as knee extension, would therefore be more likely influenced from the increase in lean tissue with supplementation than a more complex task, such as the squat exercise. Increases in 1-RM for the complex squat exercise may have been due to a learning effect, as all three groups had significant increases in squat 1-RM.

Another explanation for the differing results for knee extension peak torque and squat strength, as well as knee flexion peak torque, could be related to the different CVs for these exercises. Knee extension peak torque had a smaller CV compared to knee flexion peak torque and squat 1-RM. With a larger CV, the power to detect changes in a measure is reduced (7). For knee flexion peak torque, there tended to be an improvement with supplementation (p = .066), but for this and the squat 1-RM, the CV for repeated measures may have been too large to detect changes at an alpha level of .05 with sufficient power.

A final possibility for differences observed between squat and knee extension results may be related to the order that tests were administered. On testing days, squat exercise was performed before the isokinetic knee extension/flexion test. If supplementation allowed for an improvement in energy systems (i.e., phosphocreatine resynthesis) and an improvement in muscular endurance, then the isokinetic

Table 4 Mean ± SE Group Results for Those Subjects Completing 6 Weeks of Training With Supplementation and Then Continued Training for 6 Weeks Without Supplementation

IKET	294 ± 17 286 ± 21 267 ± 11 247 ± 11 274 ± 29 264 ± 27
IRM S	152.5 ± 11 158.5 ± 11 160.2 ± 10 163.6 ± 11 166.8 ± 11
IRM B	114.5 ± 9.9 116.2 ± 9.8 103.4 ± 6.3 102.0 ± 6.3 93.0 ± 9.4 90.5 ± 9.4
% Fat	14.0 ± 1.8 13.7 ± 1.8 17.2 ± 1.6 17.0 ± 1.6 12.2 ± 2.1 12.6 ± 2.2
Fat M	11.6 ± 1.8 11.4 ± 1.8 13.9 ± 1.6 14.0 ± 1.6 9.5 ± 2.2 9.8 ± 2.2
Lean M	67.2 ± 2.5 66.6 ± 2.5 62.3 ± 2.5 62.6 ± 2.5 61.8 ± 4.0 60.7 ± 4.0
Mass	87.6 ± 3.9 88.2 ± 3.9 85.1 ± 3.8 84.7 ± 3.8 80.0 ± 7.6 79.5 ± 7.5
Time	Posttest + 6wk Posttest + 6wk Posttest + 6wk
u	8 110
Group	M M M

Note. Mass = body mass in kg; Lean M = lean tissue mass in kg; Fat M = fat mass in kg; % Fat = percentage fat, 1RM B = 1RM bench press in kg; 1RM S = 1RM squat in kg; IKE T = isokinetic knee ext torque in Nm; + 6wk = 6 weeks after posttest with continued training but no supplementation.

tests would be more likely to improve than the squat 1-RM. We believe, however, that the order of testing had minimal influence on the results of the experiment because squat 1-RM was found in less than six attempts, and there was approximately 30-45 min rest between squat and isokinetic testing. Therefore, improvements in muscular endurance with training or supplementation would have minimal influence on the two tests.

The slightly greater response to training in the subjects receiving protein supplementation compared to those receiving placebo in the present study may be related to a greater protein need due to the substantially elevated protein turnover that results from resistance training (6, 22). Nitrogen balance studies have indicated that protein needs of individuals engaged in heavy strength training are up to two times that of sedentary individuals (21, 29). However, most strength-trained individuals (including those of the present study) consume a high protein diet that may be sufficient to meet these needs. This may account for the relatively small enhancement in lean tissue mass and knee extension peak torque and lack of enhancement beyond placebo in strength measures (bench press and squat) with protein supplementation in the present study. Previous studies of young individuals engaged in heavy resistance training over 10 to 30 days have demonstrated no effect of protein supplementation on body composition, muscle mass, and strength measures (21, 28, 29). The slightly greater response in the present study may be due to the slightly longer period of training (40 days) and possibly a higher quality of protein ingested. Two other studies have shown enhancement of muscle hypertrophy with protein supplementation during strength training (10, 23), but these were in older individuals, who may have been lacking sufficient dietary protein before the study.

This is the first study to combine creatine monohydrate and whey protein supplementation with resistance training. Creatine monohydrate may enhance protein synthesis or may allow one to train at a higher volume due to its energy system effects. Either of these effects would result in an increase in protein requirements. In fact, training volume calculations for bench press and squat indicated that the WC group was using a higher load than the W or P groups after 6 weeks of training and supplementation. The effect of creatine supplementation for improving short-term work capacity and the potential for training at a higher volume have been demonstrated repeatedly (4, 34), but its potential for stimulating protein synthesis is controversial. Ingwall (14) demonstrated that creatine stimulates in vitro biosynthesis of muscle myosin; however, others have failed to replicate these findings (11). It has been speculated that water retention during creatine supplementation (13) may act as a signal for protein synthesis within muscle cells (12). At the cellular level, creatine supplementation has been demonstrated to increase the size of muscle fibers (26, 33). Whether protein turnover is actually increased with creatine supplementation awaits further study using tracer techniques. Preliminary findings, using tracer techniques, do not support the hypothesis that creatine supplementation stimulates protein synthesis (25); however, this does not rule out the possibility of creatine acting as an anticatabolic agent.

The increase in lean tissue of the subjects in our WC group appear to be greater than that found in three other studies involving creatine or creatine and protein supplementation. With 6 weeks of training and doses of approximately 8 g creatine/day and 96 g protein/day, our subjects increased lean tissue mass by 4 kg or 6.5%. Kreider et al. (19) assessed weight-trained males over 4 weeks, supplemented with a higher dose of creatine (20 g/day) combined with 67 g protein/day, and found an

increase in lean tissue mass of 2.0 kg or 3.3%. Kreider et al. (18) assessed football players over 4 weeks, supplemented with a higher dose of creatine (15.75 g/day), and found an increase in lean tissue mass of 2.4 kg or 3.4%. Finally, Stout et al. (27) assessed football players over 8 weeks, supplemented with 10–21 g of creatine per day, and found an increase in lean tissue mass of 3.2 kg or 4.6%. They also found an increase in bench press 1-RM of 15 kg or 12%, which was similar to the increase of our WC subjects of 15 kg or 17%. Differences between studies may be due to the combination of protein supplementation with the creatine in the present study, but may also be due to differences in subject characteristics (i.e., the football players in the two studies mentioned above were heavier), training volume, or duration of training. Given the safety concerns of creatine supplementation expressed by some (15), our lower dose of creatine (approximately 8 g/day) combined with whey protein may be preferable.

A final finding of our study was that withdrawal from creatine and protein supplementation did not negatively affect lean tissue and muscle strength measures after 6 weeks. For all groups, lean tissue mass and 1-RM for bench press and squat were maintained, while knee extension and flexion peak torque was decreased. This latter finding may have been due to a reduced training volume for lower extremity exercises during this prolonged portion of the training. Training log data indicated that squat volume was lower during the last 6 weeks, although this reduction was not significant and there were no group interactions. Although not measured in the present study, others have shown that intramuscular levels of creatine are elevated with creatine supplementation and upon cessation of supplementation return to baseline values after one month (9, 13, 32). This is a time period shorter in duration than the 6 weeks cessation in the present study; therefore, we expected that lean tissue mass and muscle strength would decline. Only one study has previously assessed changes in muscular strength with cessation of creatine supplementation, finding that elevated peak torque output in a creatine-supplemented group declined to levels not different from a placebo group 4 weeks after cessation of supplementation (32). This differs from the present study, where a lack of a Group X Time interaction for muscular strength measures, during the period of supplement cessation, indicated that groups were changing at a similar rate. With a lower number of subjects participating in the extra 6 weeks, we may have lacked sufficient power to detect differences between groups.

In summary, whey protein supplementation during resistance training offers some benefit compared to resistance training alone. Specifically, males who supplemented with whey protein had a greater relative gain in lean tissue mass and knee extension peak torque with training than males who received a placebo. Other measures of muscular strength, which included 1-RM bench press, and squat and knee flexion peak torque, were unaffected by whey protein supplementation. Males who supplemented with a combination of whey protein and creatine had a greater increase in lean tissue mass and relative increase in bench press 1-RM than males who supplemented with whey protein alone or placebo. Again, squat strength and knee flexion peak torque was not influenced by supplementation. Cessation of supplementation for 6 weeks did not affect the rate of change in muscular strength measures or lean tissue mass. Supplementation in this study involved self-administration of the required substance daily with weekly monitoring. It was assumed that each subject consumed his supplement as indicated. As well, random assignment of subjects into the three groups assumes that there would be an equal number of

non-compliant subjects in each group. It was not feasible to observe each subject consuming his required supplement four times per day. We therefore relied on subjects' honesty when they reported that they had consumed their supplement. This lack of direct assessment of compliance is a limitation of the current study.

#### References

- 1. Bounous, G., G. Batist, P. Gold. Immunoenhancing property of dietary whey protein in mice: role of glutathione. *Clin. Invest. Med.* 12:154-161, 1989.
- Burke, D.G., S. Silver, L.E. Holt, T. Smith-Palmer, C.J. Culligan, and P.D. Chilibeck.
   The effect of continuous low dose creatine supplementation on force output, power output, and total work. *Int. J. Sport Nutr.* 10:235-244, 2000.
- 3. Campbell, W.W., M.C. Crim, V.R. Young, L.J. Joseph, and W.J. Evans. Effects of resistance training and dietary protein intake on protein metabolism in older adults. *Am. J. Physiol.* 268:E1143-E1153, 1995.
- 4. Casey, A., D. Contatin-Teodosiu, S. Howell, E. Hultman, and P.L. Greenhaff. Creatine ingestion favorably affects performance and muscle metabolism during maximal exercise in humans. *Am. J. Physiol.* 271:E31-E37, 1996.
- 5. Chandler, R.M., H.K. Byrne, J.G. Patterson, and J.L. Ivy. Dietary supplements affect the anabolic hormones after weight-training exercise. *J. Appl. Physiol.* 76:839-845, 1994.
- Chesley, A., J.D. MacDougall, M.A. Tarnopolsky, S.A. Atkinson, and K. Smith. Changes in human muscle protein synthesis after resistance exercise. *J. Appl. Physiol.* 73:1383-1388, 1992.
- 7. Chilibeck, P., A. Calder, D.G. Sale, and C. Webber. Reproducibility of dual energy x-ray absorptiometry. *Can. Assoc. Radiol. J.* 45:297-302, 1994.
- 8. Chilibeck, P.D., A.W. Calder, D.G. Sale, and C.E. Webber. A comparison of strength and muscle mass increases during resistance training in young women. *Eur. J. Appl. Physiol.* 77:170-175, 1998.
- 9. Febbraio, M.A., T.R. Flanagan, R.J. Snow, S. Zhao, and M.F. Carey. Effect of creatine supplementation on intramuscular TCr, metabolism and performance during intermittent, supramaximal exercise in humans. *Acta Physiol. Scand.* 155:387-395, 1995.
- Fiatarone Singh, M.A., W. Ding, T.J. Manfredi, G.S. Solares, E.F. O'Neill, K.M. Clements, N.D. Ryan, J.J. Kehayias, R.A. Fielding, and W.J. Evans. Insulin-like growth factor I in skeletal muscle after weight-lifting exercise in frail elders. *Am. J. Physiol.* 277:E135-E143, 1999.
- 11. Fry, D.M., and M.F. Morales. A reexamination of the effects of creatine on muscle protein synthesis in tissue culture. *J. Cell Biol.* 84:294-297, 1980.
- 12. Haussinger, D., E. Roth, F. Lang, and W. Gerok. Cellular hydration state: an important determinant of protein catabolism in health and disease. *Lancet* 341:1330-1332, 1993.
- 13. Hultman, E., K. Soderlund, J.A. Timmons, G. Cederblad, and P.L. Greenhaff. Muscle creatine loading in men. *J. Appl. Physiol.* 81:232-237, 1996.
- 14. Ingwall, J.S. Creatine and the control of muscle-specific protein synthesis in cardiac and skeletal muscle. *Circ. Res.* Suppl. I:I115-I123, 1976.
- 15. Juhn, M.S., and M. Tarnopolsky. Potential side effects of oral creatine supplementation: a critical review. *Clin. J. Sport Med.* 8:298-304, 1998.
- 16. Kawase, M., H. Hashimoto, M. Hosada, H. Morita, A. Hosono. Effect of administration of fermented milk containing whey protein concentrate to rats and healthy men on serum lipids and blood pressure. *J. Dairy Sci.* 83:255-263, 2000.

- 17. Kraemer, W.J., J.S. Volek, J.A. Bush, M. Putukian, and W.J. Sebastianelli. Hormonal responses to consecutive days of heavy-resistance exercise with or without nutritional supplementation. *J. Appl. Physiol.* 85:1544-1555, 1998.
- 18. Kreider, R.B., M. Ferreira, M. Wilson, P. Grindstaff, S. Plisk, J. Reinardy, E. Cantler, and A.L. Almada. Effects of creatine supplementation on body composition, strength, and sprint performance. *Med. Sci. Sports Exerc.* 30:73-82, 1998.
- Kreider, R.B., R. Klesges, K. Harmon, P. Grindstaff, L. Ramsey, D. Bullen, L. Wood, Y. Li, and A. Almada. Effects of ingesting supplements designed to promote lean tissue accretion on body composition during resistance training. *Int. J. Sport Nutr.* 6:234-246, 1996.
- 20. Lands, L.C., V.L. Gray, A.A. Smountas. Effect of supplementation with a cysteine donor on muscular performance. *J. Appl. Physiol.* 87:1381-1385, 1999.
- 21. Lemon, P.W.R., M.A. Tarnopolsky, J. D. MacDougall, and S.A. Atkinson. Protein requirements and muscle mass/strength changes during intensive training in novice bodybuilders. *J. Appl. Physiol.* 73:767-775, 1992.
- 22. MacDougall, J.D., M.J. Gibala, M.A. Tarnopolsky, J.R. MacDonald, S.A. Interisano, and K.E. Yarasheski. The time course for elevated muscle protein synthesis following heavy resistance exercise. *Can. J. Appl. Physiol.* 20:480-486, 1995.
- 23. Meredith, C.N., W.R. Frontera, K.P. O'Reilly, and W.J. Evans. Body composition in elderly men: effect of dietary modification during strength training. *J. Am. Geriatr. Soc.* 40:155-162, 1992.
- Minehira, K., S. Inoue, M. Nonaka, K. Osada, K. Yamada, M. Sugano. Effects of dietary
  protein type on oxidized cholesterol-induced alteration in age-related modulation of
  lipid metabolism and indices of immune function in rats. *Biochim. Biophys. Acta.*1483:141-153, 2000.
- 25. Parise, G., S. Mihic, D. MacLellan, K.E. Yarasheski, and M.A. Tarnopolsky. Creatine monohydrate supplementation does not increase whole body or mixed muscle fractional protein synthetic rates in males and females. *Med. Sci. Sports Exerc.* 32:S289, 2000.
- Sipila, I., J. Rapola, O. Simell, A. Vannas. Supplementary creatine as a treatment for gyrate atrophy of the choroid and retina. N. Engl. J. Med. 304:867-870, 1981.
- 27. Stout, J., J. Eckerson, D. Noonan, G. Moore, and D. Cullen. Effects of 8 weeks of creatine supplementation on exercise performance and fat-free weight in football players during training. *Nutr. Res.* 19:217-225, 1999.
- 28. Tarnopolsky, M.A., S. Atkinson, J. MacDougall, A. Chesley, S. Phillips, and H. Schwarcz. Evaluation of protein requirements for trained strength athletes. *J. Appl. Physiol.* 73:1986-1995. 1992.
- 29. Tarnopolsky, M.A., J.D. MacDougall, and S.A. Atkinson. Influence of protein intake and training status on nitrogen balance and lean body mass. *J. Appl. Physiol*, 64:187-193, 1988.
- 30. Tipton, K.D., A.A. Ferrando, S.M. Phillips, D. Doyle, and R.R. Wolfe. Postexercise net protein synthesis in human muscle from orally administered amino acids. *Am. J. Physiol.* 276:E628-E634, 1999.
- 31. Vandenberghe, K. N. Gillis, M. Van Leemputte, P. Van Hecke, F. Vanstapel, and P. Hespel. Caffeine counteracts the ergogenic action of muscle creatine loading. *J. Appl. Physiol.* 80:452-457, 1996.
- 32. Vandenberghe, K., M. Goris, P. Van Hecke, M. Van Leeputte, L. Vangerven, and P. Hespel. Long-term creatine intake is beneficial to muscle performance during resistance training. *J. Appl. Physiol.* 83:2055-2063, 1997.

- Volek, J.S., N.D. Duncan, S.A. Mazzetti, R.S. Staron, M. Putukian, A.L. Gomez, D.R. Pearson, W.J. Fink, and W.J. Kraemer. Performance and muscle fiber adaptations to creatine supplementation and heavy resistance training. *Med. Sci. Sports Exerc.* 31:1147-1156, 1999.
- 34. Volek, J.S., W.J. Kraemer, J.A. Bush, M. Boetes, T. Incledon, K.L. Clark, and K.L. Clark. Creatine supplementation enhances muscular performance during high-intensity resistance exercise. *J. Am. Diet. Assoc.* 97:765-770, 1997.
- 35. Wong, C.W., H.F. Seow, A.J. Husband, G.O. Regester, D.L. Watson. Effects of purified bovine whey factors on cellular immune functions in ruminants. *Vet. Immunopathol.* 56:85-96, 1997.

# **Acknowledgments**

 $This \ research \ was \ supported \ by \ a \ grant \ from \ Muscle Tech \ Research \ and \ Development \ Inc., Mississauga, Ontario, Canada.$