A creatine-protein-carbohydrate supplement enhances responses to resistance training

A CREATINE-PROTEIN-CARBOHYDRATE SUPPLEMENT ENHANCES RESPONSES TO RESISTANCE TRAINING

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ABSTRACT

PURPOSE: Studies attributing gains in strength and lean body mass (LBM) to creatine monohydrate (CrM) during resistance exercise (RE) training have not assessed these changes alongside cellular and sub-cellular adaptations. Additionally, CrM-treated groups have seldom been compared with a group receiving a placebo similar in nitrogen and energy. The purpose of this study was to examine the effects of a CrM-containing protein-carbohydrate (PRO-CHO) supplement in comparison to a supplement containing a similar amount of nitrogen and energy on body composition, muscle strength, fiber-specific hypertrophy and contractile protein accrual during RE training. **METHODS:** In a double-blind, randomized protocol, resistance-trained males were matched for strength and placed into one of three groups: protein (PRO), protein-carbohydrate (PRO-CHO) or the same PRO-CHO supplement (1.5g/kg body wt/day) containing CrM (Cr-PRO-CHO) (0.1g/kg body wt/day). Assessments were completed the week before and after a 10 week structured, supervised RE program; strength (1RM, three exercises), body composition (DEXA) and vastus lateralis muscle biopsies for determination of muscle fiber type (I, IIa, IIx), cross-sectional area (CSA), contractile protein and creatine content. **RESULTS**: Cr-PRO-CHO provided greater improvements in 1RM strength. At least 40% of the strength improvements could be attributed to hypertrophy of muscle involved in this exercise. Cr-PRO-CHO also resulted in greater increases in LBM, fiber CSA and contractile protein compared to PRO and PRO-CHO. **CONCLUSIONS:** In RE-trained participants, supplementation with Cr-PRO-CHO provided greater muscle hypertrophy than an equivalent dose of PRO-CHO and this response was apparent at three levels of physiology (LBM, fiber CSA and contractile protein content).

INTRODUCTION

Paragraph 1. Supplementation with creatine monohydrate (CrM) has been consistently shown to promote greater gains in lean body mass (LBM) and strength compared to placebo treated groups (20). However, in most cases, the CrM-treated group was often not compared with a group that received a placebo containing protein and an equivalent amount of energy (9; 13; 25; 27). Only one resistance exercise (RE) training study has compared the effects of a CrM-containing supplement (10g CrM, 75g CHO) with a supplement containing a similar amount of nitrogen (protein) and energy (10g milk protein, 75g CHO) (24). This study reported that CrM treatment provided no greater gain in strength, LBM or muscle fiber hypertrophy (24). However, this study utilized a group of inactive males (exercised less than twice a week prior to the study). While the influence of training status on the effects of supplementation is unknown, previous work involving CrM supplementation and RE-trained individuals has shown that treatment enabled the participants to progress at a more rapid rate, which was reflected by the larger strength gains and greater volume of work completed during the workouts (27). Therefore, unlike inexperienced participants, it may be possible that RE-trained individuals experience strength and LBM gains that are of greater magnitude during training.

Paragraph 2. Longitudinal studies that have attributed changes in LBM to supplementation during RE training seldom report these changes alongside adaptations at the cellular level (i.e., fiber-specific, type-I, IIa, IIx hypertrophy) (5; 6; 8; 13; 25). Those that have assessed fiber-specific hypertrophy in response to supplementation (24; 27) have not confirmed this response with changes at the sub-cellular level (i.e., contractile protein content). Therefore, the aim of this study was to use a group of RE-trained participants to examine the effects of a CrM-containing

protein-carbohydrate (PRO-CHO) supplement in comparison to a supplement containing a similar amount of nitrogen and energy on strength, body composition and fiber-specific (i.e., type-I, IIa, IIx) hypertrophy as well as muscle Cr and contractile protein content. The hypothesis was that in RE-trained individuals, a CrM-containing PRO-CHO supplement would provide greater benefits (i.e. muscle strength and hypertrophy) compared to a PRO-CHO supplement containing a similar amount of nitrogen and energy.

METHODS

Participants

Paragraph 3: Thirty-one recreational male bodybuilders met the requirements to commence this study that involved pre-post assessments and supplementation during 10 weeks of RE training (baseline characteristics are presented in table 1). To qualify as participants the men (a) had no current or past history of anabolic steroid use, (b) had been training consistently (i.e., 3-5 days per week) for the previous six months, (c) submitted a detailed description of their current training program, (d) had not ingested any ergogenic supplement for 12-weeks prior to the start of this investigation, and (e) agreed not to ingest any other nutritional supplements, or non-prescription drugs that may affect muscle growth or the ability to train intensely during the study. All participants were informed of the potential risks of the investigation before signing an informed consent document approved by the Human Research Ethics Committee of Victoria University and the Department of Human Services, Victoria, Australia. All procedures conformed to National Health and Medical Research Council guidelines for the involvement of human subjects for research and conformed to the policy statement regarding the use of human subjects and written informed consent published by *Medicine & Science in Sports & Exercise*®.

Paragraph 4: After baseline assessments, the men were matched for maximal strength (1RM) in three weight lifting exercises (see strength assessments) and then randomly assigned to one of three supplement groups in a double-blind fashion; protein-only (*n*=10) (PRO), protein-carbohydrate (*n*=11) (PRO-CHO), or the same protein-carbohydrate supplement that contained CrM (*n*=10) (Cr-PRO-CHO).

Supplementation

Paragraph 5. The Cr-PRO-CHO group consumed the exact same supplement as the PRO-CHO group (50% whey isolate; 50% glucose). The only difference was the Cr-PRO-CHO supplement contained a dose of CrM (0.1 g $kg^{-1}day^{-1}$). Participants were instructed to consume 1.5 grams of the supplement per kilogram of body weight per day (1.5 g $kg^{-1}day^{-1}$) for the 10 week program while maintaining their habitual daily diet. The chosen supplement dose was based on previously reported intakes of this population (14). For example, an 80kg participant in the PRO-CHO group consumed 120 g day^{-1} of a supplement that contained 52g protein, 59g carbohydrate, <0.6g fat and 1877kJ (449 kcal). An 80kg participant in the Cr-PRO-CHO group consumed 120g^{-1}day of a supplement that supplied 48g protein, 53g carbohydrate, <0.6 fat, 8.4g CrM and 1710kJ (409 kcal). Another matched group (PRO) were provided a protein only supplement (whey isolate) (1.5 g $kg^{-1}day^{-1}$) that provided an 80kg participant (120g dose) with 103g protein, <6g carbohydrate, <1.2g fat and 1864 kJ (447 kcal). All supplements were supplied by AST Sport Science, Golden, CO. USA, and were tested to comply with label claims before leaving the place of manufacture. The protein was also independently assessed by Naturalac Nutrition LTD (Level 2/18 Normanby Rd Mt Eden, New Zealand) on two separate occasions, and matched labeled ingredients on both occasions.

Paragraph 6. The participants were asked to consume their supplement dose in three equal servings throughout the day (described with measuring scoops provided). For example, one serving was consumed mid-morning, another soon after the workout in the afternoon (or similar time on non-training days), and a final serving was taken in the evening before sleep. The participants were weighed on a Seca 703 stainless steel digital medical scale (Seca, Perth, WA) every week to track body mass and shown how to adjust the supplement dose as required. The supplements were provided in identical containers with sealed, tamper-proof lids. Participants were given approximately a one-week supply of the supplement at the start of each week and asked to return the container before they received the next weeks supply as an act of compliance to the dosing procedure. In addition to having to return the container, the participants were asked to document the time of day they took the supplement in nutrition diaries that were provided. The participants' diets were monitored and assessed as previously described (7). In brief, each participant was asked to submit three written dietary recordings; one before and two during the study (each recording consisted of 3-days) for the calculation of macronutrient and energy intake. Energy intake is expressed in $kcal^{-1}kg$ of body weight per day; macronutrients are expressed in g kg^{-1} of body weight per day. The participants were asked to report any adverse events from the supplements in the nutrition diaries provided. No adverse events were reported by the participants.

Resistance training protocol

Paragraph 7. Questionnaires demonstrated that the participants had been training consistently (i.e., 3-5 days per week) for at least six months before expressing interest in this investigation. However, to ensure the participants were trained and to minimize the impact of a new program on strength and hypertrophy adaptations, all participants underwent a structured RE program for ~12

weeks that was very similar to the one used in the study (*Max-OT™*, AST Sport Science, Golden, CO, USA) (8). No supplementation was permitted during this pre-trial phase. Once the pre-trial training phase was completed, participants underwent baseline assessments. The 10 week training/supplementation program began the week immediately after baseline assessments. In brief, the program was designed specifically to increase strength and muscle size. It consisted of high-intensity (overload) workouts using mostly compound exercises with free weights. Training intensity for the program was determined initially using repetition maximums (RM) from strength tests. However, once a designated RM was achieved in each phase, the participants were encouraged by the trainer to increase the weight used. This progressive overload program was divided into 3 phases, Preparatory (weeks 1-2) (10 RM), Overload Phase-1 (weeks 3-6) (8-6 RM), and Overload Phase-2 (weeks 7-10) (6-4 RM). Qualified personnel supervised each participant on a one-to-one basis, every workout. Aside from the personal training each participant received during the 10 week program, they also kept training diaries to record exercises, sets, repetitions performed and the weight utilized throughout the program and these were viewed by the trainer on a weekly basis. The following assessments occurred in the week before and after the 10-week RE program.

Assessments

Paragraph 8. Strength assessments consisted of the maximal weight that could be lifted once (1RM) in three weight training exercises: barbell bench press, squat and cable pulldown. Recognized 1RM testing protocol and exercise execution guidelines were followed as previously documented (1). Briefly, the participant's maximal lift was determined within no more than five single repetition attempts following three progressively heavier warm up sets. Participants were required to successfully lift each weight before attempting a heavier weight. Each exercise was

completed before the next attempt and in the same order. Reproducibility for these tests was determined on 2 separate occasions; intra class correlations (ICC) and standard error of measurement (SEM) for 1RM tests were bench press $r = 0.98$, SEM 1.0kg; squat $r = 0.99$, SEM 2.5kg; pulldown $r = 0.98$, SEM 2.5kg.

Paragraph 9. Lean body mass (total fat free mass), fat mass and body fat percentage were determined using a Hologic QDR-4500 dual energy x-ray absorptiometry (DEXA) with the Hologic version V 7, REV F software (Waltham, MA). Whole body scans were performed on the same apparatus, by the same licensed operator. Quality control calibrations were performed as previously described (8). Participants were scanned at the same time of the day, that is, in the morning in a fasted state. For longitudinal studies in which relatively small changes in body composition are to be detected, whole body scanning with this instrument has been shown to be accurate and reliable (CV 0.8-2.8%) (19)

Paragraph 10. Muscle biopsies for determination of muscle fiber type, cross-sectional area (CSA), contractile protein content and Cr concentrations were taken in the week before and after the RE program. Biopsies (100-450mg) were taken using the percutaneous needle technique with suction to ensure adequate sample size (10) at a similar depth in the vastus lateralis muscle by the same medical practitioner. A small part of the sample was immediately frozen for assessment of contractile protein content and Cr. The remaining tissue was mounted using OCT medium and snap frozen in isopentane pre-cooled in liquid nitrogen and stored at -80^{o} C for histochemical analysis to classify muscle fiber types-I, IIa and IIx based on the stability of their ATPase activity, as previously described (7). Fiber type percentages and CSA were determined from sections containing a mean of 210 (range 130-400) fibers. Samples were measured on two separate occasions for day to day reproducibility ICC and SEM for fiber type distribution were

type I r = 0.82, SEM 1.8%; type IIa r = 0.94, SEM 1.3%; type IIx r = 0.94, SEM 1.2%. For mean area of fiber type I r = 0.97, SEM 87μm^2; type IIa r = 0.98, SEM 100μm^2; type IIx r = 0.97, SEM 141μm^2. Approximately 5 mg of muscle was used to determine contractile protein content as detailed by Beitzel et al. (3) and reported previously (7). Samples were run twice on two separate occasions (ICC r = 0.98, SEM 2.1 mg g^{-1}). Two mg of muscle was used to analyze Cr concentrations using fluorimetric techniques as in Harris et al. (11), with data expressed as mmol kg^{-1} dry weight (ICC r = 0.88, SEM 22).

Statistics

Paragraph 11: Statistical evaluation of the data was accomplished by two-way repeated measures analysis of variance (ANOVA) with group (supplement) and time (training) as the factors using SPSS statistical analysis software (SPSS v 11.0; Chicago, Illinois). Where significant main effects were identified by ANOVA, tukeys post hoc analysis was performed to locate differences. Deltas for each variable were analyzed with a one-way ANOVA. Preliminary power testing of expected changes in strength and body composition were based on previous data obtained by our laboratory (7; 8) and others (24; 27; 29). This testing revealed that 8 participants were required per group to obtain significance at an alpha level of 0.05 and a power of 0.8. Test-retest reliability was quantified using the intraclass correlation coefficient (ICC) two-way ANOVA (mixed effects model) and the SEM (28). Simple regression was used to determine significant relationships among the deltas for selected variables. A p value of less than 0.05 was designated to indicate statistical significance.

RESULTS

Starting characteristics

Paragraph 12. Baseline characteristics are presented in table 1. There were no differences between the groups in any variables at the start of the study ($P > 0.05$).

Dietary Analyses

Paragraph 13. Table 2 shows the average of three day written dietary recalls for energy (Kcal kg $^{-1}$ d $^{-1}$) carbohydrate and protein (g kg $^{-1}$ d $^{-1}$) of the groups before, and in the first and last week of the training program. Data does not include supplementation. No differences were identified between the groups or across time with regard to energy or macronutrient intake ($P > 0.05$).

Body Composition

Paragraph 14. Body mass and DEXA determined body composition are presented in table 3, with changes from baseline presented in figure 1. While all groups demonstrated an increase ($P < 0.05$) in body mass after the training program, a group x time interaction ($P < 0.05$) was detected; the PRO-CHO and Cr-PRO-CHO groups demonstrated a greater gain in body mass (post hoc $P < 0.05$) compared to the PRO group. All groups demonstrated an increase ($P < 0.05$) in lean mass (LBM) after the training program. However, a group x time interaction ($P < 0.01$) for LBM was detected; the Cr-PRO-CHO group showed a greater gain in LBM compared to the PRO and PRO-CHO groups (post hoc $P < 0.05$). A group x time interaction ($P < 0.05$) for fat mass and body fat percent was also observed. When compared to the PRO-CHO group, the PRO and Cr-PRO-CHO groups demonstrated a significant decrease in fat mass and body fat percent (post hoc $P < 0.05$).

Strength

Paragraph 15. Table 3 also presents the results of 1RM strength assessments and changes from baseline are presented in figure 2. All groups demonstrated an improvement ($P < 0.05$) in strength in each exercise after the training program. However, a group x time interaction ($P < 0.05$) was detected for the barbell squat, bench press and pulldown. The Cr-PRO-CHO group demonstrated a greater gain in strength in each of these exercises compared to the PRO and PRO-CHO groups (post hoc $P < 0.05$). No other differences between the groups were detected.

Muscle characteristics

Paragraph 16. There were no changes between the groups or across time with regard to fiber type proportions (table 4). All groups demonstrated an increase in CSA across all muscle fiber types ($P < 0.05$) after the training program, however a group x time interaction ($P < 0.05$) in CSA of both type-II fiber subgroups was detected (table 4). The Cr-PRO-CHO group demonstrated a greater increase in CSA in the type-IIa and IIx fibers compared to the PRO and PRO-CHO groups (post hoc $P < 0.05$) (figure 3). A group x time interaction ($P < 0.05$) was also observed for contractile protein content. The Cr-PRO-CHO group showed a greater increase in contractile protein content compared to the PRO and PRO-CHO groups (post hoc $P < 0.05$) (figure 4). Table 4 also presents muscle Cr data from samples taken before and after the training program. No differences between the groups or across time were detected.

Correlations

Paragraph 17. For all participants combined, positive correlations ($P < 0.05$) were detected between changes in CSA in the type-II fibers and strength gained in the 1RM squat exercise ($r = 0.677$) (figure 5). A correlation was also detected between the changes in contractile content (mg/g) and strength gained in squat exercise (1RM) ($r = 0.643$; $P < 0.01$) (figure 6). For all

participants combined, a positive correlation was also detected between the changes in LBM and strength (1RM) in the squat ($r = 0.661$; $P < 0.01$) (figure 7).

DISCUSSION

Paragraph 18. The most important finding of this investigation was that in RE-trained individuals, a CrM-containing PRO-CHO supplement provided significantly greater gains in 1RM strength and muscle hypertrophy compared to supplementation with an equivalent dose of PRO-CHO or PRO during 10 weeks of training. A significantly greater muscle hypertrophy response from the addition of CrM was evident at three different levels of physiology. That is, the CrM-treated group demonstrated a greater gain in LBM, hypertrophy of the type-IIa and IIx fibers, and increase in contractile protein. This is important, as we aware of no other research that has confirmed improvements in body composition via RE training and CrM supplementation with hypertrophy responses at the cellular (i.e., fiber-specific hypertrophy) and sub-cellular level (i.e., contractile protein content). Therefore, these results support the hypothesis that, in RE-trained individuals, a CrM-containing PRO-CHO supplement provides greater adaptations than a PRO-CHO supplement containing a similar amount of nitrogen and energy.

Paragraph 19. Several RE training studies have reported greater increases in strength and LBM in participants who consumed CrM as compared with a placebo (5; 6; 9; 13; 25). However, only one has compared the effects of a CrM-containing supplement with a supplement containing a similar amount of protein and energy (24). Tarnopolsky et al. (24) utilized previously inactive participants and daily supplementation with either 10g CrM + 75g CHO 1252kJ (300kcal) or 10g protein + 75g CHO 1420kJ (340kcal). When compared in this manner, Tarnopolsky et al. (24) concluded that CrM supplementation provided no greater gains in strength, LBM or muscle fiber

hypertrophy. However, whereas Tarnopolsky et al. (24) utilized previously inactive participants, the present study utilized RE-trained participants, and demonstrated significantly greater improvements in strength (three of three assessments) and muscle hypertrophy (three of three assessments) from treatment with CrM. Generally, untrained participants experience strength and lean mass changes that are of greater magnitude compared to RE-trained athletes (9). However, the influence of training status on the effects of supplements such as CrM is unknown. Previous work involving CrM supplementation and RE-trained individuals has shown that treatment enabled the participants to progress at a more rapid rate (27). This was reflected by the larger 1RM strength gains and greater volume of work completed during the workouts. I.e., more repetitions completed with heavier weight (27). Therefore, unlike inexperienced participants, it may be possible that RE-trained individuals experience strength and LBM gains that are of greater magnitude during training. Addionally, muscle Cr uptake is shown to be enhanced by macronutrient consumption (23) and post-exercise supplementation (21). In the present study, the CrM-treated participants consumed CrM with protein and carbohydrate and one of these servings were taken immediately after each workout. The results of this trial would appear to support the suggestion that CrM supplementation provides greater benefits in RE-trained individuals. However, a clear mechanism underlying these benefits remains some what elusive.

Paragraph 20. Improvements in muscular performance during high intensity contractions are associated with ATP resynthesis as a consequence of increased PCr availability in muscle via CrM supplementation (9; 11). Increasing the availability of PCr via supplementation is not only thought to enhance cellular bioenergetics of the phosphagen system but also the shuttling of high-energy phosphates between the mitochondria and cytosol to increase the availability of energy for contractile protein synthesis (2). Creatine is taken up by muscle where it appears to stimulate

transcription factors that regulate the synthesis of contractile proteins (29). Willoughby & Rosene (29) have reported an enhanced hypertrophy response from RE and supplementation (i.e., increase in strength, LBM and thigh volume) as well as alterations at the molecular level that may explain these benefits. Supplementation with CrM (6 g day $^{-1}$ for 12 weeks) resulted in a greater increase in LBM (assessed by skin fold caliper), thigh volume, (relative) muscle strength, and contractile protein content as well as up regulation of the genes and myogenic regulatory factors associated with (myosin heavy chain) contractile protein synthesis (29). An analytical review of 22 studies involving supplementation during RE training demonstrated that CrM clearly enhances maximum strength and weightlifting performance (maximal repetitions at a given percent of maximal strength) and this benefit was attributed to increased Cr availability during intense muscle contraction (20). More recently, Olsen et al. (16) reported that CrM supplementation during 16 weeks of RE amplified the training-induced increase in satellite cell number and myonuclei concentration in human skeletal muscle fibers, thereby allowing an enhanced muscle fiber growth in response to strength training. Therefore, supplementation with CrM may result in superior strength and hypertrophy responses (20) by inducing greater satellite cell number and myonuclei concentration (16) alongside transcriptional changes in muscle gene expression (29) which may contribute to, or be a product of, CrM's ability to enhance the bioenergetics of the phosphagen system (2; 11). Despite the clear beneficial effect of CrM that was observed in this study, metabolite assessments revealed no significant change in muscle Cr content at the end of the program. The CrM dose used in this study was based on others that have reported improvements in muscle hypertrophy and strength performance with small daily doses (6 g day $^{-1}$) (with no loading phase) similar to the dose utilized (0.1 g kg $^{-1}$day $^{-1}$) in this study. However, it may be that small daily doses of CrM for a prolonged duration (10 weeks) may not promote elevated

muscle Cr concentrations during intense RE training. For instance, despite a loading phase (20 g day $^{-1}$, 5 days) that provided a 25% increase in resting muscle Cr concentrations in the first week, Volek et al., (27) reported that supplementation (5 g day^{-1}) for a further 11 weeks resulted in only a ~10% increase by the end of a 12 week training/supplementation program. Van Loon et al. (26) demonstrated that a small maintenance dose (2-3 day g^{-1} for 6 weeks) in sedentary individuals failed to maintain high Cr muscle concentrations that were achieved by a CrM loading phase (20 day g^{-1}, 5days). In fact, after the 6 week maintenance phase, muscle Cr levels had returned to pre supplementation values (26). Although the results of the current investigation show clearly that CrM provided significantly greater muscle hypertrophy and strength, metabolite assessments revealed no significant change in muscle Cr content at the end of the program. The benefits of CrM are thought to be dependant on its accumulation within the cell (5; 9; 11; 20). As the advantages of supplementation may be applicable to a wide sector of the population, further studies should investigate strategies that create and maintain high muscle Cr concentrations during exercise training.

Paragraph 21. The CrM-treated group demonstrated a significantly greater increase in contractile protein content (mg g $^{-1}$ of muscle) compared to the other groups after the training program (figure 4). This result reflects the changes in CSA and LBM that were also detected. An increase in contractile protein is thought to be an important stimulus that results in an increase in muscle fiber CSA (17). RE-induced muscle fiber hypertrophy is thought to be primarily responsible for improvements in force production and strength that are observed in RE-trained participants (22). When all participants were combined, a strong relationship between changes in muscle fiber CSA of the type II fibers (IIa and IIx grouped) and strength improvements in the squat exercise were evident (figure 5). A similar relationship between changes in contractile

protein content or LBM and strength improvements in the squat was also detected (figures 6 and 7). The *r* values obtained suggest that a substantial portion (at least 40%) of the strength improvements observed across all groups could be attributed to the changes in skeletal muscle morphology. These correlations reflect a direct relationship between muscle adaptation (hypertrophy) and an improvement in functional strength. Obviously, the barbell squat exercise was the focus of these correlation assessments, simply because, unlike the bench press and pulldown exercise, the vastus lateralis is recruited heavily during this exercise and was the muscle from which the biopsy samples were obtained.

Paragraph 22. Aside from skeletal muscle morphology, the improvements in 1RM strength observed in this trial must also be attributed to the benefits of personalized coaching/supervision. Although the participants in our study were experienced, none had ever received personal training by a qualified instructor (the personal training only occurred during the 10 week trial, not the training program prior to the study). Personalized instruction of the participants was a major strength of this study as this level of supervision is shown to provide better control of workout intensity and greater strength improvements during training (15). This level of supervision was important to our hypothesis as it would ensure the best chance of enhanced physiological adaptations from an interaction between training and CrM supplementation. This is based on the premise that those taking the CrM would obtain a greater anabolic response from each workout and progress at a faster rate. It is important to remember that the instructor was blinded to the supplement groups, yet the CrM-treated group demonstrated significantly greater gains in 1RM strength (in three of three assessments) and greater muscle hypertrophy responses (in three of three assessments), thus supporting the hypothesis presented.

Paragraph 23. Another interesting finding from this study was the influence of the different supplements on body composition. While all groups demonstrated a gain in body mass after the training program, the Cr-treated group demonstrated a significantly greater gain in body mass compared to the PRO group but not the PRO-CHO group. However, there were differences in the composition of these changes in mass. Compared to the PRO-CHO group, the Cr-PRO-CHO group and the PRO groups demonstrated a decrease in fat mass and body fat percent (table 3; figure 1). The exact reasons for these different responses to the various supplements are not clear. A decrease in body fat and/or body fat percent in response to whey protein supplementation (6-10 weeks) is a phenomenon that has been reported previously in rodents (4) and humans undertaking RE training (7). Whey protein supplementation has been shown to induce greater lipid oxidation during and after exercise compared to casein and CHO; a response that resulted in a greater utilization of body fat for fuel and a reduction in body fat (4). However, this does not explain the contrasting body composition changes observed in the Cr-PRO-CHO and PRO-CHO groups. Both of these groups consumed the same supplement; the only difference being the relatively small amount of CrM present in the Cr-PRO-CHO supplement (approximately 7%). Despite this, the Cr-treated group demonstrated a reduction in fat mass (and body fat percentage) when compared to the PRO-CHO group. CrM does not appear to provide any benefit with regard to fat metabolism (12). Therefore, the improvement in body composition observed from CrM-supplementation is most like due to the large accretion of LBM that was observed in this group, which was on average, 6kgs. This extra muscle mass would almost certainly have had a positive influence on resting metabolic rate and therefore, fat metabolism, particularly in active individuals that consume the same relative energy intake (per kg of body mass) for a prolonged period of time (18), as was the case in this study. If the addition of CrM to a PRO-CHO supplement does

enhance LBM gains and improve body composition during training as observed in this study, this may have specific implications for some populations. For example, those that desire maximum gains in LBM, strength and muscle hypertrophy without an increase in fat mass will benefit from a CrM-containing PRO-CHO supplement. However, for others that desire a gain in body mass in general, CrM may not be required. Alternatively, athletes that desire strength and muscle hypertrophy with only a relatively modest increase in body mass may opt for supplementation with whey protein alone.

Paragraph 24. In conclusion, this study used a group of RE-trained participants to examine the effects of a CrM-containing (0.1 g $kg^{-1}day^{-1}$) PRO-CHO supplement in comparison to the same PRO-CHO supplement (without CrM) during 10 weeks of RE training. Although both supplements were similar in energy and nitrogen content, the group who received CrM demonstrated greater gains in 1RM strength in three exercises and these improvements were supported by a greater hypertrophy response that was apparent at three different levels; LBM, muscle fiber CSA and contractile protein content. Therefore, in RE-trained individuals, the presence of CrM in a PRO-CHO supplement results in significantly greater adaptations during RE training than supplementation without CrM.

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FIGURE CAPTIONS

Figure 1. Body mass and composition changes *Significantly different than PRO-CHO; †Significantly different than PRO ($P < 0.05$).

Figure 2. 1RM Strength Changes *Significantly different than PRO-CHO; † Significantly different than PRO ($P < 0.05$)

Figure 3. Changes in muscle fiber CSA (types-I, IIa and IIx) *Significantly different than PRO-CHO; †Significantly different than PRO ($P < 0.05$).

Figure 4. Changes in contractile protein content (mg/g of muscle) *Significantly different than PRO-CHO; †Significantly different than PRO ($P < 0.05$).

Figure 5 Relationship between type-II muscle fiber hypertrophy and 1RM strength improvements in the squat.

Figure 6. Relationship between change in contractile protein content and 1RM strength gains in the squat.

Figure 7. Relationship between change LBM and 1RM strength improvements in the squat

Figure 1

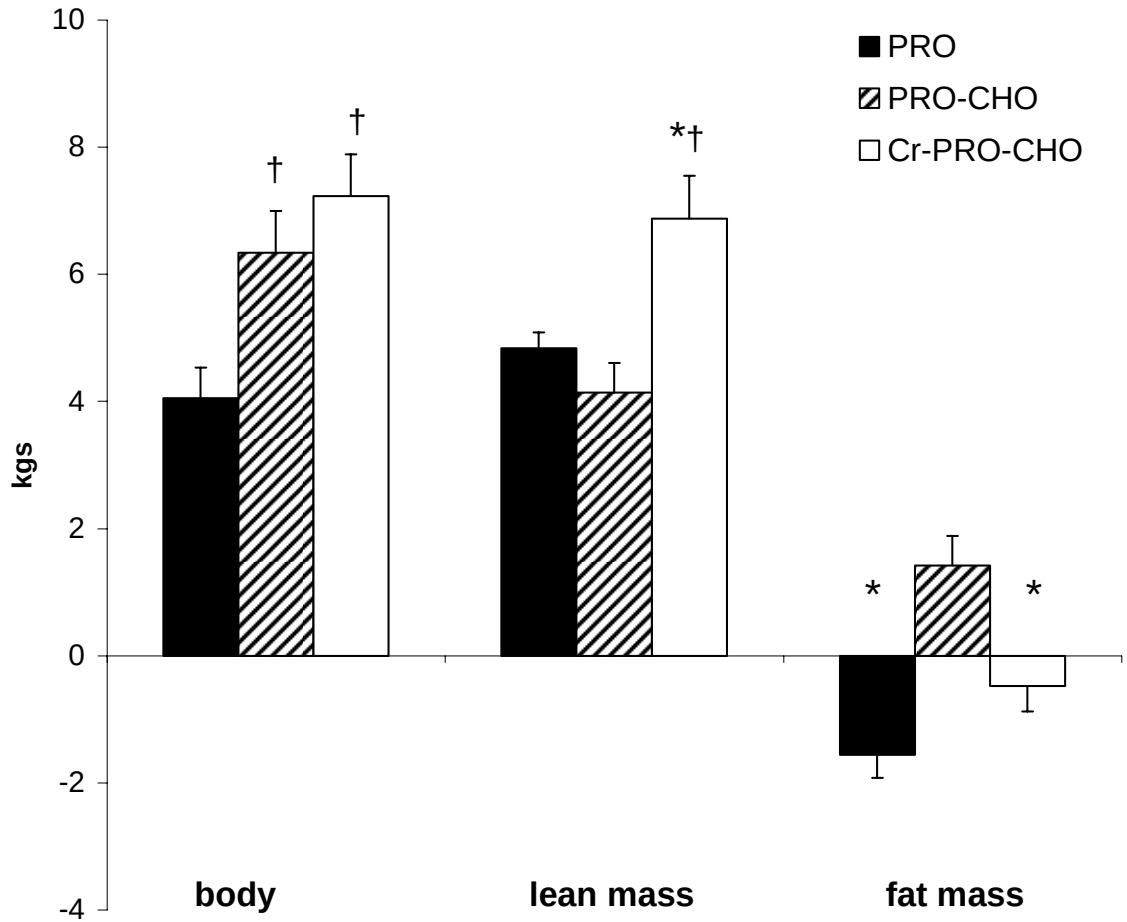


Figure 2.

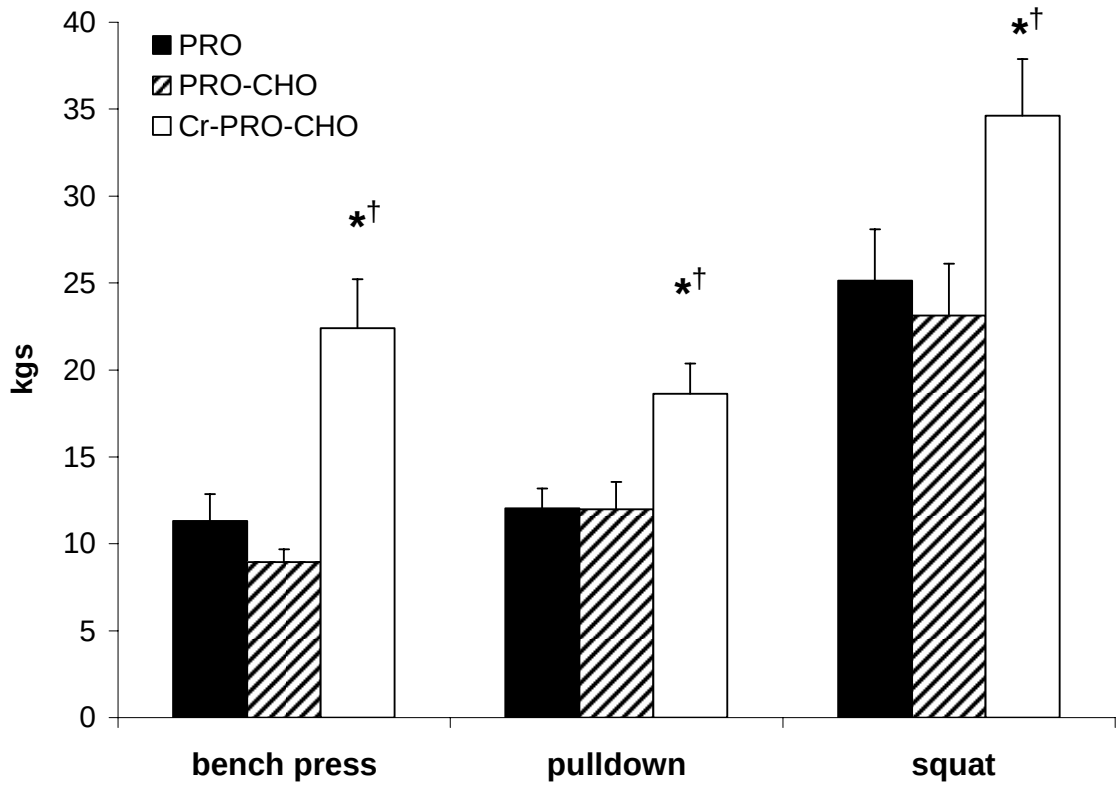

PRO
PRO-CHO
Cr-PRO-CHO
kgs
40
35
30
25
20
15
10
5
0
*†
*†
*†
bench press
pulldown
squat

Figure 3.

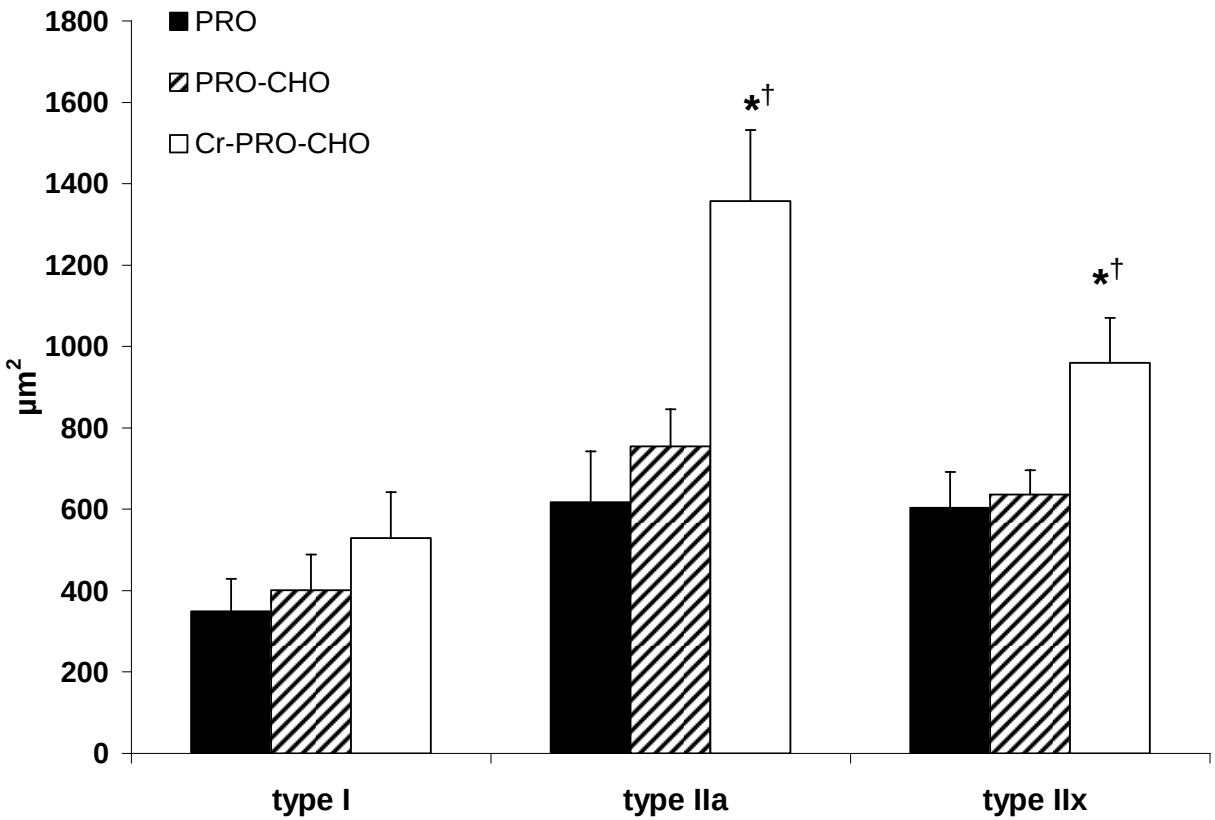

PRO
PRO-CHO
Cr-PRO-CHO
1800
1600
1400
1200
1000
800
600
400
200
0
µm2
*†
*†
type I
type IIa
type IIx

Figure 4.

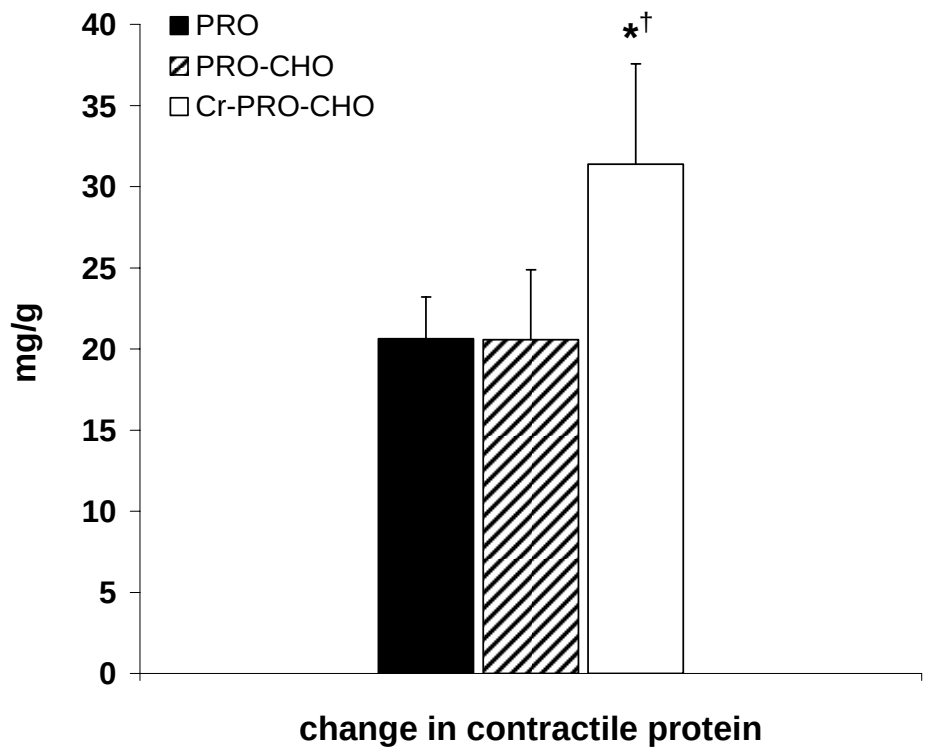

PRO
PRO-CHO
Cr-PRO-CHO
*†
40
35
30
25
20
15
10
5
0
mg/g
change in contractile protein

Figure 5.

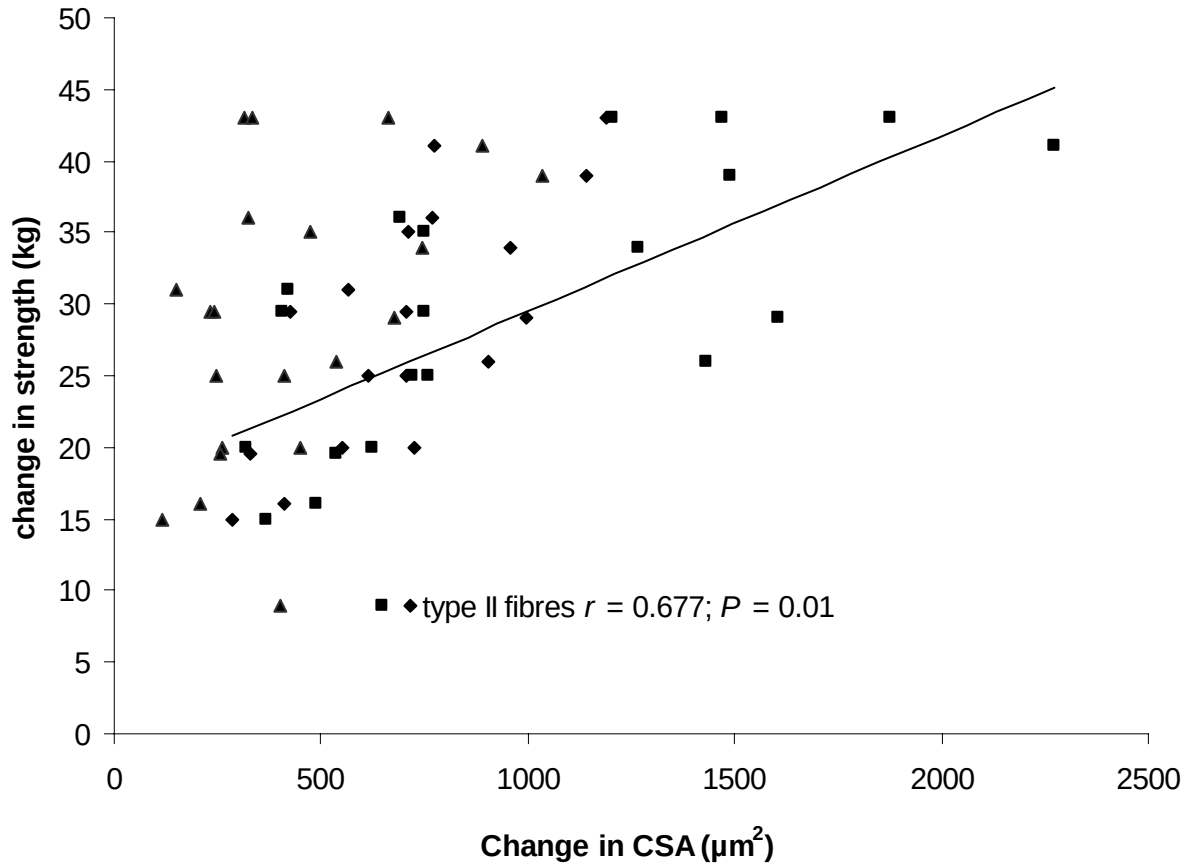

50
45
40
35
30
25
20
15
10
5
0
change in strength (kg)
0
500
1000
1500
2000
2500
Change in CSA (µm2)
type II fibres r = 0.677; P = 0.01

Figure 6.

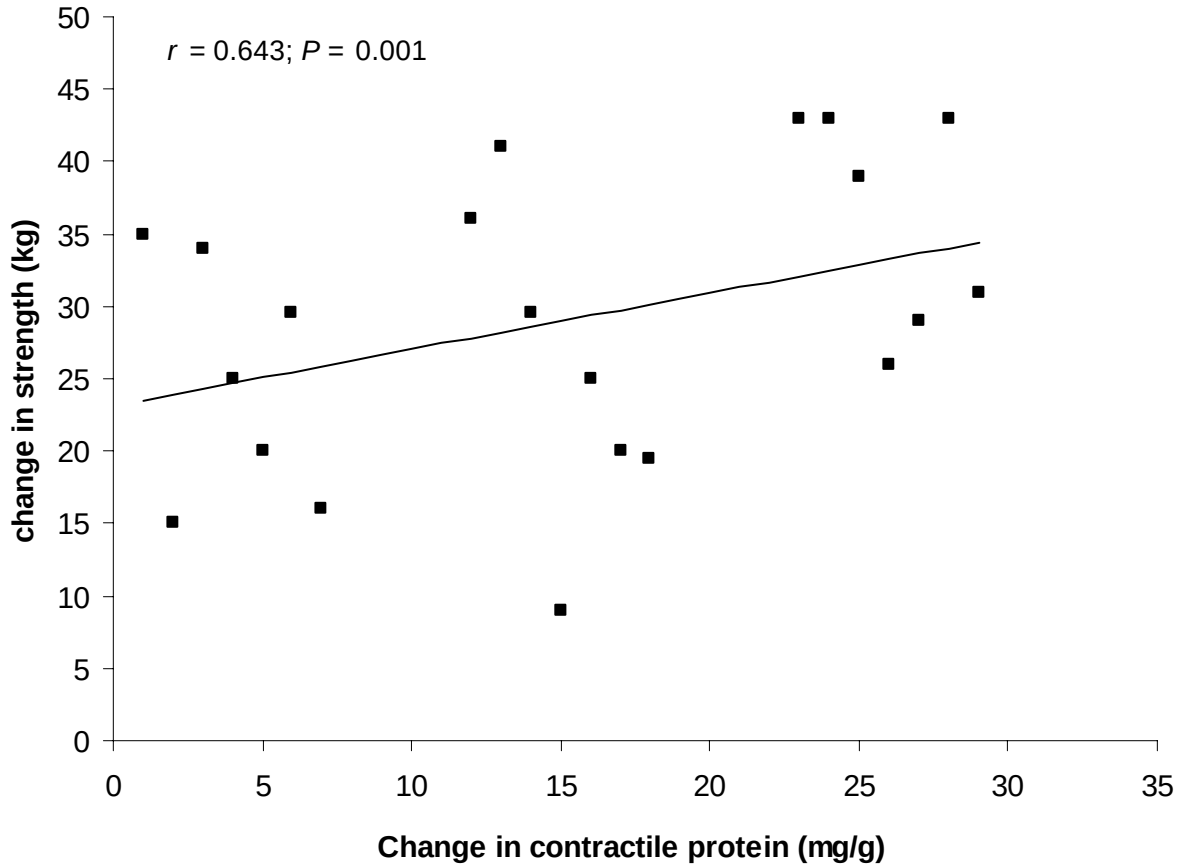
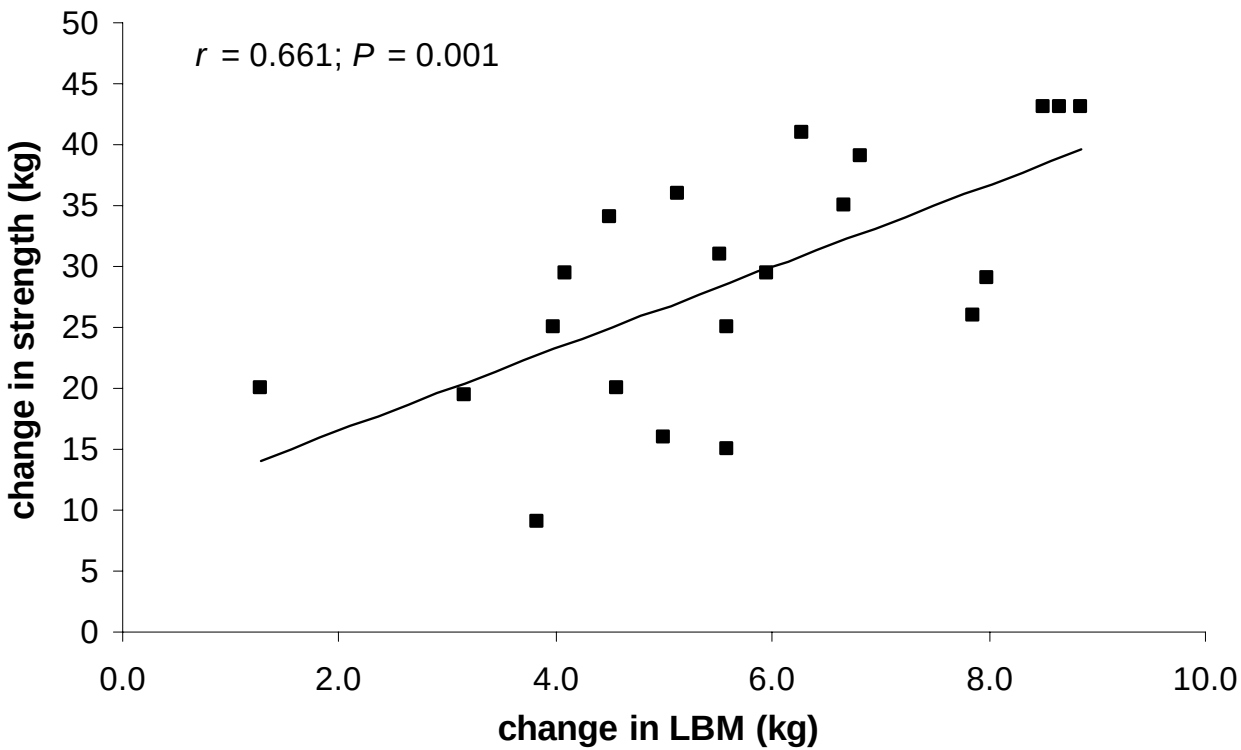

r = 0.643; P = 0.001
change in strength (kg)
Change in contractile protein (mg/g)
0
5
10
15
20
25
30
35
40
45
50

Figure 7.


r = 0.661; P = 0.001
change in strength (kg)
change in LBM (kg)
0
5
10
15
20
25
30
35
40
45
50
0.0
2.0
4.0
6.0
8.0
10.0

TABLES

Table 1. Baseline Characteristics

	PRO	**PRO-CHO**	**Cr-PRO-CHO**	***P***
Age (yrs)	25 ± 4	26 ± 3	26 ± 5	0.799
Training age (yrs)	4 ± 2	4 ± 1	4 ± 5	0.871
Height (cm)	177 ± 4	177 ± 4	179 ± 5	0.509
Lean mass (kg)	69 ± 8	67 ± 9	70 ± 12	0.729
Fat mass (kg)	16 ± 5	13 ± 5	16 ± 9	0.391
CSA type-I (μm^2)	2895 ± 511	3079 ± 1365	3129 ± 718	0.887
CSA type-IIa (μm^2)	4519 ± 639	4662 ± 1326	4528 ± 1014	0.959
CSA type-IIx (μm^2)	3798 ± 734	4370 ± 1405	3905 ± 901	0.586
1RM Bench (kg)	110 ± 13	112 ± 20	108 ± 13	0.866
1RM Squat (kg)	120 ± 15	127 ± 29	122 ± 24	0.789
1RM Pulldown (kg)	105 ± 9	108 ± 13	108 ± 15	0.834

Data presented as mean ± SD

Table 2. Dietary Analyses (means ± SD)

	PRO	PRO-CHO	Cr-PRO-CHO	*P* Group x Time
Energy intake (kJ/kg/day)				
before	135.7 ± 15.1	137.3 ± 15.5	138.2 ± 17.6	
week 1	126.0 ± 14.2	137.3 ± 13.0	126.0 ± 15.5	0.264
week 10	126.0 ± 8.0	131.5 ± 14.7	123.1 ± 10.5	
Carbohydrate (g/kg/day)				
before	3.0 ± 0.8	3.3 ± 0.6	3.1 ± 0.7	
week 1	3.0 ± 0.5	3.3 ± 0.7	2.8 ± 0.6	0.653
week 10	2.8 ± 0.5	3.2 ± 0.5	3.0 ± 0.4	
Protein (g/kg/day)				
before	2.3 ± 0.5	2.0 ± 0.8	1.8 ± 0.3	0.385
week 1	1.7 ± 0.2	1.7 ± 0.2	1.6 ± 0.3	
week 10	1.7 ± 0.2	1.8 ± 0.6	1.6 ± 0.2	

Table 3. Body mass, composition and 1RM strength (mean ± SE)

	PRO	PRO-CHO	Cr-PRO-CHO	*P* Group x Time
Body mass (kg)				
PRE	88.0 ± 3.6	82.0 ± 4.0	89.6 ± 6.5	0.001
POST #	92.2 ± 3.5	88.8 ± 3.9†	96.7 ± 2.7†	
Lean mass (kg)				
PRE	69.1 ± 2.5	66.5 ± 2.8	69.6 ± 3.8	0.001
POST #	74.0 ± 2.5	70.6 ± 2.9	76.5 ± 4.2*†	
Fat mass (kg)				
PRE	16.2 ± 1.7	12.7 ± 1.4	15.9 ± 2.8	0.01
POST	14.6 ± 1.5*	14.0 ± 1.2	15.4 ± 2.5*	
Fat %				
PRE	17.2 ± 1.5	15.1 ± 1.1	16.3 ± 0.9	0.01
POST	13.6 ± 1.2*	15.9 ± 0.8	14.1 ± 1.4*	
Squat (kg)				
PRE	119.6 ± 4.9	126.7 ± 8.7	122.2 ± 7.6	0.03
POST #	144.8 ± 4.7	149.8 ± 9.8	156.9 ± 9.6*†	
Bench press (kg)				
PRE	110.3 ± 4.0	112.0 ± 6.0	108.3 ± 4.0	0.001
POST#	121.6 ± 4.1	121.0 ± 6.0	130.7 ± 5.3*†	
Pulldown (kg)				
PRE	105.2 ± 2.9	107.8 ± 3.8	108.4 ± 4.8	0.005
POST #	117.3 ± 3.0	119.9 ± 4.8	127.1 ± 4.9*†	

*Greater change than PRO-CHO; †Greater change than PRO; # training effect all groups ($P < 0.05$)

Table 4. Muscle fibre type, CSA, contractile protein and Cr (mean ± SE)

	PRO	PRO-CHO	Cr-PRO-CHO	*P* Group x Time
%Type -1				
PRE	40.9 ± 1.9	37.9 ± 2.9	40.3 ± 1.6	0.345
POST	40.0 ± 0.9	35.7 ± 3.0	38.5 ± 2.0	
%Type-IIa				
PRE	44.0 ± 1.4	45.6 ± 2.1	47.4 ± 2.2	0.598
POST	45.1 ± 1.6	48.6 ± 2.2	51.4 ± 3.5	
%Type-IIx				
PRE	15.1 ± 1.0	16.5 ± 1.0	13.8 ± 1.0	0.410
POST	15.0 ± 1.0	15.7 ± 1.0	14.1 ± 1.0	
Type 1 CSA (μm^2)				
PRE	2895 ± 193	3079 ± 516	3129 ± 271	0.396
POST$^{\#}$	3244 ± 213	3480 ± 497	3659 ± 208	
Type IIa CSA (μm^2)				
PRE	4519 ± 242	4662 ± 501	4529 ± 383	0.002
POST$^{\#}$	5136 ± 231	5416 ± 518	5886 ± 315*†	
Type IIx CSA (μm^2)				
PRE	3798 ± 277	4370 ± 531	3905 ± 403	0.024
POST$^{\#}$	4402 ± 261	5007 ± 486	4864 ± 316*†	
Contractile protein ($mg^{-1}g$)				
PRE	57.8 ± 2.9	55.8 ± 2.0	57.1 ± 1.3	0.001
POST$^{\#}$	78.4 ± 3.1	76.3 ± 1.0	89.1 ± 1.5*†	
Total Cr ($mmol^{-1}kg$ dry wt)				
PRE	117.5 ± 2.1	119.5 ± 4.7	115.8 ± 4.4	0.289
POST	111.2 ± 6.8	109.6 ± 7.1	119.8 ± 4.2	

*Greater increase than PRO-CHO; †Greater increase than PRO; $^{\#}$ training effect all groups ($P < 0.05$)