

EFFECTS OF WHEY ISOLATE, CREATINE AND RESISTANCE TRAINING ON MUSCLE HYPERTROPHY

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Running Head: Creatine, whey isolate and resistance training

Key words: protein supplementation; histochemistry; skeletal muscle strength; fiber area, contractile protein

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ABSTRACT

PURPOSE: Studies that have attributed gains in lean body mass to dietary supplementation during RE training have not reported these changes alongside adaptations at the cellular and sub-cellular levels. Therefore, the purpose of this study was to examine the effects of two popular supplements; whey protein (WP) and creatine monohydrate (CrM) (both separately and in combination) on body composition, muscle strength, fiber-specific hypertrophy (i.e., type-I, IIa, IIx) 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 four groups: creatine/carbohydrate (CrCHO), creatine/whey protein (CrWP), WP-only or carbohydrate-only (CHO) (1.5g/kg body wt/day). All assessments were completed the week before and after an 11 week structured, supervised RE program. Assessments included 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 (Cr) content. **RESULTS:** Supplementation with CrCHO, WP and CrWP resulted in significantly greater ($P < 0.05$) 1RM strength improvements (three of three assessments) and muscle hypertrophy compared to CHO. Up to 76% of the strength improvements in the squat could be attributed to hypertrophy of muscle involved in this exercise. However, the hypertrophy responses within these groups varied at the three levels assessed (i.e., changes in lean mass, fiber-specific hypertrophy and contractile protein content). **CONCLUSIONS:** Although WP and/or CrM appear to promote greater strength gains and muscle morphology during RE training, the hypertrophy responses within the groups varied. These differences in skeletal muscle morphology may have important implications for various populations and therefore, warrant further investigation.

INTRODUCTION

Paragraph 1: Whey protein (WP) and creatine monohydrate (CrM) are two dietary supplements commonly used to promote muscle strength and hypertrophy during resistance exercise (RE) (5; 24). WP supplements generally contain a higher concentration of essential amino acids (EAA) than other protein sources (5), and have rapid absorption kinetics (9). Supplementation results in a high blood amino acid peak and stimulation of protein synthesis similar to a dose of EAA (21). WP-containing meals provide a higher postprandial leucine balance and net protein gain in young and older men compared to isonitrogenous casein meals (9). Although some studies have shown greater strength and/or lean body mass (LBM) gains with WP compared to matched groups given carbohydrate (CHO) (6) or casein (8) during RE training, no studies have assessed skeletal muscle adaptations in response to RE training and WP supplementation. The chronic use of CrM to increase muscle strength and LBM is also a common strategy among various adult populations that exercise (24). The beneficial effects of oral CrM supplementation are thought to be dependant on the extent of Cr accumulation within muscle (14). However, this response can be highly variable between subjects (17). For this reason, dietary strategies, such as combining CrM with carbohydrate (CHO) (16) or protein (27) have been used to enhance Cr uptake.

Paragraph 2: Studies that have attributed gains in LBM to dietary supplementation during RE training have not reported these changes alongside adaptations at the cellular level (i.e., fiber-specific, type-I, IIa, IIx hypertrophy) (4; 6; 8; 16; 25). Those that have reported fiber-specific hypertrophy (1; 10; 28) have not confirmed this response with changes at the sub-cellular level (i.e., contractile protein content). For example, the combination of CrM with CHO has been shown to provide greater improvements in strength and body composition (i.e. increase LBM with no increase in fat mass) compared to CHO alone (16). CrM combined with WP has also

been shown to augment muscle strength and LBM when compared to CHO or WP-only supplementation (6). However, no studies have examined the effects of CrM and WP supplementation on strength and body composition changes alongside muscle characteristics such as fiber-specific (i.e., type-I, IIa, IIx) hypertrophy and contractile protein content. Therefore, the aim of this study was to examine the effects of combining CrM with CHO and with WP during RE training in comparison to WP and CHO alone, on strength, body composition and fiber-specific (i.e., type-I, IIa, IIx) hypertrophy as well as muscle Cr and contractile protein content . The first hypothesis was that supplementation with CrM and WP or CrM and CHO would provide greater benefits than WP or CHO alone. Due to the benefits reported previously with WP (6; 8), a secondary hypothesis was that the combination of CrM and WP would provide greater benefits than the combination of CrM and CHO.

METHODS

Participants

Paragraph 3: Thirty-three recreational male bodybuilders met the requirements to commence this study that involved pre-post assessments and supplementation during 11 weeks of RE training. 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 supplementation, 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 of Technology 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 four supplement groups in a double-blind fashion; whey protein (WP), CrM and whey protein (CrWP), CrM and carbohydrate (CrCHO), or carbohydrate-only (CHO).

Supplementation

Paragraph 5: Participants were instructed to consume 1.5 grams of the supplement per kilogram of body weight per day ($1.5\text{g}^{-1}\text{kg}^{-1}\text{day}$) while maintaining their habitual daily diet. The chosen supplement dose was based on previously reported intakes of this population (18). The supplements were tested to comply with label claims before leaving the place of manufacture (AST Sports Science, Golden, CO, USA). Additionally, the WP supplement was independently assessed by Naturalac Nutrition LTD (Level 2/18 Normanby Rd Mt Eden, New Zealand) on two separate occasions, and matched labelled ingredients on both occasions. The supplements were provided in identical containers with sealed, tamper-proof lids, and they were similar in energy content on a g^{-1}kg basis. For example, an 80kg participant in the WP group consumed 120g/day of a supplement that contained approximately 103g protein, <6g carbohydrate, <1.2g fat and 1864 kJ (447 Kcal), whereas an 80kg participant in the CHO group consumed the same dose of a supplement that contained 106g carbohydrate, 0 protein or fat and 1770 kJ (424 Kcal). The Cr-containing supplements (CrCHO and CrWP) contained a 1 week loading phase with CrM (0.3g^{-1}

$^1\text{kg}^{-1}\text{day}$) that was followed by a maintenance phase ($0.1\text{g}^{-1}\text{kg}^{-1}\text{day}$) for the duration of the study (weeks 2-11) — a protocol has been shown previously to augment muscle strength and hypertrophy during RE training (28). For example, an 80kg participant in the CrCHO group consumed $120\text{g}^{-1}\text{day}$ of a loading phase supplement that contained 85g carbohydrate, 24g CrM/ and 1420 kJ (340 Kcal), and then a maintenance phase supplement (weeks 2-11), that provided 98.9g carbohydrate, 8.4g CrM and 1651 kJ (396 Kcal). A participant of the same weight in the CrWP group consumed a loading phase supplement (week 1) that contained 83g protein, <4.8g carbohydrate, <1g fat, 24g CrM and 1500 kJ (359 Kcal) followed by a maintenance phase supplement (weeks 2-11), that contained 96g protein, <5.5g carbohydrate, <1g fat, 8.4g CrM and 1729 kJ (415 Kcal).

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, the participants were asked to consume one serving mid-morning, one serving as soon as they finished each workout in the afternoon (or similar time on non-training days), and one serving 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. Where a substantial change in body mass (approximately 2 kgs) from baseline was observed, the participant was shown how to adjust the supplement dose to correspond with the increase in body weight. 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 macronutrient and energy intake. Energy intake is expressed in kcal⁻¹kg of body weight per day; protein and carbohydrate are expressed in g⁻¹kg 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, the men underwent a structured training program (similar to the one used in this study) for 8 to 12 weeks prior to commencing this trial. The 11 week RE program used in the study (*Max-OT™*, AST Sport Science, Golden, CO, USA) has been described elsewhere (7; 8) and 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 using repetition maximums (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 RE program.

Strength testing

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. A recognized 1RM testing protocol and exercise execution guidelines were followed as has been previously documented (2). 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 $r = 0.998$, SEM 1.0kg; squat $r = 0.995$, SEM 2.5kg; pulldown $r = 0.982$, SEM 2.5kg.

Body Composition

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 calibration and scanning procedures 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%) (23)

Muscle analyses

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 (12) 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°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 $r = 0.822$, SEM 1.8%; type IIa $r = 0.941$, SEM 1.3%; type IIx $r = 0.945$, SEM 1.2%. For mean area of fiber type I $r = 0.972$, SEM $87\mu\text{m}^2$; type IIa $r = 0.984$, SEM $100\mu\text{m}^2$; type IIx $r = 0.967$, SEM $141\mu\text{m}^2$. Approximately 5 mg of muscle was used to determine contractile protein content as detailed by Beitzel et al. (3) and reported previously (7). Two mg of muscle was used to analyze Cr concentrations using fluorimetric techniques as in Hultman et al., (14), data is expressed as $\text{mmol}^{-1}\text{kg}$ dry weight. Samples were run twice on two separate occasions, ICC and SEM for contractile protein content were $r = 0.984$, SEM 2.1mg/g; Cr $r = 0.881$, 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. *A priori* power testing was based on previous data on changes in strength, body composition and contractile protein data obtained by our laboratory (7; 8) and others (30). The testing indicated group sizes of between 4 and 7 participants were required to show

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 (29). 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. A p value of less than 0.09 was considered a trend.

RESULTS

Starting characteristics

Paragraph 12: Four participants did not attend the required amount of supervised training sessions (75%) or provide all dietary records. Therefore, their data was not included. Additionally, three participants chose not to return for final biopsies. This reduced the number of the groups to 7 = CHO, 5 = WP, 8 = CrCHO and 6 = CrWP. Starting characteristics of these participants are shown 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 $^{-1}$ kg $^{-1}$ d) carbohydrate and protein (g $^{-1}$ kg $^{-1}$ d) of the groups before, 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: All groups demonstrated a gain in body mass (time, $P = 0.001$) (table 3), but no group or group x time interaction was detected for body mass. No interactions for fat mass or body fat percentage were detected between the groups or across time. However, a group x time

interaction ($P = 0.043$) was observed for LBM (table 3). While each of the groups demonstrated an increase (time, $P = 0.001$) in LBM after the program (CrCHO +3.7kg), (CrWP +3.4), (WP +2.3kg), (CHO +0.7), only the CrCHO group's increase in LBM was significantly greater than the CHO group (post hoc $P < 0.05$).

Strength

Paragraph 15: 1RM strength data (kgs) barbell bench press, cable pulldown and barbell squat are presented in figures 1a, b and c respectively. All groups demonstrated an improvement in strength in each exercise after the program (time: $P = 0.0001$), and a group x time interaction ($P = 0.0001$) was observed for each exercise. The CrCHO, CrWP and WP groups demonstrated a greater increase in strength in each exercise compared to the CHO group (post hoc $P < 0.05$). However, no differences were detected between the CrCHO, CrWP and WP groups.

Muscle characteristics

Paragraph 16: No changes in fiber type proportions between the groups or across time were detected (table 4). All groups demonstrated an increase in CSA ($P < 0.05$) of the type-IIa and IIx fibers after the program. Additionally, a group x time interaction in CSA was detected for the type-1 ($P = 0.001$; figure 2a), IIa ($P = 0.001$; figure 2b) and IIx ($P = 0.001$; figure 2c) fibers. The CrCHO and CrWP groups demonstrated a greater increase in CSA in each fiber type compared to the CHO group (post hoc $P < 0.05$). The CrCHO and CrWP groups also demonstrated a greater increase in CSA in the type-I fibers when compared to the WP group (post hoc $P < 0.05$). A trend for a greater hypertrophy of the type IIa and IIx fibers ($P = 0.077$ and $P = 0.078$, respectively) was also observed in the WP group compared to the CHO group.

Paragraph 17: A group x time interaction ($P = 0.001$) for contractile (myofibrillar) protein content was also detected. The CrCHO, CrWP and WP groups each showed a greater increase in

contractile protein compared to the CHO group after the program (post hoc $P < 0.05$) (figure 2d). Additionally, the CrCHO and CrWP groups demonstrated a trend ($P = 0.07$ and $P = 0.08$, respectively) for a greater increase in myofibrillar protein content compared to the WP group.

Paragraph 18: A group difference ($P = 0.03$) was detected for the Cr-treated groups in muscle Cr (table 5). Both the CrCHO and CrWP groups showed a higher ($P < 0.05$) concentration ($\text{mmol}^{-1}\text{kg}$ dry weight) of Cr compared to the WP and CHO group after the training program, but there was no difference between the CrCHO and CrWP groups.

Correlations

Paragraph 19: For all participants combined, positive correlations ($P < 0.01$) were detected between changes in muscle fiber CSA (in all fiber types) and strength gained in the 1RM squat exercise (figure 3). A positive correlation ($P < 0.05$) was also detected between the change in contractile protein (mg/g) and (1RM) strength improvements in the squat (figure 4). Additionally, positive correlations ($P < 0.01$) were detected between the increase in contractile protein and increase in muscle fiber CSA, in all fiber types (figure 5).

DISCUSSION

Paragraph 20: The most important finding of this investigation was that although there were no differences between the groups at the start of this study and each group consumed a protein-rich diet, supplementation with CrCHO, WP and CrWP resulted in greater hypertrophy response (in at least one of three assessments) and 1RM strength gains (in three of three assessments) compared to CHO. Additionally, the changes in 1RM squat strength correlated strongly ($r \geq 0.7$; $P < 0.01$) with the changes in muscle morphology across all groups. However, when compared to CHO, the hypertrophy response from supplementation with CrCHO, WP and CrWP varied at the three levels of muscle physiology that were assessed (i.e., LBM, fiber-specific hypertrophy and contractile protein content). These findings are novel as we are aware of no other RE training studies that have reported changes in body composition from dietary intervention alongside adaptations at the cellular level (i.e., fiber -specific hypertrophy) (4; 6; 8; 16; 25) and the sub-cellular level (i.e., contractile protein content) (1; 10; 28).

Paragraph 21: Our findings only partly support the first hypothesis proposed. That is, treatment with CrCHO or CrWP provided greater improvements in strength and muscle hypertrophy when compared to CHO but not WP. Additionally, the results do not support the second hypothesis proposed. That is, no greater benefit was observed from combining CrM and WP when compared to the combination of CrM and CHO. It is possible that small number (n) in some of the groups that completed this trial may have reduced the capacity to adequately detect some differences between the groups, particularly in major variables of interest such as changes in LBM. For example, although the WP, CrCHO and CrWP groups each demonstrated relatively large changes in LBM (3.7%, 5.5% and 5%, respectively), compared to the CHO (1.1%) group, the only change in LBM deemed significantly greater than the CHO group was the CrCHO group. We

commenced this study with thirty four participants that provided similar group n 's to our previous work (7; 8) and others (28; 30) that have involved supplementation and RE training. These investigations reported significant differences between groups in LBM, strength and/or muscle hypertrophy with n 's of 9-6 in each group. For example, in a previous study completed by this laboratory (8) that utilized RE-trained participants and a similar protocol, supplementation with WP ($n = 6$) ($1.5\text{gm}^{-1} \text{kg}^{-1}$ day for 10 weeks) produced significantly greater gains in LBM and strength compared to a group given an equivalent dose of casein ($n = 7$). In another investigation that also involved RE trained participants undertaking a 10 week RE program, we were able to detect significant different gains in LBM between two groups ($n = 8$, $n = 9$) that consumed the exact same supplement at different times of the day (7). Volek et al. (28) also utilized RE-trained participants, a similar RE program and CrM supplementation protocol to the present study, and reported comparable results. That is, after the 12 week training period, CrM supplementation ($n = 9$) resulted in a significantly greater gain in LBM, 1RM squat strength and muscle fiber hypertrophy in all fiber types assessed compared to a matched placebo-treated group ($n = 10$) (28). Willoughby & Rosene (30) reported that supplementation with CrM ($n = 8$) during 12 weeks of RE resulted in a greater increase in LBM (assessed by skin fold caliper), thigh volume, (relative) muscle strength, and myofibrillar protein content than a placebo-treated group ($n=8$) and a control group ($n = 6$). Based on prior investigations (7; 8; 28; 30) it was reasonable to assume that commencing the present study with thirty-four participants would be adequate. However, a lower than anticipated finishing n in some of the groups probably reduced the capacity to detect differences between the groups in LBM. We acknowledge that the small sample size of the groups is as an important limitation of this study. Nevertheless, unlike other investigations that have reported changes in body composition from dietary intervention, the

changes in LBM in this study are supported by a number of significant differences between the groups in skeletal muscle morphology that were detected at the cellular and sub-cellular levels.

Paragraph 22: Few have used matched placebo-treated groups and quantified the extent of specific muscle fiber type (i.e., type-I, IIa, IIx) hypertrophy in response to RE training and supplementation. Volek et al. (28) reported that treatment with CrM resulted in significantly greater muscle fiber hypertrophy in all fiber types assessed compared to a matched placebo-treated group. Andersen et al. (1) reported significantly greater hypertrophy of both the type-I and II fibers as well as squat jump height in a group that received a pre- and post-workout protein supplement (25g each serving) compared to an equivalent dose of CHO during 14 weeks of RE. In the present study, significant differences between the groups in muscle fiber hypertrophy across all fiber types were detected. For example, both the CrCHO and CrWP groups demonstrated a greater increase in CSA in the type-I, IIa and IIx fibers (figures 2a, b and c) compared to the CHO group as well as a greater increase in CSA in the type-I compared to the WP group (figure 2a). However, no differences were detected between the WP, CrWP and CrCHO groups. Unlike previous studies (1; 10; 28) that have reported muscle fiber CSA changes in response to training and supplementation this study was able to confirm these hypertrophy responses with changes in contractile protein content.

Paragraph 23: The CrCHO, CrWP, WP groups in this study each demonstrated a significantly greater increase in contractile protein content (mg/g of muscle) compared to the CHO group after the training program (figure 2d). This reflects the changes in CSA that were detected, particularly in the CrCHO and CrWP groups, and to a lesser extent, the WP group; a trend ($P < 0.09$) for greater hypertrophy of the type-IIa and IIx fibers was observed for the WP group when compared to the CHO group. Although no significant differences were detected between the WP, CrCHO

and CrWP groups in LBM gains or muscle fiber hypertrophy, a trend ($P < 0.09$) for a greater increase in myofibrillar protein content was also detected in the CrCHO and CrWP groups compared to the WP group. 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 (26). An increase in contractile protein is thought to be an important stimulus that results in an increase in muscle fiber CSA (22). When all participants were combined, a strong relationship between changes in muscle fiber CSA (across all fiber types) and strength improvements in the squat exercise were evident (figure 3). A similar relationship between changes in contractile protein content and strength improvements in the squat was also detected (figure 4). Additionally, a strong relationship between changes in contractile protein content and muscle fiber hypertrophy (for all types) was observed (figure 5). The r values obtained suggest that a substantial portion (50-76%) 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 an adaptation (hypertrophy) and an improvement in functional strength. 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. Therefore, although differences between the groups in terms of changes in body composition were less evident, some statistically significant differences (and strong trends) were detected between the groups regarding muscle fiber hypertrophy and contractile protein accrual. Additionally, it was these alterations in skeletal muscle morphology that were largely responsible for the improvements in strength in an exercise involving a related muscle group. However, although these results suggest a cause-and effect-relationship between muscle hypertrophy and strength, no mechanistic assessments were attempted.

Paragraph 24: Willoughby & Rosene (30) completed one of very few studies that have linked an enhanced hypertrophy response from RE and supplementation (i.e., increase in strength, LBM and thigh volume) to alterations at the molecular level that may explain these benefits. In this study, supplementation with CrM ($6\text{g}^{-1}\text{day}$) during 12 weeks of RE resulted in a greater increase in LBM, muscle strength, and myofibrillar protein content to matched placebo-treated and control groups. These alterations corresponded with the up regulation of the genes and myogenic regulatory factors associated with (myosin heavy chain) contractile protein synthesis. A review of 22 studies involving supplementation during RE training clearly shows that CrM enhances weightlifting performance and the development of strength (24), and this is probably due to increased Cr availability during intense muscle contraction (14). More recently, Olsen et al., (20) 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 by inducing greater satellite cell number and myonuclei concentration alongside transcriptional changes in muscle gene expression which may contribute to, or be a product of, CrM's ability to enhance the bioenergetics of the phosphagen system. While these findings help to form a tempting mechanistic explanation for the greater hypertrophy responses observed in the Cr-treated groups in the present study, they do not explain the greater increases in strength and contractile protein accrual detected in the WP-supplemented group.

Paragraph 25: Although previous studies have shown that WP supplementation (1.2 to $1.5\text{g}^{-1}\text{kg}^{-1}\text{d}$) results in greater LBM and strength compared to matched CHO (6) and casein-treated groups (8), this study is the first to report changes in skeletal muscle morphology in response to RE-

training and WP supplementation. In this study, the WP group demonstrated greater improvements in 1RM strength (in all three tests) compared to the CHO-treated group (figures 1a, b, c). Based on the correlations observed, these strength improvements can be attributed mostly to skeletal muscle morphology. The protein used in this study (whey isolate) is regarded a rich source of EAA, particularly the branch chain amino acids (BCAA) (5). Supplementation with the BCAA during and after RE is shown to result in greater phosphorylation (activation) of p70^{S6k} in skeletal muscle; a rate limiting kinase in the signaling network controlling protein synthesis through translational initiation (15). More recently, supplementation with WP during RE has been shown to provide a similar effect in at least one of the signaling proteins that regulate protein synthesis through translational initiation (13). WP meals are shown to provide a high stimulation of protein synthesis and greater net postprandial protein gain compared to other high quality protein sources (9). Therefore, the frequent consumption of WP throughout the RE program in this study may have resulted in a greater anabolic response (i.e., higher rate of protein synthesis and net protein accretion) that resulted in greater synthesis of contractile protein. Although the findings with WP supplementation in this study are consistent with this theory, the mechanisms that may underline the benefits obtained from WP during RE are yet to be fully elucidated. The ability of the WP group to achieve similar strength gains without the large increase in LBM as seen in the CrCHO and CrWP groups in this study may have important sports-specific implications for individuals that compete in weight-restricted events. Therefore, further studies on the chronic effects of WP during RE are warranted, particularly at the molecular level.

Paragraph 26: Based on the mechanistic explanations that have been proposed, one may expect an additive effect from combining CrM and WP on muscle strength and hypertrophy. However, in this study, no greater effect was observed from this supplement combination compared to the

combination of CrM and CHO. One explanation for this may be the influence of the CHO (contained in CrCHO but not the CrWP supplement). For example, all groups consumed a high protein intake aside from supplementation and the results of at least one longitudinal study suggest that once dietary protein requirements appear to be met, it is the energy content of the diet that has the largest effect on hypertrophy during RE (25). In other words, when CrM is consumed in the presence of a high protein diet, the addition of CHO may be more beneficial than extra protein. However, the results also suggest that the consumption of CrM with WP provide similar benefits to that of CrM with CHO. This may have important implications for populations that desire improvements from exercise but the consumption of large amounts of glucose is undesirable, such as those with, or at risk of, type-II diabetes. As this is the only study that has compared the effects of two different CrM-containing supplements on skeletal muscle morphology during RE, the results obtained warrant further study.

Paragraph 27: Aside from the statistical evaluation of diet and the assessment of muscle hypertrophy at three levels, another strength of this investigation was the personalized training of the participants (one-to-one or one-to-two instruction of all participants during every workout). This level of supervision is shown to ensure better control of workout intensity and greater strength improvements during training (19). A personal training approach to RE supervision in RE training studies that involve supplementation is particularly important as it ensures a better chance of enhanced physiological adaptations from supplementation (28). This is based on the premise that those treated with supplements such as CrM and WP would be capable of training at a higher intensity level and progressing at a faster rate. It is important to remember that the instructors were blinded to the supplement groups, yet the WP, CrCHO and CrWP groups demonstrated significantly greater hypertrophy (in at least one of three assessments) and gains in

1RM strength (in three of three assessments) and thus, generally supports our theory. Training and dietary strategies that augment the adaptations desired from RE should continue to receive greater attention from within the scientific community as this research has important implications for an ageing population but also others that have a reduced capacity for exercise such as the frail elderly, cardiac rehabilitation patients or those living with cachectic conditions such as HIV, various forms of cancer.

Paragraph 28: In conclusion, this study examined the effects supplementation with CrCHO, CrWP, WP or CHO (1.5g/kg body wt/day) using four groups of matched, RE-trained males during 11 weeks of supervised RE training. Pre-post assessments demonstrated that supplementation with CrCHO, WP and CrWP resulted in significantly greater increases in 1RM strength (in three assessments) compared to supplementation with CHO. Up to 76% of the strength improvements in the squat could be attributed to hypertrophy of muscle involved in this exercise. However, the hypertrophy response from CrCHO, WP and CrWP varied at the three levels assessed (i.e., changes in lean mass, fiber-specific hypertrophy and contractile protein content). Therefore, although supplementation with WP and/or CrM appears to promote greater strength gains and muscle hypertrophy during RE training, the small number of participants within the groups that completed this investigation makes it difficult to draw firm conclusions with regard to the effects of the different supplement combinations used in this study, and thus warrants further investigation.

Acknowledgements

The lead investigator is a consultant to AST Sports Science. The results of the present study do not constitute endorsement of the product by the authors or ACSM.

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Captions

Figure 1a Bench Press (1RM) Strength

Training effect, *greater increase than CHO group ($P = 0.0001$, effect size = 0.585, power = 0.994) (mean \pm SE)

Figure 1b Pulldown (1RM) Strength

Training effect, *greater increase than CHO group ($P = 0.0001$, effect size = 0.585, power = 0.995) (mean \pm SE)

Figure 1c Squat (1RM) Strength

Training effect, *greater increase than CHO group ($P = 0.0001$, effect size = 0.592, power = 0.996) (mean \pm SE)

Figure 2a muscle fiber CSA type-I

Training effect *greater increase than CHO group, † greater increase than WP group ($P = 0.001$, effect size = 0.541, power = 0.983) (mean \pm SE)

Figure 2b Muscle fiber CSA type-IIa

Training effect *greater increase than CHO group ($P = 0.001$, effect size = 0.589, power = 0.995) (mean \pm SE)

Figure 2c Muscle fiber CSA type-IIx

Training effect *greater increase than CHO group ($P = 0.001$, effect size = 0.596, power = 0.996) (mean \pm SE)

Figure 2d Contractile protein (mg/g) muscle

Training effect *greater increase than CHO group ($P = 0.001$, effect size = 0.717, power = 1.00) (mean \pm SE)

Figure 3 Relationship between muscle fiber hypertrophy and 1RM strength improvements in the squat.

Figure 4. Relationship between change in contractile protein content and 1RM strength gains in the squat.

Figure 5. Relationship between contractile protein content and muscle fiber hypertrophy

Figure 1a

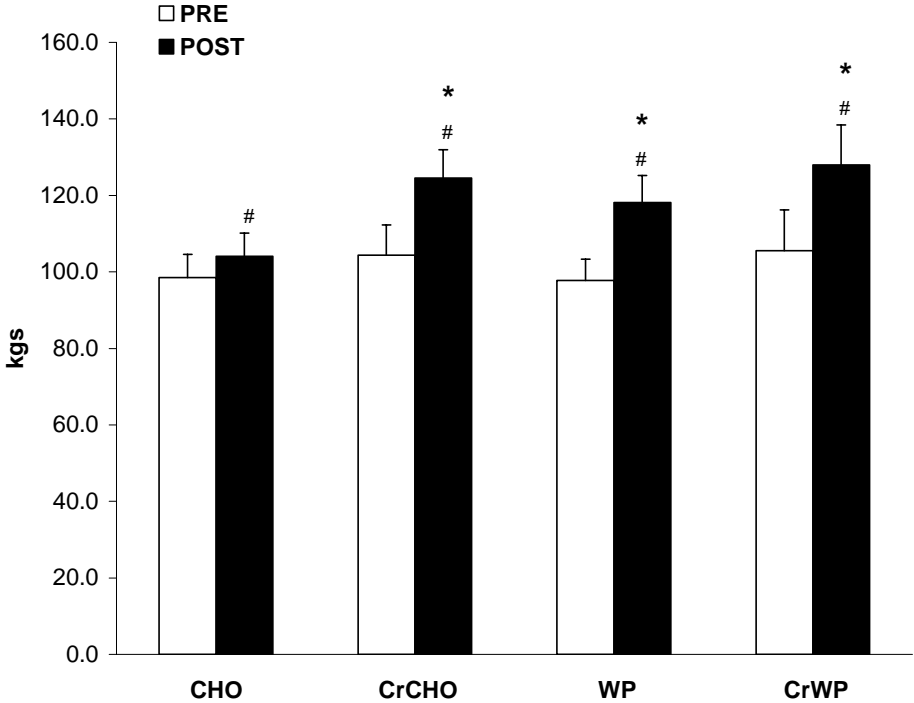


Figure 1b

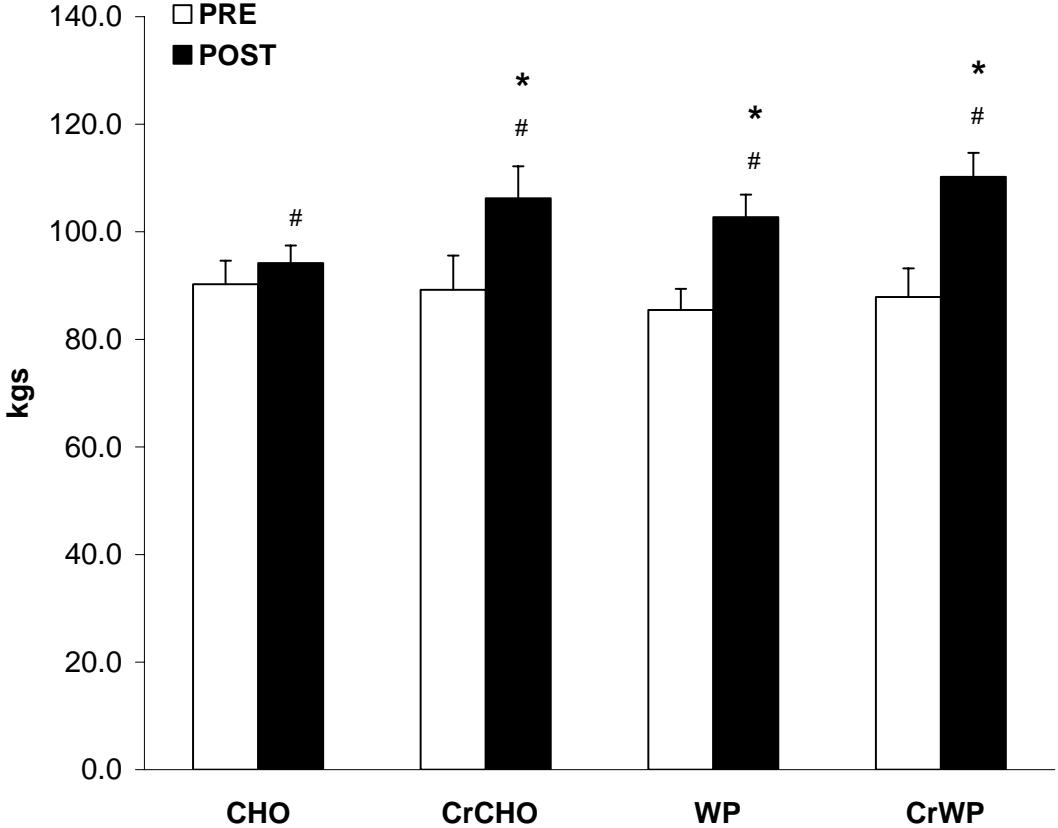


Figure 1c

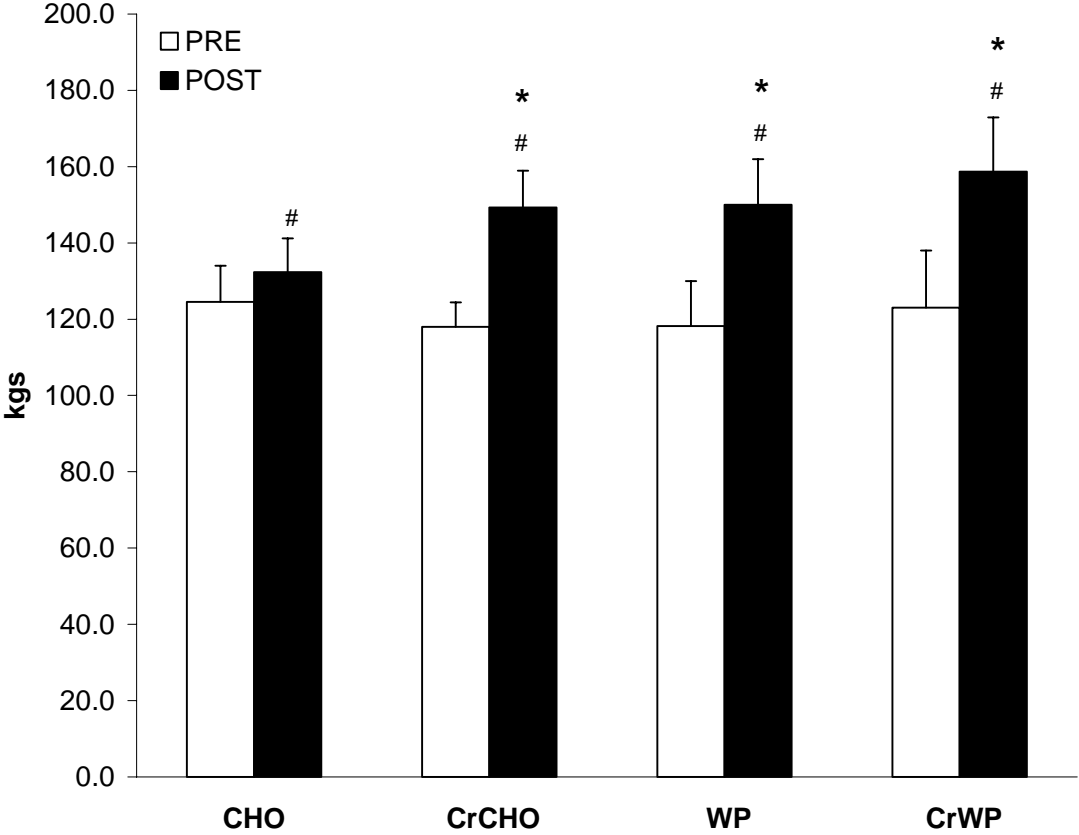


Figure 2a

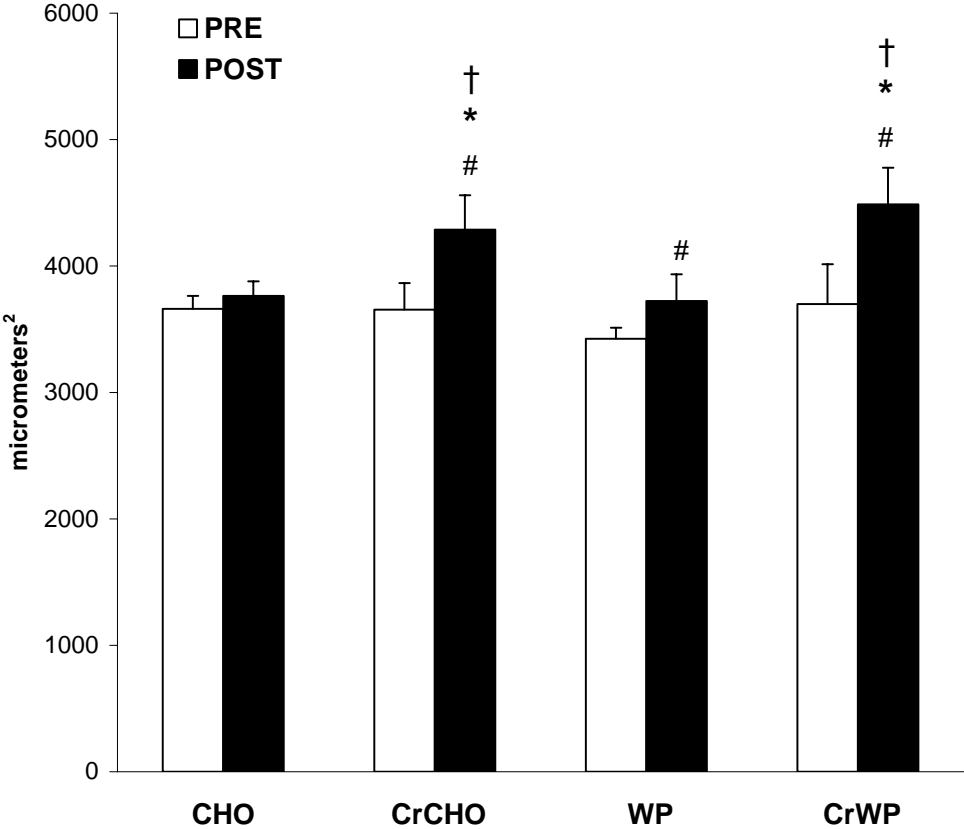


Figure 2b

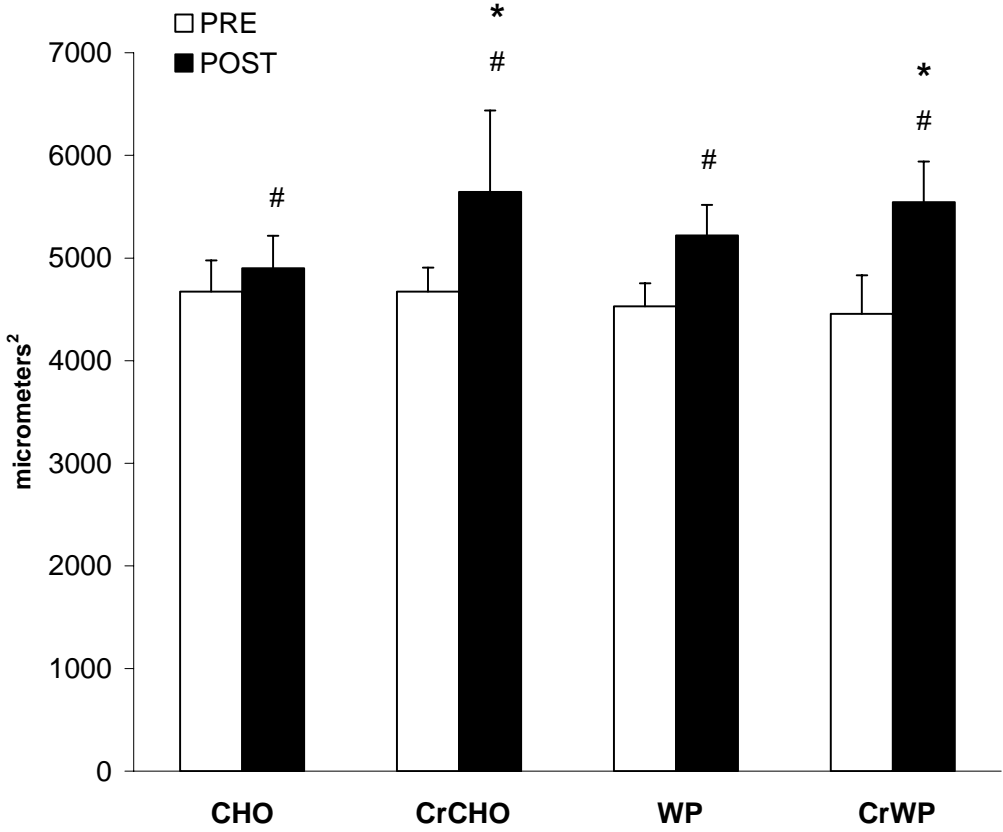


Figure 2c

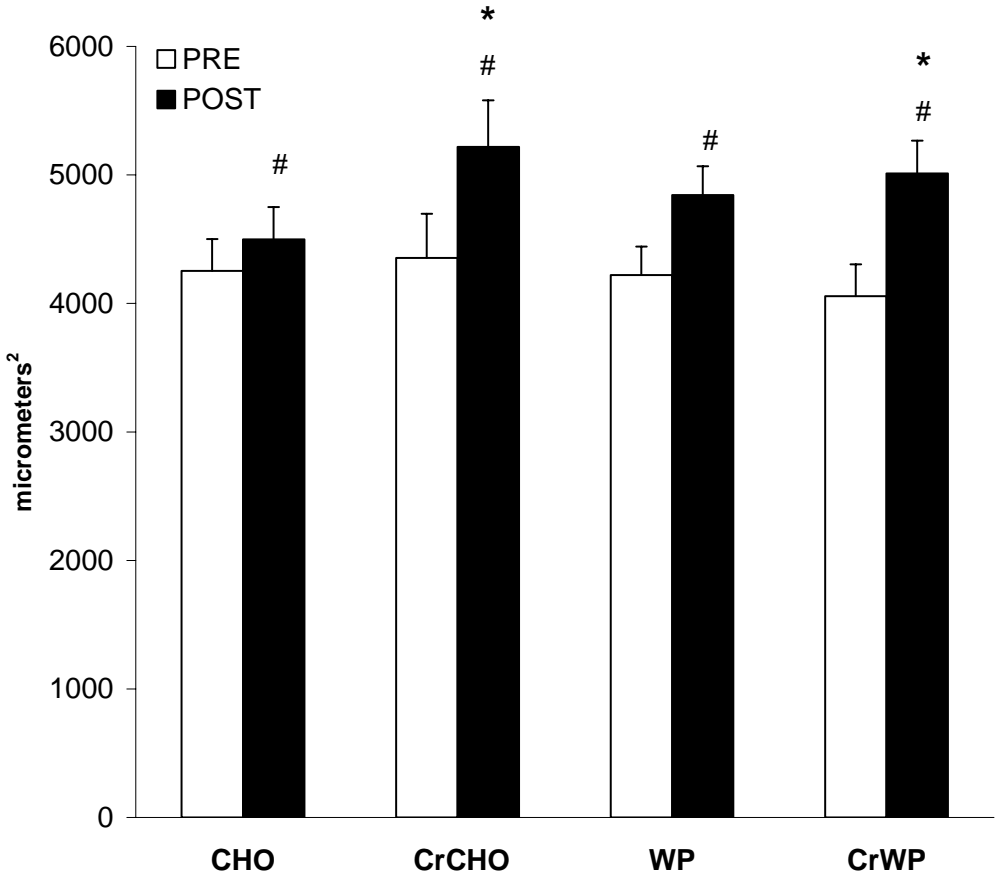


Figure 2d

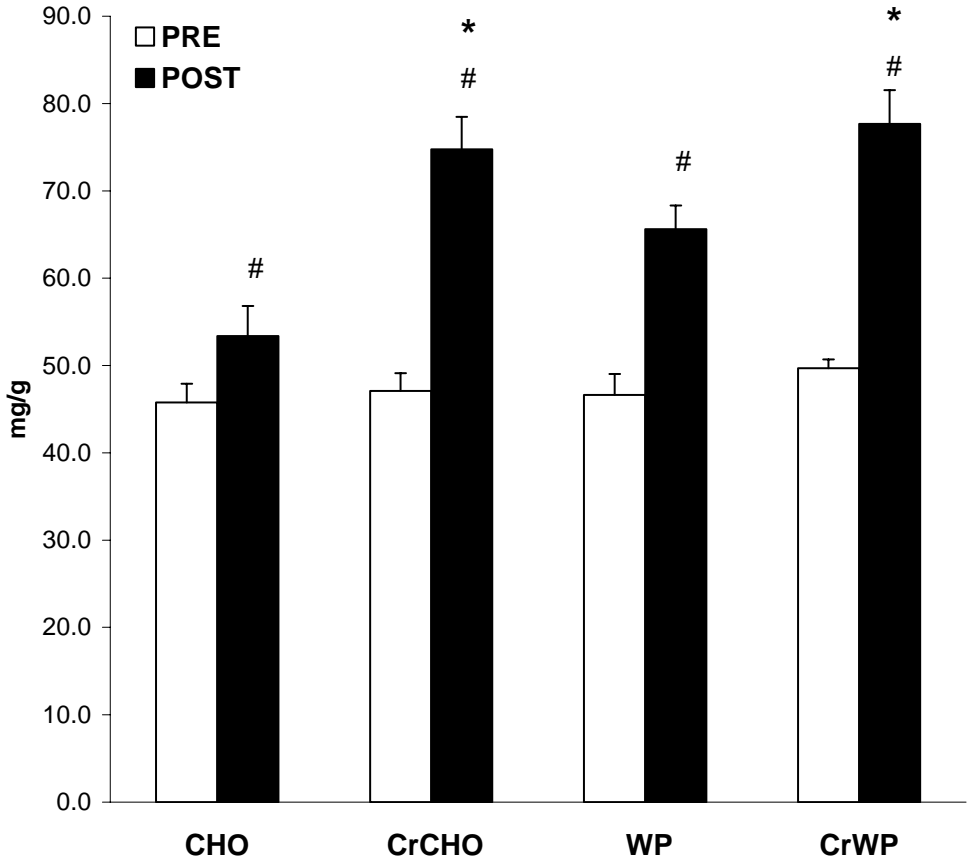


Figure 3

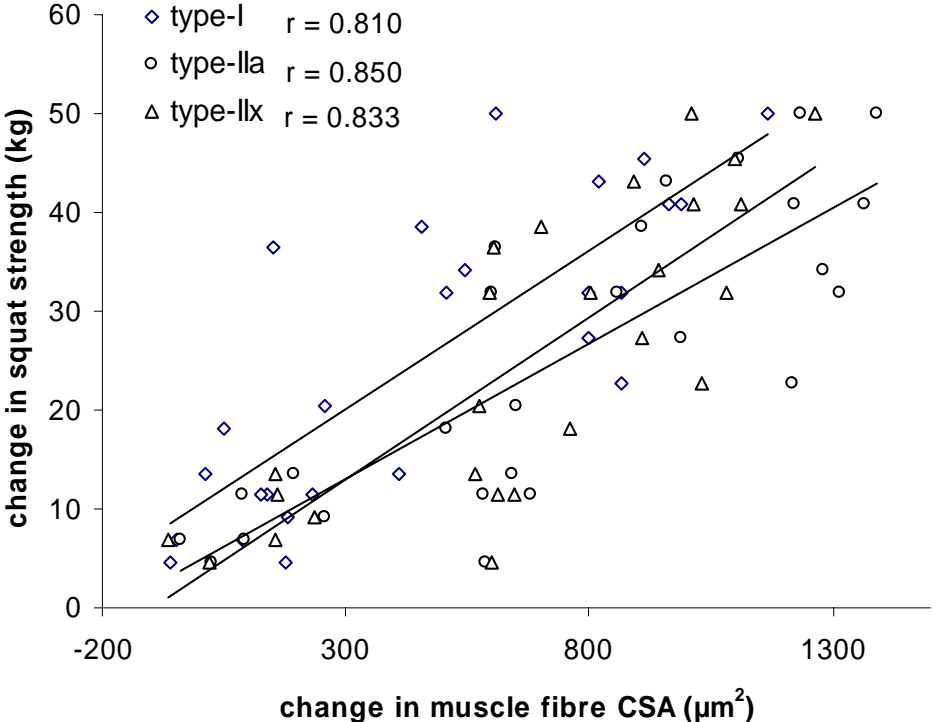


Figure 4

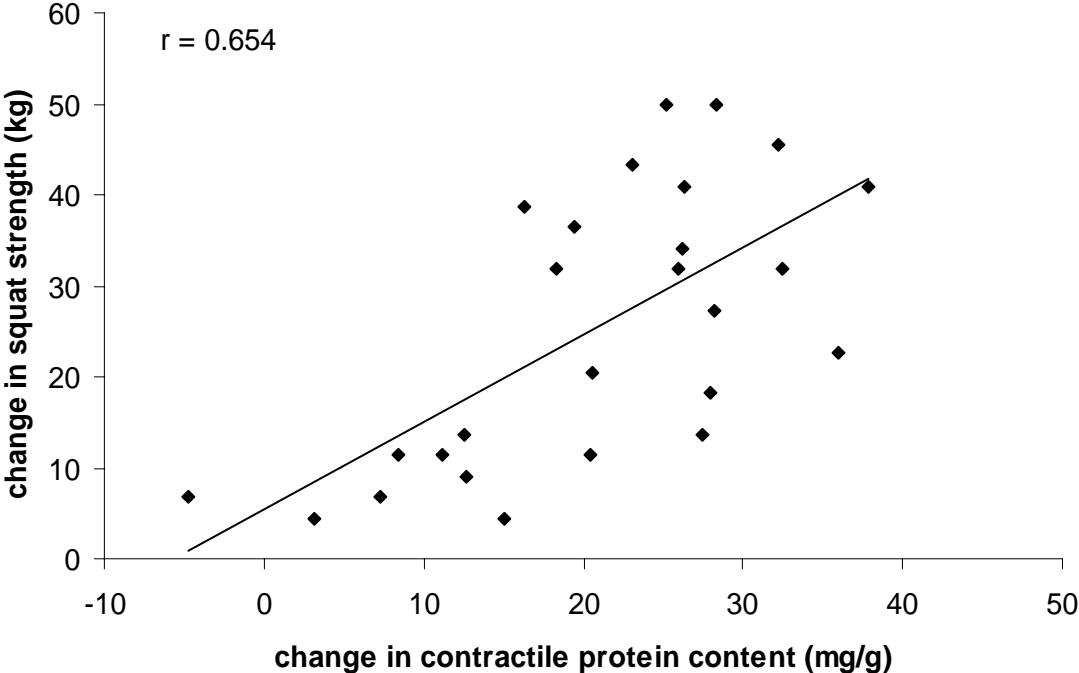


Figure 5

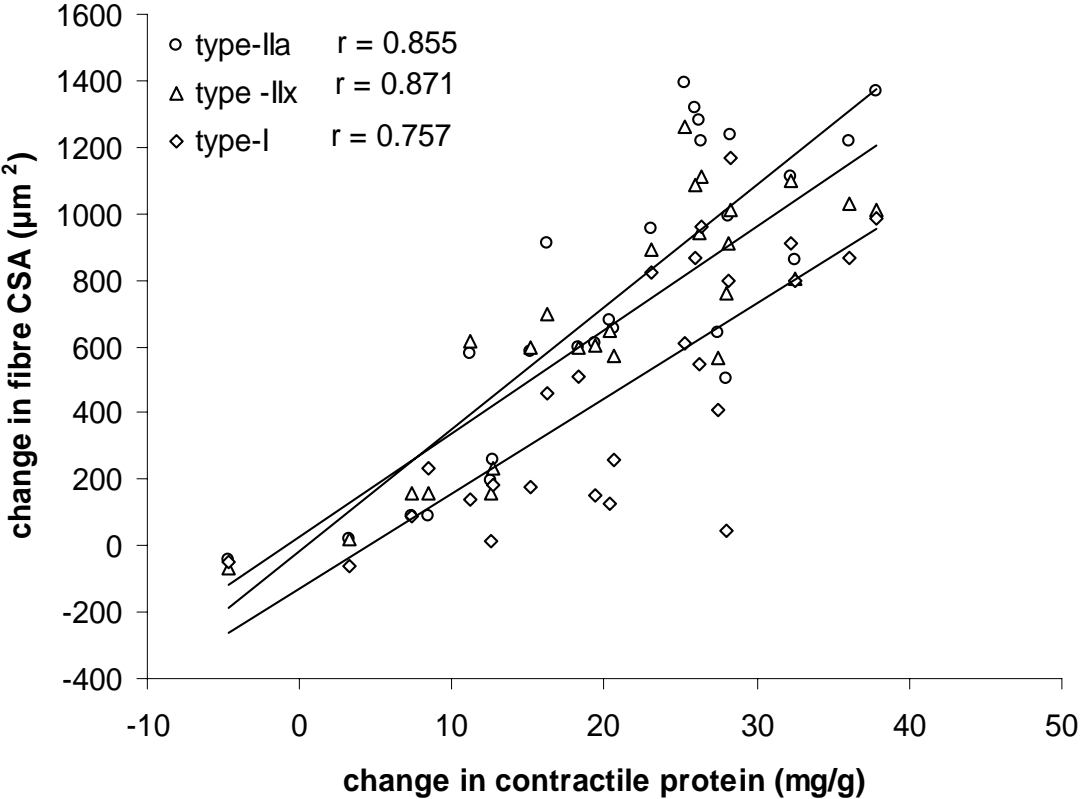


Table 1 Baseline characteristics

Characteristics	CHO	WP	CrCHO	CrWP
Age (yrs)	24 ± 7	24 ± 5	25 ± 6	25 ± 4
Training age (yrs)	6 ± 3	5 ± 2	6 ± 3	4 ± 2
Height (cm)	177 ± 5	181 ± 8	177 ± 6	190 ± 7
Body mass (kg)	76 ± 12	70 ± 11	84 ± 14	84 ± 12
Lean mass (kg)	62 ± 7	59 ± 7	67 ± 8	68 ± 6
Fat mass (kg)	13 ± 7	11 ± 4	17 ± 7	16 ± 6
CSA type-I (µm ²)	3662 ± 273	3423 ± 88	3656 ± 593	3699 ± 774
CSA type-IIa (µm ²)	4674 ± 803	4529 ± 223	4673 ± 661	4458 ± 919
CSA type-IIx (µm ²)	4253 ± 656	4220 ± 223	4354 ± 972	4057 ± 604
1RM Bench (kg)	99 ± 16	98 ± 13	104 ± 22	106 ± 26
1RM Squat (kg)	125 ± 25	118 ± 26	118 ± 18	123 ± 37
1RM Pulldown (kg)	90 ± 12	86 ± 11	89 ± 18	88 ± 13

Values are means ± SD.

Table 2 Dietary analyses

Variable	CHO	WP	CrCHO	CrWP
Energy intake (Kcal ⁻¹ kg ⁻¹ d)				
before	36.8 ± 7.2	41.6 ± 4.8	42.0 ± 6.1	40.8 ± 3.6
week 1	36.5 ± 5.3	40.5 ± 3.5	37.3 ± 3.8	39.9 ± 2.9
week 11	36.4 ± 5.9	39.1 ± 3.3	38.4 ± 4.1	39.9 ± 3
Carbohydrate (g ⁻¹ kg ⁻¹ day)				
before	2.9 ± 0.6	4.0 ± 0.6	4.4 ± 1.2	3.8 ± 1.4
week 1	2.8 ± 0.6	3.7 ± 0.4	3.7 ± 1.0	3.9 ± 1.4
week 11	2.7 ± 0.4	4.0 ± 1.2	3.7 ± 0.6	4.7 ± 1.9
Protein (g ⁻¹ kg ⁻¹ day)				
Before	1.6 ± 0.3	1.6 ± 0.2	1.5 ± 0.3	2.1 ± 1.0
week 1	1.7 ± 0.2	1.7 ± 0.2	1.5 ± 0.3	1.9 ± 0.8
week 11	1.6 ± 0.1	1.6 ± 0.1	1.5 ± 0.3	1.7 ± 0.7
Fat (g ⁻¹ kg ⁻¹ day)				
Before	2.1 ± 0.6	2.2 ± 0.4	2.0 ± 0.6	2.1 ± 1.0
week 1	2.1 ± 0.5	2.1 ± 0.4	1.9 ± 0.6	1.9 ± 0.8
week 11	2.1 ± 0.6	2.0 ± 0.3	2.0 ± 0.7	1.7 ± 0.7

values are mean ± SD

Table 3 Body Mass and Composition

Variable	CHO	WP	CrCHO	CrWP
Body mass (kg)				
PRE	75.6 ± 4.7	69.7 ± 5.0	84.2 ± 4.9	83.9 ± 4.8
POST [#]	77.0 ± 4.8	72.3 ± 4.3	88.2 ± 5.0	87.9 ± 5.0
Lean mass (kg)				
PRE	62.3 ± 2.8	59.0 ± 3.2	67.0 ± 2.6	67.9 ± 2.6
POST [#]	63.0 ± 2.7	61.3 ± 3.0	71.3 ± 3.0*	71.3 ± 2.8
Fat mass (kg)				
PRE	13.2 ± 2.8	10.6 ± 1.9	16.6 ± 2.6	15.9 ± 2.5
POST	14.0 ± 2.9	11.0 ± 1.6	17.0 ± 2.1	16.6 ± 2.6
Fat %				
PRE	16.9 ± 2.4	14.9 ± 1.7	19.1 ± 1.9	18.5 ± 1.9
POST	17.6 ± 2.5	15.0 ± 1.3	18.8 ± 1.3	18.5 ± 1.9

[#] Training effect all groups ($P = 0.001$); *greater increase than CHO group ($P = 0.043$, effect size = 0.297, power = 0.642) (mean ± SE).

Table 4 Muscle fibre type (%)

variable	CHO	WP	CrCHO	CrWP
%Type -1				
PRE	43 ± 5.9	49.9 ± 2.6	43.9 ± 2.5	41.4 ± 3.5
POST	41 ± 4.5	44.6 ± 4.3	46.7 ± 3.5	43.2 ± 3.2
%Type-IIa				
PRE	38.3 ± 5.3	30.0 ± 3.1	38.3 ± 3.3	36.9 ± 2.8
POST	39.0 ± 4.0	35.3 ± 4.0	36.7 ± 4.0	33.7 ± 2.5
%Type-IIx				
PRE	18.7 ± 2.8	18.0 ± 1.7	17.8 ± 1.8	21.6 ± 2.4
POST	20.2 ± 2.5	17.7 ± 2.7	16.5 ± 1.4	23.1 ± 1.4

(mean ± SE)

Table 5 Muscle creatine

Variable (mmol/kg dry wt)	CHO	WP	CrCHO	CrWP
Total Cr				
PRE	94.2 ± 10.1	107.1 ± 8.7	103.6 ± 8.3	109 ± 16.6
POST	95.3 ± 10.5	100.5 ± 9.5	113 ± 24.1*	125.3 ± 19.6*

*Greater than WP and CHO groups ($P = 0.03$, effect size = 0.340, power = 0.683) (mean ± SE)