International Journal of Sport Nutrition and Exercise Metabolism, 2008, 18, 389-398

Effect of Creatine Supplementation and Resistance-Exercise Training on Muscle Insulin-Like Growth Factor in Young Adults

Darren G. Burke, Darren G. Candow, Philip D. Chilibeck, Lauren G. MacNeil, Brian D. Roy, Mark A. Tarnopolsky, and Tim Ziegenfuss

The purpose of this study was to compare changes in muscle insulin-like growth factor-I (IGF-I) content resulting from resistance-exercise training (RET) and creatine supplementation (CR). Male ($n = 24$) and female ($n = 18$) participants with minimal resistance-exercise-training experience (≥1 year) who were participating in at least 30 min of structured physical activity (i.e., walking, jogging, cycling) 3–5 ×/wk volunteered for the study. Participants were randomly assigned in blocks (gender) to supplement with creatine (CR: 0.25 g/kg lean-tissue mass for 7 days; 0.06 g/kg lean-tissue mass for 49 days; $n = 22$, 12 males, 10 female) or isocaloric placebo (PL: $n = 20$, 12 male, 8 female) and engage in a whole-body RET program for 8 wk. Eighteen participants were classified as vegetarian (lacto-ovo or vegan; CR: 5 male, 5 female; PL: 3 male, 5 female). Muscle biopsies (vastus lateralis) were taken before and after the intervention and analyzed for IGF-I using standard immunohistochemical procedures. Stained muscle cross-sections were examined microscopically and IGF-I content quantified using image-analysis software. Results showed that RET increased intramuscular IGF-I content by 67%, with greater accumulation from CR (+78%) than PL (+54%; $p = .06$). There were no differences in IGF-I between vegetarians and nonvegetarians. These findings indicate that creatine supplementation during resistance-exercise training increases intramuscular IGF-I concentration in healthy men and women, independent of habitual dietary routine.

Keywords: peptide hormone, muscle biopsy, sport nutrition, vegetarians

---

Burke is with the Dept. of Human Kinetics, St. Francis Xavier University, Antigonish, NS, Canada. Candow is with the Faculty of Kinesiology and Health Studies, University of Regina, Regina, SK, Canada S4S 0A2. Chilibeck is with the College of Kinesiology, University of Saskatchewan, Saskatoon, SK, Canada S7N 5B2. MacNeil is with the Dept. of Kinesiology, and Tarnopolsky, the Dept. of Pediatrics and Medicine, McMaster University, Hamilton, ON, Canada. Roy is with the Dept. of Physical Education and Kinesiology, Brock University, St. Catharines, ON, Canada. Ziegenfuss is with the Ohio Research Group, Wadsworth Medical Center, Wadsworth, OH.

The combination of creatine supplementation and resistance training has been shown to increase lean-tissue mass (Brose, Parise, & Tarnopolsky, 2003; Burke et al., 2000, 2003; Chrusch, Chilibeck, Chad, Davison, & Burke, 2001) and muscle-fiber size (Burke et al., 2003, Volek et al., 1999). The underlying mechanisms explaining the increase in muscle mass from creatine supplementation remain to be determined; however, potential mechanisms include an increase in high-energy phosphate concentration (total creatine [TCr], phosphocreatine [PCr], and creatine [Cr]; Burke et al., 2003) and PCr resynthesis after exercise (Greenhaff, Bodin, Soderland, & Hultman, 1994), cellular hydration status (Hultman, Soderland, Timmons, Cederblad, & Greenhaff, 1996), satellite-cell activity (Dangott, Schultz, & Mozdziak, 2000; Olsen et al., 2006; Vierck, Icenoggle, Bucci, & Dodson, 2003), and myofibrillar protein kinetics (Willoughby & Rosene, 2003; Parise, Mihic, MacLennan, Yarasheski, & Tarnopolsky, 2001). Theoretically, creatine supplementation might enhance the metabolic adaptations from regular resistance-exercise-training sessions, leading to greater production of insulin-like growth factor-I (IGF-I) over time (Deldicque et al., 2005). This might help explain the increase in lean-tissue mass found in many creatine and resistance-exercise-training studies (Brose et al.; Burke et al., 2000, 2003; Chrusch et al.).

Most IGF-I production occurs in the liver in response to changes in growth-hormone concentrations and acts as an endocrine hormone, regulating tissue-specific growth and differentiation (Czerwinski, Martin, & Bechtel, 1994; Hameed, Harridge, & Goldspink, 2002). The IGF-I produced in skeletal muscle through the process of overload is an isoform of systemic IGF-I (Hameed et al.; MacGregor & Parkhouse, 1996) and controls local tissue repair and remodeling (Goldspink, 1999). Borst et al. (2001) demonstrated that resistance-exercise training resulted in a 20% increase in blood IGF-I after 13 and 25 weeks of training in young men and women, and Singh et al. (1999) reported a 500% increase in muscle stained for IGF-I in older participants after 10 weeks of resistance-exercise training. In two recent reports it was found that creatine supplementation, independent of exercise, augmented IGF-I mRNA in cultured myotubes (Louis, Van Beneden, Dehoux, Thissen, & Francaux, 2004) and in human skeletal muscle (Deldicque et al., 2005), possibly by enhancing the anabolic status of the cell involving IGF. There have been several suggestions for the possible link between muscle IGF-I activation and muscle overloading, including activation of the PI3K-Akt/PKB-mTOR-signaling pathways (Deldicque et al.) and stretch tension on the basement membrane (Goldspink) causing damage to sarcolemma and myofibrillar proteins (Bamman et al., 2001).

Creatine supplementation results in an increase in intramuscular creatine concentrations (Green, Hultman, Macdonald, Sewell, & Greenhaff, 1996; Harris, Soderland, & Hultman, 1992). Large interindividual differences, however, in baseline resting creatine concentrations and responsiveness to creatine supplementation are evident (Casey & Greenhaff, 2000; Vandenberghe et al., 1997). Participants with initially low resting creatine concentrations (i.e., vegetarians) experience the greatest increase from creatine supplementation (Casey, Constantin-Teodosiu, Howell, Hultman, & Greenhaff, 1996; Greenhaff et al., 1994; Harris et al., 1992), leading to exercise improvements (Shomrat, Weinstein, & Katz, 2000). We have previously shown that creatine supplementation during 8 weeks of whole-body resistance-exercise training increased TCr, PCr, Type II fiber area of the vastus

lateralis, bench-press strength, and isokinetic knee-flexion and -extension work over placebo (Burke et al., 2003). Vegetarians who supplemented with creatine experienced a greater increase in TCr and PCr concentration and total isokinetic work performance over nonvegetarians (Burke et al., 2003), possibly because of lower initial resting creatine concentrations leading to accelerated intramuscular creatine uptake from exogenous supplementation.

The purpose of this study was to determine the effects of creatine supplementation (8 weeks) combined with heavy resistance-exercise training (>70% 1-RM) on muscle IGF-I concentration in vegetarian and nonvegetarian participants as previously described (Burke et al., 2003). Based on our previous findings of greater adaptations from creatine supplementation, we hypothesized that creatine supplementation during resistance training would increase IGF-I over placebo, and vegetarians on creatine would experience greater gains than nonvegetarians.

Methods

Participants

Male (n = 24) and female (n = 18) participants with minimal resistance-training experience (≥1year) who were participating in at least 30 min of structured physical activity (i.e., walking, jogging, cycling) 3–5 times a week volunteered for the study. Eighteen participants were classified as vegetarian (lacto-ovo or vegan). Participants were self-described as vegetarian, whether they were lacto-ovo or vegan, and had to have been vegetarian for a minimum of 3 years. Participant exclusion criteria included a history of creatine supplementation for 6 weeks before the start of the study or any disease or medical condition that would have prevented participation in resistance training. Participants were randomly assigned (double-blind) to receive creatine or placebo in stratified blocks based on gender. All participants completed a Physical Activity Readiness Questionnaire (PAR-Q), which screens for health problems that might present a risk with physical activity. Participants who indicated a health problem were required to have medical approval before participating in the study. The study was approved by the University of Saskatchewan ethics review board for research in human participants. The participants were informed of the risks and purposes of the study before their written consent was obtained. Participant characteristics are presented in Table 1.

Supplementation

Participants were randomized (double-blind) to supplement with creatine (loading phase: 0.25 g · kg lean-tissue mass^{-1} · day^{-1} for 7 days; maintenance phase:

Table 1 Characteristics of Participants Taking Either Creatine or Placebo, *M* ± *SE*

Group	*n*, M/F	Age	Height (cm)	Weight (kg)	% Fat
Creatine	12/10	31 ± 2.6	170.3 ± 2.9	68.6 ± 4.0	20.5 ± 2.6
Placebo	12/8	37 ± 6.8	170.2 ± 2.9	69.3 ± 4.3	22.0 ± 2.6

0.06 g · kg lean-tissue mass^{-1} · day^{-1} for an additional 49 days; n = 22; 12 male [5 vegetarians], 10 female [5 vegetarians]) or placebo (maltodextrin; n = 20; 12 male [3 vegetarians], 8 female [5 vegetarians]) during 8 weeks of resistance-exercise training. The creatine loading was divided into four equal servings (~0.06 g · kg lean-tissue mass^{-1} · day^{-1}) consumed in the morning, in the afternoon or before the resistance-exercise-training session, in the evening or after the resistance-exercise training session, and before going to bed. The creatine maintenance dose of 0.06 g/kg was chosen because it has been shown to be effective for increasing muscle mass and strength (Chrusch et al., 2001). Participants were instructed to supplement with creatine immediately after each resistance-exercise-training session because creatine supplementation postexercise leads to significant muscle hypertrophy (Chilibeck, Stride, Farthing, & Burke, 2004). On nontraining days, participants were instructed to consume creatine (or placebo) in the morning or before going to bed. The average absolute daily doses of creatine for participants during loading and maintenance were 16.8 ± 0.7 and 4.2 ± 0.2 g/day, respectively. Participants mixed each supplement with ~300 ml of a fruit-flavored drink. The creatine and placebo supplements were identical in taste, texture, and appearance. Supplementation compliance was indirectly monitored by verbal communication and having participants return empty supplement bags when picking up additional supplements.

Muscle Biopsy, Histochemical Staining, and Image Analysis

Percutaneous needle biopsies were obtained from the distal third of the vastus lateralis muscle using a 5-mm Stille needle (Micrins, New York, NY) under local anesthetic with 1% lidocaine (Smith-Kline Beecham, Toronto, ON) and with suction applied via a 60-cc syringe. Participant muscle biopsies were performed 24 hr before the first training session. Target biopsy time after their last exercise session was 24 hr, with biopsies actually occurring 18–30 hr postexercise.

Preparation of staining started with fixation of the frozen section with 100% acetone at 4 °C for 10 min. The tissue was then washed in a bath with 10 mM of phosphate-buffered saline, pH 7.5, for 10 min. One hundred microliters of primary antibody (IGF-I: H-70, Santa Cruz Biotechnology, CA) was applied to each section and incubated for 30 min. The section was then washed with 10 mM of phosphate-buffer saline, pH 7.5. Then, 100 µL of biotinylated secondary antibody (Rabbit ImmunoCruz Staining System, Santa Cruz Biotechnology) was applied and incubated for 10 min, then removed and washed well with 10 mM of phosphate-buffer saline, pH 7.5. One hundred microliters of HRP-streptavidin conjugate was then added and incubated for 10 min, which was followed by the addition of concentrated DAB chromogenic substrate and an incubation of 5 min. Then, 100 µl of hematoxylin was applied and left to sit for 2 min, which was followed by dehydration with alcohol and mounting. Six to eight samples were done at a time and always included pretraining and posttraining samples for each participant. After immunoperoxidase staining, sections were mounted, and the area positively stained was analyzed using Scion Image Version Beta 4.0.2 software (Scion Corp., Frederick, MD). First, each slide was viewed under 100× magnification (Olympus BX60, Tokyo, Japan). Then, three or four pictures were taken per slide (Spot Diagnostic Instruments Inc., Sterling Heights, MI) and immediately saved

as JPEG files on a Dell Dimension XPS R450 (Dell Computer Co., Austin, TX). Approximately 100–150 muscle fibers were used to determine the area positively stained for IGF-I content (Figure 2).

Exercise Program

All participants followed the same high-volume, heavy-load (>70% 1RM) resistance-exercise-training program for 8 weeks. The program was a 4-day split routine involving whole-body musculature that was previously found to increase lean-tissue mass and strength (Burke et al., 2003; Candow, Chilibeck, Burke, Davison, & Smith-Palmer, 2001). Briefly, chest and triceps muscles were trained on Day 1 with the following exercises in order: flat bench press, incline bench press, flat dumbbell flies, incline dumbbell flies, cable triceps extensions, rope reverse triceps extensions, and French curls. On Day 2, participants trained back and biceps muscles: chin-ups, low row, lat-pull downs, alternate dumbbell row, standing EZ-curls, preacher curls, and alternate dumbbell curls. Day 3 was for legs, shoulder, and abdominal muscles and included the following exercises in order: vertical leg press, leg extension, hamstring curl, standing calf raises, seated dumbbell press, upright rows, shrugs, lateral raises, and abdominal crunches. Day 4 was a day of rest. These 4 days were considered one cycle, and the cycle was repeated continuously throughout the duration of the study. Participants performed seven cycles of 3–5 sets of 4–12 repetitions to muscle failure for each set. During Cycles 1 and 7, participants performed three sets of 10–12 repetitions, with 1-min rests between sets. For Cycles 2 and 6, participants performed three sets of 8–10 repetitions, with 1.5-min rests between sets. During Cycles 3 and 5, participants performed four sets of 6–8 repetitions, with 2-min rests between sets. For Cycle 4, participants performed five sets of 4–6 repetitions, with 3-min rests between sets. Training logs detailing the weight used and number of sets and repetitions performed for each exercise were completed for every workout. Training volume was calculated (kg × reps) for the entire resistance-exercise-training program.

Diet

Dietary intake was recorded before and after the study to assess whether there were differences in total energy and macronutrient composition between creatine and placebo. Participants were given instruction about proper portion sizes and how to accurately record all food or beverages consumed. They used a 3-day food booklet to record what they ate for 2 weekdays and 1 weekend day. Fuel Nutrition software 2.1a (LogiForm International Inc., Saint-Foy, Quebec) was used to analyze the food records for total calories and the amount of energy from carbohydrate, fat, and protein.

Statistical Analysis

A 2 (creatine vs. placebo) × 2 (vegetarian vs. nonvegetarian) × 2 (pre vs. post) ANOVA with repeated measures on the third factor was used to determine differences between the creatine and placebo groups and vegetarians and nonvegetarians

over time. Tukey's post hoc tests were used to determine differences between group means. All results are expressed as $M \pm SE$. Statistical analyses were carried out using SPSS version 10.02 for Microsoft Windows. Statistical significance was set at $p < .05$.

Results

There were no differences in total training volume between creatine and placebo over the 8 weeks of training. Creatine supplementation, however, resulted in greater training volumes at Weeks 2 and 7 ($p < .05$). Dietary analyses indicated that vegetarians consumed fewer total calories (vegetarian: pre 2,159 ± 71 kcal, post 2,213 ± 78 kcal; nonvegetarian: pre 2,638 ± 67 kcal, post 2,629 ± 61 kcal; $p < .05$) and protein (vegetarian: pre 78 ± 2 g/day, post 80 ± 2 g/day; nonvegetarian: 139 ± 2 g/day, post 138 ± 3 g/day; $p < .05$) over time, with no other differences.

At baseline the mean muscle-fiber area positively stained for IGF-I content was 4.42% (range 1.37–12.10%), and there were no significant differences between groups at baseline (CR 4.44%, PL 4.38%). The resistance-exercise-training program resulted in a significant increase of 67% in IGF-I, however, and the participants who supplemented with creatine experienced an increase of 78% in IGF-I, compared with a 55% increase exhibited by the participants who were on placebo ($p = .06$; Figure 1).

As previously reported (Burke et al., 2003), there were no significant differences between groups for body weight or lean-tissue mass at baseline. Participants supplementing with creatine, however, experienced a greater increase in body mass and lean-tissue mass than those on placebo (body mass: CR 2.2 kg or 3.2%, PL 0.6 kg or 0.9%; lean-tissue mass: CR 2.5 kg or 6%, PL 1.9 kg or 2%; $p < .05$). Vegetarians on creatine experienced an increase of 2.4 kg in lean-tissue mass, compared with an increase of 1.9 kg for nonvegetarians on creatine ($p = .06$). Vegetarians supplementing with creatine experienced a greater increase in

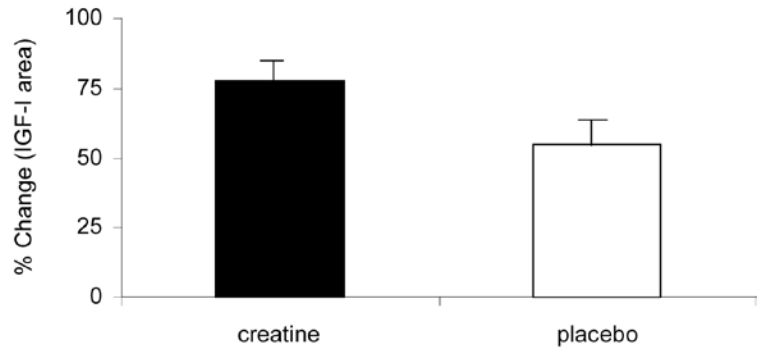

Figure 1 — Change in area positively stained for insulin-like growth factor-I (IGF-I) from before to after training and supplementation, $M \pm SE$ ($p = .06$). Area is expressed in μm^2

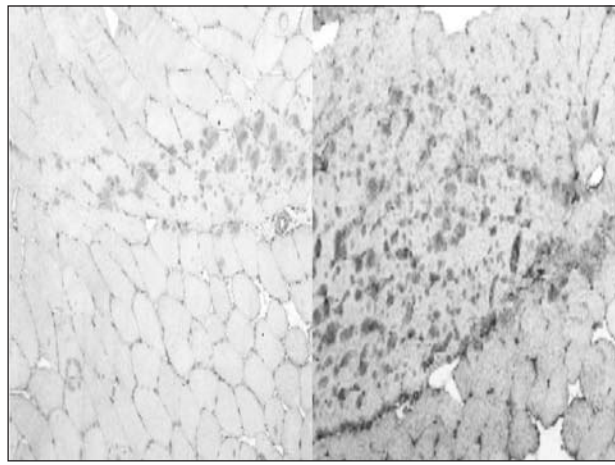

Figure 2 — An image of one participant's muscle cross-section stained for insulin-like growth factor-I presupplementation (left) and after creatine supplementation (right).

high-energy phosphate content than nonvegetarians on creatine (TCr: vegetarians 25%, nonvegetarians 7%; PCr: vegetarians 37%, nonvegetarians 11%; $p < .05$). There were no changes in TCr, PCr, or free Cr for placebo participants. Creatine supplementation increased Type II fiber area of the vastus lateralis by 28% ($p < .05$), compared with a 5% increase for placebo. The change in lean-tissue mass was significantly correlated to the change in intramuscular TCr content ($r = .61$, $p < .05$), and the change in intramuscular IGF-I content was significantly correlated to the change in intramuscular TCr content ($r = .82$, $p < .05$; Figure 2).

Discussion

The primary purpose of this study was to determine the effects of creatine supplementation and resistance-exercise training on muscle IGF-I in young adults. Results showed that muscle IGF-I content was significantly increased after high-intensity resistance-exercise training, with greater gains observed from creatine supplementation than from placebo. IGF-I has been shown to increase muscle protein synthesis and satellite-cell activity (Allen & Boxhorn, 1989) and stimulate the PI3K-Akt/PKB-mTOR-signaling pathway involved in muscle hypertrophy (Deldicque et al., 2005). In the current study, participants supplementing with creatine experienced a greater increase in IGF-I than those on placebo (CR 78%, PL 55%; $p = .06$). These results support the findings of Deldicque et al., who observed a 30% increase in IGF-I mRNA expression at rest after 5 days of creatine supplementation in young adults. Our results further suggest, however, that regular resistance-exercise-training sessions for 8 weeks increase muscle IGF-I in adult humans, with greater gains observed from creatine supplementation. Although the mechanism explaining the

increase in IGF-I from creatine remains to be elucidated, the most plausible theory involves high-energy phosphate metabolism and training intensity. As we have previously shown, creatine supplementation increased both PCr and TCr content to a greater extent than placebo (Burke et al., 2003). The increase in high-energy phosphate metabolism might have allowed resistance training to be performed with greater intensity as was observed in Weeks 2 and 7 of our resistance-exercise-training program. The higher metabolic demand from more-intense resistance-exercise-training sessions might explain the greater increase in muscle IGF-I content from creatine supplementation found in the current study.

It is unclear why vegetarians did not experience a greater increase in IGF-I than nonvegetarians. It has been shown that habitual dietary intake of reduced energy and protein might reduce serum IGF-I in humans (Thissen, Ketelslegers, & Underwood, 1994). In particular, a diet low in essential amino acids reduces IGF- I production (Harp, Goldstein, & Phillips, 1991), suggesting that essential amino acids are necessary to maximize IGF-I production. For the current study, vegetarians consumed approximately 2,200 kcal and 79 g of protein per day, compared with 2,650 kcal and 139 g of protein per day for nonvegetarians. Although we cannot differentiate between essential and nonessential amino acids in our dietary analyses, vegetarian diets tend to be low in one or more essential amino acids that have been shown to blunt IGF-I production (Clemmons, Seek, & Underwood, 1985) and might have contributed to our lack of significant findings.

As previously reported, creatine supplementation resulted in greater increases in lean-tissue mass and Type II fiber area than placebo (Burke et al., 2003). It is difficult to determine whether the greater increase in lean-tissue mass and fiber area with creatine was caused by greater muscle protein accretion. There was a trend ($p = .06$) for a greater increase in muscle IGF-I content with creatine supplementation than with placebo, suggesting a greater muscle protein synthetic response from creatine and exercise. The greater intramuscular IGF-I content (78%) from creatine supplementation than with placebo (55%) might help explain the differences in muscle mass and exercise performance as previously reported (Burke et al., 2003). The addition of creatine and subsequent increase in TCr and PCr might have directly or indirectly stimulated production of muscle IGF-I concentration and muscle protein synthesis, leading to muscle hypertrophy.

In summary, a structured resistance-exercise-training program increases IGF-I content in men and women. The addition of creatine further augments the physiological adaptations from resistance training, with no differences between vegetarians and nonvegetarians. Future research should determine the mechanisms explaining hormonal changes resulting from creatine supplementation alone and in combination with resistance-exercise training.

Acknowledgments

This study was funded by a grant from Iovate Health Research and Development and the University Council for Research, St Francis Xavier University, Canada.

References

Allen, R.E., & Boxhorn, L.K. (1989). Regulation of skeletal muscle satellite cell proliferation and differentiation by transforming growth factor-beta, insulin-like growth factor I, and fibroblast growth factor. *Journal of Cellular Physiology, 138,* 311–315.

Bamman, M.M., Shipp, J.R., Jiang, J., Gower, B.A., Hunter, G.R., Goodman, A., et al. (2001). Mechanical load increases muscle IGF-1 and androgen receptor mRNA concentrations in humans. *American Journal of Physiology. Endocrinology and Metabolism, 280,* E383–E390.

Borst, S.E., De Hoyos, D.V., Garzarella, L., Vincent, K., Pollock, B.H., Lowenthal, D.T., et al. (2001). Effects of resistance training on insulin-like growth factor-I and IGF binding proteins. *Medicine and Science in Sports and Exercise, 33,* 648–653.

Brose, A., Parise, G., & Tarnopolsky, M.A. (2003). Creatine supplementation enhances isometric strength and body composition improvements following strength exercise training in older adults. *Journals of Gerontology. Series A, Biological Sciences and Medical Sciences, 58,* 11–19.

Burke, D.G., Chilibeck, P.D., Parise, G., Candow, D.G., Mahoney, D., & Tarnopolsky, M.A. (2003). Effect of creatine and weight training on muscle creatine and performance in vegetarians. *Medicine and Science in Sports and Exercise, 35,* 1946–1955.

Burke, D.G., Silver, S., Holt, L., Smith-Palmer, T., Culligan, C., & Chilibeck, P.D. (2000). The effect of continuous low dose creatine supplementation on force, power, and total work. *International Journal of Sport Nutrition and Exercise Metabolism, 10,* 235–244.

Candow, D.G., Chilibeck, P.D., Burke, D.G., Davison, K.S., & Smith-Palmer, T. (2001). Effect of glutamine supplementation combined with resistance training in young adults. *European Journal of Applied Physiology, 86,* 142–149.

Casey, A., Constantin-Teodosiu, D., Howell, S., Hultman, E., & Greenhaff, P.L. (1996). Creatine ingestion favorably affects performance and muscle metabolism during maximal exercise in humans. *The American Journal of Physiology, 271,* E31–E37.

Casey, A., & Greenhaff, P.L. (2000). Does dietary creatine supplementation play a role in skeletal muscle metabolism and performance? *The American Journal of Clinical Nutrition, 72,* 607S–617S.

Chilibeck, P.D., Stride, D., Farthing, J.P., & Burke, D.G. (2004). Effect of creatine ingestion after exercise on muscle thickness in males and females. *Medicine and Science in Sports and Exercise, 36,* 1781–1788.

Chrusch, M.J., Chilibeck, P.D., Chad, K.E., Davison, K.S., & Burke, D.G. (2001). Creatine supplementation combined with resistance training in older men. *Medicine and Science in Sports and Exercise, 33,* 2111–2117.

Clemmons, D.R., Seek, M.M., & Underwood, L.E. (1985). Supplemental essential amino acids augment the somatomedin-C/insulin-like growth factor I response to refeeding after fasting. *Metabolism: Clinical and Experimental, 34,* 391–395.

Czerwinski, S.M., Martin, J.M., & Bechtel, P.J. (1994). Modulation of IGF mRNA abundance during stretch-induced skeletal muscle hypertrophy and regression. *Journal of Applied Physiology, 76,* 2026–2030.

Dangott, B., Schultz, E., & Mozdziak, P.E. (2000). Dietary creatine monohydrate supplementation increases satellite cell mitotic activity during compensatory hypertrophy. *International Journal of Sports Medicine, 21,* 13–16.

Deldicque, L., Louis, M., Theisen, D., Nielens, H., Dehoux, M., Thissen, J.P., et al. (2005). Increased IGF mRNA in human skeletal muscle after creatine supplementation. *Medicine and Science in Sports and Exercise, 37,* 731–736.

Goldspink, G. (1999). Changes in muscle mass and phenotype and the expression of autocrine and systemic growth factors by muscle in response to stretch and overload. *Journal of Anatomy, 194,* 323–334.

Green, A.L., Hultman, E., Macdonald, I.A., Sewell, D.A., & Greenhaff, P.L. (1996). Carbohydrate ingestion augments skeletal muscle creatine accumulation during creatine supplementation in humans. *The American Journal of Physiology, 271,* E821–E826.

Greenhaff, P.L., Bodin, K., Soderlund, K., & Hultman, E. (1994). Effect of oral creatine supplementation on skeletal muscle phosphocreatine resynthesis. *American Journal of Physiology. Endocrinology and Metabolism, 266,* E725–E730.

Hameed, M., Harridge, S.D., & Goldspink, G. (2002). Sarcopenia and hypertrophy: A role for insulin-like growth factor-1 in aged muscle? *Exercise and Sport Sciences Reviews, 30,* 15–19.

Harp, J.B., Goldstein, S., & Phillips, L.S. (1991). Nutrition and somatomedin. Molecular regulation of IGF-I by amino acid availability in cultured hepatocytes. *Diabetes, 40,* 95–101.

Harris, R.C., Soderlund, K., & Hultman, E. (1992). Elevation of creatine in resting and exercised muscle of normal subjects by creatine supplementation. *Clinical Science (London, England : 1979), 83,* 367–374.

Hultman, E., Soderlund, K., Timmons, J., Cederblad, G., & Greenhaff, P.L. (1996). Muscle creatine loading in man. *Journal of Applied Physiology, 81,* 232–237.

Louis, M., Van Beneden, R., Dehoux, M., Thissen, J.P., & Francaux, M. (2004). Creatine increases IGF-I and myogenic regulatory factor mRNA in C2C12 cells. *FEBS Letters, 557,* 243–247.

MacGregor, J., & Parkhouse, W.S. (1996). The potential role of insulin-like growth factors in skeletal muscle regeneration. *Canadian Journal of Applied Physiology, 21,* 236–250.

Olsen, S., Aagaard, P., Kadi, F., Tufekovic, G., Verney, J., Olsen, J.L., et al. (2006). Creatine supplementation augments the increase in satellite cell and myonuclei number in human skeletal muscle induced by strength training. *The Journal of Physiology, 573,* 525–534.

Parise, G., Mihic, S., MacLennan, D., Yarasheski, K.E., & Tarnopolsky, M.A. (2001). Effects of acute creatine monohydrate supplementation on leucine kinetics and mixed-muscle protein synthesis. *Journal of Applied Physiology, 91,* 1041–1047.

Shomrat, A., Weinstein, Y., & Katz, A. (2000). Effect of creatine feeding on maximal exercise performance in vegetarians. *European Journal of Applied Physiology, 82,* 321–325.

Singh, M.A., Ding, W., Manfredi, T.J., Solares, G.S., O'Neill, E.F., Clements, K.M., et al. (1999). Insulin-like growth factor I in skeletal muscle after weight-lifting exercise in frail elders. *The American Journal of Physiology, 277,* E135–E143.

Thissen, J.P., Ketelslegers, J.M., & Underwood, L.E. (1994). Nutritional regulation of the insulin-like growth factors. *Endocrinology Reviews, 15,* 80–101.

Vandenberghe, K., Goris, M., Van Hecke, P., Van Leemputte, M., Vangerven, L., & Hespel, P. (1997). Long-term creatine intake is beneficial to muscle performance during resistance training. *Journal of Applied Physiology, 83,* 2055–2063.

Vierck, J.L., Icenoggle, D.L., Bucci, L., & Dodson, M.V. (2003). The effects of ergogenic compounds on myogenic satellite cells. *Medicine and Science in Sports and Exercise, 35,* 769–776.

Volek, J.S., Duncan, N.D., Mazzetti, S.A., Staron, R.S., Putukian, M., Gomez, A.L., et al. (1999). Performance and muscle fiber adaptations to creatine supplementation and heavy resistance training. *Medicine and Science in Sports and Exercise, 31,* 1147–1156.

Willoughby, D.S., & Rosene, J.M. (2003). Effects of oral creatine and resistance training on myogenic regulatory factor expression. *Medicine and Science in Sports and Exercise, 35,* 923–929.