EFFECTS OF CREATINE SUPPLEMENTATION AND RESISTANCE TRAINING ON BONE TURNOVER MARKERS IN OLDER MEN: A PILOT STUDY

By

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ABSTRACT

EFFECTS OF CREATINE SUPPLEMENTATION AND RESISTANCE TRAINING ON BONE TURNOVER MARKERS IN OLDER MEN: A PILOT STUDY

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PURPOSE: The primary purpose of this pilot study was to determine the effect of creatine supplementation, both with and without resistance training, on human bone. Serum bone turnover markers (BTMs) for bone formation and resorption were used as outcome measures. A secondary purpose was to investigate changes in body composition and strength resulting from the supplement and resistance training regimens.

METHODS: Eight healthy older men aged 66-79 years old (71.8 ± 4.1 yr; 91.4 ± 19.3 kg; 177.9 ± 6.3 cm) were randomized into three groups: creatine only (Cr, n = 3), creatine and resistance training (Cr+RT, n = 3) and placebo (PLA, n = 2). The men underwent 12 weeks of treatment which included supplementation with creatine (0.3 g/kg for 5 days and 0.07 g/kg thereafter) or placebo. Subjects were measured pre and posttest using four serum BTMs: osteocalcin (OC), procollagen I intact n-terminal propeptide (PINP), and N and C-Telopeptide (NTx, CTx). Those in the Cr+RT group performed 12 exercises, 10 repetitions, and 3 sets session^{-1} for 12 weeks. All subjects underwent dual x-ray absorptiometry (DXA), and one-repetition maximum strength testing. **RESULTS:** Study participants in the Cr and Cr+RT groups experienced increases in osteocalcin (Cr 2.8%, Cr+RT 13.1%) as did those in PLA group (12.0%). PINP also increased in those groups

receiving creatine (Cr 2.2%, Cr+RT 3.6%), yet it decreased in the PLA group (-15.6%). Bone resorption markers (NTx and CTx) decreased in the Cr-alone group (-11.9% and -23.1%, respectively), increased somewhat in the Cr+RT group (0.8% and 10.4%, respectively), and increased by a greater amount for those in the PLA group (22.5% and 38.9%, respectively). Small changes in lean mass (Cr 1.8%, Cr+RT 3.7%, PLA -2.9%) and fat % (Cr 0.3%, Cr+RT -0.2%, PLA 2.5%) were observed. Changes in strength were greatest in the Cr+RT group, but notable increases were also seen in the Cr-alone group (leg press: Cr 20%, Cr+RT 55%, PLA 5%; knee extension: Cr 10%, Cr+RT 43%, PLA 6%; and bench press: Cr 9%, Cr+RT 27%, PLA 0%). **CONCLUSION:** Observable patterns in change in serum BTMs indicate that creatine alone, and with resistance training, may have a positive effect on bone metabolism. Body composition and strength changes in creatine-supplemented groups were consistent with prior studies using a similar cohort and methodology.

DEDICATION

This project is dedicated to the Blue Lake Rancheria Tribe of California (BLR) and its current Tribal Council. The BLR was the sole source of funding for the creatine powder, DXA scans, and serum bone turnover marker lab costs during this pilot investigation. My belief is that this project stands as a valuable example of how tribal governments can participate in research in their local area(s). It also sets an educational goal for the next generation of BLR tribal members to reach, and I challenge them to do so. I would like to thank the BLR's tribal council for having the vision, foresight, and trust in making this project possible and for supporting scientific investigation into bone and elder research.

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There are many individuals that I would like to acknowledge for their contributions to the completion of this project. First and foremost I want to express gratitude I have toward my thesis chair Dr. Tina Manos for her guidance, expertise, encouragement, and collaboration during this process. Her ability to add structure and an approach of utter tenacity towards the drafting process made completion of this project possible. I would also like to thank Dr. Thomas Koesterer, my prior thesis chair, for being a friend and for helping me develop some of the ideas expressed in this project. Thank you to my committee, Dr. Susan Rodearmel, and Dr. Jianmin Zhong, for their expertise and constructive criticism during the proposal process. It really compelled me to make the right decisions in regards to the measurements used in this project, and now it is a basis for future work. Thanks to Dr. Alan Cook, D.C., of the Osteoporosis Diagnostic Center in Eureka CA, for lending his expertise in the use of DXA and for allowing us to perform the scans in his office. Special thanks to Steven Mielke of Eureka Internal Medicine's collection lab for collaborating and coordinating the blood draws and serum preparation. A thank you also goes to HealthSport in Arcata, CA for allowing me to use their facilities to conduct the resistance training portion of this study. I would like to thank my wife Jennifer and our two sons JJ and Zac for their support, and for tolerating my absence during the process of completing this project. Thank you to my immediate family: my mother Arla, father Mike, my sister Toni, and my brother Eric who have for a long time been a direct source of inspiration and support. Thank you to the rest of my

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INTRODUCTION

In the U.S., the incidence of osteoporosis is increasing at a rate faster than would be predicted by the increase in the proportion of aged individuals (NIH, 2004). Today 1.5 million older Americans suffer from broken bones caused from osteoporosis, translating into an annual medical expense of 18 billion dollars (Kohrt et al., 2004). Osteoporotic fractures are often the start of other health complications, leading to permanent loss of function and in many cases death. Even with no change in current incidence rates, it has been estimated that the number of hip fractures will double by 2025 (2.6 million total), and continue to climb to 4.5 million by 2050, with a greater percentage increase (310%) in men than in women (240%) (Gullberg, Johnell, & Kanis, 1997).

In older men, bone thinning leading to osteoporosis and fracture is a convergence of three factors: physical inactivity, loss of muscle mass, and decreased muscular strength. Today's treatment strategies for osteoporosis are multidimensional, focusing on pharmacological treatment approaches (estrogens, bisphosphonates) that lessen bone resorption and turnover. Non-pharmacological treatment has centered around diet (calcium, Vitamin D, protein, and caffeine), weight bearing exercise, and cessation of lifestyle risk factors (smoking, alcohol consumption) (Hannan et al., 2000; Nguyen et al., 1994). A less explored, but potentially promising prophylaxis against bone loss is dietary supplementation with creatine (Candow & Chilibeck, 2010).

Creatine ($C_4H_9N_3O_2$) is a natural compound consumed at 1-2 g/day in meat-eating diets; it is also naturally produced in the liver, pancreas and kidneys (1-2 g/day) (Wyss &

Kaddurah-Daouk, 2000). Most commonly creatine supplementation is studied for its benefits to provide quick energy in skeletal muscle (Kreider et al., 1998), effects on repetitive high intensity activities (Izquierdo, Ibanez, Gonzalez-Badillo, & Gorostiaga, 2002), and relationship to athletic performance (Kreider, 2003). Creatine supplementation (in the form of creatine monohydrate) has been the most studied, most effective, and safest ergogenic aid in increasing high intensity exercise capacity and lean mass during training (Buford et al., 2007). Muscle mass and bone mass show a parallel evolution during growth, and parallel involution with age (Schoutens, Laurent, & Poortmans, 1989). Since creatine is thought to have positive energetic effects in muscle, it is postulated that creatine and resistance training (RT) may have positive effects on bone (Candow et al., 2008; Chilibeck, Chrusch, Chad, Shawn Davison, & Burke, 2005). The belief that larger muscles are able to place more mechanical stress on bone, thereby causing micro-fracture and tension on periosteal surfaces causing stimulation of bone growth, is not controversial.

Creatine supplementation increases availability of phosphocreatine (PCr), allowing for an accelerated rate of resynthesis of ATP which leads to greater muscle training adaptations, due to an enhanced quality and volume of work performed (Buford et al., 2007). Creatine may also influence muscle by other mechanisms including: an increase in cellular hydration leading to anabolic effects on myogenic transcription factors (Balsom, Soderlund, Sjodin, & Ekblom, 1995; Saab, Marsh, Casselman, & Thompson, 2002), increased satellite cell activity (Candow, 2011; Olsen et al., 2006) and myofibrillar protein kinetics (Willoughby & Rosene, 2003).

In addition to the effect on muscle, creatine may have other metabolic effects, which more directly influence bone metabolism. Evidence is mounting that creatine has a beneficial role in both accentuating the activity of chondrocytes and osteoblasts, and in potentially attenuating osteoclastic activity. Creatine appears to have effects on bone tissues on the cellular level by increasing metabolic activity and mineralization of osteoblastic-like cells (Gerber, ap Gwynn, Alini, & Wallimann, 2005) and up-regulating the expression of creatine kinase (CK) isoforms during periods of increased energy demands in osteoblasts (Wallimann, Tokarska-Schlattner, & Schlattner, 2011). Blocking CK function in chondrocytes, and hence blocking CK-catalyzed reactions, results in cellular insufficiencies in the growth plates of endochondral bone in growing rats (Funanage, Carango, Shapiro, Tokuoka, & Tuan, 1992). In an extension of the cellular research on an animal model, Antolic et al. (2007) demonstrated positive skeletal benefits in sedentary young rats supplemented with creatine monohydrate; a significant increase in bone mineral density (BMD) of the L5 vertebra, and greater maximal bone load-to-failure was observed.

Research on the effects of creatine supplementation on human bone is scant (Candow & Chilibeck, 2010). Support for the idea that creatine may favorably affect bone metabolism in the absence of resistance training comes from studies of muscular dystrophy in which boys afflicted with Duchenne muscular dystrophy were found to have an increase bone mineral density (Louis et al., 2003) and a decrease in N-telopeptide (NTx), a key index of bone resorption (M. A. Tarnopolsky et al., 2004), after supplementation with creatine monohydrate. Young and old subjects may respond

differently to creatine supplementation (Rawson, Clarkson, Price, & Miles, 2002) and it is unclear how the presence of muscular disease may have influenced the results in these studies (e.g., initial creatine levels may have been low).

Nine studies have been done in which the effect of creatine on human bone has been reported, five positive and four showing no effect (Candow & Chilibeck, 2010). These studies include younger, older, and muscular dystrophy cohorts. In seven of those studies non-diseased subjects were given creatine supplementation along with resistance training in order to determine effects on bone (Brose, Parise, & Tarnopolsky, 2003; Candow et al., 2008; Chilibeck et al., 2005; Cornish et al., 2009; Kerksick et al., 2007; Kreider et al., 1998; M. Tarnopolsky et al., 2007). Outcomes for these studies were varied and included measures of bone content, bone mass or density, or bone metabolism such as NTx (Candow & Chilibeck, 2010). Chilibeck et al. (2005) studied older men given either creatine or placebo during 12 weeks of resistance training and reported significant increases in regional bone mineral content (BMC) of the arms in the creatine group when compared to placebo. This was noteworthy, as dual x-ray absorptiometry (DXA) determined changes in bone are difficult to measure over short time frames (i.e., less than 6-8 months) (Henriksen, Leeming, Christiansen, & Karsdal, 2011). Although small changes in bone mineral density in the absence of resistance training were observed in one of the studies of boys with muscular dystrophy (Louis et al., 2003), two other studies included DXA-derived outcomes and failed to show any effect of creatine supplementation on bone in younger subjects taking creatine while doing resistance training (Kerksick et al., 2007; Kreider et al., 1998). The short duration of these studies,

or the fact that subjects had higher initial bone measures might also have made it difficult to see changes with the treatment.

A subset of the studies involved healthy exercising subjects and showed a beneficial effect, or no effect, on bone status when assessed using osteocalcin or NTx (i.e., bone turnover markers [BTMs]), (Brose et al., 2003; Candow et al., 2008; Cornish et al., 2009; M. Tarnopolsky et al., 2007). Brose et al. (2003) failed to find an effect of 14 weeks of creatine supplementation and resistance training on osteocalcin, a marker for bone formation, in older adult men and women. Cornish et al. (2009) studied young adults and administered creatine plus protein supplements with and without conjugated linoleic acid (CLA). Decreases in NTx were 3.4% in the CLA plus creatine plus protein group, and 3.9% in the creatine plus protein group. The combined results for the two groups receiving the creatine showed a trend for a significantly lower attenuation of bone resorption than for the group that had protein supplementation alone ($p = .055$) (Cornish et al., 2009). Candow et al. (2008) provided further support for the idea that creatine supplementation, when coupled with resistance training, attenuates bone loss, showing a powerful effect (ES = 2.0) in older men trained for 10 weeks. In this randomized, double-blind study the subjects received either creatine and protein, creatine alone, or placebo. NTx decreased by 27% in the combined groups receiving the creatine, while it increased 13% in the placebo group (Candow et al., 2008). M. Tarnopolsky et al. (2007) conducted the only study on healthy subjects where creatine and resistance training had not even a small effect on NTx. In this study the creatine and CLA supplement improved strength and body composition in older men and women an over 6 month time period, but failed

to change NTx (M. Tarnopolsky et al., 2007). In explaining why NTx failed to change, Dr. Tarnopolsky discussed the limitations of using a single measure of bone turnover and recommended multiple BTMs be used for resorption and synthesis in future research; he also indicated that "there were many factors--one is the confounder of the CLA" (M. Tarnopolsky, personal communication, February 25, 2013).

Factors that may explain the discrepancy of results on NTx and differences in magnitude of the effect across studies may include: initial creatine levels in subjects, dose and composition of the supplement (i.e., with or without CLA), the populations studied (differences in age, gender, disease status), measurement technique for NTx, and/or the nature of the resistance training stimulus. The effect of resistance training on aging bone by itself is inconsistent, due to differences in duration, intensity, and frequency (Candow & Chilibeck, 2010). However it is accepted that higher intensity resistance training exercise is favored over low intensity to create new bone.

Decreases in NTx is a common finding in studies of creatine supplementation, demonstrating a positive effect on bone both with, and without, the use of exercise as a treatment (Candow et al., 2008; Cornish et al., 2009; Louis et al., 2003; M. A. Tarnopolsky et al., 2004). There is only one creatine and resistance training study utilizing an older cohort in which there was not a decrease in NTx (M. Tarnopolsky et al., 2007). In a review article on the potential of creatine supplementation for improving aging bone health, Candow and Chilibeck (2010) conclude that "creatine supplementation, independent of exercise, may have a small beneficial effect on bone mineral in healthy older adults. However, the "effects of creatine on reducing bone

resorption is much greater." (p. 152). That said, much remains to be explained. Using BTMs as measures of bone turnover in creatine/exercise studies is inconsistent and when used, all of the analyses are done on urine, a less accurate method. Sometimes a single marker for bone resorption is utilized; sometimes a sole marker for bone synthesis is used (Candow & Chilibeck, 2010). Further, urine-based NTx reproducibility can vary, largely depending on the lab and specific test type, with CVs ranging from 7% to 30% (Schafer, Vittinghoff, Ramachandran, Mahmoudi, & Bauer, 2010).

Creatine has positive effects on aging bone in combination with resistance training (Chilibeck et al., 2005). A more specific summary of the attributes of studies demonstrating a positive effect from creatine supplementation on bone show beneficence when: older untrained male populations are utilized (Candow et al., 2008; Chilibeck et al., 2005), study duration is at least 10 weeks (Candow et al., 2008; Chilibeck et al., 2005; Louis et al., 2003; M. A. Tarnopolsky et al., 2004), resistance training programs contain at least 3 sets with 10 repetitions and at least 8 exercises, have a frequency of 3 times per week, and supplementation levels of creatine are at a rate of at least $0.1 g \cdot kg^{-1}$ (Candow et al., 2008; Chilibeck et al., 2005; Cornish et al., 2009). Creatine appears to have the greatest positive measureable effects on bone in subject populations that have diminished muscle mass (Candow et al., 2008; Chilibeck et al., 2005; Louis et al., 2003; M. A. Tarnopolsky et al., 2004). Creatine, in the absence of resistance training, has been shown to increase strength and decrease bone resorption markers in boys with muscle dystrophy (Louis et al., 2003; M. A. Tarnopolsky et al., 2004). Due to the intimate relationship between muscle and bone, creatine may have the greatest effect on aged bone

(Candow et al., 2008). In an older male population low-dose creatine and protein treatment combined with resistance exercise reduced muscle protein degradation and bone resorption (Candow et al., 2008).

To date, no study exists on aging bone in which researchers report on the differences between creatine supplementation alone, in the absence of resistance training. Also, no study exists to date that uses both synthesis and resorption serum BTMs in an older male population. Therefore, the primary purpose of this study was to investigate the effect of creatine supplementation on aging bone, both with and without resistance training, using serum BTMs (for bone formation and resorption). A secondary purpose was to investigate changes in body composition and strength resulting from the supplement and resistance training regimens.

METHODS

Experimental Design

A randomized, blind, repeated measures design was used to investigate the effects of 12 weeks of creatine supplementation, with and without resistance training on: bone formation and resorption turnover markers, changes in body composition, and overall strength in older men. Men were randomized into one of three groups: 1) creatine only (Cr), 2) creatine with resistance training (Cr +RT), and 3) placebo (PLA). The main dependent variables were: four bone turnover markers (BTM); DXA measurements for BMC, BMD and body composition; and strength. Markers that were used to measure bone formation were serum osteocalcin and Procollagen I Intact N-Terminal Propeptide (PINP). Resorption markers that were used to measure bone breakdown were serum (NTx) N-Telopeptide cross-linked and (CTx) C-Telopeptide beta-cross-linked. Lastly, subjects completed a three-day dietary log during the first and final week of the study to determine any nutrient differences over the course of the study within subjects and between the groups. Upon completion of the study, subjects were asked which supplement group they thought they were in. The overall study design is depicted below (see Figure 1) and a more detailed diagram of the methods is shown in Appendix A.

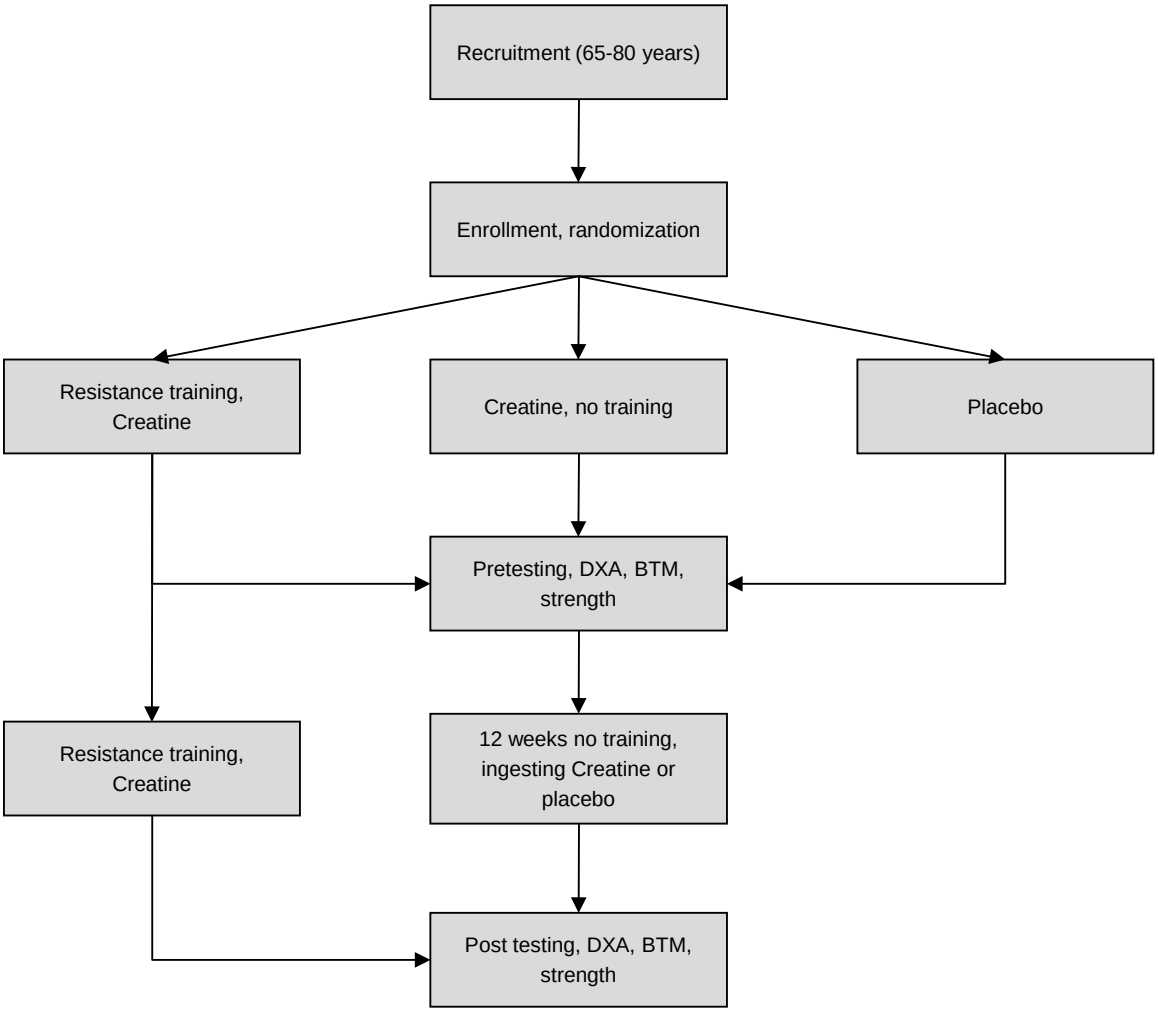

Figure 1. Experiment flow diagram

Subjects

Humboldt State University's Institutional Review Board approval (Appendix B) was obtained prior to subject recruitment. Males who were 65 to 80 years of age, healthy, and not currently involved in a resistance training program were recruited via flyers (Appendix C) posted in assisted living facilities (e.g., TimberRidge), Eureka Area 1 on Aging, Eureka Senior Resource Center, McKinleyville Senior Resource Center, local

health clinics, Blue Lake Parks and Recreation, Arcata Parks and Recreation, and the Blue Lake Rancheria's Elder Nutrition food program. A series of five project summary presentations were conducted at these facilities where approximately 250 potential participants were addressed directly. Additionally, 19 satellite Area 1 on Aging STRONG classes (Appendix D) were exposed to the recruitment materials.

A total of 10 subjects responded to the subject recruitment efforts and were screened using a preliminary intake sheet (Appendix E). Those who had a fracture during the previous year, used corticosteroids, active vitamin D therapy, bisphosphonates or calcitonin were excluded from study participation. Additionally subjects suffering gastrointestinal, renal, or liver disease or possessing any contraindications to exercise (ACSM, 2012) were excluded from participation. All 10 potential subjects passed the preliminary screening and were sent a physician's clearance form (Appendix F) to obtain permission from their personal physician before participating in the study. One of the potential subjects was not able to secure this permission, leaving 9 subjects who could participate in the study, 1 of whom dropped out before study began, citing study time requirements as being a problem. Due to the exhaustive recruitment efforts yielding only 8 suitable subjects, the study was reconfigured to be descriptive, fully recognizing insufficient power to answer the research questions posed. The names of the study participants were then coded and an individual not associated with the study randomized subjects into three groups: 1) creatine (Cr, $n = 3$), 2) creatine and resistance training (Cr+RT, $n = 3$) and, 3) placebo (PLA, $n = 2$).

Procedures

All subjects attended an orientation meeting on October 30th, 2013 at the Blue Lake Casino Hotel's fourth floor conference room. Following a detailed PowerPoint presentation (Appendix G) outlining study procedures and the risks/benefits of participation, subjects signed an Informed Consent and Release of Liability form (Appendix H). They then completed the HSU Kinesiology Medical Information and History form (Appendix I) and submitted the physician's clearance form (Appendix F), Subjects were given a 3-day diet log (Appendix J) to record food and beverage intake and were instructed to maintain their normal eating and activity levels over the duration of the study.

Serum BTM concentrations, bone content/density, body composition, and body weight were measured during the first week of the study. Additionally, pretest strength measures were obtained. Those in the Cr+RT group were then fully habituated to the RT equipment in the initial weeks of the intervention. Supplementation for all groups started the week after initial testing. During final week of the study all participants underwent all of the same testing that they had during the initial testing week.

Treatments and Measures

Supplementation. Creatine was sourced from the General Nutrition Corporation (GNC) online store. GNC "Pro Performance Creatine Monohydrate 5000" containing

99.95% pure creatine monohydrate with no other additives was used (Appendix K). Subjects in all groups ingested either the creatine supplement or placebo three times per day during the first week, the loading week. Then they ingested the treatment once per day for the remaining 11 weeks.

All treatment preparations were weighed using a Taylor stainless steel food scale to the nearest half gram. For subjects in Cr and Cr+RT groups the dosage of creatine ingested was 0.3 g creatine·kg^{-1} body weight for the first 5 days followed by 0.07 g creatine·kg^{-1} body weight for the remaining 79 days of the study (Chilibeck et al., 2005; Hultman, Soderlund, Timmons, Cederblad, & Greenhaff, 1996). The creatine was combined with an equal measure of sucrose flour for those receiving the creatine. The placebo group consumed sucrose flour with and equal portion of flour added in place of the creatine, making it indistinguishable from the creatine treatment in taste, texture and appearance.

All treatments were pre-bagged into mini Ziplock bags with the day of the week label on each mini bag. All mini bags where then distributed weekly to each subject in a larger gallon Ziplock with the subject's name on the label. Timing of the supplement and placebo ingestion during the first week was specified; clear instructions were given to subjects to ingest the treatment three times per day spaced at regular time intervals in order to minimize gastrointestinal upset during the creatine loading phase (Candow et al., 2008). Self-reported adherence to ingesting the treatment, across all groups, was above 98.0%

Strength testing. Subjects underwent pre- and post-strength testing using

procedures described by Chilibeck et al. (2005) and Chrusch, Chilibeck, Chad, Davison, and Burke (2001). All subjects completed 1-repetition max (1-RM) testing for the leg press, knee extension, and bench press exercises (Appendix L) at the beginning and again at the end of the 12-week study. These measures were utilized as indexes of muscular strength because they involve the major muscle groups of the lower and upper body and have low reported coefficients of variation of 3.8% (leg press) and 3.1% (bench press) (Candow et al., 2008). The tests were demonstrated by the researcher and subjects underwent a standard, test-specific warm up before each test. Weight was progressively increased for each 1-RM attempt, with a 2-minute rest interval between attempts. Subjects were able to reach 1-RM within 6 trials, (excluding the warm up set), for all three exercises tested.

The bilateral leg press machine (Body Master Sports Industries Inc., Rayne, LA) was used to measure overall lower body strength. Subjects were positioned so that there was a 90° angle at the knee with feet placed shoulder width apart. Subjects were then instructed to push the weight away from the body to full extension without locking their knees before returning to the starting position. Subjects were then seated on a knee extension machine (TechnoGym USA Corp, Fairfield, New Jersey). The back rest of the machine was appropriately adjusted so that the back of the subject's knees were just over the edge of the seat and the knee angle was 90º. Following trials for 1-RM of knee extension, subjects were positioned seated, with both feet flat on the floor, in a horizontal chest press machine (TechnoGym, Fairfield, New Jersey). The handles of the machine lined up at the mid chest level. For this last strength assessment the subjects were

instructed to take a comfortable grip approximately shoulder-width apart and push the handles away from the body until the arms were fully extended and then lower the weight back to the starting position.

Strength training. Participants randomized into the Cr+RT group performed a supervised whole-body resistance training program, three times a week (Monday, Wednesday, and Friday) for 12 weeks, at approximately 1 hour per session. Sessions were conducted during regular business hours at HealthSport in Arcata CA. All training sessions were supervised by the graduate student researcher acting as a group leader, as supervised sessions are likely to result in greater strength gains than unsupervised sessions (Mazzetti et al., 2000). Prior to starting the training, subjects were familiarized with the resistance training equipment and were instructed on proper form, breathing and performing a complete range of motion during the exercises.

The resistance training program consisted of 3 sets of 10 repetitions for 12 exercises and included: bench press, lat row, shoulder press, bicep curl, back extension, hip (extension, flexion, abduction, and adduction), leg flexion, knee extension, and leg press. All exercises were recorded by the researcher at the time they were performed (Appendix M). Initial machine weight settings were established as 50% 1-RM for the leg press, bench press, and knee extension. The initial weight amounts for all other exercises were established as the maximal amount a subject could perform using good form for three sets. Hip and low back exercise volume and intensity were started purposely low to minimize risk of injury (Chrusch et al., 2001).

Exercise resistance was increased by 2-5 kg when a subject could complete 10

repetitions on a third set with good form (Candow et al., 2008). There was no prescribed exercise order in completing the 12 RT exercises, although subjects were instructed to complete exercises using large muscle groups before small muscle group exercises when it was possible, depending on facility crowding. Tapering occurred during the final week of training to ensure that participants were not obstructed by training fatigue for post 1-RM testing. This exercise approach was successful in increasing muscle mass and strength (Chrusch et al., 2001), strength and lean tissue mass, and in decreasing N-telopeptides when combined with protein and creatine in older men (Candow et al., 2008). Attendance rate to the RT sessions was 94.8% over the 12-week duration of the study.

Diet records. Subjects qualifying for the study were instructed on how to properly record entries on the 3-day food record during the initial orientation meeting. Subjects were provided a 3-day diet log (Appendix J) that had an introduction describing the usefulness of accurate food recording, detailing instructions for recording food amounts, and were given a sample daily entry page to reference when recording the diet log. Participants were instructed to record their diets in the record provided for three days (two weekdays and one weekend day) during the first and final week of the study to evaluate dietary differences between the three groups and within each subject over time.

Subjects were instructed to record all foodstuffs and portion sizes consumed for those three days. Food records were analysed using the USDA's Food Tracker (USDA, 2015), (Appendix N) where foods may be entered and energy totals along with energy specifics on; carbohydrates, fats, and proteins can be determined.

DXA scans. DXA scans were conducted using a fan beam DXA scanner (Hologic, Bedford, MA) Discovery W model (S/N70284) with operating software version 12.7.4. Scans were conducted by a certified technologist at the Eureka Osteoporosis Center (2773 Harris St. Suite F, Eureka CA). When subjects arrived at the Osteoporosis Center they were taken to the scan room gowned to ensure that they had no extra metal on their body during the scan. Subjects were weighed and their height was measured using county-certified measurement equipment. Subjects were then placed on the scan table and were instructed to hold still during the scan. After the first scan, subjects were allowed to get up and walk around for 2 minutes before getting back on the table for the second scan. The total scan time was around 20 minutes.

DXA scans were used to determine a number of regional and total body parameters, but only total body BMC (bone mineral content, in grams), BMD (bone mineral density, in g/cm^2), Lean (g), and % Fat are reported in this study. Duplicate scans were repeated on all subjects again at the end of the 12-week study. Reliability of pre- and post-test measures of BMC, BMD, Lean, and % Fat measures were extremely high, at ICCs = .994 or greater (Appendix O). The average of the two pre-test measures and average of the two post-test measures was used in data analysis to ascertain changes over the 12 weeks.

Blood measures. Subjects had 12-hour fasting blood specimens taken during the week prior to the start of creatine/placebo ingestion and during week 12 at the end of the study. Specimens were obtained following standard laboratory methods for blood collection by licensed phlebotomists at the Eureka Internal Medicine's clinical laboratory

(2280 Harrison Ave., Eureka CA). Fifteen mL of blood was drawn into two separate 8.5 mL serum separator tubes (SST-Tiger Top) where they were allowed to sit for clot formation. Those specimen tubes were then centrifuged and the serum was placed in ARUP Laboratories-supplied aliquot tubes. Each subject generated eight BTM aliquot tubes both pre and posttest, as all four tests were duplicated for each subject both pre and post.

Samples were shipped in batch following ARUP Laboratories collection guidelines express overnight by FedEx in a Styrofoam cooler, on dry ice, in a box within a box arrangement with shipping peanuts surrounding the cooler and the inside box (Appendix P). All samples were frozen at Eureka Internal Medicine then transported on dry ice and stored in a -20 degree freezer at the Blue Lake Rancheria until they could be sorted, bagged and coded in a specimen test request matrix (Appendix Q).

Lab testing focused on four BTMs that measure bone synthesis and resorption. Markers that were used to measure bone formation were serum osteocalcin, which was analysed using electrochemiluminescent immunoassay (ARUP #0020728), and procollagen I intact n-terminal propeptide (PINP), which was analysed using quantitative radioimmunoassay (ARUP #0070236). Resorption markers were serum N-Telopeptide cross-linked (NTx) which was analysed using quantitative enzyme-linked immunosorbent assay and C-Telopeptide beta-cross-linked (CTx) was analysed using quantitative electrochemiluminescent immunoassay assay (ARUP #0070500 and #0070416, respectively). The automated electrochemiluminescent immunoassay assessment technique for CTx is a more sensitive test than the urine-based tests commonly used in

creatine studies examining human bone (Brown et al., 2009; Garnero, Borel, & Delmas, 2001; Herrmann & Seibel, 2008). Prior to the start of data collection, the research team requested CV data on the individual BTM tests performed by the labs. ARUP Labs supplied the following data table for intraclass coefficient of variation within the designated reference ranges (Table 1).

Two of the markers (PINP and CTx) were chosen because they represented the best BTMs for formation and resorption in bone marker chemistry currently available (Johansson et al., 2014; Lee & Vasikaran, 2012), and have been used in similarly modeled studies (Candow & Chilibeck, 2010). The International Osteoporosis Foundation, and the National Osteoporosis Foundation have also recognized the value of those two BTMs and have made efforts to standardize clinical guidelines and reference ranges for their use in fracture prediction and in the management of osteoporosis (Vasikaran et al., 2011). Furthermore, the two additional measures of each type (resorption and formation) were used following the advice of a leading researcher in a related field (M. Tarnopolsky, personal communication, February 25, 2013).

Table 1. Analytical measurement range for BTMs

ARUP Data	Osteocalcin (ng/mL)	PINP (ug/L)	CTx (pg/mL)	NTx (nm BCE)
Test Number	0020728	0070236	0070416	0070500
AMR	1-300	2-250	10-6,000	3.2-40.0
Intra CV %	2.0	8.7	4.04	11.36

Note. From ARUP Labs email transmission, Jon Lowe, Client services, Monday August 26, 2014, 12:30 pm.

Statistics

The planned statistical analyses were 3 (Cr vs. Cr+RT vs. PLA group) x 2 (pre- and post-test periods) repeated measures analyses of variance to determine mean differences in BTM, body composition and strength measures among the three groups between the two time periods. Due to the change to a more descriptive pilot study, primarily descriptive statistics and effect sizes were reported. Effect sizes were calculated using statistical techniques according to Cohen (1988), and data was input into an effect size engine, developed by Becker (1995), using the formula (Cohen's $d = M_1 - M_2 / s_{pooled}$, where $s_{pooled} = \sqrt{[(s_1^2 + s_2^2) / 2]}$). Other descriptive statistics were generated using SPSS statistical software version 22.0 (IBM-Corp, 2013). A one-way independent groups ANOVA was also conducted for body weight using SPSS. Test reproducibility was analyzed, also using SPSS, to compute the intraclass correlation coefficient (ICC) using a two-factor mixed effects model and type consistency (McGraw & Wong, 1996; Shrout & Fleiss, 1979).

Limitations and Delimitations

There were several assumptions, limitations, and delimitations to this research. It was assumed that subjects honestly reported on diet details and compliance to the supplement or placebo regimens. It was also assumed that subjects accurately reported on use of bisphosphonates or other drugs or medical conditions that might have affected bone metabolism. Clearly, the 1-RM muscle strength measurements are dependent on subject motivation. The limitations to this study were that initial body creatine levels were not measured and it was not possible to control for dietary habits and outside activity. Considerable inter-individual variability with regard to age and health status, common when using an older adult population, may have affected results. The degree of accuracy of all self-report measures and of measures using the equipment used in this study may have affected results. That said, both the Osteoporosis Center and ARUP labs reported calibration standards. This study also did not control for genetic variation between subjects even though bone mineral density, loss of bone, fracture, and many bone diseases have been shown to have strong genotype associations in family and twin studies (Ralston & Uitterlinden, 2010). Finally, the results from this study only apply to older untrained males.

RESULTS

Data from eight subjects were gathered and used in the analysis. Subjects were male Caucasians that were not participating in any exercise programs: Cr (n = 3), Cr+RT (n = 3), and PLA (n = 2). All measurements pre and posttest were conducted under the same conditions. Subject demographic data is summarized in Table 2. Age range for the subjects was between 66 and 79 years of age. Subject group mean weights were: pretest (Cr group 84.8 kg, Cr+RT group 98.6 kg, placebo group 90.5 kg), and postest (Cr group 86.2 kg, Cr+RT group 98.6 kg, placebo group 91.4 kg). There were no significant differences in body mass across groups at pretest, $F(2,5) = .308$, $p = .748$. No differences in calcium, vitamin D, or macronutrient intake between or within groups were apparent with exception of Vitamin D intake posttest in the creatine group (Table 3). All subjects reported in post study interviews that they were unable to determine what supplement group they were in.

The main dependent variables in this study were the BTM measures; descriptive statistics for BTM measures are shown in Table 4. The mean changes in the BTMs that reflect bone synthesis pre to post 12-week intervention for each group are depicted in Figure 2 and Figure 4, respectively. Individual change in osteocalcin and PINP are shown in Figure 3 and Figure 5, respectively. Osteocalcin mean lab values were greater in all three groups posttest, (Cr group 2.8% increase, Cr+RT group 13.1% increase, and placebo 12.0% increase). PINP also showed a mean increase during post testing in two of the three groups. In both Cr and Cr+RT groups there was a 2.2% and 3.6% increase

respectively, from pre to posttest; however, it was decreased in the placebo group by 15.6%. The mean changes in the BTMs that reflect bone destruction, or osteoclast activity, pre to post 12-week intervention for each group are depicted in Figure 6 and Figure 8, respectively. Individual changes in N-Telopeptide and C-Telopeptide are shown in Figure 7 and Figure 9, respectively. NTx mean lab values posttest were decreased in the Cr group, slightly increased in the Cr+RT group, and had a large increase in the placebo group, (Cr group 11.9% decrease, Cr+RT group 0.8% increase, placebo group 22.5% increase). CTx measurement followed a similar pattern, (Cr group 23.1% decrease, Cr+RT group 10.4% increase, placebo group 38.9% increase).

One set of secondary dependent variables in this study were the body composition measures. Descriptive statistics for the mean changes in body composition measures are shown in Table 5. BMC totals showed little change in the Cr group, and small positive changes in both the Cr+RT and placebo groups, (Cr group 0.6% decrease, Cr+RT group 0.8% increase, placebo group 1.8% increase). Bone mineral density was unchanged in the Cr, and Cr+RT groups, but had a slight increase in the placebo group, (Cr group 0%, Cr+RT group 0%, placebo group 0.8% increase). Total lean body tissue increased in the creatine groups and decreased in the placebo group, (Cr group 1.8% increase, Cr+RT 3.7% increase, placebo group 2.9% decrease). Body fat percentage slightly increased in the Cr group, decreased in the Cr+RT group and increased in the placebo group, (Cr group 0.3% increase, Cr+RT group 0.2% decrease, placebo group 2.5% increase).

Another set of secondary dependent variables in this study were the strength measures; descriptive statistics for the mean changes in strength measures are shown in

Table 6. The pattern of strength gain was greatest in the leg press. Leg press strength increased in all groups, but the biggest gain was in the Cr+RT group, (Cr group 20% increase, Cr+RT group 55% increase, placebo group 5% increase). Knee extension strength showed a similar pattern, (Cr group 10% increase, Cr=RT group 43% increase, placebo group 6% increase). This was also true for the bench press, except no change was observed in the placebo group, (Cr group 9% increase, Cr+RT group 27% increase, placebo group 0%). A pattern existed between the two sets of secondary dependent variables, (body composition and strength), which showed parallel changes in lean tissue and leg press strength, represented in Figure 10.

Table 2. Demographic information on subjects (mean ± *SD*)

Subject Group	Age (years)	Weight (kg)	Height (cm)
Creatine (*n* = 3)	72.0 ± 1.7	84.8 ± 9.4	179.5 ± 6.0
Creatine + RT (*n* = 3)	73.7 ± 6.1	98.6 ± 28.5	180.7 ± 6.8
Placebo (*n* = 2)	69.0 ± 2.8	90.5 ± 22.8	171.4 ± 1.8
All Subjects	71.8 ± 4.1	91.4 ± 19.3	177.9 ± 6.3

Table 3. Descriptive statistics for nutrient intake (mean ± *SD*)

Nutrient	Pretest	Posttest	% Change
Calcium (mg)			
Creatine	860.3 ± 505.3	702.8 ± 386.7	-18.3
Creatine + RT	1016.3 ± 117	1038.7 ± 101.4	2.2
Placebo*	870.7	1323.3	51.9
Vitamin D (ug)			
Creatine	1.56 ± 0.77	4.56 ± 2.55	192.3
Creatine + RT	9.44 ± 2.55	7.44 ± 3.98	-21.1
Placebo*	8.00	6.33	-20.9
Protein (g)			
Creatine	74.1 ± 19.4	75.3 ± 17.4	1.6
Creatine + RT	118.1 ± 14.8	102.8 ± 29.8	-12.9
Placebo*	111.0	102.3	-7.8
Carbohydrate(g)			
Creatine	219.7 ± 59.9	216.4 ± 92.8	-1.5
Creatine + RT	239.7 ± 35.0	255.2 ± 46.6	6.4
Placebo*	275.7	326	18.2
Fat (g)			
Creatine	72.1 ± 15.0	59.2 ± 16.6	-17.9
Creatine + RT	115.9 ± 31.9	102.9 ± 12.5	-11.2
Placebo*	143.4	140.3	-2.1
Calories (Cal)			

Creatine	1852.6 ± 495.2	1730.9 ± 541.5	-6.6
Creatine + RT	2467.3 ± 215.3	2333.5 ± 100.8	-5.4
Placebo*	2810.7	2923.0	4.0

All values are means (± *SD*) from averaged 3-day pre and post subject-submitted diet records

* Placebo represents data from a single subject

Table 4. Descriptive statistics for bone turnover markers (mean ± *SE*)

BTM	Pretest	Posttest	% Change	ES
Osteocalcin (ng/mL)				
Creatine	17.8 ± 4.8	18.3 ± 4.0	2.8	0.03
Creatine + RT	17.5 ± 2.3	19.8 ± 2.3	13.1	0.28
Placebo	15.0 ± 5.0	16.8 ± 4.8	12.0	0.13
PINP (ug/L)				
Creatine	35.7 ± 4.1	36.5 ± 4.3	2.2	0.06
Creatine + RT	36.0 ± 3.8	37.3 ± 5.5	3.6	0.08
Placebo	38.5 ±5.5	32.5 ± 2.0	-15.6	-0.46
NTx (nM BCE)*				
Creatine	15.1 ± 1.4	13.3 ± 0.9	-11.9	-0.40
Creatine + RT	12.9 ± 1.5	13.0 ± 1.2	0.8	0.01
Placebo	12.9 ± 1.2	15.8 ± 4.7	22.5	0.29
CTx (pg/mL)				
Creatine	293.7 ± 83.7	226.0 ± 66.5	-23.1	-0.25
Creatine + RT	204.2 ± 32.5	225.5 ± 36.4	10.4	0.18
Placebo	255.0 ± 69.5	354.3 ± 214.3	38.9	0.21

All values are means from averaged, duplicated pre and duplicated post blood tests.

*BCE is nanomole bone collagen equivalent

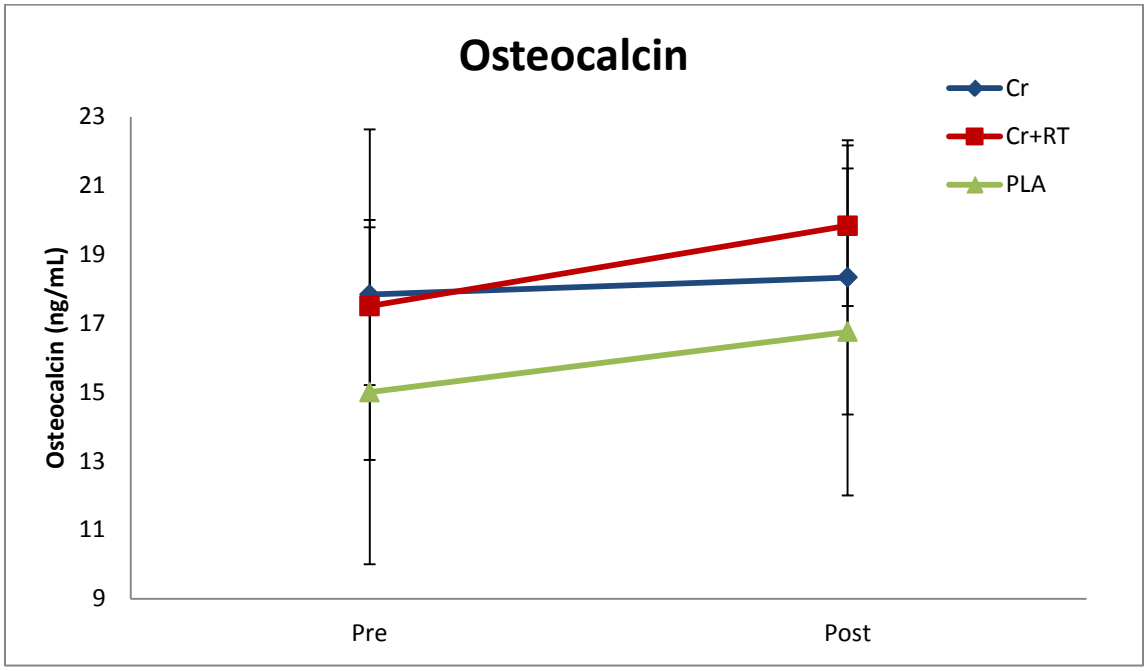

Figure 2. Mean (± *SE)* pretest and 12-week posttest values for serum osteocalcin for creatine only (Cr), creatine and resistance training (Cr+RT), or placebo (PLA) groups.

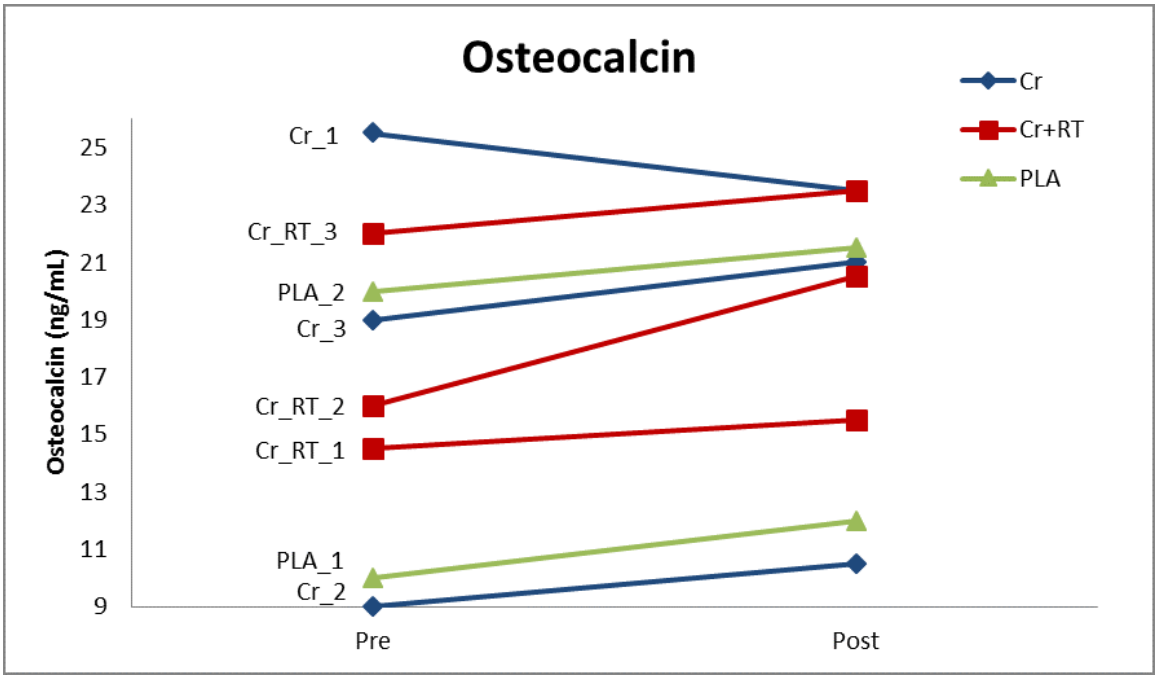

Figure 3. Individual pretest and 12-week posttest values for serum osteocalcin for creatine only (Cr), creatine and resistance training (Cr+RT), or placebo (PLA).

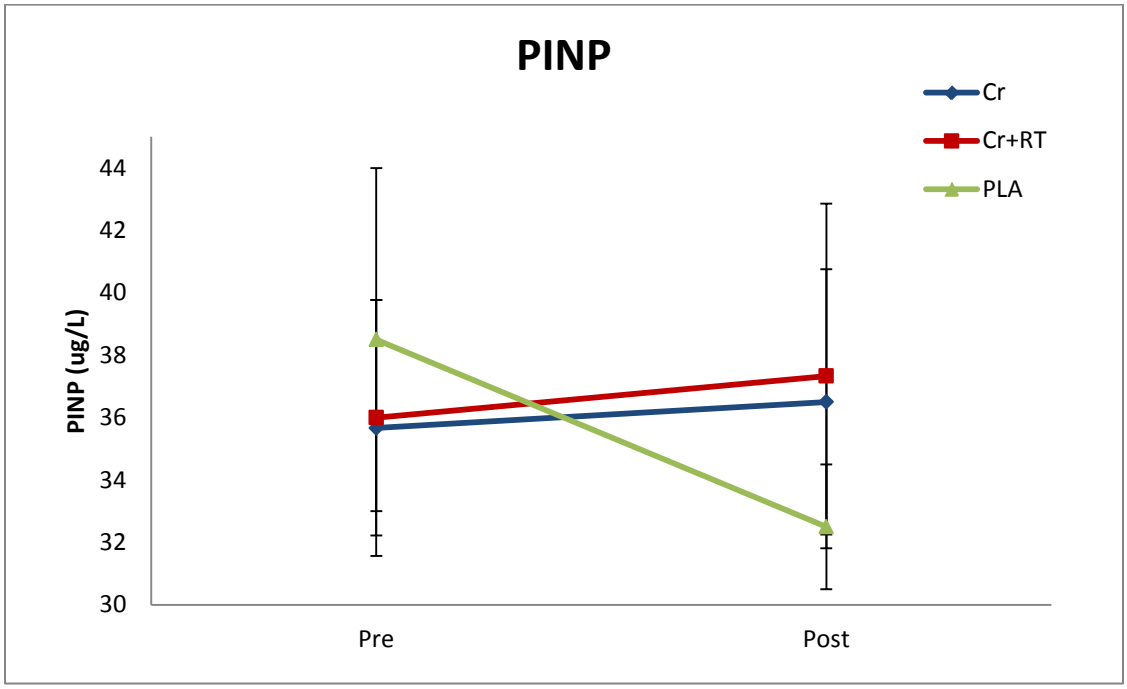

Figure 4. Mean (± *SE)* pretest and 12-week posttest values for serum PINP for creatine only (Cr), creatine and resistance training (Cr+RT), or placebo (PLA) groups.

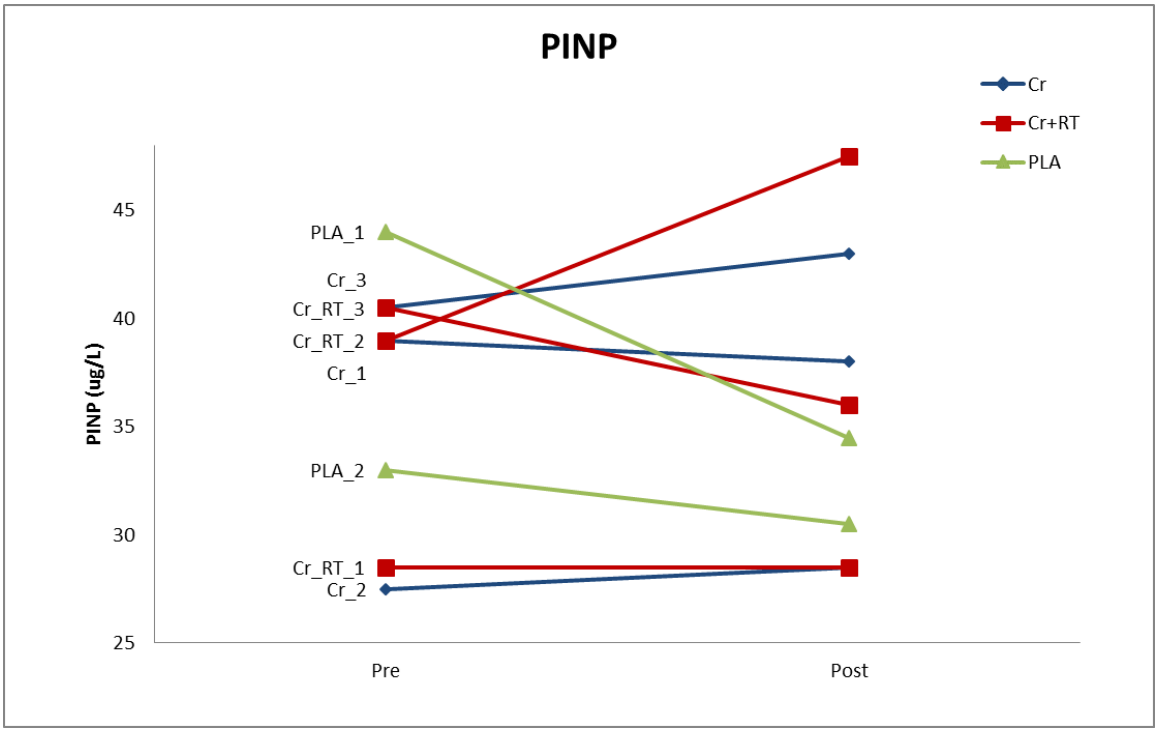

Figure 5. Individual pretest and 12-week posttest values for serum PINP for creatine only (Cr), creatine and resistance training (Cr+RT), or placebo (PLA).

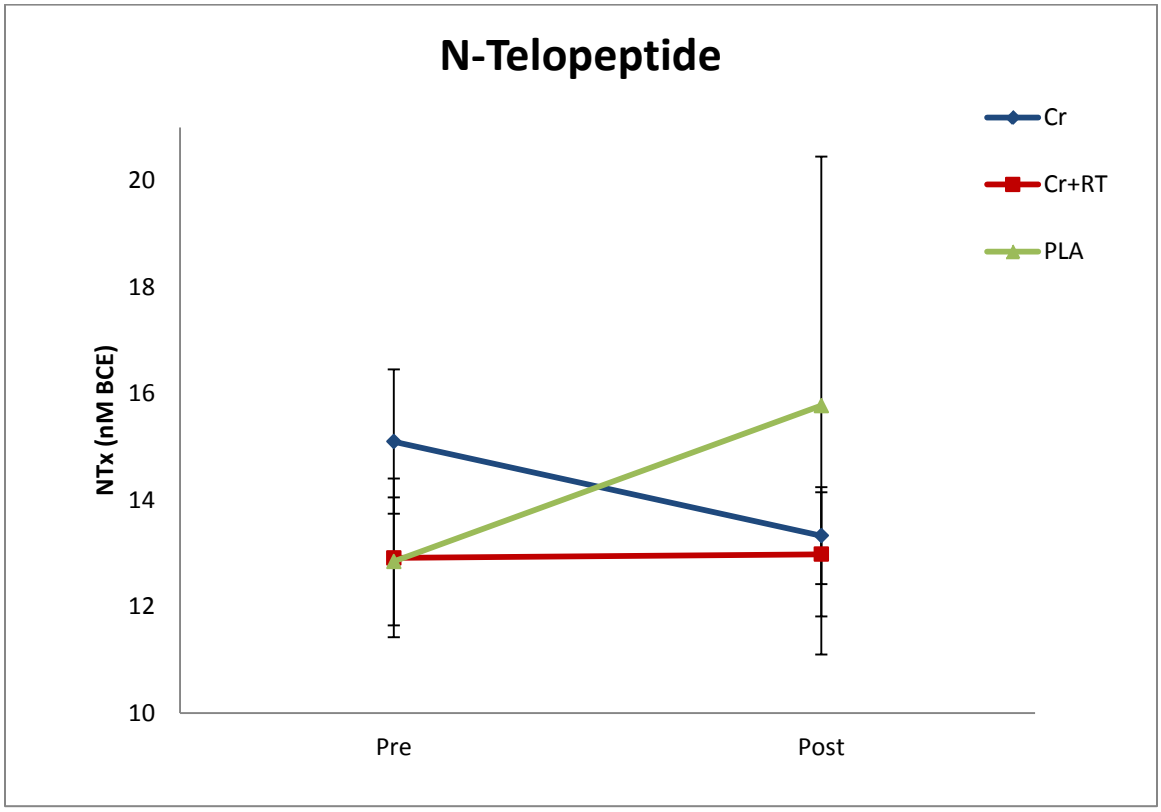

Figure 6. Mean (± *SE)* pretest and 12-week posttest values for serum NTx for creatine only (Cr), creatine and resistance training (Cr+RT), or placebo (PLA) groups.

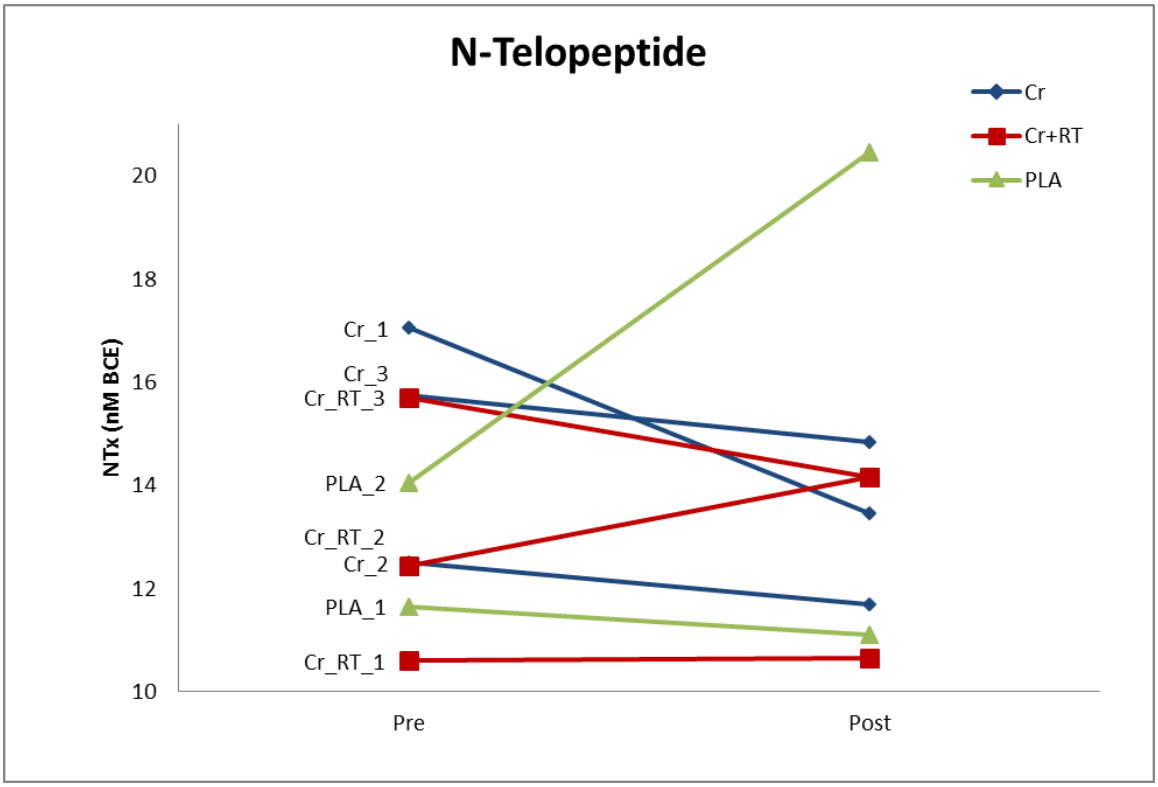

Figure 7. Individual pretest and 12-week posttest values for serum NTx for creatine only (Cr), creatine and resistance training (Cr+RT), or placebo (PLA).

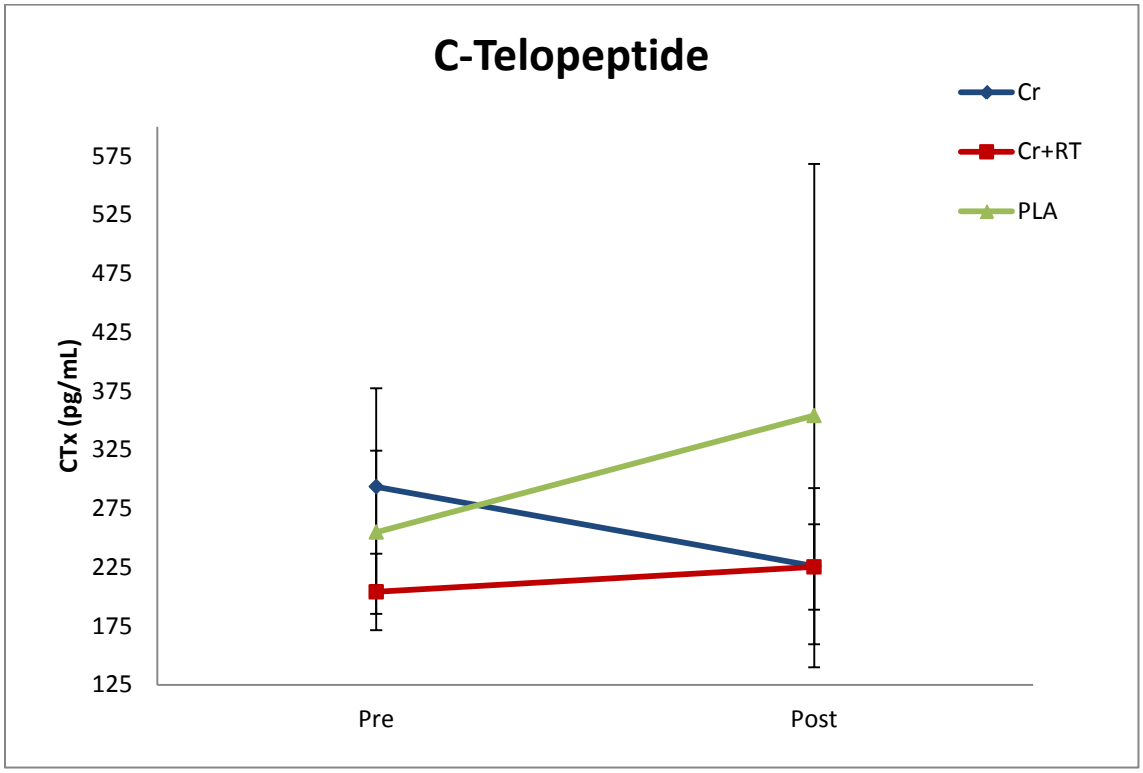

Figure 8. Mean (± *SE)* pretest and 12-week posttest values for serum CTx for creatine only (Cr), creatine and resistance training (Cr+RT), or placebo (PLA) groups.

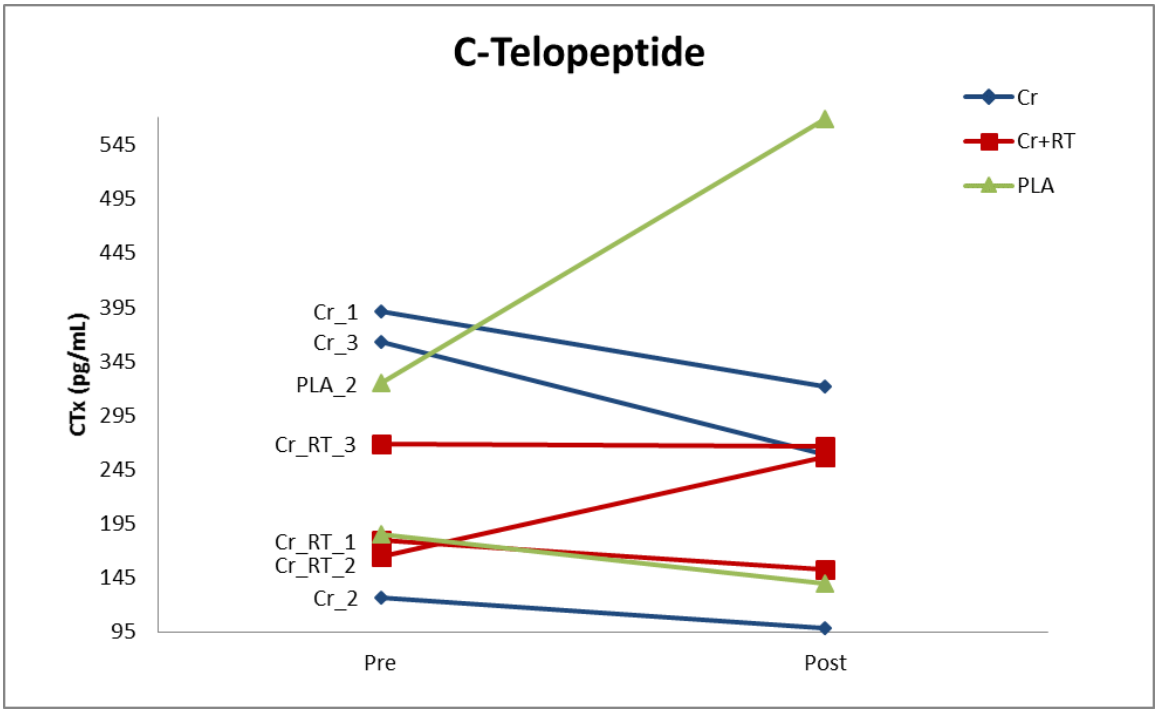

Figure 9. Individual pretest and 12-week posttest values for serum CTx for creatine only (Cr), creatine and resistance training (Cr+RT), or placebo (PLA).

Table 5. Descriptive statistics for bone quality and body composition measures obtained from DXA scans (mean ± *SE*)

DXA	Pretest	Posttest	% Change	ES
BMC Total (g)				
Creatine	3189 ± 206	3168 ± 173	-0.6	-0.03
Creatine + RT	2831 ± 359	2852 ± 351	0.8	0.02
Placebo	2530 ± 128	2576 ± 160	1.8	0.11
BMD (g/cm^3)				
Creatine	1.34 ± .07	1.34 ± .06	0	0
Creatine + RT	1.18 ± .10	1.18 ± .09	0	0
Placebo	1.18 ± .11	1.19 ± .12	0.8	0.04
Lean Total (g)				
Creatine	62713 ± 2779	63823 ± 2594	1.8	0.12
Creatine + RT	62240 ± 7743	64564 ± 7310	3.7	0.09
Placebo	56049 ± 8027	54411 ± 6770	-2.9	-0.08
Fat (%)				
Creatine	20.8 ± 3.1	21.1 ± 2.9	0.3	0.03
Creatine RT	31.0 ± 3.5	30.8 ± 2.9	-0.2	-0.01
Placebo	33.3 ± 2.4	35.8 ± 2.7	2.5	0.33

All values are means from averaged, duplicated pre and duplicated post DXA scans.

Table 6. Descriptive statistics for strength testing (mean ± *SE*)

Strength Test	Pretest	Posttest	% Change	ES
Leg Press (kg)				
Creatine	127 ± 27	152 ± 15	20	0.31
Creatine RT	122 ± 12	189 ± 1	55	0.91
Placebo	100 ± 14	105 ± 15	5	0.14
Knee Extension (kg)				
Creatine	59 ± 3	65 ± 5	10	0.38
Creatine RT	53 ± 6	76 ± 2	43	0.83
Placebo	54 ± 4	57 ± 7	6	0.14
Bench Press (kg)				
Creatine	65 ± 5	71 ± 4	9	0.34
Creatine + RT	51 ± 7	65 ± 8	27	0.47
Placebo	39 ± 11	39 ± 11	0	0

All values are means from averaged, pre and post strength tests.

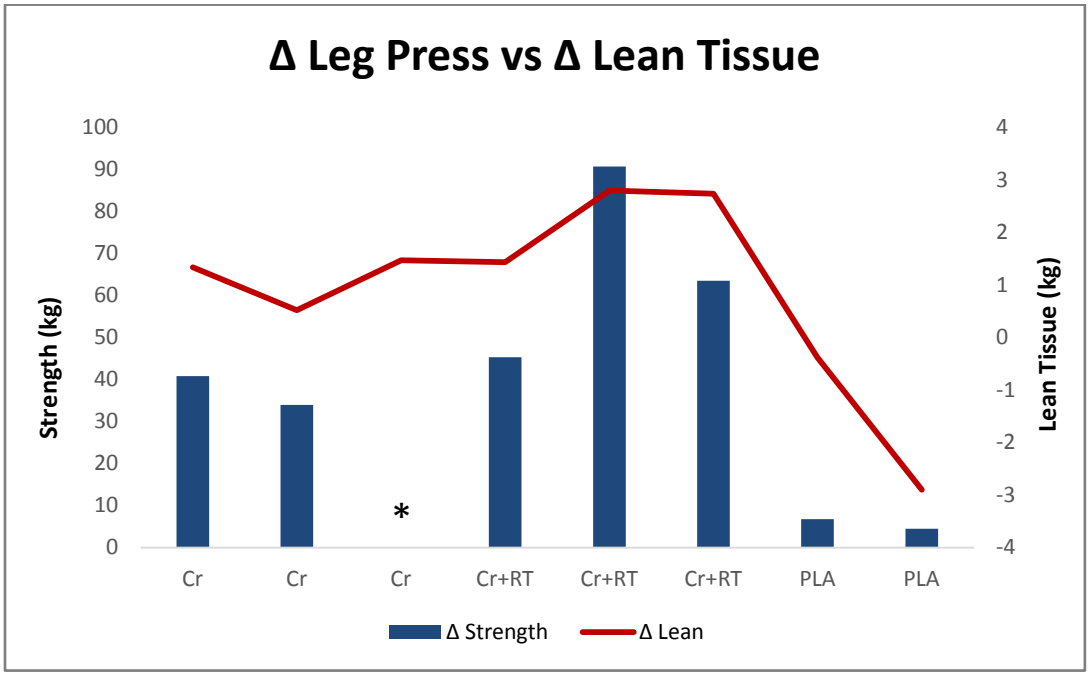

Figure 10. Individual change in leg press strength and lean tissue for 12-week supplementation for creatine only (Cr), creatine and resistance training (Cr+RT), or placebo (PLA).

*** Note:** One individual in the creatine only (Cr) group lifted the maximal weight on the leg press machine pre and posttest, and was recorded as having no change in strength.

DISCUSSION

The primary purpose of this study was to investigate creatine supplementation on aging bone, both with and without resistance training, using serum BTMs for bone formation and resorption. A secondary purpose of this study was to investigate changes in body composition and strength resulting from the creatine and resistance training regimens. This is the first investigation in which the effect of creatine ingestion on serum BTMs, both for bone synthesis and bone destruction, have been reported in this cohort. It is also the first time that a creatine only group was used, free of other confounding supplemental additives, and without the anabolic effects of RT, in a bone study using an older male cohort. That said, this being a pilot study with a low subject number, there are serious limitations to what inferences and conclusions can be drawn in regards to relationships between the independent and dependent variables in the absence of true statistical power.

The main finding of this study was observed differences in patterns of laboratory BTMs between the Cr, Cr+RT, and PLA groups. In both creatine groups, synthesis markers (osteocalcin, PINP) were greater posttest. The greatest effect was seen in osteocalcin, where there was an average of a 13% increase for those in the Cr+RT group (*ES* = 0.28); however the significance of this finding is dampened by a 12% increase in the PLA group (*ES* = 0.13). The increases in PINP for both Cr only, and Cr+RT groups (2.2%, 3.6% respectively) are especially noteworthy though in light of an almost 16%

decrease of this osteogenic marker in the corresponding time period in the placebo group. The BTMs indicating bone destruction (NTx, CTx) were similar in pattern pretest and posttest by group: decreases in the creatine group (NTx = -11.9%, *ES* = -0.40) and (CTx = -23.1%, *ES* = -0.25), unchanged or slightly elevated in the creatine plus resistance exercise group (NTx = 0.8%, *ES* = 0.01) and (CTx = 10.4%, *ES* = 0.18), and elevated in the placebo group (NTx = 22.5%, *ES* = 0.29) and (CTx = 38%, *ES* = 0.21).

The osteocalcin pattern might suggest that Cr+RT has a positive effect on bone synthesis. Osteocalcin is released by osteoblasts during bone synthesis and is often used as a general measurement of bone formation (Brown et al., 2009). Brose et al. (2003) authored the only study on an older population concerning creatine and resistance exercise that measured osteocalcin, but found no change using serum radioimmunoassay. With regard to the PINP pattern, the placebo group displayed the normal “aged bone” state, and showed a decrease in type 1 collagen production. Conversely, both creatine groups actually generated collagen that would be available for osteoid deposition. While this makes sense, and PINP has emerged as a reliable BTM in dose-response studies, this is the first time it has been used in a creatine-related bone study using the older male cohort. It is difficult to relate bone synthesis markers to the existing creatine and bone research due to the lack of comparable studies. While this is an identified limitation, there are numerous examples of studies using osteocalcin and PINP to measure bone turnover. Most recently, Johansson et al. (2014) in a meta-analysis determined that there was a significant association between both serum PINP and CTx levels, and fracture.

The observed noteworthy decrease in NTx (-11.9%, *ES* = -0.40) in those receiving

creatine alone occurred, as was hypothesized, and can be related to findings of other researchers. Studies using similar cohorts (urine based tests) have shown similar decreases in NTx in creatine groups, and increases in placebo groups, also reported as bone collagen equivalents (BCE). Candow et al. (2008) reported a creatine group having a decrease of 27% in urine NTx, while the placebo increased 13% in a RT study using a similar cohort. In a younger cohort, Cornish et al. (2009) reported a 4% decrease in urinary NTx in a creatine group and a 26% increase in the placebo. Also as stated previously, in non-exercise creatine-supplemented studies, both Louis et al. (2003) and M. A. Tarnopolsky et al. (2004) observed urinary NTx decreases (56% and 22%, respectively) in boys with Duchene's muscular dystrophy as reported by Candow and Chilibeck (2010) at a significance level ($p < 0.05$).

This investigation utilized serum NTx and CTx markers which are considered to be more accurate measurements of collagen degradation than urine measurements, due to the necessity of adjusting urine BTM measurements to total creatinine, concentration (Brown et al., 2009; Garnero et al., 2001; Herrmann & Seibel, 2008). These measures have implications for future clinical use. In a non-exercise related investigation Chandani et al. (2000) found a significant correlation between serum NTx and BMD at the femoral neck ($r = 0.26$) in elderly men. The rate of bone loss, and bone turnover, matters as we get older. Both low bone mass and rapid bone loss have been shown to be independent predictors of future fracture risk (Hansen, 1994).

In this study, large increases in NTx and CTx (NTx = 45%, CTx = 75%) were observed in subject PLA_2, much more so than in any other subject, leading to the

suspicion that the changes are more than what would be expected from the normal increase expected from aging. The observed change in bone resorption markers for this subject significantly influenced the group mean values. Midway through the experiment subject PLA_2 reported that he had been recently diagnosed with a neoplasm affecting his balance and hearing. He described his condition as requiring cranial surgery for removal and biopsy. At the time of writing this paper, he was given a tentative diagnosis of having a pituitary tumor that will require biopsy and removal. His condition would have disqualified him from participation and he would have not met the inclusion criteria for the study. However, in the interest of being complete and fully reporting the results of all subjects in the study his data was included in the analysis. Nearly every subject in the study took medication for health conditions common to the age group i.e., diabetes, blood pressure, and emphysema. It may be that the health conditions and medications confounded the results; more rigorous study inclusion criteria may be necessary in future studies. Currently, most creatine studies using this cohort only exclude participation for: diseases that may affect BMC or BMD, vitamin D therapy, bisphosphonates, high levels of calcium ingestion and bone promoting drugs.

The use of BTMs to measure the rate of bone degradation isn't without it limitations, both within, and outside this study. Collection time, fasting status, time proximity to the last exercise session, gender, age, seasonal variation, specimen handling, storage, and shipping are all considerations that must be addressed to in order to limit extraneous variability (Herrmann & Seibel, 2008). In the current study blood collection was conducted with a minimum 12 hour fasting, at least one day removed from RT, and

all samples were immediately processed, frozen at -20ºF and shipped on dry ice. Finally, international osteoporosis groups are calling for an attempt to standardize BTM usage in observational and intervention studies in order to decrease uncertainties in specific subject populations leading to greater fracture prediction and potential routine to clinical use (McCloskey, Vasikaran, Cooper, & Members, 2011).

A secondary finding of this study was a pattern of positive body composition metrics and strength increases in the two creatine groups, although the effects appeared greater in the resistance training group. As reported in other studies BMC and BMD showed little change. While DXA provides a sensitive measurement of BMD, 12 week RT studies appear to be too short in duration to observe bone changes. Kerksick et al. (2007) found no changes in BMC in a male/female cohort (18-45 yrs) RT for 12 weeks. In a 6-month RT study of older adults (men and women), M. Tarnopolsky et al. (2007) failed to observe any changes in BMD. Vincent and Braith (2002) did find a 1.96% BMD change in an older cohort after 6 months of RT without creatine supplementation. The only comparable study, supplementing with creatine, that reported positive DXA results was that done by Chilibeck et al. (2005) using a Cr+RT group for 12-weeks that observed a 3.2% increase in the BMC in the arms of RT older men.

In the current study both creatine groups had an increase in total lean body tissue while body fat percentage was nearly unchanged. Increases in lean body tissue and a decreases in body fat percentage is a common finding in creatine and RT studies using older men and women. Chilibeck et al. (2005) showed a significant correlation between

increases in lean tissue mass and BMC of the arms of older men. In a 14 week study Brose et al. (2003) also found an increase in lean tissue in a RT creatine group (1.7 ± 1.2 kg) compared to placebo (0.4 ± 0.5 kg). M. Tarnopolsky et al. (2007) observed significant positive changes in lean tissue (2.1 kg) and decreases in fat mass (1.9 kg) older men supplemented with creatine and conjugated linoleic acid.

In the current study both creatine groups also had increases in strength. Subjects in the Cr+RT group lifted on average 695 kg·session^{-1}, with 1-minute rest periods between sets. This is the first time, in a bone study using an older male cohort, that a non-exercise group taking creatine showed changes in strength. That said, the changes in the Cr+RT group were more evident with larger increases in strength, lean tissue and greater decreased percentage body fat. In contrast, the placebo group had a pattern that indicated; little to no change in strength, a loss of lean body tissue, and increase in body fat percentage. These findings in the current research indicate a pattern of increased lean body tissue, and strength in the Cr+RT group. These results find their best comparison with Chrusch et al. (2001) who studied 60 to 84 year old men and utilized the same supplement protocol, strength testing method, and resistance training protocol. The results in their study using a Cr+RT group show comparable increases in lean tissue (6.1%), leg press strength gain (50.1 kg), and knee extension strength gain (14.9 kg). More evidence linking the results of the current study to existing research comes from Candow et al. (2008) who found a comparable 5.6% increase in lean tissue and an

increase in bench press strength of 25% using a similar cohort, when supplementing with a protein and creatine mix.

The pattern observed in the current study is noteworthy—where the creatine non-exercise group appears to have emulated, to a lesser degree, the positive attributes of the creatine and resistance training group in increases in total lean mass measurements, no change in body fat percentage and increase in all strength measurements. One weakness in the current study is that the researcher supervised all of the weekly exercise sessions and strength testing. So the pattern of positive changes particularly, gains in strength for the creatine non-exercise group, can be explained by possible rater bias or retest familiarity. The changes in DXA-derived measurements are however likely to be true changes. This isn't the first time increases in strength and lean tissue findings have been observed in a creatine non-exercising cohort. M. A. Tarnopolsky et al. (2004) reported the same results in Duchene's muscular dystrophy boys. Perhaps coincidentally, the boys in his study also had a 22% decrease in urinary NTx. Likewise, Louis et al. (2003) reported 15% increases in maximal voluntary contraction, and 30% decreases in NTx in boys afflicted with muscular dystrophy who took creatine for 12 weeks but did not exercise.

If, as these results suggest, the creatine and/or creatine and resistance training did decrease bone resorption measures while increasing bone synthesis measures, there exists proposed mechanisms to explain why this is so. In the creatine-only group there was an observed pattern of increased PINP, lean body tissue, strength, as well as decreased

NTx/CTx, and body fat percentage which can be explained by the physiological tenet that muscle and bone are intimately related (Candow & Chilibeck, 2010). Many of the stimuli e.g., (chemical, neuronal, mechanical), that affect muscle, positively or negatively, also have the same effect on bone (Candow et al., 2012). Furthermore, bone and muscle follow the same pattern of accumulation during youth and involution during aging throughout the human life cycle (Schoutens et al., 1989).

Contemporary thinking is that bone tissue benefits may arise from the action of creatine on muscles causing an increase in cellular hydration leading to anabolic effects on myogenic transcription factors (Balsom et al., 1995; Saab et al., 2002), increased satellite cell activity (Candow, 2011; Olsen et al., 2006), and altered myofibrillar protein kinetics (Willoughby & Rosene, 2003). It would make reasonable sense that stronger muscles would exert greater forces on skeletal tissues and thereby stimulate additional bone growth. This stress/strain model of increased muscular force on bone would be consistent with increases in PINP, lean body tissue, increased strength and decreases in NTx/CTx.

However, the stress/strain model fails to explain the positive bone benefits observed in the creatine non-exercise group. One explanation is that creatine may also be acting indirectly as a bone signaling agent in a mechanism yet to be fully described (Candow et al., 2012). The mechanism likely involves insulin-like growth factor-1 (IGF1), and/or insulin-like binding proteins (IGF-BPs) (Candow et al., 2012; Louis et al., 2003). Post-exercise IGF mRNA has shown to be up-regulated in the skeletal muscle

tissue of subjects supplemented with creatine (Burke et al., 2008; Deldicque et al., 2005). It is known that IGF-1 stimulates satellite cell differentiation, and proliferation, and increases muscle protein synthesis in vitro (Allen & Boxhorn, 1989). The proposed link between bone tissue and IGF-1, IGF-BP comes from rat studies. McCarthy, Centrella, and Canalis (1989) showed that IGF-I and IGF-II increase bone collagen synthesis and decrease collagen degradation in cultures of rat calavariae. Additionally, a gene knockout study, also using the rat model, showed that a threshold concentration of IGF-1 was necessary to form normal bone growth and also suggested that IGF, and IGF-BP played a prominent role in pathophysiological osteoporosis (Yakar, Wu, Setser, & Rosen, 2002). Finally, most recent investigations have pointed towards the osteocyte as being a primary secretor of IGF-1 (Sheng, Lau, & Baylink, 2014), and there is an ongoing effort to use and interpret transgenic animal studies in order to fully understand IGF's relationship to skeletal growth (Mohan & Kesavan, 2012).

There is another theorized model of action involving creatine and skeletal tissue that is not in competition with ideas surrounding increased muscle strength and load, or IGF signaling. It would also account for the observations in the creatine non-exercise group in the current study. Some contend that creatine, in enhancing the available substrate in creatine phosphate reactions (ADP-ATP), is strengthening cell energetics generally (Wyss et al., 2007). Wallimann et al. (2011) assert that the creatine kinase phosphocreatine system (CK/PCr) works as a temporal and spatial energy buffering system by linking areas of ATP supply at the mitochondria with cytosolic areas of ATP

consumption. In doing so, enhancement of the CK/PCr system through oral creatine supplementation allows for a greater ability to shuttle energy to the sight of ATP consumption. This is most apparent in tissues with high-energy demands. It follows that, because skeletal muscle and bone tissue are highly energy-demanding tissues, Cr supplementation could have a positive effect on their anabolic characteristics. Evidence supporting this idea of spatial buffering and PCr/Cr shutting comes from the study of spermatozoa where CK knockout substances were added along the sperm flagella, and ATP/ADP rates were measured and timed, from the site of ATP production in the head, back towards the site of consumption in the tail. In using this method researchers were able to calculate, using distance and flagellar bending, that ADP diffusion from the tail, as well as ATP diffusion from the mitochondrial head were severely compromised when the PCr/Cr system was knocked out (Kaldis et al., 1996; Wallimann et al., 2011).

The cellular effects of Cr/PC system is an exciting area of interest and while many aspects are not completely understood thus far, there are other studies supporting the potential of creatine, and/or CK, to positively affect bone by enhancing cellular energetics. The metabolic rate and survival of cultured osteoblasts is greater when supplemented with creatine (Gerber et al., 2005). In the rat, creatine kinase is enhanced during the differentiation of osteoblasts (Ch'ng & Ibrahim, 1994). Also, sedentary young rats fed a creatine-enhanced diet were found to have greater lumbar BMD using DXA, and stronger femoral bending loads during three point load to failure testing (Antolic et al., 2007).

The preliminary positive findings in this pilot study set a course for a larger investigation to shed light on the effect of creatine on bone, body composition and strength in older men. Future researchers should use large groups of subjects and control for initial creatine levels, prescription drug BTM interactions, blood collection methods, and creatine ingestion adherence, in order to ascertain if there are statically significant differences between groups. There remains large opportunities for basic science investigations using animal models to answer fundamental physiological questions surrounding the mechanics of creatine's potential actions on bone. There are promising opportunities for applied investigations in special populations away from the traditional areas of creatine and sports performance. An ever increasing age demographic and associated increase in fracture rates in the U.S. population make older subjects a worthwhile focus. Future research using BTMs is emerging as having promise in fracture prediction and as a clinical tool to view a real-time snapshot of a subject/patient's bone status (Johansson et al., 2014). There is a clear path that exists in the current research pointing towards future studies using aged populations and creatine. Experimental designs that minimize other nutritional confounders, use uniform BTM testing methods, and take advantage of successful exercise protocols are needed in order to establish research consistency and add clarity to the handful of prior studies in this area of great public health concern.

In conclusion, supplementation with creatine both alone and with resistance training, appear to affect BTM in older men. PINP in both creatine groups slightly

increased while the placebo group had substantial decrease in that specific bone synthesis marker. With regard to the markers that that indicate bone destruction (NTx, CTx) the placebo group showed very large increases relative to those observed in both creatine groups. Creatine and resistance training also resulted in observable patterns of positive body composition, and strength measures that compared well with the few other studies sharing this design and cohort.

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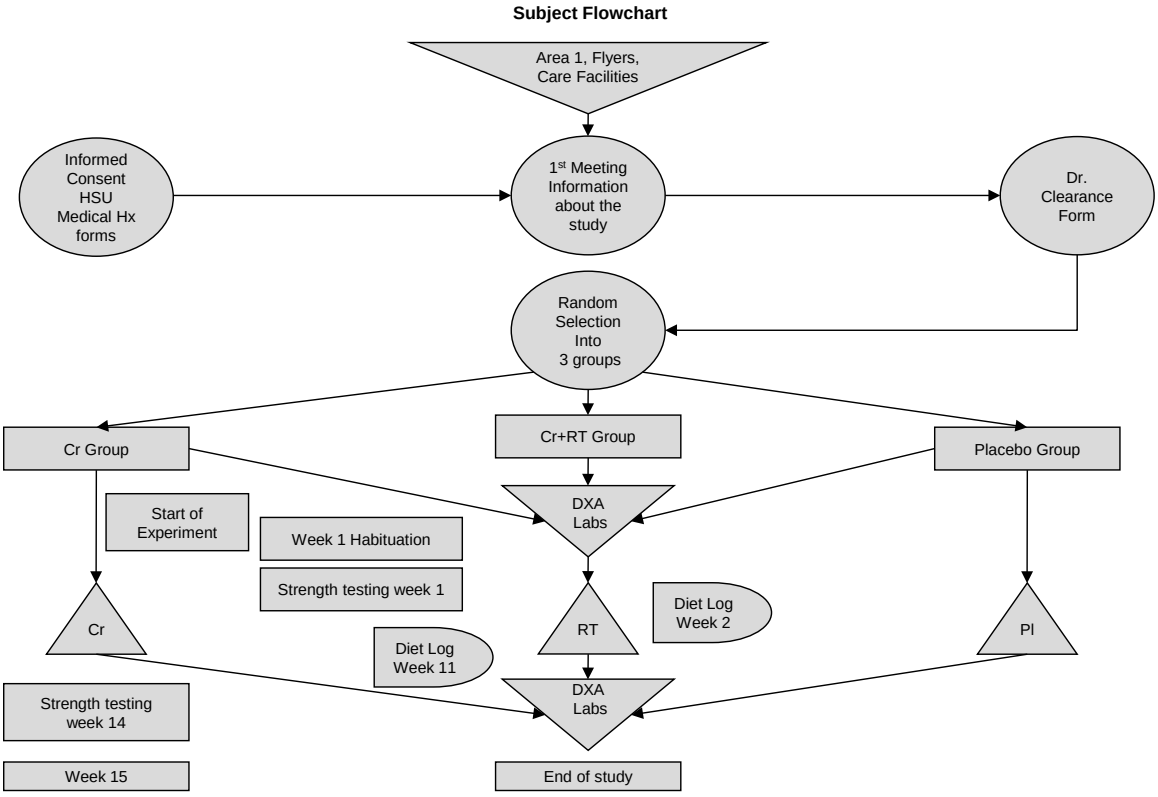
Appendix A: Experimental Design



Appendix B: IRB Approval

Date: 10/4/2013

To: Tina Manos, Jason Ramos

MEMORANDUM

From: Brian Davis
Institutional Review Board for the Protection of Human Subjects
IRB #: IRB 12-175

Title: CREATINE SUPPLEMENTION EFFECTS BONE TURNOVER MARKERS BOTH WITH, AND WITHOUT, RESISTANCE TRAINING IN OLDER MEN

Thank you for submitting your application to the Committee for the Protection of Human Subjects in Research. After reviewing your proposal and revisions, I am able to provide expedited review of your proposal because your research:

will involve the collection of blood samples by finger stick, heel stick, ear stick, or venipuncture as follows: (a) from healthy, nonpregnant adults who weigh at least 110 pounds. For these subjects, the amounts drawn will not exceed 55- ml in an 8 week period and collection will not occur more frequently than 2 times per week; or (b) for other adults and children, considering the age, weight, and health of the subjects, the collection procedure, the amount of blood to be collected, and the frequency with which it will be collected. For these subjects, the amount drawn will not exceed the lesser of 50 ml or 3 ml per kg in an 8 week and collection will not occur more frequently than 2 times

per week.

The Expedited approval of your research will expire on **5/28/2014**. By Federal Regulations, all research related to this protocol must stop on the expiration date and the IRB cannot extend a protocol that is past the expiration date. In order to prevent any interruption in your research, please submit a renewal application in time for the IRB to process, review, and extend the Expedited designation (at least one month).

Important Notes:

Any alterations to your research plan must be reviewed and approved by the IRB prior to implementation.

- Change to survey questions
- Number of subjects
- Location of data collection,
- Any other pertinent information

If Expedited approval is not extended prior to the expiration date, investigators must stop all research related to this proposal.

Any adverse events or unanticipated problems involving risks to subjects or others must be reported immediately to the IRB (irb@humboldt.edu).

The California State University

Bakersfield • Channel Islands • Chico • Dominguez Hills • East Bay • Fresno • Fullerton • Humboldt • Long Beach • Los Angeles • Maritime Academy • Monterey Bay
Northridge • Pomona •Sacramento • San Bernardino • San Diego • San Francisco • San Jose • San Luis Obispo • Sa

Appendix C: Recruitment Flyer

HUMBOLDT STATE UNIVERSITY

Volunteers Needed

Research Study

Effects of Resistance Training & Creatine Ingestion on Bone Density in Older Adults

Participants must be:

Healthy men

Age 65-80 years old

Not currently involved in a resistance training program

What you will do:

Participate in resistance training 3 times per week for 15 weeks

Ingest Creatine 3 times per day for 15 weeks

What you will receive:

Weekly gift cards totaling $300

$200 in cash for completing the study

Free bone density scans and lab work results

Contact Investigator:

Jason Ramos, DC (707) 267-4329

Master's Candidate

Department of Kinesiology

jramos@bluelakecasino.com

Appendix D: STRONG Class Schedule

STRONG

CLASS SCHEDULE

Strength Training Resources for Osteoporosis Northcoast Group

Strength Training, Flexibility and Balance Classes for general health and osteoporosis/fall prevention

STRONG and BETTER BALANCE CLASSES		
ARCATA COMMUNITY CENTER 321 Community Parkway, Arcata	**825-2027**	M, W 10:00–11:30 Fri. 10:00-11:00
BLUE LAKE PARKS & RECREATION Skinner Store (near City Hall)	**Amanda/Kara** **668-5932**	Mon., Wed., Fri. 10:00-11:00
BRIDGEVILLE COMMUNITY CENTER 38717 Kneeland Road, Bridgeville	**777-1775**	Tu., Th. 10:30
DEL NORTE SENIOR CENTER 1765 Northcrest Drive, Crescent City	**464-3069**	Mon., Wed., Fri. 10:00
EUREKA ADULT EDUCATION Winship School Campus, 2500 Cypress, Eureka	**Beth Niemeyer** **444-3387**	M, T, W, Th 1:00
FERNDALE COMMUNITY CENTER – Fortuna Senior Services End of Main Street at Fireman's Park, Ferndale	**786-4141**	Mon., Wed., Fri. 9:00
FERNDALE- ST.MARK'S LUTHERAN CHURCH 795 Berding Street, Ferndale	**786-4434 or 725-9248**	Tu., Th. 5:00
FORTUNA UNITED METHODIST CHURCH 922 N Street, Fortuna	**726-9203**	Mon., Wed. 5:00
FORTUNA PRESBYTERIAN CHURCH-go to the side of the church to the side door, 1431 Ross Hill Road, Fortuna	**(message) 726-9203**	Tu. 3:00
GENERAL HOSPITAL CAMPUS 2200 Harrison Avenue, Eureka	**441-4454**	Tu., Th. 2:30
GENERAL HOSPITAL CAMPUS 2200 Harrison Avenue, Eureka	**Joan** **442-4415**	Mon., Wed. 2:00
GARBERVILLE VETERANS HALL 483 Conger Street, Garberville	**Evelyn** **986-7230**	Mon., Th. 10:30
HOOPA SENIOR CENTER Loop Road, Hoopa	**(530) 625-4834**	Tu., Th. 1:00
HUMBOLDT SENIOR RESOURCE CENTER 1910 California Street, Eureka	**443-9747**	Mon., Wed., Th. 1:00
MC KINLEYVILLE SENIOR CENTER 1620 Pickett Road, McKinleyville	**839-0191**	Tu., Th. 9:30
MC KINLEYVILLE SENIOR CENTER – Beginning STRONG 1620 Pickett Road, McKinleyville	**839-0191**	Tu. 1:30
RIO DELL BAPTIST CHURCH 100 Butcher Street, Rio Dell	**Susan Neesen** **764-5862**	Wed., Fri. 12:00-1:00
ST. JOE'S COMMUNITY RESOURCE CENTER 38883 Hwy. 299 Willow Creek	**Tamara** **(530) 629-3141**	Monday 1:00
SOUTHERN TRINITY HEALTH SERVICES 321 Van Duzen Road, Mad River	**Cathy** **574-6616**	Tu., Th. 11:00

Coordinated by the Area 1 Agency on Aging. For Further Information call (707) 442-3763 extension 203

4-12-13

Appendix E: Study Intake Form

Study Intake Form

Thank You for calling, I would like to tell you more about study.

Name:_____

Study Design:

❑Purpose, Bone density study, Osteoporosis/Fx
❑ Longitudinal study 15 weeks
❑ Three groups (Cr+RT, Cr, Pl)
❑ Randomize, equal chance
❑ Cr ingestion (safe, well studied, ergogenic/sports aid)
❑ Bone density using DEXA, blood markers

Phone:_____

Email:_____

Add:_____

Preferred:_____

So I don't waste your time, I can't receive personal medical information until consent

Inclusion/Exclusion Criteria:

❑ 15 weeks, extended travel plans, transportation issues to and from HealthSport
❑ Hx of kidney, or liver disease
❑ Currently on Vitamin D therapy, bisphosphonates, bone medications
❑ Healthy, ambulatory,
❑ Can have osteoarthritis, old sports injuries, surgeries etc.
❑ 65-80 year of age

STOP/CONTINUE- For any of the reasons we just talked about do you think you able/unable

Subject Requirements/Expectations:

❑ RT 3X week for 14 weeks (Nov.14 through Feb. 14th)
❑ Show up to hospital for DEXA and blood draw 2 times, start finish
❑ Ingest Cr, 2-3 times per day for 12-13 weeks
❑ Exchange Cr bag, once per week (I will drop off the creatine to you)
❑ Report, or be contacted once per week
❑ Must get MD-Primary form signed (I'm going to mail/email it)

Here is the good part, what you get for being a subject

Compensation:

❑ $20 gift card once per week for 15 weeks (when I drop off Cr)
❑ $200 cash for completing the study
❑ Those not in RT group will get 3 months paid semi supervised at HealthSport
❑ An opportunity to help us add to scientific knowledge
  - ❑1.5 million Americans broken bones from Osteoporosis
  - ❑ 18 billion dollars per year

Before I continue what questions can I answer, that you might have

Time Sequence for Subjects:

❑ I'm going to send you 2 documents; Med Hx, Primary OK form
❑ Attend the informational meeting Oct. 28th Monday
  - ❑ Decide if you want to participate
  - ❑ Get MD-Primary OK, during week of Oct. 28th, (I will send you the form now)
❑ Be prepared to visit Mad River Hospital on BLANK
  - ❑ Meet in lobby at the main doors at 9 am
  - ❑ Must be fasting, no food/drink that morning, BUT yes drink water

Appendix F: Physician's Clearance Form

Physician Clearance Form

Your Patient _____ would like to participate in a research project that may involve him doing a progressive resistance training program at the local HealthSport exercise facility. We are writing to you to obtain clearance for said individual, given you are most familiar with his full medical history.

The progressive resistance training program includes: 1-2 weeks of equipment habituation, daily direct supervision by a member of the research team, and allows for a 5 minute stationary bike warm up period prior to each session. The RTP consists of a whole body 3 sets of 10 repetitions, 12-exercise strategy, conducted 3 times per week for 12 weeks. Subjects are given a 1 minute break between sets. Intensity will initially be set at 50% of a subject's 1-repetition maximum and increased progressively once the person is able to complete all 3 sets with 10 repetitions with proper form. Upon the end of the 1-2 week habituation period, strength testing measurements will be taken using a multi-trial strategy established in those prior studies. Your patient may also be asked to ingest creatine monohydrate (0.3g·kg for 7 days, 0.07g·kg for 79 days). This supplementation level is established as safe in the research for age and gender. Your patient will also undergo DEXA scans and blood will be taken to measure bone turnover markers. This program will be conducted following the guidelines of the American College of Sports Medicine (ACSM, 2014). All measures will be repeated near the end of the study.

The location for the program will be HealthSport, a non-clinical health/fitness facility. To comply with recommendations established by the American College of Sports

Medicine your patient will need to complete the medical screening questionnaires (attached). We will use this information to determine eligibility for the study. This study has been approved by the HSU Institutional Review Board (IRB 12-175).

Once completed and signed by you, your patient can return this form to us at our Oct.30th first meeting. If you have any questions, please feel free to contact me 707-267-4329 (cell number).

Thank you,

Jason Ramos, DC

HSU Kinesiology Department

Masters Candidate

Subject/Patient: _____

Please check (√) one of the following

Cleared to participate in this resistance training program

Not cleared to participate in this resistance training program

Cleared to participate in this resistance training program with the fallowing restrictions and/or limitations:

_____

_____

_____

_____

_____
Physician's Name (printed)

_____ _____
Physician's Signature Date

_____
Mailing address City State Zip

(_____) _____Phone

Appendix G: First Meeting Power Point

CREATINE SUPPLEMENTATION EFFECTS BONE TURNOVER MARKERS BOTH WITH, AND WITHOUT RESISTANCE EXERCISE

Informed Consent

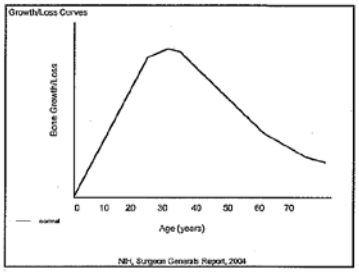
Jason Ramos, D.C.

Informed Consent

- What is Informed Consent?
  - Purpose of the study
  - Who can be a subject?
  - What we are asking you to do?
  - What is creatine monohydrate?
  - The study
  - Confidentiality and information privacy
  - Compensation
  - Voluntary participation

Purpose of the Study

- Bone Strength an Bone Density
  - Osteoporosis = "Porous bone"
- Density loss with Age
  - 5% per year
- Fracture risk with aging
  - Loss of function, ability to move
- Known treatments
  - Medications
  - Physical activity/exercise
  - Creatine Monohydrate



Purpose of the study

- Creatine Monohydrate
  - What is it?
    - Ergogenic aid
  - What does it do?
    - Known effects with exercise
    - Relationship with stronger bones
    - Acts like an anti-oxidant
    - Neuroprotection (Wallimann, 2011)

Who can be a Subject

- To be a subject in this study you must;
  - Be a man between the ages of 65-80 years old.
  - Submit the health questionnaire provided by a member of the research team.
  - Be in good general health.
  - Not have any; stomach, intestinal, liver or kidney problems.
  - Be able to walk on their own, or with minimal assistance (cane, walker).
  - Not be taking medications that increase bone strength, commonly called (bisphosphonates and statins).
  - Not be currently involved in an exercise training program.

1

What are we asking you to do?

- Total time commitment 32 hours over 12 weeks
  - Informal meeting for (1.5 hours)
  - Lab Testing, DXA scan (2-3 hours)
  - Drink the mixture 1-2 times/day, 12 weeks (10 minutes)
    - I see you once per week
  - 5 Minute phone call every week
  - Follow up testing (2-3 hours)

The Study

- What is experimental in this study?
- What are the benefits of participating?
- What are the possible risks or discomforts?
  - How can the risks/discomforts be minimized?

Confidentiality

- How will we keep your information from being seen?
  - PIN = Personal Identification Number
    - Password protection
    - Data
    - Health questionnaire
  - Storage location(s)
  - Destruction

Compensation

- What will you get for being in the study?
  - Qualify for study
    - $20 gift card each week
  - Take creatine, final tests
    - $200
  - $200 payment are made at the end of the study
- No cost to participate in study
  - Exception, transportation

Compensation for Injury

- There is no compensation for injury
- Any injury caused from study must be treated by your personal physician
- You will be responsible for the cost of treatment
  - You may bill your insurance company
  - You must pay any additional costs not covered

Participation

- Your involvement is voluntary
  - Can choose to be involved or not be involved
  - No penalty or loss of benefit
- Stopping before the end of the study
  - You are free to stop at any time
  - Researchers can end your participation
  - 12 week supply if you are selected to start

Questions so far

- Questions about the study?
- Questions about privacy of information?
- Questions about safety?
- Questions about compensation?

Contact information

- Jason Ramos
- 707-267-4329
- jramos@tgc.bluelakerancheria-nsn.gov

If you have any questions about your rights as a subject in this study, please contact the HSU Institutional Review Board for the Protection of Human Subjects in Research at irb@humboldt.edu, or 707-826-3966

Decision Time

- Do you want to participate in the study?
- Thank you for your attention and consideration.

Your job

- Read the consent carefully
- Sign consent form if you are still willing to participate
- Fill out, or turn in, the questionnaire
- Turn in physician's clearance form

Appendix H: Informed Consent Forms

Humboldt State University

Consent to Act as a Research Subject

Effect of Creatine Monohydrate on Bone Density and markers of bone metabolism in Older Men

You are being asked to take part in a research study. The following information will explain what you will be asked to do.

Before you agree to volunteer to be a subject, please:

Read the information carefully; and

Ask as many questions that you need to, so that you completely understand what you will be asked to do.

What is the Purpose of the Study?

This is a research study investigating the effects of the dietary supplement Creatine Monohydrate on the density of bone and markers of bone metabolism. At the end of the study there should be a greater understanding of if and how Creatine may lessen the net bone breakdown that occurs in older adults. The study will add some clarity to understanding Creatine's effects on bone density with and without resistance training exercise.

What Researchers are Asking You to be a Subject?

Student Researcher

Jason Ramos, DC Humboldt State University
Kinesiology & Recreation Administration
Graduate Student

Faculty Researcher

Tina Manos, Ed.D., CES, CSCS Humboldt State University
Kinesiology & Recreation Administration
Associate Professor

Who Can Be a Subject?

To participate in this study you must:

Be a man between the ages of 65-80 years old.

Submit the health questionnaire provided by a member of the research team.

Be in good general health.

Obtain your doctor's clearance

Not have any stomach, intestinal, liver or kidney problems.

Be able to walk on your own

Not be taking medications that increase bone strength, commonly called

(bisphosphonates and statins).

Not be currently involved in an exercise training program.

We are hoping to recruit 30 subjects for this study.

What Will You be Asked to Do? (Description of the Procedures)

Your total time commitment should be no longer than 63 hours over a 13-15 week period.

An informational meeting will take place at Mad River Hospital and will last approximately 1.5 hours.

- We will explain what you will be asked to do.
- You will be given time to read the consent form
- You are free to ask any questions at any time during the study.
- If you do not wish to volunteer for the study you will be free to leave at this time.
- If you wish volunteer for the study, we will ask you to sign the consent form
- We will ask you to complete two health questionnaires.
- We will review your health history for anything that may exclude you from the study.
- If you are eligible to continue in the study you will be given a form to obtain medical clearance from your doctor.

If you are cleared by your doctor to continue in the study, you will go to Mad River Hospital for a 2 hour visit. At that visit you will:

- Have the density of your hip and spine bones measured using the standard screening machine, called a DEXA (dual-energy x-ray absorptiometry). This machine will also measure how much fat is on your body.
- Be asked to provide a 30-mL blood sample (about 2 Tablespoons) in order to measure markers of your bone metabolism.
- Be given the supplement or placebo mixture and instructed on how to take it.

You will take the supplement or placebo mixture at home with water 2 or 3 times per day for 12 weeks, taking about 10 minutes each day. You will be asked to record when you take the supplement or placebo. This part of the study will require approximately 10 hours over the 12 weeks.

We will call you once a week for about 5 minutes to ask if everything is going well, and are you drinking all the required supplements.

There is a 1 in 3 chance that you will also be assigned to a group that will do a strength training program at Health Sport in Arcata. The program will take place 3 times a week for 12 weeks after an initial 1 to 2 weeks when you become used to the equipment. In total, this part of the study is estimated to take 48 hours.

A 2-hour follow-up visit, approximately 12 to 15 weeks following your initial visit, you will repeat the DEXA scan and give a 30-mL sample of blood. You also will be explained what group you were in. The results of your

individual tests for this study will be sent to you following the study.

What is Experimental in the Study?

There is some indication that Creatine may increase bone density. The experimental part of this study is measuring the effect of creatine on bone density and bone metabolism markers in the blood.

What are Your Benefits of Being in the Study?

We cannot guarantee that you will get any benefit from being in the study beyond knowing your bone mineral density when the study is over. The supplementation with creatine may increase bone density in those who get this treatment. If you are selected to participate in the resistance training you may get stronger.

What are the Possible Risks or Discomforts for You?

Potential risks with taking creatine are stomach and intestinal upset.

There is minimal risk associated with the routine medical DEXA scan. This test exposes you to low-dose x-ray with radiation exposure similar to or less than what you would get on a 1.1 hour flight from St. Louis MO to Tulsa OK for each scan. You will get 3 scans at the beginning of the study and 3 scans at the end of the study (hip, spine, and total body).

There is minimal risk associated with giving the two 30-mL blood samples. This

risk includes bruising at the sample site. There is risk of infection and transmission of blood-borne pathogens whenever blood is sampled.

If you do the 12-week resistance training program you may experience discomfort or soreness. You may injury yourself. As is true for any exercise, you may experience an abnormal heart rate, blood pressure and. in rare instances, death.

How will the Possible Risks or Discomforts to You be Minimized?

Creatine will be split into 2 or 3 doses each day and you will be asked to take the supplement with water. The smaller doses should help avoid any stomach and intestinal upset. Anyone with existing stomach, intestinal, liver or kidney conditions should not take creatine.

Trained, experienced, certified technicians from Mad River Hospital will perform the DEXA scans.

Trained, certified phlebotomists from Mad River Hospital will perform the blood draws.

Your risk of exercising will be minimized by review of your medical history; if you are at high risk for complications of exercise, you will not be able to participate. You also will be medically cleared to participate by your doctor. Proper supervision and instruction during the exercise training will be provided by experienced trainers who are certified in CPR and AED. Standardized procedures for training will be followed. Emergency equipment and trained personnel will be available to respond, should they arise during the exercise sessions.

If any new information may affect your choice to be a subject, it will be provided to you.

What Responsibilities Will You Have as a Participant in this Research?

Information you have about your health status, and current or prior cases of unusual feelings with physical effort may affect the value and safety of the exercise you will do as part of this study. You agree to report this information to the primary investigator. You also agree to report to the primary investigator any abnormal feelings you have during the tests. These feelings include extreme fatigue, shortness of breath, chest discomfort, faintness, or similar events. Finally, you agree to tell the primary investigator of any changes in medical status or medication use.

You understand that the primary investigator may stop the exercise if he or she deems that any abnormal responses are occurring.

Do You get any Compensation for being a Subject?

Some compensation is offered for taking part in the study. If you qualify for the study and begin taking the creatine, you will be given $300 in the form of weekly gift cards and cash ($20 card/week for 15 weeks), and $200 cash for completion of the study. You will also get 12 weeks of supervised resistance training at HealthSport if you are randomly selected into one of the study's non-exercise groups.

Is there Any Cost to You?

There are no direct costs to participate in this study. You will have to arrange transportation to and from the two visits to Mad River Hospital. You will have to arrange transportation three times a week to Health Sport in Arcata if selected for the exercise training group.

Will you be compensated for any injury?

You will not be compensated for any injury that may occur during this study. If you are injured form taking part in this study, we will help you find the appropriate treatment. You will be responsible for the cost of treatment. You may bill your insurance company. You will have to pay any costs not covered by your insurance. Humboldt State University will not pay for any consequences related to being a subject in this study.

How will We Keep Your Information Confidential?

We will maintain your confidentiality to the fullest extent of the law.

- We will store hardcopy information in a locked cabinet in the Human Performance Lab at Humboldt State University.
- We will store all electronic information in password-protected computers. Only the student researcher and the faculty advisor will have the passwords for these computers.
- We will only present information as group data.

- We will maintain all information for 5 years. After 5 years, all information will either be shredded or deleted from computer.
-

Inquiries:

Any questions about the procedures used in this study are encouraged. If you have concerns or questions, please ask us for further details.

Freedom of Consent:

Your participation in this study is voluntary. You are free to stop any test at any point.

Contacts:

I understand that the Investigator will answer any questions I have concerning the investigation or procedures at any time. I also understand that my participation in this study is voluntary and that I may stop at any time.

For questions about this study, please contact the Student Researcher or Faculty Research Supervisor using the contact information above.

If you have any concerns with this study, contact the IRB Chair, Dr. Ethan Gahtan, at eg51@humboldt.edu or (707) 826-4545.

If you have questions about your rights as a participant, report them to the

Humboldt State University Dean of Research, Dr. Rhea Williamson, at Rhea.Williamson@humboldt.edu or (707) 826-5169.

Signature:

Your signature below shows your voluntary agreement to participate in this study.

I,_____ have read and agree to participate in the study as described above. ***(Please PRINT Your Name Here)***

_____ _____

(Please SIGN Your Name Here) (Date)

Appendix I: HSU Medical Information Form

Humboldt State University Health and Wellness Institute

Medical Information and History and Release of Liability

Name _____

Address _____

Home Phone _____ Work Phone _____

Age _____ Date of Birth _____ Gender _____

Student ( ) Staff/Faculty ( ) Community ( ) Athlete ( )

The following questions are designed to help us tailor the health and fitness assessment and follow-up counseling to your personal situation. It is extremely important for us to know if you have any medical conditions that may affect your testing process or your progress in our program. Please take the time to answer these questions accurately.

Exercise Capacity

YES	NO	
		Are you capable of sustaining exercise at an elevated heart rate and breathing rate for at 20 minutes
		Do you participate in any upper body aerobic activity? If so, please explain:

	Do you participate in any lower body aerobic activity? If so, please explain:
	Have you been cleared by HSU to participate in athletics?

Medical History

YES	NO	In the past five years have you had:
		1 Pain or discomfort in chest, neck, jaw, or arms
		2 Shortness of breath or difficulty breathing at rest or with mild exertion (e.g., walking)
		3 Dizziness or fainting
		4 Ankle edema (swelling)
		5 Heart palpitations (forceful or rapid beating of heart)
		6 Pain, burning, or cramping in leg with walking
		7 Heart murmur
		8 Unusual fatigue with mild exertion

YES	NO	Have you ever had:
		9 Heart disease, heart attack, and/or heart surgery
		10 Abnormal EKG
		11 Stroke

	12	Uncontrolled metabolic disease (e.g., diabetes, thyrotoxicosis, or myxedema)
	13	Asthma or any other pulmonary (lung) condition
	14	Heart or blood vessel abnormality (e.g., suspected or known aneurysm)
	15	Liver or kidney disease
	16	Thyroid disorder
	17	Are you currently under the care of a physician?
	18	Do you currently have an acute systemic infection, accompanied by a fever, body aches, or swollen lymph glands?
	19	Do you have a chronic infectious disease (e.g. mononucleosis, hepatitis, AIDS)?
	20	Do you have a neuromuscular, musculoskeletal, or rheumatoid disorder that is made worse by exercise?
	21	Do you know of any reason why you should not do physical activity?

If you answered yes to any of these questions, please explain.

Risk Factors

YES	NO	DON'T KNOW		
			1	Are you a male 45 years of age or older?
			22	Are you a female 55 years of age or older
			23	Do you have a father or brother who had a heart attack or heart surgery before age 55?

	24	Do you have a mother or sister who had a heart attack or heart surgery before age 65?
	25	Do you smoke or have you quit in the past 6 months?
	26	Do you know your blood pressure? _____/_____ mmHg-Date:
	27	What is your total cholesterol? _____mg/dL-Date:
	28	Are you taking cholesterol lowering medication?
	29	Do you know your HDL cholesterol? _____mg/dL-Date:
	30	Is your HDL cholesterol > 60mg/dL?
	31	What is your fasting blood glucose? _____ mg/dL – Date:
	32	Do you exercise regularly? If so, explain.

If you answered yes to any of these questions, please explain.

_____

_____

Office Use

BP _____ Cholesterol _____ FBG _____ HDL _____ LDL _____

Health-Related Questions

YES	NO		
		2	Are you pregnant?

33	Are allergic to isopropyl alcohol (rubbing alcohol) or latex?
34	Do you have any allergies to medications, bees, foods, etc.? If so please list
35	Do you have any skin problems?
36	Do you have any other medical condition(s)/surgeries?
37	Have you had any caffeine, food, or alcohol in the past 3 hours?
38	Have you exercised today?
39	Are you feeling well and healthy today?

If you answered yes to any of these questions, please explain.

Medications

Please Select Any Medications You Are Currently Using:

□ Diuretics
□ Beta Blockers
□ Vasodilators
□ Alpha Blockers
□ Calcium Channel Blockers
□ Other Cardiovascular
□ NSAIDS/Anti-inflammatories (Motrin, Advil)
□ Cholesterol
□ Diabetes/Insulin
□ Other Drugs (record below).

Please list the specific medications that you currently take:

I certify that the information I have provided is complete and accurate to the best of my knowledge.

Date _____ Signature of Subject _____

Date _____ Signature of Witness _____

Office Use Only

____ Low Risk ____ Moderate Risk ____ High Risk

Appendix J: Diet Log

Subject Nutrient Intake

Three-Day Food Record

Name: _____

Dates: _____

Age: _____

Height: _____

Weight: _____

INTRODUCTION

This booklet is used to record your detailed daily food intake. It is meant to give the researchers some idea of your usual dietary intake. Therefore, it is very important that you do not alter your eating habits while taking part in this study. In other words, do not let the fact that you are writing down what you eat influence your choice of foods. The names of the participants in this study will be kept confidential.

The usefulness of the results of this study depends on the accuracy with which you record your daily food intake. Please write down full details on all the food and drink that you consume each day.

INSTRUCTIONS

The purpose of this diary is to record all of the food (including drinks) which you eat for a three day period. The three day period should include 2 weekdays and 1 weekend day.

Two pages are provided for each day of the three day period.

After each meal or snack that you eat, please write down in **detail** each separate food item you consumed- including the **type** of food (e.g. processed cheese) and the **amount** of food in household measures (e.g. 1 cup of cooked spaghetti). A meal will have to be listed by its separate parts (e.g. fried steak- 8 oz., French fries- 1 cup, coleslaw- 3 tbsp.)

The best way to record the information is by carrying this diary around with you wherever you go. Before going to sleep, you should look over the diary to check that you have not missed anything. Remember to included snacks!

If you eat fast food, you can just list the type of food you ate (e.g. 1 Big Mac, 1 large fries, 1 chocolate milkshake).

The following pages explain the use of household measures, and the description of foods. A sample day's diet sheet is given. Please take the time to read these pages as it will help to make your diet record more accurate.

RECORDING IN THE DIARY

1 Please use household measures. For example:

Cup: vegetables, cereal, fruit, milk, beverages

Tablespoon: sauces, fats (**Tbsp.** = Tablespoon)

Teaspoon: sugar honey, drink mix (**Tsp.** = Teaspoon)

Slices: bread, bacon

Fractions: 1/6th pie

State the **type** of food eaten. For example:

Milk: skim, goat, 1%, 2%, nonfat

Cheese: processed, Swiss, spread

Bread: enriched white, 60% whole wheat, sweet cinnamon bun, bran muffin

Cereal: Sugar Pops, Miniwheats, granola

Meat: hamburger, fried chicken-breast, scrambled eggs, cod fillets

Others: strawberry jam, Blue Bonnet margarine, Caesar dressing, oatmeal cookies

State the **amount** of food eaten. For example:

Cheese: 1 inch cube cheddar, 3 tbsp. lite cream cheese, ¼ cup 2% cottage cheese

Fruit: ½ cup canned peaches, 12 grapes, 1 medium banana

Bread: 2 slices 100% whole wheat, 1 large Kaiser roll

Cereal: ¾ cup corn flakes, 1 shredded wheat biscuit

Meat: 2 cups tuna casserole (tuna, cream of mushroom soup, noodles, peas), 4 thin slices of beef, 1 large slice of meatloaf

Include the manner of cooking: fried, boiled, raw

Remember all alcoholic drinks

Sample

Date: _____

Time	Food Description	Amount	Code
9:30am	Waffles- white flour	3, 8inch by 4 inch	
	Syrup- Maple	½ cup	
	Yogurt-peach, non-fat	125 ml	
	Coffee, 1 tsp. sugar	1 cup	
	Milk (2%)	½ cup	
12:30pm	Sandwich		
	2 slices of whole wheat bread	2 slices	
	salami	4 slices	
	lettuce	1 leaf	
	mayonnaise	1 tsp.	
5:30pm	Spaghetti	1 cup	
	Meat sauce	½ cup	
	Garlic bread	2 slices	
	Milk 2%	2 cups	

Time	Food Description	Amount	Code

Appendix K: Creatine Purity Letter

GNC
LIVE WELL.

Jun 21, 2013

Tina Manos
Dept of KRA, 1 Harpst St
Arcata, CA 95521

Dear Tina,

We appreciate your interest in our company. As the largest manufacturer and retailer of nutritional supplements, we understand that customers depend on us for quality products and quality information.

Creatine Monohydrate -The most abundant and studied form of creatine. Creatine Monohydrate is 99.95% pure combining creatine with one water molecule. Creatine Monohydrate powder is a straight formulation with no additional flavors or alcohol used inthe manufacturing process.

If you have any more questions or comments, please do not hesitate to call our Customer Service Department toll-free at 1-888-462-2548.

Thank you for your interest. We look forward to serving you in the future.

Sincerely,
Leslie
Customer Service Rep

Enclosure(s):

General Nutrition Corporation
300 Sixth Avenue, Pittsburgh, PA 15222
Tel: (412) 288-4600

Appendix L: Strength Testing Form

*first demonstrate proper exercise form and use of the machine

**allow for one warm up set and a 2 minute rest period between trials

Name:_____ Date:_____

Trial	**Leg Press**	**Bench Press**
Warm up		
Trial 1		
Trial 2		
Trial 3		
Trial 4		
Trial 5		
Trial 6		
Trial 7		
Trial 8		
Trial 9		
Trial 10		
Trial 11		
Trial 12		

Appendix M: Strength Training Log

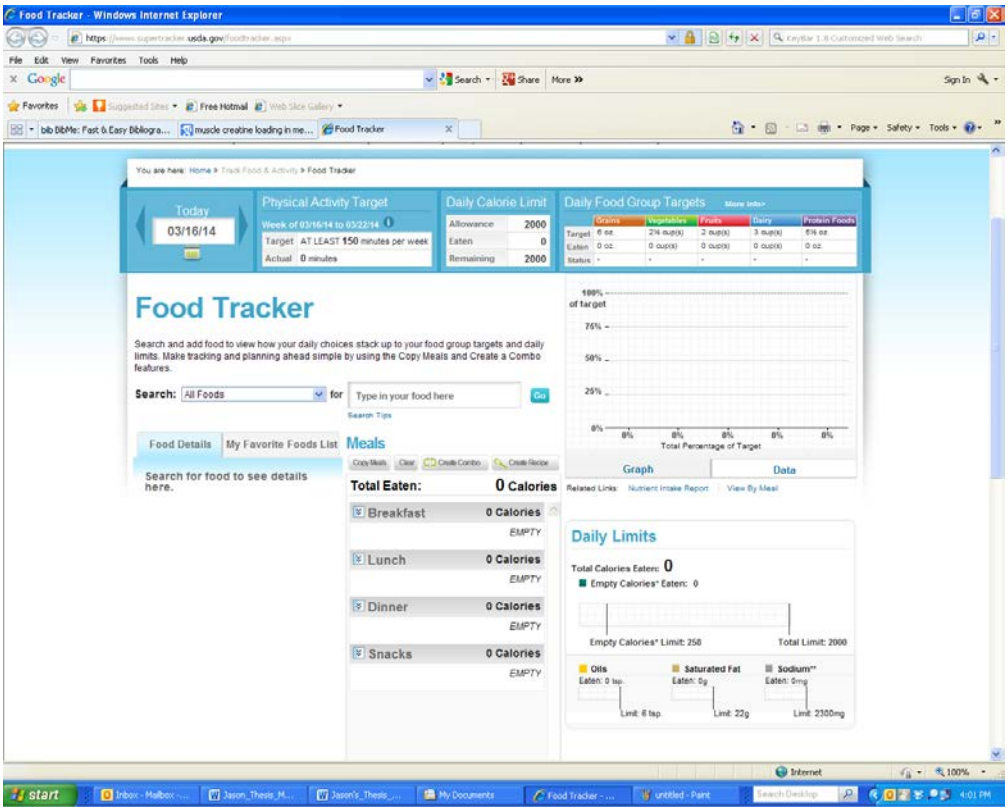
Training Log

Date: _____

Subject#: _____

Exercise	Set 1 (Wt x rep)	Set 2 (Wt x rep)	Set 3 (Wt x rep)
Leg Press			
Chest Press			
Shoulder Press			
Knee Extension			
Knee Flexion			
Hip flexion			
Hip Extension			
Lat Pull Down			
Biceps Curl			
Hip Abduction			
Hip Adduction			
Lumbar Extension			

Appendix N: USDA Food Tracker



Appendix O: Intraclass Correlation Coefficients

Intraclass correlation coefficients

DXA	Pretest	Posttest
Osteocalcin	.993	.992
PINP	.984	.983
NTx	.865	.984
CTx	.995	1.000
BMC	.998	.997
BMD	.994	.995
Lean Tissue	.999	.999
% Fat	.999	.998

Test reproducibility analyzed using SPSS (22.0 for Windows) to compute the intraclass correlation coefficient (ICC) using a two factor mixed effects model and type consistency (McGraw and Wong, 1996; Shrout and Fleiss, 1979)

Appendix P: Specimen Specifications

To see complete test information check the online test directory, www.aruplab.com. You will need to enter the ARUP test number for each assay on interest in the upper right hand corner (search box).

Osteocalcin by Electrochemiluminescent Immunoassay 0020728

Specimen Required

Patient Preparation:	
Collect:	Serum separator tube. Also acceptable: Lavender (EDTA), pink (K2EDTA), or green (sodium or lithium heparin).
Specimen Preparation:	Allow serum tube to sit for 15-20 minutes at room temperature for proper clot formation. Centrifuge and separate serum or plasma from cells ASAP or within 2 hours of collection. Transfer 0.5 mL serum or plasma to an ARUP Standard Transport Tube. (Min: 0.3 mL)
Storage/Transport Temperature:	Frozen.
Unacceptable Conditions:	Hemolyzed specimens.
Remarks:	
Stability:	After separation from cells: Ambient: 8 hours; Refrigerated: 72 hours;
Frozen:	3 months

Procollagen Type I Intact N-Terminal Propeptide 0070236

Specimen Required

Patient Preparation:	
Collect:	Serum separator tube or plain red. Collect all specimens at the same time of day; there is a diurnal variation of PINP and values are higher at night.
Specimen Preparation:	Allow serum tube to sit for 15-20 minutes at room temperature for proper clot formation. Centrifuge and separate serum from cells ASAP or within 2 hours of collection. Transfer 0.5 mL serum to an ARUP Standard Transport Tube. (Min: 0.2 mL)
Storage/Transport Temperature:	Refrigerated.
Unacceptable Conditions:	Plasma. Hemolyzed or lipemic specimens.
Remarks:	
Stability:	After separation from cells: Ambient: 24 hours; Refrigerated: 5 days;
Frozen:	2 months

C-Telopeptide, Beta-Cross-Linked, Serum 0070416

Specimen Required

Patient Preparation:	For patients receiving therapy with high biotin doses (e.g. greater than 5 mg/day), specimen should not be drawn until at least 8 hours after the last biotin administration.
Collect:	Serum separator tube, pink (K2EDTA), or green (sodium heparin).
Specimen Preparation:	Allow serum separator tube to sit for 15-20 minutes at room temperature for proper clot formation. Centrifuge and separate serum or plasma from cells ASAP or within 2 hours of collection. Transfer 1 mL serum or plasma to an ARUP Standard Transport Tube. (Min: 0.5 mL)
Storage/Transport Temperature:	Frozen.
Unacceptable Conditions:	Hemolyzed specimens.
Remarks:	
Stability:	After separation from cells: Ambient: 8 hours; Refrigerated: 8 hours;
Frozen:	3 months

N-Telopeptide, Cross-Linked, Serum 0070500

Specimen Required

Patient Preparation:	
Collect:	Plain red or serum separator tube.
Specimen Preparation:	Transfer 0.5 mL serum to an ARUP Standard Transport Tube. (Min: 0.2 mL)
Storage/Transport Temperature:	Frozen.
Unacceptable Conditions:	Severely hemolyzed specimens.
Remarks:	
Stability:	After separation from cells: Ambient: 5 hours; Refrigerated: 24 hours;
Frozen:	6 months

Appendix Q: Specimen Test Request Forms

Client Name: Humboldt - Jason Ramos						
ARUP Client Number: **121813**						
ARUP Test Number and Name	0020728 Osteocalcin by ECIA					
	0070236 PINP					
	0070416 C-Telopeptide, Beta-Cross-Linked, Serum					
	0070500 N-Telopeptide, Cross-Linked, Serum					
Specimen ID #1	**Specimen ID #2**	**Collection Date**	**Collection Time**	**Frozen**	**ARUP Test Number & Name**	**Subject**
000485218	040541	2/27/2014	8:26 AM	Yes	0020728 Osteocalcin by ECIA	1
000485218	040541	2/27/2014	8:26 AM	Yes	0070236 PINP	1
000485218	040541	2/27/2014	8:26 AM	Yes	0070416 C-Telopeptide, Beta-Cross-Linked, Serum	1
000485218	040541	2/27/2014	8:26 AM	Yes	0070500 N-Telopeptide, Cross-Linked, Serum	1
000485219	040541	2/27/2014	8:27 AM	Yes	0020728 Osteocalcin by ECIA	1
000485219	040541	2/27/2014	8:27 AM	Yes	0070236 PINP	1
000485219	040541	2/27/2014	8:27 AM	Yes	0070416 C-Telopeptide, Beta-Cross-Linked, Serum	1
000485219	040541	2/27/2014	8:27 AM	Yes	0070500 N-Telopeptide, Cross-Linked, Serum	1

Client Name: Humboldt - Jason Ramos						
000485342	052538	2/28/2014	8:48 AM	Yes	0020728 Osteocalcin by ECIA	2
000485342	052538	2/28/2014	8:48 AM	Yes	0070236 PINP	2
000485342	052538	2/28/2014	8:48 AM	Yes	0070416 C-Telopeptide, Beta-Cross-Linked, Serum	2
000485342	052538	2/28/2014	8:48 AM	Yes	0070500 N-Telopeptide, Cross-Linked, Serum	2
000485343	052538	2/28/2014	8:49 AM	Yes	0020728 Osteocalcin by ECIA	2
000485343	052538	2/28/2014	8:49 AM	Yes	0070236 PINP	2
000485343	052538	2/28/2014	8:49 AM	Yes	0070416 C-Telopeptide, Beta-Cross-Linked, Serum	2
000485343	052538	2/28/2014	8:49 AM	Yes	0070500 N-Telopeptide, Cross-Linked, Serum	2
000485344	030935	2/28/2014	8:52 AM	Yes	0020728 Osteocalcin by ECIA	3
000485344	030935	2/28/2014	8:52 AM	Yes	0070236 PINP	3
000485344	030935	2/28/2014	8:52 AM	Yes	0070416 C-Telopeptide, Beta-Cross-Linked, Serum	3
000485344	030935	2/28/2014	8:52 AM	Yes	0070500 N-Telopeptide, Cross-Linked, Serum	3
000485345	030935	2/28/2014	8:53 AM	Yes	0020728 Osteocalcin by ECIA	3
000485345	030935	2/28/2014	8:53 AM	Yes	0070236 PINP	3
000485345	030935	2/28/2014	8:53 AM	Yes	0070416 C-Telopeptide, Beta-Cross-Linked, Serum	3

Client Name: Humboldt - Jason Ramos						
000485345	030935	2/28/2014	8:53 AM	Yes	0070500 N-Telopeptide, Cross-Linked, Serum	3
000485225	031841	2/27/2014	9:11 AM	Yes	0020728 Osteocalcin by ECIA	4
000485225	031841	2/27/2014	9:11 AM	Yes	0070236 PINP	4
000485225	031841	2/27/2014	9:11 AM	Yes	0070416 C-Telopeptide, Beta-Cross-Linked, Serum	4
000485225	031841	2/27/2014	9:11 AM	Yes	0070500 N-Telopeptide, Cross-Linked, Serum	4
000485226	031841	2/27/2014	9:12 AM	Yes	0020728 Osteocalcin by ECIA	4
000485226	031841	2/27/2014	9:12 AM	Yes	0070236 PINP	4
000485226	031841	2/27/2014	9:12 AM	Yes	0070416 C-Telopeptide, Beta-Cross-Linked, Serum	4
000485226	031841	2/27/2014	9:12 AM	Yes	0070500 N-Telopeptide, Cross-Linked, Serum	4
000485329	0111443	2/28/2014	7:35 AM	Yes	0020728 Osteocalcin by ECIA	5
000485329	0111443	2/28/2014	7:35 AM	Yes	0070236 PINP	5
000485329	0111443	2/28/2014	7:35 AM	Yes	0070416 C-Telopeptide, Beta-Cross-Linked, Serum	5
000485329	0111443	2/28/2014	7:35 AM	Yes	0070500 N-Telopeptide, Cross-Linked, Serum	5
000485330	0111443	2/28/2014	7:36 AM	Yes	0020728 Osteocalcin by ECIA	5
000485330	0111443	2/28/2014	7:36 AM	Yes	0070236 PINP	5

Client Name: Humboldt - Jason Ramos						
000485330	0111443	2/28/2014	7:36 AM	Yes	0070416 C-Telopeptide, Beta-Cross-Linked, Serum	5
000485330	0111443	2/28/2014	7:36 AM	Yes	0070500 N-Telopeptide, Cross-Linked, Serum	5
000485336	021347	2/28/2014	8:30 AM	Yes	0020728 Osteocalcin by ECIA	6
000485336	021347	2/28/2014	8:30 AM	Yes	0070236 PINP	6
000485336	021347	2/28/2014	8:30 AM	Yes	0070416 C-Telopeptide, Beta-Cross-Linked, Serum	6
000485336	021347	2/28/2014	8:30 AM	Yes	0070500 N-Telopeptide, Cross-Linked, Serum	6
000485337	021347	2/28/2014	8:30 AM	Yes	0020728 Osteocalcin by ECIA	6
000485337	021347	2/28/2014	8:30 AM	Yes	0070236 PINP	6
000485337	021347	2/28/2014	8:30 AM	Yes	0070416 C-Telopeptide, Beta-Cross-Linked, Serum	6
000485337	021347	2/28/2014	8:30 AM	Yes	0070500 N-Telopeptide, Cross-Linked, Serum	6
000485339	020747	2/28/2014	8:38 AM	Yes	0020728 Osteocalcin by ECIA	7
000485339	020747	2/28/2014	8:38 AM	Yes	0070236 PINP	7
000485339	020747	2/28/2014	8:38 AM	Yes	0070416 C-Telopeptide, Beta-Cross-Linked, Serum	7
000485339	020747	2/28/2014	8:38 AM	Yes	0070500 N-Telopeptide, Cross-Linked, Serum	7

Client Name: Humboldt - Jason Ramos						
000485340	020747	2/28/2014	8:39 AM	Yes	0020728 Osteocalcin by ECIA	7
000485340	020747	2/28/2014	8:39 AM	Yes	0070236 PINP	7
000485340	020747	2/28/2014	8:39 AM	Yes	0070416 C-Telopeptide, Beta-Cross-Linked, Serum	7
000485340	020747	2/28/2014	8:39 AM	Yes	0070500 N-Telopeptide, Cross-Linked, Serum	7
000485119	0110542	2/26/2014	9:35 AM	Yes	0020728 Osteocalcin by ECIA	8
000485119	0110542	2/26/2014	9:35 AM	Yes	0070236 PINP	8
000485119	0110542	2/26/2014	9:35 AM	Yes	0070416 C-Telopeptide, Beta-Cross-Linked, Serum	8
000485119	0110542	2/26/2014	9:35 AM	Yes	0070500 N-Telopeptide, Cross-Linked, Serum	8
000485120	0110542	2/26/2014	9:36 AM	Yes	0020728 Osteocalcin by ECIA	8
000485120	0110542	2/26/2014	9:36 AM	Yes	0070236 PINP	8
000485120	0110542	2/26/2014	9:36 AM	Yes	0070416 C-Telopeptide, Beta-Cross-Linked, Serum	8
000485120	0110542	2/26/2014	9:36 AM	Yes	0070500 N-Telopeptide, Cross-Linked, Serum	8