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Nutritional augmentation of resistance training in middle aged adults

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ABSTRACT

Sarcopenia, or the loss of muscle mass, is common in the elderly, contributing to disability and increased health care costs in this population. The effectiveness of resistance training (RT) in blunting age-associated decrements in strength has been widely documented; however, resistance training alone does not appear to fully maintain or restore muscle mass with age. The purpose of this study was to examine the utility of nutritional augmentation in addition to RT in middle-aged adults, a population in which strength declines are present but are not sufficient to generate functional limitations. Thirty-three adults (14 males, 19 females) between 50 and 65 years of age were recruited and randomly placed in one of three groups: 1) exercise + placebo (PLA), 2) exercise + carbohydrate (CHO; 0.4 g/kg body weight), or 3) exercise + protein (PRO; 0.4 g/kg). Exercise consisted of a 12-week resistance training program involving 9 different exercises: shoulder press, chest press, seated row, tricep extension, bicep curl, lateral pull down, leg press, leg extension, and leg curl. During the first 3 weeks of RT, 2 sets of 10-12 repetitions (reps) of all exercises were performed at 70% (high rep, low resistance; HiRep) one rep maximum (1RM) 3 times per week. During weeks 4-12, 2 sets of 6-8 reps at 80% 1RM (moderate rep, moderate resistance; ModRep) were performed on Mondays, HiRep on Wednesdays, and 2-4 reps at 90% 1RM (low rep, high resistance; LoRep) on Fridays. Resistance was not significantly different among groups at baseline and was progressively increased at each training session as tolerated. Supplements were consumed immediately after exercise. Treatment was

double-blinded. At weeks 0, 3, 6, 9, and 12, height, weight, body mass index, and body composition (BIA) were measured. Bone mineral density and content (DXA), whole leg, leg muscle, fat and bone cross-sectional area (pQCT), dynamic strength (1RM), isokinetic dynamic strength of knee extensors and flexors (Biodex), handgrip strength, and macronutrient intake were measured at baseline and 12 weeks. Height, weight, body mass index, body composition, and dietary intake were not different among groups at baseline and significant changes were not detected post-training. Training strength averaged across all exercises increased from baseline ($P < 0.001$) during HiRep (20±3, 24±3, 33±3%; * $P < 0.08$; *p values for PRO vs. PLA), ModRep (13±3, 16±3, 22±3%; *$P < 0.04$), and LoRep (12±3, 15±3, 20±3%; * $P < 0.06$) for PLA, CHO, and PRO, respectively. The average strength gains for all three training types were 15±3, 19±3, and 25±3% for PLA, CHO, and PRO, respectively (*$P < 0.03$). The muscle cross sectional area of the thigh was similar among the groups at baseline, and following treatment, muscle cross sectional area was 35% greater in PRO than PLA ($P < 0.05$). PRO resulted in a slight but significant increase in bone cross sectional area ($P < 0.02$). These results clearly delineate the efficacy of strength training at improving muscular strength in healthy middle-aged adults. While post-exercise protein supplementation may augment strength gains associated with resistance training in this population, the magnitude of this augmentation is relatively small compared with the increases resulting from RT alone.

CHAPTER ONE: INTRODUCTION

As the baby boomer population ages, the number of elderly persons in the United States is expected to grow. This is important in light of the growing body of evidence correlating increasing age with declines in strength, muscle mass and functionality. Combined, these findings have serious implications, not only for individuals in terms of health-related quality of life, but also for the nation in the form of escalating health care costs. In light of this information, the importance of elucidating interventions effective in slowing and/or reversing age-associated declines in muscular strength, and, subsequently, maintaining functionality, independence and quality of life is evident. The overall aim of this study was to examine the interaction of nutritional and training interventions in altering body composition and muscular strength in 50-65 year old humans.

CHAPTER TWO: REVIEW OF LITERATURE

The aging population in the United States and other developed countries is growing at an alarming rate. Current projections estimate the elderly population, those 65 years of age and older, will reach 80 million in this country alone by the year 2050. By the year 2030, 1 in 5 Americans will be elderly, compared to 1 in 8 today. Increasing age is associated with a loss of independence, which is largely the result of functional declines that often accompany growing older. This is supported by data from the Survey of Income and Program Participation which revealed a strong relationship between increasing age and the need for personal assistance. In persons aged 65-69, only 9% require personal assistance; however, in the oldest old, those 85 and older – the fastest growing segment of the elderly population, over 50% require living assistance. In 1991, over 4.5 million elderly persons required some form of assistance with activities of daily living (1). As the elderly population grows, this number is expected to grow proportionally.

The aforementioned changes in demographics have the potential to impact federal and global health care costs, as the elderly require a disproportionate amount of health care and health care costs. Over one third of federal spending is directed toward programs for the care of the elderly, and, by 2015, nearly half of federal spending is expected to be used for the care of the elderly - this corresponds to $1.8 trillion per year. While social security and veterans' benefits comprise a small percentage of these costs, the majority of expenditures result from health care

costs. Last year alone, over $473 billion was spent supporting Medicare and Medicaid, and that number is expected to reach $1.2 trillion by 2015 (2).

Declines in muscular strength and size with increasing age have been well documented. The magnitude of these aging-related changes in muscle strength and size is closely related to health and quality of life (3). Decrements in muscular strength of up to 30% occur between the ages of 20 and 75 years of age, with the majority of this decline occurring after 50 years of age (4). Alterations in muscle size at both the macro- and microscopic level occur with age. After 30 years of age, the cross sectional area of the thigh and muscle density, as measured by computed tomography, decline (5), and muscle fibers taken from the vastus lateralis muscle of cadavers of older (70-73 y) men compared to young (19-37 y) men were significantly smaller (6). Type II muscle fibers appear to atrophy with age at a considerably greater degree than type I fibers. Type II fiber size is reduced by 70% in individuals over 80 years of age. These declines in fiber size have been directly linked to age-associated decrements in strength (7).

Additionally, decreased rates of protein synthesis have been observed with age (8-10). Protein synthesis in humans decreases by as much as 38% at middle age (50 yrs) and by 55% with advanced age (70+ yrs) when compared with younger individuals (~20 yrs; 9). Urinary creatinine excretion decreases by approximately 50% from 20 to 90 years of age, demonstrating declines in whole-body creatinine content and total muscle mass (11). While the exact mechanism of skeletal muscle atrophy and the associated declines in strength seen in aging populations have not been precisely identified, the underlying cause is multifactorial and appears to

involve motor unit remodeling (12), changes in hormonal activity (13), denervation, and age-related diseases (14). Age-related declines in physical activity and protein intake have also been implicated as likely contributors responsible for observed declines in muscle mass and function with age (15).

Furthermore, 28% of men and 66% of women over 74 years of age are unable to lift objects weighing more than 10 pounds (16). A similar proportion of men and women report being unable to perform certain household tasks as a result of functional limitations (17). This suggests that declines in muscular strength are closely related to reductions in the ability to execute activities of daily living. Furthermore, strong relationships between self-selected gait speed and muscle strength have been demonstrated (18). Research suggests that leg strength may provide a powerful indicator of operative capacity in the elderly (19),(20). Strong correlations between increasing age and declines in postural stability have been presented by numerous investigators (21-23), and postural instability appears to be powerfully associated with frequency of falls in the elderly (24). Fortunately, even mild exercise has been shown to be beneficial in improving balance, and, therefore, diminishing fall rates in the elderly (25), (26). Because falls often result in injury and subsequent disability, further reducing utility, the importance of improving balance and reducing frequency of falls in the elderly is clear. Furthermore, improvements in strength have been shown to improve psychological parameters, including self-efficacy, emotional health, and a number of Profile of Mood States dimensions (27).

Several strategies have been proposed to stem age-associated muscle loss, including exercise, dietary intervention, and hormone therapy. Resistance training

has been proven to be the most effective of these strategies at attenuating or reversing muscle loss (28-30). Studies examining the effects of resistance training, on average, have shown increases in strength of 2-9% per week in elderly men (31). This is similar to gains in strength seen in younger populations (32), (33). Resistance training is associated with an increase in muscle protein synthesis (Schulte, 2001 15 /id} and improvements in muscular strength and endurance (34). In older adults, resistance training also appears to improve nitrogen balance, therefore enhancing retention and/or augmentation of muscle tissues (35).

Unfortunately, resistance exercise training is only adopted by a very small percentage of elderly individuals over 65 years of age. Fear-related avoidance of activities prevents a large percentage of elderly persons not only from participating in structured exercise programs, but also reduces their tendency to participate in activities of daily living (36). Activity avoidance not only has the potential to compromise quality of life, but, ultimately, leads to further reductions in physical capabilities. These declines in physical function may lead to increased risk of falling, further fueling apprehension and activity avoidance (37). These data are supported by the findings of the 2001 National Health Initiation Survey which revealed that approximately 12% of people over 65 years of age and less than 10% of people over 75 participate in strength training activities regularly (38). This evidence suggests that regular resistance training behavior may best be learned and implemented before reaching the age of 65 years when functional limitations may inhibit the initiation of new activities. Unfortunately, there is a paucity of reports examining the impact of exercise in middle-aged adult humans.

Moreover, investigations of nitrogen balance in the elderly indicate that a large percentage of this population consume diets lacking adequate protein. The World Health Organization (WHO) has estimated protein requirements to be around 0.91 g protein per kilogram body weight per day for persons over 65 years of age. The Recommended Dietary Allowance (RDA) for protein is currently set at 0.8 g protein per kilogram body weight per day in this population. Regrettably, this information is largely based on data collected in young people. Subsequent investigations have revealed that protein requirements in the elderly may be as high as 1.25 g protein per kilogram body weight per day (39). Protein requirements may increase with age as a result of the numerous physiological alterations that occur with time. The elderly often experience a deterioration of appetite and thirst as a result of dampening of the senses, including taste, smell, and sense of thirst. Compounding this problem is the fact that digestion and absorption are curtailed due to reduced production of digestive enzymes, decreased stomach acidity, and impaired gastric motility (40), (41). Moreover, roughly 85 % of the elderly population consumes one or more prescription drugs (42). The effects of prescription drugs on nutrient metabolism and absorption are not fully known.

Despite evidence of increased protein requirements in the elderly, a study by Munro, et al. (43) revealed that of 946 elderly, free-living persons, 50% consumed diets with protein levels below those recommended by Campbell, et al (38). In addition, 25% of those persons surveyed consumed less than the amounts recommended by the WHO. Studies examining intakes of home-bound elderly persons reveal that protein intake in this population is even lower, averaging around

0.67 mg per kilogram body weight per day (44). These data suggest that a large percentage of the elderly population may be in a state of negative nitrogen balance and would benefit from protein supplementation. Data regarding protein intake in middle-aged persons is sparse, but it is possible that protein intake in this population is inadequate as well.

Although body protein stores are small, comprising about 15% of body weight, protein is continually being catabolized and anabolized, leading to a constant demand of amino acids. To maintain and/or enhance protein stores, dietary protein must be adequate. Increases in dietary protein enhance concentrations of intracellular amino acids, resulting in an escalation in rates of protein synthesis (45). During exercise, nutrients are directed toward and utilized to fuel muscle contraction, and energy, which is necessary for muscle contraction, is directed away from protein synthesis. Amino acids also are directed away from protein synthesis and are channeled through gluconeogenic processes to form glucose for additional energy. Furthermore, exercise increases amino acid oxidation and protein catabolism. Gontzea, et al examined nitrogen balance in healthy individuals who initiated an exercise program. Although nitrogen balance was steady prior to exercise, after two weeks of strength training, the subjects fell into negative nitrogen balance (46). While this evidence suggests that persons who exercise regularly have an increase in dietary protein requirements, optimal levels of protein intake in "athletes" are yet to be determined (47, 48). After exercise, amino acid availability and/or energy may be limiting for optimal muscle protein synthesis. Thus, post-exercise protein supplementation has potential to amplify the effects of a resistance training program.

Wolfe recently published data revealing a potent interaction between resistance exercise and post-exercise protein supplementation (49). In this study, rates of amino acid transport into muscle tissue and protein synthesis were observed during four different conditions: 1) rest, 2) rest plus infusion of a balanced mixture of amino acids, 3) after resistance training, and 4) after resistance training plus infusion of amino acids. While inward transport of amino acids was enhanced by exercise or amino acids availability, there appeared to be an additive effect of exercise combined with amino acid infusion. In addition, protein balance was ascertained to be negative at rest and following a bout of resistance exercise, but infusion of amino acids resulted in positive net protein synthesis. Protein synthesis was greatest when amino acids were administered following a bout of resistance exercise.

Although previous studies in our laboratory were short in duration and were conducted with young adults less than 40 years of age, increases in whole body and muscle protein accretion were noted when a protein supplement was ingested immediately following exercise (50). Further research suggested that, similar to the benefits of post-exercise carbohydrate ingestion on glycogen repletion, post-exercise protein ingestion is most beneficial to muscle protein accretion when consumed immediately after exercise versus several hours later (51). Recently, it has been suggested that protein requirements are increased in humans exercising on a regular basis and that supplemental protein with resistance exercise may positively influence muscle hypertrophy and strength in younger subjects (52). Furthermore, investigators have reported gains in muscle mass and strength when protein supplementation was provided immediately after resistance exercise versus

2 hours later in elderly humans (53). In this 12-week study with seven subjects averaging 74 years of age, resistance exercise plus supplementation of 10 g of protein immediately after exercise increased dynamic strength 46%, isokinetic strength 15%, quadriceps femoris cross-sectional area 7%, and mean muscle fiber area 24%. While these effects are certainly positive, the investigators did not define the individual contributions of protein supplementation, increased energy availability, or exercise itself. This information is necessary to allow the design of foods that would optimally augment the benefits of resistance exercise.

The benefits of resistance training extend beyond enhancements in muscle size and strength. Energy expenditure, bone density, and aerobic capacity have all been reported to increase with resistance training. With age, energy expenditure declines in sedentary individuals, contributing to the pandemic of obesity currently afflicting the country, as well as other disease states, including coronary artery disease and cancer. Resistance training is an effective channel by which energy expenditure can be increased, not only due to the caloric cost of the exercise itself, but also because prolonged resistance training results in enhancements of lean body mass, thereby increasing resting energy expenditure as well. Data collected on 18 participants initiating a resistance exercise program revealed a significant 9.3% increase in daily energy expenditure following 8 and 18 weeks of training; however, compensatory changes in energy intake were not observed (54).

Declines in bone mineral content with age have been well documented. Bone loss begins between the second and fourth decades of life at a rate of 0.5 - 1% per year (55). In women, this rate is accelerated during menopause. The stress placed

on bone during weight-bearing activities serves as an osteogenic stimulus. This effect is illustrated by evidence that athletes competing in unilateral activities, such as tennis display higher bone mineral density in their dominate compared to non-dominate arm (56), and retrospective studies have revealed that persons who regularly partake in resistance training have higher bone mineral density than those who do not (57, 58). Furthermore, a strong relationship between lean tissue mass and bone mineral density has recently been uncovered (59, 60). However, the effect of protein supplementation in conjunction with resistance training on the aforementioned parameters has not been previously examined.

Some studies have shown post-exercise protein supplementation to have little or no effect on strength and muscle mass. In a study conducted by Fiatarone, et al, examining the effect of a multi-nutrient supplement consumed immediately after resistance exercise in 100 frail nursing home residents, resistance training had a profound impact on muscular strength, with strength gains, measured by 1RM, averaging 113%, in both the supplemented and exercise only groups (61). Gait speed and stair-climbing power also increased in both groups, 11.8 and 28.4%, respectively. However, the supplement seemed to have no effect on strength, gait speed, or stair-climbing power. While these data suggest that protein supplementation may not be effective in eliciting greater strength gains in exercising elders, they also suggest that improvements in strength are, in fact, translated to improvements in functional capacity.

Because resistance training is associated with improvements in muscular strength and endurance and fat free mass, and, because protein supplementation

has the potential to further enhance these effects, the proposed study has important implications, especially in light of the growing elderly population. Furthermore, the study population we have chosen is unique and relevant. Compliance with lifestyle modifications is often negligible in the elderly. However, compliance can be expected to be much higher among 50-65 year old adults, and it is probable that the benefits of these behavioral modifications would carry over into later years. Furthermore, declines in muscular strength and endurance are significant but relatively new at this age, often prompting motivation for behavioral changes before the ability to exercise is severely limited. Finally, this population has been widely overlooked when studying the benefits of resistance training in combination with protein supplementation, because these studies have primarily been performed in either young or old populations.

The proposed study will also be unique because changes in muscle mass, strength, and function will be examined concurrently with changes in metabolic parameters. Results from this study will help establish potential mechanisms and metabolic changes by which protein supplementation may augment resistance training influences on muscle mass and function. The results of this study are expected to provide evidence whether relationships exist between the functional and metabolic changes seen as a result of exercise or exercise in combination with protein supplementation.

CHAPTER THREE: METHODS

PARTICIPANTS

Thirty-three adults (14 males, 19 females) between the ages of 50 and 65 were recruited to participate in this study. Participants were recruited by hanging fliers (Appendix 1) around the Iowa State campus and throughout the community of Ames, Iowa as well as by word of mouth. Participants were excluded if they were not generally healthy as determined by a detailed medical questionnaire (Appendix 2), if they possessed contraindications to exercise, such as recent myocardial infarction, uncontrolled arrhythmias, unstable angina, third degree heart block, or acute progressive heart failure, or if they had participated in a strength training program within the past 6 months. Subjects were asked to continue with normal activity patterns and to avoid initiating new exercise and/or eating behaviors. Prior to enrollment in the study, the study design was described and written informed consent was obtained (Appendix 3). This research was approved by the Institutional Review Board at Iowa State University. Adverse event evaluations, consisting of questionnaires (Appendix 4) and physical evaluations were performed at three-week intervals throughout the study in order to ensure the safety of the participants. No adverse events resulting from the training, testing, or supplementation protocols were reported.

Three participants were released or withdrew from the study prior to completion. One woman removed herself from the study because of time constraints at week 5, another was released when she developed severe iron

deficiency at week 1, and yet another was released when she developed fluid in her lungs at week 10. All three women were in the exercise plus protein group. Thirty participants (14 males, 16 females) completed a12-week resistance training and supplementation program, and seven served as controls.

TRAINING

The resistance training program consisted of 9 different exercises: shoulder press, chest press, seated row, tricep extension, bicep curl, lateral pull down, leg press, leg extension, and leg curl performed on Keiser® equipment (Keiser Corporation, Fresno, CA) three days per week. Prior to the onset of training, all participants were given a detailed description of equipment and the exercises they would be performing as well as stretching exercises that could be done outside of class (Appendix 5). Prior to each exercise session, all participants completed a 5-minute warm-up consisting of light stretching and aerobic activities. The warm-up was delivered and supervised by an experienced instructor. Each training session was followed by a 5-minute cool-down, consisting of walking and light stretching. During the first three weeks of RT, two sets of 10-12 repetitions (reps) of all exercises were performed on Mondays, Wednesdays, and Fridays at 70% (high rep, low resistance; HiRep) one rep maximum (1RM). During weeks 4-12, two sets of 6-8 reps at 80% 1RM (moderate rep, moderate resistance; ModRep) were performed on Mondays, HiRep on Wednesdays, and 2-4 reps at 90% 1RM (low rep, high resistance; LoRep) on Fridays (Table 1). Strength is expressed as the load that a participant was able to move for a given number of reps (4, 8, or 12 for LoRep,

ModRep, and HiRep, respectively). The percent change from baseline was then calculated. Resistance was not significantly different among groups at baseline (Figures 1 and 2) and was progressively increased at each exercise session.

SUPPLEMENTATION

Participants were matched across three treatments and one control group for age, gender, body weight, and lean mass. Following each exercise session, participants immediately consumed placebo (PLA; Splenda®, McNeil Nutritionals LLC, Ft. Washington, PA, plus Blastin' Berry Cherry Kool-Aid™, Kraft Foods Global, Inc., Glenville, IL), 0.4 g carbohydrate (CHO; sucrose plus Kool-Aid) or 0.4 g whey protein (PRO; Verry Berry Cherry Nectar™, Syntrax Innovations, Inc., Cape Girardeau, MO) per kg bodyweight. PRO consisted of a whey protein isolate free of sugar and fat that could be easily mixed with water and was closely matched for color and taste to the other treatments. Supplement nutrition facts can be found in Appendix 6 - 9. The weight to volume ratio for Splenda® and sugar was calculated, and participants receiving PLA were given a volume-matched quantity of PLA. Treatment was double-blinded. An outside investigator added berry flavoring (Kool Aid®) to Splenda® and sucrose, and supplements were weighed for each individual and placed in coded opaque 16 oz. sports bottles for distribution and consumption. Participants were instructed to add cold water to taste and consume all of the supplement immediately following the completion of each exercise session. Participants then added additional water to the bottle and drank that to ensure that

all of the supplement was consumed. All supplements were well-tolerated, and no reports of gastrointestinal distress were made.

ANTHROPOMETRIC MEASUREMENTS

Height, weight, and body mass index (BMI)

Height was measured to the nearest 0.25 cm using a wall-mounted standiometer at baseline and post-training. Weight was measured to the nearest 0.25 kg at wks 0, 3, 6, 9, and 12 using a balance scale. Shoes were removed for both measurements. BMI was calculated as weight in kilograms divided by height in meters squared at three week increments.

Circumferences

At weeks 0, 3, 6, 9, and 12, upper arm, forearm, waist, hip, and thigh circumferences were measured. All circumferences were measured in centimeters on the horizontal plane on the right side using flexible tape. The upper arm circumference was measured mid-way between the acromion and the olecranon processes. Forearm circumference was measured at the point of the greatest girth of the forearm. Waist measurements were made at the level of the umbilicus. Hip circumferences were measured at the level of greatest circumference of the buttocks, and thigh measurements were made at the level of the greatest girth of the thigh.

Bioelectrical impedance analysis (BIA)

BIA measurements were made using Quantum II equipment (RJL Systems, Clinton Twp., MI) at three-week intervals. Signal electrodes were placed on the base of the second toe and medial joint of the middle finger. Detecting electrodes were placed on the anterior portion of the ankle at the level of the medial mellealus and the posterior portion of the wrist at the level of the ulnar head. Leads were then connected to the appropriate electrodes; resistance and reactance were measured three times, averaged and entered into Cypress Software (Cypress Software, Inc., Langley, WA) along with height, weight and age to determine absolute fat and lean mass as well as percent fat and lean mass. A detailed description of these procedures can be located in Appendix 10. The Quantum II was calibrated at least once per month (Appendix 11).

Dual energy x-ray absorptiometry (DXA)

DXA measurements were taken to estimate absolute and percent lean and fat masses as well as bone mineral content and density at baseline and post-training. All whole body DXA scans were performed by a certified technician (Delphi QDR, Hologic; Bedford, MA). Prior to scanning, female participants completed a midstream urine pregnancy test, and all participants were asked to remove all metal (e.g., zippers, belts, jewelry) as well as their shoes. A technician ensured that participants were placed in the supine position on the center of scanner with arms internally rotated 90 degrees at the elbow joint and legs extended with the feet inverted medially at the ankle joint approximately 30 degrees. After positioning the

ankles, the toes of the participant were taped together to minimize movement. Participants were then instructed to lie still while the scan was conducted. Body composition and bone parameters were then determined from the scans using Hologic software, version 11.1 (Hologic; Bedford, MA). An example of data output can be found in Appendix 12.

Peripheral quantitative computed tomography (pQCT)

Cross sectional area (CSA) of the thigh as well as muscle, fat, and bone CSA were measured using pQCT (Stratec XCT3000, Stratec- A Division of Orthometrix Inc., White Plains, NY). Participants were situated in the seat of the Stratec, and their right leg was passed through the cavity of the machine. The right foot was then anchored, and the positions of the chair and foot receptacle were recorded. These positions were then reproduced during post-training testing. The site of the scan was determined by measuring the distance of the thigh from the lateral epicondyle of the patella to the inguinal crease. This distance was then divided by three to calculate one-third the length of the thigh. That distance was then measured from the lateral epicondyle of the patella to pinpoint the site of the scan (Appendix 13). The scanner was then positioned over the site, and the position of the scanner was recorded. Leg scans were then analyzed for muscle, fat, and bone (Appendix 14) using Stratec XCT version 5.5 software (Stratec - A Division of Orthometrix Inc., White Plains, NY).

STRENGTH DATA

All strength measurements were taken at baseline and after 12 weeks of resistance training.

One repetition maximum (1RM)

A 1RM was determined for chest and leg press on the same Keiser® equipment used for RT. Prior to testing, participants completed a 5-minute warm-up consisting of light resistance and aerobic exercises led by a qualified instructor. The proper seat adjustments were made to the respective machines for each participant. The appropriate resistance for a 10 repetition maximum (10RM) was then approximated based upon gender and muscle size, and the participant performed 10 repetitions at this resistance. Participants were then asked how many more repetitions they felt they could have performed at that resistance. Based upon that information, an appropriate resistance for 5RM was then approximated. The resistance was then increased to a 5 RM, then a 2 RM, and 5 and 2 reps were performed at the respective resistances. Finally, a 1 RM was attempted. All 1RMs were determined within three attempts, and participants rested for at least two minutes between efforts(62).

Knee flexion and extension strength and endurance

Isokinetic dynamic strength of the right knee flexors and extensors was measured at baseline and post-training using an isokinetic dynamometer (Multi-Joint System 3 QuickSet; Biodex Medical Systems, inc., Shirley, NY) at a fixed angular

velocity of 60 and 120 degrees per second. After situating the participant in the chair of the Biodex machine, the participant was strapped in at the waist and above the right knee joint. The right ankle was then affixed to the arm of the machine just below the calf muscle. The position of the chair and arm attachment were recorded and reproduced at week 12 testing. Participants were instructed to perform two gentle "kick and pull" motions to adjust to the speed of the machine. Then, five measurements were recorded at each velocity, and peak torque was ascertained. Muscular endurance of the right knee extensors and flexors was estimated by determining the average peak torque of 30 repetitions of knee extension and flexion on the isokinetic dynamometer at 180 degrees per second. During all measurements, except the two practice attempts, participants were encouraged to exert maximal force. An example of data obtained from testing is provided in Appendix 15.

Handgrip strength

A computer assisted, hand-held dynamometer (J Tech Medical, Heber City, UT) was used to measure handgrip strength for both hands at five different positions of phalangeal flexion. Participants were instructed to hold the dynamometer with their arm bent 90 degrees at the elbow with their shoulder relaxed. The best two of three attempts were recorded and averaged for each position on each hand.

CARDIOVASCULAR FITNESS

Cardiovascular fitness was measured using the Cooper 12-minute walk test. Participants reported to the gymnasium and were taken through a 10-minute warm-up consisting of light stretching and aerobic activities. They were then instructed to walk as quickly as possible, without running, around the gym for 12 minutes. A staggered start was used to prevent participants from walking in groups or feeling as if they were competing with each other. The total distance walked in 12 minutes was calculated and recorded. Testing was done at baseline and post-training.

DIET ANALYSIS

Participants were asked to keep a record of all foods and beverages consumed for 3 days (1 weekend day and 2 weekdays) and were given a handout (Appendix 16) to be used to assist in estimating portion sizes in addition to a food diary. All dietary intake information was collected and entered into a Nutritionist Pro® (First DataBank, Inc., San Bruno, CA) at baseline and post-training to determine macro- and micro-nutrient intakes (Appendix 17).

BLOOD AND URINE ANALYSIS

Participants were instructed to fast for 12 hours prior to blood draws and urine collection. A phlebotomist removed 10 milliliters of blood from an antecubital vein of each participant at baseline and post-training. On the same day, 10 milliliters of urine was collected and immediately refrigerated. Blood samples were centrifuged for 10 minutes at 1500 rotations per minute and refrigerated until they were shipped

along with the urine samples to LabCorp® (Laboratory Corporation of American, Burlington, NC) later that day. The commercial laboratory analyzed the blood samples to obtain a basic chemistry profile, complete blood count, lipid profile, differential and pre-albumin concentrations, and hepatic and renal enzymes. In addition, a complete urinalysis was performed on all urine samples. Basic chemistry profile included tests for blood urea nitrogen (BUN), BUN:creatinine ratio, serum calcium, carbon dioxide, serum chloride, serum creatinine, serum glucose, serum potassium, and serum sodium. The complete blood count included tests for hematocrit, hemoglobin, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), percentage and absolute differential counts, platelet count, red cell count, and white blood cell count. Measurements of total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol (calculation), triglycerides, and very low-density lipoprotein (VLDL) cholesterol (calculation) were included in the lipid profiles. Urinalysis testing included measurements of color, appearance, specific gravity, pH, protein, glucose, occult blood, ketones, leukocyte esterase, nitrite, bilirubin, urobilinogen, and microscopic examination of urine sediment.

STATISTICAL ANALYSIS

Statistical analyses were performed using SAS® statistical software (SAS Institute, Inc., Cary, NC). The effects of gender and treatment by gender and age and treatment by age interactions were evaluated. Statistical differences were not

detected in gender or age interactions, and the data were pooled for analysis. One-way analysis of variance (ANOVA) was used to detect differences among treatment groups, and means ± standard error (SE) are reported. Specific differences between treatments (PLA, CHO, PRO) were determined using the LSmeans t-test procedure. Absolute (unit) and relative (%) changes from initial to post-training were calculated, and data were analyzed similar to that above (ANOVA with LSmeans). Statistical significance was set at $P < 0.05$, and trends were identified as $P < 0.10$. Pearson's correlation coefficient was used to ascertain associations between percent body fat and absolute lean mass measured by DXA and BIA and between percent increase in training strength and change in bone mineral density.

A chronological outline of the experimental design is displayed in Table 2.

CHAPTER FOUR: RESULTS

DEMOGRAPHICS AND ANTHROPOMETRICS

Participants were closely matched for age (54.0±4.7, 55.1±5.2, and 54.0±1.8 years), weight (84.7±17.0, 85.2±14.0, and 84.4±16.7 kilograms), and lean body mass (53.1±1.1.1, 55.8±12.4, and 53.7±12.2 kilograms for PLA, CHO, and PRO, respectively) across all groups (Table 3). Similarly, percent body fat, absolute fat mass, and absolute lean mass did not differ among groups at baseline, and significant changes in the aforementioned parameters were not detected. As expected, there was a strong correlation between percent body fat and absolute lean mass measured by BIA ($r = 0.94$, $P < 0.001$) and DXA ($r = 0.96$, $P < 0.001$; Figures 3 and 4, respectively). Circumferences of the upper arm (33.6±1.1, 33.0±1.1, and 33.3±1.1 centimeters for PLA, CHO and PRO, respectively), forearm (27.7±0.9. 27.9±0.9, 27.7±1.0), waist (97.4±4.4, 100.0±4.4, 93.0±5.1), hips (110.2±3.0, 110.0±3.0, 110.4±3.7), and thighs (55.7±1.4, 58.4±1.4, 59.6±1.7) were not statistically different between the groups at baseline; however, upper arm circumference decreased in PLA (33.6±1.1 v. 32.1±1.1 at baseline and post-training, $P < 0.006$) and PRO (33.3± 1.3 v. 31.0±1.3, $P < 0.001$), and hip circumference decreased in PLA (110.2±3.0 v. 107.4±2.7, $P < 0.004$) and CHO (110.1±3.0 v. 109.5±3.5, $P < 0.01$; Table 4).

Total leg cross sectional area was not different among the three groups at baseline (18,980±934, 18,654±934, 19,992±1095 mm^2 for PLA, CHO, and PRO,

respectively) but increased in CHO with training (18,654±934 v. 19,120±885 mm^2, $P < 0.05$). Leg fat area remained unchanged in all groups. Bone mineral content decreased significantly in CHO only (2,744±186 v. 2477±228 grams, $P < 0.04$; Figure 6). Bone mineral density decreased in CHO (1.27±0.05 v. 1.23±0.05 grams per centimeter squared, $P < 0.05$) and tended to decrease in PLA (1.19±0.04 v. 1.17±0.04, $P < 0.09$), but a change in PRO was not detected (1.23±0.05 v. 1.22±0.05, Figure 7). The muscle cross sectional area of the thigh was similar among the groups at baseline (7,701±943, 9,673±943, and 10,166±1,106 millimeters squared for PLA, CHO and PRO, respectively), and, following treatment, muscle cross sectional area was greater in PRO than PLA (10,887±1032 v. 8,062±880 mm^2, $P < 0.05$; Figure 8).

STRENGTH MEASUREMENTS

Dynamic Strength

Chest press one repetition maximum increased in PLA (57.7±6.4 v. 61.2±6.9 kilograms, $P < 0.005$) and CHO (63.0±6.4 v. 66.5±6.9, $P < 0.008$), and a trend for increasing strength emerged in PRO (53.1±7.5 v. 55.7±8.1, $P < 0.08$; Table 4). Significant increases in leg press one repetition maximum were seen in PLA (438±39.8 v. 522.0±59.7 kilograms, $P < 0.02$) and PRO (378.1±46.7 v. 479±70.0, $P < 0.009$), and CHO demonstrated a trend for increased strength (417.8±39.8 v. 472.5±59.7, $P < 0.09$; Table 5).

Training strength increased for all exercises in all groups on all days, except PLA, which did not exhibit a significant increase in shoulder strength (Tables 6 - 9). Significant differences in change in average training load between PLA and PRO were seen in shoulder press (9.3±5.8 v. 32.0±6.8 % change from baseline, $P < 0.02$), seated row (11.1±3.2 v. 23.1±3.7, $P < 0.02$), leg press (11.0±2.6 v. 20.0±3.0, $P < 0.04$), and overall strength (14.7±2.8 v. 25.3±3.3, $P < 0.03$), and trends for greater strength gains in PRO compared to PLA were seen in pull downs (15.2±4.0 v. 27.6±4.3 % change, $P < 0.06$) and chest press (11.1±2.6 v. 18.2±3.0, $P < 0.09$). Percent change in training load for PRO increased significantly more than CHO in seated row (13.3±3.2 v. 23.1±3.7 % change, $P < 0.05$) and leg press (9.4±2.6 v. 20.0±3.0, $P < 0.02$). Data are displayed in Figures 9 and 10. Differences in change in training load among the treatment groups were not detected for tricep extension, bicep curl, leg extension, or leg curl; however, all training loads for all exercises increased for all groups on all exercises.

Isokinetic Strength

Over the course of the training period, isokinetic strength of the knee extensors measured at 60 degrees per second, increased in PLA and PRO (125.5±15.6 v. 146.1±15.3 Newton-meters, $P < 0.002$ and 120.2±18.3 v. 136.6±17.9, $P < 0.04$, respectively). At 120 degrees per second, knee extensor strength increased in PLA, CHO, and PRO (103.4±11.0 v. 110.0±12.2, $P < 0.008$, 87.0±11.0 v. 96.3±12.2, $P < 0.03$, and 93.9±12.9 v. 107.3±14.2, $P < 0.002$, respectively; Figure

11). Knee extensor strength was increased from baseline in all groups at 180 degrees per second (66.1±7.5 v. 85.6±9.2, 56.0±7.5 v. 68.3±9.2, and 63.1±8.7 v. 79.0±10.7 for PLA, CHO, and PRO, respectively, $P < 0.02$). At 60 degrees per second, knee flexor strength increased in PLA (77.0±9.6 v. 90.5±9.3, $P < 0.02$) and PRO (68.8±12.1 v. 85.6±11.0, $P < 0.02$), but not CHO (74.2±9.6 v. 78.2±9.3; Figure 12).

Static Strength

Peak handgrip strength increased in the right hand of PRO (24.6±5.3 v. 33.3±7.2 kilograms, $P < 0.02$). PLA and CHO did not demonstrate significant improvements in peak handgrip strength in the right (19.5±4.5 v. 26.4±6.1 and 20.5±4.5 v. 27.8±6.1, respectively) or left hand (21.0±3.8 v. 28.5±5.1 and 24.0±3.8 v. 32.5±5.1, respectively; Figure 13).

DIET COMPOSITION

Macronutrient and energy intake were similar among all groups at baseline and did not change following 12 weeks of resistance training and/or protein or carbohydrate supplementation (Table 9).

AEROBIC CAPACITY

On average, participants were able to walk 1.43±0.13 km during the Cooper 12-minute walk test. This places the participants in this study in a state of 'very poor' cardiovascular fitness for age according to averages obtained by the Institute for Aerobics Research, Dallas, TX. In other words, they were untrained. Aerobic capacity did not change in or among groups following the 12-week treatment period.

BLOOD AND URINE ANALYSIS

All blood and urine parameters were within normal ranges, and changes in these parameters were not detected at week 12. Analysis of the basic chemistry profile (Table 10), complete blood count (Table 11), lipid profile (Table 12), albumin concentrations (Table 13), and hepatic and renal enzymes (Table 14) did not reveal differences between the groups at week 12.

CHAPTER FIVE: DISCUSSION

Although middle-aged populations have been largely overlooked in the literature with regard to the benefits of strength training, the improvements in training strength as well as chest and leg press one repetition maximums in all groups in this study establishes that resistance training is an effective means of eliciting strength gains in healthy middle-aged individuals. This effect is evident whether or not nutrient supplement is provided following exercise. Similar gains in strength have been noted in both healthy young and elderly populations (63), (28), and the present data suggest that similar improvements in strength are also achievable in middle-aged persons.

Previous studies exploring the role of post-exercise protein supplementation have yielded inconsistent findings. While some researchers have found protein supplementation to have no effect on muscle size and strength (64), others have seen significant improvements in protein accretion (65) and strength. Data from the present study support the notion that post-exercise protein supplementation does impart some benefit over carbohydrate supplementation or no supplementation at all. The primary advantage of protein supplementation appears to be a gain in training strength. The average increase in average training strength was 15±3 and 18±3% for PLA and CHO, respectively, but was 25±3% for PRO. This change corresponds to a significant difference in the change in training strength observed between the PLA and PRO groups. Furthermore, the changes in training strength for all multi-joint exercises were significantly greater in PRO than PLA. While the

same pattern was not seen in single-joint exercises, strength gains seen in multi-joint exercises are apt to be more translational to changes in functionality compared to gains seen in single-joint exercises, because many activities of daily living involve the recruitment of numerous muscle groups. It is difficult to compare the effectiveness of this strength training program with that of others because of inconsistent reporting in the literature. Gains in strength are often reported as percent gains, but how that percentage is calculated is often not described. Furthermore, while the improvements in training strength were significant for all groups, improvements in chest and leg press 1RM as well as isokinetic strength were less consistent. In all likelihood, this is due to specificity of training. In other words, the participants were able to improve performance using the Keiser® equipment because this is what they trained on; however, because they were not training to improve 1RM or isokinetic strength, the improvements in these parameters were not as great.

Gontzea has previously reported an increase in muscle protein turnover, resulting in negative nitrogen balance, during the first two weeks of a resistance training program in healthy young subjects (66); however, protein balance was achieved and maintained following the first two weeks of the initialization of the program. The study protocol of the current investigation called for daily adjustments of individual training loads. Prior to the onset of the investigation, we were concerned, based upon the data reported by Gontzea (66), that the protein group could make considerable improvements in training strength within the first two to three weeks of training. This would result in higher training loads for that group for

the remainder of the training period, in turn leading to greater overall strength gains. On the contrary, as illustrated in Figures 1 and 2, while the carbohydrate and placebo groups maintained a relatively linear increase in training strength, the protein group demonstrated a sharp increase in training strength following week 6 of the training program. This suggests that increases in lean tissue may have contributed to the greater improvements in training strength seen in the group supplemented with protein. This is supported by the increases in muscle cross sectional area seen in the protein group (Figure 8).

Resistance exercise has long been touted as an effective means of improving body composition in all populations (67), (68). However, the participants in this study did not demonstrate significant improvements in body composition. Similar findings have been reported in the literature. Following 12 weeks of resistance training, Kemmler, et al. reported no significant changes in percent body fat or fat free mass in healthy, postmenopausal women (69). It is possible that the post-exercise supplementation provided in this study, which averaged 132 kilocalories, negated the caloric expenditure resulting from exercise. Beckham, et al. reported that men and women participating in heavy resistance training expended approximately 6.21 and 4.04 kilocalories per minute, respectively (70). These data suggest that participants in this study were expending between 121 and 186 kilocalories per half hour of exercise.

Numerous studies have demonstrated the benefits of resistance training on bone mineral density both acutely (71) and chronically (72). However, several other studies have reported little to no change (73) or even declines (74) (75) in bone

mineral density with resistance training. Discrepancies between studies may reflect the initial bone mineral density of the participants. Winters-Stone and Snow reported in 2003 that the greatest improvements in musculoskeletal parameters with exercise are often seen in participants with the lowest initial values. However, the changes in bone mineral density were not correlated with changes in training strength in this study (Figure 14).

The fact that bone mineral density decreased significantly in the PLA and CHO but not PRO suggests that protein supplementation may help retard bone loss in middle-aged individuals participating in a resistance training program. There are two potential mechanisms by which this may occur. The first is that the increased training load seen in the PRO group is responsible for slowing bone loss, and the second is that protein supplementation alters metabolic factors that play a role in the control of bone density. Nevertheless, the correlation between the change in training strength and the changes measured in bone mineral density is rather low ($r = 0.12$; Figure 14). Therefore, it is unlikely that training load was responsible for the deceleration in bone loss in the PRO group.

Hence, it is possible that alterations in metabolic parameters, such as Insulin-like growth factor –1 (IGF-1), may be the basis for the sparing of bone observed in PRO. These findings are supported by the work of Schurch, et al, who reported an 80% increase in circulating levels of insulin-like growth factor-1 (IGF-1) in frail elders after consuming an additional 20 grams of protein per day for six months (76). Heaney et al described a 14% rise in IGF-1 in adults consuming three extra servings of milk per day (77). IGF-1, which is stimulated by protein consumption, enhances

osteoblast-mediated bone formation, and likely plays a role in the improvements in bone mineral density with protein supplementation seen in this study and others. Other investigators have reported improvements in whole body and femoral neck bone mineral density in participants consuming supplemental protein (78). However, it should also be noted that the protein supplement contained a slightly higher amount of calcium than the other two supplements (approximately 160 versus 5.5 mg, respectively), and this could also potentially contribute to the above findings.

Prior investigations have shown that resistance exercise is an effective means of improving cholesterol profiles, specifically, by increasing concentrations of HDL cholesterol (79). This tendency, however, was not demonstrated in this study. Most likely, this is because the participants in this study had relatively normal levels of HDL cholesterol at baseline. Whereas resistance training is useful in normalizing HDL cholesterol concentrations in persons in which they are compromised, it appears that resistance training does not further improve HDL concentrations in healthy persons.

In a meta-analysis investigating the effectiveness of dietary supplements at enhancing the effects of resistance training by Nissen and Sharp, the authors noted that a major limitation of previous work done to investigate the ergogenic effect of protein is that the protein supplements given to participants are often in the form of a macronutrient mixture (80). The current study is unique in that we were able to administer and examine the effects of protein alone. Furthermore, Nissen and Sharp observed that it is difficult to produce a proper placebo when investigating protein supplements, thereby making it impossible to eliminate the prospect of the

'placebo effect.' Anecdotal evidence suggests that the majority of participants in this study were unaware of which supplement they were consuming. The color, consistency, and taste of the treatments were closely matched, and, because participants were required to consume the supplement from a sports bottle, they were unable to smell the supplement they were consuming. It is possible that the elimination of the olfactory facet of taste was sufficient to mask the smell and taste of the protein supplement used in this study.

Some investigators warn that protein supplementation may be unsafe, and investigations examining safe upper limits of protein intake should be explored further. Potential hazards associated with unwarranted protein intake include damage to the liver and kidneys, gout, calcium loss, or dehydration resulting from amplified urea output (81). However, the participants in this study were closely monitored, and no adverse events were reported, and liver and kidney enzymes were unaffected by protein supplementation at 0.4 g per kilogram body weight in addition to normal daily consumption. The average quantity of supplement provided in this study was roughly 33.8 g, or less than the amount of protein contained in 4 oz. of chicken or beef. This implies that protein supplementation at this level is safe.

In summary, PRO exhibited significantly greater improvements in strength, leg muscle cross sectional area, femur cross sectional area, and handgrip strength and maintained bone mineral density in healthy middle-aged adults. Collectively, the results of this study support the notion that protein supplementation may provide additional benefit beyond resistance training alone.

LIMITATIONS

Differences Between Genders

Statistical analyses were performed on all parameters after separating participants by gender. Differences between men and women for any parameter were not detected, which was most likely a result of small sample size.

Validation of Methodologies

The effects of prolonged protein supplementation in combination with resistance exercise are not known. It could be speculated that prolonged protein supplementation would result in an even greater augmentations muscle strength and ultimately muscle size. However, some evidence suggests that protein requirements are increased only during the first few weeks following the initiation of an exercise program (46). Additional research is needed to evaluate the long-term effects of post-exercise protein supplementation relative to resistance training alone. Carbohydrate supplementation may also be of some benefit in eliciting greater strength gains associated with exercise. While not statistically significant, the gains in training strength observed with CHO were greater than those with PLA.

Investigations into the optimal composition and dose of post-exercise protein supplementation should also be explored. Numerous studies have tested the effects of supplements containing both protein and carbohydrate, but the effects of these supplements have not been compared with the effects of protein or carbohydrate alone.

Although 24-hour food records are commonly used in research to estimate both micro- and macronutrient intake, an abundance of data exists to suggest that problems associated with underreporting and imprecision, including underestimates of energy intake, are commonly found with this form of diet assessment (82). The relatively low caloric intake reported by participants in this study coupled with high variability in the groups suggests that these data may be inadequate

Timing of Testing

Due to time restraints resulting from an impending holiday, we were unable to schedule some of the post-training testing at optimal times. Specifically, tests for isokinetic dynamic strength and post-training one repetition maximum testing were scheduled within two weeks of each other. Furthermore, all final testing was performed during the twelfth week of resistance training. It is possible that adequate recovery time was not allowed for between measurements, therefore, compromising these data.

CHAPTER SIX: CONCLUSIONS

To date, no intervention has proven to be as effective as resistance training in combating declines in muscle mass and strength associated with biological aging. However, the optimal management of sarcopenia is, most likely, a combination of exercise, nutritional, and pharmacological interventions. Further investigation is warranted to determine how best to minimize strength and functional declines associated with increasing age. Like so many prior investigations, this study clearly illustrates the effectiveness of resistance training at improving muscular strength. While the results of this study may suggest that protein supplementation provides further benefit, the magnitude of this augmentation is relatively small compared with the effects of resistance training alone, and additional examination is necessary.

CHAPTER SEVEN: TABLES AND FIGURES

TABLE 1.

Training Schedule

	Weeks 1 - 3	Weeks 4 - 12		
	Monday, Wednesday, Friday	Monday	Wednesday	Friday
Repetitions	10 - 12	6 – 8 (ModRep)	10 – 12 (HiRep)	2 – 4 (LoRep)
Resistance	70% 1RM	80% 1RM	70% 1RM	90% 1RM

Resistance training protocol: ModRep – moderate repetition, moderate resistance training day; HiRep – high repetition, low resistance training day; LoRep – low repetition, high resistance training day; 1RM – one repetition maximum.

TABLE 2.

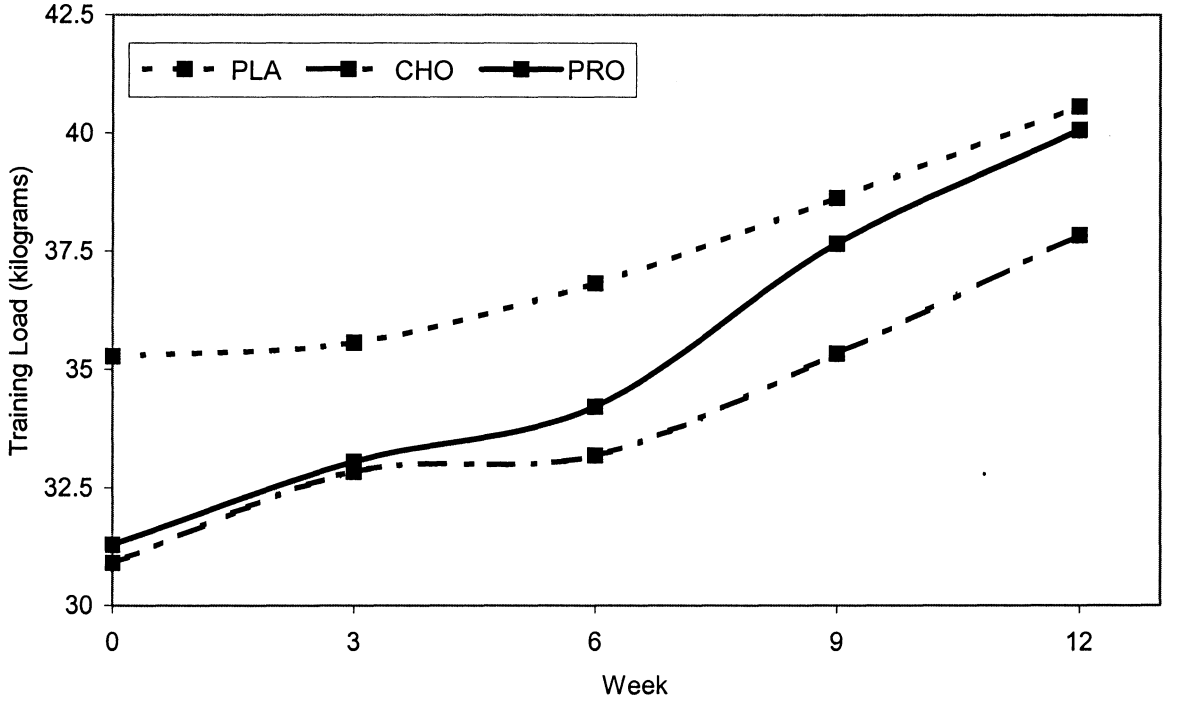
Experimental Design

Week	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13
Resistance Training			X	X	X	X	X	X	X	X	X	X	X	X	
Supplement Consumption			X	X	X	X	X	X	X	X	X	X	X	X	
1RM Testing		X												X	
Diet Record	X													X	
Activity Record	X													X	
Tracer Ingestion	X													X	
Muscle Biopsy	X														X
Ht/Wt, BIA Circum.		X			X			X			X			X	
Biodex, Handgrip		X												X	
DXA, pQCT		X												X	
Blood/Urine Sample	X														X

1RM – one repetition maximum; Ht – height; Wt – weight; BIA – bioelectrical impedance analysis; Circum. – circumferences; DXA – dual energy x-ray absorptiometry; pQCT – peripheral quantitative computed tomography.

FIGURE 1.

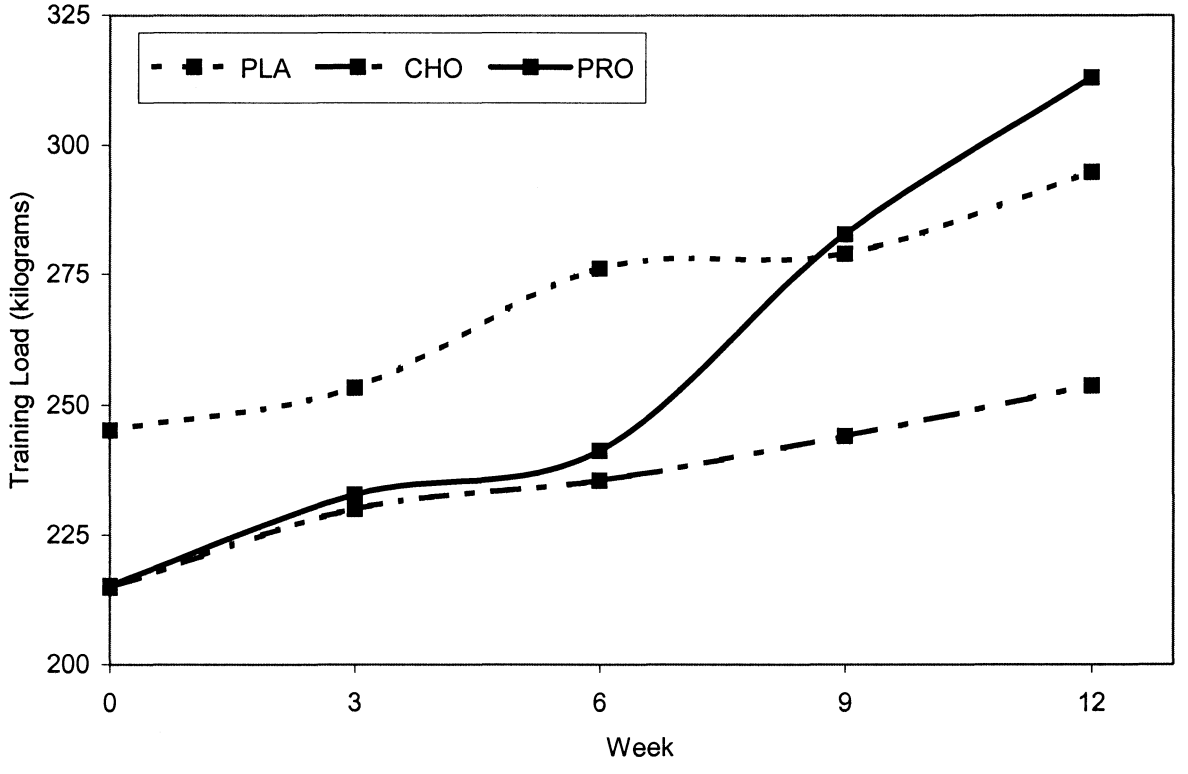
Change in training load by group – chest press



Data are expressed as means. Significant differences among the groups were not detected.

FIGURE 2.

Change in training load by group – leg press



Data are expressed as means. Significant differences among groups were not detected.

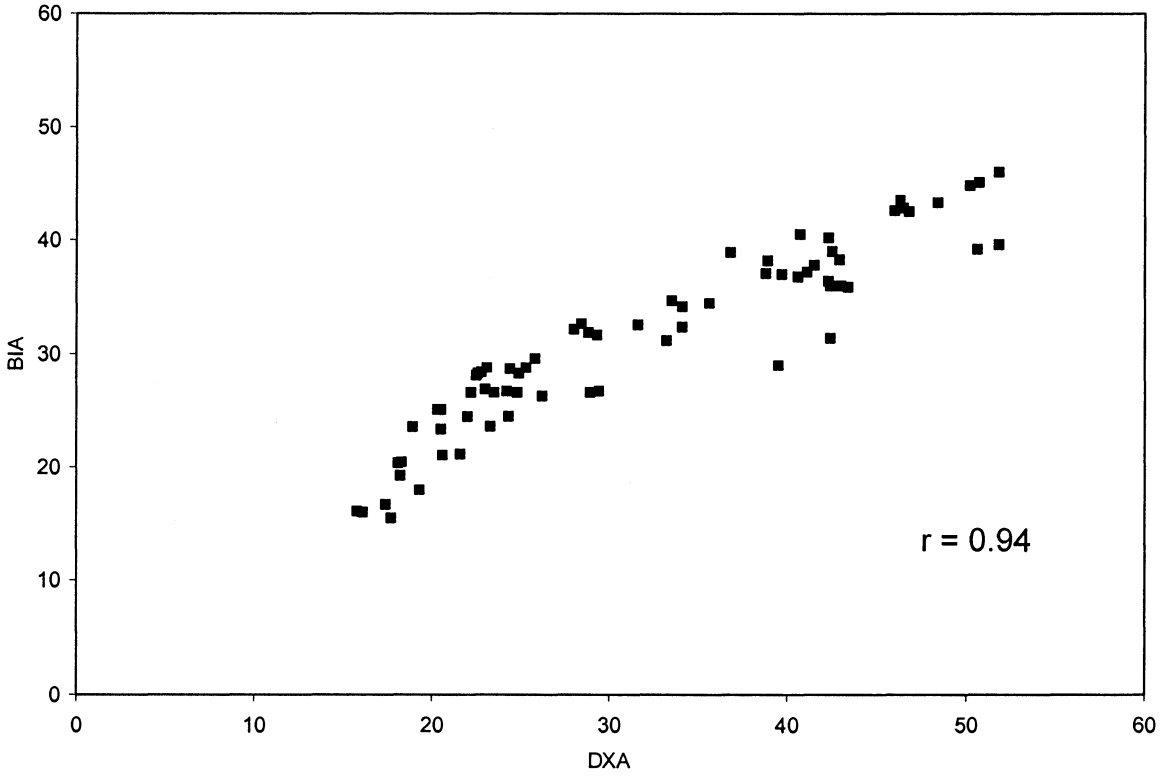
TABLE 3.

Participant Characteristics Prior to Training			
	PLA	CHO	PRO
Subjects (*n*)	11	11	8
Males (*n*)	5	5	4
Age (y)	54.0 ± 4.7	55.1 ± 5.2	54.0 ± 1.8
Body weight (kg)	84.7 ± 17.0	85.2 ± 14.0	84.4 ± 16.7
Lean mass (kg)	55.3 ± 11.1	55.8 ± 12.4	53.7 ± 12.2

Data are expressed as means ± standard errors. Significant differences among the groups were not detected.

FIGURE 3.

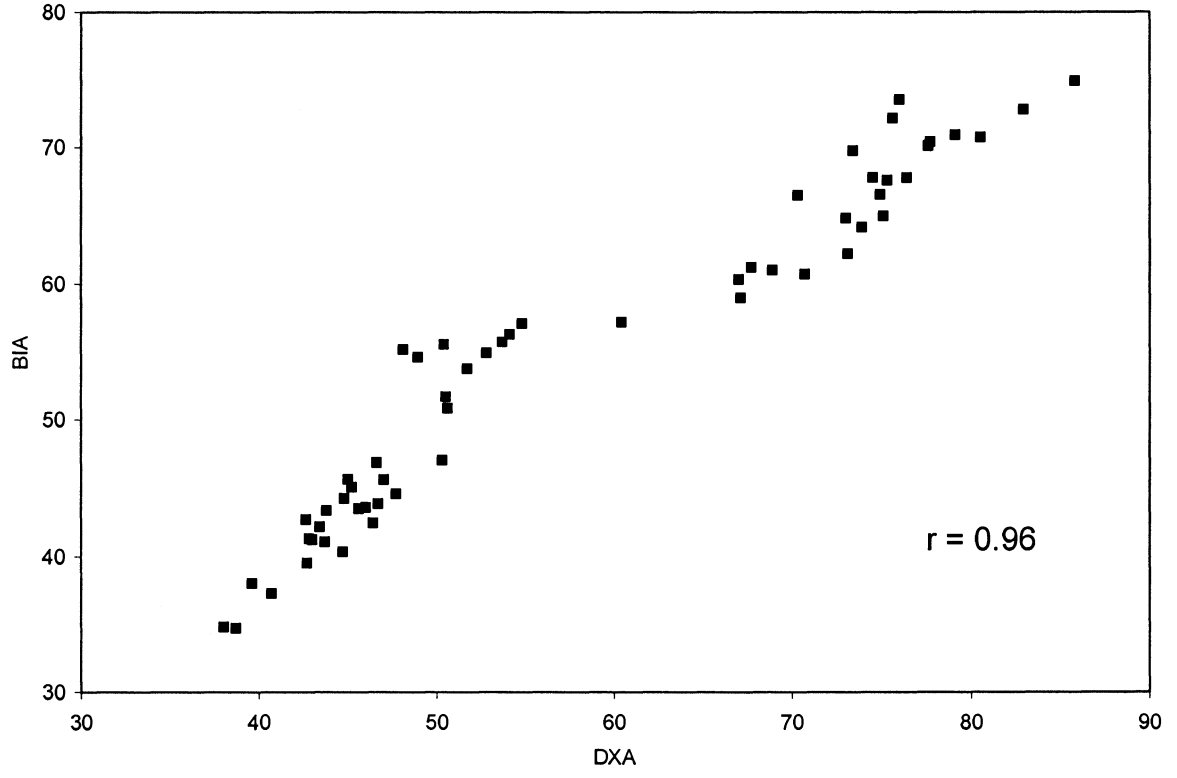
The relationship between percent body fat measured by DXA and by BIA



DXA – dual energy x-ray absorptiometry; BIA – bioelectrical impedance analysis. $P < 0.001$.

FIGURE 4.

The relationship between lean body mass (kg) measured by DXA and fat free mass measured using BIA



DXA – dual energy x-ray absorptiometry; BIA – bioelectrical impedance analysis. $P < 0.001$.

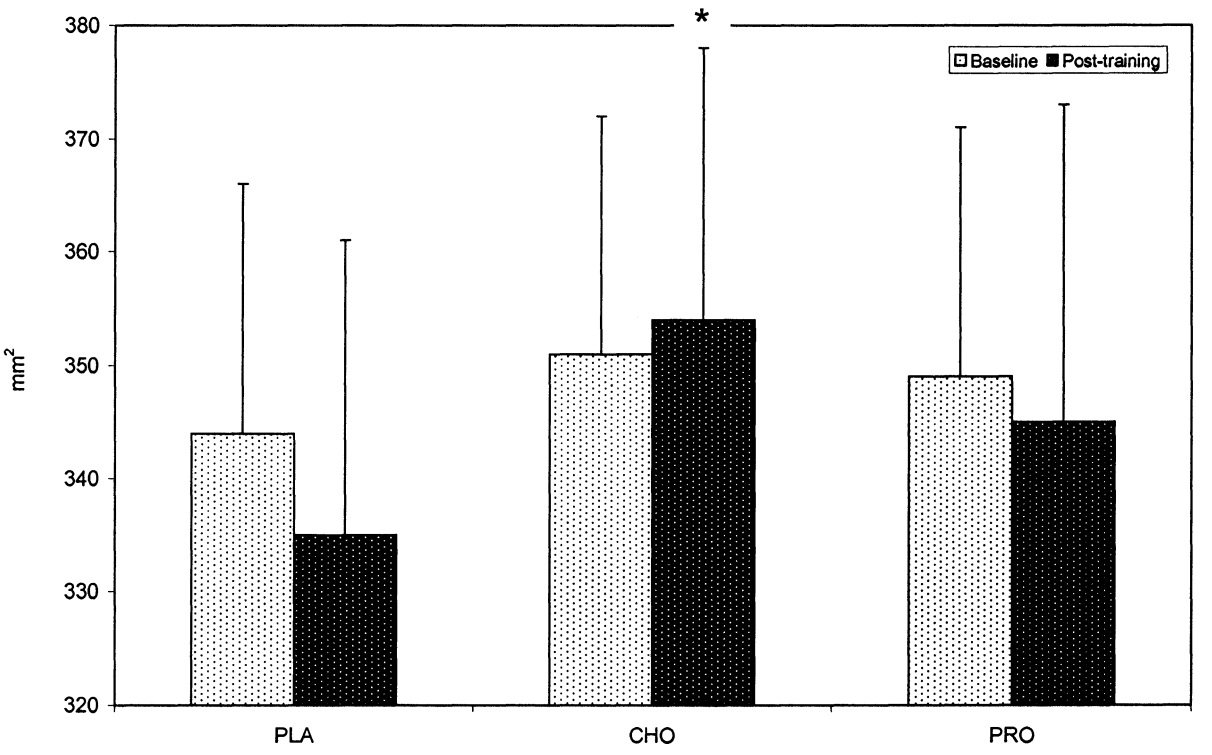
TABLE 4.

Anthropometric circumference measurements (cm)						
Treatment	PLA		CHO		PRO	
Week	0	12	0	12	0	12
Upper arm	33.6 ±1.1	32.1 ±1.1*	33.0 ±1.1	32.3 ±1.1	33.3 ±1.3	31.0 ±1.3*
Forearm	27.7 ±0.9	27.3 ±0.9	27.9 ±0.9	27.7 ±0.9	27.7 ±1.0	27.5 ±1.0
Waist	97.4 ±4.4	96.3 ±4.2	100.0 ±4.4	97.7 ±4.2	93.0 ±5.1	92.0 ±4.9
Hip	110.2 ±3.0	107.4 ±2.7*	110.1 ±3.0	109.5 ±3.5*	110.4 ±3.7	109.1 ±3.2
Thigh	55.7 ±1.4	54.5 ±1.2	58.4 ±1.4	54.5 ±1.2	59.6 ±1.7	56.5 ±1.5

Data are expressed as means ± standard errors (centimeters). * Different from baseline ($P < 0.05$).

FIGURE 5.

Cross sectional area of femur bone

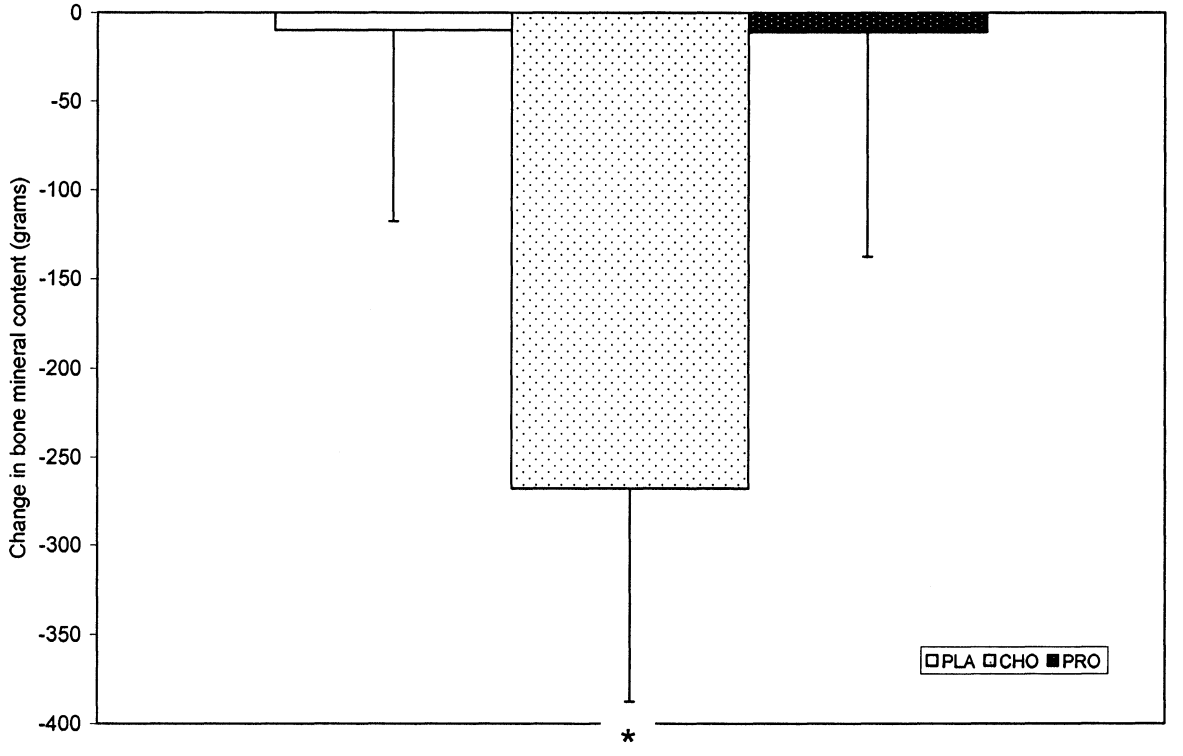


Data are expressed as means ± standard errors. mm – millimeters.

* Different from baseline ($P < 0.02$).

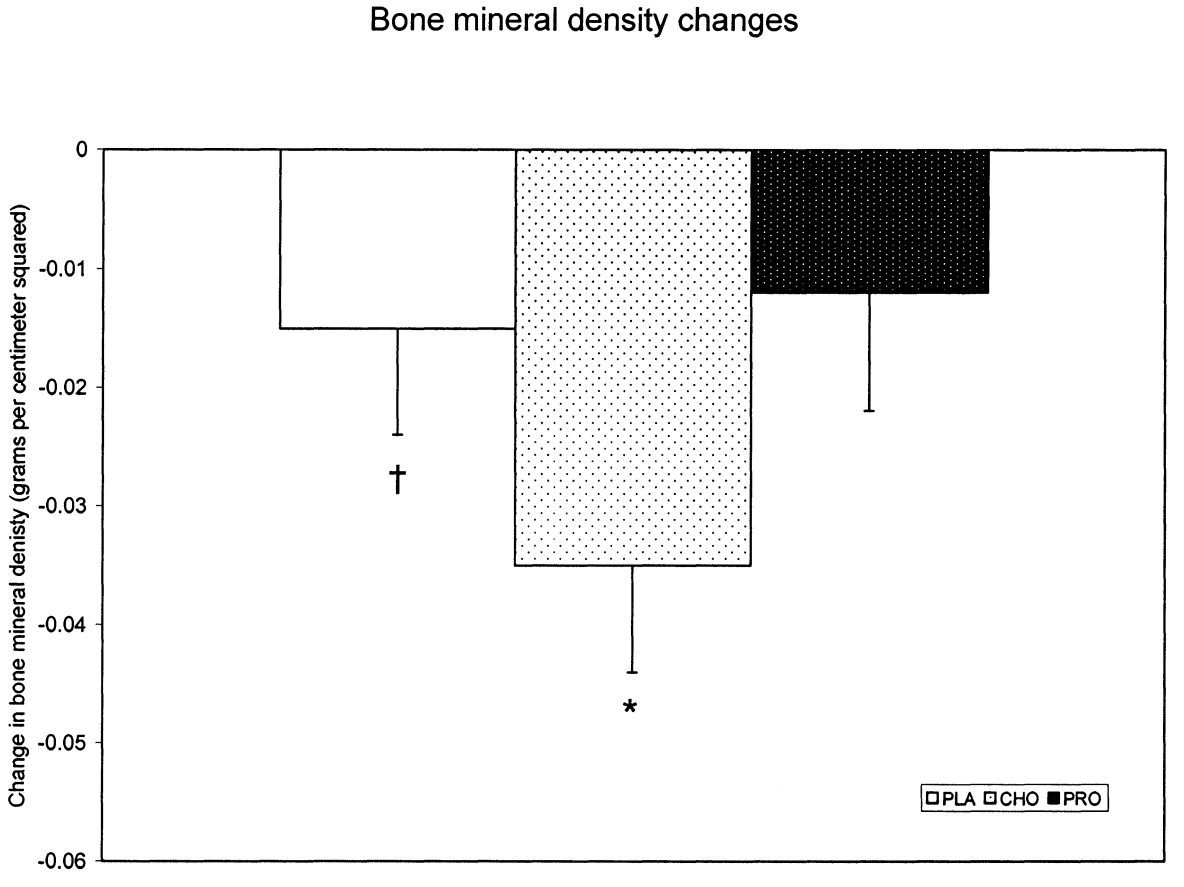
FIGURE 6.

Bone mineral content changes



Data are expressed as means ± standard errors. * Different from baseline value ($P < 0.04$). PLA – placebo group; CHO – carbohydrate group; PRO – protein group.

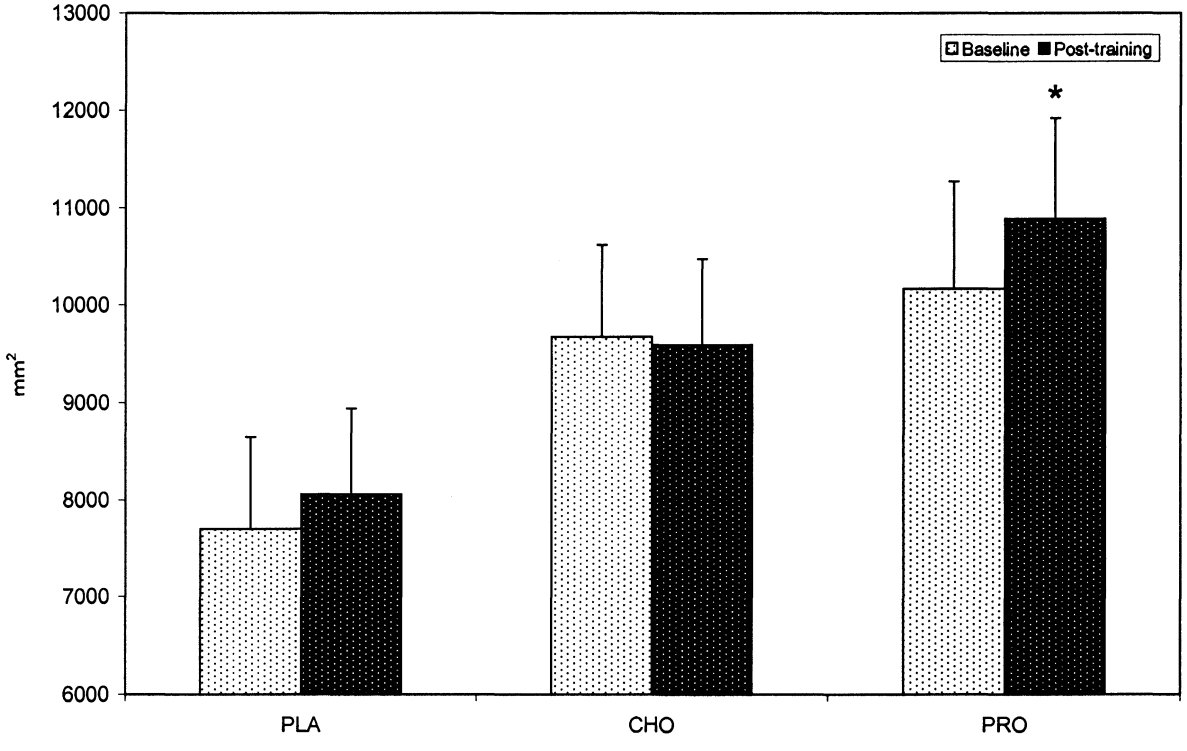
FIGURE 7.



Data are expressed as means ± standard errors. * Different from baseline ($P < 0.05$). † Different from baseline ($P < 0.09$). PLA – placebo group; CHO – carbohydrate group; PRO – protein group.

FIGURE 8.

Leg muscle cross sectional area



Data are expressed as means ± standard errors. * Change from baseline different from PLA ($P < 0.05$). PLA – placebo group; CHO – carbohydrate group; PRO – protein group.

TABLE 5.

One Repetition Maximum						
	PLA		CHO		PRO	
Week	0	12	0	12	0	12
Chest press	57.7 ±6.4	61.2 ±6.9*	63.0 ±6.4	66.5 ±6.9*	53.1 ±7.5	55.7 ±8.1†
Leg press	438.9 ±39.8	522.0 ±59.7*	417.8 ±39.8	472.5 ±59.7†	378.1 ±46.7	479.8 ±70.0*

Data are expressed as mean ± standard errors (kilograms).

* Different from pre-training measures ($P < 0.05$). † Different from pre-training measures ($P < 0.10$). PLA – placebo group; CHO – carbohydrate group; PRO – protein group.

TABLE 6.

Percent improvement in training strength – HiRep						
	Training Data			P value v. baseline		
	PLA	CHO	PRO	PLA	CHO	PRO
Shoulder press	13±12	33±12	49±14†	0.3058	0.0126	0.0023
Pull downs	23±6	28±6	40±7	0.0003	<0.0001	<0.0001
Seated row	15±6	20±6	26±7	0.0257	0.0037	0.0014
Bicep curl	35±9	23±9	35±10	0.0004	0.0128	0.0021
Tricep extension	24±11	43±11	36±13	0.0427	0.0008	0.0122
Leg curl	19±6	22±6	25±7	0.0056	0.0014	0.0022
Leg extension	17±6	22±6	33±7	0.0078	0.0010	<0.0001
Leg press	18±4	13±4	24±5	0.0005	0.0051	<0.0001
Chest press	15±5	15±5	25±6	0.0070	0.0059	0.0002
Average	20±4	25±5	33±5†	0.0001	<0.0001	<0.0001

Data are expressed as means ± standard errors (percent increase). *P* values denote difference from pre-training measures. † Different from PLA ($P < 0.10$). HiRep – high repetition, low resistance training days; PLA – placebo group; CHO – carbohydrate group; PRO – protein group.

TABLE 7.

Percent improvement in training strength –ModRep						
	Training Data			P value v. baseline		
	PLA	CHO	PRO	PLA	CHO	PRO
Shoulder press	8±4	11±4	26±5*,‡	0.0711	0.0131	<0.0001
Pull downs	15±5	23±5	22±6	0.0069	<.0001	0.0009
Seated row	8±3	9±3	22±3*,‡	0.0038	0.0015	<0.0001
Bicep curl	15±5	18±5	28±6	0.0076	0.0021	0.0001
Tricep extension	15±6	31±6	19±7	0.0102	<0.0001	0.0059
Leg curl	11±3	14±3	19±4	0.0044	0.0004	<0.0001
Leg extension	22±6	19±6	27±7	0.0006	0.0023	0.0003
Leg press	9±3	9±3	21±4*,‡	0.0065	0.0073	<0.0001
Chest press	10±3	12±3	16±3	0.001	0.0002	<0.0001
Average	13±3	16±3	22±3*	0.0001	<0.0001	<0.0001

Data are expressed as means ± standard errors (percent increase). *P* values denote difference from pre-training measures. * Different from PLA ($P < 0.05$). ‡ Different from CHO ($P < 0.05$). ModRep – moderate repetition, moderate resistance training days; PLA – placebo group; CHO – carbohydrate group; PRO – protein group.

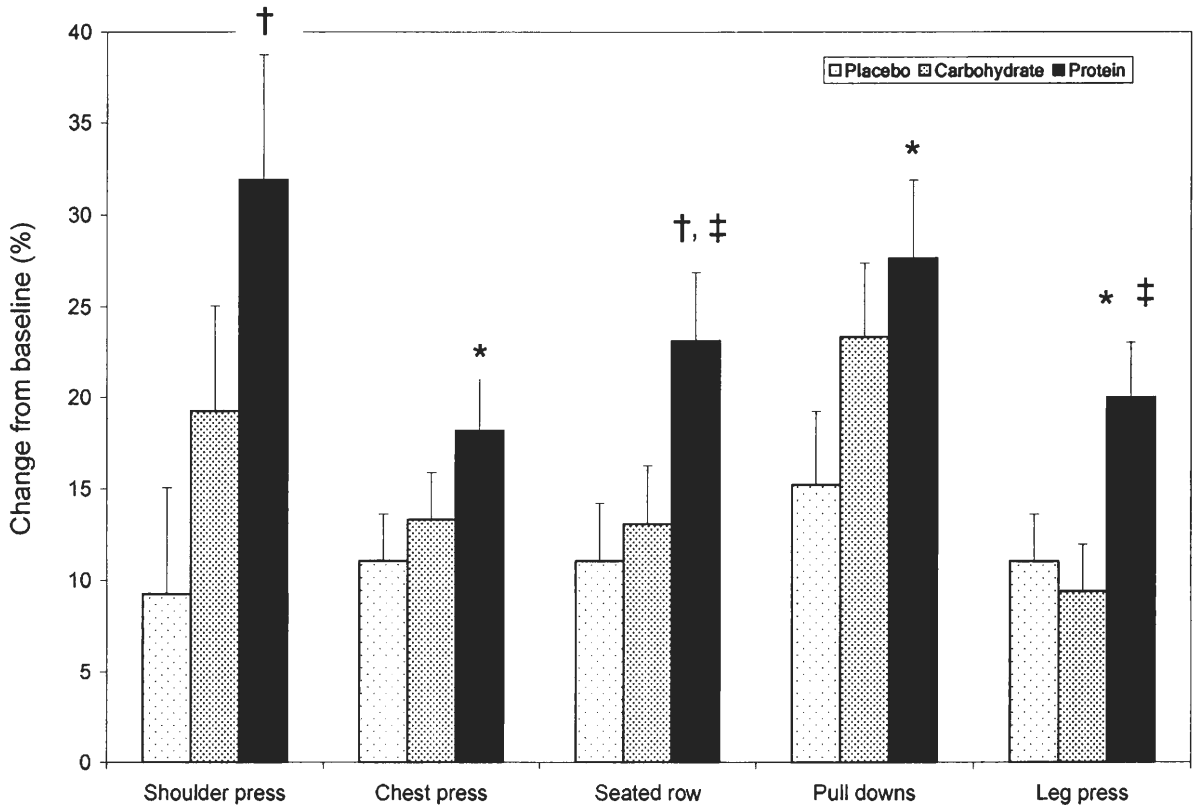
TABLE 8.

Percent improvement in training strength – LoRep						
	Training Data			P value v. baseline		
	PLA	CHO	PRO	PLA	CHO	PRO
Shoulder press	7±4	14±4	21±5*	0.1125	0.0045	0.0004
Pull downs	8±4	18±4	20±5*	0.0623	<.0001	0.0001
Seated row	10±2	10±2‡	21±3*	0.0005	0.0005	<.0001
Bicep curl	16±4	17±4	19±5	0.0003	0.0002	0.0003
Tricep extension	18±6	21±6	21±7	0.006	0.0018	0.0054
Leg curl	10±4	15±4	19±5	0.0152	0.0006	0.0003
Leg extension	24±5	18±5	30±5	<.0001	0.0006	<.0001
Leg press	6±2	6±2‡	15±2*	0.0044	0.0103	<.0001
Chest press	8±2	13±2	13±3	0.0009	<.0001	<.0001
Average	12±3	15±3	20±3†	<.0001	<.0001	<.0001

Data are expressed as means ± standard errors (percent increase). *P* values denote difference from pre-training measures. * Different from PLA ($P < 0.05$). † Different from PLA ($P < 0.10$). ‡ Different from CHO ($P < 0.05$). LoRep – low repetition, high resistance training days; PLA – placebo group; CHO – carbohydrate group; PRO – protein group.

FIGURE 9.

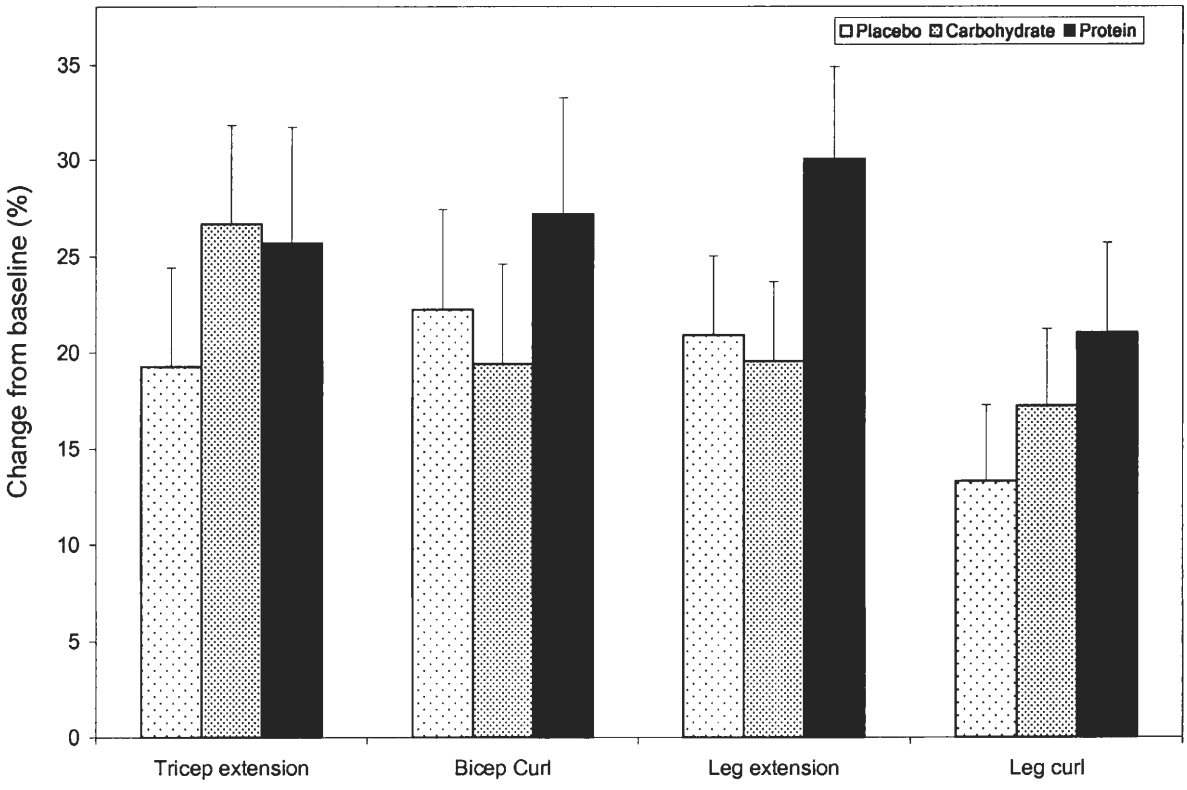
Percent change in training load for multi-joint exercises



Strength, expressed as percent increase in training load from baseline, increased for all groups ($P < 0.01$). * Different from change in PLA ($P < 0.05$). † Different from change in PLA ($P < 0.10$). ‡ Different from change in CHO ($P < 0.05$).

FIGURE 10.

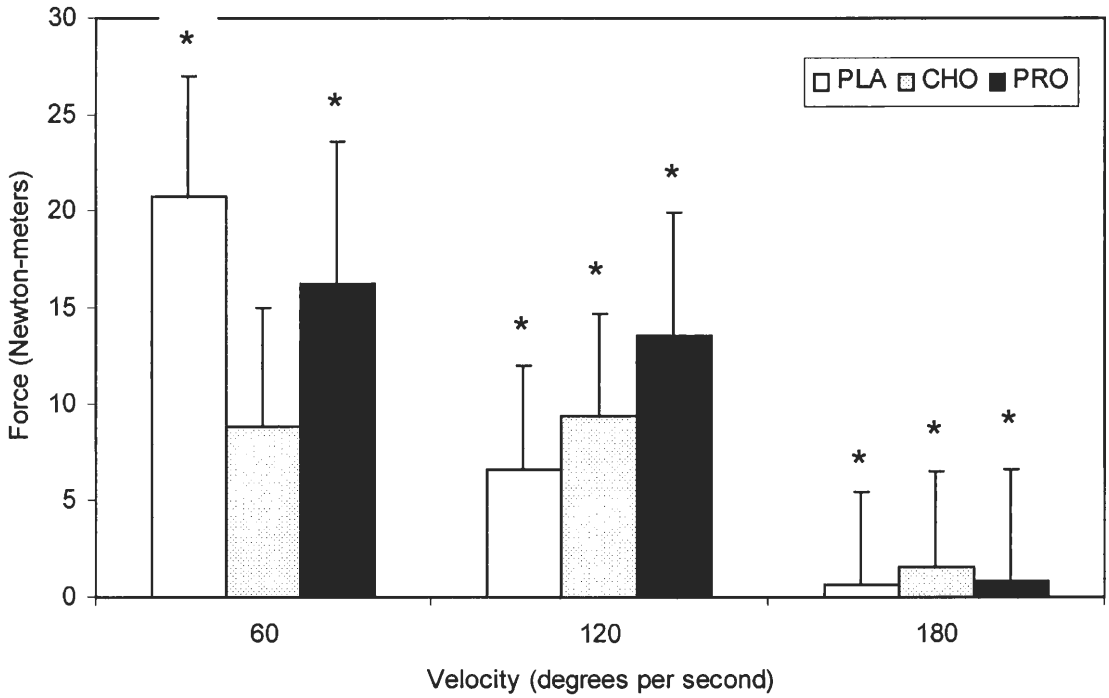
Percent change in training load for single-joint exercises



Strength, expressed as percent increase in training weight from baseline, increased for all groups ($P < 0.01$). Differences among treatment groups were not detected.

FIGURE 11.

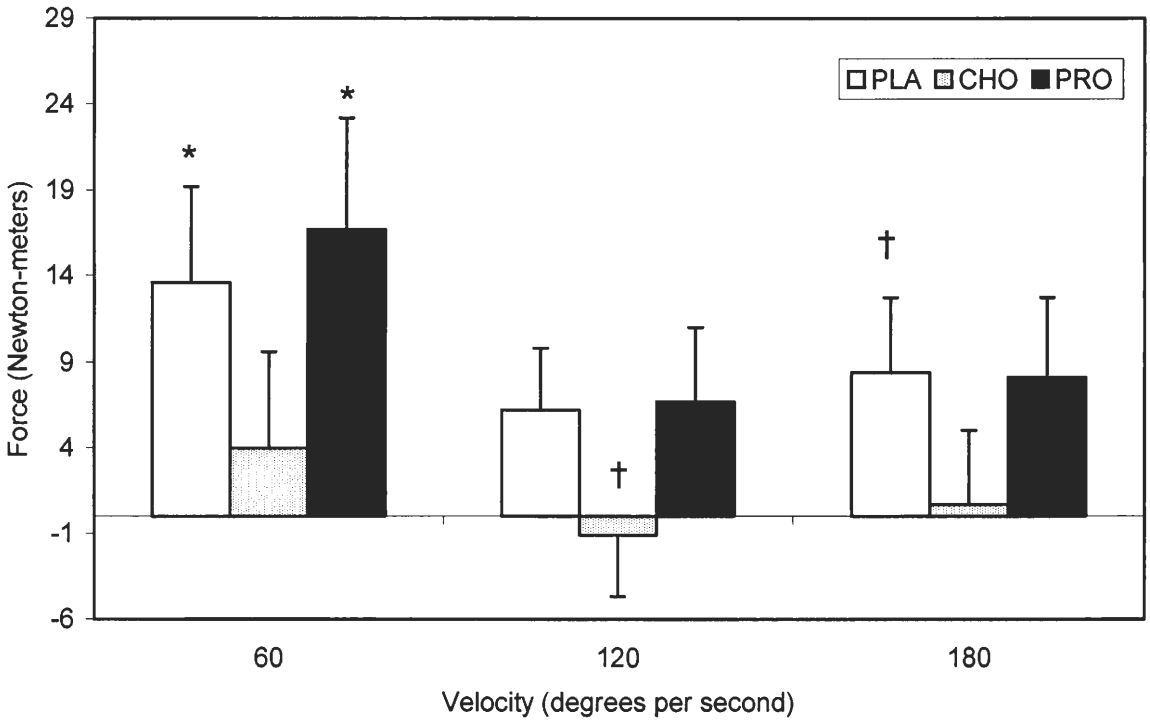
Change in peak torque (60 and 90 degrees per second) and average torque (180 degree per second) produced by knee extensors



Results are means ± standard errors. * Different from pre-training measurements ($P < 0.05$). Differences among the groups were not detected. PLA – placebo group; CHO – carbohydrate group; PRO – protein group.

FIGURE 12.

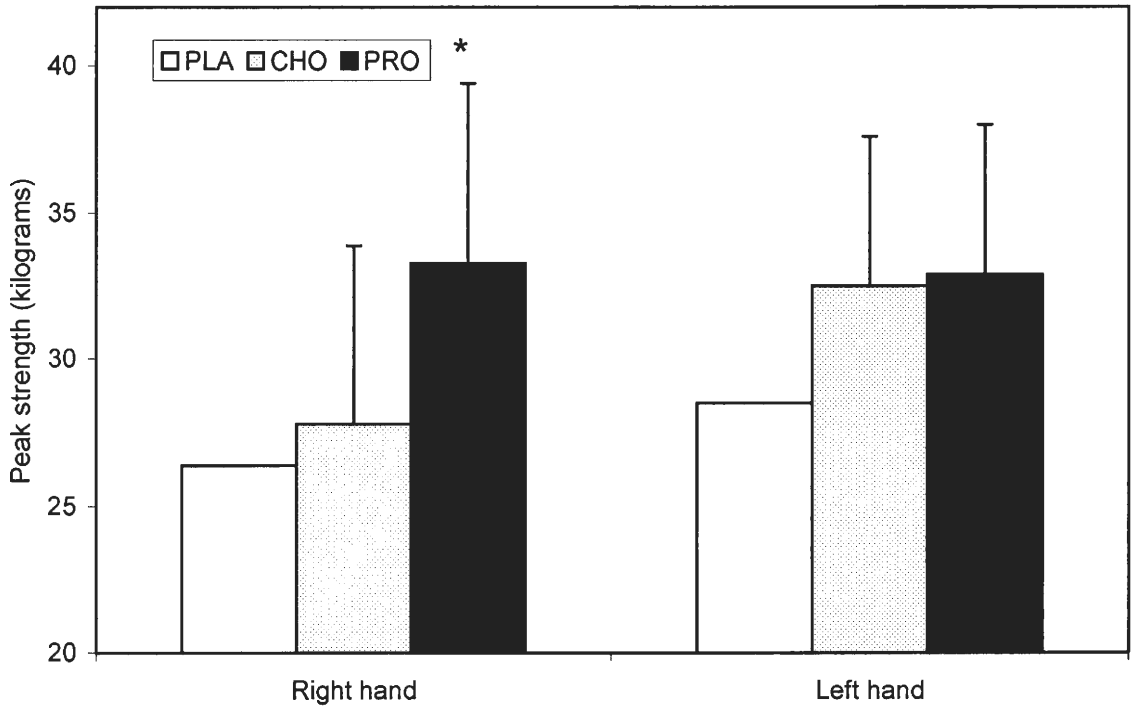
Change in peak torque (60 and 120 degrees per second) and average torque (180 degrees per second) produced by knee flexors



Results are means ± standard errors. * Different from pre-training measurements ($P < 0.05$). † Different from pre-training measures ($P < 0.10$). Differences among the groups were not detected. PLA – placebo group; CHO – carbohydrate group; PRO – protein group.

FIGURE 13.

Change in peak hand grip strength



Data are expressed as means ± standard deviations. * Difference in peak handgrip strength from 0 to 12 weeks ($P < 0.002$). Significant improvements were seen in all groups from baseline. PLA – placebo group; CHO – carbohydrate group; PRO – protein group.

TABLE 9.

Diet Composition by Group Pre- and Post-Training						
	PLA		CHO		PRO	
Week	0	12	0	12	0	12
Energy (kcals)	1988.2 ±352.1	1720.4 ±308.7	2227.8 ±741.5	2129.3 ±590.7	1754.6 ±1037.2	2098.7 ±304.2
Protein (g)	75.1 ±18.7	68.4 ±13.7	86.9 ±29.7	83.7 ±22.1	66.1 ±19.9	78.9 ±15.2
Carbohydrate (g)	232.0 ±64.3	212.0 ±55.7	262.3 ±95.5	261.6 ±77.8	277.3 ±113.6	264.5 ±32.8
Fat (g)	80.9 ±16.5	68.1 ±19.1	92.0 ±39.8	84.7 ±35.8	79.4 ±18.2	84.1 ±23.2

Data are expressed as means ± standard errors. Differences among treatment groups were not detected. kcals – kilocalories; PLA – placebo group; CHO – carbohydrate group; PRO – protein group.

TABLE 10.

Basic Chemistry Profile						
	PLA		CHO		PRO	
Week	0	12	0	12	0	12
Serum glucose (mg/dL)	89.3 ±5.7	96.5 ±2.8	81.8 ±6.3	88.9 ±3.1	92.3 ±7.7	87.3 ±3.8
Serum uric acid (mg/dL)	4.5 ±0.6	2.9 ±0.5	4.6 ±0.7	3.9 ±0.6	4.7 ±0.9	4.2 ±0.7
Blood urea nitrogen (mg/dL)	14.7 ±1.2	13.8 ±1.0	16.4 ±1.4	14.8 ±1.1	13.5 ±1.7	14.7 ±1.4
Serum creatinine (mg/dL)	0.9 ±0.1	0.9 ±0.1	0.9 ±0.1	0.9 ±0.1	0.9 ±0.1	0.9 ±0.1
BUN/creatinine ratio	17.9 ±1.5	16.5 ±1.4	17.9 ±1.6	16.7 ±1.5	16.2 ±2.0	17.0 ±1.9
Serum sodium (mmol/L)	138.2 ±0.8	139.6 ±0.6	139.2 ±0.8	139.2 ±0.7	140.0 ±1.0	139.7 ±0.8
Serum potassium (mmol/L)	4.8 ±0.4	4.3 ±0.1	4.8 ±0.4	4.3 ±0.1	4.1 ±0.5	4.3 ±0.1
Serum chloride (mmol/L)	102.7 ±0.5	103.9 ±0.5	102.1 ±0.6	102.9 ±0.5	103.8 ±0.7	103.7 ±0.7
Serum calcium (mg/dL)	9.6 ±0.1	9.6 ±0.1	9.7 ±0.1	9.7 ±0.1	9.7 ±0.2	9.7 ±0.2
Serum phosphorous (mg/dL)	4.0 ±0.3	3.8 ±0.2	3.9 ±0.3	4.0 ±0.2	3.8 ±0.3	3.6 ±0.3
Total serum protein (g/dL)	7.1 ±0.1	7.0 ±0.1	7.1 ±0.1	7.2 ±0.1	7.2 ±0.1	7.1 ±0.1
Total serum creatinine kinase (U/L)	150.5 ±36.3	142.8 ±15.5	106.7 ±40.1	125.0 ±17.9	159.3 ±49.1	146.2 ±20.9
Serum iron (µg/dL)	103.1 ±14.2	93.5 ±12.0	91.7 ±15.7	93.2 ±13.3	74.5 ±19.2	92.8 ±16.3

Data are expressed as means ± standard errors. Differences among treatment groups were not detected. PLA – placebo group; CHO – carbohydrate group; PRO – protein group.

TABLE 11.

Complete Blood Count

	PLA		CHO		PRO	
Week	0	12	0	12	0	12
White blood cell count ($x10^3/\mu L$)	5.8 ±0.4	6.4 ±0.5	6.3 ±0.4	6.1 ±0.5	5.2 ±0.5	5.6 ±0.6
Red blood cell count ($x10^6/\mu L$)	4.7 ±0.2	4.8 ±0.2	4.7 ±0.2	4.7 ±0.2	4.6 ±0.2	4.6 ±0.2
Hemoglobin (g/dL)	14.4 ±0.3	14.9 ±0.4	14.3 ±0.4	14.3 ±0.4	14.3 ±0.5	14.4 ±0.5
Hematocrit (%)	42.1 ±1.0	43.1 ±1.1	41.9 ±1.1	41.9 ±1.2	40.9 ±1.4	41.4 ±1.5
Mean corpuscular volume (fL)	89.8 ±1.8	89.5 ±1.8	90.8 ±2.1	90.1 ±2.0	87.8 ±2.6	89.0 ±2.5
Mean corpuscular hemoglobin (pg)	30.8 ±0.7	31.0 ±0.8	31.0 ±0.8	30.7 ±0.9	30.8 ±1.0	30.9 ±1.1
Mean corpuscular Hb concentration (d/dL)	34.3 ±0.3	34.6 ±0.3	34.1 ±0.3	34.0 ±0.3	35.0 ±0.4	34.8 ±0.4
Red cell distribution (%)	13.5 ±0.4	13.2 ±0.4	14.2 ±0.5	13.9 ±0.4	13.0 ±0.6	13.1 ±0.5
Platelets ($x10^3/\mu L$)	242.0 ±16.9	254.1 ±17.0	292.9 ±18.7	294.4 ±18.8	229.5 ±22.8	231.7 ±23.0
Lymphocytes (%)	34.9 ±2.7	31.1 ±2.7	35.4 ±3.0	32.3 ±3.0	41.2 ±3.7	38.7 ±3.7
Monocytes (%)	6.0 ±0.5	7.8 ±1.0	7.1 ±0.6	7.0 ±1.1	8.0 ±0.7	5.8 ±1.4
Eosiniphils (%)	3.6 ±0.6	3.0 ±0.4	2.2 ±0.6	2.1 ±0.4	2.2 ±0.8	1.8 ±0.5
Neutrophils ($x10^3/\mu L$)	52.9 ±3.1	57.6 ±2.8	54.8 ±3.4	58.3 ±3.1	47.8 ±4.1	53.2 ±3.7
Basophils (%)	0.5 ±0.1	0.5 ±0.2	0.4 ±0.2	0.2 ±0.2	0.8 ±0.2	0.5 ±0.2

Data are expressed as means ± standard errors. Differences among treatment groups were not detected. PLA – placebo group; CHO – carbohydrate group; PRO – protein group.

TABLE 12.

Lipid Profiles						
	PLA		CHO		PRO	
Week	0	12	0	12	0	12
Serum triglycerides (mg/dL)	140.1 ±19.5	198.9 ±54.4	123.3 ±21.6	117.7 ±60.1	79.0 ±26.4	88.3 ±73.6
Total cholesterol (mg/dL)	200.5 ±10.5	199.3 ±10.8	191.2 ±11.6	187.4 ±11.9	198.8 ±14.2	201.0 ±14.6
High density lipoproteins (mg/dL)	56.2 ±6.4	56.1 ±6.3	60.8 ±6.7	59.6 ±6.7	68.0 ±8.3	66.7 ±8.2
Low density lipoproteins (mg/dL)	118.0 ±9.5	120.3 ±10.1	105.7 ±10.0	104.3 ±10.7	115.0 ±12.2	118.5 ±13.1
Very low density lipoproteins (mg/dL)	26.2 ±4.0	23.4 ±3.7	24.8 ±4.2	23.6 ±3.9	15.8 ±5.1	17.5 ±4.8
Cholesterol/ HDL ratio	3.8 ±0.4	3.8 ±0.4	3.3 ±0.4	3.4 ±0.4	3.3 ±0.5	3.4 ±0.5

Data are expressed as means ± standard errors. No differences among treatment groups were detected. PLA – placebo group; CHO – carbohydrate group; PRO – protein group.

TABLE 13.

Albumin and Pre-Albumin Concentrations						
	PLA		CHO		PRO	
Week	0	12	0	12	0	12
Serum albumin (g/dL)	4.3 ±0.1	4.3 ±0.1	4.3 ±0.1	4.3 ±0.1	4.4 ±0.1	4.2 ±0.1
Serum prealbumin (mg/dL)	29.3 ±1.5	30.7 ±1.9	28.8 ±1.7	28.8 ±2.1	22.7 ±2.1	23.5 ±2.5

Data are expressed as means ± standard errors. Differences among treatment groups were not detected. PLA – placebo group; CHO – carbohydrate group; PRO – protein group.

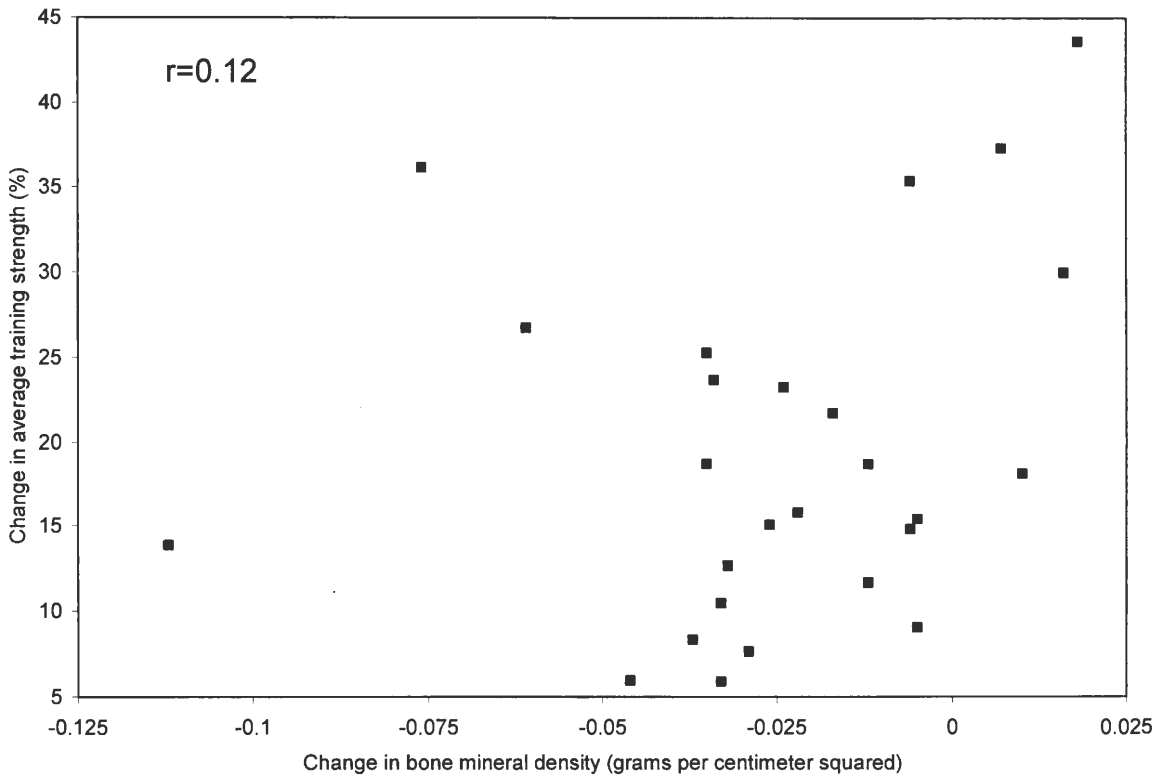
TABLE 14.

Hepatic and Renal Enzymes						
	PLA		CHO		PRO	
Week	0	12	0	12	0	12
Lactate dehydrogenase (IU/L)	186.8 ±23.7	176.4 ±7.9	214.8 ±26.3	178.0 ±8.7	183.0 ±32.2	179.3 ±10.6
Aspartate aminotransferase (IU/L)	24.3 ±2.3	24.9 ±1.1	25.6 ±2.6	22.4 ±1.2	26.2 ±3.2	24.0 ±1.5
Alanine aminotransferase (IU/L)	23.2 ±4.4	28.0 ±2.8	24.3 ±4.9	23.6 ±3.1	25.3 ±6.0	21.7 ±3.7
Gamma-glutamyl transpeptidase (IU/L)	20.8 ±6.7	22.0 ±4.5	27.2 ±7.4	22.8 ±5.0	17.7 ±9.1	16.8 ±6.1

Data are expressed as means ± standard errors. Differences among treatment groups were not detected. PLA – placebo group; CHO – carbohydrate group; PRO – protein group.

FIGURE 14.

Relationship between change in bone mineral density and change in training load



A significant correlation between change in average training strength and change in bone mineral density was not detected (r = 0.12).

APPENDIX 1: Recruitment Flier.

Adults 50-65 Years Needed for an Exercise & Nutrition Study

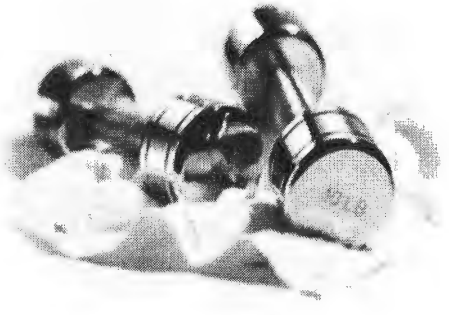
Both men and women between the ages of 50 and 65 years are needed for a study at Iowa State University consisting of:

- Three strength training sessions with a nutritional drink weekly for 12 weeks.
- Measurements of body fat, muscle mass, and strength.

For additional information or to express interest in the study, please contact:

Katie Mikus
Phone: 515-294-1216

Center For Designing Foods to Improve Nutrition
1127 Human Nutritional Sciences Building
Food Science & Human Nutrition
Ames, IA 50011



Email: ktmikus@iastate.edu

APPENDIX 2: Medical History Questionnaire.

Medical History Form

STUDY: Nutritional Augmentation of Resistance Training to Blunt Age-Related Losses of Lean Body Mass, Strength, & Functionality in Middle-Aged (50-65 Years) Humans

Subject Name:_____DATE:_____

Subject Number: _____

Home Address: _____

Street

_____

City State Zip

Home Phone:_____

Emergency Information:

Personal Physician: _____

Contact in case of Emergency:_____

Relationship: _____

Phone: _____

Personal Information:

Age:_____ Date of Birth:_____/_____/_____

Mo. Day Year

Height:_____ in. Weight: _____ lbs.

Sex: ___ male Race: ___ white

___ female ___ black

___ asian

___ hispanic

___other:_____

Marital Status:

___ single

___ married

___ divorced or separated

___ widowed

Family Health History

Date of Last Physical Exam: ____/____/____

A. If any members of your immediate family have or have had any of the following conditions, indicate their age at the time of the event:

	Father	Mother	Brother(s)	Sister(s)
Heart Attack	_____ yr	_____ yr	_____ yr	_____ yr
Stroke	_____ yr	_____ yr	_____ yr	_____ yr
Coronary Artery Disease	_____ yr	_____ yr	_____ yr	_____ yr
If deceased, age at death	_____ yr	_____ yr	_____ yr	_____ yr

B. Indicate if any members of your immediate family have or have had the following conditions by marking the appropriate lines:

	Father	Mother	Brother(s)	Sister(s)
High Blood Pressure	_____ yr	_____ yr	_____ yr	_____ yr
High Cholesterol	_____ yr	_____ yr	_____ yr	_____ yr
Diabetes	_____ yr	_____ yr	_____ yr	_____ yr
Obesity	_____ yr	_____ yr	_____ yr	_____ yr

Medical History

Answer the following questions, indicating the month and year of the event or diagnosis where appropriate.

	Yes	No	Mo./Yr.
1. Has a doctor ever told you that you have heart disease?	___	___	___/___
2. Have you ever had a heart attack?	___	___	___/___
3. Have you ever had chest pain?	___	___	___/___
4. Have you ever had cardiac catheterization?	___	___	___/___
5. Have you ever had balloon angioplasty?	___	___	___/___
6. Have you ever had coronary artery bypass graft surgery?	___	___	

If yes, list date and number of grafts:

___/___ # grafts: __ 1 __ 2 __ 3 __ 4 +

Mo./Yr.

7. Have you ever had a stroke?	___	___	___/___

8. Do you have hypertension (high blood pressure)? ___ ___ ___/___

If yes, how long have you had hypertension?

___ less than 1 year

___ 1 - 5 years

___ 6 - 10 years

___ more than 10 years

9. Do you have diabetes mellitus? ___ ___ ___/___

10. Do you take insulin for diabetes? ___ ___

If yes, how long have you taken insulin?

___ less than 1 year

___ 1 - 5 years

___ 6 - 10 years

___ more than 10 years

11. Do you take oral hypoglycemics for diabetes? ___ ___ ___/___

12. Do you have a cardiac pacemaker? ___ ___

If yes, how long have you had a cardiac pacemaker?

___ less than 1 year

___ 1 - 5 years

___ 6 - 10 years

___ more than 10 years

13. Have you had a carotid endarterectomy? ___ ___ ___/___

14. Has your doctor ever told you that you have

a heart valve problem? ___ ___ ___/___

15. Have you had a heart valve replacement surgery? ___ ___ ___/___

If yes, what heart valve was replaced? __ mitral __ aortic

16. Have you had cardiomyopathy? ___ ___ ___/___

17. Have you had a heart aneurysm? ___ ___ ___/___

18. Have you had heart failure? ___ ___ ___/___

19. Have you ever suffered cardiac arrest? ___ ___ ___/___

20. Have you ever been pregnant? ___ ___

If yes, how many children have you had?___

21. Other medical problems: Indicate if you have had any of the following medical problems:

Past	Now	
___	___	Alcoholism
___	___	Allergies
___	___	Anemia
___	___	Arthritis
___	___	Asthma
___	___	Back injury or problem
___	___	Blood clots
___	___	Bronchitis
___	___	Cirrhosis
___	___	Claudication
___	___	Elbow or shoulder problems
___	___	Emotional disorder

___	___	Eye problems
___	___	Gall bladder disease
___	___	Glaucoma
___	___	Gout
___	___	Headaches
___	___	Hemorrhoids
___	___	Hernia
___	___	Hip, knee, or ankle problems
___	___	Intestinal disorders
___	___	Kidney disease
___	___	Liver disease
___	___	Lung disease
___	___	Mental illness
___	___	Neck injury or problem
___	___	Neuralgic disorder
___	___	OB/GYN problem
___	___	Obesity/overweight
___	___	Osteoporosis
___	___	Parkinson's disease
___	___	Phlebitis
___	___	Prostate trouble
___	___	Rheumatic fever
___	___	Seizure disorder

___ ___ Stomach disease

___ ___ Thyroid disease

___ ___ Tumors or cancer -

Type:_____

___ ___ Ulcers

___ ___ Other -

Specify:_____

22. Has your doctor ever told you not to exercise for any reason?

Yes ___ No ___

If yes, please explain:

_____

_____

23. Surgical Procedures: Indicate if you have had any of the following surgeries, and if so, the approximate date.

	Yes	No	Mo. / Yr.
Adhesion repair	___	___	____/____
Appendectomy	___	___	____/____
Back surgery	___	___	____/____
Bladder surgery	___	___	____/____
Bowel surgery	___	___	____/____
Breast surgery	___	___	____/____
Cataract surgery	___	___	____/____
Gall bladder surgery	___	___	____/____

Hemorrhoid surgery	___	___	____/____
Joint surgery	___	___	____/____
Kidney surgery	___	___	____/____
Lung surgery	___	___	____/____
OB/GYN surgery	___	___	____/____
Prostate surgery	___	___	____/____
Stomach surgery	___	___	____/____
Other: _____			____/____

24. Have you ever used any tobacco product on a regular basis?___ yes___ no

If yes, what product did you use and for how long?

Product: _____ Years: _____

25. Medications: Indicate the medicines you currently use on a regular basis.

	Yes	No
Allergy medications/antihistimines	___	___
Antacids	___	___
Antibiotics	___	___
Anti-arrhythmics	___	___
Anti-inflammatory agents	___	___
Aspirin	___	___
Asthma medicines	___	___
Beta blockers	___	___
Blood pressure medicines	___	___
Blood thinners	___	___

Cortisone	___	___
Diabetes medicines/insulin	___	___
Diuretics/"water pills"	___	___
Gout medicines	___	___
Heart medicines	___	___
Hormones/estrogen	___	___
Laxatives	___	___
Nitroglycerin	___	___
Pain medicines	___	___
Psychiatric medicines/anti-depressants	___	___
Sedatives/sleeping pills	___	___
Seizure medicines	___	___
Thyroid medicines	___	___
Tranquilizers	___	___
Vitamins/iron	___	___

Other: _____

List medications you are taking now:

Name of drug	Dosage	Times/day	Duration of use
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____

Statement of Confidentiality

I understand that the information contained on this questionnaire is regarded as confidential, and will not be released without my prior written permission. The research center may, however, use the information for statistical and other research purposes.

Signature _____ Date ___/___/___

APPENDIX 3: Informed Consent Document.

INFORMED CONSENT DOCUMENT

Title of Study: Nutrition Augmentation of Resistance Training to Blunt Aging-Related Losses of Lean Body Mass, Strength, and Functionality in Middle-Aged (50-65) Humans

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This is a research study. Please take your time in deciding if you would like to participate. Please feel free to ask questions at any time.

INTRODUCTION

The purpose of this study is to determine if ingestion of protein after resistance exercise will be of benefit to people between 50 and 65 years of age. This study will provide insight into possible dietary and/or training interventions that would attenuate or reverse age-related declines in muscle mass and strength. You are being invited to participate in this study because you are a healthy middle-aged resident of central Iowa and have met the inclusion criteria for this study.

DESCRIPTION OF PROCEDURES

Before being admitted to this study, you will be asked to complete a medical history questionnaire. This is to ensure that you do not have any existing medical conditions that may affect your participation in this study. In addition, throughout the study, you will be asked to complete questionnaires to ensure that no unwanted outcomes are resulting from your participation in the study.

If you agree to participate in this study, your participation will last for approximately 15 weeks. You have a chance to be placed on one of four different treatments:

1). Exercise plus carbohydrate supplementation

2). Exercise plus protein supplementation

3). Exercise plus non-caloric supplementation

4). No exercise or supplementation

The chances of being placed on any one of these treatment groups will be random. The nutrient supplements will consist of powdered foods that you can purchase at a store. However, if you receive nutritional supplementation, you will not know which of the treatments you will receive. The supplement will be given to you as a drink to take immediately after your exercise training, and the content of the drink will not be revealed to you until after the entire study is completed. Each of the treatments has the potential to improve your exercise performance.

If you are placed in the "no exercise" group, you will be given the opportunity to participate in exercise training after the study is complete. During the study, you will be asked to maintain your normal everyday activity and not start any new exercise behavior.

Prior to and after a 12-week exercise training program, you will be asked to spend up to 4 hours in the human metabolic unit on the Iowa State University campus to obtain estimates of your strength, muscle mass, and body fat. During this time, body weight and composition will be measured using dual energy x-ray absorptiometry and bioelectrical impedance. Circumferences of the arm, forearm, thigh, waist, and hips will be measured. Peripheral quantitative computed tomography will be used to measure the muscle cross-sectional area of your mid-thigh. Knee flexion and extension strength will be assessed using a computer assisted system. Handgrip strength will also be measured, and a small amount of blood will be collected by a licensed specialist to assess your general health. Two small muscle samples will be taken, separated by at least 3 days, once at the beginning and again at the end of the study by a skilled expert. Physical activity monitors will be handed out along with instructions, and you will be asked to wear these up to three days to assess your normal, free-living physical activity and energy expenditure. You will also be given an electronic hand-held device and instructions for recording three days of food intake. Finally, you will be given a very small oral dose of a stable, non-radioactive isotope and asked to collect urine samples for 72

hours. (The non-radioactive isotope to be used in this study is 15N-glycine. This isotope is just like the glycine that is commonly found in foods that you and I consume, however, its size has been altered so that we can track its path through your body. This element is NOT radioactive and does NOT display radioactive properties.)

Prior to exercise training, you will spend approximately 2 hours in the Exercise Clinic of the Forker Building on the Iowa State University campus for two assessments:

1) The Cooper 12-minute walk test to determine your overall fitness, and
2) A One Repetition Maximum test to determine the amount of weight that you can lift for the following exercises: knee extension, knee flexion, seated bench press, seated row, leg press, biceps curl, abdominal crunch, calf press, overhead press, seated dip, lumbar extension, and lateral pull down. You also will be trained to do each of these exercises.

If you are placed on one of the three "exercising" groups, you will be asked to attend three exercise sessions per week, each lasting approximately one hour during the 12-week exercise training period. During this time, you will perform a 5 minute warm-up followed by the twelve weight lifting exercises listed above and then a 5 minute cool-down. Every other week during the 12-week period, circumferences, bioelectrical impedance analysis, heart rate, blood pressure, height and weight will be measured and recorded. On the sixth and twelfth week of

training, the one repetition maximums will be determined again. Strength, muscle mass, and body measurements will be made again after the 12-week exercise training period.

RISKS

While participating in this study you may experience the following risks:

- Venipuncture, or blood collection, is associated with hematoma, local discomfort, and on rare occasion, infection. Sterile techniques will be used by a trained professional to minimize the occurrence of undesirable outcomes.

- The muscle biopsies are associated with some risk to the subjects, including infection of the biopsy site and some degree of mild soreness on the day following the biopsy. These risks are minimized by using sterile procedures and instruments, placing slight pressure over the biopsy site for 5 minutes after biopsy, and by applying a pressure bandage over the site for 12 hours following the biopsy. Delayed and minor soreness has been reported in ~10% of the subjects. In only one instance has a subject reported soreness sufficient enough to affect their usual daily activities.

- Dual energy x-ray absorptiometry is a painless, non-invasive test. You will be asked to lie still on a quiet padded table for a few minutes. The x-ray dose you

will be exposed to is extremely low, comparable to what you would receive on a cross-country airplane flight.

- Peripheral quantitative computed tomography usually causes no discomfort. You will be exposed to a small amount of radiation, but again, this will only be a fraction of the radiation you would be exposed to when receiving a normal chest x-ray.

- Bioelectrical impedance analysis is also a painless, non-invasive test. You will be asked to lie still while technicians tape electrodes to your hand and foot. A very small electrical current will be passed through your body. You will not be able to feel the current. For this and all of the aforementioned tests, you will be asked not to wear jewelry or clothing with metal buttons or buckles, as they may cause interference with the tests.

- Assessment of knee flexion and extension strength requires you to perform maximal exertion of the quadriceps and hamstrings. You may experience some mild, temporary muscle soreness during the test. A small percentage of subjects report mild discomfort in those muscles on the following day.

- Estimation of hand grip strength requires maximal effort of the muscles in the hand and forearm. Most people experience no problems with this test, however, it may be slightly uncomfortable for people with osteoarthritis in their hands.

BENEFITS

If you decide to participate in this study there will be a direct benefit to you. Persons chosen to be included in one of the three exercising groups will receive individualized resistance training instruction and monitoring of changing in strength and body composition. All subjects will be given pre- and post-training evaluations of muscle mass, body fat, bone mineral density, leg and hand grip strength. The combined cost of these tests alone would be quite high. Furthermore, your results from the physical activity monitors and food diaries will be available to you. It is hoped that the information gained from this study will benefit society by providing insight into possible dietary and/or training interventions that would attenuate or reverse age-related declines in muscle mass and strength.

COSTS AND COMPENSATION

You will not have any costs from participating in this study. You will be compensated for participating in this study. Upon completion of the study participants will receive $200. $50 will be given for completion of baseline testing, and $50 will be given for completion of post-training testing. An additional $50 will be given for completion of both baseline muscle biopsies, and $50 will be given upon completion of both post-training muscle biopsies.

PARTICIPANT RIGHTS

Your participation in this study is completely voluntary and you may refuse to participate or leave the study at any time. If you decide to not participate in the study or leave the study early, it will not result in any penalty or loss of benefits to which you are otherwise entitled.

At any time during the study, you may withdraw your consent to participate without prejudice toward you. Such withdrawal can be for any reason you choose. Constant monitoring of all experiments will be performed by knowledgeable and CRP/AED trained individuals in an attempt to prevent any complications. Emergency first aid supplies and equipment as well as an automatic external defibulator (AED) will be immediately available.

RESEARCH INJURY

Emergency treatment of any injuries that may occur as a direct result of participation in this research is available at the Iowa State University Thomas B. Thielen Student Health Center, and/or referred to Mary Greeley Medical Center or another physician or medical facility at the location of the research activity. Compensation for any injuries will be paid if it is determined under the Iowa Tort Claims Act, Chapter 669 Iowa Code. Claims for compensation should be submitted on approved forms to the

State Appeals Board and are available from the Iowa State University Office of Risk Management and Insurance

CONFIDENTIALITY

Records identifying participants will be kept confidential to the extent permitted by applicable laws and regulations and will not be made publicly available. However, the Food and Drug Administration, Department of Health and Human Safety and the Institutional Review Board (a committee that reviews and approves human subject research studies) may inspect and/or copy your records for quality assurance and data analysis. These records may contain private information.

To ensure confidentiality to the extent permitted by law, subjects will be assigned numeric codes, and no identifying characteristics will be reported in the publication or presentation of study results. Hard copies of all data will be locked in a file cabinet and computer files will be kept in a password protected computer in the laboratories of the primary investigators.

QUESTIONS OR PROBLEMS

You are encouraged to ask questions at any time during this study. For further information about the study contact:

Katie Mikus (294-9633, ktmikus@iastate.edu)

Dr. Paul Flakoll (294-8489, flakollp@iastate.edu)

Dr. Rick Sharp (294-8429, rlsharp@iastate.edu)

If you have any questions about the rights of research subjects or research-related injury that the above individuals cannot answer, please contact the Human Subjects Research Office, 2810 Beardshear Hall, (515) 294-4566; austingr@iastate.edu or the Research Compliance Officer, Office of Research Compliance, 2810 Beardshear Hall, (515) 294-3115; dament@iastate.edu

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SUBJECT SIGNATURE

Your signature indicates that you voluntarily agree to participate in this study, that the study has been explained to you, that you have been given the time to read the document and that your questions have been satisfactorily answered. You will receive a copy of the written informed consent prior to your participation in the study.

Subject's Name (printed) _____

_____ _____

(Subject's Signature) (Date)

INVESTIGATOR STATEMENT

I certify that the participant has been given adequate time to read and learn about the study and all of their questions have been answered. It is my opinion that the participant understands the purpose, risks, benefits and the procedures that will be followed in this study and has voluntarily agreed to participate.

_____ _____

(Signature of Person Obtaining Informed Consent) (Date)

APPENDIX 4: Adverse Events Questionnaire.

Clinical Research Health Form

STUDY: Nutritional Augmentation of Resistance Training

No._____NAME: _____DATE: _____-____-____

Health Questionnaire: Have you had any of the following in the last 2-3 days?

Stomachache/pains	❒ Yes	❒ No	Diarrhea	❒ Yes	❒ No
Vomiting/Nausea	❒ Yes	❒ No	Stiff Joints	❒ Yes	❒ No
Dizziness	❒ Yes	❒ No	Nose bleeds	❒ Yes	❒ No
Coughing	❒ Yes	❒ No	Heart burn	❒ Yes	❒ No
Wheezing	❒ Yes	❒ No	Numbness	❒ Yes	❒ No
Chest pain	❒ Yes	❒ No	Nasal congestion	❒ Yes	❒ No
Weakness	❒ Yes	❒ No	Ringing in ears	❒ Yes	❒ No
Increased headache	❒ Yes	❒ No	Stress increase	❒ Yes	❒ No
Stress decrease	❒ Yes	❒ No	Decreased libido	❒ Yes	❒ No
Negative Mood	❒ Yes	❒ No	Constipation	❒ Yes	❒ No
Rash	❒ Yes	❒ No	Memory increase	❒ Yes	❒ No
Dry Scalp/hair	❒ Yes	❒ No	Fewer headaches	❒ Yes	❒ No
Excessive Dry Skin	❒ Yes	❒ No	Shortness of breath	❒ Yes	❒ No
Nail Changes	❒ Yes	❒ No	Loss of Appetite	❒ Yes	❒ No
Ear pain	❒ Yes	❒ No	Increase Appetite	❒ Yes	❒ No
Increase in libido	❒ Yes	❒ No	Loss of Energy	❒ Yes	❒ No
A decrease in Memory	❒ Yes	❒ No	Increased Energy	❒ Yes	❒ No
Itching	❒ Yes	❒ No	Blood in Urine	❒ Yes	❒ No
Swelling	❒ Yes	❒ No	Blood in Stool	❒ Yes	❒ No

APPENDIX 5: Participant Handout.

Nutritional Augmentation of Resistance Training to Blunt Age-Related Losses of Lean Body Mass, Strength, & Functionality in Middle-Aged Adults

INTRODUCTION

Research over the past decade has proven that strength training can elicit remarkable improvements in muscle mass and strength regardless of age or physical condition.

Strength training also plays an important role in the prevention and management of many chronic diseases such as osteoporosis, arthritis and diabetes. Reductions in depression, improved sleep quality, increased metabolic rate and reduced body fat are also benefits of strength training.

It is clear that strength training plays an essential role in everything from improving sports performance, to maintaining physical function and a healthy lifestyle.

GENERAL GUIDELINES

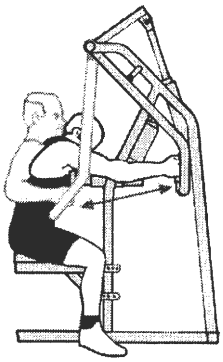
A warm-up of at least 5-10 minutes should be performed before strength training.

Strength training should be performed 3 nonconsecutive days per week.

It is important to use the proper seat, range of motion, leg and arm pad adjustments to fit body size and ensure proper body mechanics.

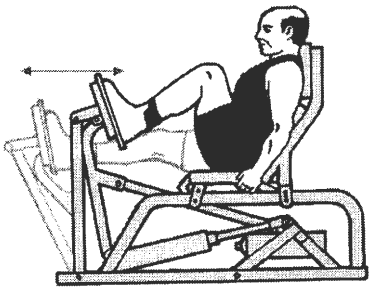
While full range of motion (ROM) should be the goal for all exercises when possible, exercises should be performed through the pain-free range of motions only. Limited ROM can still provide significant benefits with out causing injury to a painful joint.

Continuous, natural breathing patterns should be used while training. Avoid the Valsalva maneuver (holding the breath to exert more force), or prolonged holding of breath. In general, breathe out on the exertion phase of the exercise.



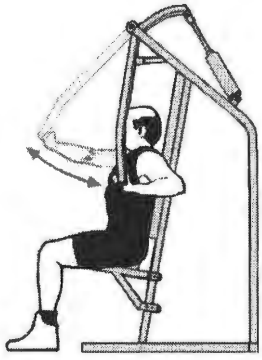
1. Upper Back

Exercise the second-largest muscle group in the body while giving the legs a rest after the cardiovascular workout. Also, exercises the biceps. *Contributes to upright posture and proper back alignment.*



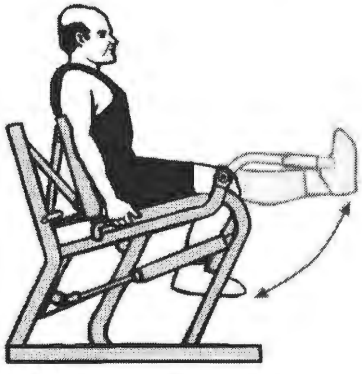
2. Leg Press

Exercise the largest muscle groups in the body qaudriceps, hamstrings and gluteals-while allowing the upper body to rest. *Contributes to walking, balance, rising and sitting*



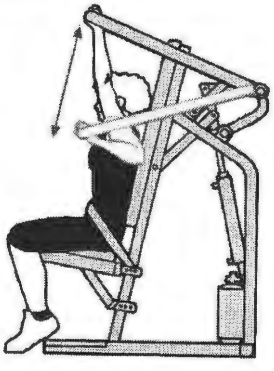
3. Seated Chest Press

Exercise the muscles in front of the chest (pectorals) as well as triceps. *Contributes to lifting and carrying.*



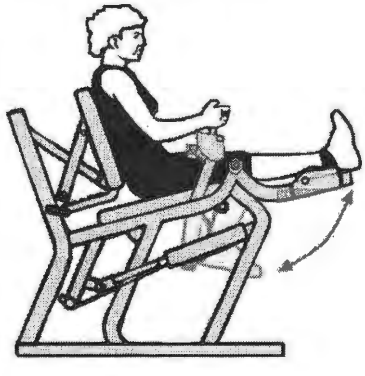
4. Leg Extension

Isolates the quadriceps, providing an intense second exercise to the lower body. *Contributes to walking, balance, and rising & sitting.*



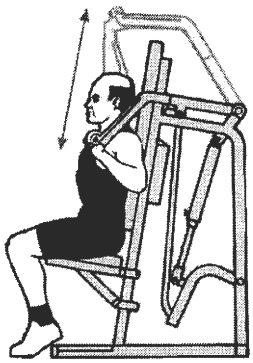
5. Lat. Pull-down

Re-exercise the body's second-largest muscle group, provides a second exercise for the biceps and engages muscles important in stabilizing the shoulder joint. *Contributes to reaching, lifting, and carrying.*



6. Leg Curl

Focuses on the hamstrings, which have been exercised once with the leg press. *Contributes to walking, balance ,rising and sitting.*

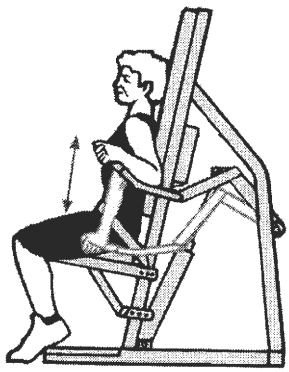


7. Shoulder (military) press

Focuses on deltoid muscles, which play an important role in stabilizing the shoulder. Proper exercise helps prevent such problems as bursitis and tendonitis. *Contributes to overhead activity, lifting, and carrying.*

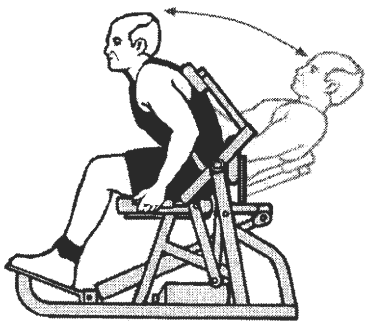
8. Abdominal

Isolates the abdominal muscles which connect the ribs to the pelvis. These muscles provide trunk (mid-body) Stability and back support. *Contributes to trunk stability and posture control.*



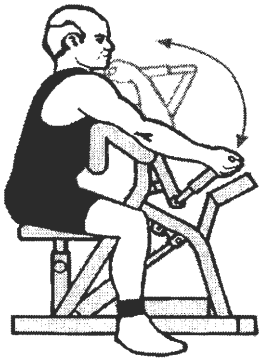
9. Triceps

The third and most focused exercise for this muscle group, which is used for assistance in rising from a chair. *Contributes to lifting, carrying, rising and sitting.*



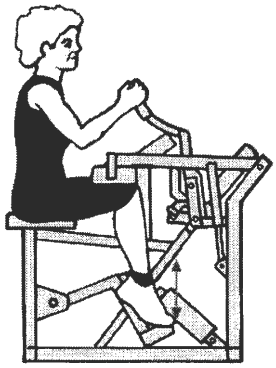
10. Lower back

Strengths the lower back to help prevent and reduce lower back pain. Anyone with a history of lower back trouble should proceed with caution. *Contributes to trunk stability, posture control.*



11. Arm Curl

Isolates the biceps for their most complete routine. *Contributes to lifting and carrying.*



12. Seated Calf

Strengthens the muscles in the calf, aiding the "push off" phase of the stride and rising onto the toes; enhances stability and mobility. *Contributes to walking and balance.*

STRETCHING

The right way to stretch is slow and relaxed. DO NOT BOUNCE. This can actually cause you to pull the muscle you are trying to stretch.

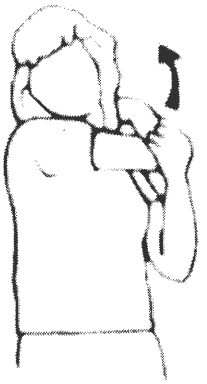
You should stretch to the point of "MILD TENSION". If you overstretch you will also cause damage. Back off if the stretch feels painful.

Hold the stretch for a minimum of 15 seconds each, without bouncing. BREATHE slowly and naturally. Do not hold your breathe while stretching. Relax the stretch. Stretch one or two more times with each stretch. Try to stretch a little further with each stretch. Again, only to the point of mild tension.

STRETCHING EXERCISES

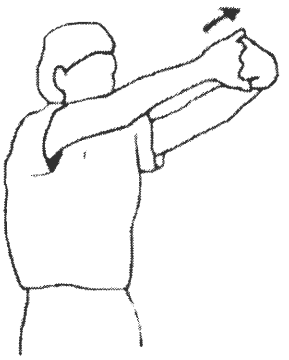
Stretches side of shoulder and back of upper arm

1. Stand or sit and place right hand on left shoulder
2. With left hand, pull right elbow across chest toward left shoulder and hold 10 to 15 seconds
3. Repeat on other side



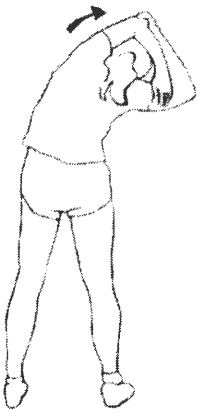
Stretches shoulder, middle back, arms, hands, fingers, wrist

1. Interlace fingers and turn palms out
2. Extend arms in front at shoulder height
3. Hold 10 to 20 seconds, relax, and repeat



Stretches triceps, top of shoulders, waist

1. Keep knees slightly flexed>
2. Stand or sit with arms overhead
3. Hold elbow with hand of opposite arm
4. Pull elbow behind head gently as you slowly lean to side until mild stretch is felt
5. Hold 10 to 15 sec
6. Repeat on other side

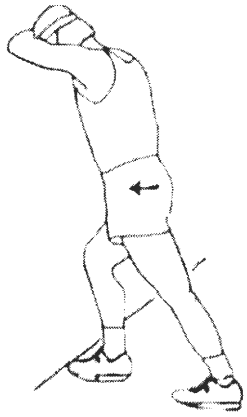


Stretches middle back

1. Stand with hands on hips
2. Gently twist torso at waist until stretch is felt
3. Hold 10 to 15 sec
4. Repeat on other side
5. Keep knees slightly flexed

Stretches calf

1. Stand a little way from wall and lean on it with forearms, head resting on hands
2. Place right foot in front of you, leg bent, left leg straight behind you
3. Slowly move hips forward until you feel stretch in calf of left leg
4. Keep left heel flat and toes pointed straight ahead
5. Hold easy stretch 10 to 20 seconds
6. Do not bounce
7. Repeat on other side
8. Do not hold breath



Stretches front on thigh (quadriceps)

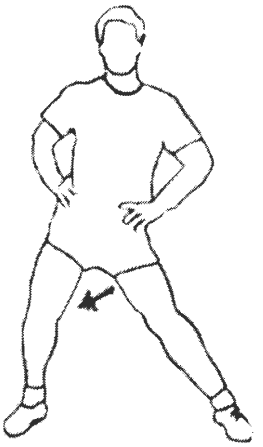
1. Stand a little a way from wall and place left hand on wall for support
2. Standing straight, grasp top of left foot with right hand
3. Pull heel toward buttock
4. hold 10 to 20 sec
5. Repeat on other side

Relaxes hamstrings, stretches calves, achilles, and ankles

1. Stand with feet shoulder-width apart
2. Keep heels flat, toes pointed straight ahead
3. Assume bent knee position (quarter squat)
4. Hold 30 sec

Stretches inner thigh, groin

1. Stand with feet pointed straight ahead, a little more than shoulder-width apart
2. Bend right knee slightly and move left hip downward toward right knee
3. Hold 10 to 15 seconds
4. Repeat on other side
5. If necessary, hold on to something (chair, etc.) for balance

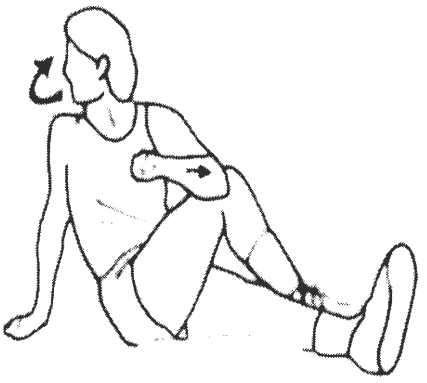


Stretches side of hip, hamstrings

1. Sit on floor with right leg straight out in front
2. Bend left leg, cross left foot over, place outside right knee
3. Pull left knee across body toward opposite shoulder
4. Hold 10 to 20 seconds
5. Repeat on other side
6. Breathe easily

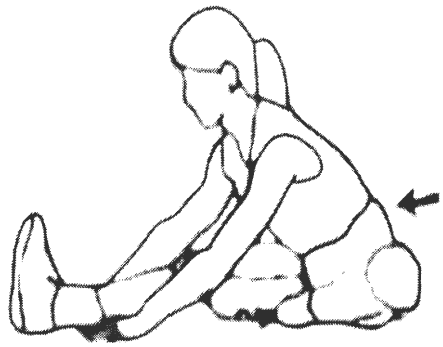
Stretches lower back, side of hip, and neck

1. Sit on floor with left leg straight out in front
2. Bend right leg, cross right foot over, place outside left knee
3. Bend left elbow and rest it outside right knee
4. Place right hand behind hips on floor
5. Turn head over right shoulder, rotate upper body right
6. Hold 10 to 15 seconds
7. Repeat on other side



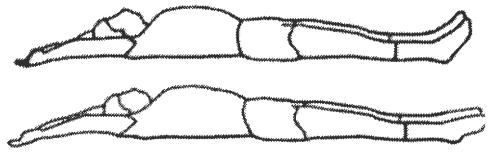
Stretches back of leg and lower back

1. Sit on floor, legs straight out at sides
2. Bend left leg in at knee
3. Slowly bend forward from hips toward foot of straight leg until you feel slight stretch
4. Do no dip head forward at start of stretch
5. Hold this developmental stretch 10 to 20 seconds
6. Repeat on other side
7. Foot of straight leg upright, ankles and toes relaxed
8. Use a towel if you cannot easily reach your feet



Stretches shoulders, arms, hands, feet and ankles

1. Lie on floor, extend arms overhead, keep legs straight
2. Reach arms and legs in opposite directions
3. Stretch 5 sec, relax



APPENDIX 6: Protein Nutrition Facts.

Supplement Nutrition Facts	
Serving Size: 1 level scoop (~27g)	
Amount Per Serving	
Calories	90
Calories from fat	0
Total Fat	0g
Saturated Fat	0g
Cholesterol	<5mg
Total Carbohydrates	0g
Sugars	0g
Sodium	60mg
Protein	23g
Calcium	160mg
Phosphorus	75mg
Magnesium	20mg

Ingredients:

PROMINA® ultrafiltered and undenatured Whey Protein Isolate (includes Beta Lactoglobulin, Alpha Lactoglobulin, Glycomacropeptides, Immunoglobulin, Bovine Serum Albumin, Protease Peptone, Lactoferrin, Lacto Peroxidase), citric acid, natural and artificial flavors, lecithin, aspartame, acesulfame-K, FD&C Yellow #5, FD&C Blue #1.

While the exact amino acid composition of this supplement is not available, whey protein is generally higher in essential amino acids, branched-chain amino acids, cystine and methionine relative to other commercially available protein sources. Below is an approximation of the amino acid concentration of whey protein isolates. For contrast, the same approximation for soy proteins is also provided. Source: US-Canada tables of feed composition (NRC, National Academy Press, Washington, D,C., 1982).

Whey, low lactose, dehyrated -as a percent of the protein is:
Arginine (3.58%), glycine (4.30), histidine (1.62), isoleucine (5.75), leucine (9.22), lysine (8.38), methionine (2.40), cystine (2.57), phenylalanine (3.30), tyrosine (2.74), serine (3.52), threonine (5.64), tryptophan (1.62), valine (5.20)

Compared with soy protein:
Arginine (8.71%), glycine (3.94), histidine (2.86), isoleucine (5.46), leucine (7.51), lysine (6.66), methionine (1.04), cystine (1.09), phenylalanine (5.13), tyrosine (3.68), serine (6.16), threonine (3.64), tryptophan (1.04), valine (5.19)

APPENDIX 7: Kool Aid® Nutrition Facts.

Kool Aid® Nutrition Facts	
Serving Size: 1 g	
Amount Per Serving	
Calories	0
Calories from fat	0
Total Fat	0g
Saturated Fat	0g
Cholesterol	0mg
Total Carbohydrates	0g
Sugars	0g
Sodium	0mg
Protein	0g
Calcium	5.5mg
Phosphorus	2.5mg
Magnesium	0mg
Ingredients: Citric Acid, Red 40, Calcium phosphate, Modified cornstarch, artificial flavor, ascorbic acid, natural flavor.	

APPENDIX 8: Sugar Nutrition Facts.

Sugar (sucrose) Nutrition Facts	
Serving Size: 3 g	
Amount Per Serving	
Calories	11
Calories from fat	0
Total Fat	0g
Saturated Fat	0g
Cholesterol	0mg
Total Carbohydrates	0g
Sugars	0g
Sodium	0mg
Protein	0g
Calcium	0mg
Phosphorus	0mg
Magnesium	0mg
Ingredients: Sugar.	

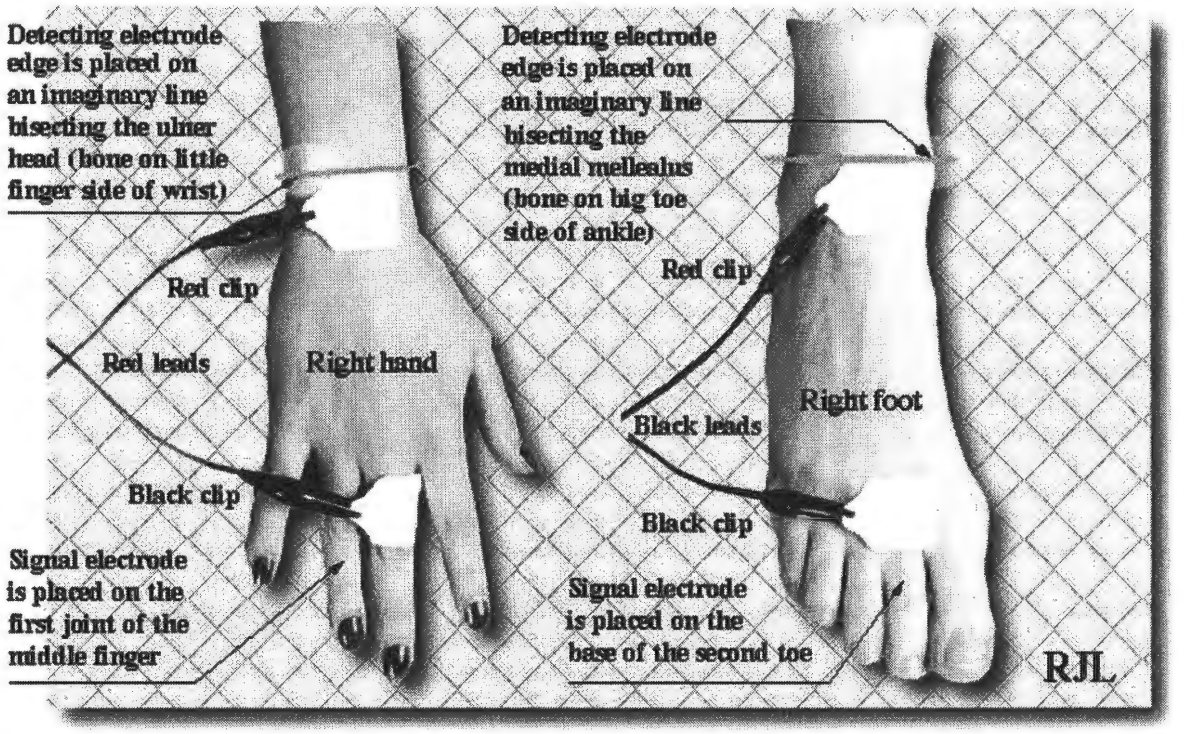
APPENDIX 9: Splenda® Nutrition Facts.

Splenda® Nutrition Facts	
Serving Size: 0.5 g	
Amount Per Serving	
Calories	0
Calories from fat	0
Total Fat	0g
Saturated Fat	0g
Cholesterol	0mg
Total Carbohydrates	0g
Sugars	0g
Sodium	0mg
Protein	0g
Calcium	0mg
Phosphorus	0mg
Magnesium	0mg
Ingredients: Maltodextrin, sucralose.	

APPENDIX 10: BIA testing procedures.

Placement of Electrodes on the Hand and Foot

BIA TESTING PROCEDURE



1. The exam area should be comfortable and free of drafts and portable electric heaters.
2. The exam table surface must be non-conductive and large enough for the subject to lie supine with the arms 30 degrees from the body and legs not in contact with each other.
3. The analyzer calibration and patient cables should be checked regularly (see manual).

SUBJECT PREPARATION

1. The subject should not have exercised or taken a sauna within 8 hours of the study.
2. The subject should refrain from alcohol intake for 12 hours prior to the study.
3. The subject's height and weight should be accurately measured and recorded.
4. The subject should lie quietly during the entire test.
5. The subject should not be wet from sweat or urine.
6. The subject should not have a fever or be in shock.
7. The study and testing procedure should be explained to the subject.

TESTING PROCEDURE

1. The subject should remove the right shoe and sock (generally the study is completed on the right side of the body). The body side (left or right) should always be used subsequently.
2. The subject should lie supine with the arms 30 degrees from the body and legs not touching and remove jewelry on the electrode side.
3. The electrode sites may be cleaned with alcohol, particularly if the skin is dry or covered with lotion.
4. Attach the electrodes and patient cables as shown in the illustration.
5. Turn the analyzer on and make sure the subject refrains from moving. When the measurements have stabilized, record the displayed Resistance (R) and Reactance (Xc) with the subject's name, age, gender, height and weight.

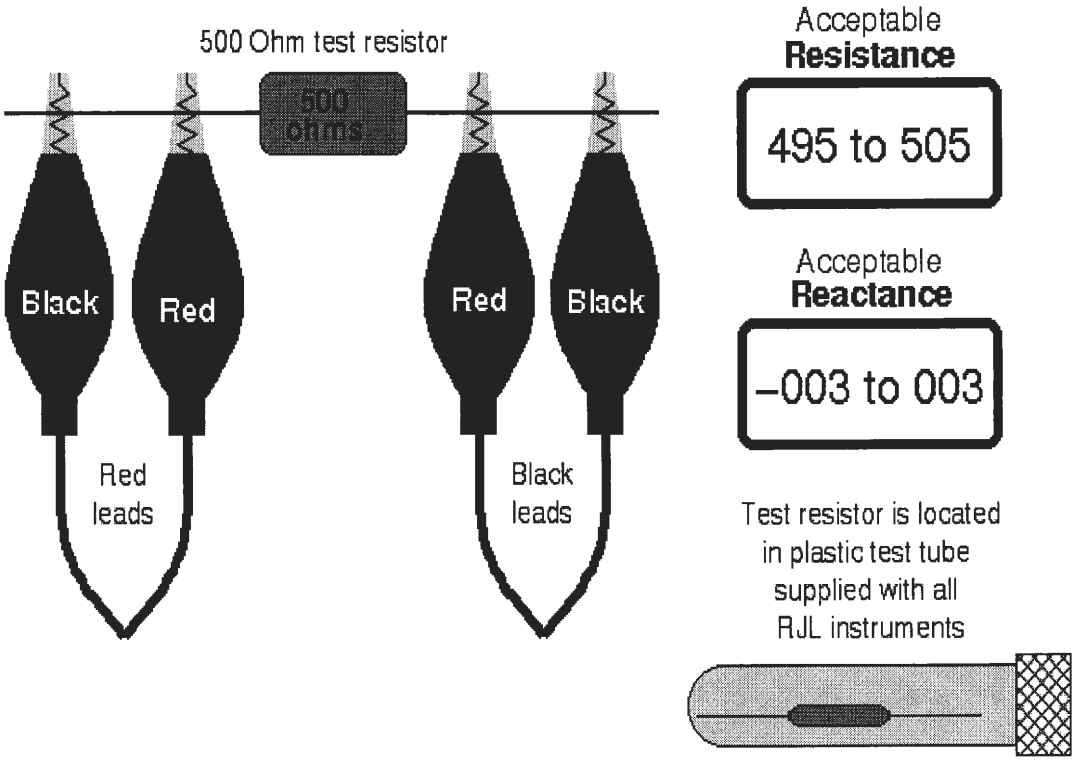
6. Remove and dispose of the electrodes, be careful not injure the subject's skin or contaminate the operator.
7. The entire testing time is less than 5 minutes - the BIA analyzer is on for less than one minute.
8. The results are available immediately from the software program.
9. The study may be repeated as often, as necessary.
10. Operator/examiners must demonstrate the following level of proficiency:
11. Two consecutive measurements made on a single, stable subject must result in values within one percent.

APPENDIX 11: BIA calibration procedures.

Testing the BIA System

The 500 ohm resistor supplied with your BIA system is used to verify the integrity of your system. The resistor is shipped in a plastic test tube and looks like a piece of wire with a small brown cylinder on the middle of it.

Connecting the leads and clips to the test resistors



The following steps will test the impedance circuitry, the subject cables, the subject cable connector and the system's batteries. Follow these steps to test your BIA system:

1. First make sure that your system is fully charged. It is very important that your system is fully charged before continuing.
2. BIA-Quantum users: make sure your system has a fresh 9 volt battery. When the BIA-Quantum batteries are low, decimal points on the impedance meter will be displayed. If this is the case, replace the battery with a fresh 9V alkaline battery by opening the cover on the bottom of the instrument with a small screw driver.
3. Disconnect the charging cord from the wall outlet. Set the resistance/reactance switch to resistance.
4. Attach the 500 ohm resistor (located in the test tube) to the subject cables. Note that the two red clips should be adjacent to the plastic middle of the resistor and that the clips of the red cable should be on one side, the black on the other (see diagram above).
5. Turn the power switch on, and note the resistance value displayed by the impedance meter. The resistance displayed should be between 495 and 505 ohms. If the resistance is in side of this range, then the impedance circuitry, cables and batteries are in good working order. If the resistance is outside of this range, record the resistance value and continue with the next steps.

6. To check the subject cable connector, press lightly on the base of the cables where they attach to the BIA system (labeled SUBJECT). If the resistance fluctuates more than 10 or 15 ohms, the connector may be damaged. Record the amount of fluctuation, and continue with the next step.
7. To check the subject cables, move the cables to different positions and note the resistance displayed on the meter. Should the resistance fluctuate more than 5 ohms, there is probably a break in the cables, and you should call RJL Systems to order a replacement subject cables.

If all the previous steps were performed with good results, your BIA system has checked out OK. If you experience unusual results while a subject is being tested, review the sections Subject Interfacing and Electrode Placement. If the results are still incorrect, call RJL Systems service department. When you call, please have the results of the above tests available for reference.

APPENDIX 12: Example DXA Data Output

Name:	Sex: Male	Height:
Patient ID: 1034	Ethnicity: White	Weight:
DOB: October 05, 1950		Age: 53

Referring Physician: Mikus

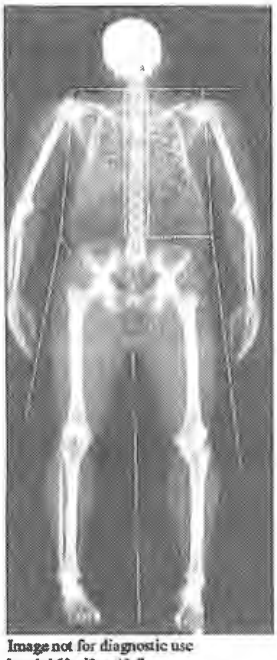


Image not for diagnostic use
k = 1.163, d0 = 43.7
318 x 150

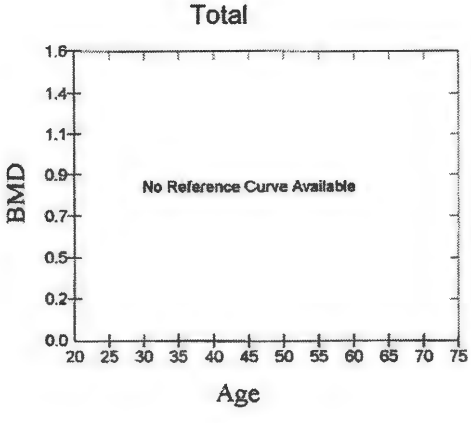
Scan Information:

Scan Date: August 02, 2004 ID: A0802040M
Scan Type: a Whole Body
Analysis: September 01, 2004 12:00 Version 11.2.1:7
Whole Body
Operator: kbh
Model: Delphi W (S/N 71258)
Comment:

DXA Results Summary:

Region	Area (cm²)	BMC (g)	BMD (g/cm²)	T - Score	PR (%)	Z - Score	AM (%)
L Arm	246.09	238.89	0.971				
R Arm	253.46	257.25	1.015				
L Ribs	163.55	120.75	0.738				
R Ribs	170.13	123.96	0.729				
T Spine	135.64	126.54	0.933				
L Spine	55.81	60.45	1.083				
Pelvis	217.03	280.93	1.294				
L Leg	433.28	654.73	1.511				
R Leg	446.07	647.22	1.451				
Subtotal	2121.06	2510.71	1.184				
Head	233.69	448.35	1.919				
Total	**2354.75**	**2959.06**	**1.257**				

Total BMD CV 1.0%, ACF = 1.022, BCF = 0.982

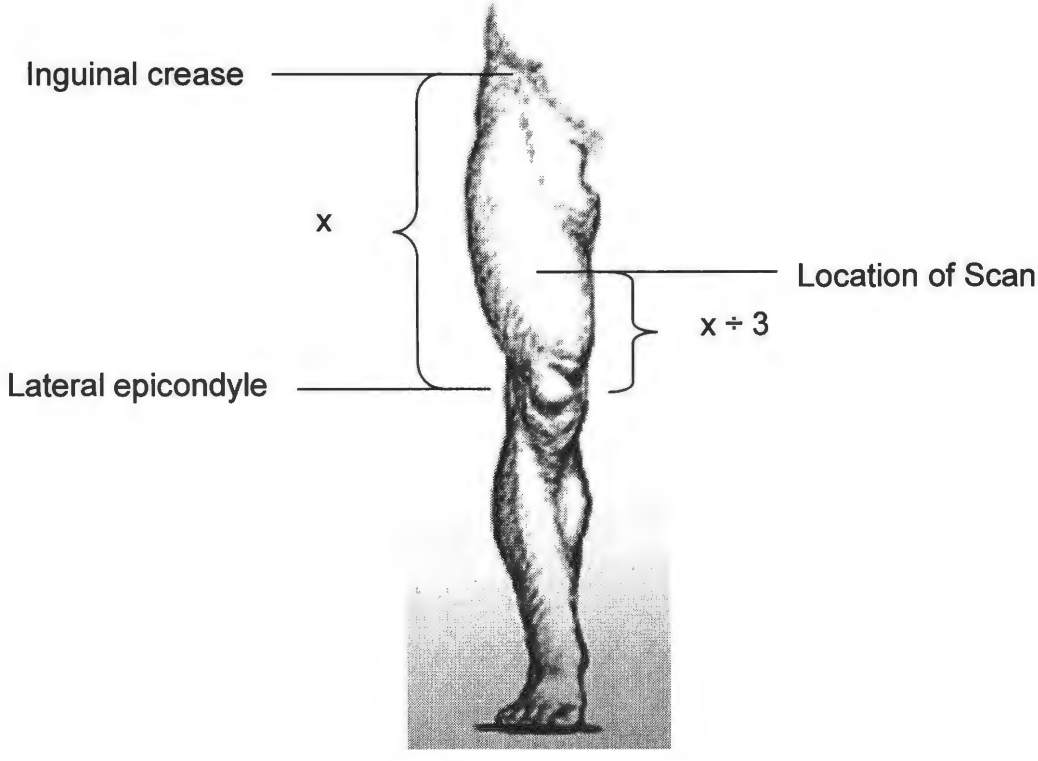


Physician's Comment:

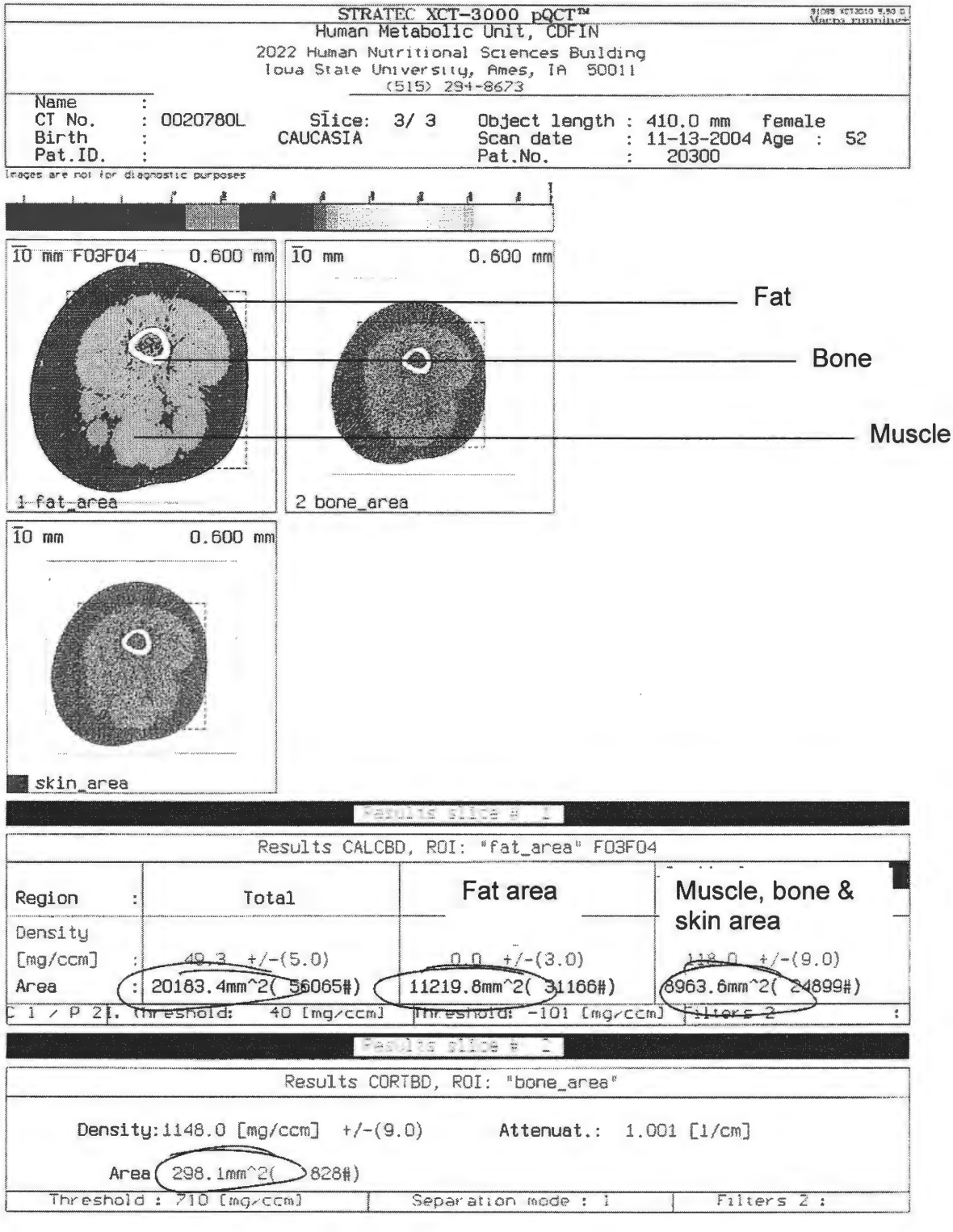
HOLOGIC

APPENDIX 13: pQCT Procedure

The distance between the lateral epicondyle of the patella and the inguinal crease was determined. That distance was then divided by three. The result number was the distance medial to the lateral epicondyle of the patella, where the scan was performed.



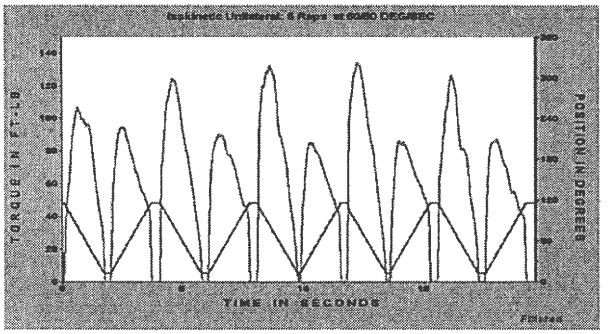
APPENDIX 14: Example pQCT Data Output

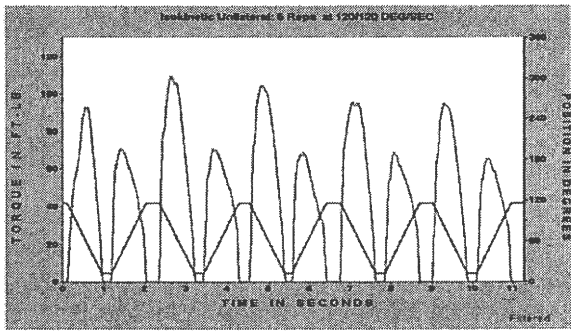


APPENDIX 15: Example Isokinetic Dynamic Strength Data

General Evaluation

Name:		Session:	8/2/04 5:06:36 PM	Windowing:	Isokinetic
ID:		Involved:	None	Protocol:	Isokinetic Unilateral
Birth Date:	(M/d/yy)	Clinician:		Pattern:	Extension/Flexion
HI:		Referral:		Mode:	Isokinetic
Wt:	100.0	Joint:	Knee	Contraction:	CON/CON
Gender:	Male	Diagnosis:		GET:	No Gravity Correction

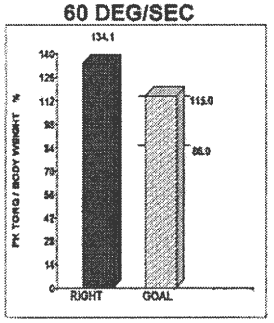




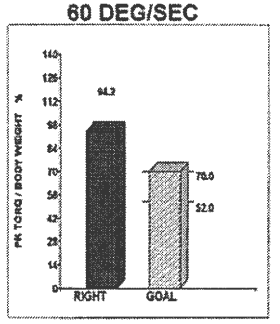
		EXTENSION 60 DEG/SEC	FLEXION 60 DEG/SEC
Side: RIGHT			
# OF REPS: 5			
PEAK TORQUE	FT-LBS	134.1	94.2
PEAK TQ/BW	%	134.1	94.2
MAX REP TOT WORK	FT-LBS	156.4	123.4
COEFF. OF VAR.	%	8.6	4.0
AVG. POWER	WATTS	106.1	78.3
ACCELERATION TIME	MSEC	50.0	50.0
DECELERATION TIME	MSEC	150.0	390.0
ROM	DEG	104.4	
AVG PEAK TQ	FT-LBS	124.8	88.4
AGON/ANTAG RATIO	%	70.3	G: 61.0

		EXTENSION 120 DEG/SEC	FLEXION 120 DEG/SE
Side: RIGHT			
# OF REPS: 5			
PEAK TORQUE	FT-LBS	109.4	70.6
PEAK TQ/BW	%	109.4	70.6
MAX REP TOT WORK	FT-LBS	136.9	93.3
COEFF. OF VAR.	%	6.8	4.2
AVG. POWER	WATTS	154.8	102.5
ACCELERATION TIME	MSEC	40.0	70.0
DECELERATION TIME	MSEC	220.0	320.0
ROM	DEG	104.3	
AVG PEAK TQ	FT-LBS	99.2	68.8
AGON/ANTAG RATIO	%	64.6	G: N/A

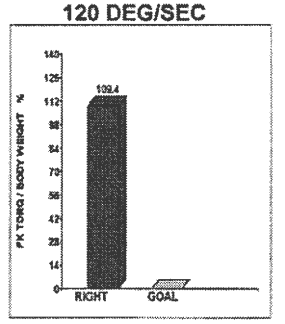
EXTENSION 60 DEG/SEC



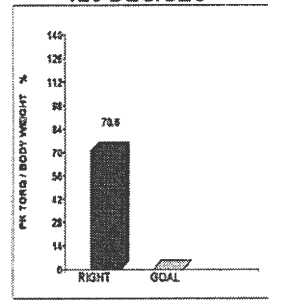
FLEXION 60 DEG/SEC



EXTENSION 120 DEG/SEC



FLEXION 120 DEG/SEC

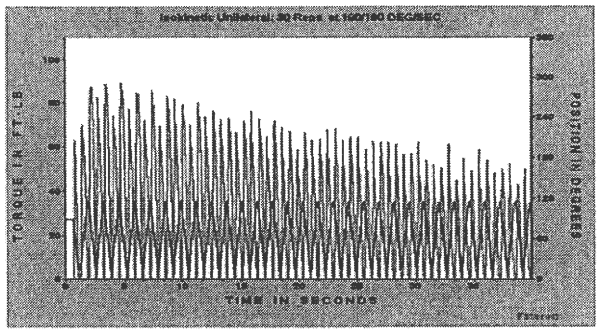


PEAK TORQUE: Highest muscular force output at any moment during a repetition. Indicative of a muscle's strength capabilities.
PEAK TQ/BW : Represented as a percentage normalized to bodyweight and compared to an established goal
MAX REP TOT WORK: Total muscular force output for the repetition with greatest amount of work. Work is indicative of a muscle's capability to produce force throughout the range of motion
[illegible] OF VAR.: Statistical representation of test validity based on reproducibility of performance. Lower values demonstrate higher reproducibility.
[illegible]WER: Total work divided by time. Power represents how quickly a muscle can produce force.
[illegible]RATION TIME: Total time to reach isokinetic speed. Indicative of a muscle's neuromuscular capabilities to move the limb at the beginning of the range of motion
[illegible]ERATION TIME: Total time to go from isokinetic speed to zero speed. Indicative of a muscle's neuromuscular capability to eccentrically control the limb at the end of the range of motion.
AGON/ANTAG RATIO: The Reciprocal muscle group ratio. Excessive imbalances may predispose a joint to injury

14

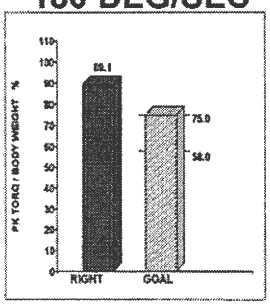
General Evaluation

Name:		Session:	7/31/04 9:09:28 AM	Windowing:	Isokinetic
ID:		Involved:	Both	Protocol:	Isokinetic Unilateral
Birth Date:	(M/d/yy)	Clinician:		Pattern:	Extension/Flexion
Ht:		Referral:		Mode:	Isokinetic
Wt:	100.0	Joint:	Knee	Contraction:	CON/CON
Gender:	Male	Diagnosis:		GET:	No Gravity Correction

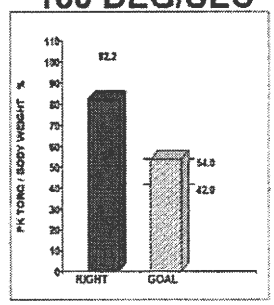


		EXTENSION 180 DEG/SEC	FLEXION 180 DEG/SEC
Side: RIGHT			
# OF REPS: 30			
PEAK TORQUE	FT-LBS	89.1	82.2
PEAK TQ/BW	%	89.1	82.2
MAX REP TOT WORK	FT-LBS	104.8	84.0
COEFF. OF VAR.	%	20.6	13.4
AVG. POWER	WATTS	138.2	116.0
ACCELERATION TIME	MSEC	50.0	50.0
DECELERATION TIME	MSEC	110.0	100.0
ROM	DEG	101.0	
AVG PEAK TQ	FT-LBS	67.1	64.9
AGON/ANTAG RATIO	%	92.2	G: 72.0

EXTENSION 180 DEG/SEC



FLEXION 180 DEG/SEC

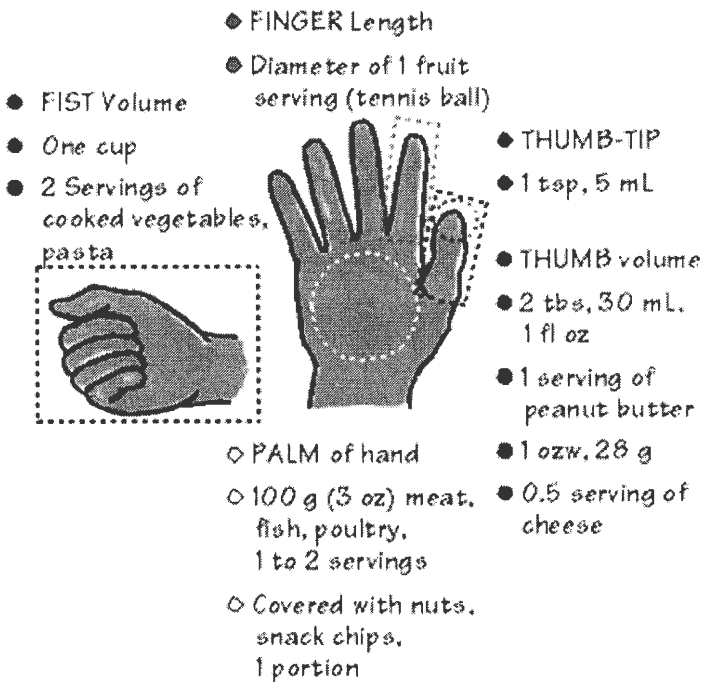


PEAK TORQUE: Highest muscular force output at any moment during a repetition. Indicative of a muscle's strength capabilities.
PEAK TQ/BW : Represented as a percentage normalized to bodyweight and compared to an established goal
MAX REP TOT WORK: Total muscular force output for the repetition with greatest amount of work. Work is indicative of a muscle's capability to produce force throughout the range of motion
[illegible]. OF VAR.: Statistical representation of test validity based on reproducibility of preformance. Lower values demonstrate higher reproducibility.
[illegible]WER: Total work divided by time. Power represents how quickly a muscle can produce force.
[illegible]ERATION TIME: Total time to reach isokinetic speed. Indicative of a muscle's neuromuscular capabilities to move the limb at the beginning of the range of motion
[illegible]ERATION TIME: Total time to go from isokinetic speed to zero speed. Indicative of a muscle's neuromuscular capability to eccentrically control the limb at the end of the range of motion.
AGON/ANTAG RATIO: The Reciprocal muscle group ratio. Excessive imbalances may predispose a joint to injury

APPENDIX 16: Diet Record Handout.

Diet Record

- Below is a rough description of commonly used portion sizes.
- Remember to record *all* foods and drinks that you consume. This includes condiments, butter, alcoholic beverages, etc. between Saturday, November 13th and Tuesday, November 16th.
- Be as specific as possible. Include brand names if possible.
- For foods such as soups or casseroles, estimate the quantity of each food item (carrots, celery, meat, etc) contained within the food.
- Do NOT alter what you are eating! Simply follow your usual routine.



Client Diet Record Nutrient Analysis

First:
Middle:
Last:
Company:

Female
Identification Number:
Date of Birth:
Height: **Weight:**

Total Days: 6
Total Foods: 85
Avg. Daily Kcals: 2561.013

Diet Name: Baseline Diet

Nutrient	Value	Unit	Goal	%
Weight	1894.983	g		
Kilocalories	2782.531	kcal		
Protein	102.686	g		
Carbohydrate	425.859	g		
Fat, Total	83.697	g		
Alcohol	0.000	g		
Cholesterol	155.721	mg		
Saturated Fat	22.759	g		
Monounsaturated Fat	36.065	g		
Polyunsaturated Fat	15.881	g		
MFA 18:1, Oleic	14.265	g		
PFA 18:2, Linoleic	2.894	g		
PFA 18:3, Linolenic	0.353	g		
PFA 20:5, EPA	0.002	g		
PFA 22:6, DHA	0.001	g		
Trans Fatty Acid	0.000	g		
Sodium	3585.043	mg		
Potassium	3226.745	mg		
Vitamin A (RE)	889.320	RE		
Vitamin A (IU)	4640.621	IU		
Vitamin A (RAE)	370.498	µg		
Beta-Carotene	780.844	µg		
Alpha-Carotene	154.090	µg		
Lutein (+ Zeaxanthin)	1699.043	µg		
Beta-Cryptoxanthin	132.744	µg		
Lycopene	3164.790	µg		
Vitamin C	105.879	mg		
Calcium	1344.141	mg		
Iron	18.920	mg		
Vitamin D (ug)	4.351	µg		
Vitamin D (IU)	180.220	IU		
Vitamin E (mg)	9.737	mg		
Vitamin E (IU)	14.508	IU		
Alpha-Tocopherol	1.342	mg		
Thiamin	2.078	mg		
Riboflavin	2.004	mg		
Niacin	15.035	mg		
Pyridoxine (Vitamin B6)	1.349	mg		
Folate (Total)	261.880	µg		
Folate (DFE)	213.426	µg		
Cobalamin (Vitamin B12)	6.791	µg		
Biotin	25.075	µg		
Pantothenic Acid	4.393	mg		
Vitamin K	60.828	µg		

Nutrient	Value	Unit	Goal	%
Phosphorus	1613.375	mg		
Iodine				
Magnesium	442.609	mg		
Zinc	17.117	mg		
Copper	1.337	mg		
Manganese	0.946	mg		
Selenium	85.963	µg		
Fluoride	354.901	µg		
Chromium	0.041	mg		
Molybdenum	27.865	µg		
Dietary Fiber, Total	29.427	g		
Soluble Fiber	2.880	g		
Insoluble Fiber	2.760	g		
Crude Fiber	3.341	g		
Sugar, Total	57.492	g		
Glucose	4.671	g		
Galactose	0.000	g		
Fructose	3.383	g		
Sucrose	12.908	g		
Lactose	25.531	g		
Maltose	0.616	g		
Sugar Alcohol				
Other Carbohydrate				
Tryptophan	861.317	mg		
Threonine	3337.105	mg		
Isoleucine	4014.798	mg		
Leucine	7439.061	mg		
Lysine	6721.169	mg		
Methionine	2074.107	mg		
Cystine	1415.124	mg		
Phenylalanine	3850.009	mg		
Tyrosine	3229.337	mg		
Valine	4421.673	mg		
Arginine	4521.557	mg		
Histidine	2610.740	mg		
Alanine	4482.381	mg		
Aspartic Acid	7438.057	mg		
Glutamic Acid	15494.480	mg		
Glycine	3521.637	mg		
Proline	5976.884	mg		
Serine	3962.872	mg		
Moisture	1183.284	g		
Ash	19.943	g		
Caffeine	0.000	mg		

Nutrient Goal Template
(Client)

Analyzed by
Selection: 8-3

Percentage of Kcals

Protein	14.3%
Carbohydrate	59.4%
Fat, total	26.3%
Alcohol	0.0%

Exchanges

Bread/Starch	3.00
Fat	7.00
Meat-Lean	2.00
Meat-Medium Fat	4.00
Meat-Very Lean	2.00
Milk-Skim	1.50
Other Carbohydrate	20.00
Vegetable	2.00

APPENDIX18: Actigraph® activity monitor.

Actigraph® activity monitor: Methods

At baseline and after 12 weeks of training, a subset of participants (n = 13) were asked to wear Actigraph® (Actigraph, LLC., Fort Walton Beach, FL) activity monitors at the level of the iliac crest in the axillary midline for 3 consecutive days (one weekend day and two weekdays). The specific instructions given to participants are located in Appendix 18. Upon return of the activity monitors, data were downloaded and analyzed using ActiSoft® (Actigraph, LLC., Fort Walton Beach, FL) software. Total counts, peak counts, and average counts were calculated for each participant.

Actigraph® monitors are uniaxial and operate by registering strain via the bending of a peizoceramic cantilever beam. It can record accelerations between 0.5 and 2 times the force of gravity (Gs), filtering vibrations made by the subject. One count is equal to 16 milliGs. Equations can then be used to convert counts to estimates of energy expenditure. These monitors are unable to detect upper body movements, rotational movements, added loads, or gradients.

Actigraph® Activity Monitor Directions

You will be wearing the monitor immediately after you wake up on Thursday, November 11th until you go to sleep for the night on Saturday, November 13th. The

monitor is to be returned to investigators along with diet records and urine as soon as possible.

The monitor attaches to your belt or clips onto your pants and should be worn on the right side just above the hip bone. The monitor has a notch on one side and should be worn with the notch facing up and out. Remember to take off the monitor before you take a shower and to record any activity you do if you forget to put the monitor back on.

Actigraph® activity monitor data.

Complications associated with acquiring data from Actigraph® activity monitors Data were collected using the Actigraph® activity monitors at baseline and 12 weeks on 21 participants. Data are only shown for seven of these participants because of obstacles associated with using these monitors. The data from one participant were not included after the participant lost the monitor given to him during his week twelve testing. The data from another participant were excluded when he attached the monitor to his calf instead of his waist, as instructed. While, MTI states that the monitors can be worn in alternative locations, the validation of this alteration in protocol has not been accomplished. One additional participant forgot to wear his monitor. The data from four other participants were not included for the reason that the monitor failed to record. Problems with the software used to download the data resulted in the absence of data from three other participants, and early termination of data collection by the monitors, perhaps due to battery death, required the removal of data from four other participants. Impediments in either baseline or post-training

data of a participant resulted in the data from that participant being removed at both time points. The data obtained from the seven participants whose data were recovered are listed in the table below. Because of variability and limited data, no conclusions were derived from these data.

Actigraph® Data									
Week	0	12	Δ	0	12	Δ	0	12	Δ
TRX	Total	Total	Total	Peak	Peak	Peak	Avg	Avg	Avg
PLA	336114	90740	245374	4720	2675	-2045	233	63	-170
PLA	204263	225570	21308	2945	3050	104	142	157	15
PLA	243316	298281	54964	4144	4506	362	169	207	38
CHO	478942	437009	41933	6748	6271	-477	333	303	-29
CHO	309432	358386	48954	3473	4655	1182	215	249	34
PRO	212998	200384	12614	5231	6540	1309	148	139	-9

Data are expressed as counts. Δ - change from baseline. PLA – placebo group; CHO – carbohydrate group; PRO – protein group.

APPENDIX 19: IDEEA® activity monitor.

Intelligent Device for Energy Expenditure and Activity (IDEEA®) monitoring: Methods

A smaller subset of participants (n = 6) wore a second activity monitor concomitantly with the first. On the day prior to the start of activity observation, participants reported to the laboratory. At that time, they were given an Actigraph® monitor and instructions along with an IDEEA® (Minisun, LLC., Fresno, CA) activity monitor and separate instructions (Appendix 19) for the second monitor. The IDEAA® activity monitors were initialized by attaching the activity monitor to the right side of the belt or pants of the participant in the auxiliary midline and attaching the leads to their respective locations. One of the five leads was attached to the ball of the right foot between the distal points of the first and second metatarsal bones. Another lead was placed at the same anatomical site on the ball of the left foot. The third and fourth leads were placed on the anterior surface at the midpoint of the right and left thigh, respectively. The fifth lead was placed on the sternum one and one half inches below the sternoclavicular joints. The skin was cleaned with an alcohol prep pad at the site of each lead, and the lead was then taped in place (Appendix 19). Participants were then asked to sit in a chair with their backs straight and their knees bent at a 90 degree angle. They were instructed to hold this position for approximately 30 seconds while the monitor was initialized using ActView® software (Minisun, LLC., Fresno, CA) to begin recording the following morning. The monitor

was then removed, and the participants were then given alcohol swabs and medical tape along with instructions for affixing the activity monitor at home.

IDEAA® monitors are more sophisticated than Actigraph monitors, and, as a result, are able to detect a much wider range of activities (Appendix 21). The monitor works by detecting signals of motion and speed from the 5 sensors placed on the body. These signals are then sent to the microprocessor worn on the waist where the combination of limb movements is analyzed and the type of activity being performed is determined. These data are stored in the microprocessor and can be later downloaded onto a computer and analyzed following each test.

IDEEA® Activity Monitor Directions

1. Attach the Recorder to the right side of your waist. Clip it to the top of your pants or belt above the hip bone.

2. The monitor has five electrodes, and you should clean the skin at the location of sensor attachment with alcohol prior to attaching them. The sensors and wires will run under all your clothes.

a. The single sensor attached to a wire must be connected to the chest or sternum, 1 ½ inches below where the clavicle bones meet. The side of the electrode labeled “Skin” must be in contact with the skin. Tape the sensor in place.

b. The sensors with dual wires are to be connected to your thighs and feet. The sensors closest to the recorder will attach to your thigh. You will connect the sensor marked “Skin Left” to your left thigh and “Skin Right” to your right thigh. The sensors will attach midway between the hipbones and the knees. Tape the sensors in place.

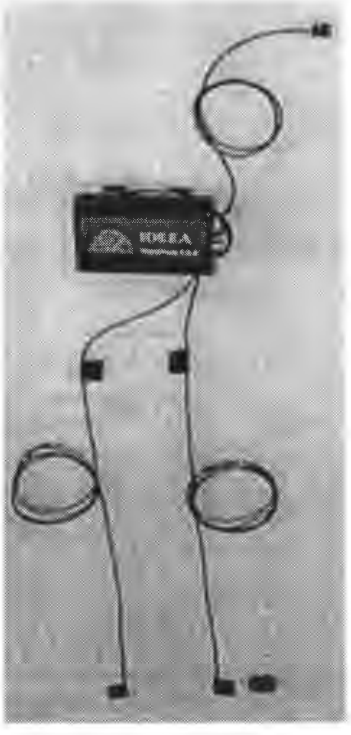
c. The smallest sensors labeled “Skin R” and “Skin L” will be taped to the bottom of your feet on the upper flat part, outside the arch. The wire should be placed so that it crosses the arch. Tape the sensors in place. Any extra length of wire can be taped to the outer side of the lower legs and/or tucked into your socks, but leave room for knee and ankle movement.

Do not wear the monitor in the shower, but wear it at all other times from the time you get out of bed to the time you go to sleep.

Placement of electrodes for activity monitoring using IDEEA®.

A. Photograph of IDEEA® activity monitor

B. Placement of electrodes for IDEEA® activity monitor

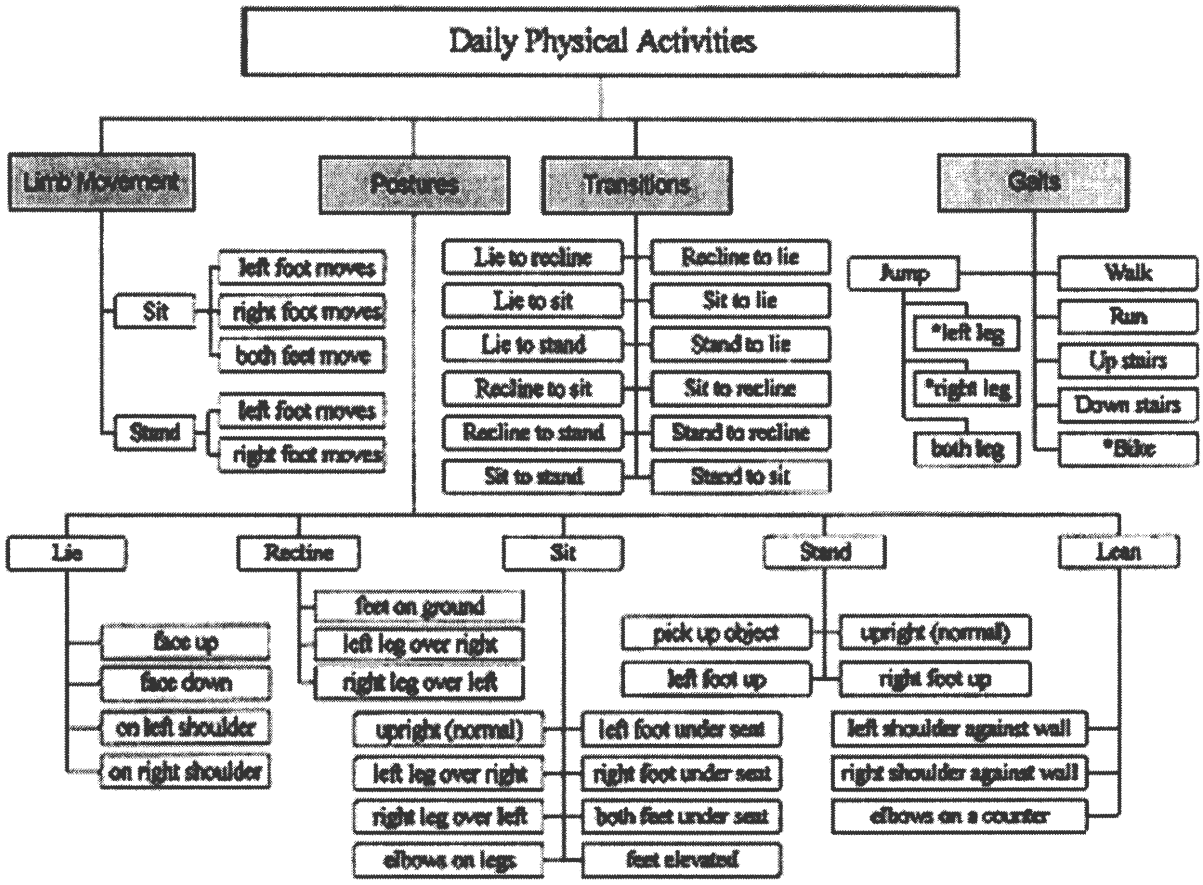


A

B

Activities detectable using IDEEA® activity monitors.

Physical activities detectable by IDEEA® activity monitors



IDEEA® Activity Monitor: Results

Complications associated with acquiring data from IDEEA® activity monitors A very small subset (*n* = 6) of participants were asked to wear IDEEA® activity monitors. The data from these monitors were to be compared from the data acquired from the Actigraph® activity monitors. However, data from four of the six

participants could not be collected post-training. Two of the female participants chosen to wear the monitors at baseline were the two participants who did not complete the study (see Methods). Two of the male participants were unable to wear the monitors during week 12. The final two participants, who wore the monitors at baseline, were two of the participants described above, whose data from the Actigraph® activity monitors were irretrievable. For this reason, none of the six participants asked to wear the monitors at baseline were asked to wear the monitors again after training was completed.

APPENDIX 20: Muscle data.

Muscle Biopsy: Methods

On the morning of the blood draw and urine collection, muscle biopsies were obtained from a subset of participants by experienced and trained scientists. Prior to the procedure, participants were asked to read and sign a separate informed consent (Appendix 22), and any questions they had were answered. The hair at the site of the biopsy was removed from male participants to minimize risk of infection. Participants were then asked to lie in the supine position on a table. The vastus lateralis muscle was located, and approximately 0.5 milliliters of 1% Xylocaine® (AstraZeneca, Waltham, MA) was injected into the skin and muscle fascia at the site where the biopsy would be obtained. After allowing approximately five minutes for the Xylocaine® to take effect, a small, one centimeter incision was made into the skin and fascia at the same site. Sterile gauze was then placed over the incision to control bleeding. After bleeding had subsided, a biopsy needle was placed into the incision and roughly 0.5 centimeters into the muscle bed. Next, suction was applied to the needle, and the blade of the biopsy needle was depressed to sever the muscle. The biopsy needle was then removed, and the muscle sample was removed from the needle. Immediately, a small portion of the sample was severed and placed in a 500 microliter polypropylene tube for later analysis. The tubes were immediately placed in liquid nitrogen to preserve the samples. The remainder of the muscle sample was positioned in Tissue Tek® (Electron Microscopy Services, Hatfield, PA) mounting medium and frozen in isopentane that was the temperature

of liquid nitrogen. All samples were then placed in a –80° Celsius freezer for storage. Three days later, after the ingestion of the N^{15}-glycine tracer solutions, this procedure was repeated. Again, at the end of the 12-week training period, two muscle biopsies were taken, separated by three days during which N^{15}-glycine tracer was ingested and urine was collected.

Following the conclusion of the study, muscle samples were oriented and cut using a Histostat (Reichert, Depew, NY) cryostat. Slices from the samples were then placed on cover slips and stained for digital imaging. The staining procedure was carried out in Columbia jars. After muscle samples were affixed to coverslips, the slips were placed in Columbia jars and incubated for five minutes at room temperature in 2.5 milliliters of 0.2 M barbital acetate buffer, five milliliters of 0.1 M hydrochloric acid and 4 milliliters of deionized water. The coverslips were then rinsed with deionized water and 10 milliliters of an incubation solution were added to the Columbia jar, and the jar was incubated in a shaker bath for 45 minutes at 37 degrees Celsius. The coverslips were then again rinsed with deionized water. A one percent calcium chloride solution was then added, and samples were allowed to sit for 3 minutes. After rinsing the coverslips with deionized water, the samples stood in a two percent cobalt chloride solution for three minutes. The samples were again rinsed with deionized water before they stood in a one percent ammonium sulfide solution for one minute. The samples were then stained with Eosin Y solution after being rinsed with deionized water. The samples were then allowed to dry, covered with a second coverslip and sent to a digital imaging center for fiber typing and determination of fiber size.

Informed consent document for muscle biopsy.

CONSENT FORM

Title of Study: Nutrition Augmentation of Resistance Training to Blunt Aging-Related Losses of Lean Body Mass, Strength, and Functionality in Middle-Aged (50-65) Humans

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You are being asked to participate as a subject for a research study. Please take your time in deciding if you would like to participate. Please feel free to ask questions at any time.

INTRODUCTION

A muscle biopsy will be performed on the lateral aspect of your vastus lateralis muscle (thigh) under local anesthetic. You will undergo a total of four muscle biopsies. Rick L. Sharp, Ph.D. or Dr. Douglas King, Ph. D. will perform the biopsies under sterile conditions. The biopsies will be used for fiber typing and for assessment of protein synthesis.

DESCRIPTION OF PROCEDURES

After your leg has been cleaned with surgical scrub, a small amount of Xylocaine (a local anesthetic) will be injected into the skin and tissue surrounding the muscle. After allowing several minutes for the anesthetic to take effect, a small incision (less than ½ inch long) will be made through your skin and connective tissue. The muscle sample will then be removed by inserting a muscle biopsy needle through the incision into the muscle and snipping off a piece of muscle that is approximately the size of a pea.

During the biopsy procedure, the most common sensation is a slight burning that occurs when the anesthetic is given, and which disappears within a few seconds. Many subjects also experience a "tugging" sensation when the piece of muscle is removed.

RISKS

While participating in this study you may experience the following risks:
Muscle biopsies are associated with some risk of infection of the biopsy site. The risk of infection is minimized by the use of sterile gloves, procedures and instruments. In the more that 3,000 muscle biopsies performed by Drs. Sharp and King, there has been only one instance of infection.

Another risk is some degree of mild soreness on the day after the biopsy. Delayed and minor soreness has been reported in ~10% of all subjects undergoing this procedure. In no instances have subjects reported an amount of soreness sufficient to affect their usual daily activities. The risk of soreness will be minimized by placing pressure over the biopsy site for 5-10 minutes after the biopsy, and by applying a pressure bandage over the site for 12 hours following the biopsy.

A very rare side effect of the muscle biopsy is lightheadedness or dizziness. This is seen in less than 1% of subjects, and subsides within a few minutes of the procedure. Elevation of the legs rapidly stops this side effect.

PARTICIPANT RIGHTS

Your participation in this study is completely voluntary and you may refuse to participate or leave the study at any time. If you decide to not participate in the study or leave the study early, it will not result in any penalty or loss of benefits to which you are otherwise entitled.

RESEARCH INJURY

Emergency treatment of any injuries that may occur as a direct result of participation in this research is available at the Iowa State University Thomas B. Thielen Student Health Center, and/or referred to Mary Greeley Medical Center or another physician

or medical facility at the location of the research activity. Compensation for any injuries will be paid if it is determined under the Iowa Tort Claims Act, Chapter 669 Iowa Code. Claims for compensation should be submitted on approved forms to the State Appeals Board and are available from the Iowa State University Office of Risk Management and Insurance.

QUESTIONS OR PROBLEMS

You are encouraged to ask questions at any time during this study. For further information about the study contact:

Katie Mikus (294-9633, ktmikus@iastate.edu)

Dr. Paul Flakoll (294-8489, flakollp@iastate.edu)

Dr. Rick Sharp (294-8429, rlsharp@iastate.edu)

If you have any questions about the rights of research subjects or research-related injury that the above individuals cannot answer, please contact the Human Subjects Research Office, 2810 Beardshear Hall, (515) 294-4566; austingr@iastate.edu or the Research Compliance Officer, Office of Research Compliance, 2810 Beardshear Hall, (515) 294-3115; dament@iastate.edu

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SUBJECT SIGNATURE

Your signature indicates that you voluntarily agree to participate in this study, that the study has been explained to you, that you have been given the time to read the document and that your questions have been satisfactorily answered. You will receive a copy of the signed and dated written informed consent prior to your participation in the study.

Subject's Name (printed) _____

_____ _____

(Subject's Signature) (Date)

INVESTIGATOR STATEMENT

I certify that the participant has been given adequate time to read and learn about the study and all of their questions have been answered. It is my opinion that the participant understands the purpose, risks, benefits and the procedures that will be followed in this study and has voluntarily agreed to participate.

_____ _____

(Signature of Person Obtaining Informed Consent) (Date)

Muscle Biopsy: Results

Muscle biopsies were obtained from 28 participants at baseline. Infiltration of the muscle sample with fat was excessive in seven of the female participants, and the procedure was not performed on those participants after 12 weeks of training. Six participants voluntarily chose not to undergo the procedure following training, and because of scheduling conflicts, four others were unable to undergo the procedure post-training. Because of the above complications, as well as others, including the dehydration of some samples, sufficient data for analysis of fiber type and size were not obtained.

A recent study published in *Metabolism* provides an indication of what we may have found. Twenty-two young males completed a 14-week resistance training program, consuming either protein or carbohydrate following each exercise session and on non-exercising days. Muscle samples were obtained at baseline and post-training and analyzed for fiber type and size (cross sectional area). While no changes were exposed in the carbohydrate supplemented group, on the contrary, type I and II fibers showed significant hypertrophy (18% and 26%, respectively) in the group supplemented with protein (83). It is likely that similar results would have been seen in this study.

Evaluation of tracer incorporation into the muscle samples revealed the data displayed in the table below. These data suggest that tracer incorporation into muscle over a three-day period is sufficient to be detected using combustion analysis.

Tracer incorporation

Participant	Avg. % N^{15}	Change in % N^{15}
1 Pre-Study-b	0.36779	
1 Pre-Study-e	0.37141	0.00362
1 Post-Study-b	0.37048	
1 Post-Study-e	0.037306	0.00258
2 Pre-Study-b	0.36936	
2 Pre-Study-e	0.37387	0.00452
2 Post-Study-b	0.37142	
2 Post-Study-e	0.37427	0.00284
3 Pre-Study-b	0.36915	
3 Pre-Study-e	0.37245	0.00330
3 Post-Study-b	0.37096	
3 Post-Study-e	0.37383	0.00287

b- denotes background enrichment before delivery of isotope. e- denotes isotopic enrichment after a 24-h oral delivery of ^{15}N-glycine.

It should be noted that these data were obtained merely to serve as a pilot study, and further investigation is needed to validate and refine this methodology.

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