EFFECT OF PROTEIN AND CREATINE SUPPLEMENTATION DURING RESISTANCE TRAINING ON MUSCLE MASS, STRENGTH, AND MUSCLE PROTEIN DEGRADATION IN OLDER MALES

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ABSTRACT

The purpose of this thesis was to determine whether nutritional supplementation combined with resistance training could maximize muscle accretion and strength in older men and whether these interventions could eliminate deficits in muscle mass and strength compared to young men. To achieve this purpose, a series of studies were performed. In the first study, the purpose was to determine differences in muscle mass, strength, and power in upper and lower body muscle groups in young and older men. These findings would determine which muscle groups were more negatively affected with age and whether nutritional supplementation and resistance training in older men could eliminate these deficits in muscle mass and strength compared to young men. Results showed that lower body measures of muscle mass, strength, and power, especially at fast velocities, is reduced more than upper body measures in older men.

In the second study, the purpose was to determine the effects of protein supplementation immediately before and after training sessions for 12 weeks in older men (59-76years), and whether this intervention could eliminate deficits in muscle mass and strength compared to young men from the first study. It was hypothesized that protein ingestion immediately before training would increase lean tissue mass and strength over protein ingestion immediately after training. Twenty-nine older men were randomized to supplement with protein or receive placebo. Results showed that the timing of protein supplementation, either before or after resistance training, had no effect on lean tissue mass, muscle thickness or strength. At the end of the study, the older group still had lower lean tissue mass, muscle thickness of the knee extensors and

flexors and ankle plantar flexors, and bench press strength compared to young men; suggesting that a longer intervention in required.

In the third study, the purpose was to determine the effects of creatine and protein supplementation during resistance training in older men. In addition, these results, combined with the results of 17 subjects from the second study, would determine if 22 weeks of resistance training could eliminate remaining deficits in muscle mass and strength compared to young men. It was hypothesized that creatine and protein together would increase muscle mass and strength over creatine supplementation and placebo during training. Older men (59-77years) were randomized to receive creatine and protein, creatine, or placebo on training days (i.e.3x/week) during 10 weeks of training. Subjects who supplemented with creatine experienced greater gains in total muscle thickness ($p<0.05$), and the addition of protein to creatine significantly increased lean tissue mass compared to placebo. Following 22 weeks of resistance training, deficits in muscle mass and strength were no longer evident compared to the young. Based on this series of studies, muscle mass, strength, and power are significantly reduced in older compared to younger men. Twenty-two weeks of resistance training in older men is sufficient to eliminate deficits in muscle mass and strength compared to young men. These results have application for health and research professionals for the design of nutritional supplementation and exercise interventions. For optimal increase in muscle mass, one should consume creatine and protein on training days during a resistance training program that incorporates power and strength exercises emphasizing lower body muscle groups.

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LIST OF ABREVIATIONS

α	alpha
α- KG	alpha-ketoglutarate
α-KIC	alpha-ketoisocaproate
α- KMV	alpha-keto-β-methylvalerate
AB	Alberta
ADP	adenosine diphosphate
AGAT	arginine: glycine amidinotransferase
Arg	arginine
ATP	adenosine triphosphate
bHLH	basic helix-loop-helix
C	carbon
CA	California
CH_2	methylene group
CH_3	methyl group
CK	creatine kinase
cm	centimeter
CO	Colorado
CO_2	carbon dioxide
COO	carboxyl group
CP	creatine protein group
Cr	creatine
CR	creatine group

Crn	creatinine
CreaT	creatine transporter
CT	Connecticut
d	day
DNA	deoxyribonucleic acid
DXA	dual energy X-ray absorptiometry
g	gram
GAA	guanidinoacetate
GAMT	guanidinoacetate *N*-methyltransferase
GFR	glomerular filtration rate
glu	glutamate
Gly	glycine
H	hydrogen
H_2O	water
Il	Illinois
kcal	kilocalorie
kg	kilogram
L	liter
LTM	lean tissue mass
M	molar
MA	Massachusetts
MB	Manitoba
Met	methionine

mg	milligram
MH	methylhistidine
MHz	megahertz
mL	milliliter
mm	millimeter
mmol	millimol
MRF-4	myogenic transcription factor-4
MRI	magnetic resonance imaging
mRNA	messenger ribonucleic acid
N	nitrogen
NACL	sodium chloride
NaOH	sodium hydroxide
NA_2HPO_4	disodium phosphate
NH_3	amino group
nm	nanometer
NPN	non-protein nitrogen
NTX	cross-linked N-telopeptides of Type I collagen
O	oxygen
ON	Ontario
PCr	phosphocreatine
PLA	placebo
PRO-A	protein after group
PRO-B	protein before group

Ra	rate of tracer appearance
rad	radian
Rd	rate of tracer disappearance
RDA	recommended dietary allowance
RM	repetition maximum
RNA	ribonucleic acid
rRNA	ribosomal ribonucleic acid
s	second
SPSS	statistical package for the social sciences
tRNA	transfer ribonucleic acid
μ	micro
Vb	body density
yr	year

Chapter 1

Introduction to the Study

1.1 Introduction

Sarcopenia refers to the age-related loss of muscle mass which subsequently has a negative effect on strength, power, and functional ability (Evans, 1995). It is estimated that annual health care costs related to sarcopenia approach $300 billion in the USA (Booth et al., 2000). Contributing factors to sarcopenia include changes in muscle function, physical inactivity, and undernutrition (Tipton, 2001).

It is estimated that physical inactivity results in a 50% reduction in muscle mass in older individuals (Smith & Gilligan, 1983). Resistance training has a positive effect on muscle accretion in the aging population (Rennie & Tipton, 2000; Schulte & Yarasheski, 2001; Tipton, 2001). However, muscle loss is still observed in older adults who maintain physical activity (Starling et al., 1999) and weight bearing exercise (i.e. master athletes) (Hameed et al., 2002; Trappe, 2001). Therefore, other factors such as nutrition must contribute to the age-related loss in muscle.

Protein supplementation has been used as an ergogenic aid due to the belief that individuals who engage in resistance type training may benefit from additional dietary protein. The breakdown of contractile protein during heavy resistance training suggests that protein needs may exceed that of basal to supply additional amino acids for metabolic demand (Rennie & Tipton, 2000). It has therefore been speculated that additional dietary protein during resistance training would have a beneficial effect on body composition and exercise performance during resistance training (Lemon, 1998).

Creatine is among the most widely used and researched ergogenic aids to date (Jacobs, 1999). Creatine is readily found in red meat and seafood and is endogenously synthesized by humans in the liver and pancreas. The majority of creatine is stored in

skeletal muscle as phosphocreatine (PCr). During intense muscle contraction, ADP is rapidly phosphorylated by PCr to produce ATP which is utilized within the myofibrils of skeletal muscle for muscle contraction. The basis behind the ergogenic effects of creatine is an increased initial storage pool of PCr in skeletal muscle (Brose et al., 2003), an enhanced resynthesis of PCr during recovery periods from intense muscle contraction (Greenhaff et al., 1994), and possibly increased muscle protein synthesis (Willoughby & Rosene, 2003) and decreased protein breakdown (Parise et al., 2001).

It is well known that muscle mass and strength decrease with age. However, the loss of muscle mass and strength appears to be greater in lower body muscle groups compared to upper body muscle groups in older individuals. For example, when assessing muscle mass and strength in the elbow and knee flexors and extensors between young and older men and women, Lynch et al. (1999) found that arm and leg muscle mass and strength decreased with age but the decrease was significantly greater in the knee flexors and extensors compared to the elbow flexors and extensors. Although the mechanism for this greater loss of muscle mass and strength in lower body muscle groups with age is not fully known, it is theorized that muscle fiber morphology may be involved. Muscle samples obtained at autopsy from 6 males (17-30 years) showed a greater amount of fast-twitch II muscle fibers in lower body muscle groups compared to upper body muscle groups (Johnson et al., 1973). Since there is a gradual dissapperance in fast-twitch (type II) muscle fibers with age (Ansved & Larrson, 1989), lower body muscle groups may atropy at a faster rate than upper body muscle groups. In addition, a further fast-to-slow transformation process resulting in an increased number of intermediate (i.e. type IIa) and slow (i.e. type I) muscle fibers have also been reported with age (Bass et al., 1975;

Boreham et al., 1988; Caccia et al., 1979). This fast-to-slow transititon may have a negative effect on the ability to generate force at higher limb speeds in older individuals (Jubrias et al., 1997). For example, Overend et al. (1992a) found a reduction in force production of the knee extensors at a fast (120^0/s) vs. slow (0^0/s) velocitity in older men. The authors propose that the reduction in force production at higher velocities may be the result of muscle atrophy of type II muscle fibers. In addition, age is a significant determinant of ankle plantar flexion torque at high velocities (180^0/s), but not at slower velocities (30^0/s); suggesting a relative loss of high-speed strength in the ankle plantar flexors in older individuals (Cunningham et al., 1987).

Another factor which may contribute to the greater loss of muscle mass and strength in lower body muscle groups in older individuals involves physical inactivity. For example, Klitgaard et al. (1990) found a significant reduction in knee extensor force production in sedentary older individuals compared to older individuals who had a long history of strength and endurance training. The authors suggest that a protective effect of exercise on maintaining muscle mass and strength in the knee extensors may only appear when exercise is performed at high intensities which primarily recruit fast-twitch type II muscle fibers.

Most studies comparing deficits in force production at different velocities have concentrated on lower body muscle groups (Cunningham et al., 1987; Overend et al., 1992a). It is important to determine if strength at faster velocities (i.e. power) is reduced similarily between upper and lower body muscle groups for the design of optimal exercise interventions which include power-type training in older individuals. Power is important in older individuals, as it is a significant predictor of performance in functional

tasks such as rising from a chair, stair climbing, and walking (Bassey et al., 1992). If the deficits in strength at faster velocities are greater than strength at slower velocities, this may indicate that resistance training programs for older individuals should emphasize power in addition to strength. Recent studies have shown that explosive power training in older individuals can be safe and effective (Hakkinen et al., 1998; 2002). The purpose of the first study was to compare lean tissue mass, muscle thickness, strength, and power at different velocities in the elbow and knee flexors and extensors and ankle plantar flexors between young and older men. These findings would determine which muscle groups were more negatively affected with age for the design of optimal exercise interventions which put more training emphasis on those muscle groups with lower mass and strength. In addition, power is important in older individuals. If the deficits in strength at faster velocities are greater than strength at slower velocities, this may indicate that resistance training programs for older individuals should emphasize power in addition to strength.

Along with resistance training, protein supplementation (Bos et al., 2000; Esmarck et al., 2001; Meredith et al., 1992) and creatine supplementation (Brose et al., 2003; Chrusch et al., 2001; Gotshalk et al., 2002) have proven effective for increasing muscle mass and strength in older individuals. Aging is associated with a reduction in whole body protein synthesis (Schulte & Yarasheski, 2001) possibly through a decrease in myofibrillar protein turnover (Hameed et al., 2002). Muscle loss may be the result of a decrease in protein synthesis, an increase in protein breakdown, or both (Tipton, 2001). Net muscle protein balance (i.e. synthesis-breakdown) becomes positive (i.e. synthesis > breakdown) in the presence of amino acids (Phillips et al., 1997; Tipton et al., 2001; Wolfe, 2001). Amino acid ingestion at rest, before, and following exercise increases

protein synthesis and attenuates protein breakdown (Tipton et al., 1999, 2001; Wolfe, 2001). Amino acids are selected for protein synthesis by binding with transfer RNA (tRNA). The information and order of amino acid sequence for each protein is governed by messenger RNA (mRNA) that is produced from DNA through transcription (Houston, 2001). An increase in amino acid availability through additional dietary protein could potentially increase the combination of tRNA with an amino acid for translocation to ribosomal RNA (rRNA) (i.e. increased translational efficiency) located on the ribosome organelle where protein synthesis occurs. Increased translational efficiency, in the presence of increased amino acid availability, could potentially increase muscle protein synthesis during resistance training (Welle et al, 1994, 1995; Houston, 2001) leading to greater muscle mass and strength (Figure 1.1).

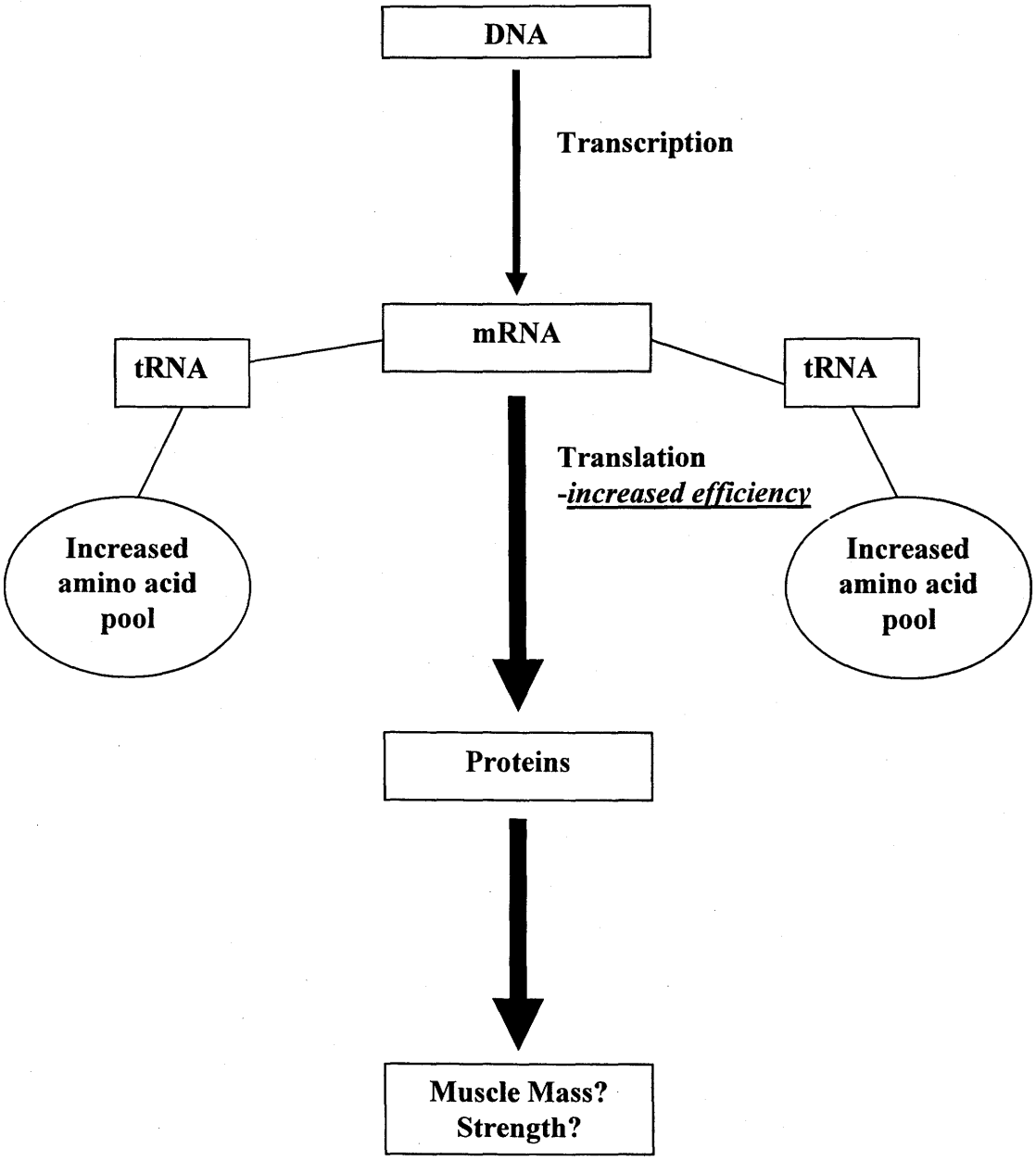

Figure 1.1 Schemtic diagram of increased translational efficiency leading to greater muscle mass and strength.

In examining the effects of protein (~ 30g/day; 10 days) supplementation on body composition and whole-body protein kinetics in 17 malnourished elderly subjects, Bos et al. (2000) found a significant increase in muscle protein synthesis and fat-free mass from protein supplementation. It is important to note that malnourished older individuals may have higher protein turnover rates and a greater loss of muscle protein than healthy older individuals as a result of a hypercatabolic state (Beaumont et al., 1989). On the other hand, amino acid ingestion during 12 weeks of resistance training did not increase muscle mass and strength to a greater extent than resistance training alone in older men (Godard et al., 2002). Furthermore, high protein meals (~28% of energy intake/meal) did not enhance the increase in myofibrillar protein synthesis induced by resistance training alone in sedentary older men and women (Welle & Thornton, 1998); suggesting that the additional amino acids may not be incorporated into muscle protein (Volpi et al., 1999). Therefore, the quantity of dietary protein during resistance may not be a key regulator for increasing muscle mass and strength in healthy older individuals.

Research suggests that the timing of protein ingestion is crucial for improving muscle mass and strength (Phillips, 2004). In assessing the effects of protein versus carbohydrate supplementation before (~ 25 g) and after (~ 25g) lower body resistance training sessions for 14 weeks in young males, Andersen et al. (2005) found a significant increase in muscle cross-sectional area of type I (~18%) and type II (~26%) muscle fibers of the vastus lateralis in the protein group with no effect in the carbohydrate group. Therefore, protein supplementation before and after resistance training sessions induces an anabolic signal for muscle growth. A limitation of the study by Andersen et al. (2005) is that no comparision was made between protein supplementation immediately before

compared to immediately after resistance training sessions. However, in young adults, ingesting an amino acid solution (~ 6 g essential amino acids) immediately before a bout of acute heavy resistance training resulted in a greater increase in muscle protein synthesis compared to consuming the amino acid solution immediately after resistance training. The authors suggest that the greater increase in muscle protein synthesis from essential amino acid ingestion prior to exercise may be the result of increased amino acid delivery to working muscle from exercise induced blood flow (Tipton et al., 2001). Unfortunately, muscle mass and strength were not assessed in the Tipton et al. (2001) study. Therefore, it is unknown if the increase in muscle protein synthesis from ingestion of essential amino acids before and after an acute bout of resistance training would lead to greater muscle hypertrophy during regular resistance training sessions. On the other hand, older males who ingested protein (~ 10 g) immediately after resistance training sessions for 10 weeks had significant increases in muscle size and strength over protein ingestion two hours post-exercise (Esmarck et al., 2001). Consuming protein one to three hours post-exercise did not alter muscle protein synthesis (Rasmussen et al., 2000) or muscular strength (Esmarck et al., 2001). Therefore, the timing of protein ingestion is important for creating an anabolic environment for muscle growth (Tipton et al., 2001), with protein supplementation immediately before (Andersen et al., 2005; Tipton et al., 2001) and immediately after resistance training (Esmarck et al., 2001) appearing optimal. However, it is unknown if advantages exist in consuming protein before compared to after resistance training sessions in older individuals. Therefore, the purpose of the second study was to determine the optimal time to consume dietary protein, either before or after training sessions, to maximize muscle mass and strength in older men. These

findings would also determine if the timing of protein supplementation during resistance training is beneficial for reducing age-related deficits in muscle mass and strength between young and older men.

Research on older individuals has reported decreases in muscle mass (Lindle et al., 1997; Tzankoff & Norris, 1977), strength (Aniansson et al., 1986; Larsson et al., 1979) and high-energy phosphate metabolism (Smith et al., 1998). Creatine supplementation increases intramuscular total creatine (i.e. creatine and phosphocreatine) in older individuals (Brose et al., 2003). The increase in high energy phosphates could allow one to train with a greater volume of resistance exercise leading to increased lean tissue mass and muscular strength (Chrusch et al., 2001). Several studies have reported that creatine supplementation increases muscle mass and strength in older individuals (Brose et al., 2003; Chrusch et al., 2001; Gotshalk et al., 2002), while others report minimal or no benefit (Bermon et al., 1998; Eijnde et al., 2003). These differing results may be due to the inherent variability in older populations, with some studies demonstrating impaired metabolism of high energy phosphates with aging (McCully & Posner, 1992; Smith et al., 1998) while others have not observed the same impairments (Chilibeck et al., 1998b; Kent-Braun & Ng, 2000).

Resistance training results in significant muscle protein turnover (i.e. protein degradation and synthesis) leading to muscle hypertrophy. Greater muscle hypertrophy may imply enhanced protein synthesis and/or reduced protein degradation. Protein or amino acid supplementation enhances the ratio of protein synthesis to degradation post-exercise (Biolo et al., 1997). Creatine supplementation has been shown to increase protein synthesis (Willoughby & Rosene, 2003) and reduce protein degradation (Parise et

al., 2001). Therefore, combining creatine and protein supplementation during resistance training may augment protein synthesis and attenuate protein degradation leading to greater muscle mass and strength over creatine and protein supplementation alone. For example, college-aged athletes who supplemented with a combination of creatine (0.1g/kg body mass/day) and protein (1.2g/kg body mass/day) every day during six weeks of heavy resistance training experienced significant gains in lean tissue mass and muscular strength over protein supplementation or resistance training alone (Burke et al., 2001). Furthermore, young males who consumed a commercial creatine (~ 20 g) and protein (~ 67g) supplement every day during 4-weeks of resistance training experienced significant gains in lean tissue mass (Kreider et al., 1996). The increase in lean tissue mass with the combination group (i.e. creatine + protein supplementation) was greater than the increase typically shown when supplementation is with creatine alone (Kreider et al., 1998). The purpose of the third study was to determine the effects of creatine and protein supplementation during resistance training in older men. With the age-related loss of muscle mass and strength, combining creatine and protein supplementation during resistance training may serve as a more effective intervention to increase muscle mass and strength over creatine supplementation alone during resistance training in older individuals. These findings would also determine if protein and creatine supplementation during resistance training in older men was effective for eliminating age-related deficits in muscle mass and strength compared to young men.

The overall purpose of this thesis was to determine how to maximize muscle accretion and strength through the combination of nutritional supplementation and resistance training in older men and to determine if these interventions could eliminate

deficits in muscle mass and strength compared to young men. To achieve this purpose, a series of studies were performed. In the first study, the purpose was to determine differences in muscle mass, strength, and power in upper and lower body muscle groups in young and older men. These findings would determine which muscle groups were more negatively affected with age for the future design of optimal exercise interventions which put more training emphasis on those muscle groups with low mass, strength, and power. In addition, these results would be used in the second and third study to determine if nutritional supplementation and resistance training in older men could eliminate deficits in muscle mass and strength compared to young men. In the second study, the purpose was to determine if the timing of protein ingestion, either before or after resistance training sessions, had a greater effect on muscle mass, strength, and muscle protein degradation over resistance training alone in older men. These findings would also determine if 12 weeks of nutritional supplementation (i.e. protein and carbohydrate) and resistance training in older men could reduce age-related deficits in muscle mass and strength compared to young men. The third study was designed to determine the effectiveness of protein combined with creatine supplementation during 10 weeks of resistance training in older men. Results from this study would determine whether creatine and protein supplementation is more effective for increasing muscle mass and strength and reducing muscle protein degradation over creatine or resistance training alone. In addition, these results, combined with the results from the second study, would determine whether 22 weeks of nutritional supplementation (i.e. creatine, protein, carbohydrate) and resistance training in older men could eliminate age-related deficits in muscle mass and strength compared to young men.

1.2 Review of Literature

The review of literature includes sections on protein and creatine supplementation and resistance training and their potential for increasing muscle mass and strength in older individuals. Differences in muscle mass and strength in upper and lower body muscle groups between young and older individuals is reviewed because it is important to determine the effect that aging has on different muscle groups for the design of optimal exercise training programs (i.e. programs that would emphasize those muscle groups with low mass, strength, and power). Protein turnover and metabolism are reviewed because of the critical role that amino acids play in stimulating muscle protein synthesis leading to muscle hypertrophy. Resistance training and the timing of protein ingestion are reviewed to provide background of the current research findings relevant to this study. The role and effects of creatine supplementation, combined with protein, are reviewed to provide insight into the possible mechanisms for how creatine and protein together increase muscle mass and strength over creatine or protein supplementation alone. Finally, side effects from protein and creatine supplementation are reviewed to provide background information regarding dosing practices that appear most effective with the least amount of reported side effects.

1.2.1 Age-Related Differences in Muscle Mass and Strength

The loss of muscle strength with age is thought to be secondary to the decline in muscle mass. What is controversial is whether muscle and strength loss, especially at fast velocities, is similar in upper and lower body muscle groups in older individuals. For example, in comparing muscle size differences of the elbow flexors and extensors in

older (76-95 yr, n=13) men, Klein et al. (2001) found muscle size of the elbow extensors to be 11% lower compared to the elbow flexors. The authors suggest that the greater reduction in muscle size of the elbow extensors compared to the elbow flexors may be due to differences in activity level between muscle groups (i.e. intensity of contractions). In addition, the elbow extensors appear to atrophy at a faster rate than the elbow flexors (MacDougall et al., 1980; 1984), indicating that muscle loss among different muscle groups in the same limb may differ in older individuals. Regarding lower body muscle groups, Overend et al. (1992a) observed a significant reduction in muscle cross-sectional area of the knee flexors and extensors, and knee extensor strength at fast (120°/s) vs. slow (0°/s) velocities in old (65-77yr, n=12) versus younger (19-34 yr, n=13) men. The authors suggest these differences may be due to the atrophy of fast contracting type II muscle fibers in the knee extensors. In addition, Klitgaard et al. (1990) reported a significant decrease in speed of contraction (20-26%) of the knee extensors in elderly men when linked to a relative increase in type I myosin heavy chains. It has also been shown that age is a significant determinant of ankle plantar flexion torque at high velocities (180°/s), but not at slower velocities (30°/s) (Cuningham et al., 1987); supporting the suggestion that there is a relative loss of high-speed strength in older individuals in lower body muscle groups. Taken together, these findings suggest that upper and lower body muscle groups may be affected differently with age. However, no comparision of the deficits in upper and lower body muscle groups in the same older individuals were made in these studies. In addressing this limitation, Lynch et al. (1999) found that arm and leg muscle mass and strength decreased with age but the decrease was significantly greater in the knee flexors and extensors compared to the elbow flexors and extensors. However, no

determination of strength at different velocities was performed in this study. The determination of upper and lower body muscle groups affected the most by age is important for the design of optimal exercise interventions which put more emphasis on those muscle groups with low mass and strength. In addition, power is important in older individuals, as it is a significant predictor of performance in functional tasks such as rising from a chair, stair climbing, and walking (Bassey et al., 1992). If the deficits in strength at faster velocities are greater than strength at slower velocities, this may indicate that resistance training programs for older individuals should emphasize power in addition to strength.

1.2.2 Potential Mechanisms for the Age-Related Loss of Muscle Mass

The loss of muscle mass with age is primarily caused by a reduction in muscle fiber number (Trappe, 2001), although fiber atrophy, particually among type II fibers, is also involved (Larsson et al., 2001). During the aging process, there is spatial rearrangement of motor unit fibers and an increased number of muscle fibers per motor unit area (Larsson et al., 2001). Consequently, total muscle fiber number typically decreases indicating a denervation-reinnervation process (Ansved & Edstrom, 1990). In relation to this spatial reorganization of motor unit fibers, changes in myofibrillar protein isoform expression has also been observed both in slow-twitch and fast-twitch muscles. There appears to be a gradual disappearance in fast-twitch (type II) muscle fibers with age which preceeds the age-related loss of total muscle fiber number (Ansved & Larrson, 1989). A further fast-to-slow transformation process resulting in an increased number of

intermediate (i.e. type IIa) and slow muscle fibers (i.e. type I) have also been reported with age (Bass et al., 1975; Boreham et al., 1988; Caccia et al., 1979).

Another possible mechanism for the loss of muscle mass with age involves muscle contractile properities (Larsson et al., 2001). The age-related slowing of twitch properities in motor units of both fast and slow-twitch muscle fibers is thought to be caused by structural (Decoster et al. 1981), functional (Larsson & Salviati, 1989), and biochemical (Viner et al., 1996) changes in the sarcoplasmic reticulum. Sarcoplasmic reticulum properties are the strongest determinants of speed of contraction whereas myosin heavy chain isoforms influence maximum shortening velocity (Brody, 1976). It has been shown that age has a negative effect on sarcoplasmic reticulum protein function (Larsson et al., 2001). A progressive decline in maximum contractile force in skeletal muscle is common with age. Although the mechanism for this loss of force production is not fully known, possible explanations include a decrease in the number of cross-bridges in the driving stroke per muscle fiber volume, a decrease in force generated by each cross-bridge cycle, or a combination of both (Larsson et al., 2001). At the cellular level, there is a significant reduction in myosin per muscle fiber volume in old age (Marx et al., 2002). Myosin is the main myofibrillar protein responsile for force production in skeletal muscle. Using a rat model, Larsson et al. (2001) found a two-fold decrease in speed of contraction between old and young isolated muscle fibers suggesting an accelerated decline in myosin function with advanced age.

Muscle loss with age may also be related to a decrease in the production of anabolic hormones such as testosterone, growth hormone, and insulin-like growth factor - 1 which have a negative effect on the capacity of skeletal muscle to incorporate amino

acids into proteins or through an increase in catabolic agents such as interleukin-6 which increases the rate of muscle protein loss (Deschenes, 2004).

1.2.3 Proteins

Proteins are assemblages of individual amino acids. Amino acids contain an amino group (NH_3^+) chemically linked to the carboxyl group (COO^-) of other amino acids (Brooks et al., 2000). Amino acids provide energy during times of need (i.e. starvation, extended exercise) and support the utilization of fats and carbohydrates (Wagenmakers, 1998). Enzymes are protein molecules that selectively combine with other molecules and catalyze chemical reactions. Proteins play a role in hormone control and regulation. Structural proteins include connective and fibrous tissue (i.e. collagen, elastin) and the contractile proteins, actin and myosin (Marks et al., 1996). Proteins combine with other substances in the blood (i.e. albumin, hemoglobin, myoglobin) to provide a vehicle of transport. Proteins also regulate antibody production and immune system response (Marks et al., 1996).

1.2.4 Protein and the Amino Acid Pool

Proteins are abundant in meat, legumes, vegetables, and dairy products. Protein digestion begins in the stomach under the regulation of two digestive enzymes, pepsin and hydrochloric acid (Brooks et al., 2000). Very little amino acid absorption occurs in the stomach; the majority takes place in the small intestine. Amino acid absorption occurs through the mucosal cells lining the small intestine by specific carrier proteins (Marks et al., 1996). Carrier proteins lie on the brush border of the intestinal villa with active sites

for amino acids and sodium. Operating against a concentration gradient, sodium pulls the carrier protein and attached amino acids into the mucosal cells. From there, amino acids diffuse into circulation. Once in circulation, amino acids enter one of the most important and highly controlled environments in the body, the amino acid pool (Wagenmakers, 1998). The amino acid pool is comprised of the three compartments; blood, liver, and peripheral tissues (i.e. skeletal muscle). Amino acids in one compartment are in constant equilibrium with another. Elimination of nitrogenous products from the amino acid pool through protein and amino acid catabolism requires a constant influx of new protein (Rennie & Tipton, 2000). There is constant protein turnover (i.e. protein degradation and synthesis). Some of the amino acids released during protein degradation will be recycled to make new proteins or utilized in the amino acid pool (Wagenmakers, 1998). The liver is capable of synthesizing most amino acids needed to build proteins, referred to as dispensable amino acids (Elliott & Elliott, 2001). Indispensable amino acids cannot be endogenously synthesized and must be supplied in the diet (Marks et al., 1996). Amino acids synthesized in the liver are utilized for various physiological processes or get released in the blood. Therefore, the amino acid pool may contain amino acids resulting from dietary input, catabolism of cellular proteins, or amino acids synthesized and released from the liver (Houston, 2001).

Humans do not have the ability to store amino acids; rather we store amino acids as proteins. Depending on the physiological status of the individual, amino acids not utilized for the synthesis of proteins or other nitrogen containing compounds (i.e. creatine) will loose their amino group and the resulting carbon skeleton can be used to

make glucose in the liver (e.g. gluconeogenesis), converted into fat for storage, or be oxidized to ATP, CO_2 and H_2O (Houston, 2001).

Resistance training causes an increase in protein synthesis and protein breakdown (Tipton et al., 1999). Consequently, the net balance between protein synthesis and protein breakdown improves after resistance training, but in the post-absorptive state, remains negative (i.e. rate of protein breakdown > protein synthesis) (Wolfe, 2001). Amino acid ingestion is known to be a potent stimulator of protein synthesis (Tipton et al., 2001). Amino acid ingestion at rest and following exercise increases protein synthesis and attenuates protein breakdown (Tipton et al., 2001; Wolfe, 2001). Whereas resistance training can diminish the net breakdown of muscle protein in the absence of amino acids, net gain of muscle protein occurs in the presence of amino acids (Wolfe, 2001). Therefore, an increase in amino acid availability from protein supplementation during resistance training could potentially increase muscle protein synthesis leading to greater muscle mass over resistance training alone.

1.2.5 Protein Metabolism

Skeletal muscle is capable of oxidizing six amino acids (Chang & Goldberg, 1978). These six are leucine, isoleucine, valine, glutamate, asparatate, and asparagine (Wagenmakers, 1998) (Figure 1.2).

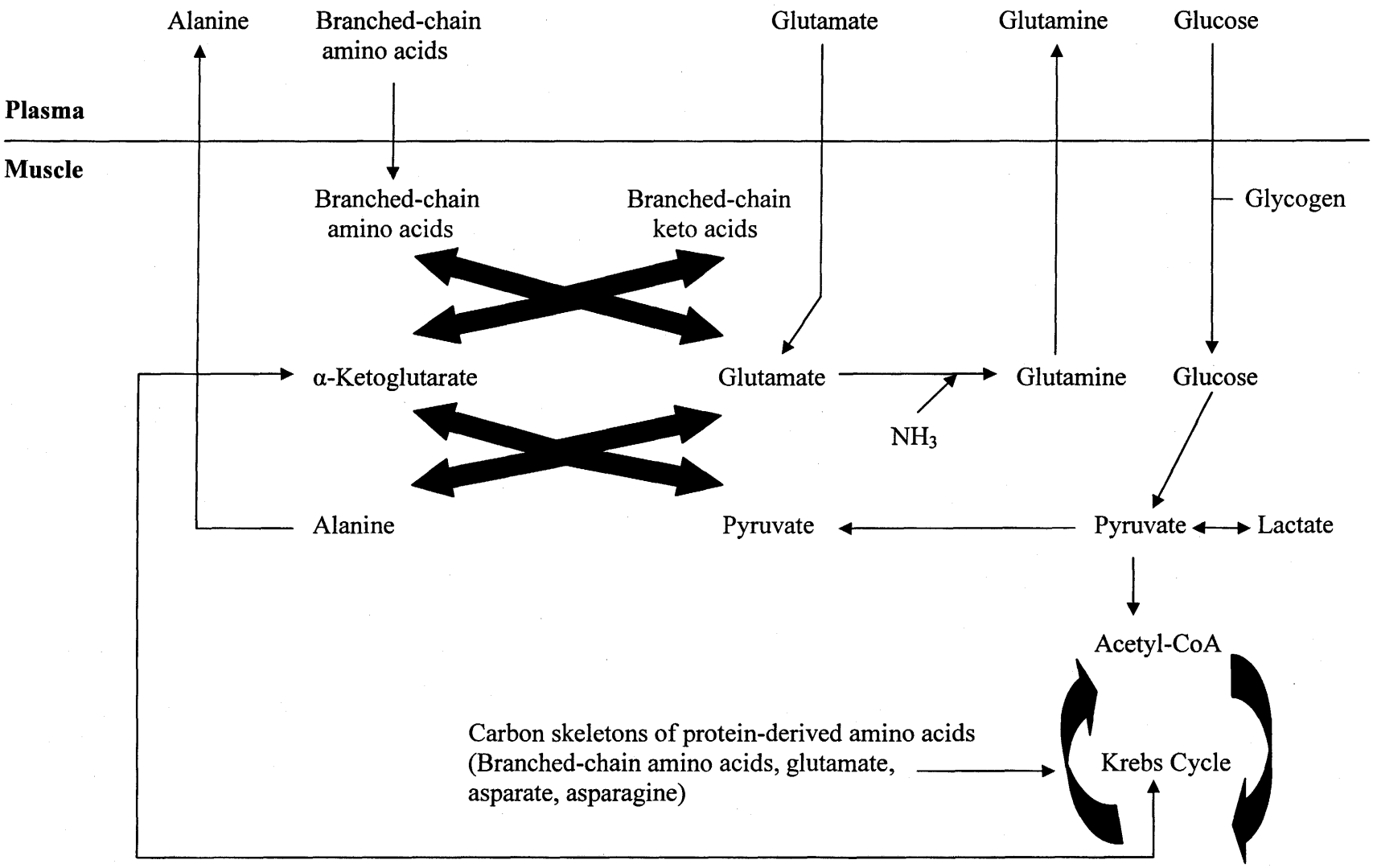

Figure 1.2 Schematic representation of muscle amino acid metabolism.

In the post-absorptive state, net muscle protein balance remains negative (i.e. degradation > synthesis) (Tipton et al., 1999). This results in the release of the branched-chain amino acids, glutamine, and alanine (Wagenmakers, 1998). In the reaction catalyzed by branched-chain aminotransferase, the amino group is donated to α-ketoglutarate (Krebs cycle intermediate) to form glutamine and a branched-chain α -keto acid (Brooks et al., 2000). In the reaction catalyzed by glutamine synthase, glutamate reacts with ammonia to form glutamine. Glutamine may donate its amino group to pyruvate to form alanine and regenerate α-ketoglutarate (Wagenmakers, 1998). These reactions provide a mechanism for the elimination of amino groups in the form of non-toxic nitrogen carriers glutamine and alanine (Marks et al., 1996). Amino acids not metabolized will be oxidized in proportion to their occurrence in muscle protein (Wagenmakers, 1998). Glutamine, having two nitrogen atoms per molecule, is dominant for amino acid nitrogen release from skeletal muscle (Candow et al., 2001a). The branched-chain amino acids, glutamate, asparatate, and asparagine are released in smaller amounts and help synthesize glutamine and alanine (Marks et al., 1996).

1.2.6 Protein Requirements

When dietary protein intake equals excretion, nitrogen balance is achieved. When dietary intake exceeds excretion, the individual enters positive nitrogen balance. This indicates amino acids are being stored and lean tissue synthesized (Rennie & Tipton, 2000). When dietary intake is less than excretion, the individual will enter negative nitrogen balance and loose muscle protein (Butterfield & Calloway, 1984). Nitrogen balance depends on the quantity of dietary protein and total energy input. Despite

adequate protein, if total energy intake is insufficient to meet physiological demand, the individual will enter negative nitrogen balance (Butterfield & Calloway, 1984).

According to the Dietary Reference Intake guidelines, the Recommended Dietary Allowance (RDA) for dietary protein is 0.8g/kg body mass/day for sedentary and physically active individuals. However, some research suggests that a protein intake below 1.0g/kg body mass/day is insufficient to maintain nitrogen balance in young, physically active individuals (Tarnopolsky et al., 1992). Consensus from several research groups suggests that exercising individuals consuming dietary protein in the range of 1.0-1.9g/kg body mass/day will achieve nitrogen balance (Lemon, 1998; Lemon et al., 1992; Tarnopolsky et al., 1992; 1988). However, it has been suggested that amino acid and protein requirements based on nitrogen balance methodologies may underestimate metabolic demand (Zello et al., 1995).

Research is lacking as to the effect of additional dietary protein in older individuals. Older individuals may increase total energy intake through additional dietary protein. Protein requirements for older individuals (0.6-2.0 g/kg body mass/day) (Campbell et al., 1994; Cheng et al., 1978; Gersovitz et al., 1982; Zanni et al., 1979) depend on nutritional status and health. Cheng et al. (1978) concluded that 0.8g/kg body mass/day of dietary protein was adequate to maintain nitrogen balance in older men; however, 0.8g/kg body mass/day of dietary protein for 30 days was inadequate to achieve nitrogen balance in fifteen elderly men and women (Gersovitz et al., 1982). Therefore, it is clear that the optimal level of dietary protein needed to acheive nitrogen balance and preserve lean tissue mass in older individuals is controversial (Campbell et al., 1994).

1.2.7 Resistance Training in Older Individuals

Muscle accretion reflects net balance between muscle protein synthesis and protein breakdown (Balagopal et al., 1997). Muscle loss may be the result of a decrease in protein synthesis, an increase in protein breakdown, or both (Tipton et al., 2001). In older men and women (> 60 years), significant reductions in muscle mass (Klitgaard et al., 1990), whole body protein synthesis (43%), and myofibrillar protein synthesis (28%) were observed (Welle et al., 1994; 1993). Myosin heavy chain II is an important contractile protein and its synthesis declines with age (Balagopal et al., 1997). The diminished ability to remodel this contractile protein may contribute to the age-related loss of muscle (Balagopal et al., 1997).

Resistance training has a positive effect on muscle protein turnover and muscle accretion in the aging population (Rennie & Tipton, 2000; Schulte & Yarasheski, 2001; Tipton, 2001). Untrained volunteers (60-72 yr) who performed isokinetic knee extension and flexion exercise for twelve weeks had significant gains in muscle cross-sectional area (5-11%), muscle fiber area (28-34%), and muscle protein turnover (41%) (Frontera et al., 1988). Even in the frail elderly (90-92 yr, n=10), eight weeks of intense resistance training significantly increased knee extensor muscle mass (9.0 ± 4.5%) (Fiatarone et al., 1990).

Approximately 30-40% of strength loss occurs after the third decade (Schulte & Yarasheski, 2001). Resistance training has been shown to increase muscle strength in older individuals (Fiatarone et al., 1994; 1990; Yarasheski et al., 1993). In older men and women (72-98 yr), ten weeks of lower body resistance training significantly improved leg strength (113%) compared to non-exercising individuals (Fiatarone et al., 1994). In older

men and women (> 60 yr), whole body resistance training (12 weeks; 3 times/week, 65-100% 1-RM) significantly increased knee extension torque (Yarasheski et al., 1993) and in the frail elderly (90-92 yr, n=10), eight weeks of intense resistance training improved knee extensor muscle strength by 174-200% (Fiatarone et al., 1990). Therefore, older individuals, independent of functional ability, respond positively to resistance training (Tipton, 2001).

1.2.8 Resistance Training and Protein Supplementation in Older Individuals

Research is limited regarding additional dietary protein and resistance training in older individuals. Although muscle protein turnover may be reduced in older individuals (Campbell et al., 1995), the capacity to respond to resistance training and additional dietary protein is similar to that of the young (Rennie & Tipton, 2000). For example, hospitalized older males (61-72 yr) undergoing physical rehabilitation who supplemented with protein (~ 24 g/day) during twelve weeks of knee flexor and extensor strength training experienced greater gains in midthigh muscle mass over strength training alone (Meredith et al., 1992). In examining the effects of protein (~ 30g/day; 10 days) supplementation on body composition and whole-body protein kinetics in 17 malnourished elderly subjects, Bos et al. (2000) found a significant increase in muscle protein synthesis and fat-free mass from protein supplementation. It is important to note that malnourished older individuals may have higher protein turnover rates and a greater loss of muscle protein than healthy older individuals as a result of a hypercatabolic state (Beaumont et al., 1989). In addition, there may be increased reliance of the splanchnic tissues in the regulation of muscle protein turonover in older individuals which could

limit the availability of amino acids to peripheral tissues (i.e. muscle, gut) for protein synthesis (Volpi et al., 1999). Splanchnic tissues are responsible for the uptake and release of amino acids to the peripheral tissues. If the splanchnic tissues utilize more amino acids in older individuals, less amino acids will be available for other tissues (Boirie et al., 1997). Therefore, if the increase in first-pass splanchnic extraction attenuates amino acid availability to the peripheral tissues, the response of muscle protein synthesis to oral ingestion of amino acids from protein supplementation may be reduced in older individuals. However, amino acid ingestion in older adults caused a significant increase in first-pass splanchnic extraction and amino acid delivery to skeletal muscle which resulted in a gradual increase in muscle protein synthesis (Volpi et al., 1999). The authors suggest it is unlikely that the loss of muscle mass with age is due to an age-related increase in splanchnic utilization of ingested amino acids (Volpi et al., 1999).

In contrast to the findings of Bos et al. (2000) and Meredith et al. (1992), others have found no benefit from protein supplementation during resistance training in healthy older adults. For example, in healthy older men (65-80 yr) who consumed an amino acid solution immediately following knee extension exercise (12 weeks), no improvements in whole muscle strength or muscle cross-sectional area were observed (Godard et al., 2002). In addition, healthy older men and women (62-75 yr) who increased dietary protein intake (28% of energy intake/meal) during unilateral knee extension exercise had no greater increase in myofibrillar protein synthesis over exercise alone (Welle & Thornton, 1998). The authors suggest that this slower rate of protein synthesis may be caused by a reduction in translational efficiency in older muscle.

1.2.9 Timing of Protein Supplementation

Muscle hypertrophy following resistance training requires net protein synthesis of myofibrillar proteins and therefore, maximal stimulation of muscle protein synthesis is required for the development of muscle mass in older individuals (Esmarck et al., 2001). It is well known that resistance training results in increased protein degradation and protein synthesis (Phillips, 2004). Evidence exists that the rate of protein synthesis exceeds the rate of protein degradation when protein or amino acids are ingested at rest (Biolo et al., 1997). As protein or amino acid ingestion is critical for creating an optimal effect on net muscle protein synthesis, intake of protein before and after exercise is likely important. During resistance training, there may be a net loss of muscle protein, because muscle protein synthesis is either decreased (Bylund-Fellenius et al., 1984) or unchanged (Carraro et al., 1990), whereas protein degradation is elevated (Rennie et al., 1981). Although the machinery for stimulating muscle protein synthesis is increased after resistance training (Welle & Thornton, 1998), it appears that this response may not be increased until some time after the resistance training session (Tipton et al., 2001). Therefore, protein ingestion before resistance training sessions may counter the net loss of muscle protein, thereby creating an anabolic environment for muscle growth (Tipton et al., 2001). For example, six healthy young men and women who ingested an amino acid solution (6g essential amino acids) immediately before an acute bout of resistance training experienced a greater increase in muscle protein synthesis compared to consuming the amino acid solution immediately after training (Tipton et al., 2001). The authors suggest that the greater rate of net muscle protein syntheis to consuming the essential amino acids immediately before rather than after exercise was due to increased

amino acid delivery to muscle from exercise increased blood flow. On the other hand, the rate of muscle protein degradation exceeds the rate of muscle protein synthesis after resistance training in the postabsorptive state (Phillips et al., 1997). However, amino acid ingestion postexercise has been shown to have a positive effect on the rate of muscle protein synthesis (Tipton et al., 1999). The stimulation of protein synthesis following resistance training probably follows a specific time course (Esmarck et al., 2001) as it has been observed that the rate of muscle protein synthesis is greater one hour compared to 24 and 48 hours post exercise (Phillips et al., 1997). Interestingly, older males who ingested protein immediately following resistance training sessions over a 12-week period experienced significant gains in muscle cross-sectional area, mean fiber area, and muscular strength over protein ingestion two hours post-exercise (Esmarck et al., 2001). Consuming protein one to three hours post-exercise did not alter muscle protein synthesis (Rasmussen et al., 2000) or muscular strength (Esmarck et al., 2001). Therefore, the timing of protein ingestion is crucial for creating an anabolic environment for muscle growth (Tipton et al., 2001), with either protein supplementation immediately before (Tipton et al., 2001) or immediately after resistance training (Esmarck et al., 2001) appearing optimal. However, it is unknown if advantages exist in consuming protein before compared to after resistance training sessions in older men. Therefore, the purpose of the second study was to determine the effects of protein supplementation before and after resistance training sessions on muscle mass and strength in healthy older men.

1.2.10 Creatine

Creatine is a nitrogen-containing compound naturally produced in the body and/or consumed in the diet and derived from reactions involving the amino acids glycine, arginine, and methionine (Wyss & Kaddurah- Daouk, 2000). In the human body creatine is primarily produced in a two step process starting in the kidney and finishing in the liver, but can also be synthesized entirely in the pancreas or liver (Loike et al., 1988). In the first step in creatine synthesis, the enzyme arginine: glycine amidinotransferase (AGAT) reversibly catalyzes the transfer of an amidine group from arginine to glycine to produce guanidinoacetate (Wyss & Kaddurah-Daouk, 2000). Guanidinoacetate is then transferred in the blood to the liver (Wyss & Kaddurah-Daouk, 2000). Once in the liver, the methyl group from methionine known as S-adenosylmethionine, is donated to guanidinoacetate by S-adenosylmethionine: guanidinoacetate N- methyltransferase (GAMT) (Persky & Brazeau, 2001; Walker, 1979) which results in the formation of creatine (Hunter, 1922). The rate-limiting step in creatine biosynthesis is the formation of guandinoacetate by AGAT (Walker, 1979; Wyss & Kaddurah-Daouk, 2000). A diagram of the chemical structure of creatine and a schematic representation of its synthesis and storage in the body is presented in Figure 1.3 and Figure 1.4 respectively.

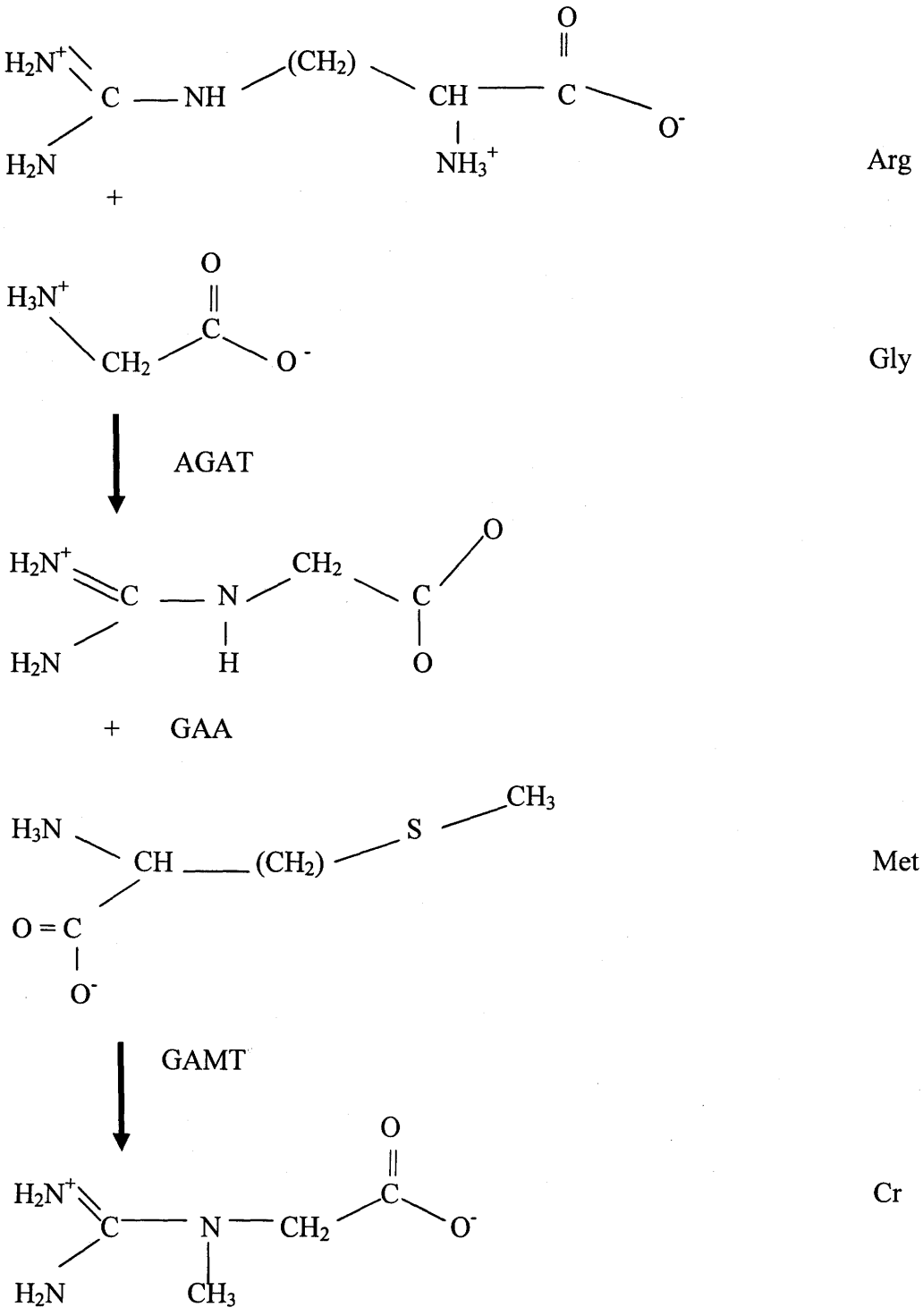

Figure 1.3 Chemical structure of creatine.

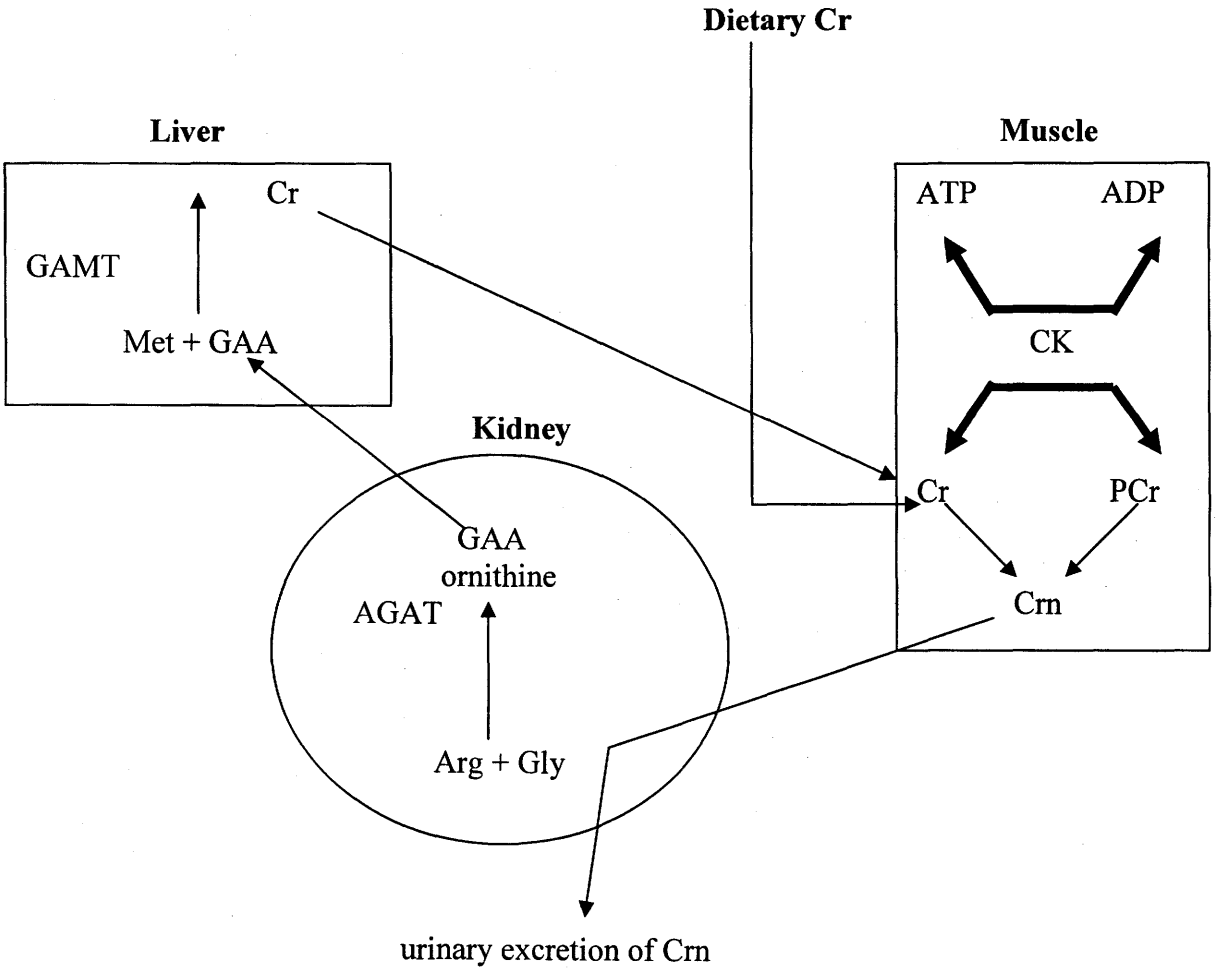

Figure 1.4 Schematic representation of creatine synthesis and storage in the body.

Creatine is obtained at a rate of 1-2 g/day in an omnivore's diet (Greenhaff et al., 1993; Persky & Brazeau, 2001). Typically, dietary creatine matches endogenous creatine degradation, resulting in the formation of creatinine (Walker, 1979). Based on a 70-kg human with a total creatine pool of 120 g, creatinine excretion amounts to 2g/day (Persky & Brazeau, 2001; Walker, 1979). Dietary creatine enters circulation and is transported to skeletal muscle, liver, kidney, and the brain (Guerro-Ontiveros & Wallimann, 1998). Dietary creatine has been shown to reduce endogenous creatine synthesis but values return to normal with creatine cessation (Walker, 1979). Very little creatine is retained at the site of production. The majority of creatine is transported from areas of synthesis (i.e. liver, kidney, pancreas) to areas of storage and utilization (i.e. skeletal muscle) (Persky & Brazeau, 2001). Skeletal muscle accounts for approximately 95% of all creatine stores in the body (Green et al., 1996; Greenhaff et al., 1993). Creatine uptake is facilitated by the creatine transport protein, CreaT (Guerro-Ontiveros & Wallimann, 1998). CreaT expression matches that of creatine kinase (CK): high in areas of creatine storage and utilization (i.e. skeletal muscle) and low in areas of creatine synthesis (i.e. liver, kidney, pancreas). Skeletal muscle creatine content is dependent on muscle fiber composition (Persky and Brazeau, 2001). Type II muscle fibers have high levels of free creatine (Cr) and phosphocreatine (PCr). Intramuscular creatine stores range from 120-160 mmol/ kg dry muscle (Casey et al., 1996) with approximately 60% being PCr (Casey et al., 1996; Harris et al., 1992). With age, there is a progressive decline in skeletal muscle mass and strength (Evans, 1995). Speculation exists that reduced high-energy phosphate metabolism may play a role in these metabolic changes with age. Since 90-95% of PCr is housed in skeletal muscle (Terjung et al., 2000) and PCr is needed to resynthesize ATP

(Greenhaff et al., 1994), one would assume that a progressive decrease in skeletal muscle with age would be associated with a reduction in PCr. An increase in intramuscular creatine from creatine supplementation should theoretically increase PCr resynthesis during muscle contraction leading to greater muscle mass and strength. Therefore, a purpose of the third study was to determine the effects of creatine supplementation during resistance training on muscle mass and strength in older men.

1.3 Creatine Supplementation in Older Individuals

Research showing a beneficial effect from creatine supplementation in the aging population is equivicol (Bermon et al., 1998; Brose et al., 2003; Candow et al., 2004; Chrusch et al., 2001; Eijnde et al., 2003; Gotshalk et al., 2002, Jakobi et al., 2001; Rawson et al., 1999; Rawson & Clarkson, 2000; Smith et al., 1998). Aging may be associated with a reduction in muscle mass, muscle protein turnover, and high-energy phosphate metabolism. The mechanism causing these physiological impairments is not fully known (Smith et al., 1998). Evidence exists that older individuals may have reduced phosphocreatine (PCr) stores (Smith et al., 1998); for example, the resynthesis rate of PCr declines approximately 8% every 10 years after the third decade (McCully & Posner, 1992). Creatine supplementation increases intramuscular total creatine (i.e. creatine and phosphocreatine) in older individuals (Brose et al., 2003). The increase in high energy phosphates could allow one to train with a greater volume of resistance exercise leading to increased lean tissue mass and muscular strength (Chrusch et al., 2001). For example, Brose et al. (2003) found a significant increase in intramuscular total creatine, strength, and lean tissue mass (1.7 ± 1.2 kg) in older adults from creatine supplementation (5g/day)

during 14 weeks of resistance training and Gotshalk et al. (2002) found a significant increase in lean tissue mass (2.2 kg) after seven days of creatine supplementation (0.3g/kg body mass/day) in older men (59-77 years). In addition, Chrusch et al. (2001) reported a significant increase in lean tissue mass of 3.3 ± 1.6 kg following 12 weeks of creatine supplementation and resistance training in older men. The increase in lean tissue mass from creatine supplementation across studies was greater than groups that ingested placebo. The exact mechanism as to how creatine may act to increase muscle mass is not fully known. However, it has been theorized that creatine has the ability to regulate osmosis within the working cell and could potentially elevate intracellular osmolarity (Balsom et al., 1995). The anabolic signal induced by cellular hydration may increase the expression of myogenic transcription factors such as MRF-4 and myogenin (Balsom et al., 1995; Francaux & Poortmans, 1999; Kreider et al., 1998) or alter the level of charged tRNAs which are specific for myofibrillar protein synthesis (Ingwall, 1974). Myogenic transcription factors are members of basic helix-loop-helix (bHLH) proteins that function as transcription activators based on their inherient properties as DNA-binding proteins (Willoughby & Rosene, 2003). As such, they initiate transcription and regulate gene expression by binding to specific regions of a DNA sequence located on the promoter and enhancer regions downstream of muscle specific genes such as myosin heavy chain (Willoughby & Rosene, 2003). Creatine may increase the expression of myogenic transcription factors and facilitate the up-regulation of muscle specific-genes such as myosin heavy chain, thereby facilitating an increase in muscle mass and strength (Willoughby & Rosene, 2003). For example, in young healthy volunteers, creatine supplementation (20g/day) during two weeks of leg immobilization followed by ten

weeks of rehabilitation training significantly increased the expression of myogenic transcription factor MRF-4. The change in MRF-4 expression was correlated with an increase in muscle cross-sectional area (Hespel et al., 2001). In addition, creatine supplementation (6g/day) combined with 12-weeks of heavy resistance training significantly increased mRNA and protein expression of myogenin and MRF-4 in young male subjects (Willoughby & Rosene, 2003). Myogenin and MRF-4 function as positive transcription activators and regulate gene expression. It has been hypothesized that creatine supplementation may increase mRNA template availability in muscle undergoing hypertrophy, resulting from changes in transcriptional capacity, translational efficiency, and/or mRNA stability which may depend on the up-regulation of myogenin and MRF-4 (Willoughby & Rosene, 2003). However, in contrast to these findings, Bermon et al. (1998) failed to observe an increase in lower limb muscle mass after 8 weeks of creatine supplementation and resistance training in older men and women. Furthermore, creatine supplementation for up to one year failed to increase fat free mass in older men (Eijnde et al., 2003). In other studies, creatine supplementation in older males did not improve physical performance, muscle mass (Rawson et al. 1999; Rawson & Clarkson, 2000), or neuromuscular fatigue (Jakobi et al. 2001). Based on these equivicol results, future studies will have to determine the potential of creatine supplementation to influence high-energy phosphate metabolism, intracellular cell volume, gene expression, and muscle protein turnover in older individuals.

1.3.1 Protein and Creatine

Based on the ergogenic properties of creatine and protein supplementation, it has been speculated that creatine and protein supplementation together may promote muscle mass and strength over creatine or protein alone. In a study by Burke et al. (2001), college-aged athletes who supplemented with creatine (0.1g/kg body mass/day) and protein (1.2g/kg body mass/day) everyday during six weeks of heavy resistance training experienced significant gains in lean tissue mass and muscular strength over protein supplementation or resistance training alone. In addition, young males who ingested a commercial creatine (~20 g) and protein (~ 67g) supplement everyday during 4-weeks of resistance training experienced significant gains in lean tissue mass (Kreider et al., 1996). The mechanism for the greater increase in muscle mass from creatine and protein supplementation is not fully known. However, possible factors include cellular hydration and protein kinetics. Creatine has the ability to regulate osmosis within the working cell and could potentially increase intracellular osmolarity (Balsom et al., 1995) and up-regulate myogenic transcription factors such as MRF-4 and myogenin (Balsom et al., 1995; Francaux & Poortmans, 1999; Kreider et al., 1998), or alter the level of charged tRNAs which are specific for myofibrillar protein synthesis (Ingwall, 1974). It is well established that muscle protein synthesis proceeds in the presence of amino acids (Tipton, 2001). An increase in amino acid availability through additional dietary protein could potentially increase the combination of tRNA with an amino acid for translocation to ribosomal RNA (rRNA) (i.e. increased translational efficiency, see Figure 1.1) located on the ribosome organelle where protein synthesis occurs. Increased translational efficiency from protein supplementation could potentially increase muscle protein synthesis with

subsequent resistance training sessions (Houston, 2001; Welle et al., 1994, 1995) leading to greater muscle mass and strength. The up-regulation of transcription factors from creatine supplementation, combined with increased translational efficiency from protein supplementation, may have an additive effect on protein kinetics leading to greater muscle mass and strength in older individuals.

1.3.2 Influence of Creatine and Protein Supplementation on Protein Degradation

Protein degradation plays many essential roles in the functioning of organisms, but only in the past few decades has scientific advances enabled us to quantify protein degradation in humans.

The most popular method used to measure muscle protein degradation involves isotopic tracer technology using radioactive or stable isotopic tracers such as leucine, glycine, or phenylalanine provided as a continuous or primed intravenous infusion (Fouillet et al., 2002). With the continuous intravenous tracer infusion method, arterial plasma tracer enrichment activity reaches a plateau at approximately two hours (Liu & Barrett, 2002). At steady state, whole body protein turnover equals the rate of tracer appearance (Ra) and tracer disappearance (Rd) from the free amino acid pool. The net balance of the amino acid tracer reflects the difference in uptake for protein synthesis and its release from degraded protein (Liu & Barrett, 2002). The amino acid selected as the tracer is dictated somewhat by the tissue being studied. If labeled leucine is used as the tracer and the loss of labeled CO_2 is measured, this approach provides an accurate method to simultaneously estimate whole body protein synthesis and degradation (Liu & Barrett, 2002). However, when assessing skeletal muscle protein turnover, phenylalanine

appears most appropriate. Phenylalanine is neither synthesized nor oxidized in muscle (Tipton et al., 2001). An increase in the rate of phenylalanine disappearance (Rd) from plasma represents the sum of amino acid oxidation to CO_2 and nonoxidative disposal, thereby indicating protein synthesis and an increase in plasma phenylalanine appearance (Ra) reflects the inflow of unlabeled amino acids into blood derived from protein degradation and dietary intake (Liu & Barrett, 2002).

The major advantage in using isotopic tracers is that it allows simultaneous estimation of both protein synthesis and degradation (Liu & Barrett, 2002). Repeated samples can easily be performed in a short period of time to assess changes in synthesis and degradation (Liu & Barrett, 2002). However, besides its cost, this technique has several limitations. An important issue relative to amino acid tracer studies is access to the amino acid precursor pool of substrates used directly for protein utilization (Fouillet et al., 2002). Knowledge of the precise isotopic enrichment is crucial for accurately calculating the amino acid flux and measurement of the true precursor amino acid (i.e. amino acyl transfer RNA) is virtually impossible (Fouillet et al., 2002). The most accessible amino acid pool (i.e. plasma amino acids) is far from providing a satisfactory measurement of intracellular enrichments (Garlick et al., 1994) and leads to an underestimation of protein turnover. Steady state conditions are critical in examining postprandial metabolism. It has been shown that plasma amino acid levels differ in response to changes in food intake (i.e. small vs. large meals) (Elia et al., 1989). Synthetic administration of amino acids do not elevate plasma amino acid and insulin concentrations to the same level as dietary protein (Elia et al., 1989). Therefore, the acute

effect of an increase in amino acid supply may be diminished or suppressed depending on the type and timing of amino acid ingestion.

Another important limitation is the discrepancy between findings obtained using different methodologies and different amino acid tracers, which may lead to differing physiological interpretations of the effects of dietary factors on protein metabolism (Fouillet et al., 2002). For example, the effect of increasing habitual dietary protein intake on whole-body protein kinetics in elderly women differed depending on whether the ^{13}C-leucine or ^{15}N-glycine method was used (Pannemans et al., 1997). Even when using the same precursor method, the use of ^{13}C-leucine or $^{2}H_5$-phenylalanine as a tracer to detect the effects of feeding subjects carbohydrates alone or carbohydrates combined with protein have produced different results (Wagenmakers, 1999). The feeding of carbohydrate and protein together resulted in a significant increase in muscle protein synthesis when $^{2}H_5$-phenylalanine was used as the tracer but not ^{13}C-leucine, leading to a greater improvement in net protein balance in the phenylalanine trial (Fouillet et al., 2002).

Another method used to assess muscle protein degradation involves measuring an amino acid in the urine that is neither metabolized nor reincorporated into protein once released from muscle, such as 3-methylhistidine (Fouillet et al., 2002). Measurement of 3-methylhistidine was used as an index of skeletal muscle protein degradation in this thesis. This measurement and the potential for protein and creatine to affect this measurement are reviewed. 3-methylhistidine is an amino acid found in actin and the heavy chain of myosin in skeletal muscle (Rennie & Millward, 1983) and when measured in urine, is considered to be an indicator of skeletal muscle catabolism (Frontera et al.,

1998; Lukaski et al., 1981). However, 3-methylhistidine excretion can only provide an estimate of the relative change in muscle protein degradation over time, not the absolute change. It is produced by the posttranslational methylation of specific histidine residues in actin and myosin and is neither reutilized for protein synthesis nor metabolized oxidativey but is excreted in the urine (Lukaski et al., 1981).Therefore, an increase in urinary 3-methylhistidine would potentially represent an increase in muscle protein turnover, while a decrease in urinary 3-methylhistidine would represent a decrease in muscle protein turnover. Resistance training results in significant muscle protein turnover (Pivarnik et al., 1989). Therefore, resistance training should result in an increase in urinary 3-methylhisitine. In eleven college-aged males who participated in total body resistance training at moderate intensity (i.e. 70 % 1-RM) for eleven days, significant elevations in urinary 3-methylhistidine levels were observed by the third day of training (Pivarnik et al., 1989). The authors suggest that there is a short time lag (i.e. 72 hours) between the initiation of resistance training and evidence of muscle protein degradation. In contrast, Dohm et al. (1982) reported increased levels of urinary 3-methylhisitine after one day of intense power-lifting training in young adults. Differences between studies may be related to the intensity and duration of training sessions. Evans et al. (1986) had untrained subjects perform a single bout of eccentric cycle ergometer exercise and found urinary 3-meyhylhistidine was significantly increased 10 days after the training session. This was in relation to a subsequent increase in plasma creatine kinase levels indicating muscle damage. A progressive increase in 3-methylhistidine over time may be the result of muscle hypertrophy, which is accompanied by an increase in myofibrillar protein turnover (Frontera et al., 1988). For example, six weeks of resistance training in young

adults significantly increased lean tissue mass and daily excretion of 3-methylhistidine (Candow et al., 2001a).

Disagreement regarding the validity of 3-methylhisitdine excretion as an indicator of muscle protein degradation relates primarily to the contribution of non-muscle sources (i.e. skin, gut). Other areas of concern are the failure to account for inter-individual differences in the ratio of non-myofibrillar/myofibrillar contributions (Liu & Barrett, 2002) and lack of dietary control (Pivarnik et al., 1989). For example, it has been shown that meat consumption increases urinary 3-methylhistidine values and may falsely represent an increase in myofibrillar protein turnover. Therefore, three days of a meat-free diet are required to return 3-methylhistidine levels to baseline (Lukaski et al., 1981). However, even though criticism exists as to the accuracy of 3-methylhistidine for assessing myofibrillar degradation (Rennie & Millward, 1983), recent evidence has shown that 3-methylhistidine release into the interstitum after resistance training is not increased (Trappe et al., 2004). Therefore, most of the increase in 3-methylhistidine after resistance training most likely arises from skeletal muscle. Urinary 3-methylhistidine excretion was used as an indicator of muscle protein degradation in this thesis because it has been shown to be a valid indicator of muscle protein degradation (Welle et al., 1995).

Resistance training results in significant muscle protein degradation (Tipton et al., 2001). Protein or amino acid supplementation enhances the ratio of protein synthesis to degradation post-exercise (Biolo et al., 1995). Therefore, protein supplementation during resistance training should theoretically attenuate the rise in urinary 3-methylhistidine induced by resistance training alone. The normal rise in muscle protein degradation following resistance training was reduced with amino acid supplementation in young

males (Campbell et al., 1995). However, others have found no effect from high protein intake (1.6-2.4 g/kg body mass/day) during resistance training on 3-methylhistidine excretion in young men (Hickson & Hinkelmann, 1985). Possible differences beween studies include meat-free dietary control, habitual protein intake, age, and resistance training design (Pivarnik et al., 1989).

Creatine supplementation has been shown to reduce protein degradation in healthy individuals (Parise et al., 2001). With subsequent resistance training sessions, creatine may reduce muscle protein degradation resulting in a decrease in urinary 3-methylhistidine excretion. However, research is limited examining the effects of creatine supplementation on urinary 3-methylhistidine in healthy individuals. In young males suffering from Duschenne muscular dystrophy, characterized by an increase in protein breakdown, creatine supplementation for 16 weeks did not reduce urinary 3-methylhistidine excretion (Tarnopolsky et al., 2004). However, it is unknown whether creatine supplementation combined with resistance training will reduce urinary 3-methylhistidine excretion in healthy older men.

1.3.3 Protein and Creatine Safety

Since protein and creatine supplements were used, the safety profiles of each are reviewed here. The kidney looses approximately 30% of its weight after the third decade, which corresponds to a 50% reduction in glomerular filtration rate (GFR) and renal blood flow (Fujita, 1990). Individuals with abnormal kidney function have significant elevations in non-protein nitrogen (NPN) when consuming excess protein. In older adults, Kountz et al., (1951) showed that serum NPN increased with protein intake, indicating reduced amino acid incorporation into muscle protein. However, in healthy

individuals, there is no convincing evidence that high protein diets (e.g. >2.0g/kg body mass/day) have an adverse effect on kidney function (Lemon, 1998). Further, high protein intake (>80% total energy input) for more than half its lifespan in a rodent did not produce any negative complications (Zaragoza et al., 1987).

Protein and calcium are essential for bone maintenance. However, high protein diets have been shown to increase the acid load of the urine requiring the body's calcium as a buffer (Packard & Heany, 1997). High protein diets may increase urinary calcium excretion, leading to bone loss (Heaney & Recker, 1982; Itoh et al., 1998). However, others have found a positive correlation between protein intake and bone mineral density (Cooper et al., 1996).

Before dietary protein can be utilized as fuel, the nitrogen-containing amino group must be removed (Brooks et al., 2000). This elevates the urea cycle and leads to water excretion (Lemon, 1998). Water loss could be a concern for aging individuals who have decreased thirst sensation (Morely, 2001). However, there is no evidence of protein-induced dehydration. As long as fluid consumption matches fluid loss, no adverse health complications should occur (Lemon, 1998).

There is limited research on the potential health risks associated with creatine supplementation. Side effects reported involve gastrointestinal irritation, nausea, vomiting, diarrhea, and excessive thirst (Juhn & Tarnopolsky, 1998a; Terjung et al., 2000). Anecdotal reports of physical distress from creatine supplementation usually occur during creatine loading (i.e. 15-20g/day, 1-2 weeks days). The average person consumes 1-2 grams of creatine per day (Juhn & Tarnopolsky, 1998b). Therefore, it is reasonable to suspect that an abnormal response to creatine loading may occur (Schilling et al., 2001).

Using a retrospective questionnaire, Chrusch et al. (2001) reported that twelve weeks of creatine supplementation (0.3 g/kg body mass/day x 5 days; 0.07 g/kg body mass/day thereafter) combined with resistance training in older men caused increased incidence of loose stools, muscle pulls, and muscle strains over resistance training alone. However, using a similar questionnaire, Chilibeck et al. (2004) and Volek et al. (2001) did not report any side effects in young subjects using a similar dose of creatine during resistance training. Others have also shown no ill effect from creatine loading (Volek et al., 1999). In elite male handball players who supplemented with creatine (20g/day x 5 days) during interval training, no medical symptoms were reported (Izquierdo et al., 2002). In college football players, creatine supplementation (16g/day x 28 days) was tolerated with no report of muscle cramps, gastrointestinal irritation or other symptoms (Kreider et al., 1998).

Initial weight gain from creatine supplementation may be the result of enhanced intramuscular creatine stores (Francaux & Poortmans, 1999). Creatine has the ability to regulate osmosis within the myocyte and could potentially elevate intracellular osmolarity (Ingwall, 1976). Thus, water would enter the cell and increase surface volume (Francaux & Poortmans, 1999; Ingwall, 1976; Kreider et al., 1998). It is unknown if cellular hydration will lead to impaired muscle function (Hultman et al., 1996). Hultman et al. (1996) showed that creatine loading in young males caused a significant reduction in urine volume through net body water retention. However, creatine did not adversely affect muscle function. In older men (59-72 yr) who supplemented with creatine (0.3g/kg body mass/day; 7 days), no reports of muscle cramping were observed (Gotshalk et al., 2002).

There is no direct link between creatine supplementation and dehydration (Juhn & Tarnopolsky, 1998a; Terjung et al., 2000). Speculation surrounding creatine-induced dehydration stemmed from the death of three college aged wrestlers who were using severe weight reduction practices and fluid restriction measures (Terjung et al., 2000). All three wrestlers were supplementing with creatine at the time of their death but no evidence has been put forth implicating creatine (Terjung et al., 2000).

Creatine is a nitrogenous containing amine compound that is readily converted to creatinine and excreted through the kidneys (Poortmans et al., 1997). An increase in dietary creatine may cause unwanted stress to kidney function (Jacobs, 1999). However, Poortmans et al., (1997) examined the effect of creatine supplementation (20g/day x 5 days) in young healthy males and found no effect on kidney function. Blood and urine analysis showed an increase in arterial creatine and urinary creatine content with supplementation. Arterial and urinary creatinine was not affected. Therefore, creatine supplementation had no effect on glomerular filtration rate. To further support their findings, Poortmans and Francaux, (1999) showed that creatine supplementation (10 months- 5 years) at various doses (1-80g/day) had no effect on plasma albumin, urinary creatinine, or urea.

Creatine is naturally found in the brain and cerebrospinal fluid (Horn et al., 1998). Speculation exists that creatine supplementation may increase creatine transport across the blood brain barrier and increase brain creatine content (Horn et al., 1998; Terjung et al., 2000). Rats fed creatine-enriched chow for 40 days experienced no change in brain creatine content (Horn et al., 1998). Creatine supplementation may serve a neuroprotective effect in individuals suffering from various neurological disorders

(Matthews et al., 1998). In patients suffering from multiple sclerosis and mitochondrial cytopathy, creatine supplementation (10g/day x 5 days; 5g/day x 5 days) significantly improved neuromuscular fatigue (Tarnopolsky & Martin, 1999). In a series of animal studies, creatine supplementation attenuated drug induced oxidative stress and neuronal-drop out in the corpus striatum (Matthews et al., 1998). In mice suffering from amyotrophic lateral sclerosis, creatine feeding reduced oxidative stress and improved the survival rate of motor cortex and alpha-motor neurons (Kliveni et al., 1999). In a transgenic mouse model with Huntington's disease, creatine supplementation improved survival rate and reduced motor impairment and brain atrophy (Andreassen et al., 2001). Creatine supplementation also increased energy metabolism (Carter et al., 1995) and synaptic nerve transmission in incubated anoxia hippocampal slices (Whittingham & Lipton, 1981). The authors suggest that creatine supplementation may be neuroprotective for individuals at risk of seizure and stroke.

Long term investigation regarding creatine supplementation is scarce. In highly trained athletes, creatine supplementation (1-80g/day; 10 months-5 yr) combined with strenuous physical activity (12-18 hours/week), did not affect glomerular filtration rate (creatinine clearance), tubular reabsorption (urea clearance), or glomerular membrane permeability (albumin clearance) (Poortmans & Francaux, 2000). In patients suffering from gyrate atrophy, creatine supplementation (1.5g/day; >1 yr) increased muscle fiber area, body weight (10%), and strength (Silpa et al., 1981) with no apparent side-effects. In the third study of this thesis, potential side-effects from creatine supplementation were assessed through a questionnaire we have previously used to examine the effects of creatine supplementation during resistance training in older men (Chrusch et al., 2001).

Protein and creatine were chosen for my nutritional intervention because protein and creatine alone have been shown to have a positive effect on muscle mass and strength in the aging population (Bos et al., 2000; Brose et al., 2003; Candow et al. 2004; Chrusch et al., 2001; Gotshalk et al., 2002; Meredith et al., 1992). Protein and creatine together may therefore serve as an effective intervention to increase muscle mass and strength in the aging population.

1.3.4 Summary of the Review

Sarcopenia is a serious health concern. Contributing factors for sarcopenia include changes in muscle function, physical inactivity, and undernutrition. Muscle and strength loss may be prevented through adequate nutrition and resistance type exercise. Resistance training has a positive effect on muscle accretion in the aging population (Rennie & Tipton, 2000; Schulte & Yarasheski, 2001; Tipton, 2001). However, muscle loss is still observed in older adults who maintain weight-bearing exercise (i.e. master athletes) (Hameed et al., 2002; Trappe, 2001). Therefore, other factors such as nutrition must contribute to the loss of muscle with age.

It is well known that muscle mass and strength decreases with age. However, the loss of muscle mass and strength appears to be greater in lower body muscle groups compared to upper body muscle groups in older individuals. For example, in comparing muscle mass and strength of the elbow and knee flexors and extensors between young and older men and women, Lynch et al. (1999) found that arm and leg muscle mass and strength decreased with age but the decrease was significantly greater in the knee flexors and extensors compared to the elbow flexors and extensors. Most studies comparing

deficits in upper and lower body muscle groups in older individuals have made separate comparisons between upper and lower body muscle groups between young and older individuals. The determination of muscle groups affected the most by age is important for the design of optimal exercise interventions which put more emphasis on those muscle groups with low mass and strength. Although the mechanism for the greater loss of muscle mass and strength in lower body muscle groups with age is not fully known, it is theorized that there is a gradual dissapperance in fast-twitch (type II) muscle fibers with age (Ansved & Larrson, 1989). A further fast-to-slow transformation process resulting in an increased number of intermediate (i.e. type IIa) and slow (i.e. type I) muscle fibers have also been reported with age (Bass et al., 1975; Boreham et al., 1988; Caccia et al., 1979). This fast-to-slow transititon may have a negative effect on the ability to generate force at higher limb speeds (Jubrias et al., 1997). The purpose of the first study was to compare lean tissue mass, muscle thickness, strength, and power at different velocities in the elbow and knee flexors and extensors and ankle plantar flexors between young and older men. These findings would determine which muscle groups were more negatively affected with age for the design of optimal exercise interventions which put more training emphasis on those muscle groups with lower mass and strength. In addition, power is important in older individuals, as it is a significant predictor of performance in functional tasks such as rising from a chair, stair climbing, and walking (Bassey et al., 1992). If the deficits in strength at faster velocities are greater than strength at slower velocities, this may indicate that resistance training programs for older individuals should emphasize power in addition to strength.

Resistance training causes an increase in protein synthesis and protein breakdown (Tipton et al. 1999). Consequently, the net balance between protein synthesis and protein breakdown improves after resistance training, but in the post-absorptive state, remains negative (i.e. rate of protein breakdown exceeds protein synthesis) (Wolfe, 2001). Amino acid intake at rest and following exercise increases protein synthesis and attenuates protein breakdown (Tipton et al. 2001; Wolfe, 2001). For example, in examining the effects of protein (~ 30g/day; 10 days) supplementation on body composition and whole-body protein kinetics in 17 malnourished elderly subjects, Bos et al. (2000) found a significant increase in muscle protein synthesis and fat-free mass from supplementation. It is important to note that malnourished older individuals may have higher protein turnover rates and a greater loss of muscle protein than healthy older individuals as a result of a hypercatabolic state (Beaumont et al., 1989). On the other hand, high protein meals (~28% of energy intake/meal) did not enhance the increase in myofibrillar protein synthesis induced by resistance training in sedentary older men and women (Welle & Thornton, 1998); suggesting that the additional amino acids may not be incorporated into muscle protein (Volpi et al., 1999). Therefore, the quantity of dietary protein during resistance may not be a key regulator for increasing muscle mass and strength in healthy older individuals.

Research suggests that the timing of protein ingestion is crucial for improving muscle mass and strength (Phillips, 2004). Young athletes who ingested an amino acid solution immediately before a bout of acute heavy resistance training had a greater increase in muscle protein synthesis compared to consuming the amino acid solution immediately after resistance training (Tipton et al., 2001). Older males who ingested

protein immediately after resistance training sessions for 10 weeks had significant increases in muscle size and strength over protein ingestion two hours post-exercise (Esmarck et al., 2001). Consuming protein one to three hours post-exercise did not alter muscle protein synthesis (Rasmussen et al., 2000) or muscular strength (Esmarck et al., 2001). Therefore, the timing of protein ingestion is important for creating an anabolic environment for muscle growth (Tipton et al., 2001), with protein supplementation immediately before (Tipton et al., 2001) and immediately after resistance training (Esmarck et al., 2001) appearing optimal, possibly through an increase in amino acid delivery to working muscle from exercise increased blood flow (Tipton et al., 2001). However, it is unknown if advantages exist in consuming protein immediately before compared to immediately after resistance training sessions. The purpose of the second study was to determine the optimal time to consume dietary protein, either before or after training sessions, to maximize muscle mass and strength in older men. These findings would also determine if the timing of protein supplementation during resistance training is beneficial for reducing age-related deficits in muscle mass and strength between young and older men.

Research on older individuals has reported decreases in muscle mass (Lindle et al., 1997; Tzankoff & Norris, 1977), strength (Aniansson et al., 1986; Larsson et al., 1979) and high-energy phosphate metabolism (Smith et al., 1998). Creatine supplementation increases intramuscular total creatine (i.e. creatine and phosphocreatine) in older individuals (Brose et al., 2003). The increase in high energy phosphates could allow one to train with a greater volume of resistance exercise leading to increased lean tissue mass and muscular strength (Chrusch et al., 2001). Several studies have reported

that creatine supplementation increases muscle mass and strength in older individuals (Brose et al., 2003; Chrusch et al., 2001; Gotshalk et al., 2002), while others report minimal or no benefit (Bermon et al., 1998; Eijnde et al., 2003). These differing results may be due to the inherent variability in older populations, with some studies demonstrating impaired metabolism of high energy phosphates with aging (McCully & Posner, 1992; Smith et al., 1998) while others have not observed the same impairments (Chilibeck et al., 1998b; Kent-Braun & Ng, 2000).

Resistance training results in significant muscle protein turnover (i.e. protein degradation and synthesis) leading to muscle hypertrophy. Greater muscle hypertrophy may imply enhanced protein synthesis and/or reduced degradation. Protein or amino acid supplementation enhances the ratio of protein synthesis to degradation post-exercise (Biolo et al., 1997). Creatine supplementation has been shown to increase protein synthesis (Willoughby & Rosene, 2003) and reduce protein degradation (Parise et al., 2001). Therefore, combining creatine and protein supplementation during resistance training may augment protein synthesis and attenuate protein degradation leading to a greater increase in muscle mass and strength over protein and creatine supplementation alone. For example, college-aged athletes who supplemented with a combination of creatine (0.1g/kg body mass/day) and protein (1.2g/kg body mass/day) every day during six weeks of heavy resistance training experienced significant gains in lean tissue mass and muscular strength over protein supplementation or resistance training alone (Burke et al., 2001). The purpose of the third study was to determine the effects of creatine and protein supplementation during resistance training in older men. With the age-related loss of muscle mass and strength, combining creatine and protein supplementation during

resistance training may serve as a more effective intervention to increase muscle mass and strength over creatine supplementation alone during resistance training in older individuals. These findings, combined with the results from Study 2, would determine whether 22 weeks of nutritional supplementation (i.e. creatine, protein, carbohydrate) and resistance training in older men could eliminate age-related deficits in muscle mass and strength compared to younge men.

The overall purpose of this thesis was to determine how to maximize muscle accretion and strength through the combination of nutritional supplementation and resistance training in older men and to determine if these interventions could eliminate deficits in muscle mass and strength compared to young men. This purpose can be achieved by determining differences in muscle mass and strength in upper and lower body muscle groups between young and older men (Study 1), by investigating the effects of protein supplementation before and after resistance training sessions in older men (Study 2), by investigating the effects of creatine combined with protein supplementation in older men (Study 3), and by combining the results from Study 2 and Study 3 to determine whether nutritional supplementation (i.e. creatine, protein, carbohydrate) and resistance training in older men could eliminate age-related deficits in muscle mass and strength compared to younger men.

Purpose and Hypotheses

The purpose of this thesis was to determine how to maximize muscle accretion and strength through the combination of nutritional supplementation and resistance

training in older men and to determine if these interventions could eliminate deficits in muscle mass and strength compared to young men.

1.3.5 Purpose and Hypothesis Study 1

The purpose of Study 1 was to compare lean tissue mass, muscle thickness, strength, and power at different velocities in the elbow and knee flexors and extensors and ankle plantar flexors between young and older men. This allowed determination of which muscle groups were most affected by the aging process. It was hypothesized that young men would have greater lean tissue mass, muscle thickness, strength, and power for all muscle groups versus older men. It was also hypothesized that lower body muscle groups would be more affected than upper body muscle groups (Lynch et al., 1999), and strength and power at fast velocities would be more affected than at slower velocities (Overend et al., 1992a, Cunningham, 1987).

1.3.6 Purpose and Hypothesis Study 2

The primary purpose of Study 2 was to determine the optimal time of protein ingestion to maximize muscle mass and strength in older men. A secondary purpose was to determine if this intervention could reduce age-related deficits in muscle mass and strength between young and older men. Based on the findings of Tipton et al. (2001) who found young athletes who ingested an amino acid solution immediately before a bout of heavy resistance training had a greater increase in muscle protein synthesis compared to consuming the amino acid solution immediately after resistance training, it was hypothesized that protein ingestion immediately before resistance training would increase

muscle mass, strength, and attenuate muscle protein degradation over protein ingestion immediately after resistance training.

1.3.7 Purpose and Hypothesis Study 3

The primary purpose of Study 3 was to determine changes in muscle mass, strength, and muscle protein degradation resulting from combining creatine and protein supplementation during resistance training in older men. A secondary purpose was to determine if this intervention, combined with the results from the second study, could eliminate deficits in muscle mass and strength between young and older men. The rate of muscle protein synthesis is elevated with ingestion of amino acids (Tipton et al., 2001), while ingestion of creatine has the potential to either increase protein synthesis (Willoughby & Rosene, 2003) or reduce the rate of protein degradation (Parise et al., 2001). The combination of creatine and protein during resistance training produced greater gains in lean tissue mass and strength over protein supplementation or resistance training alone in young men (Burke et al., 2001). The increase in lean tissue mass with the combination of creatine and protein was also greater than the increase typically shown when supplementation is with creatine alone (Kreider et al., 1998). Based on these findings, it was hypothesized that protein and creatine supplementation together during resistance training would increase muscle mass, strength, and attenuate muscle protein degradation over creatine supplementation and resistance training or resistance training alone. It was also hypothesized that creatine supplementation combined with resistance would increase muscle mass, strength and muscle protein degradation over resistance training alone.

1.3.8 Limitations

(1) Results obtained from this study can only be applied to the specific populations from which the subjects were drawn from.

(2) The primary measure of muscle strength was dependent on subject motivation, time of testing, resistance training compliance, and supplementation.

(3) Muscle thickness measurements (pre and post) of the elbow, knee, and ankle flexors and extensors can only be assumed to have come from the same anatomical site.

(4) Twenty-four hour urine samples were collected by subjects in the second and third study and are dependent on subject adherence to the designated urine collection procedure throughout the day. Human error may have occurred during sample collection.

(5) Dietary habits were not completely controlled since the subjects were free-living individuals. Energy intake estimation relies upon the ability of the subjects to report accurate portion sizes and frequency of food intake as well as their loyalty in filling out the 3-day food records.

(6) Without controlling for diet, the 72-hour meat free period may not have been adhered to accurately. Meat-based products (i.e. pork, poultry, seafood, beef, processed meats) may elevate 3-methylhistidine levels above normal and may produce inaccurate measures of skeletal muscle protein breakdown (Lukaski et al., 1981).

1.3.9 Delimitations

(1) Results from this study apply to the specific age range of the subjects, their training status, health, and diet since all of these variables may affect the results.

(2) Since the majority of the analyses performed involved the use of technical equipment, it can only be assumed that the information provided was accurate.

(3) Since the study was conducted under laboratory conditions, it is difficult to predict if nutritional supplementation during resistance training would have an effect on daily living.

1.3.10 Thesis Structure

This thesis involved three separate but connected studies. For ease of understanding and clarity, the methodology section has been divided into the three studies. In the next chapter, each study is written as a complete and separate study which includes an introduction, methods, results, and discussion section along with respective tables and figures. A complete reference list of all cited manuscripts and other documented literature in each respective study is included at the end of the document.

Chapter 2

STUDIES 1, 2, & 3

Study 1

Differences in Size, Strength, and Power of Upper and Lower Body Muscle Groups in Young and Older Men

Introduction

It is well known that muscle mass and strength decrease with age. However, the loss of muscle mass and strength appears to be greater in lower body muscle groups compared to upper body muscle groups in older individuals. For example, when comparing muscle mass and strength in the elbow and knee flexors and extensors between young and older men and women, Lynch et al. (1999) found that arm and leg muscle mass and strength decreased with age but the decrease was significantly greater in the knee flexors and extensors compared to the elbow flexors and extensors. Most studies comparing deficits in upper and lower body muscle groups in older individuals have made separate comparisons between upper and lower body muscle groups (Kent-Braun & Ng, 1999; Klein et al., 2001; Overend et al., 1992a, 1992b). This can only indirectly answer the question of which muscle groups are most affected by the aging process. A comparison of the relative deficits between muscle groups within older individuals is needed to determine which muscle groups are most affected by age. The determination of muscle groups affected the most by age is important for the design of optimal exercise interventions which put more emphasis on those muscle groups with low mass and strength. The primary purpose of this study was to determine the relative deficits in muscle thickness, torque, normalized torque (i.e. torque relative to muscle thickness), and power between upper and lower body muscle groups within older men. Based on the findings of Lynch et al. (1999), it was hypothesized that lower body muscle groups would show greater deficits compared to upper body muscle groups within older men.

There appears to be a gradual dissapperance in fast-twitch (type II) muscle fibers with age (Ansved & Larrson, 1989). A further fast-to-slow transformation process

resulting in an increased number of intermediate (i.e. type IIa) and slow (i.e. type I) muscle fibers have also been reported with age (Bass et al., 1975; Boreham et al., 1988; Caccia et al., 1979). This fast-to-slow transititon may have a negative effect on the ability to generate force at higher limb speeds (Jubrias et al., 1997). For example, Overend et al. (1992a) found a reduction in normalized force production of the knee extensors at a fast (120^0/s) vs. slow (0^0/s) velocity in older men. The authors propose that the loss of normalized force at higher speeds in the knee extensors is the result of atrophy of type II fibers. Another factor which may contribute to the greater loss of muscle mass and strength in lower body muscle groups in older individuals is physical inactivity. For example, Klitgaard et al. (1990) reported a significant reduction in knee extensor force production in sedentary older individuals compared to older individuals who had a long history of strength and endurance training. The authors suggest that a protective effect of exercise on maintaining muscle mass and strength may only appear when exercise is performed at high intensities which primarily recruits fast-twitch type II muscle fibers.

Most studies comparing deficits in force production at different velocities have concentrated on lower body muscle groups. It is important to determine if strength at faster velocities (i.e. power) is reduced similarily between upper and lower body muscle groups for the design of optimal exercise interventions which include power-type training. Power is important in older individuals, as it is a significant predictor of performance in functional tasks such as rising from a chair, stair climbing, and walking (Bassey et al., 1992). If the deficits in strength at faster velocities are greater than strength at slower velocities, this may indicate that resistance training programs for older individuals should emphasize power in addition to strength. Recent studies have shown

that explosive power training in older individuals is safe and effective (Hakkinen et al., 1998; 2002). A secondary purpose was to compare torque, normalized torque, and power at slow (i.e. 60°/s [1.05 rad/s]) and fast (i.e. 180°/s [3.14 rad/s]) velocities for the elbow and knee flexors and extensors and for the ankle plantar flexors. Due to the reduction and atrophy of fast-twitch type II muscle fibers (Ansved & Larrson, 1989) and subsequent reduction in intense physical activity participation (Klitgaard et al., 1990), it was hypothesized that torque, normalized torque, and power at the faster velocity would be more affected in the older group.

Methods

Subjects

Fifty men (18-76 yr) who were not participating in resistance type training volunteered for the study through a newspaper advertisement. Subjects between the ages of 18-33 were classified as young and those between the ages of 59-77 were considered old. Age ranges were based on the results of Reimers et al. (1998) who found significant differences in muscle mass between young (20-30 yr) and older individuals (60-80 yr). Fifty subjects were required to achieve 80% power as determined using the nomogram of Day and Graham, (1990) based on an ankle plantar flexor muscle thickness of 3.7 cm (Reimers et al., 1998) with a standard deviation from the means of 4.4, assuming a difference parameter (i.e. standard deviation of the means / standard deviation of measurements) of 0.68 at an alpha value of 0.05. All subjects less than 70 years of age were required to fill out a Physical Activity Readiness Questionnaire (Appendix A), which screens for health problems that may present a risk with performance of physical

activity (Thomas et al., 1992). Subjects who indicated a health problem and all subjects 70 years of age or older were required to have medical approval before participating in the study. Subject characteristics are presented in Table 3.1. The study was approved by the University Ethics Review Board for Research in Human Subjects (Appendix A). The subjects were informed of the risks and purposes of the study before their written consents were obtained (Appendix A).

Experimental Protocol

Each subject visited the laboratory on two occasions, once to become familiar with the Biodex isokinetic dynamometer and again, one week later, for actual testing. During testing, isokinetic concentric torque and power were assessed by three sets of one-repetition maximal (1-RM) elbow flexion and extension, knee flexion and extension, and ankle plantar flexion on the right side of the body for simplification of testing. Muscle thickness of the elbow flexors and extensors, knee flexors and extensors, and ankle plantar flexors was assessed by ultrasound. Normalized torque was calculated as the ratio of torque to muscle thickness (Nm/cm). Whole-body lean tissue mass was assessed by air-displacement plethysmography.

Torque/Power

Torque and power were measured in the elbow flexors and extensors, knee flexors and extensors, and ankle plantar flexors using an isokinetic dynamometer (Biodex System 3, Biodex Medical Systems Inc; Appendix G). For all tests, subjects were positioned against the backrest of the stationary seat with a hip angle of 85^0 and stabilizing belts were placed across the subject's chest and waist. The dynamometer was

set in concentric mode for elbow flexion and extension, knee flexion and extension, and ankle plantar flexion at angular velocities of 1.05 rad/s and 3.14 rad/s. For elbow flexion and extension, a strap was placed across the subject's upper right arm to keep the elbow axis of rotation in the correct position. Torque measures were corrected for gravity on the lever arm and the handle of the dynamometer, and for each subject's individual limb weight. The elbow flexion and extension attachment on the dynamometer was set to a range of motion (60^0 – 160^0) for each subject and for each testing condition, where 0^0 was in the farthest arm flexion (60^0) and 100^0 was in full arm extension (160^0) (Farthing & Chilibeck, 2003). One repetition of elbow flexion, separated by a 3 second pause, followed by elbow extension, at maximal effort was repeated three times. A one-minute rest was given between repetitions. Testing was done at each velocity of 1.05 rad/s and 3.14 rad/s in random order. There was a 3 second pause between agonist and antagonist muscle contractions to help reduce stretch-reflex muscle shortening which could have influenced torque (Bosco et al., 1982). The highest torque and power obtained during the three repetitions at each velocity were used for analyses.

Knee flexion and extension torque and power were measured through a range of motion of 90^0 -170^0 of knee flexion (internal angle). A stabilizing belt was placed across the distal one-third of the right thigh. Torque measures were corrected for the effects of gravity on the lower leg and the dynamometer's resistance pad. The rotational axis of the dynamometer was positioned to be coaxial with the knee axis (lateral condyle) during testing (Candow et al., 2001a).

Ankle plantar flexion torque and power were measured with belts placed across the distal part of the femur and across the top of the forefoot and midfoot. The foot was

positioned in the dynamometer attachment (footplate) so that the axis of rotation of the ankle was aligned with the axis of the lever arm. A pad was placed under the right distal part of the femur so that knee flexion in the exercised leg was 20° from horizontal (i.e. 160 ° knee extension). The knee of the non-exercised leg was flexed at 90° with the foot resting on a "t-bar" which was attached to the Biodex chair. Subjects moved the ankle through a range of motion from 20° dorsi flexion to 40° plantar flexion.

Reproducibility of torque and power measurements was determined by testing 28 subjects (12 young, 16 older) one week apart, and measuring the coefficient of variation, defined as the square root of the between-test variance (standard deviation), divided by the combined (marginal) mean of the test results for days 1 and 2, multiplied by 100 (to produce a percentage). The coefficients of variation for the young and older men are presented in Table 3.2.

Table 3.1. Subject characteristics of young and older men.

Group	Age (yr)	Height (cm)	Weight (kg)	Lean tissue mass (kg)
Young (n=22)	23.0 ± 0.7	179 ± 1.1	80.8 ± 3.0	64.3 ± 1.5*
Older (n=28)	65.5 ± 0.9	176 ± 2.3	84.9 ± 2.6	58.5 ± 1.2

Values are means ± standard error. * Indicates young men had more lean tissue mass compared to older men ($F [1, 47] = 8.2$, $p<0.05$; $\eta^2 = 0.15$).

Table 3.2. Coefficients of variation (%) from duplicate measures of torque and power in young (n=22) and older men (n=28).

	1.05 rad/s		3.14 rad/s	
	Young	Older	Young	Older
Torque				
Elbow flexors	3.6	5.9	4.3	3.6
Elbow extensors	4.3	3.2	4.0	2.2
Knee flexors	2.0	5.4	3.1	2.1
Knee extensors	2.2	4.3	2.7	2.3
Ankle plantar flexors	4.0	2.5	3.1	2.6
Power				
Elbow flexors	4.7	10.3	6.2	11.2
Elbow extensors	3.2	6.3	4.5	7.0
Knee flexors	9.5	13.1	11.8	23.0
Knee extensors	5.7	8.4	3.5	9.7
Ankle plantar flexors	5.3	8.5	10.8	14.6

Muscle Thickness

Muscle thickness of the elbow and knee flexors and extensors and ankle plantar flexors was measured using B-Mode ultrasound (Aloka SSD-500 Tokyo, Japan). To measure elbow flexor and extensor muscle thickness, a small mark was drawn on the

lateral side of the right arm to indicate 65% of the distance down from the acromion process to the olecranon process (Farthing & Chilibeck, 2003). A tape measure was wrapped around the arm at the 65% mark and was used as a reference, and another mark was placed on the bulk of the biceps and triceps where the center of the ultrasound probe was placed. To measure elbow flexor muscle thickness, each subject placed his arm flat on the table with the belly of the biceps facing upwards and the forearm supinated. To measure elbow extensor muscle thickness, subjects stood with their back facing the researcher and elbows relaxed and extended.

To measure knee flexor and extensor muscle thickness, a small mark was drawn on the lateral side of the right leg to indicate 70% of the distance down from the greater trochanter to the lateral epicondyle of the tibia (Abe et al., 2001). A tape measure was wrapped around the right leg at the 70% mark and was used to mark another reference point on the bulk of the vastus lateralis and biceps femoris where the center of the ultrasound was placed. To measure knee extensor muscle thickness, each subject was placed on a table in a seated position with his right leg extended and relaxed. To measure knee flexor muscle thickness, each subject was prone on the table with both legs extended and relaxed.

To measure ankle plantar flexor muscle thickness, a small mark was drawn on the lateral side of the right leg to indicate 30% of the distance down from the lateral condyle of the tibia to the lateral malleolus of the fibula (Abe et al., 2001). A tape measure was wrapped around the leg at the 30% mark and was used to mark another reference point on the bulk of the gastrocnemius where the center of the ultrasound was placed. To measure

ankle plantar flexor muscle thickness, each subject was prone on the table with right leg fully extended and relaxed.

A 5-MHz scanning transducer head was placed perpendicular to the muscle area interface. The scanning head was coated with water-soluble transmission gel to provide acoustic contact with the muscle surface. When the image produced on the screen was visible, the image on the monitor was frozen. With the image frozen, a cursor was enabled to quantify muscle thickness (cm) at three sites: the proximal, the mid, and the distal, as determined by divisions (1cm) on the monitor. The distal and proximal sites were 6 cm apart, with the mid site located 3 cm between them. The mid site corresponded to where the reference mark was drawn over the measured muscle. Muscle thickness measurements were extrapolated from the monitor screen by measuring the distance from the bottom of the subcutaneous adipose layer to the surface of the humerus for elbow flexor and extensor muscle thickness, to the surface of the femur for knee flexor and extensor muscle thickness, and to the surface of the tibia for ankle plantar flexor muscle thickness. Three muscle thickness measurements were taken at each of the three sites. The closest two values were then taken and averaged to achieve a final muscle thickness value for that site. The values for all three sites were then averaged to achieve one global muscle thickness score for each muscle group. Reproducibility of muscle thickness measurements was determined by testing 28 subjects (12 young, 16 older) one week apart. For each muscle thickness measurement precise markings on the skin were taken using overhead transparency film to ensure that identical sites were measured on each occasion (Farthing & Chilibeck, 2003). The coefficients of variation for muscle thickness measurements in the young group were 2.6% (elbow flexors), 1.7% (elbow extensors),

3.1% (knee flexors), 0.9% (knee extensors), and 2.1% (ankle plantar flexors). The coefficients of variation for muscle thickness measurements in the older group were 2.5% (elbow flexors), 2.2% (elbow extensors), 3.6% (knee flexors), 2.1% (knee extensors), and 3.3% (ankle plantar flexors). Muscle thickness measurements for upper and lower body muscle groups have been validated with magnetic resonance imaging (MRI). Muscle thickness of the knee extensors is a significant predictor of knee extensor volume as measured by MRI (r=0.91) (Miyatani et al., 2002), and muscle thickness of the elbow flexors and extensors are significant predictors of elbow flexors and extensors volume as measured by MRI (r=0.96) (Miyatani et al., 2000).

Lean Tissue Mass Assessment

Whole-body lean tissue mass was assessed by air-displacement plethysmography (BOD POD, Life Measurement Inc., Concord, CA; Appendix H).The BOD POD measures body volume, but by displacing air rather than water (i.e. hydrostatic weighing) from which body density and percent body fat can be estimated (Vescovi et al., 2001). Once V_b is determined, densitometry principles are used to determine body composition from body density (body mass/ V_b). The BOD POD offers an advantage over hydrostatic weighing in that it is simpler to use, subject compliance is higher because head submersion under water in unnecessary, subjects appear to tolerate the test more easily, the chamber is less intimidating than the hydrostatic tank (Fields & Goran, 2000), and is more applicable to a wider population (McCrory et al., 1998).

Prior to measurements all subjects were instructed to refrain from physical activity for 24 hours to reduce fluctuations in breathing patterns from elevated

metabolism (Fields et al., 2002) and food and drink for 3 hours. Food and fluid consumption will be held primarily within the stomach and bladder. It has recently been shown that air-displacement plethysmography interprets the gain in body mass from fluid ingestion shortly before testing as fat mass (Vukovich & Peeters, 2003). Subjects were also instructed to shave all excess hair that is part of their normal routine as body hair may reduce body volume by increasing the amount of isothermal air (i.e. air that is more compressible at a constant temperature) near the surface of the body. Subjects were weighed to the nearest 0.1 kg and measured to the nearest 0.1 cm wearing Lycra shorts and a swim cap after voiding their bladder to reduce excess fluid retention. Excess clothing causes a significant underestimation of body volume because air that comes into contact with cloth will remain isothermal as pressure fluctuates (Fields et al., 2002). Subjects were then seated in the BOD POD chamber, and the chamber was sealed so that measurements of whole body volume could be made. Subjects were instructed to relax, breathe normally, and sit still during the 20-second measurement. Repeated trials were completed until consistent results were achieved as determined by the BOD POD software (Life Measurement Instruments, Software Version 1.69, Concord, CA). When the test was complete (2-5 minutes), body density was calculated by dividing the individual's mass by body volume, corrected for estimated lung volume. Percent body fat (% fat) was derived using the Siri equation (% fat = 495/ Density – 450; see Siri, 1996). Lean tissue mass was then determined by the equation: total body mass – (% fat x total body mass). Reproducibility was assessed by testing 29 subjects (12 young, 17 older) one week apart. The coefficient of variation for lean tissue mass was 0.80% in the young group and 0.84% in the older group. The validity of our measurements using the BOD

POD was estimated by measuring 12 young and 15 older subjects in the BOD POD and by using dual-energy X-ray absorptiometry (QDR 2000, Hologic, Waltham, MA). Correlation coefficients between BOD POD and dual-energy X-ray absorptiometry measurements of lean tissue mass were 0.98 ($p<0.05$) for the young group and 0.96 ($p<0.05$) for the older subjects.

Physical Activity

Physical activity was assessed by having each subject fill out a leisure time exercise questionnaire (Godin & Shephard, 1985), in which he indicated the number of times on average per week he participated in strenuous exercise (e.g. running, jogging, bicycling), moderate exercise (e.g. fast walking, tennis, badminton), and mild exercise (e.g. yoga, gardening) (Apendix B). This questionnaire has been validated in male subjects of similar ages to those in the current study against measures of metabolic equivalents by accelerometry, the four-week physical activity history from the Minnesota Leisure-Time Physical Activity Questionnaire, body fatness, and maximal oxygen consumption (correlations ranged from 0.21-0.38; $p<0.05$) (Godin & Shephard, 1985; Jacobs et al., 1993).

Statistical Analyses

There were four separate analyses performed in this study. In the first analysis, a one-factor ANOVA was used to determine differences in lean tissue mass and muscle thickness of the elbow flexors and extensors, knee flexors and extensors, and ankle plantar flexors between young and older men.

In the second analysis, a 2 (young vs. older) × 2 (1.05 rad/s and 3.14 rad/s) analysis of variance (ANOVA) with repeated measures on the second factor was used to determine whether there were any differences in torque, normalized torque, or power for the elbow flexors and extensors, knee flexors and extensors, and ankle plantar flexors between young and older men. Whenever significance was evident, Tukey post hoc tests were performed.

In the third analysis, a one-factor repeated measures ANOVA was used to compare muscle groups to determine which had the greatest relative deficit compared to the young. To determine which muscles were smallest relative to the young group, each older subject's muscle thickness score was divided by the mean of the young group and multiplied by 100% (Hortobagyi et al., 1996). An average for each of these relative scores was then computed for each muscle group.

In the fourth analysis, a 5 (elbow flexors, elbow extensors, knee flexors, knee extensors, and ankle plantar flexors) x 2 (1.05 rad/s and 3.14 rad/s) repeated measures ANOVA was used to determine which muscles had the lowest torque, normalized torque, and power relative to the young group. A Tukey's post-hoc test was used to determine differences between means when interactions were significant from the ANOVAs.

In the fifth analysis, Chi-square classification was used to assess differences in physical activity level between young and older men. All results are expressed as means ± standard error. The magnitude of the difference between significant means (i.e. effect size) was determined by eta squared (η^2). Eta squared is a measure of the proportion of the total variance that is explained by the treatment effects. An η^2 value of .15 represents large differences, .06 represents medium differences, and .01 represents small

differences. Significance was set at $p<0.05$. Statistical analyses were carried out using SPSS version 11.5 for Windows XP (SPSS, Chicago, IL) (Appendix J). Experimental design is shown in Figure 3.1.

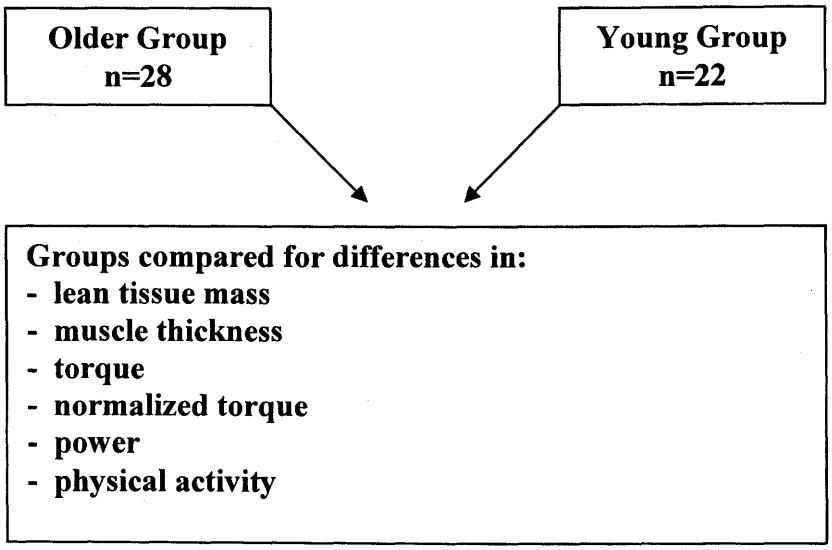

Figure 3.1 Experimental design.

Results

I. Torque

Results for torque are presented in Table 3.3. For all muscle groups there was a group main effect ($F [1, 48] = 5.3$, $p<0.05$; $\eta^2 = 0.25$), with the young group having greater torque than the older group and a velocity main effect, with slow-velocity torque greater than fast-velocity torque ($F [1, 48] = 134.4$, $p<0.05$; $\eta^2 = 0.94$).

The torque values for muscle groups from the older subjects, expressed as a percentage of the peak torque of the young group, are presented in Figure 3.2. There was a muscle group x velocity interaction ($F [4, 104] = 9.0$, $p<0.05$) when comparing these percentages between muscle groups and velocities. Differences specific to each muscle group among the muscles and velocities from the older men are outlined in Figure 3.2.

To summarize, relative torques for lower body measures at 3.14 rad/s were lower than relative torques for elbow extension at both velocities and elbow flexion at 1.05 rad/s.

Table 3.3. Peak torque (Nm) during slow (1.05 rad/s) and fast (3.14 rad/s) contractions for the elbow flexors and extensors, knee flexors and extensors, and ankle plantar flexors in young (n=22) and older men (n=28).

	1.05 rad/s		3.14 rad/s	
Muscle Group	Young	Older	Young	Older
Elbow flexors	60.5 ± 1.5*	49.6 ± 1.6	52.9 ± 1.5*	40.4 ± 1.2
Elbow extensors	60.2 ± 1.4*	47.6 ± 1.3	55.3 ± 1.5*	46.5 ± 1.2
Knee flexors	146.0 ± 2.0*	103.1 ± 2.9	127 ± 3.6*	81.9 ± 2.7
Knee extensors	217.1 ± 4.8*	162.1 ± 5.8	163.7 ± 6.9*	100.3 ± 4.0
Ankle plantar flexors	118.7 ± 3.0*	86.4 ± 3.8	69.1 ± 3.0*	42.8 ± 3.2

Values are means ± standard error. * Indicates torque is greater in young vs. older men across velocities ($p<0.05$).

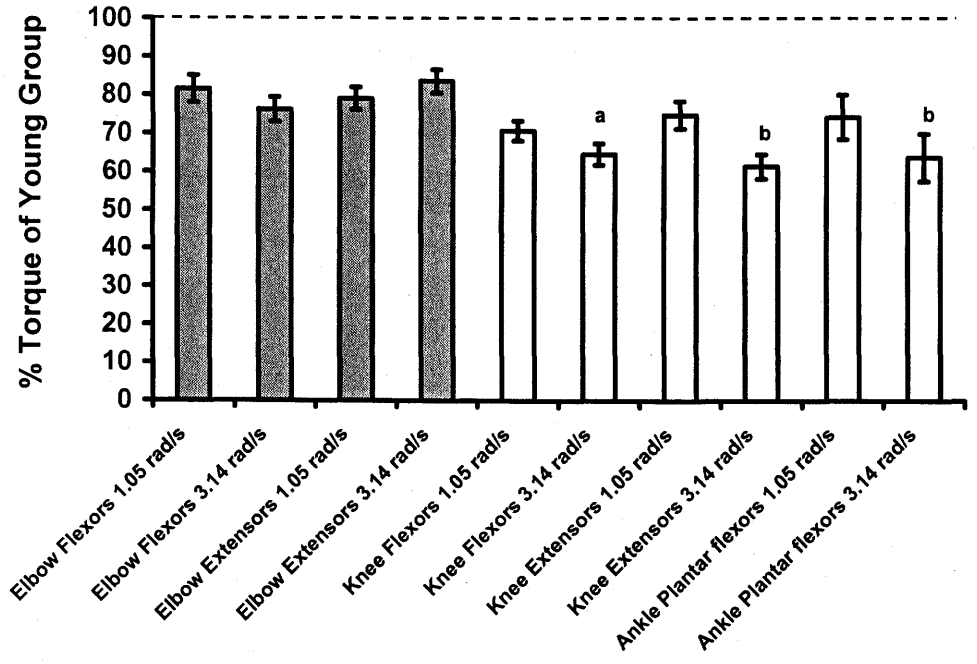

Figure 3.2 Graph of torque for older men expressed relative to mean torque of young men. Filled columns represent upper body muscle groups and open columns represent lower body muscle groups. Results are means ± standard error. **a,** Indicates relative knee flexion torque at 3.14 rad/s < relative elbow flexion torque at 1.05 rad/s and relative elbow extension torque at 3.14 rad/s ($p<0.05$). **b,** Indicates relative knee extension and ankle plantar flexion torques at 3.14 rad/s < relative elbow flexion torque at 1.05 rad/s and relative elbow extension torque at 1.05 rad/s and 3.14 rad/s ($p<0.05$).

II. Muscle Thickness

Ultrasound images for the elbow and knee flexors and extensors and the ankle plantar flexors are shown in Figure 3.3 for a young and an older subject.

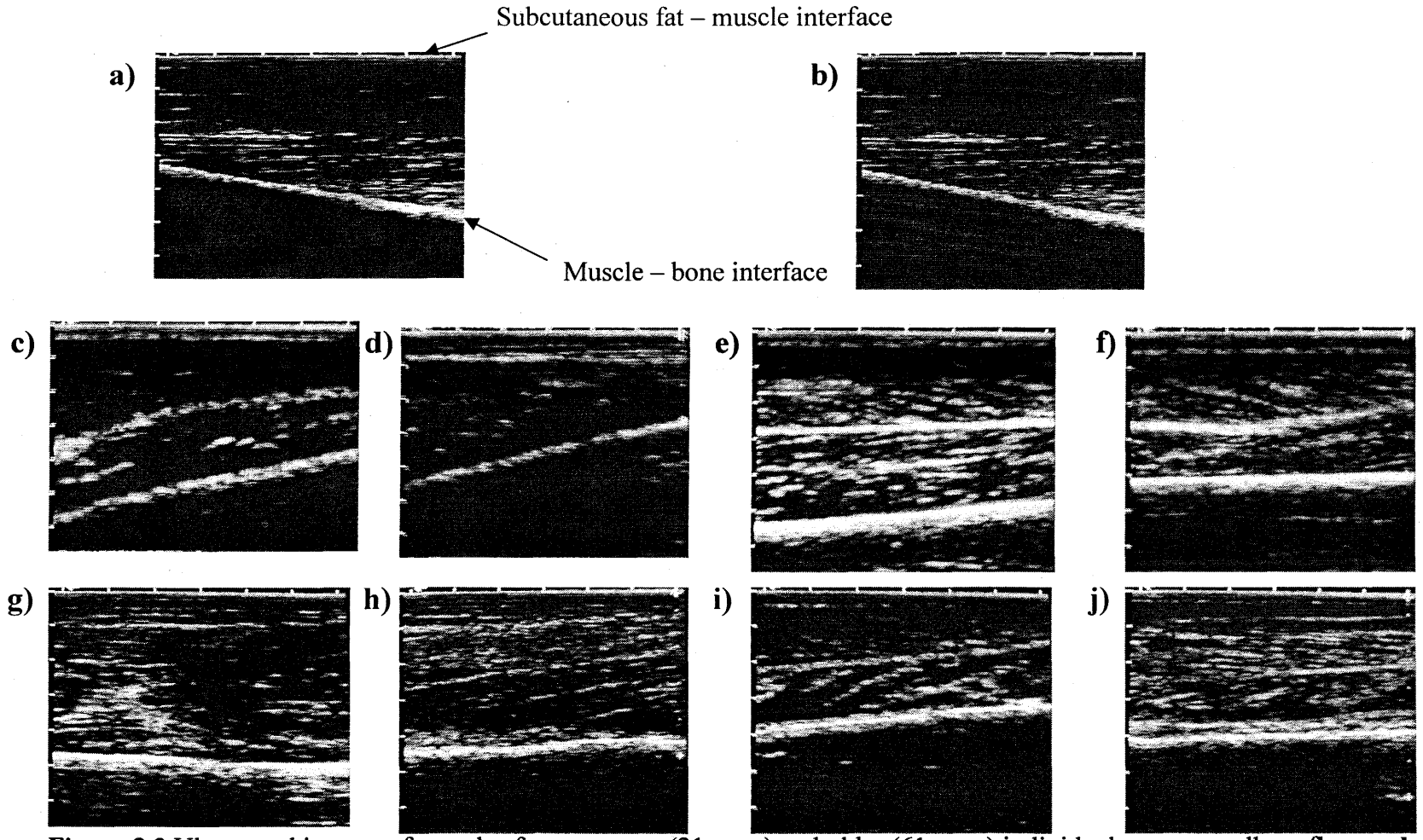

Figure 3.3 Ultrasound images of muscles from a young (21years) and older (61years) individual. **a**, young elbow flexors; **b**, older elbow flexors; **c**, young elbow extensors; **d**, older elbow extensors; **e**, young knee extensors; **f**, older knee extensors; **g**, young knee flexors; **h**, older knee flexors; **i**, young plantar flexors, and **j**, older plantar flexors.

Muscle thickness measurements were significantly greater in young vs. older men for all muscle groups except the elbow extensors ($F [1, 48] = 0.2$, $p=0.69$, $\eta^2 = 0.21$; Table 3.4). The thicknesses for muscle groups from the older subjects, expressed as a percentage of the mean muscle thickness of the young group, are presented in the last column of Table 3.4. Relative thickness of the elbow extensors was greater than that of all other muscle groups ($F [4, 108] = 12.1$, $p<0.05$) and the relative thickness of the elbow flexors was greater than that of the ankle plantar flexors ($p<0.05$).

III. Normalized Torque

Results for normalized torque are presented in Table 3.5. For all muscle groups, there was a velocity main effect, with slow-velocity normalized torque greater than fast-velocity normalized torque ($F [1, 48] = 109$, $p<0.05$, $\eta^2= 0.92$). For the elbow extensors ($F [1, 48] = 7.3$, $p<0.05$, $\eta^2= 0.22$) and knee flexors ($F [1, 48] = 78.7$, $p<0.05$, $\eta^2= 0.13$), there was a group main effect, with the young group having greater normalized torque than the older group across velocities. There was a group x velocity interaction for the knee extensors, with the normalized torque at the higher velocity being greater in the young compared to the older group ($F [1, 48] = 4.4.$, $p<0.05$, $\eta^2 = 0.09$).

Table 3.4. Muscle thickness (cm) for the elbow flexors and extensors, knee flexors and extensors, and ankle plantar flexors in young and older men.

	Young (n=22)	Older (n=28)	Older relative to Young (%)
Muscle Group			
Elbow flexors	3.2 ± 0.1*	2.8 ± 0.1	86.6 ± 3.1**
Elbow extensors	4.1 ± 0.1	3.9 ± 0.1	98.6 ± 2.7***
Knee flexors	5.5 ± 0.1*	4.5 ± 0.1	82.8 ± 2.9
Knee extensors	4.2 ± 0.1*	3.5 ± 0.1	80.4 ± 3.8
Ankle plantar flexors	4.4 ± 0.3*	3.2 ± 0.2	74.7 ± 4.2

Values are means ± standard error. * Indicates young men had greater muscle thickness vs. older men ($p<0.05$). ** Indicates elbow flexors relative muscle thickness > ankle plantar flexors relative muscle thickness ($p<0.05$). *** Indicates elbow extensors relative muscle thickness > all others ($p<0.05$).

Table 3.5. Normalized torque (Nm/cm muscle thickness) during slow (1.05 rad/s) and fast (3.14 rad/s) contractions for the elbow flexors and extensors, knee flexors and extensors, and ankle plantar flexors in young (n=22) and older men (n=28).

	1.05 rad/s		3.14 rad/s	
Muscle Group	Young	Older	Young	Older
Elbow flexors	19.0 ± 0.6	18.5 ± 1.1	16.7 ± 0.7	15.2 ± 0.9
Elbow extensors	15.0 ± 0.5*	12.1 ± 0.5	13.7 ± 0.4*	11.8 ± 0.6
Knee flexors	26.7 ± 1.0*	23.0 ± 0.6	23.0 ± 0.8*	18.4 ± 1.1
Knee extensors	52.2 ± 2.0	48.8 ± 2.5	39.2 ± 2.3*	30.4 ± 1.8
Ankle plantar flexors	29.2 ± 1.9	28.8 ± 2.8	17.1 ± 1.4	14.0 ± 1.6

Values are means ± standard error. * Indicates normalized strength is greater in young vs. older men at the same velocity ($p<0.05$).

The normalized torque values for muscle groups from the older subjects, expressed as a percentage of the mean normalized torque of the young group, are presented in Figure 3.4. There was a muscle group x velocity interaction when comparing these percentages between muscle groups and velocities ($F [4, 104] = 8.2$, $p<0.05$). Specific differences between the older muscle groups and velocities are outlined in Figure 3.4. In general, the lowest values relative to the young group were for elbow extension at 1.05 rad/s and knee extension and flexion at 3.14 rad/s. The highest value relative to the young group was for ankle plantar flexion at 1.05 rad/s.

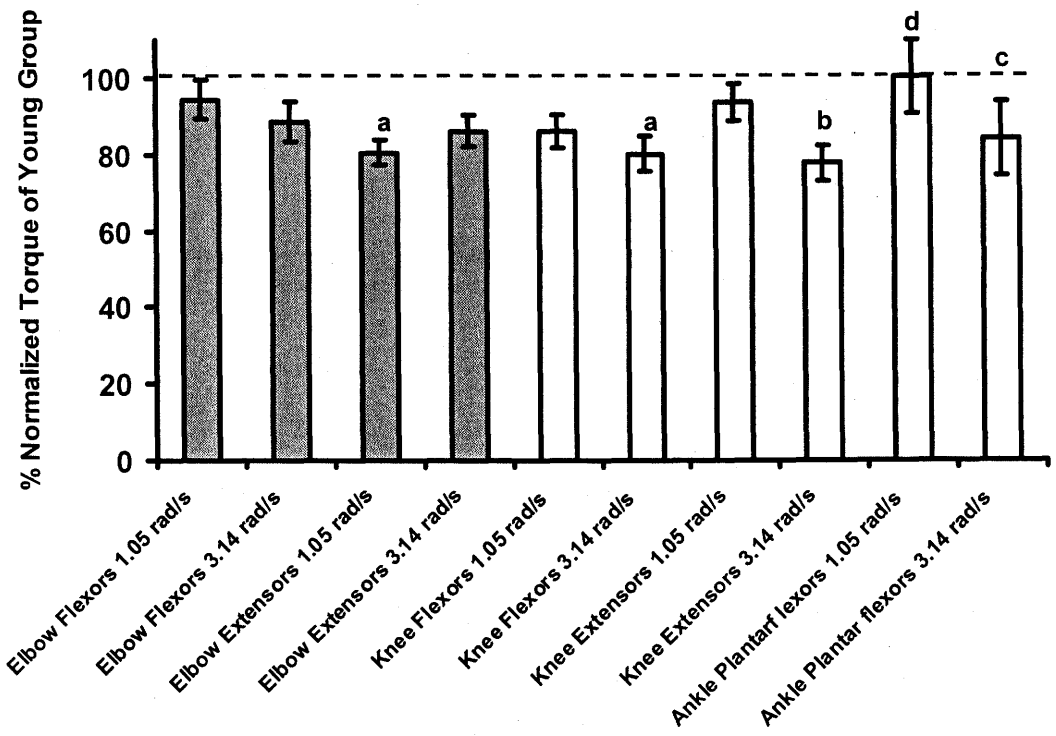

Figure 3.4 Graph of normalized torque (Nm / cm muscle thickness) for older men expressed relative to mean normalized torque of young men. Filled columns represent upper body muscle groups and open columns represent lower body muscle groups. Results are means ± standard error. **a**, Indicates relative elbow extension normalized torque at 1.05 rad/s and relative knee flexion normalized torque at 3.14 rad/s < relative elbow flexion, knee extension, and ankle plantar flexion normalized torques at 1.05 rad/s ($p<0.05$). **b**, Indicates relative knee extension normalized torque at 3.14 rad/s < relative elbow flexion, knee extension, and ankle plantar flexion normalized torques at 1.05 rad/s, and relative elbow flexion normalized torque at 3.14 rad/s ($p<0.05$). **c**, Indicates relative ankle plantar flexion normalized torque at 3.14 rad/s ($p<0.05$) < relative elbow flexion and ankle plantar flexion normalized torques at 1.05 rad/s. **d**, Indicates relative ankle plantar flexion normalized torque at 1.05 rad/s > all other normalized torques except elbow flexion and knee extension at 1.05 rad/s ($p<0.05$).

IV. Power

Results for power are presented in Table 3.6. There was a group main effect with the young men having greater power than the older men for all muscle groups at both velocities ($F [1, 48] = 23.3$, $p<0.05$, $\eta^2 = 0.83$). There was a group x velocity interaction for the elbow extensors ($F [1, 47] = 14.7$, $p<0.05$, $\eta^2 = 0.32$), knee flexors ($F [1, 47] = 6.3$, $p<0.05$, $\eta^2 = 0.19$), and knee extensors ($F [1, 47] = 12.1$, $p<0.05$, $\eta^2 = 0.23$). The young group had greater power than the older group at both velocities for all these contractions ($p<0.05$), but the differences between young and older men were greater at the higher velocity.

Power for muscle groups from the older subjects expressed as a percentage of the mean power of the young group, is presented in Figure 3.5. There was a muscle group x velocity interaction ($F [4, 104] = 4.7$, $p<0.05$) when comparing these percentages between muscle groups and velocities in the older group. Specific differences between the older muscle groups and velocities are outlined in Figure 3.5. In general, the lowest values relative to the young group were for knee flexion at 3.14 rad/s and ankle plantar flexion at 3.14 rad/s.

Table 3.6. Power (watts) during slow (1.05 rad/s) and fast (3.14 rad/s) contractions for the elbow flexors and extensors, knee flexors and extensors, and ankle plantar flexors in young (n=22) and older men (n=28).

	1.05 rad/s		3.14 rad/s	
Muscle Group	Young	Older	Young	Older
Elbow flexors	48.8 ± 5.9*	31.8 ± 1.7	57.9 ± 3.2*	41.6 ± 3.4
Elbow extensors	49.0 ± 1.5*	34.9 ± 1.4	92.2 ± 4.4* ¶	58.2 ± 3.9
Knee flexors	90.5 ± 5.2*	56.2 ± 3.2	113.4 ± 12.1* ¶	52.4 ± 3.8
Knee extensors	138.7 ± 6.8*	97.8 ± 5.1	201.7 ± 11.2* ¶	126.1 ± 8.3
Ankle plantar flexors	71.3 ± 2.8*	47.0 ± 4.8	75.8 ± 5.2*	42.0 ± 5.1

Values are means ± standard error. * Indicates power is greater in young vs. older men at the same velocity ($p<0.05$). ¶ Indicates the difference between young and older men at the fast velocity is greater than the difference between young and older men at the slow velocity (group × velocity; $p<0.05$).

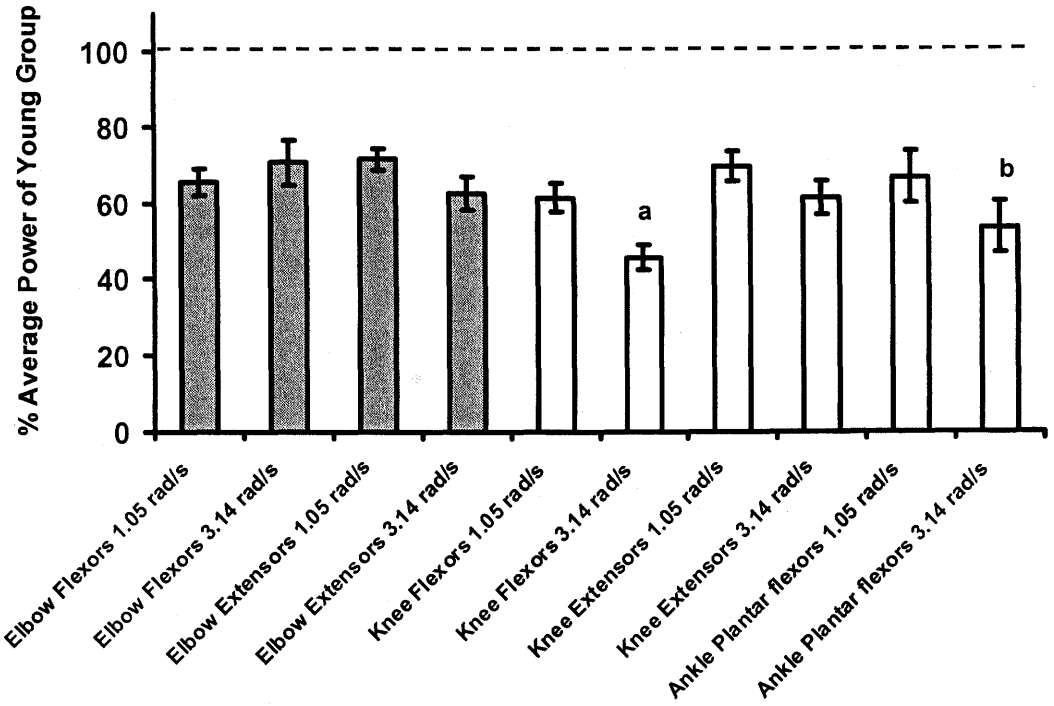

Figure 3.5 Graph of power for older men expressed relative to mean power of young men. Filled columns represent upper body muscle groups and open columns represent lower body muscle groups. Results are means ± standard error. **a**, Indicates relative knee flexion power at 3.14 rad/s < relative power for all others except plantar flexion at 3.14 rad/s ($p<0.05$). **b**, Indicates relative plantar flexion power at 3.14 rad/s < relative elbow flexion power at 3.14 rad/s and elbow flexion, elbow extension, knee extension, and ankle plantar flexion at 1.05 rad/s ($p<0.05$).

V. Physical Activity

Young men participated more in strenuous-intensity exercise (Young = 3.1 ± 4.2, Older = 0.8 ± 1.6, $p<0.05$) on average per week compared to older men. There was no difference between young and older subjects for light-intensity and moderate-intensity exercise.

Discussion

Results showed that muscle thickness, torque, and power in lower body muscle groups is more affected by age than upper body muscle groups. These torque measurements are very similar to measurements for young and older subjects of similar age in previous studies of the elbow flexors (Hughes et al., 2001), elbow extensors (Hughes et al., 2001; Poulin et al. 1992), knee extensors (Horstmann et al., 1999; Hughes et al., 2001; Poulin et al. 1992), knee flexors (Horstmann et al., 1999; Hughes et al., 2001), and ankle plantar flexors (Horstmann et al., 1999; Thelen et al., 1996). Older men had lower torque values relative to young men for knee flexion and extension and ankle plantar flexion when compared to upper-body measures. These findings are in agreement with those of others who found lower body torque to be reduced with age while upper body torque was maintained (Frontera et al., 1991; Klitgaard et al., 1990; Lynch et al., 1999). Most studies have compared upper or lower body muscle groups between young and older individuals without comparing the relative deficits in strength within the older individuals' muscle groups. This study is unique in that deficits of torque, power, and size between muscle groups within the older subjects were determined by expressing each older subject's torque, power, or muscle thickness relative to the young group mean.

This allowed a statistical comparison between older muscle groups for torque, normalized torque, power, and size deficits relative to the young group.

The lowest muscle thickness measurements in the older men relative to the young men were for the lower body measurements. These results are in agreement with others who found muscle mass to be significantly reduced in the lower body, but relatively well-maintained in the upper body with age (Janssen et al., 2000; Kubo et al., 2003; Reimers et al., 1998). A potential limitation of this study was that ultrasound muscle thickness measurements cannot differentiate between muscle and non-muscle tissue (i.e. connective tissue and intramuscular fat). Intramuscular fat and/or connective tissue measurements may be higher in older compared to young individuals for the elbow flexors and extensors (Klein et al., 2001), knee flexors and extensors (Overend et al., 1992a, 1992b), and ankle plantar flexors (Rice et al., 1989) as assessed by MRI or computed tomography scanning. However, muscle thickness measurements for upper and lower body muscle groups have been validated with magnetic resonance imaging (MRI). Muscle thickness of the knee extensors is a significant predictor of knee extensor volume as measured by MRI ($r=0.91$) (Miyatani et al., 2002), and muscle thickness of the elbow flexors and extensors are significant predictors of elbow flexors and extensors volume as measured by MRI ($r=0.96$) (Miyatani et al., 2000). The only muscle thickness measurement that did not differ between young and older men was for the elbow extensors. This is in agreement with one other study that assessed muscle thickness of the elbow extensors in young and older individuals (Kubo et al., 2003). It is difficult to explain why the elbow extensors would be maintained with age, while elbow flexors muscle thickness is lower but it may be related to changes in ability to activate the flexors and extensors with age. Jakobi and

Rice (2002) found that the ability to activate the elbow extensors was maintained to a greater degree than the ability to activate the elbow flexors in older versus younger males. These differences between muscle groups may be the result of a greater age-related decrease in motor unit discharge rates in the elbow flexors, compared to the elbow extensors (Jakobi & Rice, 2001) because suboptimal discharge rates may be a possible factor leading to a decrease in muscle activation (Herbert & Gandevia, 1999).

Although loss of strength with aging is largely due to loss of muscle mass, often strength is lost to a greater degree than muscle mass. This implies that loss of strength may also be due to decreased ability to fully recruit motor units (Jakobi & Rice, 2002) or alterations in muscle contractile properties (Frontera et al., 2000). For example, the age-related slowing of twitch properities in motor units of both fast and slow-twitch muscle fibers is thought to be caused by structural (Decoster et al. 1981), functional (Larsson & Salviati, 1989), and biochemical (Viner et al., 1996) changes in the sarcoplasmic reticulum. Sarcoplasmic reticulum properties are the strongest determinants of speed of contraction whereas myosin heavy chain isoforms influence maximum shortening velocity (Brody, 1976). It has been shown that age has a negative effect on sarcoplasmic reticulum protein function (Larsson et al., 2001). The decrease in force production with age may also be related to the decrease in the number of cross-bridges in the driving stroke per muscle fiber volume, a decrease in force generated by each cross-bridge cycle, or a combination of both (Larsson et al., 2001). Results indicated that strength expressed relative to muscle mass by ultrasound (i.e. normalized torque) was lowest for the elbow extensors at the slow velocity, and knee extensors and flexors at the fast velocity for the older group when expressed relative to the young group. Results of decreased normalized

torque, especially at fast velocities, for the knee flexors and extensors are in agreement with Jubrias et al. (1997) and Overend et al. (1992) who also reported a decrease in normalized torque at fast velocities for the knee flexors and extensors in older individuals. It is speculated that the greater deficits in force at faster velocities may be related to a reduction in type II muscle fibers with age (Jubrias et al., 1997). In addition, myosin is the main myofibrillar protein responsible for force production in skeletal muscle. At the cellular level, there is a significant reduction in the amount of myosin per muscle fiber volume in old age (Marx et al., 2002). For example, Larsson et al. (2001) reported a two-fold decrease in speed of contraction between young and old isolated muscle fibers; suggesting an accelerated decline in myosin function with age. Results of decreased normalized torque for the elbow extensors, but not the elbow flexors in the older group are in contrast to those of one recent study that showed a reduction in normalized torque of the elbow flexors, but not the elbow extensors in older individuals (Klein et al., 2001). As mentioned above, a limitation of the ultrasound measurements used in the current study is that noncontractile tissue (i.e. fat and connective tissue) cannot be differentiated from contractile tissue in the ultrasound images. The study of Klein et al. (2001) used MRI, which can differentiate between contractile and noncontractile tissue. Torque in their study was normalized relative to contractile tissue; whereas torque in this study was normalized to contractile and noncontractile tissue. Klein et al. (2001) found a greater amount of noncontractile tissue in the elbow extensors, compared to flexors in their older group. If this was the case in my older group, then their elbow extensor normalized torque would be underestimated because noncontractile tissue would be included in the denominator of this measurement.

Similar to torque measurements, relative deficits for power were greatest in the lower body (specifically the knee flexors and ankle plantar flexors at fast velocities) of the older group. In general, power was affected more than torque in the older group. This is in agreement with one other study that found greater deficits in upper and lower body power compared to strength measurements in older individuals (Izquierdo et al., 1999). Power is important in older individuals, as it is a significant predictor of performance in functional tasks such as rising from a chair, stair climbing, and walking (Bassey et al., 1992).Therefore, the greater deficits in power would indicate that resistance training programs for older individuals should emphasize power in addition to strength. Research has shown that explosive power training in older individuals is effective for increasing muscle mass, strength, and functional ability (Hakkinen et al., 1998; 2002).

Physical activity levels could account for some of the differences between muscle groups in this study. For example, heavy-intensity activity levels were lower in the older men. Heavy- intensity activities such as running and jumping would involve muscle groups such as the knee flexors and extensors, and contractions at fast velocities, which were most affected in the older men. Interestingly, Klitgaard et al. (1990) found that sedentary older individuals showed a significant decline in leg extensor strength, but older individuals who had a history of resistance or endurance training had strength values similar to that of the young. The findings of Klitgaard et al. (1990) combined with the results of this study suggest a protective effect of exercise on muscle strength may only appear when exercise is performed at high intensities which primarily recruit fast-twitch type II muscle fibers.

For all muscle groups, torque at the fast and slow velocity was lower in the older men. The absolute differences between older and young men were similar for the slow versus fast velocity torques within each muscle group. These findings are in agreement with several studies that also showed no differences in the amount of torque reduction with age for fast versus slow velocity movements within specific muscle groups (Borges, 1989; Horstmann et al., 1999; Poulin et al., 1992). However, when comparing different muscle groups within the older men, the torques relative to those in the young men were lower at the fast velocities for the knee flexors and extensors and ankle plantar flexors when compared to the relative torques at the slow velocities for the elbow flexors and extensors. In other words, for the older men, the greatest torque deficits relative to the young men were for the knee flexors and extensors and ankle plantar flexors at the fast velocity. Others have also found a greater reduction in torque at fast velocities with age for the knee flexors (Overend et al., 1992a), knee extensors (Jubrias et al., 1997; Lanza et al., 2003; Overend et al., 1992a), and ankle plantar flexors (Cunningham et al., 1987). When examining power measurements, it was found that the effect of contraction speed is more evident, with power at the fast velocities more affected in the older group than power at slow velocities. When power was expressed relative to the mean of the young group, the measurements that were most affected in the older men were the knee flexors and ankle plantar flexors at the fast velocity. There are several possible reasons for the greater reduction in force production at faster velocities in the lower body in the older group. For example, several studies have demonstrated a slowing of muscle twitch properities with age which may result from a decrease in fast-twitch type II muscle fibers (Narici et al., 1991) and the diminished ability to generate force at fast velocities.

However, in examining the relative contributions of muscle atrophy to decreased force production of the knee extensors between young and older adults, Jubrias et al. (1997) found a significant reduction in force output at faster (i.e. 180°/s), but not at slower (60°/s) velocities. In addition, myosin heavy chain IIb was significantly reduced but this isoform was not related to force production. The authors suggest that the decrement in force production at faster velocities may not be totally explained by changes in muscle fiber composition and that alterations in muscle cell function may also be involved. For example, there is a reduction in calcium release from the sarcoplasmic reticulum in fast twitch muscle fibers with age (Delbono et al., 1995). A reduction in calcium release from the sarcoplasmic reticulum could possibily result in fewer crossbridge cycles and a reduction in force production with age (Jubrias et al., 1997).

There were several limitations to this study. One limitation was the use of ultrasound for determing muscle thickness. Ultrasound does not differentate between contractile and non-contractile tissue (i.e. intramuscular adipose tissue, connective tissue). Therefore, muscle size measurements from ultrasound may have been overestimated. It would be necessary to replicate this experiment using MRI to differentate between contractile and non-contractile tissue. A second limitation was the use of the leisure time exercise questionnaire to assess physical activity. The primary purpose of the first study was to determine differences in muscle mass, strength, and power between upper and lower body muscle groups in young and older men. This questionnaire did not assess physical activity in upper and lower body muscle groups separately. An assessment tool that separates activities for upper (i.e. shuffle board) and lower (i.e. stationary cycling) body muscle groups should have been used. A third

limitation was the use of an isokinetic dynamometer to determine torque and power. An isokinetic dynamometer provides an upper limit to the velocity that can be attained by the subject but does not guaranttee that the subject reaches the predetermined speed. It was therefore possible for subjects to complete contractions and generate torque and power without achieving a constant velocity. Finally, there was no measure of contractile or mechanical properties for each muscle group assessed. Future research should investigate age-related differences in muscle architecture, such as fiber pennation, to help explain muscle and strength loss with age. In addition, determing the effects of gender, chronic disease, progressive physical inactivity, decreasing motivation, alterations in muscle fiber capacity, motor neuron abnormalities, and hormonal differences should also be determined.

In conclusion, it was hypothesized that lower body muscle measures would be more affected with age than upper body measures. Results showed that muscle thickness, strength, and power of the knee flexors and extersors and ankle plantar flexors was more affected by age than measures for the elbow flexors and extensors. A secondary hypothesis was that strength and power at fast velocities would be more affected than at slower velocities, especially in lower body muscle groups. Results showed that strength of the knee flexors and extensors and ankle plantar flexors and power of the knee flexors and ankle plantar flexors at the fast velocities were more affected with age than these measures at the slower velocity. These findings have immediate application for health and research professionls as it indicates that exercise programs for older men should include power and strength training, especially in lower body muscle groups, to maximize muscle mass and strength.

Study 2

Effects of Protein Supplementation Before and After Resistance Training in Older Men

Introduction

Aging is associated with a loss of muscle mass and a subsequent reduction in muscle strength. Results from the first study showed an age-related reduction in lean tissue mass and muscle thickness of the elbow flexors, knee flexors and extensors, and ankle plantar flexors between young and older men. Resistance training increases muscle mass in healthy older individuals (Chrusch et al., 2001); however, it is unknown if other interventions, such as nutritional supplementation (i.e. protein and carbohydrate) and resistance training, can reduce age-related deficits in muscle mass between young and older men.

Resistance training results in significant muscle protein turnover. Amino acid ingestion at rest, before, and following exercise increases protein synthesis and attenuates protein breakdown (Tipton et al., 2001; Wolfe, 2001). Amino acids are selected for protein synthesis by binding with transfer RNA (tRNA). The information and order of amino acid sequence for each protein is governed by messenger RNA (mRNA) that is produced from DNA through transcription (Houston, 2001). An increase in amino acid availability through additional dietary protein could potentially increase the combination of tRNA with an amino acid for translocation to ribosomal RNA (rRNA) (i.e. increased translational efficiency; see Figure 1.1) located on the ribosome organelle where protein synthesis occurs. Increased translational efficiency, in the presence of increased amino acid availability from protein supplementation, could potentially increase muscle protein synthesis during resistance training (Welle et al, 1994, 1995; Houston, 2001) leading to greater muscle mass and strength. For example, older males (61-72 years) who consumed a nutritional supplement drink (~506 kcal, 22 g/protein) during 12 weeks of knee flexor

and extensor resistance training experienced significant gains in muscle mass over training alone (Meredith et al., 1992). In examining the effects of protein (~ 30g/day; 10 days) supplementation on body composition and whole-body protein kinetics in 17 malnourished elderly subjects, Bos et al (2000) found a significant increase in muscle protein synthesis and fat-free mass from supplementation. It is important to note that malnourished older individuals may have higher protein turnover rates and a greater loss of muscle protein than healthy older individuals as a result of a hypercatabolic state (Beaumont et al., 1989). Therefore, a greater protein requirement for these unhealthy older individuals could have accounted for the benefit from increased protein intake. On the other hand, amino acid ingestion during 12 weeks of resistance training did not increase muscle mass and strength to a greater extent than resistance training alone in healthy older men (Godard et al., 2002). Furthermore, high protein meals (~28% of energy intake/meal) did not enhance the increase in myofibrillar protein synthesis induced by resistance training in sedentary healthy older men and women (Welle & Thornton, 1998); suggesting that the additional amino acids may not be incorporated into muscle protein (Volpi et al., 1999). These results suggest that the quantity of dietary protein during resistance training may not be a key regulator for increasing muscle mass and strength in healthy older individuals

Research suggests that the timing of protein ingestion is crucial for improving muscle mass and strength (Phillips, 2004). In assessing the effects of protein versus carbohydrate supplementation before (~ 25 g) and after (~ 25g) lower body resistance training sessions for 14 weeks in young males, Andersen et al. (2005) found a significant increase in muscle cross-sectional area of type I (~18%) and type II (~26%) muscle fibers

of the vastus lateralis in the protein group with no effect in the carbohydrate group. Therefore, protein supplementation before and after resistance training sessions induces an anabolic signal for muscle growth. A limitation of the study by Andersen et al. (2005) is that no comparison was made between protein supplementation immediately before compared to immediately after resistance training sessions. However, in young adults, ingesting an amino acid solution (~ 6 g essential amino acids) immediately before a bout of acute heavy resistance training resulted in a greater increase in muscle protein synthesis compared to consuming the amino acid solution immediately after resistance training. The authors suggest that the greater increase in muscle protein synthesis from essential amino acid ingestion prior to exercise may be the result of increased amino acid delivery to working muscle from exercise induced blood flow (Tipton et al., 2001). Unfortunately, muscle mass and strength were not assessed in the Tipton et al. (2001) study. Therefore, it is unknown if the increase in muscle protein synthesis from ingestion of essential amino acids before and after an acute bout of resistance training would lead to greater muscle hypertrophy during regular resistance training sessions. On the other hand, older males who ingested protein (~ 10 g) immediately after resistance training sessions for 10 weeks had significant increases in muscle size and strength over protein ingestion two hours post-exercise (Esmarck et al., 2001). Consuming protein one to three hours post-exercise did not alter muscle protein synthesis (Rasmussen et al., 2000) or muscular strength (Esmarck et al., 2001). Therefore, the timing of protein ingestion is important for creating an anabolic environment for muscle growth (Tipton et al., 2001), with protein supplementation immediately before (Andersen et al., 2005; Tipton et al., 2001) and immediately after resistance training (Esmarck et al., 2001) appearing optimal.

However, it is unknown if advantages exist in consuming protein before compared to after resistance training sessions in older individuals.

The primary purpose of this study was to examine the effect of protein supplementation immediately before and immediately after resistance training on lean tissue mass, muscle thickness, and muscular strength in older men. Based on the potential for increased blood flow and amino acid delivery to skeletal muscle during exercise (Tipton et al., 2001), it was hypothesized that protein ingestion immediately before resistance training would increase lean tissue mass and strength over protein ingestion immediately after resistance training. It was also hypothesized that protein ingestion, independent of timing, combined with resistance training would have a greater effect than resistance training alone. Results from the first study showed that older men had lower lean tissue mass and muscle thickness for the elbow flexors, knee flexors and extensors, and ankle plantar flexors compared to younger men. A secondary purpose of this study was to determine whether 12 weeks of nutritional supplementation (i.e. protein and carbohydrate) and resistance training in older men was sufficient to eliminte deficits in muscle mass and strength compared to reference groups of young men.

3-methylhistidine is an amino acid found in actin and the heavy chain of myosin in skeletal muscle (Rennie & Millward, 1983) and when measured in urine, is considered to be an indicator of skeletal muscle catabolism (Frontera et al., 1988; Lukaski et al., 1981). It is produced by the posttranslational methylation of specific histidine residues in actin and myosin and is neither reutilized for protein synthesis nor metabolized oxidatively but is excreted in the urine (Lukaski et al., 1981).Therefore, an increase in urinary 3-methylhistidine would potentially represent an increase in muscle protein

degradation while a decrease in urinary 3-methylhistidine would represent a decrease in muscle protein degradation. Resistance training results in significant muscle protein degradation (Pivarnik et al., 1989). Protein or amino acid supplementation enhances the ratio of protein synthesis to degradation post-exercise (Biolo et al., 1997). Therefore, protein supplementation during resistance training should theoretically attenuate the rise in urinary 3-methylhistidine induced by resistance training alone. A progressive increase in 3-methylhistidine over time may be the result of muscle hypertrophy, which is accompanied by an increase in myofibrillar protein turnover (Frontera et al., 1998). Recent evidence has shown that 3-methylhistidine release into the interstitum after resistance training is not increased (Trappe et al., 2004). Therefore, most of the increase in 3-methylhistidine after resistance training most likely arises from skeletal muscle and is a valid indicator of muscle protein degradation (Welle et al., 1995). It was hypothesized that protein supplementation during resistance training would decrease urinary 3-methylhistine excretion over resistance training alone.

Methods

Subjects

A group of 38 men (65 ± 1.0 yr; 173.8 ± 1.3 cm; 87 ± 3.7 kg) who were not engaged in resistance type training volunteered for the study through a newspaper advertisement. Of the 38 men, 22 had previously participated in Study 1. Thirty-six subjects were needed to achieve 80% power as determined using the nomogram of Day and Graham, (1990) based on a mean lean tissue mass of 53.5 kg (Chrusch et al., 2001; Esmarck et al., 2001, Tipton et al., 2001) with a standard deviation from the means of

1.8, assuming a difference parameter (i.e. standard deviation of the means / standard deviation of measurements) of 0.7 at an alpha value of 0.05. Subjects less than 70 years of age were required to fill out a Physical Activity Readiness Questionnaire (PAR-Q), which screens for health problems that may present a risk with performance of physical activity (Thomas et al., 1992). Subjects that indicated a health problem and all subjects over 69 years of age were required to have medical approval before participating in the study. The study was approved by the University Ethics Review Board for Research in Human Subjects (Appendix A). The subjects were informed of the risks and purposes of the study before their written consents were obtained (Appendix A).

Study Design

The study used a double-blind repeated measures design in which every subject participated in resistance training and were randomized to a protein treatment condition or placebo for 12 weeks. Prior to the first visit to the laboratory for initial testing and data collection, all subjects were instructed to refrain from physical activity for 48 hours as it has been shown that muscle protein synthesis and degradation is elevated for 48 hours post-exercise (Phillips et al., 1997). The dependent variables measured before and after 12 weeks of nutritional supplementation and resistance training were (1) lean tissue mass, (2) muscle thickness of the elbow, knee, and ankle flexors and extensors, (3) strength (leg press and bench press one repetition maximum; 1-RM), and (4) urinary 3-methylhistidine excretion (an index of myofibrillar protein degradation). In addition, subjects completed dietary records for 3 days during the first and final week of resistance training and supplementation to assess nutrient differences between groups. At the end of the study,

subjects were asked whether they perceived they were on the protein supplement or placebo.

In addition to measuring changes in lean tissue mass, muscle thickness, and strength before and after training and supplementation, these pre- and post-training measures from the older subjects were compared to three reference groups of moderately active young males; one for comparison of lean tissue mass (n = 57; age = 24.5 ± 0.6 yr; mass = 81.6 ± 1.8 kg; height = 178 ± 1 cm), one for comparison of muscle thickness obtained from the first study (n = 22; age = 22.0 ± 0.7 yr; mass = 80.8 ± 3.0 kg; height =179 ± 1 cm); and one for comparisons of bench press and leg press strength (n = 60; age = 26.6 ± 0.8 yr; mass = 80.9 ± 1.9 kg; height = 178 ± 1 cm) to determine if the age-related deficits in lean tissue mass, muscle thickness, and strength of the older men could be overcome with this intervention.

Randomization and Supplementation

An individual, who was not involved in the study, was responsible for randomizing the subjects and coding the supplements to ensure all subjects and investigators remained blinded throughout the study. Entry and analysis of data was performed by analyzing coded groups. After matching subjects for age and body mass to minimize differences between groups, each subject was randomly assigned to supplement orally with either protein (0.3 g protein/kg body mass; contained in 0.54 g/kg body mass of Myoplex®, Experimental and Applied Sciences, Inc., Golden, CO and 0.09 g/kg body mass chocolate cocoa; see Table 1 for Myoplex® ingredients), dissolved in ~ 300 ml of water, immediately before (i.e. 5 minutes) resistance training sessions (PRO-B) and

placebo (PLA, maltodextrin/sucrose/chocolate cocoa, 0.63 g/kg body mass) immediately after (i.e. 5 minutes) resistance training sessions; placebo immediately before and protein immediately after resistance training sessions (PRO-A); or placebo immediately before and after resistance training sessions. Myoplex® has been approved by ConsumerLab.com®, an independent organization which provides test results on nutritional products for the purity and lack of containments (i.e. products not listed on the label).

This combination of Myoplex® and chocolate cocoa was used for the protein supplement and maltodextrin/sucrose/chocolate cocoa for the placebo because it was effective for matching the protein and placebo for energy content, taste, texture, color, and appearance. The protein dose of 0.3 g/kg body mass was chosen because it is an approximate amount shown to increase muscle mass during resistance training (Burke et al., 2001; Esmarck et al., 2001). Subjects were instructed to arrive for their training sessions in a fasted state (~ at least three hours).

Table 4.1. Ingredients in Myoplex® supplement (g/kg body mass).

Protein	3.0×10^{-1}
Carbohydrates	1.7×10^{-1}
Fat	1.8×10^{-2}
Cholesterol	1.1×10^{-4}
Sodium	2.9×10^{-3}
Calcium	3.6×10^{-3}
Iron	1.0×10^{-5}
Vitamin A	2.1×10^{-6}

Resistance Training Program

Prior to the start of the study, each subject familiarized themselves with the resistance training equipment by participating in supervised resistance training sessions (3x/week; 2 weeks) in our research weight training room located in the Education Building, University of Saskatchewan. Familiarization with the resistance training equipment helped decrease the amount of learning (i.e. rapid improvement in the ability to perform a training exercise) which may contribute to the increase in strength during the initial stages of resistance training (Chilibeck et al., 1998a). All subjects followed the same high volume, resistance training program combined with the protein supplements or placebo for 12 weeks. Prior to training sessions, but after the supplement drink was consumed, each subject warmed up for 10 minutes on a stationary Monarch (Ergomedic 818E; Stockholm, Sweden) cycle ergometer and completed light stretching. Training sessions were supervised as previous research has demonstrated greater gains compared to unsupervised training (Mazzetti et al., 2000). Training sessions were completed at the convenience of each subject and were approximately 60 minutes in duration. There was no prescribed order to completing their weekly sessions, however subjects were encouraged to take at least one day rest between subsequent training days to reduce the chance of injury and minimize fatigue. Subjects trained 3 days/week for 3 sets of 10 repetitions with two-minute rest between sets for each exercise at an intensity corresponding to approximately 70% 1-RM for the leg press and bench press and a weight corresponding to their 10 repetition maximum for other exercises. We have previously used a similar resistance training program successfully to increase muscle mass and strength in older men (Chrusch et al., 2001). Resistance exercises included leg

press, leg (knee) extension, leg curl (knee flexion), and calf press using Hammer Strength equipment (Life Fitness; Franklin Park, IL), and bench press, shoulder press, lat pull down, and biceps curl using Lever equipment (Pulse Fitness Systems; Winnipeg, MB), and triceps extension using Paramount Fitness equipment (Apple Fitness; Edmonton, AB). Subjects maintained daily training logs where average training volume per session (weight x sets x repetitions) was determined for each subject. Resistance was increased by 2-5 kg once a subject completed 3 sets of 10 repetitions for an exercise.

Lean Tissue Mass

Lean tissue mass was assessed by air-displacement plethysmography (BOD POD, Life Measurement Inc., Concord, CA; Appendix H), as previously described in the first study. Reproducibility was assessed by testing 17 subjects one week apart. The coefficient of variation for lean tissue mass was 0.84%. The validity of the BOD POD was checked by measuring 15 of the subjects on the BOD POD and by dual-energy X-ray absorptiometry (DXA; Hologic QDR 2000, Waltham, MA). The correlation coefficient between BOD POD and DXA measurements was 0.96 ($p<0.05$).

Muscle Thickness

Muscle thickness of the elbow and knee flexors and extensors and ankle plantar flexors was measured using B-Mode ultrasound (Aloka SSD-500, Tokyo, Japan) as previously described in the first study. For ankle dorsi flexor muscle thickness, a small mark was drawn on the lateral side of the right leg to indicate exactly 30% of the distance down from the lateral condyle of the tibia to the lateral malleolus of the fibula (Abe et al.

2001). A tape measure was then wrapped around the leg at the 30% mark and used to mark another reference point on the bulk of the gastrocnemius and tibialis anterior muscle where the center of the ultrasound was placed. To measure ankle dorsi flexor muscle thickness, each subject was placed on a table in a seated position with their right leg extended and relaxed. Reproducibility of muscle thickness measurements was determined by testing 16 subjects one week apart. The coefficients of variation for muscle thickness measurements were: 2.5% (elbow flexors) 1.7% (elbow extensors), 3.1 % (knee flexors), 2.1% (knee extensors), 3.2% (ankle plantar flexors) and 4.0% (ankle dorsi flexors).

Muscular Strength

Leg press and bench press strength was assessed using a 1-repetition maximum (1-RM) standard testing procedure (Chrusch et al., 2001) prior to and following nutritional supplementation and resistance training. To measure the 1-RM leg press, a bilateral, leg press machine (Hammer Strength; Winnipeg, MB) was used. Following a demonstration, subjects were positioned in the leg press so that a 90^0 angle at the knee was achieved and feet placed shoulder width apart. Subjects were instructed to push the weight away from their body to full extension without locking the knees before returning to the starting position. A warm-up consisted of 10 repetitions of leg press using a weight determined by each subject to be comfortable. Weight was then progressively increased for each subsequent 1-RM attempt with a two-minute rest interval. The 1-RM was usually reached in four to six trials, including the warm up set.

For 1-RM bench press, subjects were positioned in a bilateral, bench press machine (Lever Pulse Fitness; Winnipeg, MB) with both feet on the floor. Following a demonstration, subjects were instructed not to lift their buttocks off the bench or arch their back during the lift. Subjects were positioned in the bench press machine so that the adjacent bars lined up mid-chest level. Subjects were instructed to grasp the bars (overhand grip) approximately shoulder width apart and push the weight away from the body until full extension and then lower the weight back to the starting position. A warm-up consisted of 10 repetitions with a comfortable starting weight as determined by each subject. Weight was then progressively increased for each subsequent 1-RM attempt with a two-minute rest interval. The 1-RM was usually reached in four to six trials, including the warm up set.

These two exercises were chosen as an index of muscular strength because they involve the major muscle groups in the lower and upper body. Reproducibility of the strength measures was assessed on ten subjects, one week apart. The leg press and bench press strength measures had coefficients of variation of 3.8% and 3.1% respectively.

Urinary 3-methylhistidine

For the measurement of 3-methylhistidine, an index of myofibrillar protein degradation, urine was collected during the last 24-hours of a 72-hour meat free diet immediately before and immediately after the study. A meat-free diet was implemented because meat consumption increases urinary 3-methylhistidine values and may falsely represent an increase in myofibrillar protein turnover (Lukaski et al., 1981). Three days of a meat-free diet are required to return 3-methylhistidine levels to baseline (Lukaski et

al., 1981). The designated urine collection procedure was to discard the first urination upon waking in the morning and then collect all urine samples for 24 hours, including the first urination upon waking the following morning (Appendix F). Urine samples were brought to the researcher where the subject's urine volume was recorded. Aliquots of each urine sample were drawn off from the 24 hour collection and stored at -20° Celsius until analyzed. The concentration of 3-methylhistidine was determined by high-performance liquid chromatography (3 mm Chromsep ODS-2 column, Varian Inc., Mississauga, ON; flow rate 1.0 mL/min) and 2475 multi-wavelength fluorescence detection (Waters, Mississauga, ON) using the methods of Wassner et al., (1980) with modification for sample volumes. Derivatization was completed by placing 200 μL of diluted (10 times with 0.9% NaCl) urine samples or 3-methylhistidine standards (Pfaltz and Bauer, Waterbury, CT), 1 mL of borate buffer (0.25 M boric acid, adjusted to pH 9.5 with NaOH), 1 mL of fluorescamine reagent (acetonitrile containing 1 mg fluorescamine per mL) in glass autosampler vials, which were then mixed and allowed to stand at room temperature for 5 minutes. Two hundred microlitres of 70% perchloric acid was added to the vials, which were capped with teflon-lined seals and heated at 80° C for 1 hour. After cooling to room temperature, samples were filtered using Acrodisc® 13 mm syringe filters with 0.2 Supor® membrane (Pall Corporation, MI, USA). Filtered samples were injected (20μL) with an autosampler (715 Ultra WISP autoinjector, Waters). The mobile phase was 23% acetonitrile and 77% 20 mM Na_2HPO_4 adjusted to pH 7.2 with NaOH. Peaks were monitored at 365 nm (excitation) and 460 nm (emission) and integrated with chromatography software (Millenium chromatography manager Millenium^{32}, version 4, Waters). The intra-assay coefficient of variation from duplicate samples was 5.1%. The

daily amount of 3-methylhistidine excreted by each subject was determined by multiplying the concentration by the 24-hour urine volume. This amount of 3-methylhistidine was then expressed relative to lean tissue mass (μmol/kg) (Candow et al. 2001a).

Dietary Intake

Dietary intake was recorded during the first and final week of nutritional supplementation and resistance training to assess whether there were differences in total energy and macronutrient composition between the protein treatment conditions and placebo (Appendix D). Dietary intake was not recorded immediately before and immediately following the study because habitual dietary intake would be altered due to the 72-hour meat free diet required for determination of urinary 3-methylhsitidine. Subjects used a 3-day food booklet to record what they ate for 2 weekdays and 1 weekend day. Subjects were instructed to record all food items, including portion sizes consumed for the 3 designated days. The Interactive Healthy Eating Index (Center for Nutrition Policy and Promotion, United States Department of Agriculture; http://www.usda.gov/cnpp/) was used to analyze 3-day food records. Each food item was entered and the program provided total energy consumption on average over the 3 days as well as energy from carbohydrates, fats, and proteins individually. We have previously used these food records in our college for assessing energy and macronutrient consumption (Burke et al., 2001; Candow et al., 2001a; Chrusch et al. 2001).

Statistical Analyses

There were five separate analyses performed in this study. In the first analysis, a A 3 (PRO-B vs. PRO-A vs. PLA group) × 2 (pre- and post-test periods) doubly multivariate analysis of variance (ANOVA), appropriate for analysis of designs with multiple time points (i.e. pre- and post-test periods) and multiple dependent variables was used to determine differences between the protein and placebo groups over time for the dependent variables of lean tissue mass, muscle thickness, strength, and 3-methylhistidine. Doubly multivariate analysis of variance protects against Type I error and provides insight into an effect of an intervention on multiple dependent variables which may be overlooked when dependent variables are assessed individually. In the second analysis, a 3 (PRO-B vs. PRO-A vs. PLA) x 2 (pre- and post-test periods) analysis of variance (ANOVA) with repeated measures on the second factor was used to determine differences in energy and macronutrient contents between groups during the first and final week of supplementation and training. In the third analysis, a one-factor ANOVA was used to evaluate change scores for the dependent variables and to determine differences between the protein groups combined and the placebo group. A one-factor ANOVA was used in the fourth analysis to determine differences in average training volume (kilogram x sets x repetitions) per session between the protein and placebo groups and to determine whether there were differences in baseline measurements between groups. A Tukey's post-hoc test was used to identify differences between means when interactions were found. In the fifth analysis, separate one-factor ANOVAs were used to compare pre- and post-training values for lean tissue mass, muscle thicknesses, and strength of the older group to young reference groups. All results

are expressed as means ± standard error. The magnitude of the difference between significant means (i.e. effect size) was determined by eta squared (η^2). Eta squared is a measure of the proportion of the total variance that is explained by the treatment effects. An η^2 value of .15 represents large differences, .06 represents medium differences, and .01 represents small differences. Statistical analyses were carried out using SPSS version 11.5 for Windows XP (SPSS, Chicago, IL) (Appendix J). Significance was set at $p<0.05$. Experimental design is shown in Figure 4.1.

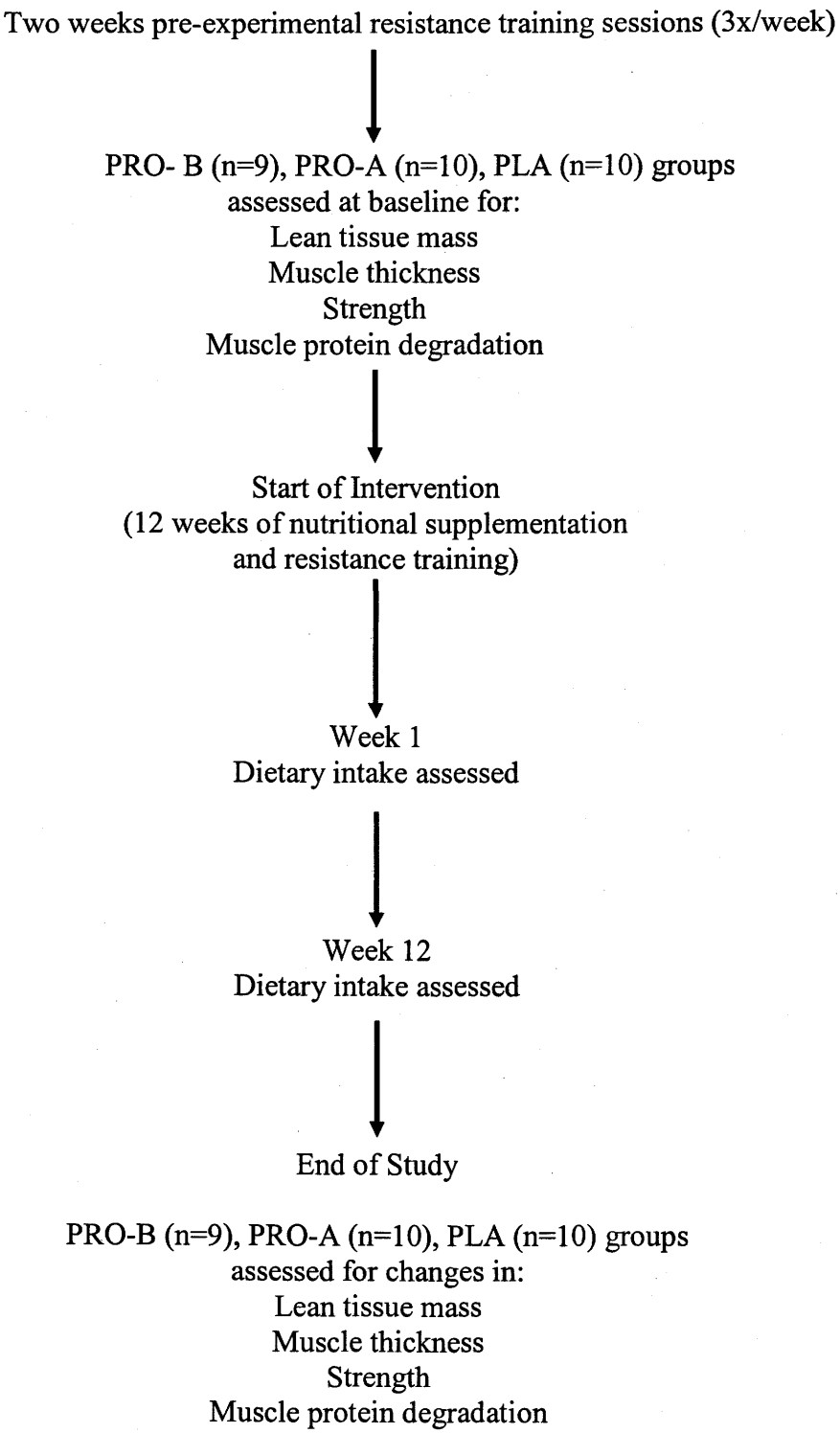

Figure 4.1 Experimental design.

Results

Subjects

Of the original 38 subjects who volunteered, 29 subjects completed the study. Of the original subjects, one subject in the PLA group withdrew because of cataract surgery. Two subjects (one from the PRO-B and one from the PRO-A) withdrew because of shoulder and knee pain. There were six subjects (two from each group) who withdrew due to time constraints. There were 27 subjects (eight in PRO-B group, 10 in PRO-A group, and nine in the PLA group) who were able to provide urine samples for analysis of 3-methylhistidine. Twenty-eight subjects (nine in PRO-B group, 10 in PRO-A group, and nine in the PLA group) were able to provide 3-day food records during the first and final week of training. Five subjects (one in PRO-B group, two in PRO-A group, and two in the PLA group) were correct in perceiving they were on the protein supplement or placebo, with the remaining subjects not knowing whether they were on the protein supplement or placebo. Baseline characteristics of subjects who completed the study are shown in Table 4.2. There were no differences between the protein and placebo groups for any of the baseline measurements.

Table 4.2. Subject characteristics at baseline for protein before (PRO-B), protein after (PRO-A), and placebo (PLA) groups.

Group	Age (yr)	Mass (kg)	Height (cm)
PRO-B (n=9)	63.3 ± 1.1	87.5 ± 6.4	176 ± 2.0
PRO-A (n=10)	66.5 ± 1.7	85.3 ± 3.6	173 ± 2.0
PLA (n=10)	64.6 ± 1.3	87.2 ± 5.8	173 ± 1.0

All values are means ± standard error.

I. Lean Tissue Mass

There was a significant time main effect, ($F [1, 26] = 10.0$, $p<0.05$, $\eta^2 = 0.13$) for lean tissue mass but no differences among groups (Figure 4.2). The relative increase in lean tissue mass for the PRO-B, PRO-A, and PLA groups were 1.2%, 1.7%, and 1.0% respectively. Lean tissue mass of the combined groups of older men before (58.3 ± 1.3 kg) ($F [1, 83] = 12.1$, $p<0.05$) and after (59.0 ± 1.2 kg) ($F [1, 83] = 9.8$, $p<0.05$) training was lower than younger men (64.3 ± 1.0 kg) from the database.

II. Muscular Strength

There was a significant time main effect for leg press ($F [1, 26] = 93.2$, $p<0.05$, $\eta^2 = 0.79$) and bench press ($F [1, 26] = 81.0$, $p<0.05$, $\eta^2 = 0.76$) strength with training (Figures 4.3 and 4.4; $p<0.05$). The relative increases in leg press 1-RM for the PRO-B, PRO-A, and the PLA groups were 31%, 25%, and 22%, respectively. The relative increases for bench press 1-RM for the PRO-B, PRO-A, and PLA groups were 28%, 23%, and 28%, respectively. There were no differences among groups for changes in strength with training. Bench press strength of the combined groups of older men before (77 ± 4 kg) and after training (95 ± 4 kg) were lower than younger men (121 ± 4 kg) ($F [1, 86] = 18.2$, $p<0.05$) from the database. Leg press strength of the combined groups of older men before training (169 ± 7 kg) was lower than the younger men (231 ± 7kg) ($F [1, 86] = 27.4$, $p<0.05$). After training, leg press strength of the older men (210 ± 8 kg) did not differ from the younger men.

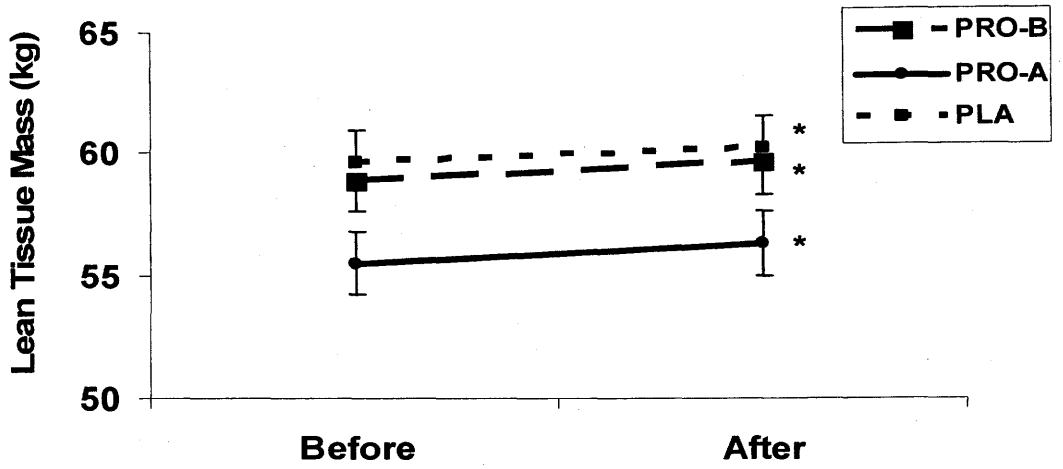

Figure 4.2 Graph of lean tissue mass before and after 12 weeks of nutritional supplementation and resistance training for PRO-B (n=9), PRO-A (n=10), and PLA (n=10) groups. Values are means (kg) ± standard error. * Indicates all groups increased lean tissue mass with training ($p<0.05$), with no significant differences between groups.

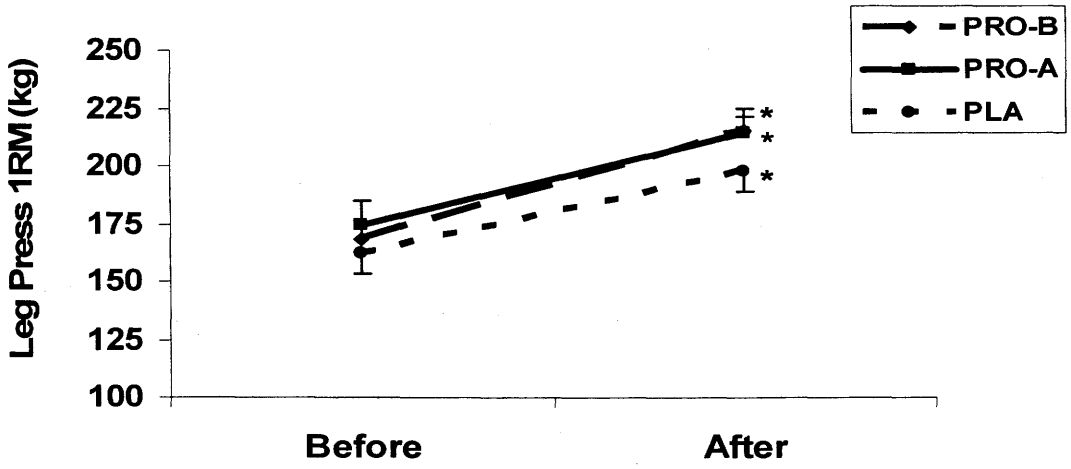

Figure 4.3 Graph of leg press strength (1-RM) before and after 12 weeks of nutritional supplementation and resistance training for PRO-B (n=9), PRO-A (n=10), and PLA (n=10) groups. Values are means (kg) ± standard error. * Indicates all groups increased strength with training ($p<0.05$), with no significant differences between groups.

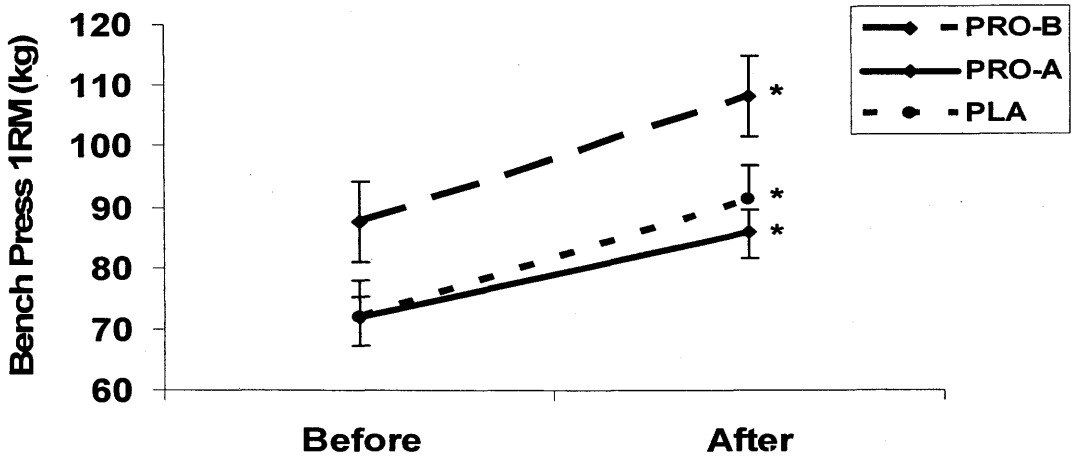

Figure 4.4 Graph of bench press strength (1-RM) before and after 12 weeks of nutritional supplementation and resistance training for PRO-B (n=9), PRO-A (n=10), and PLA (n=10) groups. Values are means (kg) ± standard error. * Indicates all groups increased strength with training ($p<0.05$), with no significant differences between groups.

III. Muscle Thickness

A significant increase in muscle thickness for all muscle groups was observed with training. There was a greater increase in muscle thickness of the knee extensors ($F [2, 26] = 3.9$, $p<0.05$, $\eta^2 = 0.18$) for the PRO-B group vs. PLA group, with no other differences (Table 4.3). The increase in muscle thickness for the six muscle groups combined was 3.3 cm (18.2%) for the PRO-B group, 2.4 cm (13%) for the PRO-A group, and 2.6 cm (15%) for the PLA group.

Before the training program, muscle thickness of the older group was significantly less than the young group for all sites, except the elbow extensors (Table 4.4). Following 12 weeks of nutritional supplementation and resistance training in the older men, muscle thickness of the elbow flexors ($F [1, 48] = 0.3$, $p<0.05$) and ankle dorsi flexors ($F [1, 48] = 2.9$, $p<0.05$) was no longer different compared to the young, and elbow extensor muscle thickness was greater in the older vs. young men ($F [1, 48] = 17.1$, $p<0.05$; Table 4.4).

IV. 3-Methylhistidine

There were no differences in urinary 3-methylhistidine levels over time in any group. The PRO-B group had values of 4.4 ± 0.9 and 4.4 ± 0.6 μmol/kg lean tissue mass, the PRO-A group had values of 5.8 ± 0.9 and 5.4 ± 1.0 μmol/kg lean tissue mass, and the PLA group had values of 2.7 ± 0.3 and 3.2 ± 0.5 μmol/kg lean tissue mass before and after training respectively.

Table 4.3. Muscle thickness measurements (cm) for the elbow and knee flexors and extensors, and ankle plantar flexors and dorsi flexors in older men before and after 12 weeks of supplementation and resistance training.

	PRO-B (n=9)			PRO-A (n=10)			PLA (n=10)		
Muscle Group	Pre	Post	%	Pre	Post	%	Pre	Post	%
Elbow flexors	2.9 ± 0.2	3.5 ± 0.1*	22.3 ± 5.1	2.8 ± 0.2	3.3 ± 0.1*	20.4 ± 3.9	2.7 ± 0.1	3.1 ± 0.1*	16.3 ± 3.9
Elbow extensors	4.1 ± 0.2	5.1 ± 0.2*	24.7 ± 4.0	3.9 ± 0.2	4.6 ± 0.3*	16.1 ± 3.8	3.9 ± 0.2	4.7 ± 0.2*	25.0 ± 5.0
Knee flexors	4.4 ± 0.3	5.0 ± 0.2*	16.7 ± 8.7	4.4 ± 0.2	4.7 ± 0.2*	8.2 ± 4.8	4.5 ± 0.4	5.0 ± 0.4*	14.9 ± 4.5
Knee extensors	3.1 ± 0.2	3.7 ± 0.2*	21.0 ± 6.1**	3.4 ± 0.1	3.8 ± 0.2*	11.9 ± 4.3	3.6 ± 0.3	3.8 ± 0.4*	4.9 ± 2.3
Ankle plantar flexors	3.1 ± 0.2	3.5 ± 0.2*	12.1 ± 4.3	3.5 ± 0.4	4.1 ± 0.4*	18.1 ± 8.3	3.0 ± 0.2	3.3 ± 0.2*	14.6 ± 6.7
Ankle dorsi flexors	2.2 ± 0.2	2.4 ± 0.1*	11.6 ± 7.9	2.4 ± 0.1	2.5 ± 0.2*	3.5 ± 4.4	2.2 ± 0.1	2.5 ± 0.2 *	12.0 ± 8.7
Ave. Total Change			18.2 ± 5.3			13.0 ± 4.6			15.0 ± 4.1

Values are means (cm) ± standard error. %= percent change over time.* Indicates significantly different after training ($p<0.05$).
** Indicates PRO-B had greater gains in knee extensor muscle thickness vs. PLA ($p<0.05$).

V. Training Volume

There were no differences in average training volume per session between the PRO-B, PRO-A, and the PLA groups. The PRO-B group had a mean average volume of 14,897 ± 1205 kg per training session, the PRO-A group had an average volume of 14,278 ± 1185 kg per training session, while the PLA group had an average volume of 13,310 ± 1022 kg per training session.

VI. Diet

From analysis of 3-day dietary intakes, all groups increased total caloric intake with training ($F [1, 25] = 4.6$, $p<0.05$, Table 4.5). There was a significant group x time interaction for dietary protein intake. Post hoc analysis indicated that the PRO-B group consumed significantly more dietary protein before training compared to the PRO-A group ($F [2, 25] = 4.2$, $p<0.05$, $\eta^2 = 0.25$), but the difference between groups was not significant at the end of training. There were no differences between the protein groups and the placebo group.

Table 4.4. Muscle thickness measurements (cm) for the elbow and knee flexors and extensors and ankle plantar flexors and dorsi flexors in older men before and after 12 weeks of supplementation and resistance training compared to young men.

	Older (n=22)		Young (n=22)
Muscle Group	Before	After	
Elbow flexors	2.8 ± 0.1*	3.3 ± 0.1	3.2 ± 0.1
Elbow extensors	4.1 ± 0.1	4.8 ± 0.1**	4.0 ± 0.1
Knee flexors	4.5 ± 0.1*	4.9 ± 0.1*	5.5 ± 0.1
Knee extensors	3.5 ± 0.2*	3.8 ± 0.2*	4.2 ± 0.2
Ankle plantar flexors	3.3 ± 0.2*	3.6 ± 0.2*	4.4 ± 0.3
Ankle dorsi flexors	2.3 ± 0.1*	2.5 ± 0.1	2.7 ± 0.1

Values are means (cm) ± standard error.* Indicates values for older men are less than for young men ($p<0.05$). ** Indicates older men had greater muscle thickness after training compared to young men ($p<0.05$)

Table 4.5. Total calories (kcal/d) and macronutrient (g/kg/day) content of PRO-B, PRO-A, and PLA groups for 3 days during the first and final week of nutritional supplementation and training.

	PRO-B		PRO-A		PLA	
	Week 1	Week 12	Week 1	Week 12	Week 1	Week 12
Kilocalories Per day	2310 ± 162	2350 ± 215*	2150 ± 176	2756 ± 396*	2387 ± 225	2769 ± 293*
Carbohydrates	2.7 ± 1.2	2.7 ± 1.3	3.1 ± 1.8	3.9 ± 1.1	3.1 ± 1.1	3.5 ± 1.1
Fat	1.1 ± 1.0	1.1 ± 1.5	1.0 ± 0.4	1.3 ± 0.6	1.1 ± 0.4	1.3 ± 0.2
Protein	1.5 ± 1.0**	1.4 ± 0.6	1.2 ± 0.6	1.4 ± 0.3	1.3 ± 0.2	1.5 ± 0.7

Values are means ± standard error. Data is based on the average for one day from 3-day food records. * Indicates significantly greater after training ($p<0.05$). ** Indicates PRO-B consumed more dietary protein before training compared to PRO-A ($p<0.05$).

Discussion

This was the first study to compare the effects of protein supplementation consumed immediately before and immediately after resistance training sessions for 12 weeks in healthy older men. It was hypothesized that protein supplementation immediately before resistance training would increase muscle mass and strength and attenuate myofibrillar protein breakdown over protein ingestion immediately following resistance training. It was also hypothesized that protein supplementation during resistance training, independent of timing, would have a greater effect than resistance training alone. Results showed that protein supplementation either before or after resistance training had no effect on muscle mass, strength, or muscle protein degradation; with the exception that protein supplementation before training sessions resulted in a greater increase in knee extensor muscle thickness over placebo. Despite the lack of benefit from protein supplementation, a unique and important finding of this study was that only 12 weeks of resistance training in these older men was sufficient for eliminating muscle size deficits of the elbow flexors and ankle dorsi flexors compared to young men from the first study. However, at the conclusion of resistance training, the older group still had significantly lower lean tissue, bench press strength, and muscle thickness of the knee extensors and flexors and ankle plantar flexors compared to young men. The greatest deficits for muscle size in older compared to younger individuals are for lower-body muscle groups (Janssen et al., 2000). These deficits may be too large to overcome with 12 weeks of resistance training. Despite these deficits in lower body muscle groups, the older group was able to increase leg press strength to a similar level as the young reference group. This suggests that the improvement in leg press strength with training

may be caused by neural, rather than muscular, adaptations (Chilibeck et al., 1998a). Complex exercises, such as those involving movement at one or more joint (i.e. leg press), may involve a longer initial neural adaptation compared to single-joint exercises (i.e. arm curl); resulting in delayed muscle hypertrophy (Chilibeck et al., 1998a). For example, Rutherford & Jones (1986) suggested that during the leg press exercise, learning and coordination plays a greater role early in training compared to the arm curl exercise. The authors further state that with complex exercises such as the leg press, support muscles may have to increase in strength or improve their ability to activate muscle contraction. In a study by Chilibeck et al. (1998a), leg press strength was significantly increased after 10-weeks of resistance training in young women, with no concurrent gain in leg muscle mass. In addition, young males who performed the leg press exercise for 7-16 weeks experienced significant gains in muscle strength with no increase in muscle cross-sectional area of the knee extensors (Dons et al., 1979). Longer training periods (i.e. > 12 weeks) than the one used in this study may be required to increase lower body muscle mass because of increased motor unit number and innervation ratios (muscle fibers/ motor unit) in the muscle groups involved in the leg press exercise (Chilibeck et al., 1998a). For example, the major muscle recruited during the leg press exercise is the vastus lateralis, which has been shown to have a larger number of motor units and higher innervation ratios compared to the biceps brachii, the major muscle recruited in the arm curl exercise (Galea et al., 1991).

Results of no effect from protein supplementation on muscle mass and strength are in agreement with a recent study by Godard et al. (2002) who found amino acid ingestion during 12 weeks of resistance training did not increase muscle mass and

strength to a greater extent than resistance training alone in healthy older men. The authors suggest that the response of muscle protein synthesis to oral ingestion of amino acids may be impaired in older individuals due to alterations in their metabolic response to exercise. Furthermore, high protein meals (28% of energy intake/meal) did not enhance the increase in myofibrillar protein synthesis induced by resistance training in sedentary healthy older men and women (Welle & Thornton, 1998). Results showed that dietary protein intake was well above (~ 1.3g/kg/day) the recommended level of 0.8g/kg/day; suggesting that the additional amino acids supplied from increased protein intake were not incorporated into muscle protein. On the other hand, in examining the effects of protein (~ 30g/day; 10 days) supplementation on body composition and whole-body protein kinetics in 17 malnourished elderly subjects, Bos et al. (2000) found a significant increase in muscle protein synthesis and fat-free mass from supplementation. It is important to note that malnourished older individuals may have higher protein turnover rates and a greater loss of muscle protein than healthy older individuals as a result of a hypercatabolic state (Beaumont et al., 1989). Therefore, a greater protein requirement for these unhealthy older individuals may have accounted for the differences between studies. Our results showed that all groups were consuming adequate protein in their diet

Results from the present study showed a significant increase in leg extensor muscle thickness when protein was ingested immediately before resistance training sessions. These results support that of Tipton et al. (2001) who found greater amino acid incorporation into muscle protein of the vastus lateralis when amino acids were consumed immediately before an acute bout of resistance training. During resistance

training, there may be a net loss of muscle protein, because muscle protein synthesis is either decreased (Bylund-Fellenius et al., 1984) or unchanged (Carraro et al., 1990), whereas protein degradation is elevated (Rennie et al., 1981). Although the machinery for stimulating muscle protein synthesis is increased after resistance training (Welle & Thornton, 1998), it appears that this response may not be increased until some time after the resistance training session (Tipton et al., 2001). Based on the results of the present study and that of Tipton et al. (2001), protein ingestion immediately before resistance training sessions may counter the net loss of muscle protein in the knee extensors by creating an anabolic environment for muscle growth; possibly through increased amino acid delivery to muscle from exercise increased blood flow (Tipton et al., 2001).

The majority of research on the timing of protein ingestion has examined its effect in the postexercise period. Results from the present study do not support an ergogenic effect from protein supplementation (~28g) consumed immediately after total body resistance training in healthy older men. These results are in agreement with Godard et al. (2002) who found no effect from essential amino acid supplementation (~12 g/day) during 12-weeks of lower body resistance training on muscle size and strength in healthy older men. However, Esmarck et al. (2001) showed that protein (~10 g) consumed immediately following total body resistance training sessions for 12 weeks was effective for increasing muscle mass in healthy older individuals. Discrepancies between studies are mostly likely related to methodological differences. In particular, differences in supplement composition (~28g protein vs. ~10g protein vs. ~12 g essential amino acids) and the type of resistance training (total body compared to lower body resistance training) may account for these inconsistent findings.

Three-methylhistidine is an amino acid located primarily in skeletal muscle from the post-translation modification of specific histidine residues in myofibrillar proteins (Lukaski, 1997). During muscle protein catabolism, the released 3-methylhistidine is neither re-utilized for protein synthesis nor metabolized oxidatively but, instead, is quantitatively excreted in the urine (Lukaski, 1997); therefore, it serves as a useful indicator of myofibrillar protein degradation (Pivarnik et al., 1989). Results showing no effect from protein supplementation on urinary 3-methylhistidine excretion are in agreement with Campbell et al. (1995) who also found no effect from protein supplementation (0.8-1.6 g/kg/day) during 12 weeks of resistance training on urinary 3-methylhistidine levels in healthy older adults. It has been suggested that during the initial stages of resistance training, dietary protein needs are elevated for muscle protein synthesis and muscle recovery (Rennie & Tipton, 2000). However, regular resistance training sessions may decrease dietary protein requirements (Tarnopolsky et al., 1992) because a continuum of training stimuli will result in a series of muscle protein turnover responses each of which is progressively reduced (Figure 4.5) (Rennie & Tipon, 2000).

This theoretical model was supported by Farrell et al. (1999) who found the rate of muscle protein turnover following 8 weeks of resistance training was significantly reduced in trained versus untrained rats. Based on these findings, protein requirements may decrease with regular resistance training sessions (i.e. down regulation adaptation), possibly due to net protein utilization and reduced muscle protein turnover (Butterfield, 1987). A reduction in muscle protein turnover with regular resistance training sessions would theoretically result in a decrease in urinary 3-methylhistidine.

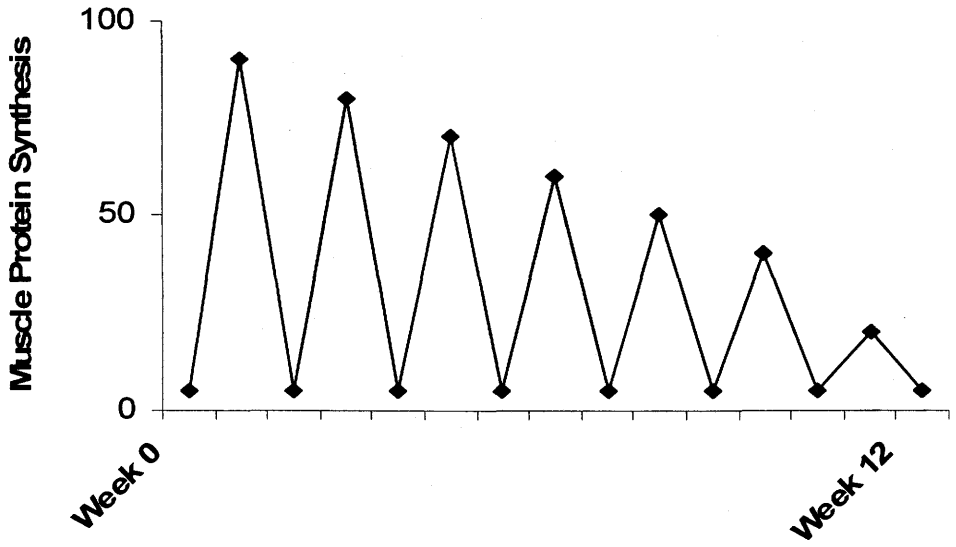

Figure 4.5 Hypothetical response of muscle protein synthesis to repeated bouts of resistance training training.

There were several limitations to this study. One limitation was sample size. To acheive 80% power, thirty-six subjects were required. Unfortunately, twenty-nine subjects completed the study. This reduction in statistical power could have resulted in Type II error; the inability to detect a statistically significant treatment effect. A second limitation was individual nutritional status. Factors such as lifestyle, nutrient-nutrient, drug-nutrient, and disease may have had an adverse effect on the treatment effect. For example, smoking results in a decrease in macronutrient consumption and alcohol has a negative effect on muscle protein turnover. Regarding nutrient-nutrient interaction, one nutrient may adversely affect the bioavailabilty or metabolism of another nutrient. For instance, high protein intake has the potential to negatively affect body calcium stores. In addition, many over the counter prescriptions have adverse effects on nutrient metabolism and function. Finally, disease may increase nutrient requirements for immune system function, muscle preservation, and recovery. It would be necessary to determine the potential effect that each of these variables may have on a protein treatment effect before replicating this experiment. A third limitation was methodology. Air-displacement plethysmography was used to determine lean tissue mass. Although each subject served as their own control, if the pre-test requirements of no physical activity for 24 hours and no food and drink for 3 hours were not adhered to, error may have occurred. Ultrasound was used to determine muscle thickness of the elbow and knee flexors and extensors and ankle plantar flexors and dorsi flexors. Ultrasound does not differentate between contractile and non-contractile tissue. It would be necessary to replicate this experiment using MRI to accurately measure muscle size. In addition, subject motivation, time of day testing, and recovery from exercise may have affected strength results. Muscle protein

degradation was assessed by 3-methylhistidine. Disagreement regarding the validity of 3-methylhisitdine excretion as an indicator of muscle protein degradation relates primarily to the contribution of non-muscle sources (i.e. skin, gut) and failure to account for inter-individual differences in the ratio of non-myofibrillar/myofibrillar and lack of dietary control. Without controlling for diet, the 72-hour meat free period required for estimation of 3-methylhistidine may not have been adhered to accurately. Future research should use isotopic tracer methodology which allows simultaneous estimation of both protein synthesis and degradation. A fourth limitation was dietary assessment. Three-day food records were used to assess energy and macronutrient consumption. Disadvantages in using 3-day food records include failure to recall accurate food portion sizes and frequency of eating. In addition, motivation, writing skills, honesty, and cooperation in filling out the records may have affected results for diet. It would be necessary to control for diet when replicating this study. Another limitation was that potential side effects from protein supplementation were not assessed. Increased protein intake may have an adverse effect on liver and kidney function, bone loss, and dehydration. When duplicating this experiment, liver and kidney enzymes, n-telopeptides (markers of bone resorption), blood calcium, and hydration status should be assessed to determine the safety of additional dietary protein intake during a resistance training program. Finally, no measure of muscle protein kinetics was made in this study. Future research should assess transcriptional and translational efficiency through isotopic tracer or muscle biopsy technique to help understand the mechanism of an anabolic response from amino acids. In addition, future research should also determine the effect that changes in body

composition, physical functional ability, physical activity, food intake patterns, and frequency of disease may have on protein kinetcs in older men and women.

In summary, 12 weeks of supervised resistance training is an effective way to increase muscle mass and strength in healthy older men. This intervention is effective for overcoming deficits in leg press strength and muscle size deficits of the elbow flexors and ankle dorsi flexors between young and older men. Unfortunately, at the conclusion of resistance training, the older group still had significantly lower lean tissue mass, muscle thickness of the knee extensors and flexors and ankle plantar flexors, and bench press strength compared to young men. Therefore, these age-related deficits in muscle mass and strength may be too large to overcome in older individuals with 12 weeks of resistance training and a longer intervention (i.e. > 12 weeks) is required.

Study 3

Effect of Creatine and Protein Supplementation Combined With Resistance Training in Older Men

Introduction

Creatine supplementation increases intramuscular total creatine (i.e. creatine and phosphocreatine) in older individuals (Brose et al., 2003). The increase in high energy phosphates could allow one to train with a greater volume of resistance exercise leading to increased lean tissue mass and muscular strength (Chrusch et al., 2001). For example, Brose et al. (2003) found a significant increase in intramuscular total creatine, strength, and lean tissue mass (1.7 ± 1.2 kg) in healthy older adults from creatine supplementation (5g/day) during 14 weeks of resistance training. In addition, Chrusch et al. (2001) reported a significant increase in lean tissue mass of 3.3 ± 1.6 kg following 12 weeks of creatine supplementation and resistance training in older men. The underlying mechanisms explaining the increase in muscle mass from creatine supplementation remains to be determined; however, potential mechanisms include an increase in cellular hydration (Hultman et al., 1996; Saab et al., 2002) and myofibrillar mRNA kinetics (Willoughby & Rosene, 2001), and a reduction in protein degradation (Parise et al., 2001). Creatine has the ability to regulate osmosis within the working cell and potentially elevate intracellular osmolarity leading to cellular hydration (Balsom et al., 1995). The anabolic signal induced by cellular hydration may increase myogenic transcription factors such as MRF-4 and myogenin (Balsom et al., 1995; Francaux & Poortmans, 1999; Kreider et al., 1998) or alter the level of charged tRNAs which are specific for myofibrillar protein synthesis (Ingwall, 1974). Myogenic transcription factors are members of basic helix-loop-helix (bHLH) proteins that function as transcription activators based on their inherent properties as DNA-binding proteins (Willoughby & Rosene, 2003). As such, they initiate transcription and regulate gene expression by

binding to specific regions of a DNA sequence located on the promoter and enhancer regions downstream of muscle specific genes such as myosin heavy chain (Willoughby & Rosene, 2003). Creatine may increase the expression of myogenic transcription factors and facilitate the up-regulation of muscle specific-genes such as myosin heavy chain, thereby facilitating repetitive increases in muscle mass and strength (Willoughby & Rosene, 2003). For example, in young healthy volunteers, creatine supplementation (20g/day) during two weeks of leg immobilization followed by ten weeks of rehabilitation training significantly increased the expression of myogenic transcription factor MRF-4. The increase in MRF-4 expression was correlated with an increase in muscle cross-sectional area (Hespel et al., 2001). In addition, creatine supplementation (6g/day) combined with 12-weeks of heavy resistance training significantly increased mRNA and protein expression of myogenin and MRF-4 in young male subjects (Willoughby & Rosene, 2003). Myogenin and MRF-4 function as positive transcription activators. It was speculated that creatine supplementation may increase mRNA template availability in muscle undergoing hypertrophy, resulting from changes in transcriptional capacity, translational efficiency, and/or mRNA stability which may depend on the up-regulation of myogenin and MRF-4 (Willoughby & Rosene, 2003).

It is well established that muscle protein synthesis proceeds in the presence of amino acids (Tipton, 2001). An increase in muscle protein synthesis over time may lead to an increase in muscle size and strength. Amino acids are selected for protein synthesis by binding with transfer RNA (tRNA). The information and order of amino acid sequence for each protein is governed by messenger RNA (mRNA) that is produced from DNA through transcription (Houston, 2001). An increase in amino acid availability

through additional dietary protein could potentially increase the combination of tRNA with an amino acid for translocation to ribosomal RNA (rRNA) (i.e. increased translational efficiency; see Figure 1.1) located on the ribosome organelle where protein synthesis occurs. Increased translational efficiency, in the presence of increased amino acid availability from protein supplementation, could potentially increase muscle protein synthesis with subsequent resistance training sessions (Houston, 2001; Welle et al., 1994, 1995) leading to greater muscle mass and strength. Therefore, with the up-regulation of transcription factors from creatine supplementation, combined with increased translational efficiency from protein supplementation, the combination of creatine and protein supplementation during resistance training may have an additive effect on protein kinetics leading to greater muscle mass and strength in older individuals.

The purpose of this study was to compare changes in lean tissue mass, muscle thickness, strength, and muscle protein degradation from creatine and protein supplementation to supplementation with creatine or placebo during resistance training in older men. Given the potential of increased phosphocreatine for exercise improvement (i.e. allowing one to train with a greater volume during resistance exercise) (Chrusch et al., 2001) and amino acid availability for protein synthesis (Tipton et al., 2001), it was hypothesized that creatine and protein together would increase lean tissue mass and strength over creatine supplementation and placebo. Secondary measures in the current study included markers of muscle protein turnover (myofibrillar protein degradation, by 3-methylhistidine). Creatine has been shown to increase protein synthesis (Ingwall et al., 1974) and reduce protein degradation (Parise et al., 2001). Protein or amino acid supplementation enhances the ratio of protein synthesis to degradation post-exercise

(Biolo et al., 1997). It was therefore hypothesized that creatine and protein supplementation would decrease myofibrillar protein degradation to a greater extent than either creatine supplementation or placebo during resistance training.

In the first study, muscle mass and strength were determined between young and older men. Older men had lower lean tissue mass and muscle thickness for the elbow flexors, knee flexors and extensors, and ankle plantar flexors compared to younger men. Following 12 weeks resistance training in older men in the second study, elbow flexor and ankle dorsi flexor muscle thickness was similar between age groups and elbow extensor muscle thickness was actually greater in the older vs. young men ($p<0.05$). The 12 weeks of resistance training was also effective for eliminating the older subject's deficits in leg press strength; however, lean tissue mass, bench press strength, and muscle thicknesses of the knee flexors and extensors and ankle plantar flexors was still lower compared to young reference groups. Seventeen of the older men who participated in the second study also participated in this study to determine whether a total of 22 weeks of resistance training could eliminate remaining deficits in lean tissue mass, bench press strength, and muscle thicknesses of the knee flexors and extensors and ankle plantar flexors compared to younger men.

Methods

Subjects

A group of 40 men (59-77years) volunteered for the study through a newspaper advertisement. Of the 40 men who volunteered, 17 previously participated in Study 2. The older subjects familiarized themselves with the resistance training equipment by participating in supervised resistance training sessions (3x/week; 2 weeks) in our research weight training room located in the Education Building, University of Saskatchewan. Twenty-eight subjects were needed to achieve 80% power as determined using the nomogram by Day and Graham, (1990) based on a mean lean tissue mass of 55.7 kg (Burke et al., 2001; Chrusch et al., 2001; Esmarck et al., 2001, Tipton et al., 2001) with a standard deviation from the means of 2.0, assuming a difference parameter (i.e. standard deviation of the means / standard deviation of measurements) of 0.77 at an alpha value of 0.05. The study was approved by the University Ethics Review Board for Research in Human Subjects (Appendix A). The subjects were informed of the risks and purposes of the study before their written consents were obtained (Appendix A).

Experimental Design

Detailed procedures for subject randomization (page 101), supplementation (page 101), and urine collection (page 107) are provided in the second study. After matching subjects for age and body mass to minimize differences between groups, each subject was randomly assigned to supplement with creatine and protein (CP) (0.1 g creatine /kg body mass/day; contained in 0.45g/kg SyntheVol™ 2 HP, Experimental and Applied Sciences, Inc., Golden, CO [see Table 5.1 for complete ingredients], combined with 0.3 g

protein/kg body mass/day; contained in 0.55 g/kg Myoplex®; Experimental and Applied Sciences, Inc., Golden, CO [see Table 1 for complete ingredients], and 0.2 g/kg body mass of a chocolate and cherry-flavored sucrose powder), creatine (CR) (0.1 g creatine/kg body mass/day and 1.1 g/kg body mass of chocolate-cherry flavored sucrose powder); or placebo (PLA) (1.2g/kg body mass/day of chocolate-cherry flavored sucrose powder), consumed in three equal doses throughout each training day (i.e. 0.4g/kg body mass of supplement powder dissolved in ~300 ml of water 5 minutes before their training session, 0.4g/kg body mass of supplement powder dissolved in ~300 of water 5 minutes after their training session, and 0.4g/kg body mass of supplement powder dissolved in ~300 ml of water before going to bed). SyntheVol™ 2 HP has also been approved by ConsumerLab.com®, an independent company that assesses nutritional supplements for purity and lack of containments.

The supplements were mixed with the chocolate-cherry flavored sucrose power to ensure that supplements and placebo were similar in energy content, taste, texture, and appearance. However, the creatine and protein supplement was more nitrogenous because it contained protein. Subjects were instructed to mix their supplement in water and not in a caffeinated beverage as caffeine may negate the ergogenic effects of creatine on skeletal muscle (Vandenberghe et al., 1996). Subjects fasted for at least 3 hours prior to each training session. The supplement was provided immediately before (i.e. 5 minutes) and immediately after (i.e. 5 minutes) each training session as it has been shown that the timing of either protein or creatine ingestion is crucial for creating an anabolic environment for muscle growth, with ingestion immediately before (Andersen et al., 2005; Tipton et al., 2001) and immediately after resistance training (Andersen et al.,

2005; Chilibeck et al., 2004, Esmarck et al., 2001) appearing optimal. Subjects were instructed to consume the supplement before going to bed to help promote net muscle protein balance (Tipton et al., 1999). The creatine dose of 0.1g/kg body mass was chosen because it has been shown to be effective when combined with protein (Burke et al., 2001) or when given on its own in young subjects (Burke et al., 2000). The protein dose of 0.3 g/kg body mass was chosen because it is an approximate amount shown to increase muscle mass during resistance training (Burke et al., 2001, Esmarck et al., 2001). Creatine supplementation occurred three days per week (i.e. only on training days) for two reasons: daily creatine supplementation in older men (i.e. 0.3 g/kg body mass creatine for 5 days and 0.07g/kg body mass thereafter for 12 weeks) resulted in minor side-effects (i.e. loose stools, muscle cramping, and muscle pulls/strains) (Chrusch et al., 2001) and supplementation with creatine only on training days (i.e. 2 days per week) was effective for increasing lean tissue mass, muscle thickness, and strength in young individuals (Chilibeck et al., 2004). All supplementation was double blind. To achieve the double blind, an individual who was not involved in the study was responsible for packaging the supplements according to the randomization list. Compliance with the supplementation protocol was monitored by having subjects return empty supplement bags when picking up additional supplements.

Table 5.1. Ingredients in Myoplex® and SyntheVol™ 2 HP supplements (g/kg body mass).

Ingredient	Myoplex®	Ingredient	SyntheVol™ 2 HP
Protein	3.0×10^{-1}	Protein	4.0×10^{-2}
Carbohydrates	1.7×10^{-1}	Carbohydrates	2.7×10^{-1}
Fat	1.8×10^{-2}	Aspartame	1.5×10^{-3}
Cholesterol	1.0×10^{-4}	Creatine Monohydrate	1.0×10^{-1}
Sodium	2.9×10^{-3}	Glutamine Peptides	6.0×10^{-2}
Calcium	3.6×10^{-3}	Inzitol® (D-Pinitol)	8.0×10^{-4}
Iron	1.0×10^{-5}		
Vitamin A	2.1×10^{-6}		

The dependent variables measured before and after training and supplementation were: (1) lean tissue mass, (2) muscle thicknesses of the elbow and knee flexors and extensors and ankle plantar flexors and dorsi flexors, (3) leg press and bench press strength, and (4) urinary 3-methylhistidine excretion (index of myofibrillar protein degradation). In addition, subjects completed dietary records for 3 days during the first and final week of training and supplementation to assess nutrient differences between groups. Detailed descriptions for the resistance training program (page 104), and methods for lean tissue mass (page 105), muscle thickness (page 105), strength (page 106), urinary 3-methylhisitidne (page 107), and dietary intake assessments (page 109), are provided in the second study.

Retrospective Creatine Side Effects and Treatment Identification Assessment

A retrospective creatine side-effect assessment (Chrusch et al., 2001) was administered to all subjects upon completion of the study (Appendix C) to determine if supplementing with creatine on training days was well tolerated in healthy older males. These findings would provide information regarding effective creatine dosing practices with minimal side effects. The retrospective assessment consisted of yes or no responses concerning energy level, strength, muscle fatigue and soreness, stiffness or tightness and pulls or strains, joint soreness, emotional states, headaches, feelings of physical appearance, sleep quality, appetite, thirst, sex drive, and gastrointestinal function abnormalities (Chrusch et al., 2001).

Comparisons to Young Reference Groups

Of the 40 men (age = 66 ± 2.9 years, mass = 82.6 ± 4.6 kg, height = 177 ± 1.7 cm) who volunteered for this study, 17 previously participated in the second study examining the effects of nutritional supplementation (i.e. protein and carbohydrate) during 12 weeks of resistance training. Individual results of these 17 men (age = 63.8 ± 1.0 yr, mass = 87.9 ± 4.6 kg; height = 176 ± 4.3 cm) from the second study were combined with their results from this study to determine if a total of 22 weeks of resistance training was effective for eliminating remaining deficits in lean tissue mass, bench press strength, and muscle thicknesses of the knee flexors and extensors and ankle plantar flexors compared to reference groups of young men. Of the 17 older men, four were in the CP group in the current study, six were in the CR group, and seven were in the PLA group. Ten had previously received protein supplementation and seven received placebo in the second study of this thesis. Three reference groups of young males were used for comparisons; one for comparison of lean tissue mass (n = 57; age = 24.5 ± 0.6 yr; mass = 81.6 ± 1.8 kg; height = 178 ± 1 cm), one for comparison of muscle thicknesses (n = 22; age= 22.0 ± 0.7 yr; mass = 80.8 ± 3.0 kg; height = 179 ± 1 cm); and one for comparisons of bench press and leg press strength (n = 60; age = 26.6 ± 0.8 yr; mass = 80.9 ± 1.9 kg; height = 178 ± 1 cm).

Statistical Analyses

There were six separate analyses performed in this study. In the first analysis, a 3 (CP vs. CR vs. PLA) x 2 (pre- and post training and supplementation) doubly multivariate analysis of variance (ANOVA), appropriate for analysis of designs with

multiple time points (i.e. pre- and post-test periods) and multiple dependent variables was used to assess changes in lean tissue mass, muscle thickness, strength, and 3-methylhistidine. In the second analysis, a 3 (CP vs. CR vs. PLA) x 2 (pre- and post training and supplementation) analysis of variance (ANOVA) with repeated measures on the second factor was used to assess changes in energy and macronutrient contents during the first and final week of supplementation and training. In the third analysis, a 2 (CP + CR vs. PLA) x 2 (pre- and post training and supplementation) analysis of variance (ANOVA) with repeated measures on the second factor was used to assess changes in the dependent variables between the creatine groups combined and placebo. In the fourth analysis, Chi-square analysis was used to assess retrospective creatine side effects. In the fifth analysis, a one-factor ANOVA was used to determine differences in change scores between groups and to determine differences in average training volume (kilogram x sets x repetitions) per session between groups. In addition, a one-factor ANOVA was used to determine whether there were differences in baseline measurements between groups. In the sixth analysis, separate one-factor ANOVAs were used to compare lean tissue mass, muscle thicknesses, and strength before and after 22 weeks of nutritional supplementation and resistance training in the 17 older men who participated in the second and third study to young reference groups. A Tukey's post-hoc test was used to test for differences between means when interactions were found. All results are expressed as means ± standard error. The magnitude of the difference between significant means (i.e. effect size) was determined by eta squared (η^2). Eta squared is a measure of the proportion of the total variance that is explained by the treatment effects. An η^2 value of .15 represents large differences, .06 represents medium differences, and .01 represents small

differences. Significance was set at $p<0.05$. Statistical analyses were carried out using SPSS version 11.5 for Windows XP (SPSS Inc., Chicago, IL) (Appendix J).

Experimental design is shown in Figure 5.1.

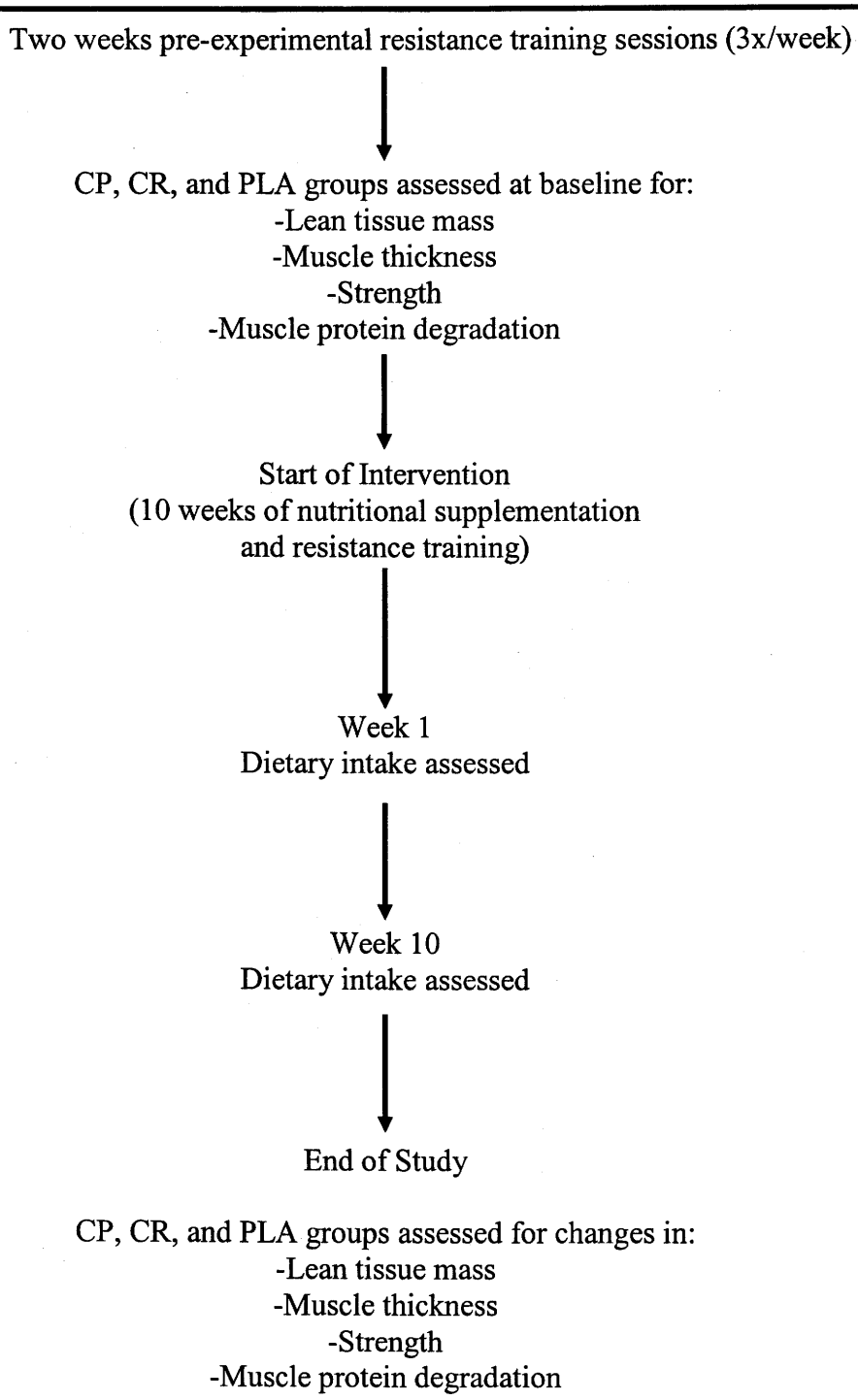

Figure 5.1 Experimental design.

Results

Subjects

Of the original 40 subjects who volunteered for the study, 35 subjects completed the study. Of the original subjects, two from the PLA group withdrew due to shoulder and hip pain. There was one subject in the CP group who withdrew because of gall bladder surgery and two others in the CP group who withdrew because of time constraints. There were 34 subjects (9 in the CP group, 13 in the CR group, and 12 in the PLA group) who were able to provide urine samples for analysis of 3-methylhistidine. Two subjects in the CR group were unable to complete bench press testing because of shoulder problems. Twenty-eight subjects (nine in the CP group, 10 in the CR group, and nine in the PLA group) were able to provide 3-day food records during the first and final week of training. Baseline characteristics of subjects who completed the study are shown in Table 5.2.

There were no differences among groups in any of the baseline measurements. Supplementation compliance, based on the number of empty supplement bags returned, was similar (CP 94%, CR 93%, PLA 95%) between groups.

I. Lean Tissue Mass

There was a significant group x time interaction for lean tissue mass ($F [2, 32] = 4.7$, $p<0.05$, $\eta^2 = 0.23$). Post hoc analysis indicated that lean tissue mass significantly increased in all groups with training ($F [1, 32] = 24.2$, $p<0.05$; $\eta^2 = 0.43$; Figure 5.2). The gain was greater in the CP group (+ 3.2 kg or 5.6%) compared to the PLA group (+0.6 kg or 1.0 %) ($p<0.05$). The increase in body mass in the subjects who supplemented with

creatine (i.e. CP + C) was greater (+0.6 kg or +0.8%) compared to placebo (-0.5kg or -0.8%) ($F [1, 36] = 4.8$, $p<0.05$).

II. Muscle Thickness

A significant increase in muscle thickness for all muscle groups ($p<0.05$, Table 5.3), except the ankle dorsi flexors, was observed for all groups with training. The increase in total muscle thickness for the six muscle groups combined was significantly greater in the subjects who supplemented with creatine (CP and CR groups combined; + 1.9 cm or 8.1%) compared to placebo (+0.8 cm or 3.7%) ($F [1, 33] = 4.4$, $p<0.05$, $\eta^2 = 0.12$).

III. Muscular Strength

There was a significant time main effect for leg press ($F [1, 32] = 44.8$, $p<0.05$; $\eta^2 = 0.60$) and bench press ($F [1, 32] = 38.3$, $p<0.05$; $\eta^2 = 0.55$) strength for all groups with training (Figures 5.3 and 5.4). The increases in leg press 1-RM for the CP, CR, and the PLA groups were 20.4 kg or 11%, 19.5 kg or 10%, and 21.3 kg or 10.1%, respectively. The increases for bench press 1-RM for the CP, CR, and PLA groups were 18.6 kg or 19.5%, 10 kg or 11.1% and 9.5 kg or 9.3 % respectively.

Table 5.2. Subject characteristics at baseline for creatine and protein (CP), creatine (CR) and placebo (PLA).

Group	Age (yr)	Mass (kg)	Height (cm)
CP (n=10)	67.3 ± 3.1	82.5 ± 2.8	177 ± 2.0
CR (n=13)	65.5 ± 2.7	79.0 ± 5.4	175 ± 2.0
PLA (n=12)	64.1 ± 3.1	86.3 ± 5.5	178 ± 1.0

Values are means ± standard error.

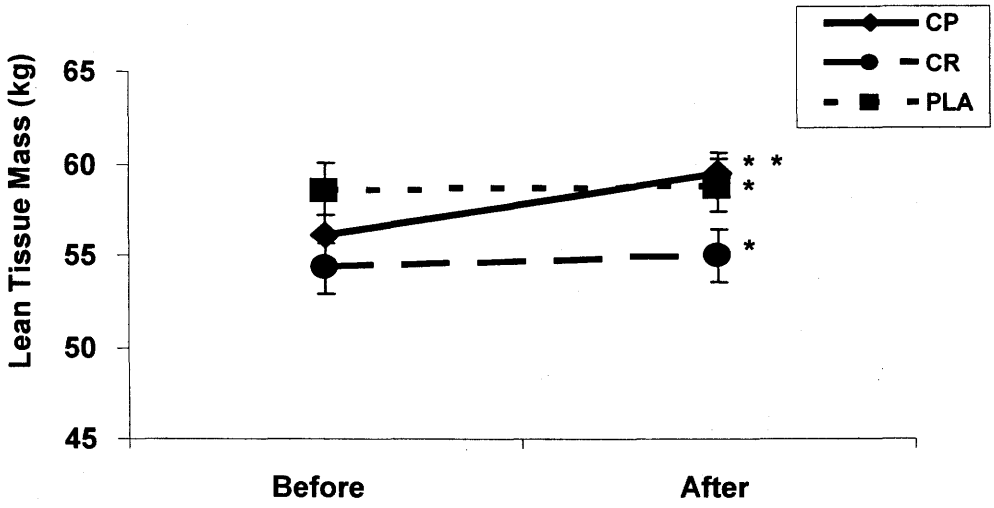

Figure 5.2 Graph of lean tissue mass before and after 10 weeks of nutritional supplementation and resistance training for CP (n=10), CR (n=13), and PLA (n=12) groups. Values are means ± standard error.* Indicates all groups increased lean tissue mass with training ($p<0.05$). ** Indicates CP group increased lean tissue mass more than PLA with training ($p<0.05$).

Table 5.3. Muscle thickness measurements (cm) for the elbow and knee flexors and extensors and ankle plantar flexors and dorsi flexors before and after 10 weeks of nutritional supplementation and resistance training.

	CP (n=10)			CR (n=13)			PLA (n=12)		
Muscle Group	Pre	Post	%	Pre	Post	%	Pre	Post	%
Elbow flexors	2.9 ± 0.2	3.1 ± 0.2*	9.0 ± 6.0	2.8 ± 0.2	3.0 ± 0.2*	8.8 ± 3.7	3.0 ± 0.2	3.2 ± 0.1*	8.0 ± 5.3
Elbow extensors	4.3 ± 0.3	4.7 ± 0.3*	1.6 ± 5.0	4.1 ± 0.2	4.5 ± 0.2*	11.4 ± 4.3	4.7 ± 0.1	4.8 ± 0.2*	1.4 ± 3.6
Knee flexors	4.9 ± 0.3	5.2 ± 0.2*	7.0 ± 5.2	4.5 ± 0.2	4.8 ± 0.2*	9.4 ± 3.6	4.8 ± 0.2	4.9 ± 0.2*	3.2 ± 2.9
Knee extensors	3.3 ± 0.3	3.8 ± 0.3*	13.6 ± 4.7	3.3 ± 0.2	3.6 ± 0.2*	11.3 ± 3.7	3.7 ± 0.3	3.9 ± 0.2*	5.8 ± 3.6
Ankle plantar flexors	3.8 ± 0.3	4.3 ± 0.2*	14.1 ± 5.0	3.5 ± 0.2	3.9 ± 0.3*	13.8 ± 4.0	3.9 ± 0.2	4.2 ± 0.2*	8.0 ± 6.4
Ankle dorsi flexors	2.3 ± 0.1	2.5 ± 0.3	4.6 ± 5.1	2.1 ± 0.1	2.3 ± 0.2	7.9 ± 6.7	2.3 ± 0.2	2.4 ± 0.2	6.8 ± 5.6
Ave. Total Change			10 ± 5.2			10.4 ± 4.3			5.5 ± 4.6

Values are means ± standard error. %= percent change over time.*Significantly different after training ($p<0.05$).

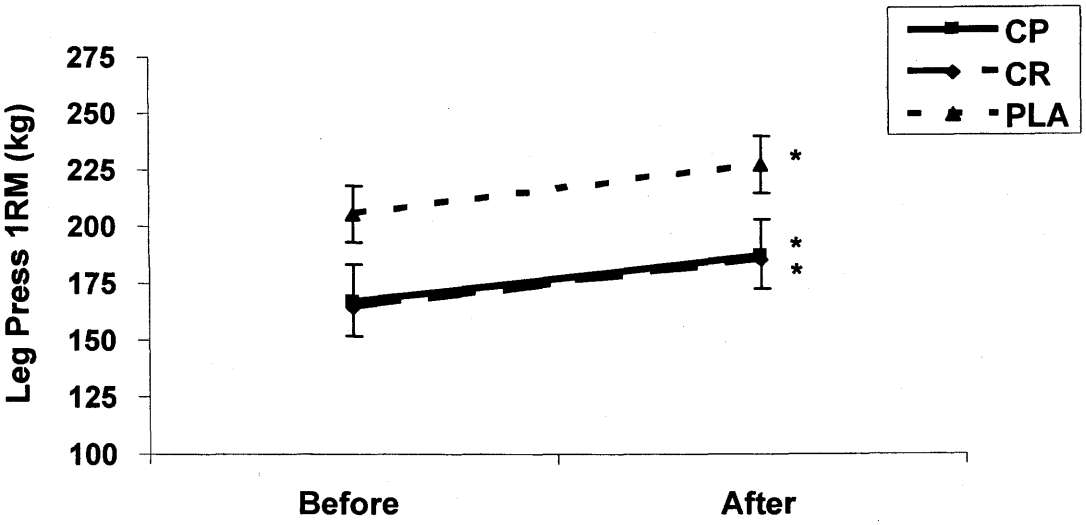

Figure 5.3 Graph of leg press strength (1-RM) before and after 10 weeks of nutritional supplementation and resistance training for CP (n=10), CR (n=13), and PLA (n=12) groups. Values are means ± standard error. * Indicates all groups increased strength with training ($p<0.05$), with no differences between groups.

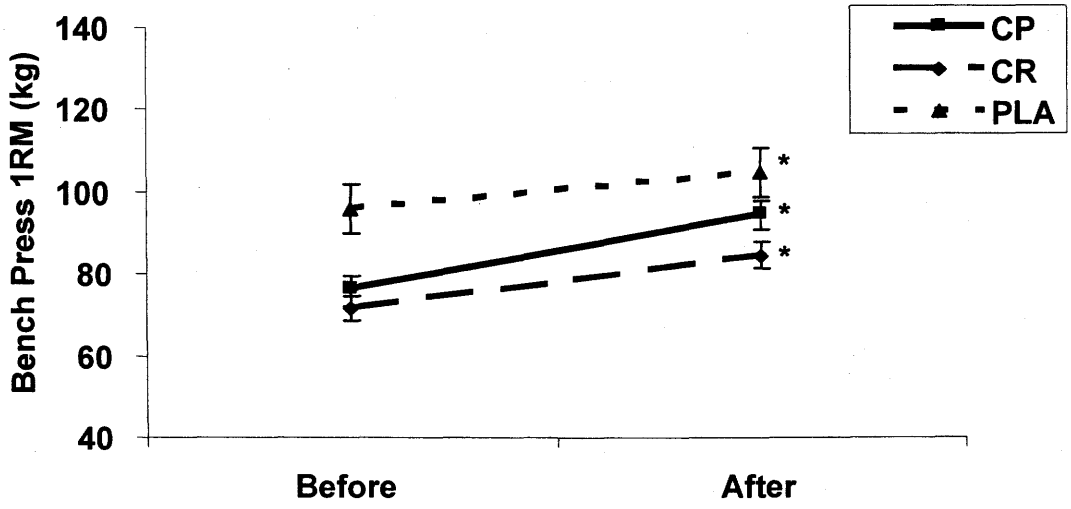

Figure 5.4 Graph of bench press strength (1-RM) before and after 10 weeks of nutritional supplementation and resistance training for CP (n=10), CR (n=11), and PLA (n=12) groups. Values are means ± standard error. * Indicates all groups increased strength with training ($p<0.05$), with no differences between groups.

IV. 3-methylhistidine

There were no differences in urinary 3-methylhistidine levels between groups over time. The CP group had values of 3.0 ± 0.3 and 2.2 ± 0.9 μmol/kg lean tissue mass, the CR group had values of 3.6 ± 1.2 and 2.1 ± 1.0 μmol/kg lean tissue mass, and the PLA group had values of 3.5 ± 0.5 and 3.4 ± 0.8 μmol/kg lean tissue mass before and after training respectively.

V. Training Volume

There were no differences in average training volume per session between the CP, CR, and PLA groups. The CP group had a mean average volume of 14,249 ± 1736 kg per training session, the CR group had an average volume of 14,748 ± 1298 kg per session, while the PLA group had an average volume of 15,145 ± 1156 kg per session.

VI. Diet

Dietary intake did not differ significantly between groups and did not differ significantly over the course of training (Table 5.4).

VII. Side Effects and Treatment Identification

There were no significant side effects reported within groups over time. Two subjects in the PLA group and one subject in the CR group reported increased muscle soreness and stiffness during weeks four through seven of the resistance training program. Correct treatment identification for the CP, CR and PLA groups were 10%, 8% and 17%, respectively.

Table 5.4. Total calories (kcal/day) and macronutrient (g/kg/day) content of CP, CR, and PLA groups for 3 days during the first and final week of nutritional supplementation and resistance training.

	CP (n=9)		CR (n=10)		PLA (n=9)	
	Week 1	Week 10	Week 1	Week 10	Week 1	Week 10
Kilocalories Per day	2543 ± 232	2368 ± 122	2558 ± 198	2513 ± 204	2162 ± 126	2195 ± 128
Carbohydrates	3.4 ± 1.0	3.0 ± 0.9	4.0 ± 1.1	3.5 ± 1.0	2.7 ± 1.0	2.52 ± 1.2
Fat	1.3 ± 0.6	1.2 ± 0.5	1.3 ± 0.8	1.3 ± 0.7	1.0 ± 0.4	1.1 ± 0.2
Protein	1.3 ± 0.5	1.2 ± 0.4	1.5 ± 0.9	1.5 ± 0.7	1.3 ± 0.5	1.2 ± 0.5

Values are means ± standard error. Data is based on the average for one day from 3-day food records.

VIII. Comparison of Older and Younger Males

The 17 older men who participated in both the second and third study for a total of 22 weeks of resistance training had lower muscle thicknesses (Table 5.5), lean tissue mass (58.4 ± 1.7 kg), leg press strength (168 ± 8 kg), and bench press strength (75 ± 4 kg) before training compared to the young reference groups (lean tissue mass = 64.3 ± 1.0 kg; leg press strength = 231 ± 7 kg; bench press strength = 121 ± 4 kg). After 22 weeks of resistance training, the older group no longer differed from the young reference groups for lean tissue mass (60.5 ± 1.9 kg), muscle thickness of the knee flexors (5.1 ± .2 cm), knee extensors (4.0 ± .2 cm), and ankle plantar flexors (4.2 ± .2cm) and bench press strength (107 ± 5 kg).

Table 5.5. Muscle thickness measurements (cm) for the elbow and knee flexors and extensors and ankle plantar flexors and dorsi flexors in older men before and after 22 weeks of resistance training compared to young men.

	Older (n=17)		Young (n=22)
Muscle Group	Before	After	
Elbow flexors	2.8 ± 0.1*	3.3 ± 0.1	3.2 ± 0.1
Elbow extensors	4.1 ± 0.1	4.9 ± 0.2**	4.0 ± 0.1
Knee flexors	4.7 ± 0.2*	5.1 ± 0.2	5.5 ± 0.1
Knee extensors	3.6 ± 0.2*	4.0 ± 0.2	4.2 ± 0.2
Ankle plantar flexors	3.2 ± 0.2*	4.2 ± 0.2	4.4 ± 0.3
Ankle dorsi flexors	2.2 ± 0.1*	2.5 ± 0.1	2.7 ± 0.1

Values are means ± standard error.* Muscle thickness in older men before training is less than for young men ($p<0.05$). ** Older men had greater muscle thickness after training compared to young men ($p<0.05$).

Discussion

Based on previous findings in young men (Burke et al., 2001), it was hypothesized that creatine and protein supplementation during resistance training would increase lean tissue mass and strength over creatine and resistance training alone. Results showed that subjects who supplemented with creatine experienced greater gains in body mass and muscle thickness, and the addition of protein to creatine increased lean tissue mass to a greater extent than resistance training alone.

Results of a significant increase in lean tissue mass from creatine and protein supplementation in older men is similar to findings in young men (Burke et al., 2001). Although the mechanism for the greater increase in lean tissue mass from creatine and protein supplementation is not fully known, possible factors include cellular hydration and protein kinetics. Creatine has the ability to regulate osmosis within the working cell and could potentially increase intracellular osmolarity (Balsom et al., 1995) and up-regulate myogenic transcription factors such as MRF-4 and myogenin (Balsom et al., 1995; Francaux & Poortmans, 1999; Kreider et al., 1998). It is well established that muscle protein synthesis proceeds in the presence of amino acids (Tipton, 2001). An increase in amino acid availability through additional dietary protein could potentially increase the combination of tRNA with an amino acid for translocation to ribosomal RNA (rRNA) (i.e. increased translational efficiency, see Figure 1.1) located on the ribosome organelle where protein synthesis occurs. Increased translational efficiency, in the presence of increased amino acid availability from protein supplementation, could potentially increase muscle protein synthesis with subsequent resistance training (Houston, 2001; Welle et al., 1994, 1995) leading to greater muscle mass and strength.

The up-regulation of transcription factors from creatine supplementation, combined with increased translational efficiency from protein supplementation, may explain the greater increase in lean tissue mass from creatine and protein supplementation during resistance training over training alone.

Results showed older men who supplemented with creatine (~8g) and protein (~30g) three times per week increased lean tissue mass by 3.2 kg or 5.6% compared to 4 kg or 6.5% experienced by young men who supplemented with a similar dose of creatine but a much higher dose of protein (~96 g) everyday during six weeks of resistance training (Burke et al. 2001). The increase in lean tissue mass in the older men who supplemented with creatine and protein was also greater than the increase observed in young men (2.0 kg or 3.2%) ingesting a commercial creatine (~ 20g) and protein (~ 67g) supplement (Phosphagain™, Experimental and Applied Sciences, Inc., Golden, CO) everyday during 4 weeks of resistance training (Kreider et al., 1996). A unique aspect of this study was that supplementation occurred only on training days. Justification for this protocol was based on a recent study by Chilibeck et al. (2004) who found supplementing with creatine only on training days was effective for increasing lean tissue mass and muscle thickness in young men. Also, it has been shown that protein requirements for the elderly are probably not increased above normal dietary levels on non-training days (Welle & Thornton, 1998). Therefore, it is only necessary to provide protein supplementation on training days to augment muscle protein synthesis (Esmarck et al., 2001). Based on these results, older men experience similar gains in lean tissue mass as their younger counterparts when supplementing with a lower dose of creatine and protein on training days during a resistance training program. This is important as compliance

may be higher and costs lower when smaller, less frequent doses of supplement are consumed.

Results of a significant increase in body mass and muscle thickness in subjects who supplemented with creatine support the findings of an increase in lean tissue mass. Creatine may up-regulate the expression of myogenic transcription factors and muscle specific-genes such as myosin heavy chain, thereby facilitating repetitive increases in muscle mass and strength (Willoughby & Rosene, 2003). However, the possibility of creatine resulting in intracellular water retention could have influenced these results. For example, Powers et al. (2003) observed a significant increase in net body water retention which correlated to an increase in body mass in young adults after supplementing with creatine (25g/day for the first 7 days; 5g/day for the remaining 21 days) for 28 days. However, lean tissue mass was not assessed in this study. Burke et al. (2003) reported an increase in intracellular water retention, as assessed by bioelectrical impedance, in subjects supplementing with creatine or placebo during eight weeks of resistance training However, the percentage of intracellular water to body weight did not differ in subjects supplementing with creatine; indicating that the increase in intracellular water retention paralleled an increase in dry muscle mass. Lean tissue mass was greater in subjects supplementing with creatine compared to placebo. These combined results of net water retention and lean tissue mass indicate that creatine supplementation during resistance training resulted in dry muscle mass accretion (Burke et al., 2003).

There was no greater effect from creatine supplementation on lean tissue mass over resistance training alone. These findings are in agreement with Bermon et al. (1998) who failed to observe an increase in lower limb muscle mass after 8 weeks of creatine

supplementation and resistance training in older men and women. Furthermore, creatine supplementation for up to one year failed to increase fat free mass in older men (Eijnde et al., 2003). However, Brose et al. (2003) found a significant increase in lean tissue mass (1.7 ± 1.2 kg) in older adults from creatine supplementation (5g/day) during 14 weeks of resistance training, and Gotshalk et al. (2002) found a significant increase in fat-free mass (2.2 kg) after seven days of creatine supplementation (0.3g/kg/day) in older men (59-77 y). In addition, Chrusch et al. (2001) reported a significant increase in lean tissue mass of 3.3 ± 1.6 kg following 12 weeks of creatine supplementation and resistance training in older men. These inconsistent results between studies warrant future research to determine the potential of creatine supplementation to influence high-energy phosphate metabolism, intracellular cell volume, gene expression, and muscle protein turnover in older individuals.

There was no significant increase in muscle strength from creatine and protein supplementation compared to placebo. Results of no greater increase in lower body strength from creatine and protein supplementation are similar to findings in young men (Burke et al., 2001). One possible explanation may involve the complexity of the exercise involved. Complex exercises, such as those involving movement at one or more joint (i.e. leg press), involves greater learning and coordination compared to the single joint exercises. Therefore, the observed increases in strength may have been due to learning, as all three groups increased strength over time.

Muscle protein degradation, as measured by urinary 3-methylhistidine excretion, was not affected by creatine supplementation in the present study. These results are in agreement with Tarnopolsky et al. (2004) who found no effect from 16 weeks of creatine

supplementation on urinary 3-methylhistidine excretion in young males suffering from Duschenne muscular dystrophy, a disease characterized by an increase in protein breakdown. Interestingly, short term creatine supplementation decreased whole body protein breakdown (~ plasma leucine rate of appearance) in young men (Parise et al., 2001). However, muscle represents 20-30% of whole body protein breakdown and no measure of myofibrillar protein breakdown was made in this study. Taken together, these results suggest that creatine supplementation reduces whole-body protein degradation but has little effect on myofibrillar protein degradation.

An important finding of the current study is that 22 weeks of resistance training in older men is sufficient to eliminate deficits in lean tissue mass, muscle thickness, and strength compared to young groups.

In addition to the limitations from the second study for methodology, individual nutritional status, and dietary assessment, there are several other limitations to this study. One limitation was the assumption that all subjects would respond similarily to creatine supplementation. Research suggests that a main determinant of any response from creatine supplementation is initial muscle creatine concentration which is quite variable. When replicating this experiment, it would be necessary to measure initial muscle creatine concentration using magnetic resonance spectroscopy or muscle biopsy to determine individual variation in muscle creatine accumulation in older individuals. Additionaly, without controlling for diet, high red meat and seafood intake may have saturated muscles with creatine thereby invalidating a creatine treatment effect. A second limitation was that no measure of water rentention or hydration status was made. Creatine supplementation has been shown to increase water retention and hydration status. An

increase in water retention may have altered lean tissue mass and muscle thickness results. Water retention and hydration status should be measured when duplicating this study to ensure that any increase in muscle mass from creatine supplementation is the result on an increase in contractile protein. A third limitation was that muscle fiber type compositon, muscle cross-sectional area, myogenic transcription factors, muscle protein synthesis, or hormonal properties were not measured in this study. Future research is needed to determine the effects of creatine supplementation on muscle protein kinetics, gene expression, and hormonal properties in males and females.

In summary, older men who supplement with creatine during a 10-week supervised resistance training program experience significant gains in body mass and muscle thickness and the addition of protein to creatine further increases lean tissue mass. Twenty-two weeks of resistance training is sufficient to eliminate deficits in lean tissue mass, muscle thickness, and strength compared to young reference groups. These findings have immediate application for research and health professionals for the design of optimal nutritional supplement and exercise interventions for older individuals. For example, emphasizing food products that contain dietary creatine and protein (i.e. red meat, seafood) on training days during a resistance training program may increase muscle mass to a greater extent than resistance training alone.

Chapter 3

General Discussion

Discussion

Sarcopenia, defined as the age-related loss of muscle, is a serious health concern (Evans, 1995). Contributing factors to the age-related loss of muscle include physical inactivity and undernutrition (Tipton, 2001). It is estimated that physical inactivity results in a significant loss of muscle mass in older individuals. Although resistance training has a positive effect on muscle mass in older individuals (Chrusch et al., 2001), muscle loss is still evident in older individuals who perform regular resistance type activities (Hameed et al., 2002; Starling et al., 1999; Trappe, 2001). Therefore, other factors such as nutrition must contribute to the age-related loss in muscle.

The loss of muscle mass and strength appears to be greater in lower body muscle groups compared to upper body muscle groups in older individuals. For example, in comparing muscle size and strength of the elbow and knee flexors and extensors between young and older men and women, Lynch et al. (1999) found arm and leg muscle size and strength decreased with age but the decrease was significantly greater in the knee flexors and extensors compared to the elbow flexors and extensors. Although the mechanism for the greater loss of muscle mass and strength in lower body muscle groups with age is not fully known, it is theorized that a reduction in muscle fiber size and number (Trappe, 2001), particularly among type II fibers, is involved (Larsson et al., 2001). This fast-to-slow transition may have a negative effect on the ability to generate force at higher limb speeds (Jubrias et al., 1997). Therefore, it is important to determine if strength at faster velocities (i.e. power) is reduced similarly between upper and lower body muscle groups for the design of optimal exercise interventions which include power-type training. If the deficits in strength at faster velocities are greater than strength at slower velocities, this

may indicate that resistance training programs for older individuals should emphasize power in addition to strength.

In the first study, differences in muscle mass, strength, and power between upper and lower body muscle groups between young and older men were determined. Most studies comparing deficits in upper and lower body muscle groups in older individuals have made separate comparisons between upper and lower body measures which only indirectly answers the question of which muscles are most affected by the aging process. A comparison of the relative deficits between muscle groups within older individuals was needed to determine which muscle groups are most affected by age. It was hypothesized that muscle mass, strength, and power of lower body muscle groups would be more affected than upper body muscle groups. Also, the loss of muscle mass with age is primarily due to a reduction in size and number of type II muscle fibers whereas the size and number of type I muscle fibers are maintained (Jubrias et al., 1997; Klitgaard et al., 1990; Lexell, 1993). Therefore, strength and power at faster velocities, which primarily involves fast twitch muscle fibers, would be more affected than strength at slower velocities.

Results showed that muscle thickness, torque, and power in the lower body were more affected by age than upper body measures. Older men had lower torque values relative to young men for knee flexion and extension and ankle plantar flexion when compared to upper-body measures. Torque expressed relative to muscle mass (i.e. normalized torque) was lowest for the knee flexors and extensors at the fast velocity for the older group when expressed relative to the young group. These results are in agreement with Jubrias et al. (1997) and Overend et al. (1992a) who reported a

significant decrease in knee flexor and extensor normalized torque at fast velocities in older individuals. Similar to torque measurements, relative deficits for power were greatest in the lower body (specifically the knee flexors and ankle plantar flexors at fast velocities) of the older group.

When different muscle groups were compared within older men relative to those in young men, torque was lower at the fast velocity for the knee flexors and extensors and ankle plantar flexors compared to the slow velocity for the elbow flexors and extensors. In other words, for the older men, the greatest torque deficits relative to the young men were for the knee flexors and extensors and ankle plantar flexors at the fast velocity. In addition, power at the fast velocity was more affected in the older group than power at the slow velocity. Based on these findings, lower body muscle mass, and torque and power are affected more by aging than upper body measures at fast velocities. These findings have immediate application for health and research professionals as it suggests that resistance training programs should include power training, in addition to strength, to maximize muscle mass and strength in older individuals.

Resistance training increases muscle mass in healthy older individuals; however, it is unknown if the timing of protein ingestion, either immediately before or immediately after resistance training sessions, is of greater benefit than training alone in older men and whether this intervention eliminates age-related deficits in muscle mass and strength compared to young men. Muscle loss may be the result of a decrease in protein synthesis, an increase in protein breakdown, or both (Tipton et al., 2001). Amino acid ingestion following exercise increases protein synthesis and attenuates protein breakdown (Tipton et al., 2001; Wolfe, 2001). In recent years, the question as to when is the optimal time to

consume dietary protein during resistance training has gained considerable attention. In young athletes who ingested an amino acid solution immediately before and immediately after an acute bout of heavy resistance training, greater gains in muscle protein synthesis were observed when the amino acid solution was consumed before exercise (Tipton et al., 2001). However, muscle mass and strength were not assessed in this study. On the other hand, older males who ingested protein immediately after resistance training experienced significant gains in muscle size and strength compared to waiting two hours post-exercise to supplement with protein (Esmarck et al., 2001). Therefore, the timing of protein ingestion appears crucial for creating an anabolic environment for muscle growth (Tipton et al., 2001), with protein supplementation immediately before (Andersen et al., 2005; Tipton et al., 2001) and immediately after resistance training (Andersen et al., 2005; Esmarck et al., 2001) appearing optimal. The second study was designed to determine if advantages exist from protein supplementation immediately before compared to immediately after resistance training in healthy older men. Based on previous research showing that amino acid ingestion before exercise resulted in greater gains in muscle protein synthesis compared to amino acid ingestion after exercise (Tipton et al., 2001), it was hypothesized that protein supplementation before exercise would be more beneficial than protein supplementation after exercise. However, results showed that the timing of protein supplementation, either before or after resistance training, had no effect on lean tissue mass, muscle thickness, or strength; with the exception that protein supplementation before training sessions resulted in a greater increase in knee extensor muscle thickness over placebo. Despite the lack of benefit from protein supplementation, a unique and important finding of this study was that 12 weeks of resistance training was

sufficient for eliminating deficits in elbow flexor and ankle dorsi flexor muscle thickness and leg press strength compared to young men. However, at the conclusion of resistance training, the older group still had significantly lower lean tissue mass, bench press strength, and muscle thickness of the knee flexors and extensors and ankle plantar flexors compared to young men. Despite the deficits in these lower body muscle groups after 12 weeks of resistance training, leg press strength in the older men was comparable to a young reference group; suggesting that the improvement in leg press strength with training may be caused by neural, rather than muscular, adaptations (Chilibeck et al., 1998a). Complex exercises, such as those involving movement at one or more joint (i.e. leg press), may involve a longer initial neural adaptation compared to single-joint exercises (i.e. arm curl), resulting in delayed muscle hypertrophy (Chilibeck et al., 1998a). Increasing the duration (i.e. > 12 weeks) of resistance training is required to eliminate deficits in lean tissue mass, strength, and muscle thickness between young and older men.

The purpose of the third study was to determine the effects of creatine and protein supplementation during 10 weeks of resistance training on muscle mass, strength, and muscle protein degradation in older men. In addition, these results, combined with the results of 17 subjects from the second study, would determine if 22 weeks of resistance training could eliminate deficits in lean tissue mass, muscle thickness, and strength between young and older men. The rate of muscle protein synthesis is elevated with oral ingestion of amino acids (Tipton et al., 2001), while ingestion of creatine has the potential to increase protein synthesis (Willoughby & Rosene, 2003) or attenuate the rate of protein degradation (Parise et al., 2001). It was therefore hypothesized that creatine,

combined with protein supplementation, would be more ergogenic than creatine or resistance training alone. Results showed that creatine supplementation increased body mass and muscle thickness and the addition of protein to creatine further increased lean tissue mass. Results of 17 subjects who participated in the second and third study suggest that 22 weeks of resistance training in older men is sufficient to eliminate deficits in lean tissue mass, muscle thickness, and strength compared to young men.

The overall purpose of this thesis was to determine whether nutritional supplementation combined with resistance training could maximize muscle accretion and strength in older men and whether these interventions could eliminate deficits in muscle mass and strength compared to young men. In the first study, it was determined that lower body measures of muscle mass, strength, and power, especially at fast velocities, is reduced more than upper body measures in older men. These findings suggest that exercise programs for older individuals should include power and strength training, especially in lower body muscle groups, to maximize muscle mass and strength. In the second study, it was found that the timing of protein supplementation, either before or after resistance training sessions, had no effect on lean tissue mass, muscle thickness, or strength. Despite these results, an important finding of this study was that 12 weeks of resistance training was sufficient for eliminating deficits in elbow flexor and ankle dorsi flexor muscle thickness and leg press strength compared to young men. However, the older group still had significantly lower lean tissue mass, bench press strength, and muscle thickness of the knee flexors and extensors and ankle plantar flexors compared to young men; suggesting that a longer intervention was required. In the third study, it was determined that subjects who supplemented with creatine experienced greater gains in

total muscle thickness and the addition of protein to creatine significantly increased lean tissue mass compared to placebo. Following 22 weeks of training, deficits in muscle mass and strength were no longer evident compared to the young.

Based on this series of studies, muscle mass, strength, and power is significantly reduced in older compared to younger men. Twenty-two weeks of resistance training in older men is sufficient to eliminate deficits in muscle mass and strength compared to young men. The combination of creatine and protein had a greater effect on muscle mass over creatine or protein alone. These results have immediate application for health and research professionals for the design of nutritional supplementation and resistance training interventions which emphasize dietary creatine and protein intake on training days during a resistance training program which includes power and strength exercises of lower body muscle groups.

Chapter 4

General Conclusions

Conclusions

Based on the results of this series of studies, there are a number of conclusions that can be drawn regarding nutritional supplementation and resistance training in older men. In the first study, it was determined that lower body muscle mass, strength, and power is affected more by aging than upper body measures. Strength and power at fast velocities, specifically in the lower body, are affected more by aging than measurements at slow velocities. These findings suggest that older men should participate in power and strength training, especially in lower body muscle groups, to maximize muscle mass and strength.

In the second study, protein supplementation before and after resistance training sessions for 12 weeks had no greater effect on muscle mass and strength over resistance training alone in older men; except that knee extensor muscle thickness was greater in subjects who consumed protein before training sessions compared to placebo. Despite the lack of benefit from protein supplementation, 12 weeks of resistance training was sufficient for eliminating deficits in elbow flexor and ankle dorsi flexor muscle thickness and leg press strength compared to young men. However, at the conclusion of resistance training, the older group still had significantly lower lean tissue mass, bench press strength, and muscle thickness of the knee flexors and extensors and ankle plantar flexors compared to young men. These deficits may be too large to overcome in older individuals with 12 weeks of resistance training and a longer intervention (i.e. > 12 weeks) is required.

In the third study, creatine supplementation during 10 weeks of resistance training in older men increased body mass and muscle thickness; and the addition of protein to

creatine further augmented lean tissue mass in older men. These findings have immediate application for health and research professionals for the design of optimal nutritional supplement and exercise interventions for older individuals. For example, emphasizing food products that contain dietary creatine and protein (i.e. red meat, seafood) on training days during a resistance training program will increase muscle mass to a greater extent than resistance training alone. In addition, these results, combined with the results from 17 subjects from the second study, suggest that 22 weeks of resistance training in older men is sufficient to eliminate deficits in lean tissue mass, muscle thickness, and strength compared to young men.

In summary, muscle mass, strength, and power is significantly reduced in older compared to younger men. Twenty-two weeks of resistance training in older men is sufficient to eliminate these deficits in muscle mass and strength compared to young men. The combination of creatine and protein had a greater effect on muscle mass over creatine or protein alone. These results have immediate application for health and research professionals for the design of nutritional supplementation and resistance training interventions which emphasize dietary creatine and protein intake on training days during a resistance training program which includes power and strength exercises of lower body muscle groups.

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APPENDIX A

Ethics Approval

Consent Forms

PAR-Q

Certificate of Approval

PRINCIPAL INVESTIGATOR	DEPARTMENT	BMC #
P. Chilibeck	Kinesiology	03-996

INSTITUTION (S) WHERE RESEARCH WILL BE CARRIED OUT

University of Saskatchewan

Saskatoon SK

SPONSORING AGENCIES

UNFUNDED

TITLE:

Protocol Title: The Influence of Age on Size and Strength of Specific Muscle Groups

ORIGINAL APPROVAL DATE	CURRENT EXPIRY DATE
02-Jun-2003	01-Jun-2004

CERTIFICATION UPDATE	APPROVED ON
Revised Consent Form (rec'd 26 Sept 03)	02-Oct-2003

CERTIFICATION

The University of Saskatchewan Biomedical Research Ethics Board (Bio- REB) has reviewed the above-named research project including the protocol and consent form, where applicable. The proposal was found to be acceptable on ethical grounds. The principal investigator has the responsibility of ensuring that the authorized research is carried out according to governing law. This Approval is valid for the above time period provided there is no change in experimental protocol or in the consent process.

ONGOING REVIEW REQUIREMENT(S) / REB ATTESTATION

In order to receive annual renewal, a status report must be submitted to the Chair for Committee consideration within one month of the current expiry date each year the study remains open, and upon study completion. Please refer to the following website for further instructions: http://www.usask.ca/research/ethics.shtml. In respect to clinical trials, the University of Saskatchewan Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 of the Food and Drug Regulations and carries out its functions in a manner consistent with Good Clinical Practices. This approval and the views of this REB have been documented in writing.

APPROVED

Barry D. McLennan, Ph.D., Chair
University of Saskatchewan
Biomedical Research Ethics Board

Certificate of Approval

PRINCIPAL INVESTIGATOR	DEPARTMENT	BMC #
Philip D. Chilibeck	Kinesiology	03-874

INSTITUTION (S) WHERE RESEARCH WILL BE CARRIED OUT

College of Kinesiology
105 Gymnasium Place
Saskatoon SK S7N 5C2

SPONSORING AGENCIES

AMERICAN COLLEGE OF SPORTS MEDICINE FOUNDATION

TITLE:

Timing of Protein Ingestion Combined with Resistance Training in Older Men

ORIGINAL APPROVAL DATE	CURRENT EXPIRY DATE	APPROVAL OF
03-Mar-2003	01-Mar-2004	Protocol as submitted Consent Form as submitted

CERTIFICATION

The University of Saskatchewan Biomedical Research Ethics Board has reviewed the above-named research project at a full-board meeting (any research classified as minimal risk is reviewed through the expedited review process). The proposal was found to be acceptable on ethical grounds. The principal investigator has the responsibility for any other administrative or regulatory approvals that may pertain to this research project, and for ensuring that the authorized research is carried out according to governing law. This Approval is valid for the above time period provided there is no change in experimental protocol or in the consent process.

ONGOING REVIEW REQUIREMENTS/REB ATTESTATION

In order to receive annual renewal, a status report must be submitted to the Chair for Committee consideration within one month of the current expiry date each year the study remains open, and upon study completion. Please refer to the following website for further instructions: http://www.usask.ca/research/ethics.shtml. In respect to clinical trials, the University of Saskatchewan Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 of the Food and Drug Regulations and carries out its functions in a manner consistent with Good Clinical Practices. This approval and the views of this REB have been documented in writing.

APPROVED.

Barry D. McLennan, Ph.D., Chair
University of Saskatchewan
Biomedical Research Ethics Board

University of Saskatchewan
Biomedical Research Ethics Board (Bio-REB)

03-Mar-2003

Certificate of Approval

PRINCIPAL INVESTIGATOR	DEPARTMENT	BMC #
Philip D. Chilibeck	Kinesiology	03-875

INSTITUTION (S) WHERE RESEARCH WILL BE CARRIED OUT

College of Kinesiology
105 Gymnasium Place
Saskatoon SK S7N 5C2

SPONSORING AGENCIES

AMERICAN COLLEGE OF SPORTS MEDICINE FOUNDATION

TITLE:

Effect of Protein and Creatine Combined with Resistance Training in Older Men

ORIGINAL APPROVAL DATE	CURRENT EXPIRY DATE	APPROVAL OF
03-Mar-2003	01-Mar-2004	Protocol as submitted Consent Form as submitted

CERTIFICATION

The University of Saskatchewan Biomedical Research Ethics Board has reviewed the above-named research project at a full-board meeting (any research classified as minimal risk is reviewed through the expedited review process). The proposal was found to be acceptable on ethical grounds. The principal investigator has the responsibility for any other administrative or regulatory approvals that may pertain to this research project, and for ensuring that the authorized research is carried out according to governing law. This Approval is valid for the above time period provided there is no change in experimental protocol or in the consent process.

ONGOING REVIEW REQUIREMENTS/REB ATTESTATION

In order to receive annual renewal, a status report must be submitted to the Chair for Committee consideration within one month of the current expiry date each year the study remains open, and upon study completion. Please refer to the following website for further instructions: http://www.usask.ca/research/ethics.shtml. In respect to clinical trials, the University of Saskatchewan Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 of the Food and Drug Regulations and carries out its functions in a manner consistent with Good Clinical Practices. This approval and the views of this REB have been documented in writing.

APPROVED.

Barry D. McLennan, Ph.D., Chair
University of Saskatchewan
Biomedical Research Ethics Board

University of Saskatchewan
Biomedical Research Ethics Board (Bio-REB)

22-Jul-2004

Certificate of Approval

PRINCIPAL INVESTIGATOR	DEPARTMENT	Bio #
Philip D. Chilibeck	Kinesiology	04-154

INSTITUTION (S) WHERE RESEARCH WILL BE CARRIED OUT

University of Saskatchewan

Saskatoon SK

SUB-INVESTIGATOR(S)

Darren Candow

SPONSORING AGENCIES

UNIVERSITY OF SASKATCHEWAN

TITLE:

Validation of Air-Displacement Plethysmography for Determining Body Composition in Older Indviduals

ORIGINAL APPROVAL DATE	CURRENT EXPIRY DATE	APPROVAL OF
22-Jul-2004	01-Jul-2005	Protocol as submitted Revised Consent Form (17 July 04)

CERTIFICATION

The University of Saskatchewan Biomedical Research Ethics Board has reviewed the above-named research project at a full-board meeting (any research classified as minimal risk is reviewed through the expedited review process). The proposal was found to be acceptable on ethical grounds. The principal investigator has the responsibility for any other administrative or regulatory approvals that may pertain to this research project, and for ensuring that the authorized research is carried out according to governing law. This Approval is valid for the above time period. Any amendments or modifications to the approved protocol must be submitted for REB approval review and approval prior to implementation.

ONGOING REVIEW REQUIREMENTS/REB ATTESTATION

In order to receive annual renewal, a status report must be submitted to the Chair for Board consideration within one month of the current expiry date each year the study remains open, and upon study completion. Please refer to the following website for further instructions: http://www.usask.ca/research/ethics.shtml. In respect to clinical trials, the University of Saskatchewan Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 of the Food and Drug Regulations and carries out its functions in a manner consistent with Good Clinical Practices. This approval and the views of this REB have been documented in writing.

APPROVED.

Barry D. McLennan, Ph.D., Chair
University of Saskatchewan
Biomedical Research Ethics Board (Bio-REB)

Consent Form

Title of the study:
The influence of age on size and strength of specific muscle groups

Names of Researchers: Philip D. Chilibeck, Ph.D., College of Kinesiology, University of Saskatchewan, phone: 966-1072, Darren Candow, M.Sc. (student researcher), College of Kinesiology, University of Saskatchewan.

Purpose of the study: The purpose of the study is to compare the size and strength of six different muscle groups between older and younger men.

Possible benefits of the study: You will receive an evaluation of your muscular strength and size and body composition. The results of the study can be useful to others for designing future strength training programs. These benefits are not guaranteed.

Procedures: You will initially be given a questionnaire (the physical activity readiness questionnaire) which assesses whether you are at a health risk from participating in exercise testing. If there are possible health risks, we will require you to have permission from your family physician to participate in the study.

The strength of six of your muscle groups (the front and back of your upper and lower leg and upper arm) will be assessed using a specialized device called an isokinetic dynamometer. This dynamometer is set at different speeds. You will be required to exert maximal force at the speed set on the dynamometer (i.e. a fast speed and a slow speed). This procedure will take one hour.

The size of the same muscle groups will be assessed by measuring the thickness of these muscles with an ultrasound probe. This procedure will take 30-45 minutes.

Body composition will be assessed by a technique called "air displacement plythesmography". This measurement requires that you sit still in a chamber. Your body density will be determined by the amount of air displaced from the chamber. Your body density is then used to estimate your lean tissue and fat mass. This measurement takes about 10 minutes.

To test the reliability of these measurements you will be asked to return to our lab after one week to have these measurements re-assessed.

Foreseeable risks, side effects or discomfort:
The strength testing may result in muscle pulls or strains. You will be given a proper warm-up and this will minimize this risk. You may feel claustrophobic inside the body composition chamber, but there is a window in the chamber, through which you can look. This will minimize this risk. There may be unknown or unforeseen risks associated with the strength testing.

Alternatives to this study:
You do not have to participate in this study to have an assessment of your muscular strength and size or body composition. You could make an appointment with the College of Kinesiology to have these measurements taken without participating in this study.

You are free to withdraw from this study at any time and this withdrawal will not affect your academic status or access to health care or other services.

Precautions will be taken to protect your anonymity during the study. All data collected will be stored in a locked office in the College of Kinesiology. The results of the study will be published in a student's thesis and a journal article, but only aggregate data will be reported and you will be unidentifiable.

If you have questions concerning the study you can contact Dr. Philip Chilibeck at 966-1072 or 343-6577 or Darren Candow (student researcher) at 227-0513.

If you have questions about your rights as a research subject, you can contact the Office of Research Services at the University of Saskatchewan at 966-4053.

We will advise you of any new information that will have a bearing on your decision to continue in the study.

We will advise you of your own results and the overall results of the study at the completion of the study.

By signing below, you acknowledge that the study and contents of the consent have been explained to you and that you understand the contents, and have received a copy of the consent for your own records.

Participant's Signature: _____ Date: _____

Researcher's Signature: _____ Date: _____

Witness' Signature: _____ Date: _____

Consent Form

Title of the study: Timing of protein ingestion combined with resistance training in older men

Names of Researchers: Philip D. Chilibeck, Ph.D., College of Kinesiology, University of Saskatchewan, phone: 966-1072, Darren Candow, M.Sc. (student researcher), College of Kinesiology, University of Saskatchewan, phone: 966-1099.

Purpose of the study: The purpose of the study is to compare the effects of protein ingestion immediately before training sessions to protein ingestion immediately following training sessions, on muscle mass and strength, during 12 weeks of strength training.

Possible benefits of the study: You might increase your muscle mass and strength by participating in this study. These benefits are not guaranteed.

Procedures: You will initially be given a questionnaire (the physical activity readiness questionnaire) which assesses whether you are at a health risk from participating in exercise training. If there are possible health risks, we will require you to have permission from your family physician to participate in the study.

You will be randomized (by chance) into one of three groups: Group 1 will receive 0.3 g / kg body mass of protein supplementation immediately before exercise training sessions (three times per week for 12 weeks) and placebo (sucrose) immediately after training sessions. Group 2 will receive placebo immediately before exercise training sessions and 0.3 g / kg body mass of protein supplementation immediately after training sessions. Group 3 will receive placebo before and after training sessions. The protein will be mixed with sucrose to mask its taste. Neither you nor the researchers will know which group you are in until the end of the study.

All groups will participate in 14 weeks of strength training. The first two weeks will serve as a familiarization period, during which you will be taught how to use the exercise equipment. Supplementation with protein or placebo will be given after the first two weeks for the remainder of the study. Training will be 3 days per week and each training session will last for 1-1.5 hours and will require you to perform 13 different exercises designed to train all your major muscle groups.

Your muscular strength will be measured for two different exercises at the start of the study, at 2 weeks, and after the end of the training (14 weeks).

Your body composition (lean tissue and fat mass) will be measured twice: after the first 2 weeks of training (familiarization period) and at the end of training (14 weeks). Body composition will be assessed by a technique called "air displacement plythesmography". This measurement requires that you sit still in a chamber. Your body density will be

determined by the amount of air displaced from the chamber. Your body density is then used to estimate your lean tissue and fat mass. This measurement takes about 10 minutes.

Your muscle thickness will be measured twice: after the first 2 weeks of training (familiarization period) and at the end of training (14 weeks). Muscle thickness will be measured using ultrasound by placing a gel over your skin and applying a probe to your skin surface. Muscle thickness will be measured at the front and back of your upper arms, thigh, and lower leg. This procedure will take 30-45 minutes.

You will be required to collect urine for 24 hours at two time points: after the first 2 weeks of training (familiarization period) and at the end of training (14 weeks). Prior to this urine collection, you will have to consume a meat-free diet for 3 days. The purpose of the urine collection is to measure a marker of muscle protein breakdown. Meat consumption affects the level of this marker; therefore, three days without meat is required.

You will be required to record all the food you eat in a food diary, for three days, at the start and end of the 14 week study.

Foreseeable risks, side effects or discomfort:
The exercise training and strength testing may result in muscle pulls or strains. You will be given a proper warm-up prior to exercising and this will minimize this risk. You may feel claustrophobic inside the body composition chamber, but there is a window in the chamber, through which you can look. This will minimize this risk. There may be unknown or unforeseen risks associated with the training or supplementation.

Alternatives to this study:
You do not have to participate in this study to increase your muscle mass and strength. You can perform alternative exercises (i.e. free-body exercises such as push-ups or chin-ups instead of the exercise program in this study). You could also increase your protein consumption from your diet by consuming more high-protein sources, such as meats, milk, and eggs instead of receiving the protein supplementation in this study.

You are free to withdraw from this study at any time and this withdrawal will not affect your access to health care or other services.

Precautions will be taken to protect your anonymity during the study. All data collected will be stored in a locked office in the College of Kinesiology. The results of the study will be published in a student's thesis and a journal article, but only aggregate data will be reported and you will be unidentifiable.

If you have questions concerning the study you can contact Dr. Philip Chilibeck at 966-1072 or 343-6577 or Darren Candow (student researcher) at 966-1099.

If you have questions about your rights as a research subject, you can contact the Office of Research Services at the University of Saskatchewan at 966-4053.

Consent Form

Title of the study: Effect of protein and creatine combined with resistance training in older men.

Names of Researchers: Philip D. Chilibeck, Ph.D., College of Kinesiology, University of Saskatchewan, phone: 966-1072, Darren Candow, M.Sc. (student researcher), College of Kinesiology, University of Saskatchewan, phone: 966-1099, fax: 966-6464

Purpose of the study: The purpose of the study is to compare the effects of protein combined with creatine ingestion to creatine ingestion alone on muscle mass and strength, during 10 weeks of strength training. Creatine monohydrate is a substance found in meat products which, when given in higher amounts than usually consumed in the diet, has been shown to increase lean tissue mass.

Possible benefits of the study: You might increase your muscle mass and strength by participating in this study. These benefits are not guaranteed.

Procedures: You will initially be given a questionnaire (the physical activity readiness questionnaire) which assesses whether you are at a health risk from participating in exercise training. If there are possible health risks, we will require you to have permission from your family physician to participate in the study.

You will be randomized (by chance) into one of three groups: Group 1 will receive 0.3 g / kg body mass of protein supplementation and 0.1 g / kg body mass of creatine monohydrate (three times per week for 10 weeks). Group 2 will only receive the 0.1 g/kg body mass creatine monohydrate (three times per week for 10 weeks). Group 3 will receive placebo (sucrose; three times per week for 10 weeks). The creatine monohydrate and protein will be mixed with sucrose to mask their taste. Neither you nor the researchers will know which group you are in until the end of the study.

All groups will participate in 12 weeks of strength training. The first two weeks will serve as a familiarization period, during which you will be taught how to use the exercise equipment. Supplementation with protein and creatine, creatine, or placebo will be given after the first two weeks for the remainder of the study, on the days that you train. Training will be 3 days per week and each training session will last for 1-1.5 hours and will require you to perform 9 different exercises designed to train all your major muscle groups.

Your muscular strength will be measured for two different exercises after the first 2 weeks of training (familiarization period) and at the end of the training (12 weeks).

Your body composition (lean tissue and fat mass) will be measured twice: after the first 2 weeks of training (familiarization period) and at the end of training (12 weeks). Body composition will be assessed by a technique called "air displacement plythesmography". This measurement requires that you sit still in a chamber. Your body density will be

determined by the amount of air displaced from the chamber. Your body density is then used to estimate your lean tissue and fat mass. This measurement takes about 10 minutes.

Your muscle thickness will be measured twice: after the first 2 weeks of training (familiarization period) and at the end of training (12 weeks). Muscle thickness will be measured using ultrasound by placing a gel over your skin and applying a probe to your skin surface. Muscle thickness will be measured at the front and back of your upper arms, thigh, and lower leg. This procedure will take 30-45 minutes.

You will be required to collect urine for 24 hours at two time points: after the first 2 weeks of training (familiarization period) and at the end of training (12 weeks). Prior to this urine collection, you will have to consume a meat-free diet for 3 days. The purpose of the urine collection is to measure a marker of muscle protein breakdown. Meat consumption affects the level of this marker; therefore, three days without meat is required.

You will be required to record all the food you eat in a food diary, for three days, at the start and end of the 12 week study.

Foreseeable risks, side effects or discomfort:
The exercise training and strength testing may result in muscle pulls or strains. You will be given a proper warm-up prior to exercising and this will minimize this risk. You may feel claustrophobic inside the body composition chamber, but there is a window in the chamber, through which you can look. This will minimize this risk.

Creatine supplementation has been shown to be associated with minimal side effects, especially with the low dose given in this study. There have been anecdotal reports of increased muscle cramping or muscle pulls during creatine supplementation, but when this is compared to subjects receiving placebo, there is no differences in rates of occurrence of muscle cramping or pulls. Creatine supplementation has been shown, on two occasions, to worsen kidney function in individuals who already had kidney disease. If you have any problems with kidney function you should not participate in this study.

There may be unknown or unforeseen risks associated with the training or the protein and creatine supplementation.

Alternatives to this study:
You do not have to participate in this study to increase your muscle mass and strength. You can perform alternative exercises (i.e. free-body exercises such as push-ups or chin-ups instead of the exercise program in this study). You could also increase your protein and creatine consumption from your diet by consuming more high-protein sources, such as meats, milk, and eggs instead of receiving the protein and creatine supplementation in this study.

You are free to withdraw from this study at any time and this withdrawal will not affect your access to health care or other services.

Precautions will be taken to protect your anonymity during the study. All data collected will be stored in a locked office in the College of Kinesiology. The results of the study will be published in a student's thesis and a journal article, but only aggregate data will be reported and you will be unidentifiable.

If you have questions concerning the study you can contact Dr. Philip Chilibeck at 966-1072 or 343-6577 or Darren Candow (student researcher) at 966-1099.

If you have questions about your rights as a research subject, you can contact the Office of Research Services at the University of Saskatchewan at 966-4053.

We will advise you of any new information that will have a bearing on your decision to continue in the study.

We will advise you of your own results and the overall results of the study at the completion of the study.

By signing below, you acknowledge that the study and contents of the consent have been explained to you and that you understand the contents, and have received a copy of the consent for your own records.

Participant's Signature:_____ Date: _____

Researcher's Signature:_____ Date: _____

Witness' Signature: _____ Date: _____

Consent Form

Title: Validation of air-displacement plethysmography for determining body composition in older individuals

Names of Researchers: Philip D. Chilibeck, Ph.D., College of Kinesiology, University of Saskatchewan, phone: 966-1072 or 343-6577, Darren Candow, M.Sc. (student researcher), College of Kinesiology, University of Saskatchewan, phone: 966-1099 or 249-4853

Purpose of the study: The purpose of the study is to validate lean tissue and fat mass measurements by air displacement plethysmography by comparing these to lean tissue and fat mass measurements by dual energy X-ray absorptiometry.

Possible benefits of the study: You will receive information on your body composition. These benefits are not guaranteed.

Procedures: In addition to the body composition measurement by air displacement plesthysmography, for this study we are also assessing body composition by dual energy X-ray absorptiometry. This involves lying still on a table for approximately 10 minutes while your body composition is assessed.

Foreseeable risks, side effects or discomfort: There is radiation exposure from the dual energy X-ray absorptiometry but this is considered minimal.

There may be unforeseen and unknown risks during the study, or after the study has been completed.

Alternatives to this study:
Your body composition can be assessed by alternative methods, such as by skin-fold calipers.

Research-Related Injury: There will be no cost to you for participation in this study. You will not be charged for any research procedures. In the event you become ill or injured as a result of participating in this study, necessary medical treatment will be made available at no additional cost to you.

Confidentiality: Precautions will be taken to protect your anonymity during the study. All data collected will be stored in a locked office in the College of Kinesiology. The results of the study will be published in a student's thesis and a journal article, but only aggregate data will be reported and you will be unidentifiable.

If you have questions concerning the study you can contact Dr. Philip Chilibeck at 966-1072 or 343-6577 or Darren Candow (student researcher) at 966-1099 or 249-4853.

If you have questions about your rights as a research subject, you can contact the Office of Research Services at the University of Saskatchewan at 966-4053.

We will advise you of any new information that will have a bearing on your decision to continue in the study.

You are free to withdraw from this study at any time and this withdrawal will not affect your access to health care or other services.

By signing below, you acknowledge that the study and contents of the consent have been explained to you and that you understand the contents, and have received a copy of the consent for your own records.

Participant's Signature:_____ Date: _____

Researcher's Signature:_____ Date: _____

Witness' Signature: _____ Date: _____

APPENDIX B

Physical Activity Questionnaire

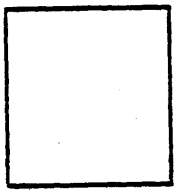
Please do not include activities performed from our strength training program.

Godin Leisure-Time Exercise Questionnaire

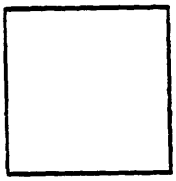
Considering a 7-Day period (one week), how many times on the average do you do the following kinds of exercise for more than 15 minutes during your free time (write on each line the appropriate number).

Times Per Week

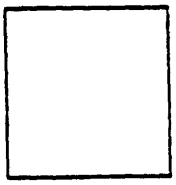
A) Strenuous Exercise
(Heart Beats Rapidly)
(i.e. Running, jogging, squash, roller skating, vigorous swimming, vigorous long distance bicycling)



B) Moderate Exercise
(Not Exhausting)
(i.e. fast walking, tennis, easy bicycling, badminton, easy swimming, popular and folk dancing)



Mild Exercise
(Minimal Effort)
(i.e. yoga archery, gardening, house work, bowling, horseshoes, golf, easy walking)



APPENDIX C

Creatine Questionnaire

Retrospective Creatine Side Effects Assessment

Circle yes or no for ALL of the side effects listed. Answer yes for each side effect that you experienced and attribute to your participation in this study. Answer the *Magnitude Scale* and the *Occurrence Scale* sections if you circled yes to a side effect.

Side-Effects (Page 1 of 2)			Magnitude Scale To what extent?					Occurrence Scale Which WEEK of the supplementation period did it begin?										
	YES	NO	Small		Moderate		Large											
1) Increased Energy in Performing Every Day Activities	Yes	No	1	2	3	4	5	1	2	3	4	5	6	7	8	9	10	11
2) Increased Physical Strength	Yes	No	1	2	3	4	5	1	2	3	4	5	6	7	8	9	10	11
3) Increased Muscle Fatigue or Soreness	Yes	No	1	2	3	4	5	1	2	3	4	5	6	7	8	9	10	11
4) Decreased Muscle Fatigue or Soreness	Yes	No	1	2	3	4	5	1	2	3	4	5	6	7	8	9	10	11
5) Increased Joint Soreness	Yes	No	1	2	3	4	5	1	2	3	4	5	6	7	8	9	10	11
6) Decreased Joint Soreness	Yes	No	1	2	3	4	5	1	2	3	4	5	6	7	8	9	10	11
7) Increased Muscle Stiffness or Tightness	Yes	No	1	2	3	4	5	1	2	3	4	5	6	7	8	9	10	11
8) Decreased Muscle Stiffness or Tightness	Yes	No	1	2	3	4	5	1	2	3	4	5	6	7	8	9	10	11
9) Increased Muscle Cramping	Yes	No	1	2	3	4	5	1	2	3	4	5	6	7	8	9	10	11
10) Decreased Muscle Cramping	Yes	No	1	2	3	4	5	1	2	3	4	5	6	7	8	9	10	11
11) Muscle Pull or Strain with Pain in Moving Muscle	Yes	No	1	2	3	4	5	1	2	3	4	5	6	7	8	9	10	11
12) Headaches	Yes	No	1	2	3	4	5	1	2	3	4	5	6	7	8	9	10	11
13) Increased Sad Feelings	Yes	No	1	2	3	4	5	1	2	3	4	5	6	7	8	9	10	11
14) Increased Happy Feelings	Yes	No	1	2	3	4	5	1	2	3	4	5	6	7	8	9	10	11
15) Feel Worse About Your Physical Appearance	Yes	No	1	2	3	4	5	1	2	3	4	5	6	7	8	9	10	11
16) Feel Better About Physical Appearance	Yes	No	1	2	3	4	5	1	2	3	4	5	6	7	8	9	10	11
17) Increased Irritability	Yes	No	1	2	3	4	5	1	2	3	4	5	6	7	8	9	10	11
18) Decreased Irritability	Yes	No	1	2	3	4	5	1	2	3	4	5	6	7	8	9	10	11
19) Increased Anxiety	Yes	No	1	2	3	4	5	1	2	3	4	5	6	7	8	9	10	11
20) Decreased Anxiety	Yes	No	1	2	3	4	5	1	2	3	4	5	6	7	8	9	10	11
21) Improved Sleep Quality	Yes	No	1	2	3	4	5	1	2	3	4	5	6	7	8	9	10	11

Side-Effects (Page 2 of 2)			Magnitude Scale To what extent?					Occurrence Scale Which WEEK of the supplementation period did it begin?										
	YES	NO	Small		Moderate		Large											
22) Decreased Sleep Quality	Yes	No	1	2	3	4	5	1	2	3	4	5	6	7	8	9	10	11
23) Nausea	Yes	No	1	2	3	4	5	1	2	3	4	5	6	7	8	9	10	11
24) Vomiting	Yes	No	1	2	3	4	5	1	2	3	4	5	6	7	8	9	10	11
25) Loose Stools	Yes	No	1	2	3	4	5	1	2	3	4	5	6	7	8	9	10	11
26) Constipation	Yes	No	1	2	3	4	5	1	2	3	4	5	6	7	8	9	10	11
27) Stomach Upset (Cramping and/or Gas)	Yes	No	1	2	3	4	5	1	2	3	4	5	6	7	8	9	10	11
28) Increased Appetite	Yes	No	1	2	3	4	5	1	2	3	4	5	6	7	8	9	10	11
29) Decreased Appetite	Yes	No	1	2	3	4	5	1	2	3	4	5	6	7	8	9	10	11
30) Increased Thirst	Yes	No	1	2	3	4	5	1	2	3	4	5	6	7	8	9	10	11
31) Decreased Thirst	Yes	No	1	2	3	4	5	1	2	3	4	5	6	7	8	9	10	11
32) Increased Sex Drive	Yes	No	1	2	3	4	5	1	2	3	4	5	6	7	8	9	10	11
33) Decreased Sex Drive	Yes	No	1	2	3	4	5	1	2	3	4	5	6	7	8	9	10	11

APPENDIX D

Three-day Food Records

Subject Nutrient Intake

College of Kinesiology
University of Saskatchewan

THREE-DAY FOOD RECORD

NAME: _____

DATES: _____

SPORT: _____

AGE: _____

HEIGHT: _____

WEIGHT: _____

INTRODUCTION

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

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

INSTRUCTIONS

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

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

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

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

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

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

RECORDING IN THE DIARY

1) Please use household measures. For example:

cup: vegetables, cereal, fruit, milk, beverages
tablespoon: sauces, fats
teaspoon: sugar, honey, drink mix
slices: bread, bacon
fractions: 1/6 pie.

2) State the type of food eaten. For example:

Milk: homo, 2%, 1%, skim, goat's
Cheese: processed, Swiss, spread
Bread: enriched white, 60% whole wheat, sweet cinnamon bun, bran muffin
Cereal: Sugar Pops, Miniwheats, granola
Meat: hamburger, fried chicken - breasts, scrambled eggs, cod fillets
Others: strawberry jam, Becel margarine, Caesar dressing, oatmeal cookies.

3) State the amount of food eaten. For example:

Cheese: 1" cube cheddar
3 tbsp lite cream cheese
1/4 cup 2% creamed cottage cheese
Fruit: 1/2 cup canned peaches (in heavy syrup)
12 grapes
1 medium banana
Bread: 2 slices 100% whole wheat
1 large kaiser
Cereal: 3/4 cup corn flakes
1 shredded wheat biscuit

Meat: 1 cup baked beans with pork
2 cups tuna casserole (tuna, cream of mushroom soup, noodles, peas)
4 thin slices roast beef

Vegetables: 2 slices cucumber
1/2 cup boiled cabbage

4) Include manner of cooking: fried, boiled, raw.

5) Remember all alcoholic drinks.

Here is a Sample: Date: Sat., Dec. 14th (Day 3)

Time	Food Description	Amount	Code
9:30a.m.	Waffles-white flour	3, 8"x4"	ea.
	syrup-Aunt Jemima	1/2 cup	
	yogurt-peach	125ml	
	coffee, 1 tsp. sugar	1 cup	
	milk (2%)	1/4 cup	
10:30	Chocolate chip cookies.	3	
	coffee, 1 tsp. sugar	1 cup	
	milk (Half & Half-10%)	1/4 cup	
12:30	Sandwich		
	-2 slices whole wheat bread	2 slices	
	-mozarella cheese (3"x1/4"x2")	2 slices	
	-salami	4 slices	
	-lettuce	1 leaf	
	-butter	1 tsp.	
	-mayonaise	1 tsp.	
5:30	Spaghetti	1 cup	
	meat sauce	1/2 cup	
	garlic toast	2 slices	
	(Continue on the next page if your need		
	it) Leave Code column blank.		

Date: _____

Time	Food Description	Amount	Code

Nutrient Intakes For

Nutrient	Your Intake	Recommendation	Percent Recommen
Food Energy (kcals)	3315	2900	114%
Protein (gm)	184	63	293%
Carbohydrate (gm)	330	**	**
Dietary Fiber (gm)	60	25	241%
Total fat (gm)	143	**	**
Saturated fat (gm)	49	**	**
Monounsaturated fat (gm)	60	**	**
Polyunsaturated fat (gm)	23	**	**
Cholesterol (mg)	852	<=300mg	♠
Vitamin A (RE)	1298.6	900	144%
Vitamin E (α-TE)	15.3	15	102%
Vitamin C (mg)	186.2	90	207%
Thiamin (mg)	4.8	1.2	403%
Riboflavin (mg)	3.6	1.3	278%
Niacin (mg)	47.2	16	295%
Folate (mcg)	220	400	55%
Vitamin B-6 (mg)	3.1	1.3	236%
Vitamin B-12 (mcg)	5.9	2.4	244%
Calcium (mg)	681.1	1000	68%
Iron (mg)	25.5	8	318%
Magnesium (mg)	388.2	420	92%
Phosphorus (mg)	?267.9	700	324%
Zinc (mg)	20.2	11	184%
Potassium (mg)	5475	*	*
Sodium (mg)	10311	<=2400mg	♠

♠ Not Calculated.

* Nutrient has no established Recommendation.

APPENDIX E

Training Log

Date: _____

Perform 3 Sets of 8-10 repetitions

Exercise	Set 1 (Wt x rep)	Set 2 (Wt x rep)	Set 3 (Wt x rep)
Leg Press			
Calf Press			
Chest Press			
Leg Extension			
Lat Pull Down			
Leg Curl			
Shoulder Press			
Biceps Curl			
Triceps Press			

Date: _____

Perform 3 Sets of 8-10 repetitions

Exercise	Set 1 (Wt x rep)	Set 2 (Wt x rep)	Set 3 (Wt x rep)
Leg Press			
Calf Press			
Chest Press			
Leg Extension			
Lat Pull Down			
Leg Curl			
Shoulder Press			
Biceps Curl			
Triceps Press			

APPENDIX F

Urine Collection Procedures

Urine Collection Instructions

1. Follow a meat-free diet (i.e. no fish, chicken, pork, beef, or processed meats) for 3 days.

2. On the 3rd day of being meat free, collect all urinations in the orange container for a period of 24 hours.

3. Discard the first urine in the morning of the 3rd day of being meat free, but collect all other urinations during that day, including the first urine when you wake up the 4th day.

4. Please keep urine refrigerated or stored in a cold place.

Questions:

Dr. Phil Chilibeck 966-1072

Darren Candow 966-1099, 249-4853

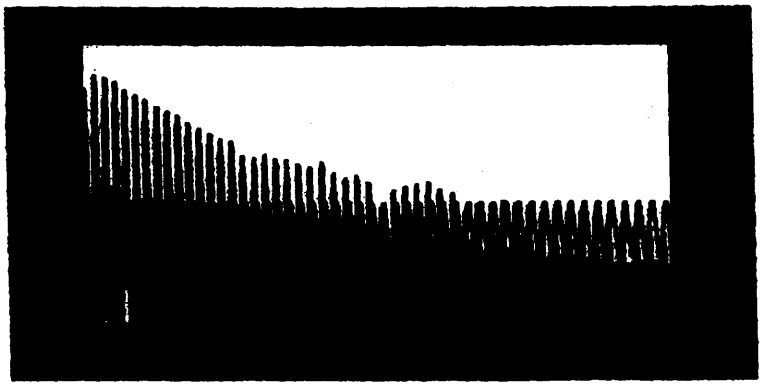
APPENDIX G

Biodex Output

Comprehensive Evaluation

Name:		Session:	[redacted]	Windowing:	Isokinetic
ID:	[redacted]	Involved:	None	Protocol:	Isokinetic Unilateral
Birth Date:	(MM/yy)	Clinician:		Pattern:	Extension/Flexion
Ht:		Referred:		Mode:	Isokinetic
Wt:		Joint:	Knee	Contraction:	Con/Con
Gender:		Diagnosis:		CET:	10 N-M at 167 Degrees

		EXTENSION 180 DEG/SEC	FLEXION 180 DEG/SEC
Side: LEFT			
# OF REPS: 09			
PEAK TORQUE	N-M	99.9	59.3
PEAK TQ/BW	%	169.3	100.5
TIME TO PK TQ	MSEC	200.0	160.0
ANGLE OF PK TQ	DEG	124.0	136.0
TORQ @ 20 DEG	N-M	0.0	0.0
TORQ @ 0.10 SEC	N-M	98.4	51.1
COEFF. OF VAR.	%	28.5	26.2
MAX REP TOT WORK	J	89.6	47.1
MAX WORK REP'S	#	2	2
WRK/BODYWEIGHT	%	151.8	79.9
TOTAL WORK	J	2761.3	1561.3
WORK FIRST THIRD	J	1397.4	735.6
WORK LAST THIRD	J	534.8	318.2
WORK FATIGUE	%	61.7	56.7
AVG. POWER	WATTS	110.6	61.9
ACCELERATION TIME	MSEC	20.0	70.0
DECELERATION TIME	MSEC	100.0	60.0
ROM	DEG	69.1	
MAX/AVE PEAK TQ		63.7	40.0
AGONIST/ANTAG RATIO	%	59.4	Goal 72.0



APPENDIX H

BOD POD Output

Body Composition Test Results

SUBJECT DATA

Name :
Age :
Gender :
Technician: darren

Date :
Height : 71 ins (180 cms)
Model : Siri
Density: 1.044 kg/l

RESULTS

Percent Fat : 24.1 %
Percent Lean : 75.9 %

Fat Weight : 19.1 kgs
Lean Weight : 60.1 kgs
Total Weight : 79.1 kgs

Body Fat: A certain amount of fat is absolutely necessary for good health. Fat plays an important role in protecting internal organs, providing energy and regulating hormones. For men, the minimal amount of "essential fat" is approximately 3-5%. For women, "essential fat" is approximately 12-15%. If too much fat accumulates over the years, health may be compromised (see table below).

Lean Mass: Lean mass is everything except the fat. It includes muscle, water, bone, and internal organs. Muscle is the "metabolic engine" of the body that burns calories (fat) and plays an important role in maintaining strength and energy. Healthy levels of lean mass contribute to physical fitness and may prevent conditions such as osteoporosis.

✓	Body Fat Rating	Men	Women	Explanation
	Risky (high body fat)	>30%	>40%	Too much body fat can pose serious health risks. Ask your health care professional about how to safely modify your body composition.
	Excess Fat	21-30%	31-40%	Indicates an excess accumulation of fat over time.
	Moderately Lean	13-20%	23-30%	Fat level is acceptable for good health.
	Lean	9-12%	19-22%	Lower body fat levels than many people. This range is excellent for health and longevity.
	Ultra Lean	5-8%	15-18%	Fat levels sometimes found in elite athletes.
	Risky (low body fat)	<5%	<15%	Too little body fat can present health risks, especially for women. If in doubt, check with your health care professional.

APPENDIX I

DXA Output

University of Saskatchewan, College Of Kinesiology
87 Campus Drive
Saskatoon, SK S7N 5B2

)hone: 306-966-1072

ıe:	Sex: .	Height: .
	Ethnicity:	Weight:
		Age:

ırring Physician: College Of Kinesiology

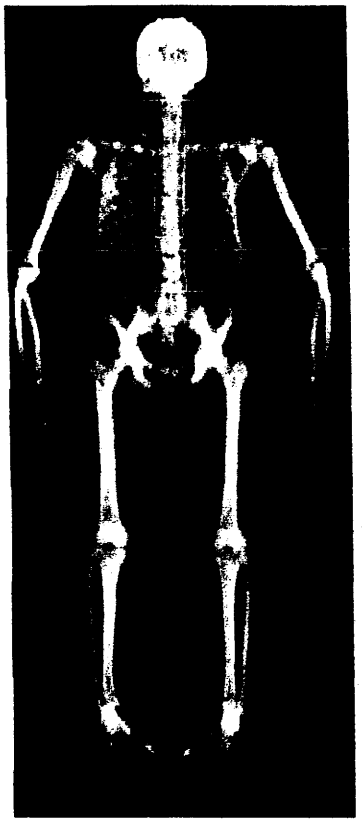


Image not for diagnostic use
318 x 150

Scan Information:

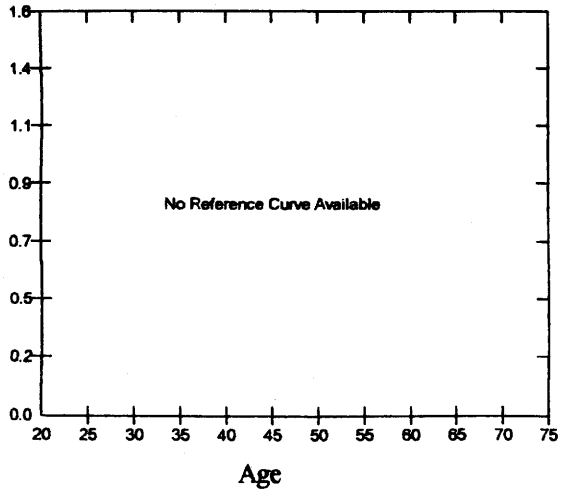
Scan Date: November 23, 2004 ID: A1123040C
Scan Type: a Whole Body
Analysis: November 23, 2004 19:16 Version 12.3
Auto Whole Body
Operator: NR
Model: Discovery W (S/N 80964)
Comment:

DXA Results Summary:

Region	Area (cm²)	BMC (g)	BMD (g/cm²)	T - Score	Z - Score
L Arm	247.72	256.71	1.036		
R Arm	268.07	278.00	1.037		
L Ribs	130.32	131.64	1.010		
R Ribs	128.75	123.74	0.961		
T Spine	150.67	156.53	1.039		
L Spine	57.53	74.84	1.301		
Pelvis	279.42	486.52	1.741		
L Leg	403.47	576.31	1.428		
R Leg	435.95	626.64	1.437		
Subtotal	2101.89	2710.94	1.290		
Head	229.72	539.49	2.349		
Total	**2331.61**	**3250.43**	**1.394**		

Total BMD CV 1.0%

Total



Physician's Comment:

HOLOGIC

Study 1
Differences in Size, Strength, and Power of Upper and Lower Body Muscle Groups in Young and Older Men

Univariate Tests of Significance for Lean Tissue Mass and Muscle Thickness

	SS	df	MS	F	p
Lean Tissue Mass					
Group	433.10	1	433.10	8.2	.006
Error	2467.64	47	52.503		
Elbow Flexors					
Group	2.254	1	2.254	8.7	.005
Error	12.129	47			
Elbow Extensors					
Group	.058	1	.058	.161	.690
Error	16.8	47	.359		
Knee Flexors					
Group	11.421	1	11.421	19	.000
Error	28.224	47	.601		
Knee Extensors					
Group	7.202	1	7.202	10.9	.002
Error	31.078	47	.661		
Ankle Plantar Flexors					
Group	15.707	1	15.707	12.256	.001
Error	60.238	47	1.282		

Univariate Tests of Significance for Torque

	SS	df	MS	F	p
Elbow Flexors					
Group	171.562	1	1717.562	108.2	.000
Error	671.998	48	15.875		
Elbow Extensors					
Group	219.605	1	219.605	7.4	.009
Error	1417.635	48	29.534		
Knee Flexors					
Group	9638.01	1	963.01	85.9	.000
Error	5387.391	48	112.273		
Knee Extensors					
Group	78264.291	1	78264.291	159.3	.000
Error	23581.119	48	491.273		
Ankle Plantar Flexors					
Group	53669.156	1	53669.156	311.2	
Error	8278.284	48	172.464		

Univariate Tests of Significance for Normalized Torque

	SS	df	MS	F	p
Elbow Flexors					
Velocity	196.327	1	196.327	109.728	.000
Error	85.882	48	1.789		
Elbow Extensors					
Velocity	13.259	1	13.259	7.3	.010
Error	87.463	48	1.822		
Knee Flexors					
Velocity	413.521	1	413.521	78.7	.000
Error	252.287	48	5.256		
Knee Extensors					
Group x Velocity	139.634	1	139.634	4.4	.041
Velocity	5823.942	1	5823.942	184.9	.000
Error	1511.943	48	31.499		
Ankle Plantar Flexors					
Velocity	4450.961	1	4450.961	163.0	.000
Error	1310.360	48	27.299		

Univariate Tests of Significance for Power

	SS	df	MS	F	p
Elbow Flexors					
Velocity	2130.320	1	2130.320	8.6	.005
Error	11623.539	47	247.309		
Elbow Extensors					
Velocity	26506.907	1	26506.907	165.5	.000
Group x Velocity	2361.300	1	2361.300	14.7	.000
Error	7526.583	47	160.140		
Knee Flexors					
Group x Velocity	4185.532	1	4185.532	6.3	.015
Error	31122.014	47	662.171		
Knee Extensors					
Velocity	47406.561	1	47406.561	69.9	.000
Group x Velocity	8246. 804	1	8246.804	12.1	.000
Error	31919.795	47	679.145		

Torque for Older Group Relative to Young Across Muscle Groups and Velocities

1-MUSCLE, 2-VELOCITY						
	df	MS	df	MS		
	Effect	Effect	Error	Error	F	p
1	4	2383.397	104	440.3867	5.412055	0.000534
2	1	2589.021	26	107.2063	24.1499	4.21E-05
12	4	632.9148	104	70.47887	8.980206	2.86E-06

Muscle Thickness of Older Group Relative to Young Across Muscle Groups

1-MUSCLE						
	df	MS	df	MS		
	Effect	Effect	Error	Error	F	p
1	4	2234.845	108	185.0189	12.079	3.77E-08

Normalized Torque for Older Group Relative to Young Across Muscle Groups

1-MUSCLE, 2-VELOCITY						
	df	MS	df	MS		
	Effect	Effect	Error	Error	F	p
1	4	1048.339	104	1104.957	0.94876	0.439029
2	1	4012.382	26	185.8305	21.59162	8.54E-05
12	4	1089.041	104	133.2545	8.172639	9.03E-06

Power for Older Group Relative to Young Across Muscle Groups

1-MUSCLE, 2-VELOCITY						
	df	MS	df	MS		
	Effect	Effect	Error	Error	F	p
1	4	1969.417	104	582.005	3.383848	0.01206
2	1	4545.642	26	273.1527	16.64139	0.00038
12	4	855.2059	104	183.7238	4.654845	0.001698

Study 2
Effect of Protein Supplementation Before and After Resistance Training in Older Men

Univariate Tests of Significance for Lean Tissue Mass, Strength and Muscle Thickness.

	SS	df	MS	F	p
Lean Tissue Mass					
Time	13.965	1	13.965	9.9	.004
Error	36.320	26	1.397		
Leg Press					
Time	116775.317	1	116775.317	93.2	.000
Error	32581.111	26	1253.120		
Chest Press					
Time	23164.291	1	23164.291	80.9	.000
Error	7447.361	26	286.437		
Elbow Flexors					
Time	3.755	1	3.755	74.675	.000
Error	1.307	26	.050		
Elbow Extensors					
Time	9.39	1	9.639	84.6	.000
Error	2.965	26	.114		
Knee Flexors					
Time	3.042	1	3.042	17.0	.000
Error	4.659	26	.179		
Knee Extensors					
Time	2.222	1	2.222	26.187	.000
Error	2.206	26	.085		
Ankle Plantar Flexors					
Time	2.279	1	2.279	14.6	.001
Error	4.064	26	.156		
Ankle Dorsi Flexors					
Time	.389	1	.389	4.4	.046
Error	2.305	26	.089		

Analysis of Variance Tests of Significance for % changes.

	SS	df	MS	F	p
Knee Extensors					
Group	1186.508	2	593.254	3.9	.033
Error	3938.758	26	151.491		

Univariate Tests of Significance for Diet.

	SS	df	MS	F	p
Calories					
Time	1619749.629	1	1619749.629	4.6	.042
Error	8771816.313	25	350872.653		
Protein					
Group x Time	4224.485	2	2112.242	4.2	.027
Error	12662.934	25	506.517		

Lean Tissue Mass of Older vs. Younger Men Before Training

1-GROUP						
	df	MS	df	MS		
	Effect	Effect	Error	Error	F	p
1	1	668.3369	83	55.27893	12.09027	0.000809

Lean Tissue Mass of Older vs. Younger Men After Training

1-GROUP						
	df	MS	df	MS		
	Effect	Effect	Error	Error	F	p
1	1	524.2434	83	53.2762	9.840106	0.002363

Bench Press of Older vs. Younger Men Before Training

1-GROUP						
	df	MS	df	MS		
	Effect	Effect	Error	Error	F	p
1	1	179551.4	86	3555.891	50.49408	3.27E-10

Bench Press of Older vs. Younger Men After Training

1-GROUP						
	df	MS	df	MS		
	Effect	Effect	Error	Error	F	p
1	1	63546.06	86	3501.156	18.15002	5.2E-05

Leg Press of Older vs. Younger Men Before Training

1-GROUP						
	df	MS	df	MS		
	Effect	Effect	Error	Error	F	p
1	1	361000	86	13191.54	27.36602	1.17E-06

Leg Press of Older vs. Younger Men After Training

1-GROUP						
	df	MS	df	MS		
	Effect	Effect	Error	Error	F	p
1	1	40542.86	86	14071.27	2.88125	0.093233

Elbow Flexor Muscle Thickness of Older vs. Younger Men After Training

1-GROUP						
	df	MS	df	MS		
	Effect	Effect	Error	Error	F	p
1	1	0.089284	48	0.281456	0.317222	0.575904

Ankle Dorsi Flexor Muscle Thickness of Older vs. Younger Men After Training

1-GROUP						
	df	MS	df	MS		
	Effect	Effect	Error	Error	F	p-level
1	1	0.853972	48	0.296538	2.879812	0.096173

Elbow Extensor Muscle Thickness of Older vs. Younger Men After Training

1-GROUP						
	df	MS	df	MS		
	Effect	Effect	Error	Error	F	p-level
1	1	6.99013	48	0.408664	17.10484	0.000141

Study 3
Effect of Creatine and Protein Supplementation Combined With Resistance Training in Older Men

Univariate Tests of Significance for Lean Tissue Mass, Strength, and Muscle Thickness

	SS	df	MS	F	p
Lean Tissue Mass					
Time	50.274	1	50.274	24.2	.000
Group x Time	19.411	2	9.705	4.7	.017
Error	66.489	32	2.078		
Leg Press					
Time	34880.668	1	34880.668	44.8	.000
Error	24896.795	32	778.025		
Chest Press					
Time	13230.936	1	13230.936	38.3	.000
Error	11044.679	32	345.146		
Elbow Flexors					
Time	.650	1	.650	7.3	.011
Error	2.843	32	.089		
Elbow Extensors					
Time	1.795	1	1.795	10.3	.003
Error	5.599	32	.175		
Knee Flexors					
Time	1.412	1	1.412	7.1	.012
Error	6.390	32	.200		
Knee Extensors					
Time	1.545	1	1.545	17.1	.000
Error	2.889	32	.090		
Ankle Plantar Flexors					
Time	2.765	1	2.765	11.9	.002
Error	7.406	32	.231		