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The effects of red meat consumption and
high-intensity resistance training of
skeletal muscle strength, muscle mass and
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Irene Fe Gutteridge
University of Wollongong

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**THE EFFECTS OF RED MEAT CONSUMPTION AND
HIGH-INTENSITY RESISTANCE TRAINING ON SKELETAL
MUSCLE STRENGTH, MUSCLE MASS AND FUNCTIONAL
STATUS IN HEALTHY OLDER ADULTS.**

A thesis submitted in partial fulfillment of the requirements for the award of the degree

Master of Science (Research)

from

The University of Wollongong

by

Irene Fe Gutteridge, BSc (Kinesiology)

School of Health Sciences

2008

I, Irene Fe Gutteridge, declare that this thesis, submitted in partial fulfillment of the requirements for the award of Master of Science, in the School of Health Science, University of Wollongong, is wholly my own work unless otherwise referenced or acknowledged. The document has not been submitted for qualifications at any other academic institution.

Irene Fey Gutteridge

27th August 2008

**THE EFFECTS OF RED MEAT CONSUMPTION AND HIGH-INTENSITY
RESISTANCE TRAINING ON SKELETAL MUSCLE STRENGTH,
MUSCLE MASS AND FUNCTIONAL STATUS IN HEALTHY OLDER
ADULTS.**

ABSTRACT

With the older adult representing an increasingly large percentage of the Western world, attempts are being sought to improve their healthy aging through various modes of prevention. The age-associated declines that occur in the physiological and functional systems along with levels of physical activity and quality of life have the potential to be attenuated and ameliorated with various forms of health-related interventions. It has been suggested that intake of dietary protein in the elderly may be too low to sustain normal muscle mass and red meat intake declines in the over 65 y age group in Australia.

The present study examined the effects of a high-intensity resistance training program and two levels of red meat intake on skeletal muscle strength, body composition and other health-related markers in healthy, community-dwelling older adults. Twenty-eight healthy male and female subjects with mean age (\pm SD) of 67 \pm 3 y and randomized to either a moderate (400g/wk) or high (800g/wk) red meat diet, completed a supervised twice weekly, twelve week high-intensity resistance training program of the lower extremities. The moderate meat diet represented the usual intake for older Australians over 65 y. Diet histories, body composition assessments, mid-thigh CT scans, grip strength, lower extremity performance, physical activity levels, one-mile walk test, fasted blood samples and morning urine samples were taken at baseline and twelve weeks. Four-repetition maximum strength testing of the lower extremity was undertaken at pre-, mid- and post-intervention.

Leg strength was greater in males than in females and this was strongly associated with their muscle mass and stature independent of gender. Age related declines in grip strength and leg muscle strength were evident at baseline. Resistance training significantly increased leg muscle strength >50% ($p<0.001$) irrespective of gender and age but grip strength (not targeted by the training program) remained unchanged. In subjects on the higher meat diet, mid-intervention leg press strength improvements were greater than those seen with the moderate meat diet ($p<0.01$), although significant differences between diets were not sustained at week twelve. The sum of seven skinfolds (mm) decreased significantly in all subjects with training (131.2 ± 8.8 to 119.9 ± 7.3 , $p<0.001$) and significant improvement to the proportion of cross-sectional area of thigh muscle and thigh fat were measured in the non-dominant leg ($p<0.05$). Physical activity levels and lower extremity performance remained unchanged. The high red meat diet provided additional short-term benefits for building muscle strength without compromising cardiovascular disease risk factors, but in the longer term had no additional beneficial effects to strength and functional parameters.

In summary, healthy older adults exhibit an age related decline in strength, yet all have the capacity to greatly increase strength with muscle specific exercise training. Marked increases in strength can be rapidly achieved with short term high intensity resistance training. Resistance training is well tolerated and can be recommended for improving strength and enhancing other health-related parameters as part of a prevention based healthy aging strategy.

Acknowledgments

This project was an amazing experience in so many ways!

I am privileged to have witnessed such change and improvement in the lives of the study volunteers over the course of the project. Without the study volunteers, none of this would have been possible. Their enthusiasm and commitment to the study was relentless. It is without a doubt a period in my life that I will never forget and will continue to inspire me into my own “older” years. Dr. Peter McLennan was a key figure throughout this journey. He took me under his wing when I had nowhere else to go and handed me this project with great trust, encouragement and supported me right to the very end! I am forever thankful to Andrew Frith, not only did his assistance during the testing and intervention prove to be stupendous, he was dedicated to ensuring “my subjects” were taken care of and looked after. Acknowledgment must go to the Exercise Science students from the School of Health Sciences. Their punctuality and dedication were critical to the exercise intervention running smoothly. Sheena McGhee was integral in many aspects. Her skilled hands and demeanor was essential to testing procedures, as was her hospitality during the write-up stages. Dr. Alice Owen was integral with supervision, guidance and early manuscript proofing as well as key in biochemical assessments. Dr. Gregory Peoples was a great co-supervisor during the final stages of the write-up and was a breath of fresh air as our conversations often diverted to many other interesting ideas about human function and performance. I am thankful for Herb Groeller who reminded me that “less is more” in the early stages of this project. Thanks must go to Dr. Dennis Calvert and Marc Brown for their assistance with the medical screening and the ECG evaluations. The collection of dietary data was possible due to the assistance of Lynda Gillen and Anne McMahon within the Smart Foods Centre and the prompt and friendly deliveries of high quality meat from Dorahy Meats, Unanderra. The one mile walk test was made possible due to the assistance from the members of the Cardiovascular Lab from the School of Health Sciences. The use of the Wollongong City Council Gymnasium was essential to the success of the study and served as a perfect setting for the exercise intervention. A big thank-you must go to all the postgraduate students, staff and friends within the Department of Biomedical Sciences who provided many friendly smiles and open ears for someone so far from her roots and loved ones.

*“Do not say at the start what the final stage will be.”
Moshe-Pinhas Feldenkrais*

Dedication

*This thesis is dedicated to my first teachers, my parents.
Their parenting has been the best and possibly the
hardest kind of teaching to do for a child.
They taught me without parenting and have provided me
with amazing opportunities in which to learn, grow and
develop into my own being.*

For this I am forever grateful!

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Abbreviations

ACSM/AHA	American college of sports medicine and the American heart association
ADL	Activities of daily living
BIA	Bioelectrical impedance
BMI	Body mass index
CT	Computerized tomography
CSA	Cross-sectional area
ECG	Electrocardiogram
LEP	Lower extremity performance
MWT	One mile walk test
PASE	Physical activity scale for the elderly
RDA	Recommended daily allowance
RM	Repetition maximum
RPE	Ratings of perceived exertion
RT	Resistance Training
SPPB	Short physical performance battery
VO _{2max}	Maximal oxygen consumption

Glossary of Terms

Aerobic capacity

The maximum amount of oxygen the body can use during a specified period, such as in physical exercise. It describes the functional status of the cardiorespiratory system (the heart, lungs and blood vessels) and the ability to remove and utilize oxygen from circulating blood.

Concentric muscle contraction.

Phase of movement in which the joint angle of the joint being used is decreased as the prime muscle being used is actively shortened.

Eccentric muscle contraction

Phase of movement in which the joint angle of the joint being used is increased as the prime muscle being used is actively lengthened.

Fall

An event which results in a person coming to rest unintentionally on the ground or other lower level, not as a result of a major intrinsic event (such as a stroke or syncope) or overwhelming hazard.

Muscular hypertrophy

Muscular growth in which there are increases in the synthesis of contractile proteins (actin and myosin) within the myofibril and increases of myofibrils with a muscle fiber.

Periodization

Planned variation in an exercise regime such as resistance training with the purpose to offer greater gains in performance-related variables such as strength and endurance.

Physical activity

Any bodily movement either planned or non-planned that results in an expenditure of energy.

Physical exercise

Partaking in an activity with the intent to develop or maintain physical fitness and overall health, such as resistance or endurance training.

Progressive overload

Practice of the gradual and continual increase of stress placed on the physical body during exercise. Training variables (e.g., resistance, number of sets and repetitions, rest periods, time) are manipulated to offer a progressive overload to the body.

Repetition

One complete movement of an exercise that consists of a concentric muscle contraction and an eccentric muscle contraction

Repetition maximum (RM)

Maximal number of repetitions performed per set for a given resistance training exercise. A 1RM is classified as the heaviest resistance that an individual can perform once with proper lifting technique.

Rest period

Time that occurs between exercise sets to allow for recovery. The length of rest is highly dependent on factors such as: training goals, load lifted, experience of the person training

Sarcopenia

Derived from the Greek “Sarco” denoting “flesh”, and “penia” which indicates deficiency, hence a deficiency of flesh or muscle. It refers to the involuntary and gradual loss of muscle mass and strength that occur with advancing age.

Set

A group of repetitions completed together without stopping.

Specificity

Training in a specific manner to produce specific adaptations or training outcomes.

Successful aging

Maintaining and even enhancing functionality and quality of life into older ages through maintenance of factors such as physical activity levels, muscular strength and muscle mass etc.

Training frequency

Number of training sessions in a given time period.

Usual aging

Occurs when factors of lifestyle or environment intensify the common age related changes such as diminished muscle strength and muscle mass, increases in body fat, lower levels of physical activity etc.

Scientific Communications

1. McLennan PL, Tapsell LC, Owen AJ, Gutteridge IF. The influence of red meat intake upon the response to a resistance exercise-training program in older Australians. Nutrition Society of Australia. Hobart, 2003.
2. Owen AJ, Gutteridge IF, McLennan PL, Tapsell LC. Growing old powerfully: The combined value of red meat & exercise for older Australians. Nutrition Society of Australia, Wollongong section. Wollongong, 2003.
3. Owen AJ, McLennan PL, Gutteridge IF. The influence of red meat intake upon the response to a resistance exercise training program in older Australians. Council on the Ageing. Melbourne, 2004.

Chapter One

*-Introduction
and
Literature Review-*

“Every organized being forms a whole, a unique and closed system, of which all parts mutually correspond by reciprocal reaction for the same definite end. None of these parts can change without the others changing also, consequently each of them taken separately, presents and postulates the others.”

George Cuvier

1.1 INTRODUCTION

The World Health Organization predicts that by 2050 those aged 60 and over will represent two billion of the world's population compared to 600 million seen in 2000 (World Health Organization, 2008). Similar trends are predicted for individual developed countries such as Canada, Australia and the United States. In Canada the 2001 census reports seniors aged 65 and over to be 13% of the country's population and that by 2011 this will increase to 15%, with those aged 80 years and over representing the largest population of seniors (Statistics Canada, 2001). According to the Australian Bureau of Statistics by 2051 those aged 65 years and older will represent a greater proportion of the Australian population with the proportion of this age group representing 26% to 28% of the population as compared to 13% which was recorded in 2004, with those 85 years and greater making up a greater percentage of the population in 2051 (6-8%) when compared to 2004 (1.5%) (Australian Bureau of Statistics, 2008). In the United States, those aged 65 years and over represented 13% of the total American population (35 million) in 2000, and by the year 2030 it is projected this number will double to 70 million (U.S Census Bureau, 2000). Preparations to accommodate this growing aged population in the Western world are essential. Modes of prevention that will maintain and even improve the health and quality of life of this aging population that are simple, effective and affordable are necessary in order to take the burden off of the health care systems and create sustainable solutions for future populations.

One factor contributing to this increase in the older adult population is a substantial decrease in mortality rates from all causes over the past two decades. In the United

States those women and men aged 65-74 years have decreased rates of mortality of 6 and 19 % respectively, with those aged 75-84 years also decreasing (8 and 16% for women and men, respectively)(Sahyoun et al., 2001). With this lower mortality there is a possibility of increased morbidity associated with such a growing populations of older adults. Rather than merely sustaining a persistently disabled growing population of older adults it is imperative that the most practical and affordable modes of prevention are sought to avoid escalating levels of morbidity. Primary prevention as a means of compressing such morbidity has been proposed through the adoption of certain lifestyle habits (Hubert et al., 2002). Identifying prominent factors that are associated with healthy aging and enhanced quality of life should be key in preparing for the potential impact of this growing population on health care systems.

The muscular system represents one of the largest organ systems in the body. The ubiquitous functionality of muscle as a major organ is well established as it: provides movement of the human body; is a major site for fuel deposition and utilization; and provides a reserve of tissue when the human body is faced with unfavourable conditions (McComas, 1996). It is one body system that if either nurtured or neglected will greatly influence the quality of aging that an adult experiences.

It is well established that the mass and strength of the muscular system undergoes a rapid decline between the ages of 65-70 (Brooks and Faulkner, 1994). Both have been shown to be representative of one another, in that lower levels of muscular strength are associated with lower levels of muscle mass and vice versa (Frontera et al., 1991). Such losses could potentially lead to the inability to maintain activity

levels sufficient for skeletal muscle to maintain physiological adaptability and functionality which is crucial for optimal health and successful human aging.

Sarcopenia, a term used to depict involuntary age-associated declines within the muscular system, (loss of skeletal muscle mass and strength) results from a myriad of factors (Rosenberg, 1989).

Diagram 1. Potential Factors Contributing to Sarcopenia

Adapted from Doherty (2003) and Roubenhoff and Hughes (2000)

Declines in physical activity and structured exercise are typically associated with aging (Sims et al., 2007) and have negative consequence on the entire human body. Even athletically-inclined older adults such as Masters Athletes experience some degree of age-related losses. While aerobic training has gained great popularity in the prevention of heart disease and obesity, specific attention is now being placed on resistance training. The usefulness of such training regimes with older populations, and more specifically, high intensity resistance training to counteract the negative consequences of sarcopenia is being explored extensively. The primary purpose of resistance training is to improve various aspects of skeletal muscle structure and function which are lost with age-induced sarcopenia. There is sufficient data to suggest that this mode of exercise is a positive and safe stimulus for an older adult (Porter, 2001; Breen et al., 2007).

Decreasing the age-related losses of the muscular system, thus allowing for consistent involvement in physical activity has potential to prevent early disability into older ages. Many studies have investigated the usefulness of high-intensity resistance training with frail and functionally compromised elderly (Fiatarone et al., 1994) in order to aid and restore severely declined levels of functional capacity, mobility and cognitive-related factors. Although such populations deserve focus and study, another sub-population that may be overlooked are those older adults that are active, healthy and community-dwelling. Older and healthy community-dwelling adults are prime candidates for primary prevention. Preserving and further improving the health of this active and healthy older population has the potential to greatly aid the impact of the growing older adult population which is projected within the next century.

There is great variation within intervention-based studies aimed at improving the musculoskeletal system of the older adult. Assessments of physiological outcomes are increasingly thorough and comprehensive. Now not only are outcomes specifically related to the intervention being studied (e.g., improved muscle strength, muscle mass), but factors that are non-specific to the intervention, such as physical activity levels and functional status, are becoming “gold standard” in geriatric research as they are indicative of future disability and early mortality (Guralnik et al., 1995; Paffenbarger et al., 1997; Sui et al., 2007).

The American College of Sports Medicine and the American Heart Association (ACSM/AHA) have published guidelines aimed at older adults in order to educate individuals about the importance of maintaining physical activity and muscular strength. The importance of resistance training during the older years is stressed with recommendation for older adults to train all major muscle groups in a progressive overload fashion (Nelson et al., 2007). These recommendations have been used in resistance training interventions with healthy older adults (Nelson et al., 1994; Taaffe et al., 1999), and differ from earlier studies that have focused on older and frailer populations, in which interventions were aimed solely towards exercising the lower extremities (Fiatarone et al., 1990). Nonetheless such exercise specificity is critical for the aged, as future disability is highly associated with the lower extremity and its strength (Guralnik et al., 1995). Often these studies examine the effects on improved muscle strength, body composition and lower extremity performance. Earlier high-intensity resistance training interventions in healthy older adults focused on primarily exercising the thigh musculature (Frontera et al., 1988; Frontera et al., 1990). These protocols were very similar to many of the interventions given to the frail elderly, however at the time little

attention was placed towards other characteristics such as lower extremity performance and physical activity status in these healthier older adults.

The usefulness of high-intensity resistance training aimed solely towards the lower extremity in healthy adults warrants study as it may be a very simple mode of preserving muscle strength and mass, as well as functional status. Observations made with Master Athletes and their inevitable loss of muscle mass and aerobic capacity, even with higher levels of physical activity deserves mention. Could it be a lack of specified stimulus, such as resistance training, that targets muscle strength and accretion of muscle mass? Or could it be that the inability to preserve muscle mass and aerobic capacity is due to other environmental factors such as nutrition?

Nutrition is an important lifestyle factor closely related to human performance and has also been studied in combination with resistance training interventions. Protein nutrition is a factor closely related to the accretion of skeletal muscle, as dietary protein provides the essential amino acids needed for the building of muscle tissue. For an increase in muscle mass to occur as a result of resistance training, a period of enhanced muscle protein synthesis and reduced muscle protein breakdown must occur, along with positive muscle amino acid balance (Yarasheski et al., 1993).

Controversy still exists pertaining to the protein needs for individuals and athletes who exercise and are involved in various forms of resistance training. The consumption of extra protein is not recommended for younger athletes as they typically consume a diet that far exceeds protein recommendations for athletic endeavor (Phillips, 2004). However, older adults may need a greater daily allowance of protein to maintain muscle mass (Campbell et al., 2001) and the

source of protein, namely animal protein (Campbell et al., 1999) may play a role in the accretion of muscle mass. The *per capita* meat consumption of Australians aged over 65 years is the lowest of any adult age group in the country, and approximately one third that of the intake in early adulthood (25-44 year olds) (Australian National Nutrition Survey, 1995). As the average age of our population grows, this could translate into a decline in average adult red meat intake. This may be due to a perception that meat is unhealthy, but lean red meat provides not only an important source of protein, but also many other nutrients including iron, zinc, and a small amount of omega-3 fatty acids. The effects of animal protein and its influence on skeletal muscle strength, mass and function in an intervention setting has yet to be firmly established. The relationships between dietary protein and resistance training with older adults remains open for debate due to the conflicting results throughout the literature (Campbell and Leidy, 2007). It may be that the type of protein consumed is important owing to the fact that different sources of protein contain different micronutrients and non-nutrients.

The lack of consistency in combined dietary and resistance training interventions in healthy older adults suggests that it may be the specificity of exercise, diet or another parameter of human aging not yet rigorously investigated that is responsible for enhancement of skeletal muscle function and successful aging. While many high-intensity resistance training interventions study older adults who are frail and functionally compromised, the effectiveness of similar intervention schemes on strength, body composition, lower-extremity performance and physical activity status in healthy older adults is less clear.

Therefore this study will examine the role of high-intensity strength training (specific to the lower extremities) and the efficacy of animal protein as a dietary intervention in promoting the improvement of body composition, skeletal muscle strength, lower extremity performance and physical activity status in healthy older adults.

1.1.1 AGE-ASSOCIATED DECLINES

Generally there is 30-40% loss of strength over the adult life span with the greatest declines occurring after the age of 45 years (Aoyagi and Shepard, 1992). Such age-related losses are ubiquitous (Frontera et al., 1991; Evans 1995; Frontera et al., 2000; Goodpaster et al., 2006) and there is much interest in determining the underlying mechanisms that cause such involuntary and inevitable changes with aging. The withdrawal of anabolic stimuli, such as decreased physical activity levels, reduced protein intake, and various hormonal factors along with events that create catabolic stimuli such as sub-clinical inflammation and catabolic cytokines have been suggested as other possible factors (Roubenoff and Hughes, 2000). In general, such mechanisms could potentially be responsible for age-associated declines seen in skeletal muscle mass and strength.

Explanations for loss in muscle strength and mass include: an overall reduction of whole muscle cross-sectional area which is thought to be caused by the actual reduction in muscle fibre cross-sectional area; change in proportion of muscle fibre type; loss of individual fibres; degeneration of the peripheral nerves; and environmental factors such as declines in physical activity (for review Aoyagi and Shepard, 1992; Faulkner et al., 2007).

Both cross-sectional studies (Frontera et al., 1991; Metter et al., 1997; Frontera et al., 2000) and longitudinal studies (Frontera et al., 2000; Goodpaster et al., 2006; Frontera et al., 2008) verify such changes. Metter et al., (1997) found that between the ages of 20 and 80 years both men and women demonstrate age-associated declines in strength and muscle mass (Metter et al., 1997). Frontera et al., (1991) has also linked the decrements seen in muscle strength and muscle mass when assessing older men and women over three age groups (45-54 years, 55-64 years and 65-78 years) (Frontera et al., 1991). When strength of the oldest subgroup (68-78 years) was compared to the youngest subgroup (45-54 years) the oldest exhibited significantly lower absolute values for strength in all muscles tested. When strength was corrected for muscle mass, the significant age-related differences in all muscle groups were abolished, suggesting that muscle mass is a major independent factor of muscular strength.

To explore these age-associated factors longitudinally, the same research group used the previous subjects (Frontera et al., 1991) and noted decreases in strength to be 60% greater than the observations in cross-sectional analysis (Hughes et al., 2001). Although the decrement in muscle strength was affected by trends in body composition change with age, muscle mass only contributed a small percentage (5%) of the variance in knee extensor and flexor strength. Similar longitudinal results have shown that strength declines three fold in comparison to muscle losses annually (Goodpaster et al., 2006).

This change is small in comparison to the impact of muscle mass towards muscle strength in the previous cross-sectional study (Frontera et al., 1991), as well as another longitudinal study in which muscle cross-sectional area over twelve years

accounted for 90% of the variability in follow-up strength suggesting that muscle mass and strength are intimately related to one another (Frontera et al., 2000).

Skeletal muscle mass shows the greatest declines within the lower body region, namely the legs, for both men and women, with independently living healthy older adults, free of disease and severe weight loss, losing skeletal muscle mass over a five year period with the most significant area of loss being in the legs (Gallagher et al., 2000). One possible explanation for these decreases in lower body muscle mass is that the function of the lower body does not maintain fast twitch muscle fibre characteristics while the upper body does (Aoyagi and Shepard, 1992).

More recently, another longitudinal study showed significant losses in muscle strength specific to the anterior portion of the thigh (knee extensor musculature of the thigh), but not the posterior (Frontera et al., 2008). Decline in muscle cross-sectional area followed a similar pattern with decreases being most prominent within the anterior region and not the posterior. This signifies specific decline within the quadriceps muscle, which functionally is critical for lower extremity performance. Activities such as walking and standing up from a chair, are two functional activities that are linked with future risk of disability (Guralnik et al., 1995) and early mortality (Laukkanen et al., 1995) in the aged.

The extent to which a loss of muscle mass contributes to declines in strength is up for debate. Even those older adults that are deemed higher functioning, such as Master Athletes, lose muscle mass and strength into their older years similarly to their sedentary counterparts (Hawkins et al., 2003). What is of interest is that older, strength-trained subjects maintain muscle and strength characteristics similar to

younger untrained subjects (Klitgaard et al., 1990). Nevertheless, age does appear to be an independent predictor of strength, and in older adults strength is associated with muscle mass (Hakkinen et al., 1998; Newman et al., 2003).

Frail elders who have greater baseline levels of muscle mass are able to accrue greater strength when under going resistance training intervention (Fiatarone et al., 1994) and having greater levels of strength is positively associated with greater functional status and exercise capacity in older adults (Baker et al., 2007).

Therefore, despite the uncertain and inexact interactions between the declines in skeletal muscle strength and muscle mass, it is obvious that ameliorating both are essential for the continual high functioning of the aging adult.

Along with skeletal muscle mass, body fat is another component of body composition having significant inverse associations with muscular strength (Newman et al., 2003). With age, there is a general increase in body fat (Durnin and Womersley, 1974) with greater accumulation of fat internally and within the truncal regions (Borkan et al., 1985; Durnin and Womersley, 1974). Such changes in body fat and body fat distribution have been associated with increased risk for cardiovascular disease and its associated risk factors such as insulin resistance and hypercholesterolemia (Despres et al., 1990). When comparing younger populations with older men and women, along with less skeletal muscle mass, older groups have slightly higher body mass indices and higher body fat percentages than their younger counterparts (Baumgartner et al., 1998). Reasons for such changes in body fat levels are partially synonymous to the declines of strength and muscle mass seen with age, namely physical activity levels.

Adequate levels of physical activity have been shown to counteract typical changes seen with body fat levels in older adults (Kohrt et al., 1992). Due to the fact that body fat level is an important risk factor for various morbidities in older adults, attempts to maintain adequate functional status to maintain physical activity levels are essential for the older adult not only to avoid risk factors associated with high body fat but also to preserve skeletal muscle strength and mass.

1.1.2 MUSCLE STRENGTH AND FUNCTIONAL STATUS

Functional status, functional capacity and functional limitations, have been used interchangeably in reporting the health status of the aged. Determining the status of physical ability and mobility of older adults allows health care professionals to intervene if necessary to aid in the avoidance of future disability in older adults.

Muscular strength and mobility-related functions are associated with one another and have been shown to predict early mortality in older adults (Laukkanen et al., 1995). Factors such as walking speed and repeated chair stands are some common mobility-related functions that have been used to examine an individual's functional limitations in daily life. These deceptively simple, yet essential tasks of daily life have been highly associated with leg extensor (Visser et al., 2005) and hand grip strength in older adults (Visser et al., 2000). Such factors have been shown repeatedly to predict future disability and dependence into older ages, with specific attention being directed towards the usefulness of lower extremity performance to singly predict such declines (Guralnik et al., 1995; Rantanen et al., 1999; Guralnik et al., 2000; Penninx et al., 2000; Shinkai et al., 2000; Forrest, 2006).

Lower extremity function is useful when assessing the effects of an exercise intervention program (resistance training plus balance training) on fear of falling with older adults (Arai et al., 2007). The Falls Efficacy Scale (Tinetti et al., 1990), used to measure self-reported fear of falling, revealed the study population to be already highly functioning (according to the high score). Scores did not change as a result of the exercise intervention, however when examining baseline levels of maximum walking velocity (as measured by the time to walk a track length of 16 meters) and knee extensor strength, they found significant negative correlations with the Falls Efficacy Scale (Arai et al., 2007). This suggests that greater functional ability and strength will have a positive impact towards reducing the risk of falling in the elderly. This is essential, as falls in the aged, and the subsequent risk of fracture, can lead to morbidity and early mortality (Tinetti et al., 1988). Additionally, the subjects experienced improvements in lower extremity performance, when measured by the timed “Up & Go” test (measures the time taken to stand up from chair, walk three meters, turn, walk back and sit down without falling or being assisted by another individual (Podsiadlo and Richardson, 1991)). This study exemplifies the need to address the heterogeneity of older adults as the Falls Efficacy self-report scale did not reveal changes, whereas the objective measurements of functional mobility, as shown with the time “Up & Go” chair stand test did.

A ten year of follow-up of older women (65-91 years) showed that despite having higher levels of lower extremity performance in younger “old” years (65-69 years), greater declines were seen at ten year follow-up in the high functioning older women as compared to their less functionally able counterparts (Forrest et al., 2006). This suggests that adults who exhibited already low levels of functional

status were somehow maintaining themselves functionally over the ten year period, whereas the already higher functioning older adults neglected to maintain functionality, and may potentially have had a complacent nature towards undergoing further activity to maintain their functional status.

1.1.3 PHYSICAL ACTIVITY LEVELS AND THE OLDER ADULT

One of the greatest priorities of health organizations is to instill adequate levels of physical activity amongst older adults (Nelson et al., 2007). Compression of morbidity and protection of early mortality occurs in those who maintain chronic high intensity exercise regimes into older ages (Wang et al., 2002). The body's 'need to move' (and move often) is supported from epidemiological investigations that have consistent reports of physical activity levels being inversely related to many unfavourable outcomes seen throughout aging (Fletcher et al., 2001).

Epidemiological investigations, such as the College Alumni Studies (Paffenbarger et al., 1978; Paffenbarger, 1984; Paffenbarger, et al., 1994; Paffenbarger, 1997) and the Women's Health Initiative Observational Study (Manson et al., 2004) demonstrated that chronic disease and mortality are influenced by lifestyle factors such as physical activity levels. The original thought was that past exercise history was the main predictor of age-related chronic disease (Paffenbarger et al., 1984), but now current physical activity patterns show significant benefit when physical activity levels are increased in the older years (Paffenbarger et al., 1997). Intensity of activity is also of importance as those that partake in a regular walking regime, along with engaging in vigorous physical activity have greater reduction from cardiovascular risk as compared to either activity alone; with walking pace (i.e.,

those walking at increasingly faster paces) being a significant contributor to lowered risk (Manson et al., 2004).

These two investigations reveal the importance of maintaining regular physical activity into the older years. They also reveal the need to maintain sufficient physical capacity to undertake intermittent activity that is more intense in nature. These relationships have led to the promotion of physical activity and structured exercise regimes by organizations that promote the prevention of age-related diseases such as coronary heart disease, diabetes mellitus and osteoporosis (Pate et al., 1995; Nelson et al., 2007).

Exercise and physical activity have been considered a possible biomarker of aging in that a lack of them and subsequent declines in aerobic capacity and other physiological processes may lead to a vicious cycle of less activity and further functional decline (Bortz and Bortz, 1996). Physical activity has been suggested to be a prime anabolic stimulus for skeletal muscle and the prevention of sarcopenia (Roubenoff and Hughes, 2000).

The age-associated decline of physical activity levels is partially responsible for losses of physiological capacity with age, along with accompanied reductions in muscle mass, strength and aerobic capacity (Fleg and Lakata, 1988). It may be that the age-associated decline in aerobic capacity is not restricted to aging but to habitual inactivity or disuse (Bortz, 1989). However, even with higher levels of physical activity declines in aerobic capacity persist (Fleg et al., 2005; Tanaka and Seals, 2008). Fleg et al., (2005) found longitudinal declines of peak aerobic capacity in healthy older adults still occurred regardless of higher levels of physical

activity over a 20-year period (Fleg et al., 2005). Master Athlete's who maintain vigorous levels of aerobic activity into older ages, still experience the age-related declines in aerobic capacity (Tanaka and Seals, 2008). As 35% of the decline in aerobic capacity of trained and sedentary men is due to age-related decreases in muscle mass (Rosen et al., 1998), it could be that the involuntary loss of muscle mass with aging, or sarcopenia, is the culprit for these inevitable declines in VO_{2max} .

Given that skeletal muscle is the primary oxygen consuming organ during maximal exercise, and given that skeletal muscle mass decreases with age along with maximal aerobic capacity, this could suggest improving muscle mass as a prime target to ameliorate such declines (Fleg and Lakata, 1988). Other factors such as basal oxygen consumption (Tzankoff and Norris, 1977) and glucose tolerance (Kolterman et al., 1980) are affected by the age-associated declines of skeletal muscle mass. Therefore methods to improved muscle mass and associated strength are important from a physical, functional and metabolic perspective in older adults.

This complex interdependence among factors such as physical activity levels, sarcopenia, functional ability and aerobic capacity support the importance of moderate to high levels of physical activity for avoidance of age-associated morbidities and improved quality of life. This demonstrates the multi-dimensional nature of choosing the most appropriate interventions for the aging adult.

1.2 INTERVENTION

Not all age-related bodily changes are necessarily inevitable. General thinking has gone from the inevitable loss of muscular strength, mass and function in aging

populations, to the ability to reverse and ameliorate these common traits by lifestyle choice and intervention. There is increasing evidence pointing to the multi-dimensional benefits accrued when engaging in various forms of physical activity and even more so with structured higher intensity exercise.

Older adults that are involved in some form of structured exercise have shown enhanced benefits such as greater muscular strength (Ringsberg et al., 2001), less body fat mass (Kohrt et al., 1992), enhanced skeletal muscle morphology (Klitgaard et al., 1990), improved lower extremity performance and decreased prevalence of osteoporosis (Brach et al., 2004) as compared to their sedentary and even physically active but untrained counterparts. Elderly women who participated in a structured exercise class of one hour per week over a 20-year period demonstrated greater quadriceps strength, grip strength, and improved balance and gait (Ringsberg et al., 2001). Other investigations comparing not only the differences between active and sedentary older subjects, but also the difference in training method (Klitgaard et al., 1990) and exercise background (Brach et al., 2004) in active older adults have demonstrated that specificity of exercise is important into older ages (Brooks et al., 1996) and that structured exercise (i.e., strength training and higher intensity aerobic training) provides the best solution for maintaining muscle mass and its associated strength.

Although increasing caloric output in the form of physical activity has been shown to decrease the risk of premature mortality (Paffenbarger et al., 1997), when older adults expend the same amounts of calories in a given week, those who formally exercise train (aerobic dance, weight lifting, jogging, swimming) had higher scores on functional tests that measure lower extremity performance, such as one-leg

standing balance, chair sit-to-stands and gait speed tests compared to those who were active by daily pursuits such as walking, gardening and volunteer work (Brach et al., 2004). Those who formally exercise trained showed the lowest disease rates for heart disease, diabetes mellitus and osteoporosis versus those who were habitually active.

Earlier research-based interventions aimed at the aging population were targeted around endurance-based activities as they were seen as easy, effective and required little to no instruction (Meredith et al., 1989; Coggan et al., 1992; Green et al., 1995). It is now accepted that structured exercise, and that of higher intensity, is a necessity for all adults and especially the aged in order to optimize the aging process. It is possible that if strength levels are already substantially reduced with an older adult due to habitual disuse, exercise training that targets primarily muscular strength, namely resistance training, may be required before they can participate meaningfully in aerobic exercise (Fletcher et al., 2001). Exercise training in the form of resistance training is now being seen as a form of structured exercised that will greatly benefit the older adult.

1.2.1 RESISTANCE TRAINING

Structured exercise, specifically in the form of resistance training is becoming increasingly accepted and practiced in older adult populations. Once thought to be primarily for bodybuilding, sports enhancement and orthopaedic rehabilitation, it is becoming a common tool used by the general population for general health and touted as essential for older populations (Nelson et al., 2007).

Also called strength training or weight training, resistance training is a term used to depict a form of exercise that entails movement of the body, via its musculature, against an opposing force with the use of devices (e.g., machines, free weights, elastic tubing) that provide such opposing forces (Evans et al., 1999; Fleck and Kraemer, 2004). The principles and practices of resistance training are vast; for reference and explicit description of various systems and protocols see (Baechle and Earle, 2000; Fleck and Kraemer, 2004).

In summary many variables are to be considered when undergoing resistance training regimes. Length of training schedule, frequency of training, intensity, sets, repetitions, rest periods, and speed of movement will vary depending on the desired outcomes. Improvements in muscular strength, endurance, hypertrophy and power are some of the more common outcomes achieved through various resistance training modalities. Each outcome requires a varied manipulation of training variables (e.g., frequency of training, reps, sets, rest period, speed).

Adaptations made as a result of resistance training are extensive (for review - Folland and Williams, 2007). Within the muscle morphological changes that occur such as increases in whole muscle size and increased muscle cross-sectional area are believed to be due to muscle fibre hypertrophy, with preferential hypertrophy of Type II fibres, changes to architectural characteristics of the muscle and hyperplasia (Folland and Williams, 2007). For review see McDonagh and Davies (1984). Equally as important are neurological adaptations made during resistance training. Disproportionate increases in strength that are typically seen in the early training stages (6-10 weeks) attest to such adaptations. The additional effects of cross-over training and strength improvements that are seen in non-exercised limbs,

as well as improvement in strength with imagined training stimulus, all validate the importance of motor learning and enhanced coordination during resistance training (Folland and Williams, 2007).

1.2.2 RESISTANCE TRAINING AND THE OLDER ADULT

The benefits of resistance training interventions with older populations on factors such as skeletal muscle strength and mass, the prevention of osteoporosis and balance performance, along with the efficacy of combined resistance training interventions that incorporate aerobic and/or dietary interventions have been extensively reviewed (Fielding, 1995; Layne and Nelson, 1999; Porter, 2001; Benjamin and Carson, 2004; Cyarto et al., 2004; Breen et al., 2007; Campbell and Leidy, 2007; Orr et al., 2008).

Along with enhancement of physical improvements, older adults benefit from exercise through improvement in other health-related parameters such as aerobic capacity (Frontera et al., 1990), cognitive function (Singh et al., 1997) enhanced lipoprotein profiles (Fahlman et al., 2002), dynamic balance and spontaneous levels of physical activity (Nelson et al., 1991; Fiatarone et al., 1994).

There is still debate as to the most ideal and realistic training stimulus for an older adult to accrue substantial gains in muscular strength and mass that will allow them to maintain physical and functional capacities needed to maintain independent living and a high quality of life. Additionally, due to the importance of resistance training as a form of structured exercised, various guidelines and recommendations for older adult populations have been published (Feigenbaum and Pollock, 1997;

Mazzeo et al., 1998; Evans et al., 1999; Pollock et al. 2000; Fletcher et al., 2001; Nelson et al., 2007; Williams et al., 2007).

Most commonly, they recommend moderate intensities of effort (10-15 reps or ~65-75% 1RM) that would equate to one set of ten to 15 repetitions for older adults, performed twice per week using major muscle groups. They further stress the importance of allowing for adaptations to take place within the musculoskeletal system, the practice of proper technique and the use of moderate levels of intensity to maximize the potential for exercise sets to be completed. To facilitate improvements in strength and endurance over time, the manipulation of variables such as number of repetitions, sets, increasing load and decreasing the rest period between sets is suggested. Increasing number of repetitions is recommended as the initial variable to manipulate, with training load increasing after the individual can perform twelve to 15 repetitions comfortably. High-intensity resistance training (80% 1 RM) has been suggested as an option for older adults that possess: sufficient levels of fitness; are supervised; and have appropriate experience and knowledge of the exercises (Nelson et al., 2007).

The American College of Sports Medicine (ACSM) and the American Heart Association (AHA) have published the most up to date recommendations for physical activity in older adults, and these include resistance training (Nelson et al., 2007). A summary of the guidelines can be found in Appendix H

1.2.3 RESISTANCE TRAINING INTERVENTIONS AND THE OLDER ADULT

Resistance training interventions have been used with a variety of older adult populations having a range of levels of health and functional status (e.g., healthy (Hebuterne et al., 2001), sedentary (Nelson et al., 1994), frail (Fiatarone et al., 1994), depressed (Singh et al., 2005), low bone mass (Liu-Ambrose et al., 2004), chronic heart failure (Pu et al., 2001) and renal insufficiency (Castenada et al., 2001)) using an array of training protocols and intensities.

In addition to the vast nature of training variables that can be manipulated within interventions, assessing improvements and other health-related factors associated within them is equally diverse. In the past, changes in muscular strength, muscle mass and body composition were mainly addressed. Now measurements of physical activity status and lower extremity performance (LEP) are becoming almost a “gold standard” in investigating these populations due to their strong association with disability and early mortality (Guralnik et al., 1994).

Frontera and coworkers administered high-intensity resistance training to older men over a period of twelve weeks at a time when such high intensity protocols were not the norm with older adults (Frontera et al., 1988). Subjects underwent supervised and progressive resistance training at high-intensity (80% 1RM, 3 sets, 8 reps, 3 times per week) for the knee flexors and extensors. Significant improvements in muscle strength and muscle mass were found through assessment of one-repetition maximum (1RM) and computed tomography, respectively. At study completion strength of the knee extensors and flexors had increased by 107% and 227%, respectively. Changes within the thigh musculature showed increases of

5%, 11%, 9% in total thigh area, total muscle area and quadriceps area, respectively.

Since then, other resistance training interventions have followed similar protocols in healthy individuals (Frontera et al., 1990). In an attempt to elucidate the most appropriate training stimulus and volume for this older population, a range of training variables have been manipulated such as familiarization periods prior to strength testing (Henwood et al., 2008), training duration (Henwood and Taaffe, 2006), training intensity (Vincent et al., 2002), sets performed (Galvao and Taaffe, 2005), frequency per week (DiFrancisco-Donoghue et al., 2007), use of periodization (Esmarck et al., 2001; Izquierdo et al., 2004), alone and in combination with endurance (Izquierdo et al., 2004) and functional training regimes (Arai et al., 2007).

The ability to detect changes within body composition as a result of high-intensity resistance training interventions is challenging, but nonetheless important. To compare and contrast various body composition measurement techniques Nelson et al., (1996) studied postmenopausal women after a one year strength training intervention using an array of techniques specifically designed to assess various aspects of body composition (Nelson et al., 1996). A large variation was found in the ability of various techniques to detect change over this intervention period (Nelson et al., 1996). (For detailed explanation and description of techniques see Lohman's *Advances in Body Composition Assessment* (Lohman, 1992) as such review goes beyond the scope of this study). Computed tomography (CT) of the mid-thigh and the 24-hour creatinine method were found to be the most sensitive in detecting specific increases in muscle mass, whereas anthropometry was unable to

detect changes to gross body composition (percentage body fat). Other whole body analysis methods involving measurement of bodily constituents, such as potassium, which assumes cellular potassium concentration to be a constant component of lean body mass, revealed an increase in total body potassium within the strength trained group as compared to the control group therefore signifying an increase in lean tissue.

Although such highly sensitive techniques are useful for detecting change, they are often not feasible to use in “real-life” situations (for example, in public fitness and gym-type settings where older adults may undertake a structured resistance training program). Therefore, it may be valuable to determine if standard methods of body composition measurement are still warranted, despite reports of such crude measurements not being sufficiently sensitive to detect changes.

Izquierdo et al., (2004) found that percentage body fat, as estimated by skinfold thickness, decreased during 16 weeks of strength training with healthy older men (Izquierdo et al., 2004). Training was twice weekly, whole body and progressive with the first eight weeks consisting of low intensity resistance training and the last 8 weeks being of high intensity resistance training. Interestingly, they compared this strength trained group with a cycling endurance group and a combined strength + endurance group (strength one day and endurance protocol one day over a week). In contrast to the strength only group the percentage body fat of the other groups remained unchanged with these protocols. Muscle cross-sectional area and strength increases were similar in both strength and combined groups, with increases of 11% recorded in quadricep muscle cross-sectional area.

Hagberg et al., (1989) also found an amelioration of body composition status as assessed by anthropometry during a resistance training intervention in older adults (Hagberg et al., 1989). Sum of seven skinfolds (mm) decreased from 158 to 148 when older men and women underwent whole body resistance training of moderate intensity over a 26-week period (Hagberg et al., 1989).

In contrast to the two aforementioned studies, no changes to body composition were recorded with healthy older men who underwent ten weeks of whole body resistance training when assessments were made by anthropometry (Hakkinen et al., 1998). However, muscular strength and mass both increased (maximal isometric peak force, 16.5% and quadricep cross-sectional area, 8.5%). Training was slightly different (3 times per week: hypertrophy (8-10 RM) training one day, strength (3-5RM) training one day, and power (15RM, high velocity, 6-8 reps) training on the third day), with sets increasing from three to six over the course of the ten weeks (Hakkinen et al., 1998).

The shorter ten week training duration illustrates that changes are occurring to muscle by this time (Hakkinen et al., 1998), however their failure to find changes in body composition could have been due to this shorter intervention period not inducing measurable change and not the failure of anthropometry to detect changes. Hagberg et al., (1989) and Izquierdo et al., (2004) found changes with longer training periods.

Regional specific changes have been observed in older women and men, with increases in mid-thigh cross-sectional area and decreased in mid-thigh subcutaneous fat in response to 16 weeks of whole body resistance training (Treuth

and Hunter, 1995; Treuth et al., 1994). These two studies varied in training intensities, however the older women experienced decreased mid-thigh subcutaneous adipose tissue of 5.8% (Treuth and Hunter, 1995) and older men experienced similar changes with decreased mid-thigh subcutaneous adipose tissue of 9%. Additionally the women experienced decreased intra-abdominal adipose tissue of 9.7% (Treuth and Hunter, 1995). Anthropometric changes were not observed in the mid-thigh using either girth or skinfold measurements.

Along with variations in training periods with the above studies, other reasons for conflicting results seen in body composition as assessed by anthropometry could be due to variations with training protocols or lack of precision in skinfold measurement technique.

In shorter resistance training interventions (eight to twelve weeks) (Frontera et al., 1988; Frontera et al., 1990;) significant strength and muscle mass gains are achieved with high-intensity training. Some studies have shown that lower intensities can improve strength and mobility-related variables, such as lower extremity performance, to the same extent, however these interventions are of longer duration (24 weeks) (Vincent et al., 2002; Fatouros et al., 2005).

Differences between strength gains and lower extremity performance were not detected by stair climbing, in comparing the effects of either low or high resistance training in healthy older adults over a 24 week period (Vincent et al., 2002).

However, in healthy older men who underwent 24 weeks of whole body resistance training of either high intensity resistance training or low intensity resistance training, both groups had significant improvements in all variables, yet the high

intensity group experienced greater gains (63-91% strength, 17-55% anaerobic power, 9-14% mobility) in comparison to the low intensity group (42-66% strength, 10% anaerobic power, 5-7% mobility) (Fatouros et al., 2005). Interestingly, they studied a 48-week detraining period immediately after the intervention and those in the high intensity group maintained their improvements in strength, peak power and lower extremity performance, while the low intensity resistance training group had lost the improvements gained during the intervention period (Fatouros et al., 2005).

These two studies suggest that although low intensity training does elicit positive and significant changes to muscle strength and functional parameters in older adults, high intensity resistance training offers added strength reserves when resistance training is not regularly practiced (i.e., during a detraining period). The subjects of Vincent et al., (2002) and Fatouros et al., (2005) underwent whole body resistance training as recommended by experts (Nelson et al., 2007), however there is a possibility that such comprehensive programs could deter the older adult from pursuing resistance training as a structured regular form of exercise. With the older adult unaccustomed to resistance training and the importance of adequate training adherence for gains to be made, higher intensities seem to offer the best outcomes, with shorter bouts of intervention being equally beneficial (Frontera et al., 1988; Frontera et al., 1990).

In early studies subjects used only lower extremity exercises (leg extension and flexion) (Frontera et al., 1988; Frontera et al., 1990) and it is unknown if the improved measures related to lower extremity performance measures, which are now commonly assessed in older adults due to their ability to predict future

disability (Guralnik et al., 1995). Assessing lower extremity performance as opposed to solely measuring improvements in muscular strength specific to the exercise performed is becoming more common in order to determine grand scale effects of interventions on other facets of daily living with older adults.

Walking speed, as measured over a ten meter walking course, improved when older women underwent 18 weeks of progressive overload resistance training (Familiarization week *plus* week 1:60% 1RM, week 2-12: 70% 1RM, week 13-16: 75% 1RM, 3-4 sets, 8 reps, three times per week) using solely lower extremity exercises (leg press, leg extension curl, calf/heel raise) (Sipila et al., 1996). This was in addition to increased isometric knee strength (knee extension and flexion), Improved lower extremity performance has been reported in cohorts that have lower functional capacity and muscle reserve (Fiatarone et al., 1994) as well as in already highly functioning older adults (Arai et al., 2007). Frail elderly subjects who undertook a ten week high intensity resistance training intervention solely targeting lower extremity musculature, improved muscle strength and muscle mass as well as lower extremity performance assessments when compared to non-exercised subjects (Fiatarone et al., 1994).

Healthy older adults who undertook a combined twelve week resistance training and balance training program that focused mainly on lower body musculature twice per week had improvements in lower extremity performance as measured by the timed “Up & Go” test (Arai et al., 2007). This test measures the time taken to stand up from a chair, walk three meters, turn, walk back and sit down without falling or being assisted by another individual (Podsiadlo and Richardson, 1991). Maximum walking velocity (as measured by the time to walk a track length of 16

meters) and knee extensor strength were also correlated negatively with the falls efficacy scale, a subjective measure for the fear of falling in the elderly (Tinetti et al., 1990), which again confirms the importance of lower extremity function for enhanced quality of life and avoidance of disability.

Lower extremity performance was also improved in older adults (60-90 years) with shorter intervention periods of eight weeks that followed progressive overload principles (Schot et al., 2003). Details of exact time points for increasing intensity were not reported, however after a familiarization period of lower intensity training, training intensity was increased with the final three weeks of intervention being solely high intensity. Total body and lower body strength increased by 161% and 196% respectively and sit-to-stand performance improved.

Use of a familiarization phase prior to commencing the high intensity phase (Sipila et al., 1996; Schot et al., 2003) is another key element that differs among studies. Again, such periodized and progressive approaches with older adults may be more indicative of real-life situations (e.g., as would occur if an older adult was to commence a resistance training program at a gymnasium or recreation centre) in contrast to studies that encourage the loads of high intensity to be attained by the third or fourth training session (Fiatarone et al., 1990; Baker et al., 2007).

The ACSM/AHA guidelines for resistance training and the older adults now encourage such familiarization practices (Nelson et al., 2007). They are used to minimize risk of injury and allow for adaptations to take place gradually with inexperienced older populations. Given the large neural gains typically seen in strength during the first few weeks of resistance training (Sale, 1988) the accuracy

of strength gains can be greatly skewed if strength testing occurs before a proper familiarization period and technique is optimized..

One study used a familiarization period that consisted of a two week conditioning phase of lower to moderate intensity followed by strength testing prior the start of their higher intensity intervention over 24 weeks (Henwood et al., 2008). In a similar study, subjects underwent six weeks of progressive and moderate resistance training followed by six weeks of high intensity resistance training (Esmarck et al., 2001). Despite being only half the duration, this twelve week intervention produced significant increases in strength for their subjects with the assistance of a familiarization period and a shorter bout of high-intensity resistance training.

The ACSM/AHA guidelines also suggest that older adults engage in a minimum of two resistance training sessions per week (Nelson et al., 2007). The manipulation of training volume (days trained per week, number of sets) has been studied, with three days per week being more commonly prescribed. Programs of one and two day per week may be equally effective when training at higher intensity (Taaffe et al., 1999). Groups undergoing high-intensity resistance training one, two or three times per week for 24 weeks had no differences in strength gains. Lower extremity performance measured by the time to rise from a chair also improved significantly in all three groups. Chair rise time was also significantly associated with changes seen in quadricep strength and in lean muscle mass (Taaffe et al., 1999).

The same research group found similar improvements in lower extremity performance and significant strength gains with twice weekly higher intensity training over 24 weeks (Henwood et al., 2008). Similarly, a one year, twice weekly,

high-intensity resistance training intervention with women produced improvements that were indirectly related to the training stimulus (Nelson et al., 1994).

Two separate one year exercise interventions; one involving a walking program (Nelson et al., 1991) and the other involving a whole body high-intensity resistance training program (Nelson et al., 1994) revealed improvements in bone mineral density (the main focus of both studies). Several other changes were revealed after the resistance program only. Activity levels as determined by energy expenditures increased in the resistance training group by 27%, with controls decreasing by 25%. The time to complete a dynamic backward tandem walk improved in the resistance training group, and total body muscle mass increased. The resistance training group gained 1.2 kilograms of total body muscle mass as measured by urinary creatinine excretion method, whereas the controls lost 0.5 kilograms over the year. Therefore, even though the one year walking intervention was of higher intensity and greater volume than the high-intensity strength training regime, the resistance training intervention had other health and mobility-related benefits that were non-specific to the intervention.

Increased voluntary physical activity was also seen in frail elderly subjects undergoing strictly lower body and high-intensity resistance training (Fiatarone et al., 1994). Although studying different factors comparable improvements in mid-thigh muscle cross-sectional area were also found with either whole body or lower body high-intensity resistance training with older men and women over a twelve week period (Campbell et al., 2002).

Benefits of resistance training can therefore be demonstrated not only with longer training protocols (24–52 weeks) using five to six whole body exercises (Nelson et al., 1994; Taaffe et al., 1999; Henwood et al., 2008), but also with shorter intervention periods and strictly lower body exercises (Frontera et al., 1988; Frontera et al., 1990; Fiatarone et al., 1994; Campbell et al., 2002).

From the diverse evidence above, it is without a doubt that complexity exists in terms of resistance training and what it can offer the older adult. It is important to consider the ability of one form of structured exercise to benefit such a wide range of health-related factors associated with successful aging. Thought must be put towards the types of interventions that will provided the best outcomes for the success of the aging adult, while also being realistic towards what a normal everyday population can and will pursue when not in a research setting. With shorter, lower extremity based training regimes providing improvements in muscle composition, strength (substantiated in a systematic review and meta-analysis of more than 40 studies, most of 8-12 weeks in duration (Latham et al., 2004)) and other aspects of body composition, it may be that such shorter and concise practices would be of greater appeal to older adults who are inexperienced and new to such structured exercise. It may also be of interest to investigate other lifestyle related factors, such as nutrition, that may enhance the ability for improvements in muscle strength and mass to occur while undergoing such training interventions.

1.2.4 PROTEIN NUTRITION AND RESISTANCE TRAINING

INTERVENTIONS

Nutrition is an important lifestyle factor closely related to human performance.

Athletes often use dietary practices to gain a competitive edge and often turn to such practices when training levels have reached optimal levels and body

composition can't be further enhanced (Burke and Deakin, 2000). Older adults who are not at peak levels of physical performance and are less physically conditioned may also benefit by turning to practices that enhance nutritional status and improve muscle strength and mass when undergoing training protocols.

It has been suggested that the recommended daily allowance (RDA) for protein is not sufficient for older adults to maintain skeletal muscle reserves (Campbell et al., 2001). Over a 14-week period, older men and women given a diet that provided the RDA for protein of 0.8 g/kg/day, plus adequate levels of carbohydrate and fat, exhibited decreased mid-thigh muscle mass, although subjects maintained their strength.

There is also a question as to whether or not protein requirements are greater for older adults undergoing resistance training in order to maximize muscle accretion, hence leading to other enhancements in whole body function. One study of high-intensity resistance training plus a metabolically controlled dietary intervention of either the RDA for protein 0.8g/kg/d, or twice the RDA 1.6g/kg/day controlled for increases in energy expended in resistance training by providing extra energy in the form of low-protein foods beginning on the first day of resistance training (Campbell et al., 1994). After twelve weeks there were significant increases in dynamic muscular strength of the lower extremities as well as increases in fat-free

mass that were similar in both protein groups. Therefore the high protein diet did not enhance body composition status.

However these two studies were based on strictly lacto-ovo-vegetarian foods, therefore protein in the form of animal striated tissue (i.e. beef, poultry, fish, pork) was not consumed during the controlled 14 week period.

Nutritional supplementation can benefit older adult men in conjunction with high-intensity resistance training (Meredith et al., 1992). While training with or without supplementation produced similar increases in strength, the supplemented group had significant increases in thigh girth with only a small increase in the control group. Due to the mixed nature of the supplement it was not possible to determine the actual nutrient that was responsible for the gain in skeletal muscle.

Two studies that attempted to determine whether or not animal versus lacto-ovo protein sources will influence outcome on strength and muscle have yielded conflicting results (Campbell et al., 1999; Haub et al., 2002). The difference between type of protein was explored by comparing an omnivorous diet with a lacto-ovo-vegetarian diet in conjunction with combined high-intensity resistance training in older men (Campbell et al., 1999). Strength improved in both dietary groups and was independent of diet. Body composition changes were different among those eating animal products in the self-selected diet versus those eating lacto-ovo-vegetarian based protein. Whole body muscle mass and density along with fat-free mass increased in the omnivorous group whereas these values decreased in the lacto-ovo-vegetarian group. There was a trend of decreased percentage body fat in the omnivorous group while it increased in the lacto-ovo-

vegetarian group. Significant changes seen in muscle composition showed increases in type II muscle fibre area of 16% in the omnivorous group and 7% in the lacto-ovo-vegetarian group. They suggested that some unique properties of meat may contribute to this change, however they were not able to distinguish the difference because of the contributions of different meat sources.

A contrasting study found no difference in strength or muscle cross-sectional area gains, when comparing a lacto-ovo-vegetarian diet with a beef-containing diet while older men underwent twelve weeks of high-intensity resistance training (Haub et al., 2002).

1.3 SUMMARY

If interventions such as high intensity resistance training can enhance physiological function and are safe for older adults, then focusing primarily on enhancing skeletal muscle function through more structured activity, such as resistance training may serve as a foundation for healthy older individuals to maintain and improve other parameters health. If interactions do exist between skeletal strength, muscle mass, physical activity levels, aerobic capacity, functional ability and dietary factors, then study of these interactions, be it complex, is warranted. The proposal that protein is an important contributor to new muscle accretion and strength considered together with the reported low red meat intake of older Australians suggests that a combination of resistance training and increased meat intake could provide additional benefit.

1.4 AIMS AND HYPOTHESIS

In order to advance preventative aspects of healthy aging, there is a need to investigate shorter, progressive, high-intensity and lower extremity based resistance training interventions with healthy older adults to complement the research conducted on the frail elderly and observations from other younger adult populations. It is equally important to examine the effects of such interventions on broad components of physical health such as physical activity status and lower extremity performance. Due to the conflicting evidence of the role of protein nutrition in the improvement of muscular strength, mass and body composition in the older adult this study was designed to address the ability for animal protein and associated nutrients, namely from red meat, to affect skeletal muscle strength, mass and performance in healthy older adults.

It was hypothesized that:

- i. Red meat intake in older adults is low compared to published intakes for younger adults.
- ii. Red meat as a dietary intervention of moderate to high levels will be well tolerated by the subject population of older adults.
- iii. Resistance exercise training of higher intensity will be well tolerated by older adults and will improve their muscular strength and muscle mass.
- iv. Resistance exercise training will improve body composition status in healthy older adults.
- v. High meat eaters would have significantly greater improvements in muscular strength and muscle mass.
- vi. Strength and muscle mass will be greater in males compared to females.
- vii. Resistance exercise training will improve functional status and physical activity levels in healthy older adults.

Chapter Two

-Methods-

*"That which is used develops, and that which is not used wastes away."
Hippocrates*

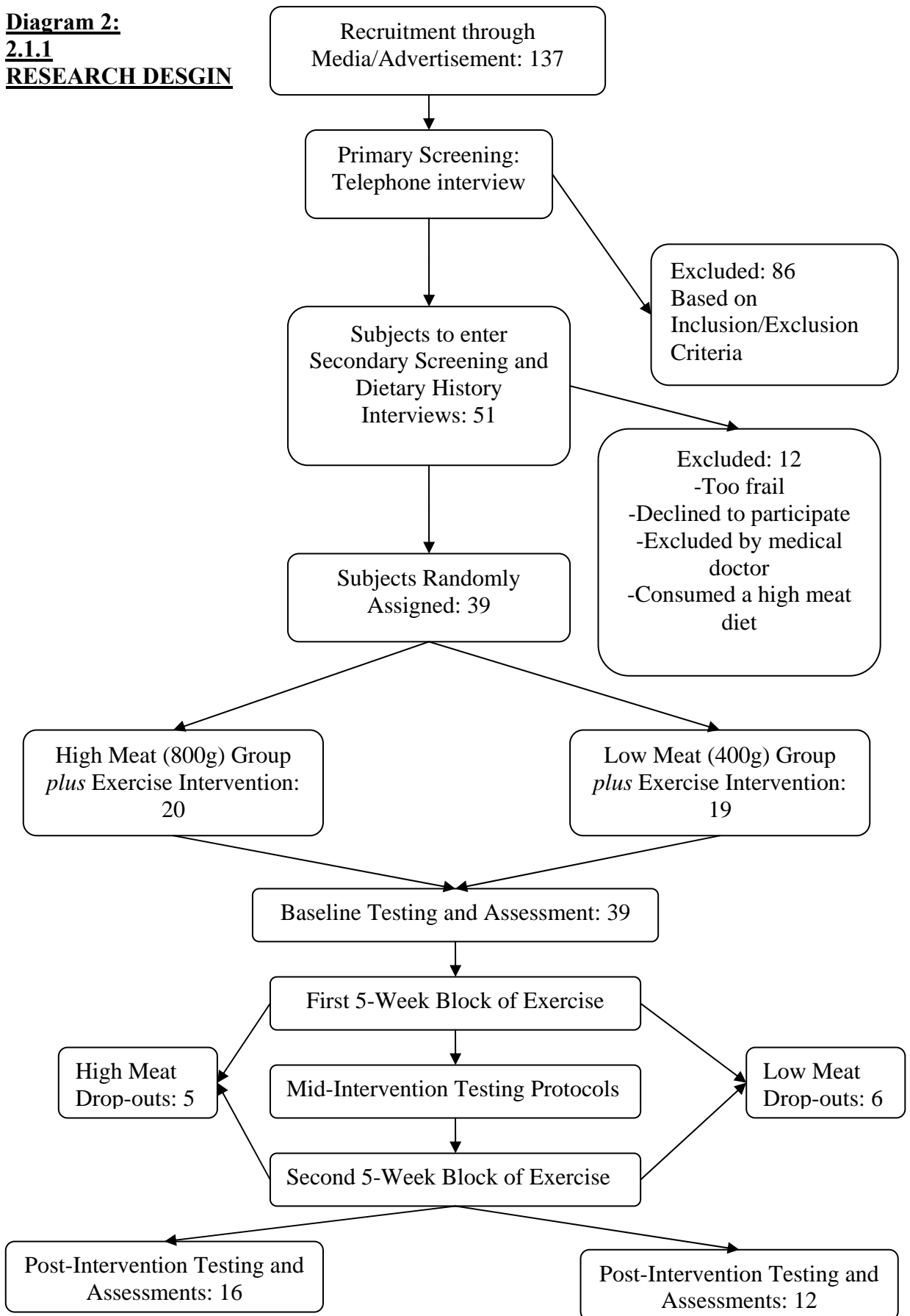
2.1 INTRODUCTION

The combined exercise and dietary intervention was conducted through the University of Wollongong's Department of Biomedical Science and Smart Foods Centre. The research design conducted over a twelve week period assessed the effectiveness of a combined resistance training and dietary intervention in improving skeletal muscle structure and function. Physical testing procedures, assessments and intervention protocols were administered between the months of July and December.

Due to the comprehensive nature of the methodology separate introductions will be used to describe the purpose of each section. Assessments used covered a broad range of health and physiological parameters in accordance with past research, access to resources and time limitations.

See Diagram 2 on the following page which depicts the general design of the methodology from pre- to post-intervention testing protocols.

Diagram 2:
2.1.1
RESEARCH DESIGN



2.2 SUBJECTS

2.2.1 SUBJECT RECRUITMENT

Community-dwelling retired individuals between the ages of 65-80 years were the population sought for this study and were recruited from community-dwelling individuals in the Illawarra area of New South Wales, Australia. Recruitment was made through a local newspaper article and radio advertisement. Involvement of couples (i.e. Married couples, siblings living together) was encouraged during the recruitment process; however individual volunteers were accepted as well. The rationale for encouraging involvement of couples was to aid compliance with meat consumption and administration of the study as intervention protocols required constant contact with subjects for scheduling purposes as well as the weekly delivery of the dietary intervention, red meat.

Past studies using this specific population in resistance training interventions have proven to be safe and effective for improving various aspects of physiological function, namely improved muscular strength. However, due to the high intensity nature of the intervention and the potential for musculoskeletal injury and cardiovascular strain it was necessary to perform a screening protocol that would identify those volunteers that could potentially be at risk during exercise training.

The study was conducted within the University of Wollongong's Department of Biomedical Science and Smart Foods Centre and was approved by the University of Wollongong/Illawarra Area Health Service Human Research Ethics Committee under ethics CT KM HE02/069.

2.2.2 SCREENING

2.2.2.1 Primary Screening

Potential subjects were required to be healthy, independently living individuals and were considered if they satisfied our inclusion/exclusion criteria. This primary screening was conducted by telephone. If interested volunteers fulfilled our criteria and remained willing to participate, a Subject Information Package (Appendix A) was sent to them detailing the study requirements. They were then scheduled to attend the remaining screening procedures at the University of Wollongong.

Inclusion/Exclusion Criteria

Inclusion:

- Age; between 65-80 years.
- They consumed red meat (beef and/or lamb).
- They regularly consumed a *low/moderate amount of red meat per week.
- Willingness to increase or decrease their red meat intake for the duration of the study.
- Not currently or recently involved (not in past 12 months) in any resistance training.
- Ability to commit to 12 weeks of intervention and the additional screening and testing procedures for pre and post-intervention.
- Ability to transport themselves to and from study and testing locations.
- Ability to cook personal meals.

Exclusion:

- They regularly consumed a **high amount of red meat per week.
- Any known bone, muscle or joint problems that may be aggravated by resistance training.
- Having fractured any bones in the past 6 months.
- Any other medical/health condition that may prevent a consistent resistance -training regimen.

*Low was classified as having equal to or less than three main meals per week that were based around red meat.

** High was classified as having greater than three main meals per week that were based around red meat.

-Red meat was defined as beef, veal or lamb.

2.2.2.2 Secondary Screening

The secondary screening procedures were administered over two days and included; medical contraindications to undertaking an exercise training and evaluation program. It was conducted through medical questionnaire, electrocardiogram (ECG), fasting blood, urinalysis, and with physician oversight and approval.

During the first day each potential subject was met by the primary investigator and a thorough review of the subject information package took place while addressing any questions or concerns that arose during this time. If the volunteers were still eager to participate they were asked to read and sign an informed consent form (Appendix B).

Subjects then filled out a medical questionnaire specifically designed for older adults embarking on a vigorous exercise training regime (Evans et al., 1999) (Appendix C). This medical questionnaire was developed by Maria Fiatarone, M.D., and has been used effectively for identifying high risk individuals who are over the age of fifty. Two twelve lead resting ECG (supine and standing positions), fasting blood and morning urine samples were taken by a qualified individual familiar and experienced with such techniques. The Faculty of Health and Behavioural Science's medical doctor assessed results and volunteers were excluded based on the exclusion criteria for contraindications to exercise from the American Heart Association's Exercise Standards for Testing and Training (Fletcher et al., 2001) (Appendix D).

Once volunteers satisfied the above criteria they returned to the University of Wollongong for the second day of screening procedures. This involved meeting with the Faculty's medical doctor and reviewing the information presented on the medical questionnaire as well as the results of the twelve lead ECG, blood and urine analysis. Upon approval from the Faculty's medical doctor the subjects were required to successfully complete the One Mile Walk Test (MWT) which is an indirect sub maximal oxygen consumption test (Kline et al., 1987) that was to be used as one of the physical testing procedure. (See section 2.4.2.3 - MWT protocol). On completion of the MWT test potential subjects were assessed with dietary history interviews to for the final screening stage of study eligibility.

2.2.2.2.1 Final Subject Break-Down

A total of one hundred and thirty eight (n=137) individuals contacted the primary investigator through our recruitment process of local newspaper and radio advertisements. Upon completion of the primary screening protocol (initial telephone interview), eighty-six (n=86) were excluded as they did not satisfy the inclusion/exclusion criteria (page 43). The major exclusion criteria related to health problems that might be aggravated by resistance training and the inability to commit to regular attendance for the full duration of the study. Fifty-one (N=51) underwent secondary screening protocols and twelve (n=12) were further excluded due to: electrocardiogram abnormalities (n=8); severe frailty (n=2); husband not participating (n=1); and baseline meat intake being too high (n=1). Thirty-nine (N=39) remained and were matched and randomized into experimental (n=20) and control (n=19) groups.

Cited medical ailments of the 39 subjects included: vision and hearing problems (n=10), unusual musculoskeletal pain in the past six months (n=3), chronic musculoskeletal problems such as osteoporosis and arthritis (n=6), fracture or joint replacement (n=1) and prescription medications for high cholesterol (n=7), hypertension (n=5) and hormone replacement therapy (n=1). See Diagram 2.

2.2.2.2.2 Dietary History Interviews

Each subject underwent open-ended diet history interviews that were conducted by qualified registered dietitians working within the University of Wollongong's Smart Foods Centre. The diet history interview was chosen as the most suitable method of assessment due to its comprehensive nature. Subjects who participated as a couple were interviewed together with their partner. The format of the diet history interview was specifically adapted to allow for a more comprehensive assessment of red meat consumption (Appendix E).

Diet history interviews were used to assess usual dietary intakes in terms of energy and macronutrients while making specific enquiry on consumption patterns and amounts of red meat (beef, veal and lamb) eaten on a weekly basis. As the preliminary screening criteria were crude and did not allow for an exact determination of the low self-reported amounts of red meat eaten per week it was necessary to establish the actual quantity and types of red meat regularly eaten by the recruited volunteers. Upon analysis of the baseline diet history interviews the design of the dietary intervention and grouping of subjects could be accomplished (See section 2.3.1 - Design and randomization).

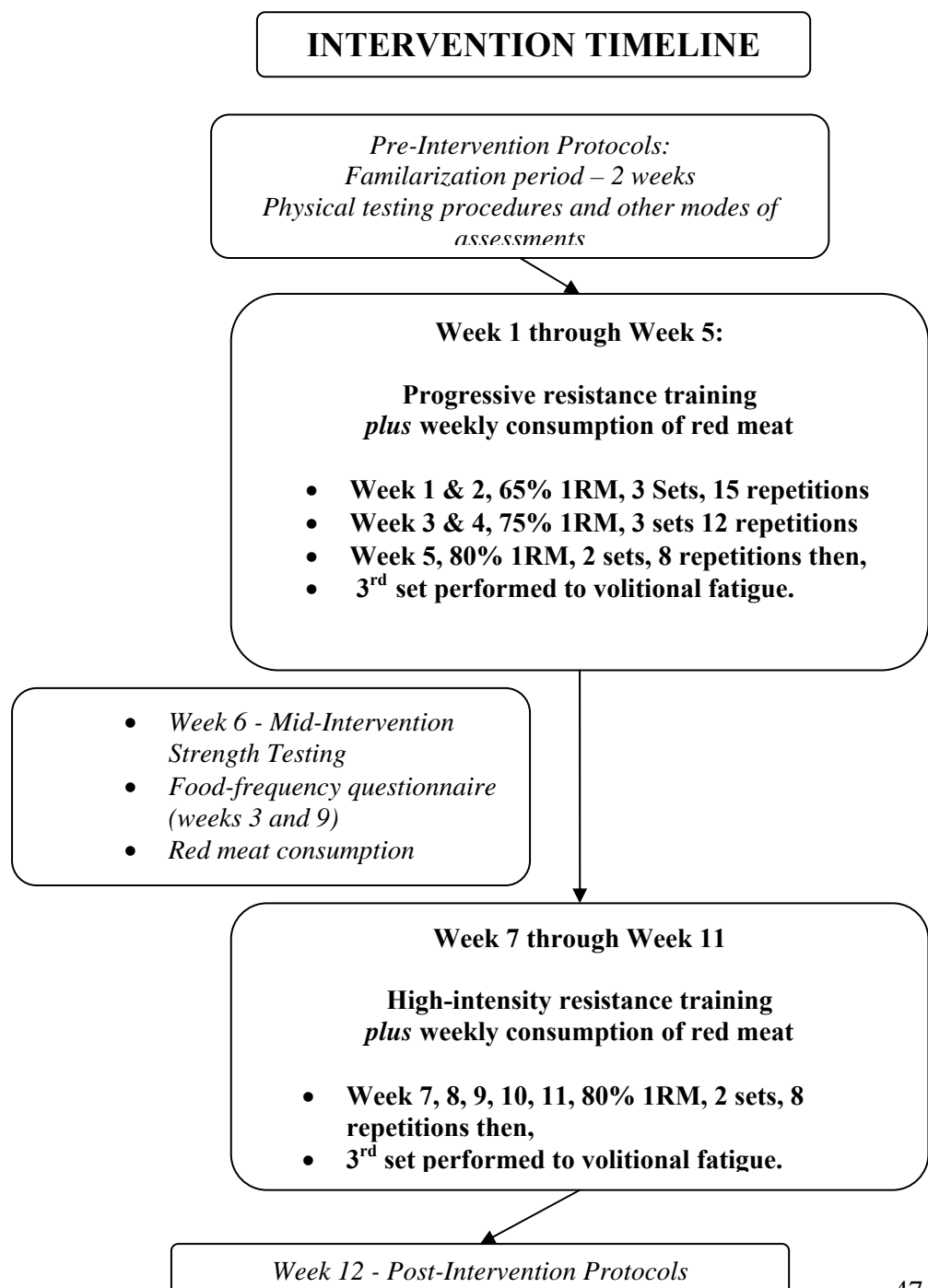
Diet history interviews were done again at post-intervention to assess any change in energy or macronutrient intake from baseline to study completion. It also assessed subject compliance with red meat intake.

2.2.3 SUBJECT DROP-OUT

After commencement of the study, a total of eleven subjects terminated the intervention during the twelve weeks. Five subjects terminated in the first six weeks and six subjects terminated during the second six weeks. Of the eleven, six terminated from the low meat-control group and five from the high meat group. In the low meat-control group there were five females and one male and within the high meat group there were three females and two males that terminated. Cause of termination included, musculoskeletal ailments occurring as a result of the training (n=5), personal obligations (n=3), re-occurring injury (n=1), gastrointestinal discomfort as a result of the higher intake of meat (n=1), flu and reports of the intervention being too intense (n=1). See Diagram 2.

2.3 INTERVENTION

This twelve week combined exercise and dietary intervention involved familiarization period to training sessions, weekly provision of red meat to subjects, progressive and high-intensity resistance training twice weekly. Additional assessments were made throughout the twelve week period to assess compliance to the intervention and physical improvements of the subjects. The detailed outline of the intervention timeline: Diagram 3



2.3.1 DESIGN AND RANDOMIZATION

Baseline dietary history interviews provided the reference point for dietary intervention and the randomization of subjects to either control or experimental group. The median baseline consumption of red meat was provided to the control group. The experimental group received double the median baseline level of red meat intake.

The data collected from the baseline diet history revealed the baseline median intake of the entire group (n=39) to be 400g/week. Subjects were divided into two groups matched for mean weekly red meat intake by the method of block randomization (Bowling, 1997). To facilitate intervention procedures with red meat consumption, couples were randomized into the same group (control or experimental). Groups were equalized based on age, body mass index (kg/m^2) and strength. Grip strength was used as a general predictor of overall body strength (Rantanen et al., 1994). (See section 2.4.2.2 - Grip strength testing procedures). The control group received 400 g of red meat per week and the experimental group received 800 g red meat per week.

2.3.2 DIETARY INTERVENTION

Combined exercise and dietary interventions often utilize nutrients that are in supplemental forms and can potentially disrupt customary dietary eating patterns. The use of such forms of supplementation can be not only unpractical in terms of cost, but also ethically unsound for making health recommendations.

This present study is distinct in that it enabled the implementation of a unique intervention that utilized a single whole food, namely red meat, as part of a normal

balanced diet. As well as being a source of dietary protein for the older adult it provided other essential micronutrients and non-nutrients that are important for various physiological functions. Therefore using such a food product has numerous potential benefits in achieving improvements in health.

2.3.2.1 Supplying Red Meat

Appropriate quantities of red meat were supplied each week by a retail butcher (Dorahy Meats, Unanderra, New South Wales). Various cuts of red meat to provide meal variation (steak, chops, schnitzel, sausages, lean mince) were vacuumed packaged (unfrozen) in portions provided to each subject according to their randomization into control (400g) or experimental (800g) groups. The food was delivered to the subjects chilled in insulated bags during their exercise training sessions at the Wollongong City Council Gymnasium. Each subject was instructed to consume the allotted portions of red meat over the following six days. Subjects were advised to maintain their normal diets while utilizing the portions of red meat as they would normally during their daily meals. They were asked to avoid the consumption of additional red meat products throughout the study period.

The ability to blind the subjects was limited due to the obvious quantities red meat provided. However while subjects were informed that controlled red meat intake was part of the study, they were not informed as to how many intervention groups were involved or whether they were designated into high or low groups. All subjects were provided with equivalent resistance training sessions in small groups providing little opportunity for discussion of their relative meat provisions. Subjects were encouraged to avoid discussing the dietary intervention during contact with other participants or researchers. Subjects were assigned numerical codes and the primary investigator and all personnel involved with measurement

and training procedures (i.e., exercise training staff, CT scan technicians, laboratory personnel, etc.) were blinded as to the meat distribution amongst participants.

2.3.2.2. Assessment of Compliance and Subject Satisfaction

Compliance with the dietary intervention was assessed with a food-frequency questionnaire (Appendix F) and was completed by the subjects at two time-points (week 3 and week 9). Additionally subjects were given a “cut-preference” questionnaire (Appendix G) to ensure they received cuts of meat they preferred, hence aiding compliance with red meat consumption.

2.3.3 EXERCISE TRAINING

All subjects underwent identical exercise training in the form of progressive resistance and high-intensity resistance training (Fleck and Kraemer, 2004). This method of training incorporated the basic principles of resistance training along with guidelines and recommendations for improving skeletal muscle structure and function in older individuals (Evans et al., 1999; Nelson et al., 2007) (See Appendix H for complete summary of guidelines). The 2007 recommendations suggests the use of high-intensity training for older adults that are fit, are familiar with training procedures and are supervised (Nelson et al., 2007). Due to the inexperienced nature of the subjects to resistance training a familiarization period took place for two weeks prior to the start of the intervention, along with a progressive resistance training program for five weeks prior to the use of high-intensity training.

All training sessions, as well as muscular strength testing were performed at the Wollongong City Council Gym. All sessions were fully supervised by either the

primary investigator or assistants trained specifically by the primary investigator in weight room. Subjects were scheduled to train at set scheduled times twice weekly.

2.3.3.1 Resistance Training Exercises

Strictly lower body exercises were performed due to their importance in functional daily activities such as walking and standing up from a chair (Bassey, 1992). Such functional movements that utilize mainly the lower extremities have been associated with future institutionalization and mortality in older adults (Guralnik et al., 1994). Along with the functional consequences and importance of lower limb strength, skeletal muscle is a major contributor to whole body oxygen consumption (Tzankoff and Norris, 1977) therefore there is metabolic significance in targeting this larger bulk of muscle mass.

The original intent was to utilize four exercises that provided a well-rounded program of resistance training for the lower extremity musculature; however upon familiarization with the intended resistance training machines, two of the machines were inappropriate for the majority of the subjects, especially the women. The calf machine that targets the gastrocnemius and soleus muscles caused discomfort with the mode in which it rested on the shoulders of the individuals. Secondly, the prone hamstring machine that targets the hamstring muscle group was not appropriately designed for individuals of smaller stature; hence the females and smaller males of the group were unable to perform the exercise. Therefore the use of two variable-resistance training machines (seated bi-lateral leg press and extension) that focused primarily on quadriceps musculature was used.

Exercise 1) Seated bi-lateral leg press, involves flexion and extension of the upper and lower leg at the hip and knee joint, respectively. The prime movers recruited

for these actions include the gluteus maximus and biceps femoris muscles at the hip joint and quadriceps muscles (rectus femoris, vastus lateralis, vastus medialis, vastus intermedius) at the knee joint. Exercise 2) Seated bi-lateral leg extension, involves flexion and extension at the knee joint with the prime mover solely being the quadriceps muscles (rectus femoris, vastus lateralis, vastus medialis, vastus intermedius). There are additional muscles that aid in stabilizing the body during these exercises some of which are the deep external rotators of the hip joint (piriformis, gluteus minimus, quadrator femoris, gemmelli and obturator muscles) and trunk musculature (iliopsoas, abdominal musculature).

2.3.3.2 Familiarization Period

The purpose of the familiarization period was two-fold: to introduce the subjects to the practice of resistance training and secondly, we took into consideration the potential for improvements in strength to occur within the first weeks simply due to subjects experiencing neuromuscular adaptations (Sale, 1988) to the given exercises, as well as becoming more comfortable in a foreign environment. During these sessions the basic principles of resistance training were taught. This included teaching subjects how to subjectively rate their exercise intensity using ratings of perceived exertion (RPE), proper exercise execution, warm-up and cool-down protocols and proper breathing instruction. It also provided a time to answer any questions and concerns that may arise during the intervention period.

Each subject attended two sessions during a two-week period and subjects were shown the facility and location of amenities such as toilets, water fountains, telephones and necessary exits.

2.3.3.3 Borg Ratings of Perceived Exertion (RPE)

Borg's ratings of perceived exertion, or RPE (Borg, 1962) was used throughout the familiarization, training sessions and other physical testing procedures to record the subjective intensity experience by the subject while exercising. RPE are a useful tool for assessing the subjective intensity of a person while performing physical work and are based on physical sensations experienced by the individual such as increased heart rate, respiration, sweat rate and muscle fatigue. The original scale that was developed ranged from six to 20, with six representing the intensity of "no exertion at all" to 20 representing the intensity "maximal exertion". Since its original development a category scale was developed that uses a scale ranging from zero to ten, with zero representing the intensity of "nothing at all" to ten representing the intensity of "very, very strong" (Borg, 1982) (See Appendix I for complete scale). Category scale was used due to its simpler nature for associating with subjective intensity. Subjects were trained in the usage of the scale by being shown a diagrammatic scale with appropriate levels of intensity corresponding to the numbers zero through ten.

2.3.3.4 Exercise Execution and Technique Instruction

A thorough demonstration of each piece of exercise equipment was provided for the subjects before they familiarized themselves with the equipment. Each subject familiarized themselves with all pieces of exercise equipment that would be used in the intervention. Proper body form and the importance of slow controlled movements were emphasized during the familiarization sessions. Low intensities were utilized during the familiarization period (RPE ~2, considered "weak" or "light", or ~40% of the subjects 1 RM for ~ 20 repetitions) (Taaffe et al., 1999) and

provided for neuromuscular adaptations (Sale, 1988) to be made while teaching proper exercise execution and technique for all exercises.

The stringency of speed was reinforced, as the training effect would vary if the speed of movement was not held consistent. Resistance training guidelines for older adult populations have suggested using a speed of exercise execution that consists of four seconds on the concentric contraction and six seconds on the eccentric contraction (Evans et al., 1999). We choose to simplify this and used a two second count for both contractions.

2.3.3.5 Warm-up Protocol

The purpose of the warm-up was to increase metabolic processes within the body such as increasing core body temperature and increasing the supply of oxygen to working muscles (Woods et al., 2007). It also served to motivate the subjects for the training session and lower the risk of injury during the training (Kallinen and Markku, 1995). The warm-up included five minutes of circuit-type movements that focused on the lower body musculature that was to be exercised. The circuit was formatted to provide low intensity movements that consisted of stepping, marching and walking. This was followed by ten minutes of flexibility exercises targeting the main joint areas that were to be actively involved during the training sessions. The calf (gastrocnemius and soleus), quadriceps (rectus femoris, vastus lateralis, vastus medialis, vastus intermedius), hamstring (biceps femoris, semitendinosus, semimembranosus) and gluteal musculature (gluteus maximus) were the main muscle groups targeted. There was one flexibility exercise for each major muscle group. Each exercise was repeated twice and bi-laterally, with a gentle, low intensity holding position for thirty seconds.

2.3.3.6 Cool-down Protocol

Although there is a lack of evidence supporting a decrease in delayed onset muscle soreness with an active recovery period following exercise training (Barnett, 2006), the American Heart Association (Fletcher et al., 2001) recommends such practices for an elderly population due the potential risk for cardiovascular abnormalities (e.g., arrhythmias, post-exercise hypotension) following resistance training, therefore we choose to administer a protocol of active recovery for the subjects as a final endpoint to the training session. This protocol was identical to the warm-up protocol as described above.

2.3.3.7 Proper Breathing Instruction

Breathing techniques were taught to ensure minimal stress to the cardiovascular system during all training and testing procedures (Evans et al., 1999). Subjects were instructed to perform an inhalation prior to the exercise being executed, followed by an exhalation during the concentric contraction. They were then instructed to perform an inhalation during the eccentric contraction. They were asked to avoid performing the Valsalva manoeuvre while performing the exercises, which involves performing a forced exhalation with a closed glottis and leads to changes in blood pressure responses (Harman et al., 1988). When it proved to be challenging for the subjects to coordinate their breathing as required they were instructed to maintain a normal breathing pattern and avoid straining whenever possible (ACSM, 2000).

2.3.4 TRAINING PROTOCOL

2.3.4.1 Typical Training Session

Each training session had a pre-determined timeline which consisted of a set routine that was expected of each subject upon arrival at the gymnasium. Ninety minutes was allotted for each session and this proved to be ample time to answer questions or concerns from the subjects, warm-up, undertake training protocols, cool-down and collect red meat for the following week. Subjects worked in pairs during the exercise session and rested for two minutes between each set of exercise. There was not a specified order in which the exercises were performed as we were limited to one seated bi-lateral leg press and one seated bi-lateral leg extension machine. Whenever possible we alternated the piece of equipment that the subjects used on the second day of training, therefore if the subject's first exercise for their first training day of the week was the leg press, on the second training day we attempted to have them commence with the leg extension. They were permitted a warm-up exercise set of ten repetitions that was performed for each of the exercises (leg press and leg extension) at low intensity (RPE of ~2, considered "weak" or "light", or ~ 40% of 1 RM) (Taaffe et al., 1999) which preceded the true exercise set.

2.3.4.2 Intervention and Testing Structure

The training structure was divided into two blocks of five weeks per block with one week for testing at the end of each block (see Diagram 3). Throughout the twelve weeks subjects trained twice weekly with 48 hours scheduled between each session allowing for recovery time between the first and second training session of the

week. The starting load lifted by the subjects for each exercise was determined by their 4RM strength testing results taken at during the pre-intervention. The equipment was unsuited to a direct 1RM measure in these subjects therefore a converting factor was used to convert 4RM values to 1RM values ($1RM = 4RM \text{ weight} \times 1.13$) (Jubrias et al., 2001) and then a percentage of the 1RM was used to determine the starting load for the first week of block one.

The first five weeks consisted of a progressive and periodized approach to resistance training (Fleck and Kraemer, 2004). Such protocols have been used in past resistance training interventions to offer graded adaptation to untrained individuals (Esmarck et al., 2001; Henwood and Taaffe, 2006). During week one and two the subjects performed three sets of 15 repetitions at 65% of their estimated 1RM. The third and fourth week consisted of three sets of twelve repetitions at 75% of their estimated 1RM. The fifth week consisted of two sets of eight repetitions at their estimated 1RM with the third set being performed to volitional fatigue. This served as another method for determining the load adjustments to be made for the next session.

During week six, subjects underwent the mid-intervention strength testing. This testing week was not considered a training week, although it did provide a stimulus to the subject of higher intensity and others have reported such stimulus (i.e., strength testing) to be warranted as a training day (Taaffe et al., 1999). The following five weeks followed progressive high-intensity resistance training that has been used extensively in the past with older adults (Frontera et al., 1988; Taaffe et al., 1999; Baker et al., 2007). Subjects completed two sets of eight repetitions at

80% of their estimated 1RM. The third set was performed to volitional fatigue and if the subject was able to complete twelve or more repetitions we increased their training load the following session (Jozsi et al., 1999). This enabled a continual overload and ensured subjects were training at high-intensity. Intensity progressively increased throughout the twelve week intervention and new lifting loads were increased during the actual training session if subjective intensity of the subject did not match the required training intensity. This was based on the subject's RPE and the ability to complete the final exercise set for the specific exercise with ease (i.e. not reaching volitional fatigue). Adjustments to the load lifted on the exercise equipment were limited to 1.25 pounds. Subjects were encouraged to attempt the new set load in the following session and were instructed to maintain proper exercise form and execution. If they were not able to execute the movement properly then the load was decreased accordingly. Likewise, load was decreased if any acute physical pain was experienced from the subject. When subjects did not experience RPE 7 (very strong) to 10 (very, very strong) then the load was increased accordingly.

Appendix J is the detailed recording sheet used by the primary investigator and/or assistants to record measurement details (set, repetitions, mass lifted, RPE) during training sessions.

2.4 PHYSICAL TESTING PROCEDURES AND ASSESSMENTS

A comprehensive battery of physical testing procedures and assessments was administered at pre-, mid- and post-intervention to assess intervention effects. This included tests of muscular strength and aerobic capacity, assessments of physical activity levels, functional lower body performance, and body composition status.

2.4.1 EXERCISE TEST TERMINATION CRITERIA

During all physical testing procedures subjects were given the right to refuse or terminate a test. As well, researchers reserved the right to refuse or terminate a test based on their own subjective observations of the subject. Test termination criteria that were adhered to for terminating any of the physical testing procedures, as well as training sessions, were adapted from the American College of Sports Medicine's Guidelines for Exercise Testing and Prescription (ACSM, 2000). They include; onset of angina or angina-like symptoms, signs of poor perfusion such as light-headedness, confusion, ataxia, pallor, cyanosis, nausea or cold clammy skin, failure of heart rate to increase with increased exercise intensity, subject requests to stop, physical or verbal manifestations of severe fatigue, and failure of the testing equipment.

2.4.2 PHYSICAL TESTING PROCEDURES

With the exception of the 4RM strength testing that was performed at the Wollongong and the Mid-thigh computerized tomography that was performed at Wollongong Hospital's Radiology Department all other procedures were conducted at the University of Wollongong's Exercise Science and Rehabilitation Centre

2.4.2.1 Four-repetition Maximum (4RM)

Four-repetition maximum (4RM) strength testing was used to assess pre-intervention muscular strength and to monitor strength changes at mid- and post-intervention. The 4RM was defined as the maximal load lifted in four consecutive repetitions at a slow and controlled movement while maintaining proper technique. Due to the inexperience of these volunteers, their older age and equipment used for testing, a sub-maximal strength test that involved a 4RM protocol was used (Jubrias

et al., 2001) as apposed to the conventional 1RM protocol for strength testing (ACSM, 2000).

In total there were six 4RM strength testing days for each subject at pre-, mid- and post-intervention. Subjects were tested on machines identical to those used during the training sessions. They completed either the seated bi-lateral leg press or seated bi-lateral leg extension strength tests at two separate occasions during the testing week, with a minimum of 36 hours in between tests at pre-, mid- and post-intervention. Testing was administered and supervised by the primary investigator and/or by trained individuals instructed by the primary investigator.

Improvements in strength that occur within the first three to four weeks of resistance training are mainly due to neural adaptations (Sale, 1988); namely improved motor recruitment, learning and coordination of the specific exercise, therefore the first 4RM measurements were administered following the two-week familiarization. This two-week familiarization period also helped to minimize any potential for musculoskeletal injury (Pollock et al., 1991).

The 4RM protocol followed the same protocol as the traditional 1RM strength test (ACSM, 2000). Prior to testing, subjects performed a warm-up protocol that consisted of a general warm-up (Section 2.3.3.5) with an addition of ten repetitions of the specific movement to be tested at low intensity or RPE of ~2 or ~40% of their 1RM. Rest was permitted for two minutes and then they were instructed to perform the predicted 4RM. If the subjects did not reach fatigue with the predicted 4RM value and they perceived that they could perform a fifth repetition, they rested for two minutes and attempted another 4RM set with an added load. The goal was

to attain the 4RM within four attempts. Appendix K is the recording sheet used by either the primary investigator or the assistants during the 4RM testing procedures.

2.4.2.2 Grip Strength

Grip strength was assessed as a secondary measure of muscular strength that was not specific to the resistance training protocol. It was completed at pre- and post-intervention using a hand-grip dynamometer (Original Smedley's™ Dynamometer 100 kg, Tokyo). Assessment of such movements will aid in determining if the experimental group (High Meat Group) gained improvements apart from the areas targeted. Grip strength also aided in matching and equalizing control and experimental groups during the randomization process.

The subjects chosen dominant hand was used and the apparatus was adjusted to accommodate the various hand sizes of the subjects. Subjects performed three trials by beginning the movement with the arm horizontally abducted and perpendicular to their body. They were instructed to grip the apparatus as forcefully as possible while bringing the arm down to anatomical position (i.e. arm to the side of the body). They were allowed a one minute rest between each trial and the highest value in kilograms was recorded. The primary investigator administered the test at all times.

2.4.2.3 One Mile Walk Test (MWT)

The decline in aerobic capacity with age and its association with the loss of skeletal muscle mass (Fleg and Lakata, 1988) is another key factor in the aging process. However the measurement of direct aerobic capacity is laborious to perform and for our purposes was beyond the scope of this study. Therefore a field test was used

that has been previously validated against more direct measurements of aerobic capacity (Kline et al., 1987). Field tests are an alternative method of measuring aerobic capacity indirectly (Williams et al., 2007). This mode of assessment was ideal due to the need for minimal equipment, expense, ability to administer in a group setting and was completed using a measured indoor track at University of Wollongong's Recreation Centre.

The One Mile Walk Test (MWT) has been proven to be a safe field test to administer to elderly individuals (Bazzano et al., 1998). It was used in accordance with its previous use with older adults to estimate maximal oxygen consumption (Kline et al., 1987). Its use at pre-intervention served dually as a secondary screening procedure upon the request of the Faculty's supervising medical doctor.

Polar Beat™ heart rate monitors were used to record heart rate during at the completion of each lap, as well as immediately before and immediately upon completion of the walk in a standing position. Six-digit LCD stopwatches were used to record the total time taken to complete the walk. Before and immediately upon completion of the walk a qualified nurse assessed blood pressure with a DINAMAP®PRO series monitor (GE Medical Systems, Sydney, Australia).

Subjects performed a warm-up that involved walking at low intensity (RPE of ~2, considered "weak" or "light") for two laps or ~ 200 meters. They were then instructed to walk 15 laps (equating to 1 mile, or 1.6 kilometers) as fast as possible without compromising proper walking form or beginning to run. RPE were used throughout and at completion of the walk to assess the subject's subjective exercise

intensity. After a five minute cool-down period of light walking, or until subject's intensity was light (RPE=2) to minimal intensity (RPE= < 1), final post-walk blood pressure and heart rate measurements were taken to ensure recovery reached pre-exercise levels. Both blood pressure measurements and post-walk heart rate measurements were taken with the subjects seated.

Estimated maximal oxygen uptake was calculated using regression equations formulated by Kline et al., (1987). This prediction was calculated with the use of the subject's age, sex, body weight, time to complete one-mile and final heart rate recording at walk completion. Values for predicted maximal oxygen uptake are reported as an absolute value in litres per minutes (l/min) and as a relative value in milliliters per kilogram of body weight per minute (ml/kg/min). See Appendix L for regression equations.

2.4.3 Physical Activity and Functional Performance Assessments

The assessment of physical activity status and functional performance was done using tools (questionnaire & objective performance tests) that have been used previously in similar intervention settings. They were selected due to the simplicity of test administration as well for their capacity to detect a change over a shorter period of time (e.g. 12-week time frame) (Foldvari et al., 2000).

2.4.3.1 Physical Activity Scale for the Elderly (PASE)

Physical activity status and the ease of performing habitual daily tasks are factors crucial for successful aging as well as the prediction of future health outcomes such as disability and frailty (Guralnik et al., 1994)

The Physical Activity Scale for the Elderly (PASE) (Washburn and Ficker, 1999) was used at pre- and post-intervention to assess self-report levels of physical activity. It questions the individual in a questionnaire format specifically asking the frequency and amount of time one would spend doing various activities (occupational, household and leisure) over a one week period. It has been previously validated in the assessment of physical activity status of older individuals (Foldvari et al., 2000).

The format of the questionnaire requires the individual to answer ten questions pertaining to the listed activities. Below outlines these ten questions. It is then tabulated and a score is determined for the individual. PASE scores range from zero to 400. A higher score depicts greater levels of physical activity. Appendix M represents the scoring scheme and full PASE Booklet (New England Research Institutes, 1991 New England Research Institutes, Inc.).

Questions from the Physical Activity Scale for the Elderly (PASE)*

Adapted from New England Research Institutes, 1991 New England Research Institutes, Inc.

*

**Permission obtained from New England Research Institutes, Inc. by
purchase of the product for this study.**

2.4.3.2 Short Physical Performance Battery (SPPB)

The Short Physical Performance Battery (SPPB) (Guralnik et al., 1994) was performed at pre- and post-intervention as an assessment of functional lower extremity physical performance. It was chosen due to its simple yet comprehensive nature in assessing objective lower extremity function. The SPPB consists of three tests of standing balance, usual walking speed, and the ability to rise from a chair.

Standing balance included three separate tests which were first demonstrated by the investigator. The first test was the semi-tandem stance, in which the heel of one foot was put to the side of the first toe of the opposite foot. The subjects chose which foot was either forward or back. If the subject could not hold the semi-tandem stance for ten seconds, they were instructed to perform the side-by-side stance which involved standing with feet together. If the subject could hold the semi-tandem stance for ten seconds they were instructed to perform the full tandem stance which involved having the heel of one foot directly in front of the toes of the back foot. The subject was timed to a maximum of ten seconds.

Usual walking speed was tested over an eight foot walking course with a foot margin at either end allowing ample time for them to slow down and stop. No instructions were given and subjects were asked to “walk to the other end of the course at your usual speed, just as if you were walking down the street to go to the store”. They performed this twice and the fastest time was used for recording purposes.

The ability to stand up from a chair, also termed the “chair stand”, consisted of a straight backed chair positioned next to a wall. Subjects were instructed to cross their arms over their chest and stand up one time. If this was successful, they were then timed in their ability to stand up and sit down five times by starting in a sitting position and ending in the standing position on the fifth chair stand.

Each separate test was graded based on the level reached within the specific movement and the recorded time or ability within each test. Each test was assigned a score ranging from zero to four, with zero being the lowest possible score and four being the highest. The scores were then summed for a total score of zero to twelve. Each subject was given a demonstration by the primary investigator to ensure a full understanding of the required movements. The scoring scheme is provided in Appendix N.

2.4.4 BODY COMPOSITION ASSESSMENT

The associations between declining muscular strength and that of muscle mass make body composition assessments an essential component in studying the effects of interventions with older adults. Proper quantification of these body composition changes is challenging and often requires more than one technique (Nelson et al., 1996). Therefore a comprehensive anthropometric profile and computed tomography assessments were done to assess general as well as specific aspects of body composition at pre- and post-intervention.

2.4.4.1 Anthropometric profile

An anthropometric profile was completed by a single qualified individual experienced in anthropometric profiling (Level 1 – International Society for the Advancement of Kinanthropometry) at pre- and post-intervention. Height and weight of each subject was measured using a wall-mounted stadiometer and an electronic scale, respectively, while barefoot and wearing light clothing. These measurements also served to calculate body mass index (kg/m^2) which was used to equalize groups during the randomization process.

Skinfolds were assessed using a Harpenden Skinfold Caliper (John Ball British Indicators Ltd.) and girths measured with a steel measuring tape (KDS 2 meter metal tape). All measurements were made on right side of the body. The sum of seven skinfolds (biceps, triceps, subscapular, supraspinale, abdominal, front thigh and medial calf) was used to assess overall change in regional body fatness.

Separate sum of skinfolds for three landmarks on the thigh were used to assess changes specific to the training regime and location (Andersen and Saltin, 1985; Saltin, 1985). Landmarks for measurement sites and measurements were taken according to the anthropometric standards put in place by the International Society for the Advancement of Kinanthropometry (ISAK) (Norton, 2000). See Appendix O for the complete description of antropometric sites and measurements.

Readings were made with a one to two second delay while the calliper was maintained on the skinfold. Skinfold sites were measured twice with a third measurement taken if the first two measurements were not within one millimeter of each other. All girths were measured twice with a third measurement taken if the

first two measurements were not within seven millimeters of each other. All measurements were done in a rotating fashion.

2.4.4.2 Bioelectrical Impedance (BIA)

Bioelectrical impedance (BIA) is based upon the conductive and dielectric properties of various biological tissues at different frequencies, and has the advantage of being a safe, non-invasive method for measuring fat-free mass (Lee et al., 2001). Body composition measurements of fat free mass and percentage body fat was determined by BIA using an IMP5 single frequency analyzer (Impedimed, QLD, Australia) and prediction equation which has been validated in an older-aged population group was used in the BIA analysis (Kyle et al., 2001).

Subjects underwent BIA in the morning and were instructed to: not eat or drink within four hours of the assessment, avoid moderate or vigorous physical activity within twelve hours of the assessment, not consume alcohol within 48 hours of the assessment, and have not ingested any diuretic agents, including caffeine, prior to the assessment unless prescribed by a physician. See Appendix P for equation and protocol used by investigator to take measurements and record data.

2.4.4.3 Mid-Thigh Computed Tomography

Mid-thigh single slice cross-sectional computed tomography (CT) scans were performed at the Radiology Department, Wollongong Hospital using a Somatom Plus CT System (Siemens Medical Solutions, Lavern, PA, USA). These were completed at pre- and post-intervention by a qualified radiographer and the scans analyzed using 'Image Pro' software (Media Cybernetics, MD, USA).

Total thigh cross-sectional area, muscle cross-sectional area, thigh circumference, percentage muscle and fat were assessed for the both right and left legs. To differentiate between changes to dominant and non-dominant legs due to intervention procedures, subjects were asked to simply “stand on one leg”. This preferred leg for standing was recorded and was deemed their dominant leg. See Appendix Q for complete details of scan analysis.

2.5 STATISTICAL ANALYSES

In estimating the required sample size, the published literature suggests that studies of exercise training in older adults, conducted over 12-weeks, commonly produce significant enhancement of muscle mass and strength, using sample sizes of N=17-32. (The proposed sample size therefore intended to exceed N=32). One study has further shown benefits of exercise combined with omnivorous diet compared with a lactoovovegetarian diet over 12 weeks (N=19) (Campbell et al., 1999).

Data analysis was performed using Statistix version 7.0 (FL, USA) and SPSS version 11.0 (IL, USA) software packages. Comparisons between baseline and post-intervention values were made using paired t-tests. Comparisons between dietary groups were made using unpaired t-tests. Non-parametric tests (Mann-Whitney U and Wilcoxon signed ranks tests) were used to analyse data not normally distributed. Pearson and Spearman’s correlations were performed to examine the relationships between variables. Two-way analysis of variance (ANOVA) was used to examine the relationships between gender and diet and their effects on response to the training. Differences in leg muscle strength measures at the three timepoints (weeks 0,6 and 12) were analysed using repeated measures ANOVA and repeated measures ANCOVA. Data is given as Mean \pm SEM unless otherwise stated.

Chapter Three

-Results-

*“However beautiful the strategy, you should occasionally look at the
results.”*

Sir Winston Churchill

3.1 DIET HISTORY

The high and low meat groups did not differ at baseline or over the course of the study in energy intake or macronutrient intake, including total protein (Table 1). The red meat intake of the low meat group at study completion did not differ from baseline. The high meat group reported consuming significantly more red meat at week twelve ($P < 0.01$) than the low meat group and as compared to their intake at baseline signifying compliance to dietary intervention (Table 1). At baseline all subjects had very stable eating patterns and consumed red meat as part of evening meals approximately three times per week. The average baseline frequency of red meat consumption per week was 3.4 ± 1.8 day per week. Food frequency questionnaires administered at week three and nine revealed that red meat was consumed by all subjects, with it mainly being eaten as part of the evening meal (data not shown).

Nutrient	All subjects N=28		Low Meat Group n=12		High Meat Group n=16	
	PRE	POST	PRE	POST	PRE	POST
Total Energy (MJ)	8.6 ± 1.9	8.6 ± 2.5	8.7 ± 2.2	8.6 ± 2.5	8.5 ± 1.7	8.5 ± 2.5
Carbohydrate(%En)*	44.7 ± 7.5	42.4 ± 8.8	44.8 ± 7.7	44.2 ± 7.4	44.7 ± 7.5	41.0 ± 9.7
Protein (%En)*	19.8 ± 4.9	19.7 ± 3.8	18.8 ± 2.5	19.4 ± 3.5	20.5 ± 6.1	19.9 ± 4.2
Total Fat (%En)*	27.4 ± 6.6	29.1 ± 5.9	27.9 ± 6.9	29.0 ± 6.3	27.1 ± 6.6	29.1 ± 5.9
Saturated fat (%En)*	10.4 ± 3.5	11.2 ± 3.1	10.5 ± 4.1	10.9 ± 3.5	10.3 ± 3.2	11.4 ± 2.8
Red Meat (g/week)	555 ± 317	755 ± 350 [#]	547 ± 249	553 ± 175	561 ± 368	907 ± 375 ^{#§}

Table 1. Dietary macronutrient and red meat consumption at pre- and post-intervention.

Values given as mean ± SD. *Macronutrients given as percentage of total energy intake (%En). [#] Different to pre-intervention, $P < 0.01$. [§] Different to low meat group post-intervention, $P < 0.01$.

3.2 SAFETY OF RED MEAT

3.2.1 Iron Status

Ferritin levels tended to decrease slightly, but not significantly, over the course of the intervention. There was no significant difference between the dietary groups in the effect of resistance training or red meat intake on iron status (Table 2). When gender was examined separately the reduction almost achieved significance ($p=0.07$) in females.

3.2.2 Urinary Protein

There were no significant changes in urinary protein excretion over the course of the study in either dietary group (Table 2).

3.2.3 Markers of Oxidative Damage

Protein carbonyls as a measure of the oxidative damage to protein, did not differ between groups over the course of the intervention and were within the normal range for plasma protein carbonyl (< 0.10 nmol/mg) (Table 2).

Oxidative damage to DNA as measured by urinary levels of the product 8-hydroxy-2 deoxyguanosine (8-OHdG) in morning urine samples was expressed relative to urinary creatinine levels to account for differences in urinary concentration. Levels of 8-OHdG did not change significantly over the 12 week study period in either the low or high meat groups (Table 2).

Oxidative damage to lipids as measured by plasma concentration of the lipid oxidation product malondialdehyde (MDA) in fasted blood samples did not change significantly over the course of the study, and did not differ between groups (Table 2).

Table 2. The effect of diet and exercise intervention upon iron status, urinary protein and oxidative stress markers

	All subjects		LOW meat group		HIGH meat group	
	<i>Pre</i>	<i>Post</i>	<i>Pre</i>	<i>Post</i>	<i>Pre</i>	<i>Post</i>
Ferritin (ng/ml)	173.04 ± 27.83	158.53 ± 25.98	143.21 ± 28.09	123.98 ± 27.52	195.42 ± 43.91	184.45 ± 40.06
Urinary Protein/ Creatinine (mg/g)	1.37 ± 0.08	1.36 ± 0.08	1.21 ± 0.13	1.23 ± 0.14	1.49 ± 0.10	1.46 ± 0.10
Urinary 8-OHdG (µg/g creatinine)	11.20 ± 0.66	11.23 ± 0.81	11.65 ± 1.03	12.98 ± 1.13	10.86 ± 0.88	9.92 ± 1.04
Plasma protein carbonyl (nmol/mg)	0.058 ± 0.006	0.065 ± 0.007	0.049 ± 0.009	0.057 ± 0.013	0.064 ± 0.009	0.071 ± 0.009
Plasma [MDA] (µM)	0.09 ± 0.01	0.10 ± 0.01	0.11 ± 0.02	0.12 ± 0.02	0.08 ± 0.01	0.09 ± 0.01

Values given as Mean ± SEM

3.3 EXERCISE INTERVENTION AND ASSESSMENT PROCEDURES

There were a total of twenty-seven sessions that included: familiarization sessions (n=2); strength testing (n=6); and resistance training (n=19). Both familiarization sessions were attended by all subjects at pre-intervention and were well tolerated by all subjects. This familiarization period proved to be essential for the success of the actual intervention as it provided physical and psychological preparation for the upcoming twelve week intervention. Over the twelve week period, subjects found the ratings of perceived exertion (RPE) to be easy to learn and provided an excellent means of communication between them and the investigators. Warm-up and cool-down protocols were equally well received and were integral in maintaining participant morale during the 90 minute sessions. Proper breathing cues and speed to complete each exercise repetition were followed by most subjects. When attention to proper breathing form or speed of movement was neglected on their part the individual supervising each subject promptly reinforced them to proper protocol. All subjects who completed the twelve weeks were present for all strength testing days with the exception of one subject who did not complete the post-strength testing of the seated bi-lateral leg press due to specific discomfort with this exercise on the day of testing.

Subject adherence to the training protocol was high over the twelve weeks with eighteen completing all sessions, seven completing 94.7% and one completing 89.4% of the training sessions. During week five there was only one training session due to a statutory holiday. All intensities of training were adhered to as training intensities increased. If subjects were unable to make designated training sessions, rescheduling was allowed as there were additional training blocks to

accommodate such occurrences. The recruitment of couples aided the adherence to intervention procedures (diet and exercise) as partner support was evident on a week-to-week basis. In all cases in which one partner needed to terminate their involvement with the study the remaining partner continued.

3.4 EFFECT OF INTERVENTION

3.4.1 All Subjects

Of the 39 subjects enrolled in the study, 28 completed intervention and final testing. Reasons for discontinuation were various and included admission for surgery after a two-year wait, illness and family obligations (details in methods Chapter 2). The drop outs occurred at varied times between weeks two and twelve of the intervention. Hereafter results are presented for the subjects who completed the intervention and final testing (N=28).

Leg press strength increased significantly for all subjects from pre-intervention (109±8 kg), to mid- (142±8) and post-intervention (164±8) (P<0.001) (Figure 1).

Leg extension strength increased significantly for all subjects from pre-intervention (30±2 kg) to mid- (40±3) and post-intervention (48±3) (P<0.001) (Figure 2).

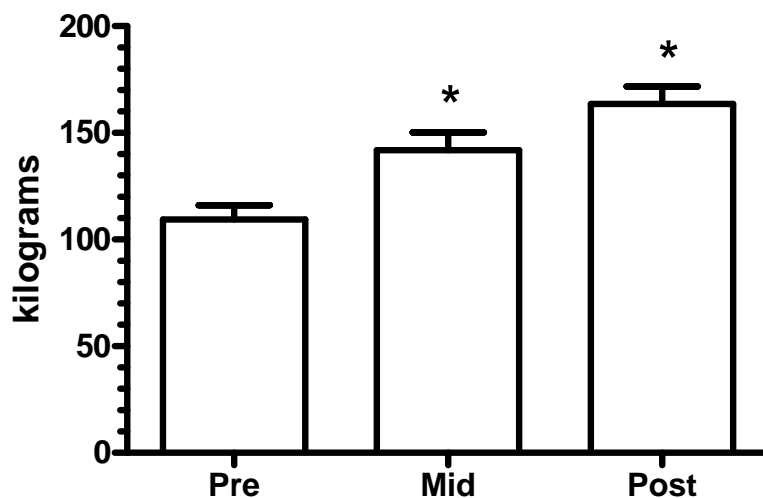


Figure 1. Leg press strength (4RM absolute values) for all subjects at pre-, mid- and post-intervention. * Significantly different from pre-intervention, P<0.001. Values are means ± SEM. N = 28.

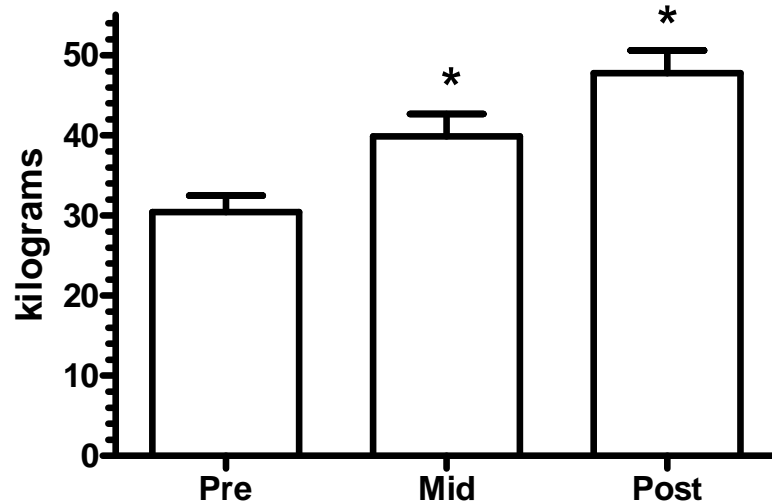


Figure 2. Leg extension strength (4RM absolute values) for all subjects at pre-, mid- and post-intervention. * Significantly different from pre-intervention, $P < 0.001$. Values are means \pm SEM. $N = 28$.

The sum of seven skinfolds and the sum of three thigh skinfolds decreased significantly from pre- to post-intervention across all subjects ($P < 0.001$) (Table 3).

No significant differences were found for grip strength, BMI, thigh girth, % body fat, fat-free mass, physical activity scale for the elderly, short physical performance battery or maximal oxygen uptake as estimated from the one-mile walk test between measurements taken pre- and post-intervention, (Table 3).

Table 3. Primary and secondary characteristics for all subjects pre- and post-intervention

	Pre-Intervention	Post-Intervention
Age (years) (N=28)	67.4 ± 0.7	67.4 ± 0.7
BMI (kg/m²) (N=28)	27.3 ± 0.8	27.2 ± 0.8
Grip strength (kg) (N=28)	36.5 ± 1.7	36.7 ± 1.5
One-mile walk test Estimated O₂ uptake (ml/kg/min) (N=26)	30.75 ± 1.0	29.61 ± 1.5
PASE (N=28)	176 ± 10	179 ± 10
SPPB (N=27)	11.5 ± 0.2	11.7 ± 0.1
Sum of 7 skinfolds (mm) (N=19)	131.2 ± 8.8	119.9 ± 7.3[*]
Thigh girth (cm) (N=25)	55.4 ± 1.2	56.3 ± 1.3
Sum of 3 thigh skinfolds (mm) (N=27)	64.7 ± 5.9	55.0 ± 4.7[*]
Fat free mass (kg) (N=24)	54.23 ± 2.03	54.27 ± 1.95
Body fat (%) (N=24)	30.0 ± 1.5	29.5 ± 1.4

PASE: Physical activity scale for the elderly; SPPB: Short physical performance battery. Sum of 7 skinfolds: sum of biceps, triceps, subscapular, supraspinale, abdominal, front thigh and medical calf skinfolds. Thigh girth: taken 1cm below the gluteal furrow. Sum of 3 thigh skinfolds: sum of three locations on anterior thigh. See Appendix O for full description of landmarks. Fat free mass and % body fat are values from bioelectrical impedance analysis. Values are means ± SEM. N=28 except where both pre- and post intervention values were not available as indicated. ^{*} Significantly different to pre-intervention, p<0.001.

The mid-thigh CT scan cross-sectional area of fat for all subjects decreased significantly from pre- to post-intervention ($P < 0.05$) (Table 4). The thigh girth, CT scan thigh circumference, and mid-thigh cross-sectional area of muscle did not differ from pre- to post-intervention.

Table 4. Right thigh characteristics pre- and post-intervention for all subjects and subdivided into high and low meat groups.

	All Subjects		Low Meat		High Meat	
	Pre	Post	Pre	Post	Pre	Post
Thigh girth (cm)	55.4 ± 1.2	56.3 ± 1.3	56.2 ± 1.0	55.9 ± 1.3	54.8 ± 1.3	55.6 ± 1.3
Sum of 3 skinfolds (mm)	64.7 ± 5.9	55.0 ± 4.7*	56.4 ± 7.6	51.2 ± 6.9 §	70.8 ± 8.5	57.7 ± 6.5*
CT scan thigh circumference (cm)	52.8 ± 1.1	52.0 ± 1.0	53.6 ± 1.5	53.0 ± 1.4	52.2 ± 1.5	51.4 ± 1.3
CT scan thigh fat (cm ²)	85.6 ± 10.0	80.3 ± 8.7§	85.3 ± 17.4	81.0 ± 16.8	85.8 ± 11.3	79.8 ± 9.5
CT scan thigh muscle (cm ²)	128.4 ± 5.9	128.6 ± 5.6	131.5 ± 9.3	132.3 ± 9.5	126.3 ± 7.9	126.0 ± 7.2

Thigh girth and sum of 3 skinfolds as assessed by anthropometry for all subjects (N=26); low meat (n=11); high meat (n=15). Thigh girth: taken 1cm below the gluteal furrow. Sum of 3 skinfolds: Sum of three locations on anterior thigh. See Appendix O for full description of landmarks. CT scan measurements for all subjects (N=24), low meat (n=10) and high meat (n=14) groups. Values are means ± SEM. * Significantly difference from pre-intervention, $P < 0.001$. § Significantly different from pre-intervention, $P < 0.05$.

The middle of three thigh skinfolds, (that best corresponded to the mid-thigh CT scan location) correlated with mid-thigh fat cross-sectional area determined from CT scan ($r^2 = 0.716$, slope $P < 0.0001$) (Figure 3).

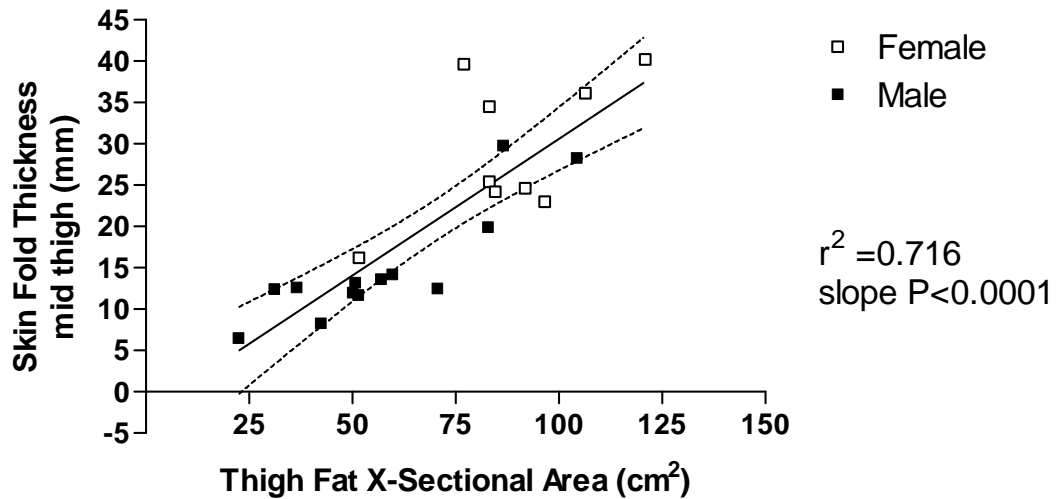


Figure 3. Relationship between CT cross-sectional area of the thigh fat and mid thigh (of three) skinfolds, pre-intervention. N=22.

When the CT scans were analyzed separately for dominant and non-dominant legs (determined by the leg preferred for standing during the balance tests), the proportion of thigh muscle cross-sectional area in the non-dominant leg was significantly greater post-intervention compared to pre-intervention and the proportion of cross-sectional area as fat was lower for all subjects ($P < 0.05$) (Table 5). No significant changes were seen in the dominant leg. The ratio of muscle to fat significantly increased in the non-dominant leg from pre- to post- intervention ($P < 0.05$).

Table 5. Thigh muscle and fat cross-sectional area proportions pre- and post-intervention in non-dominant and dominant legs for all subjects and subdivided according to meat intake.

		All subjects		Low Meat		High Meat	
		Pre	Post	Pre	Post	Pre	Post
Non-Dominant Leg	% Thigh Muscle	58.8 ± 2.9	60.4 ± 2.8 *	60.6±5.2	61.7±5.2	57.5±3.4	59.5±3.2 *
	% Thigh Fat	37.7 ± 3.0	36.0 ± 2.9 *	35.8±5.4	34.7±5.5	39.1±3.6	36.9±3.3 *
	Muscle/Fat Ratio	2.05 ± 0.29	2.22 ±0.33 *	2.28 ± 0.46	2.43 ± 0.49	1.88 ± 0.39	2.07 ± 0.45
Dominant Leg	% Thigh Muscle	59.4 ± 2.9	60.2 ± 2.7	60.2 ± 5.5	60.7 ± 5.2	58.9 ± 3.2	59.9 ± 3.0
	% Thigh Fat	37.2 ± 3.0	36.3 ± 2.8	36.4 ± 5.7	35.9 ± 5.4	37.7 ± 3.3	36.6 ± 3.1
	Muscle/Fat Ratio	2.08 ± 0.29	2.13 ± 0.30	2.29 ± 0.49	2.23 ± 0.43	1.93 ± 0.37	2.06 ± 0.44

All subjects N=24; low meat, n=10; high meat, n=14. Values are means ± SEM.

* Significantly different to pre-intervention, P<0.05.

3.4.2 Effect of Diet Meat Intervention

Neither the increase in leg press strength (Figure 4) nor the increase in leg extension strength (Figure 5) differed between high and low meat diet groups after twelve weeks exercise intervention. A significant increase in the absolute change of leg press strength between pre- and mid-intervention was seen in the high meat group compared to the low meat group (P=0.01), but this was not sustained throughout the intervention.

The sum of three thigh skinfolds was significantly decreased post-intervention in both the low meat group (P<0.01) and the high meat group (P<0.05) (Table 4).

From CT scans the high meat group had significantly increased percentage of thigh cross-sectional area as muscle in the non-dominant leg post-intervention (P<0.05), and significantly decreased percentage of thigh cross-sectional area as fat (Table 5).

Leg Press

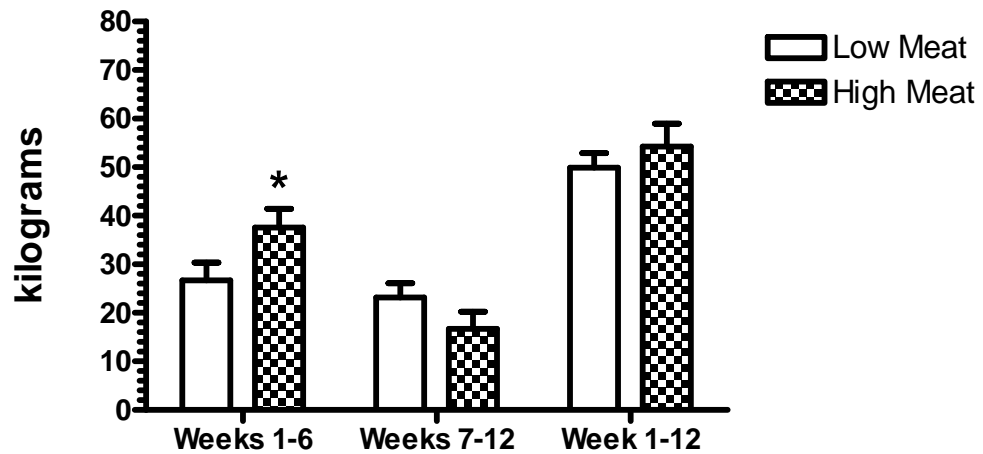


Figure 4. The effect of low and high meat diet on the absolute change in leg press strength in response to resistance training between pre- to mid-intervention (week 1-6), mid- to post-intervention (week 7-12) and pre- to post-intervention (week 1-12).
* Significantly different from pre- to mid-intervention, $P < 0.05$. Mean \pm SEM.

Leg Extension

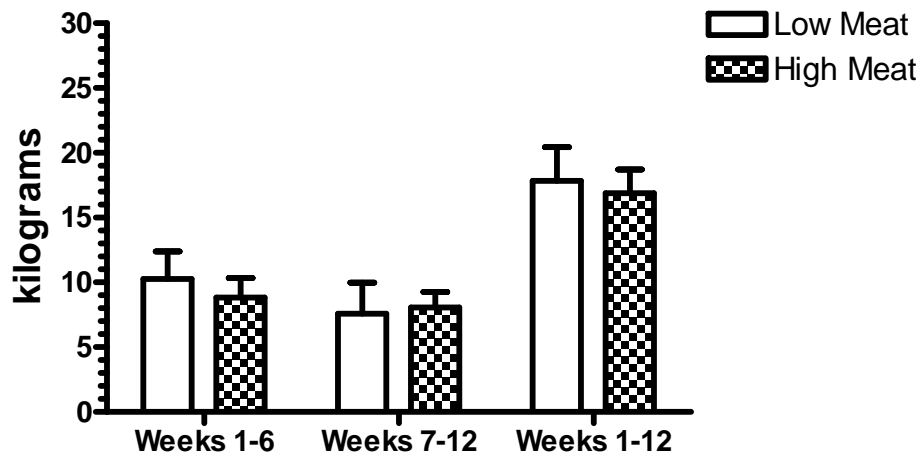


Figure 5. The effect of low and high meat diet on the absolute change in leg extension strength in response to resistance training between pre- to mid-intervention (week 1-6), mid- to post-intervention (weeks 6-12) and from pre- to post-intervention (weeks 1-12). Mean \pm SEM.

No significant differences were found from pre- to post-intervention between high and low meat groups for measurements for grip strength, BMI, thigh girth, body fat percentage, fat-free mass, physical activity scale for the elderly, short physical performance battery or estimated maximal oxygen uptake (Table 3), mid-thigh cross-sectional area of muscle or CT thigh circumference (Table 4).

3.4.3 Gender Differences

At pre-intervention, males (n=17) were significantly heavier (82.8 ± 2.1 kg) ($P < 0.002$) and taller (171.5 ± 1.3 cm) ($P < 0.0001$) compared to females (n=11) (66.2 ± 4.0 kg; 160.0 ± 1.7 cm). Males had significantly greater grip strength (41.6 ± 1.7 kg) compared to females (28.6 ± 1.2) ($P < 0.0001$). Leg press strength (figure 6) and leg extension strength (figure 7) at pre-, mid- and post-intervention were all significantly greater in males compared to females (Table 6).

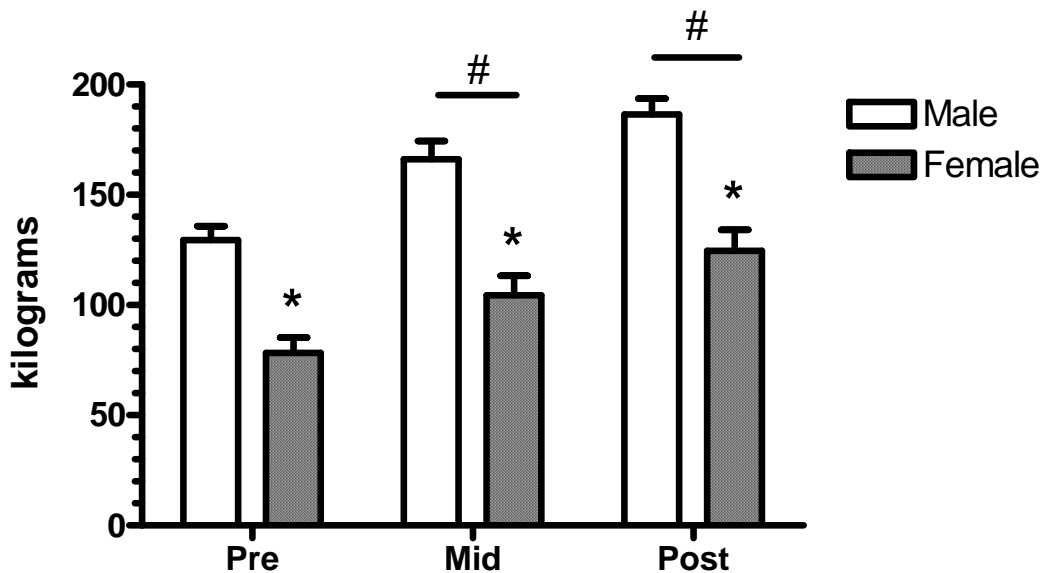


Figure 6. Effect of gender and training on leg press strength at pre-, mid- and post-intervention. Values are means \pm SEM.

* Significantly different from male.

Significantly different from pre-intervention.

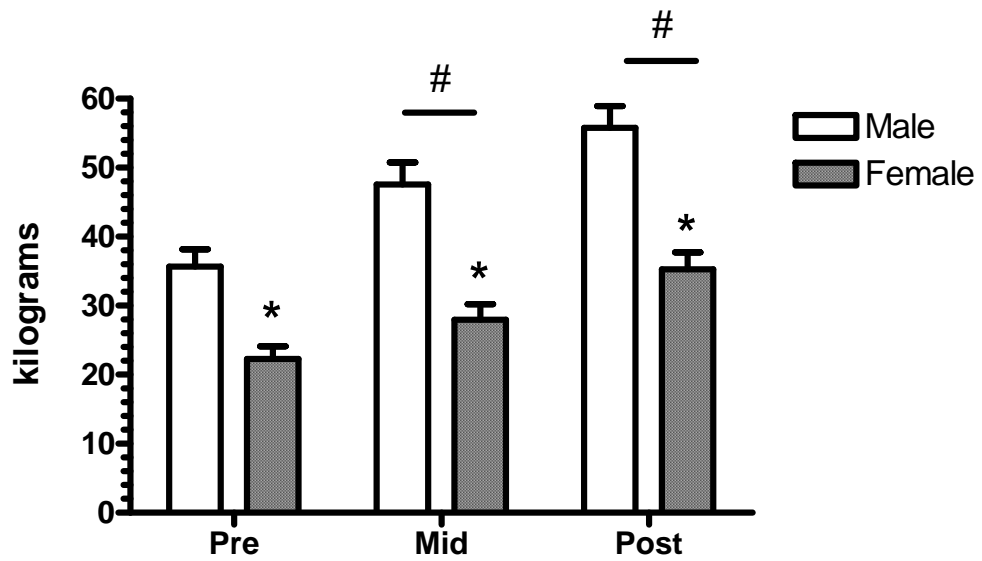


Figure 7. Effect of gender and training on leg extension strength at pre-, mid- and post-intervention. Values are means \pm SEM.
 * Significantly different from male.
 # Significantly different from pre-intervention.

Table 6. Gender comparison of absolute measurements for leg press and leg extension strength at pre-, mid- and post-intervention.

Gender	Leg press			Leg extension		
	Pre	Mid	Post	Pre	Mid	Post
Male (n=17)	129 \pm 6	166 \pm 8	186 \pm 7	36 \pm 2	48 \pm 3	56 \pm 3
Female (n=11)	78 \pm 7*	104 \pm 9*	125 \pm 9*	22 \pm 2*	28 \pm 2*	35 \pm 2*

*Significantly different from male, P<0.001

Table 7. Mid-thigh CT scan values from a males and female subject

Mid-thigh CT Scan	Male subject		Female subject	
	Left leg	Right leg	Left leg	Right leg
Total x-sectional area (cm ²)	208	207	191	200
Muscle x-sectional area (cm ²)	146	140	105	109
% Muscle	70.2	67.6	55.2	54.3
% Fat	26.3	28.9	41.4	42.3
Thigh circumference (cm)	52.1	52.1	49.3	48.3
Skin fold thickness		52.8		54.1

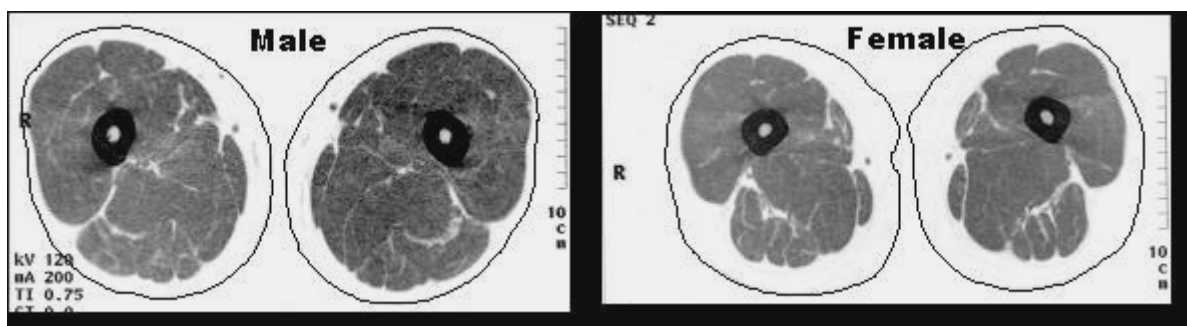


Figure 8. Examples of CT scan analysis from a male and a female subject.

These mid-thigh cross-sectional scans illustrate the greater muscle mass and lower percent fat for a male compared with a female of comparable leg x-sectional area.

Female subjects (Table 8) commenced at pre-intervention with significantly greater sum of three thigh skinfolds, greater percentage cross sectional area as fat and less percentage thigh cross-sectional area as muscle than males (Table 9) and remained significantly different post-intervention ($P < 0.05$). An example CT scan and measurements illustrating the commonly observed differences between male and female subjects is provided in Figure 8.

Table 8. Female thigh muscle and fat cross-sectional area proportions pre- and post-intervention for all subjects and subdivided according to meat intake.

Females	All		Low Meat		High Meat	
	Pre	Post	Pre	Post	Pre	Post
Thigh girth(cm)	56.4 ± 1.9	58.4 ± 2.0	59.6 ± 3.4	61.0 ± 3.1	55.3 ± 2.2	57.4 ± 2.5
Σ of 3 thigh skinfolds(mm)	93.0 ± 10.1	78.5 ± 7.5*	97.0 ± 24.3	87.9 ± 23.7	91.9 ± 12.0	75.8 ± 8.0*
% Thigh muscle	47.2 ± 2.8	48.9 ± 2.6	39.6 ± 6.3	40.1 ± 5.5	50.1 ± 2.6	52.2 ± 2.0*
% Thigh fat	49.7 ± 3.0	48.1 ± 2.7	57.8 ± 6.5	57.2 ± 5.7	46.7 ± 2.8	44.6 ± 2.2

All subjects (n=11), low meat (n=3), high meat (n=8). All anthropometric measures were taken on the right leg only. Values are as Means ± SEM. * Significantly different from pre-intervention, p<0.05

The sum of three thigh skinfolds decreased significantly from pre- to post-intervention in both female and male subjects (P< 0.05). The reduced skinfolds were significant within the high meat groups but not the low meat groups for both male (Table 9) and female (Table 8). The percentage of thigh cross-sectional area as muscle increased significantly from pre- to post-intervention in the females high meat group only (P<0.05), while no difference was found within all females or in males.

Table 9. Male thigh muscle and fat cross-sectional area proportions pre- and post-intervention for all subjects and subdivided according to meat intake.

Males	All		Low Meat		High Meat	
	Pre	Post	Pre	Post	Pre	Post
Thigh girth(cm)	55.5 ± 0.8	55.6 ± 0.9	55.4 ± 1.2	54.5 ± 1.4	55.5 ± 1.2	56.9 ± 1.1
Σ of 3 thigh skinfolds(mm)	49.7 ± 4.1	42.5 ± 3.3*	47.4 ± 4.0	43.1 ± 3.4	52.4 ± 7.7	41.9 ± 6.1*
% Thigh muscle	69.5 ± 2.2	70.2 ± 2.2	69.4 ± 3.4	70.6 ± 2.9	69.5 ± 2.9	69.7 ± 3.4
% Thigh fat	26.8 ± 2.2	25.9 ± 2.3	26.7 ± 3.5	25.4 ± 3.2	26.9 ± 3.0	26.5 ± 3.5

All subjects (n=16), low meat (n=8) and high meat (n=8). All anthropometric measures were taken on the right leg only. Values are means ± SEM. * Significantly different from pre-intervention, p<0.05

3.4.4 Associations Between Muscle Cross Sectional Area and Strength.

Leg strength correlated with total thigh muscle cross-sectional area for leg press strength pre-intervention ($r^2 = 0.801$) and post-intervention ($r^2 = 0.756$) (Figure 9).

The slopes did not differ but the elevations of the regression lines were significantly different ($P < 0.0001$).

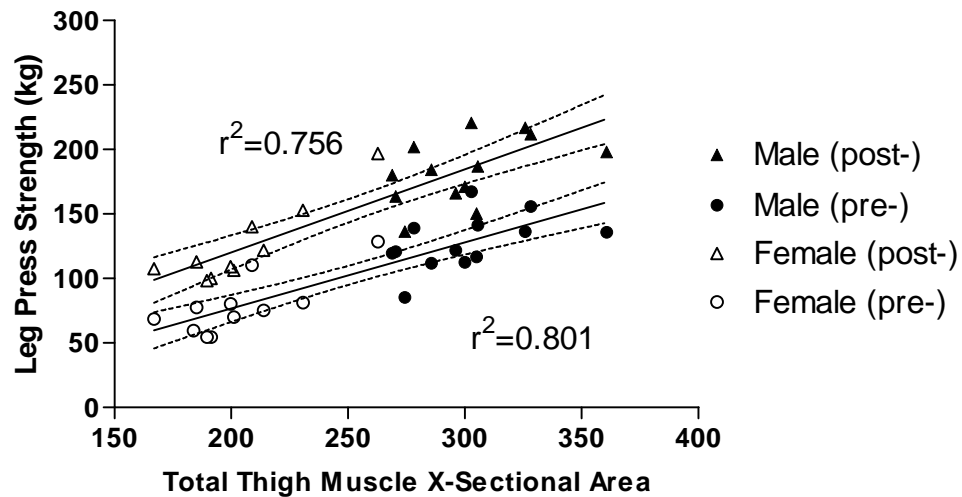


Figure 9. Baseline relationship between muscle cross-sectional area (cm^2) and leg press strength pre- and post intervention. Total thigh muscle cross-sectional area is for both left and right thighs. $N = 24$

Leg strength correlated with total thigh muscle cross-sectional area for leg extension pre-intervention ($r^2 = 0.426$) and post-intervention ($r^2 = 0.517$) (Figure 10). The slopes did not differ but the elevations of the regression lines were significantly different ($P < 0.0001$).

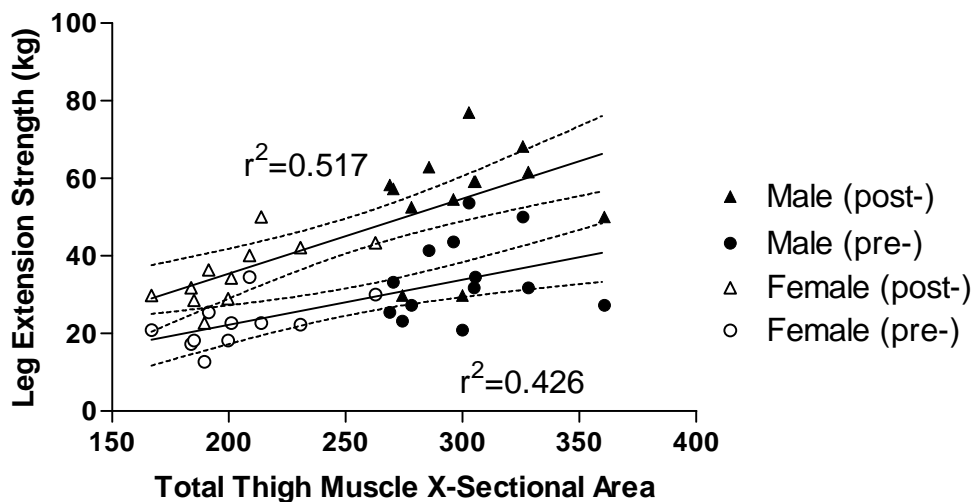


Figure 10. Baseline relationship between muscle cross-sectional area (cm^2) and leg extension strength. Total thigh muscle cross-sectional area is for both left and right thighs. $N = 24$.

3.4.5 Association Between Age and Muscle Strength

Grip strength at baseline declined in association with increased age, producing a significant negative correlation for the subject group as a whole (age v grip strength weighted for gender: Pearson $r = -0.5013$, $P = 0.040$) (Figure 11).

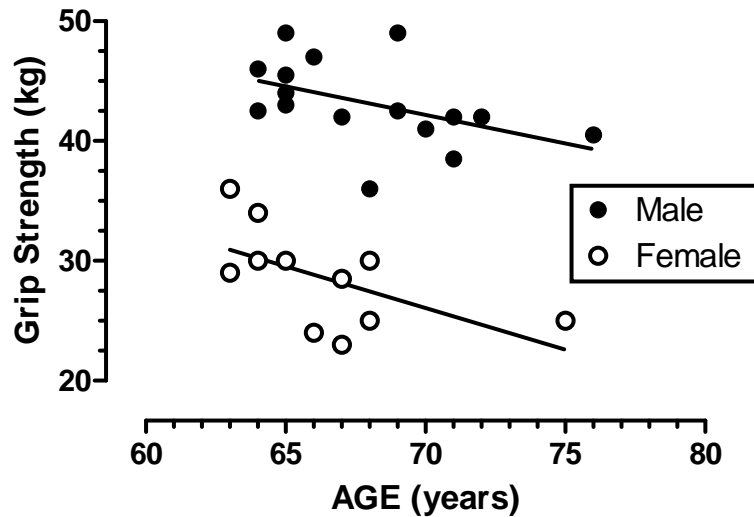


Figure 11. Relationship between age and grip strength at baseline. N= 28.

Leg press strength also declined with age producing a significant negative correlation (age v leg press strength weighted for gender: Pearson $r = -0.5092$, $P = 0.037$) (Fig 12). Leg extension strength was not significantly associated with age (Pearson $r = 0.2025$) (data not shown).

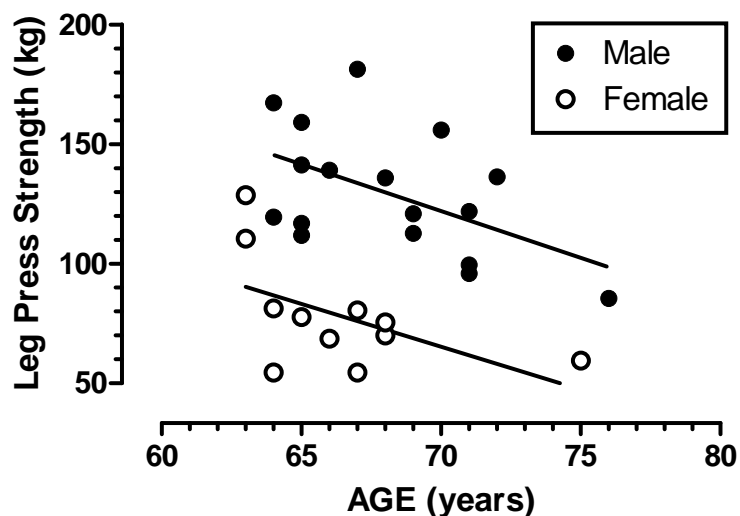


Figure 12. Relationship between age and leg press strength at baseline. N= 28.

Chapter Four

*-Discussion
&
Future
Recommendations-*

*“The important thing is to not stop questioning. Curiosity has its own
reason for existing.”
Albert Einstein*

Discussion

With an increasing number of older adults set to inhabit the Western world over the next century and the health care expenses that go towards typical age-associated morbidities, there is a definite need to seek out preventative measures that are easily administered and have positive impact on the typical age-related declines that occur with the aging process. It is apparent from the increasing research and scientific dedication to the study of such preventative measures that the interest exists; the key is ensuring the Western world at large is educated about findings that are significant and relevant for the success of aging. This study has confirmed and added to the body of research aimed at improving and enhancing the physical function and quality of life of the aging population. The results of the present study illustrate the marked improvements in leg muscle strength that can be achieved by targeted lower-body resistance training in older adults who are not incapacitated by age-related disability. In addition to the improvements in strength, there were significant reductions in body fat and increases in muscle mass in the legs, most evident in the non-dominant (weaker) leg. In conjunction with the resistance training, a higher intake of red meat appeared to be associated with a greater short-term improvement in muscle strength when compared to the moderate meat diet, but the differences between diets were not maintained longer-term. The significance of morphological changes in leg fat and muscle were also restricted to the higher meat group, but there was no effect of dietary red meat or lower limb resistance training on the strength of untrained muscles of the hand.

Higher functioning older adults represent a segment of the aging population that could potentially go unnoticed and not received adequate guidance toward maintaining proper strength and muscle reserves into older ages. Sarcopenia has been termed “clinically silent” (Gallagher et al., 2000) as the common age related declines of muscle mass can be coupled with increased body fat levels, leaving a deceptively “healthy” body mass to present itself. Such declines may go completely unnoticed by health care providers as routine yearly physical and medical check-ups rarely, if at all, assess functional strength and mobility of their aging patients. Lower reserves and the typical losses seen within the musculoskeletal system may not even be addressed until a debilitating event occurs, such as a fall. It could be only after such an event that attention is first paid towards the maintenance of muscle mass, strength and other preventative measures.

In the face of these typical signs of the aging process, three possible forms of prevention are recognized; primary; secondary; and tertiary. Primary prevention involves complete avoidance of an initial event (e.g., fall due to poor balance and agility). Secondary prevention occurs when a condition (e.g., sarcopenia or osteoporosis) is recognized and treated before a debilitating event occurs, and tertiary prevention occurs after a debilitating event, and then interventions are designed to prevent another mishap (Jette and Branch, 1981). Methods must be sought that have potential to counteract the vicious cycle of diminished strength, muscle mass and functional capacity.

Due to the healthy, high functioning nature of our subjects at commencement, the study interventions were aimed towards primary prevention, namely

improving components of skeletal muscle strength and mass along with dietary intervention that would have positive effects on other parameters of health.

Human skeletal muscle demonstrates considerable plasticity and adaptive capabilities (Reeves et al., 2006) in response strength training stimuli.

Older strength trained adults are able to maintain the same level of muscle structure and function as younger counterparts as seen in morphological aspects of muscle fibres, cross-sectional muscle fiber area and distribution of myosin isoforms, which are normally decreased and altered in aging human muscle (Klitgaard et al., 1990). It is clear that with strength training neither age (Sharman et al., 2001) nor gender (Roth et al., 2001) are limiting factors for improvements to be made when undergoing resistance training interventions.

As predicted, all subjects gained significant improvements in leg strength specific to the intervention exercises over the course of the twelve weeks. This is consistent with studies that show undeniable strength gains with a variety of older adults who undertake higher intensity resistance training interventions (Fiatarone et al., 1990; Frontera et al., 1990; Hakkinen et al., 1998; Castenada et al., 2001; Singh et al., 2005; Henwood et al., 2008).

The most recent Australian national nutrition survey suggests that older Australians (65y +) are eating on average approximately 350g of red meat per week (Australia National Nutrition Survey, 1995) and this was borne out in the baseline assessment of red meat intake. The higher red meat diet in this

study represents more than double the average intake for this age group and more closely approximates the intake of younger Australian adults (Australia National Nutrition Survey). Nevertheless, there was little difference between the high and low meat groups after twelve weeks of intervention. This could be due to a number of factors. Our study population was clearly not malnourished and their eating patterns demonstrated a balanced diet of all major nutrients of carbohydrate, protein and fat. It could be that despite having red meat intakes consistent with their age group and less than younger adults (Australian National Nutrition Survey 1995), their diets were already well suited to the nutritional requirements that would be needed to gain lean body mass (Grandjean et al., 1999). Therefore, increasing the quantity of protein within their currently high nutrient quality diets could have little effect. A recent review concluded that having a dietary intake moderately above ($1.0\text{g protein} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ or $\sim 125\%$ of the RDA) the $0.8\text{g protein} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ recommended daily allowance of protein is important, but that neither type of protein, nor protein-enriched supplements, nor manipulating the timing of protein ingestion after an exercise stimulus further increased muscle strength and mass as a result of resistance training in the aged (Campbell and Leidy, 2007). Resistance training, especially of higher intensity appears to be such a potent anabolic stimulus that its effects surpass any further enhancements that may occur as a result of nutrient specific intervention within healthy populations. But an overriding observation is that the total dietary protein did not differ significantly between the two levels of red meat intake.

Body iron stores tend to increase with age and there is some suggestion that moderate elevations in body iron stores may be associated with increased risk

for developing cancer and cardiovascular disease (Steven et al., 1988; Salonen et al., 1992). A high red meat diet is likely to provide a greater amount of bioavailable iron, but Garry and colleagues found no association between dietary heme iron and body iron stores in a cross-sectional study of healthy elderly men and women (Garry et al. 2000). Whether a high red meat protein diet has the potential to adversely affect body iron stores in the elderly has yet to be clearly established. Conversely, resistance training is thought to decrease body iron stores in elderly men and women decreasing serum ferritin in older men (Wells et al., 2003) and women (Murray-Kolb et al., 2001), although it appears that there may be sex specific differences in the effect of resistance training on iron status (Murray Kolb et al., 2001). Despite a downward trend there were no significant changes to plasma ferritin in the current study suggesting that short term increases in dietary heme iron in association with an exercise training program do not have adverse effects on body iron stores in older adults. The numbers in this study were small and the lack of significance may be related to this, but it is also possible that the adequacy of iron intake from even the lower level of meat consumption in this study counteracted the effect of resistance training on ferritin.

Urinary protein excretion remained unchanged over the course of the study in both dietary group which suggests that both levels of dietary protein were well tolerated in this older-aged subject group which was on average slightly hypertensive and overweight thus putting it at greater risk of developing microalbuminuria (Wang et al., 2006). The relationship between dietary protein intake and proteinuria is still unclear, but in those with high BMI in association with hypertension or type II diabetes the risk of development of

microalbuminuria and progression to overt proteinuria is elevated, and there is a paucity of research examining the effects of high protein diets on renal function. However in this population, normal-high protein diets may be useful in both weight and blood glucose management. In one pilot study examining protein intake and renal function in type II diabetics it was found that urinary protein increased with soy protein diets, while it remained stable with a diet containing red-meat (Anderson et al., 1998). Clearly more research is needed to examine the potential role for meat within high-protein weight-loss diets in people with metabolic syndrome (the cluster of high BMI, insulin resistance and other cardiovascular disease risk factors). Nevertheless, the safety of red meat in moderate to higher doses in healthy older individuals was apparent from the stability seen with the biochemical measures of iron status, urinary protein excretion and protein and lipid oxidation markers in blood and urine over the course of the intervention with our subjects.

Females on average have lower absolute total body strength when compared to males. Body size and other factors such as cultural norms are some factors associated with the greater total body muscular strength that males typically exhibit when compared to females (Walter et al., 1988). For example grip strength is largely associated with stature in both men and women (Syddal et al., 2003) and our female subjects displayed lesser values for grip strength when compared to the males while also being significantly smaller in stature when compared to the males. The strong correlation between thigh muscle cross sectional area and leg press strength in this study confirmed that stature and muscle mass are the major determinants of absolute strength independent of gender.

Males have a higher fat-free body mass relative to women. This is attributable to the large quantity of total skeletal muscle mass as a result of hormonal factors, such as higher testosterone concentrations (Wilmore, 1982) as well as larger cross-sectional area of the individual muscle fibres (Miller et al., 1993) and a greater number of muscle fibres (Sale, 1999). Gender differences have also been explained by muscle architecture with differences in the muscle fibre pennation angle and fibre length (Abe et al., 1998) which greatly affects the ability to generate muscle force and velocity. Males have larger pennation angles allowing for a greater degree of packing within the fibre, which is thought to permit greater production of muscular force for the same given muscle volume (Chow et al., 2000). This is demonstrated in our study with females significantly weaker than males across all time points for leg and grip strength.

Regional differences in relative strength are also seen between genders, with lower extremities strength in females maintained at about 72% that of males whereas the difference in upper body strength is greater at only 55% that of males (Hoffman et al., 1979; Maughan et al., 1983). Joszi et al., (1999) found that when assessing the strength increases in older men and women after a twelve week resistance training intervention, men exhibited larger strength gains in all the tested exercises with the exception of leg press (Joszi et al., 1999). These regional specific differences between strength of the upper and lower body could be attributed to gender differences in physical and labour-based activities as well as altered regional distribution of muscle mass (Wilmore et al., 1974; Kraemer and Koziris 1994). The gender differences between the upper and lower body is in accordance with our subjects as

discussed in regards to the lesser values for grip strength with our female group, however at the completion of the twelve weeks, our females displayed similar leg strength values to what the males exhibited at baseline suggesting their ability to gain strength significantly and consistently over the intervention period. Moreover, the new levels of strength attainment remained highly correlated with muscle cross-sectional area independent of gender. This is accordance with other documented ability for older females to gain strength and muscle volume similar to that of their males counterparts in response to resistance training intervention (Roth et al., 2001).

Even though both males and females gained significant strength for leg exercises across the intervention grip strength remained unchanged for all our subjects and within gender groups. Grip strength is often used to assess overall body strength (Basse, 1992) and is an effective assessment to identify frailty among older adults and predict early mortality (Laukkanen et al., 1995). The grip strength of our subjects indicated our cohort to be higher functioning when comparing to other intervention-based or cross-sectional studies. At 42 kg for males and 29 kg for females the grip strength in this study was higher than the 39 kg and 23kg (Syddall et al., 2003), and 28 and 18kg (Snih et al., 2002) reported for similar age groups of males and females, respectively.

There were no improvements in grip strength from pre- to post-intervention in our study group whereas an increase by 4% occurred with a group of healthy older women undergoing a whole body resistance training class over a period of twelve weeks compared to non-exercising controls (Skelton et al., 1995). However, although the use of body weight, rice bags and elastic tubing would

be deemed as low intensity when compared to machine-based resistance machines that can facilitate significant loads of weight, the specificity of exercises using rice bags and elastic tubing would necessitate the use of forearm musculature and grip strength to successfully perform such exercises. It is likely that clear lower-body specificity of our exercise intervention contributed to the lack of grip-strength gain in our group. The lack of improvement in grip strength between the high and low meat groups also confirms that diet did not individually influence overall whole body muscular strength independent of training. It is clear that muscular strength outcomes are highly specific to the chosen form of intervention. Choosing interventions that are suitable for the subject populations and providing ample stimulus that will appropriately challenge and stimulate whole body improvement is important, while not undermining the importance of targeting specific areas that are critical for functional living.

The failure of skeletal muscle mass to increase as assessed by mid-thigh computed tomography was not unforeseen despite the significant increases in leg strength. The reported non-linearity between losses in muscle strength and mass into older years which have been previously discussed (Hughes et al., 2001; Goodpaster et al., 2006) could account for the lack of change to muscle mass. Perhaps strength is a more potent factor to assess, as leg extensor strength is independently related to lower-extremity performance (when using repeated chair stands as a measure) in cross-sectional examination of older men and women, whereas leg muscle mass is not (Visser et al., 2000b). Other explanations for the lack of change to muscle mass could have been the non-regional specific assessment of thigh composition as selective hypertrophy

within the thigh has been reported with strength training of the legs (Narici et al., 1996). Our assessment of mid-thigh total cross-sectional area may not have been comprehensive enough to detect selective changes within thigh musculature, namely the quadriceps, which was the primary muscle targeted during our intervention.

Measuring specific changes to this muscular region may be of importance in future investigations. Selective decreases in cross-sectional area of the anterior portion of the thigh coincided with decreased knee extensor (quadricep) strength, whereas the posterior thigh cross-sectional area and knee flexor (hamstring) strength both remained unchanged over a 8.9 year longitudinal study (Frontera et al., 2008). This specific decrease within the knee extensor (anterior thigh) is important to note as the ability of these muscles to improve with high-intensity resistance training interventions targeting lower extremity musculature is significantly associated with functional performance measures such as chair stands and walking speed (Taaffe et al., 1999) which offer protection against future disability and early mortality (Guralnik et al., 1995). Age-related decrease in muscle strength (Frederiksen et al., 2002) was reflected in this study by the significant negative correlations of age with grip and leg press strength but interestingly not in leg extension strength.

Often people are not symmetrical in their functionality and actions, and people may have a preferred leg that they use for stability and strength. In the present study preferred, or as we termed it, dominant leg was determined by the chosen foot that the subject stood on when simply asked to arbitrarily stand on one foot for the balance test. All exercises were provided bilaterally, which is

contrary to daily use where the dominant leg may be favoured for unilateral tasks that require muscular strength and stability. It appeared that the greatest changes to muscle and fat cross-sectional area were seen in the non-dominant leg. The non-dominant leg muscle cross-sectional area tended to be lower than the other leg at baseline but with training there was a significant increase in muscle and decrease in fat cross-sectional area only in the dominant leg. The muscle strength and endurance tests were completed bilaterally so the non-dominant leg strength could not be separately discerned. However, this finding suggests that lesser functioning muscle may benefit more from resistance training and confirms the usefulness of high-intensity training that is structured solely towards the lower extremities. Weak quadricep strength has been associated with osteoporosis (Liu-Ambrose et al., 2003) and risk of osteoporotic fracture can be reduced by improving leg strength. Being caught in circumstances such as losing balance on uneven terrain or being hustled by other individuals in a crowded environment has the potential to create a perfect opportunity for falls and therefore increasing the risk of fracture. By enabling an older adult to be more prepared for recovering and stabilizing themselves in such unforeseen situations, they are more likely to be better prepared to avoid a fall. Although there is still debate as to the effectiveness of high-intensity resistance training at improving balance in the elderly (Orr and Fiatarone-Singh, 2008) and potentially lessening fall risk in the elderly, there is no harm in improving the symmetry of strength with both legs in an older adult population.

Another potential reason for the lack of increase in muscle mass could have been specific variables within the exercise training. Many studies that report

significant changes to muscle mass use protocols that are longer in duration, start the high intensity component earlier within the intervention, or manipulate training variables specifically to elicit hypertrophy-based responses. Although our protocol did impose a high-intensity stimulus, it may have lacked diversity within the stimulus, as substantial muscular hypertrophy along with strength has been reported with more varied protocols (Tracy et al., 1999). Bodybuilders commonly vary the training stimulus within each performed set in order to fatigue the muscle and facilitate further muscle fibre recruitment and a similar practice imposed on healthy older men and women produced significant change to thigh muscle volume after only 9 weeks of training that involved unilateral resistance training of the quadriceps (Tracy et al., 1999). For example, a subject performs four to five 4-5 repetitions at high-intensity (5RM), then in the same exercise set the resistance is reduced so an additional one to two repetitions are performed. This is then repeated within the same exercise set until a total of 15 repetitions are performed with the process then repeated for the following sets.

A varied approach to training stimulus, three days per week with a different training stimulus each day (e.g. day 1: strength, 3-5RM, 5 reps; day 2: hypertrophy, 8-10RM, 10 reps; day 3: power, 15RM with high velocity, 6-8 reps) produced significant changes to strength and muscle mass in a short intervention of only ten weeks (Hakkinen et al., 1998). Over the course of the ten weeks, training volume was augmented from three to six sets and it produced increases in cross-sectional area of quadriceps together with decreased proportion of type IIb fibres and increases in mean fibre area of type I and type IIa. This shows that with shorter periods of training an actual

change is occurring within the muscle, and strength gains can be attributed to more than just neural adaptations.

Perhaps higher functioning older adults like our cohort, who have the physical capacity to significantly improve strength with basic, high-intensity resistance training regimes may require diversity within the training stimulus to elicit marked changes in muscle mass. Further to this, diet intervention and the effect of protein source may play a larger role for the significant accretion of muscle mass with such training protocols.

Muscle mass may also be more likely to increase over shorter intervention periods when high intensity is imposed within the first few weeks of training such as when subjects commenced high-intensity resistance only after a maximum of three practice sessions in a twelve week program (Frontera et al., 1988) or even with short training periods of eight weeks when starting higher intensities with minimal conditioning or familiarization periods (Fiatarone et al., 1990). High strength gains are not unexpected with minimal familiarization, which was the case in both studies (Frontera et al., 1988; Fiatarone et al., 1990), however the improvements in muscle mass are a surprising outcome from such short periods of training. One group of subjects was frail, institutionalized and older (86-96 years) (Fiatarone et al., 1990), suggesting that improvements could be attributed to low functional status and muscular reserve at baseline. However, the other group of subjects was healthy and younger (60-72 years) (Frontera et al., 1988), similar to our study population. Perhaps an aggressive stimulus at the outset along with minimal

physiological preparation creates an environment in which skeletal muscle accretion is enhanced?

The latter two studies performed training three days per week as opposed to our twice weekly program, which raises another potential difference. However twice per week resistance training can improve muscle cross-sectional area as well as ameliorate other body composition measures such as percent body fat (Hakkinen et al., 1998). Body fat levels declined when a group underwent resistance training alone in contrast to the lack of changes to body composition with resistance+endurance or endurance training alone (Izquierdo et al., 2004). As they did not report physical activity levels it is unknown if this resistance trained group increased their physical activity levels as a result of the intervention, owing to the decrease in body fat levels, or if it was a true effect of the resistance training

This decrease of body fat is important to note as we found a significant decrease in the sum of seven skinfolds as well as the sum of three thigh skinfolds in our subjects at the end of the twelve weeks, whereas longer training periods but similar training volume have not produced changes to body composition, even with whole body regimes (Galvao and Taaffe, 2005).

Interestingly, both Izquierdo et al., (2004) and this study found changes when body composition was assessed using anthropometry, which is not commonly praised as a reliable method to assess changes in body composition with older adults (Nelson et al., 1996). Conversely, we did not find changes when body fat or fat free mass was measured with bioelectrical impedance, which

signifies the range of sensitivity and accuracy of various methods used to measure body composition with older populations. Factors such as hydration and prior activity greatly affect bioelectrical impedance (Kyle et al., 2004; Sergi et al., 2006) measurements, therefore variations in subjects compliance to pre-testing instructions, suggested by personal communication with subjects, may have influenced our measures.

Reductions of body fat with resistance training have been acknowledged and considered an important effect of resistance training, however the exact mechanisms behind such decreases are not defined (Fielding, 1995). Total energy expenditure increases along with decreased percentage body fat in older adults undergoing resistance training intervention, even after adjusting for the energy cost of resistance training (Hunter et al., 2000). Resistance training also increases energy requirements of older adults (Campbell et al., 1994), therefore it seems natural that body fat levels would be affected as a result of resistance training. If the significant decrease in skinfold measurement is a true effect in our study population then the stability of our subjects in regards to weight and BMI could indicate that an increase in muscle mass did occur, but was not identified with our measurement techniques. Campbell et al., (1994) found significant decreases in the sum of seven skinfolds, but no change to mid-thigh circumference when assessing older adults undergoing twelve weeks of resistance training using similar protocols to our study (Campbell et al., 1994), however they performed body composition measurements that were more comprehensive as compared to our study and found significant decreases in percentage body fat and fat mass, along with significant increases to fat-free mass.

The maintenance of a healthy body composition is important for all ages, and unfavourable body fat levels are a risk for certain age-associated morbidities such as cardiovascular disease, diabetes and quality of life in older adults (Han et al., 1998). Adequate levels of physical activity are important and such health behaviours are stressed by health organizations because the amount of physical activity (as measured by calories expended per week) is inversely proportional to morbidity (Spirduso et al., 2001). New recommendations from the ACSM/AHA have acknowledged this dose-response effect and suggest that aerobic and resistance training activities be performed above the minimum recommended amounts in order to gain additional health benefits (Nelson et al., 2007).

Unfortunately, many populations in the Western world do not achieve adequate levels of physical activity. Nine out of ten older adults in England are not meeting the guidelines for the recommended level of exercise for adults as prescribed by the ACSM/AHA (Pate et al., 1995) putting them at risk for increasing levels of morbidity and early mortality (Taylor et al., 2004). Similarly, in 2001, 55% of adults in the United States did not engage in sufficient physical activity to meet the same recommended guidelines of 30 minutes of moderate intensity exercise on most days of the week (Center for Disease Control and Prevention, 2001). Only half of older community-dwelling adults studied in a cross-sectional examination in Victoria, Australia were reported to be exercising at “sufficient” levels to provide health benefits (sufficient was the accumulation of at least 150 minutes of activity over 1 week; or the accumulation of at least 150 minutes of activity and at least 5 sessions of activity over 1 week) (Sims et al., 2007). However, 81% of

subjects reported no moderate activity, with 71% of those aged 65-69 years and 79% of those 85+ years reporting no vigorous physical activity (Sims et al., 2007). Given that low physical fitness (which results from low levels of physical activity) is a prominent risk factor in the functional decline of older adults (Morey et al., 1998), and that physical activity levels are independently associated with self-report assessment of functional status (Foldvari et al., 2000) it seems fitting to assume that preserving and potentially increasing physical activity levels into older age is paramount to attenuate the usual declines seen within the aging muscular system.

Our study population displayed higher levels of physical activity compared to other studies when using the PASE scores (baseline scores for our subjects: 176; versus 144 (Washburn and Ficker, 1999); and 85 (Schuit et al., 1997) and still gained significant strength and improved body composition measures and quality of life (based on anecdotal evidence). This demonstrated that even highly active older individuals have the potential to further improve their physiological and functional capacity.

The self-reported physical activity levels of our subjects did not change over the course of the intervention and although this has been reported before (Henwood et al., 2008) PASE scores have increased in other studies that have used this tool for assessing physical activity status in exercise based interventions (Reeder et al., 2008). However such differences could be due to variation in the health status of the subjects. For example, physical activity levels increased by 11% when using PASE as an assessment tool with older adults that had at least one chronic condition (overweight, hypertension, type 2

diabetes mellitus, dyslipidemia, osteoarthritis) while undergoing a 3 month class-based exercise intervention (Reeder et al., 2008). These subjects were younger (~60 years) than our study population, which in a healthy population of older adults would be expected to exhibit higher activity levels. However due to the study requirements, their chronic conditions resulted in lower levels of activity when assessed by PASE scores as compared to our higher functioning and more active yet older group.

Conversely, PASE scores did not change after healthy, older, independent, community- dwelling men and women underwent either strength-based or high-velocity-based resistance training interventions for twenty-four weeks (Henwood et al., 2008). Higher baseline PASE scores were also seen within their intervention and control groups, signifying a higher functioning cohort of older adults. Our study population even displayed slightly higher PASE scores at baseline demonstrating again the high functioning status of our subjects.

Such discrepancy in responses of PASE scores across subject populations could signify its use as a tool for monitoring self-report physical activity levels is better suited for lower functioning older adults, however it has been validated with older healthy men and women using sensitive measurements of energy expenditure through the doubly labeled water method (Schuit et al., 1997) and portable accelerometer (Washburn and Ficker, 1999), which both support its use as tool to measure physical activity in older adults.

Given the validity of the PASE and the failure of activity levels to increase within our study and others (Henwood et al., 2008), this could also signify that the study intervention (i.e., resistance training) is actually the true basis for other changes that are occurring within the intervention period, and they are not due to changes to physical activity status outside the intervention. If the decrease in sum of seven skinfolds is a true effect of the intervention, then this signifies the importance of such intervention procedures for improving body composition status. It also shows that it can be achieved with a stimulus that doubly serves to improve other aspects of physical function such as increased muscular strength which is essential for the older adult. Significant improvements in body composition (decrease fat, increase lean mass) occur along with quality of life and improvement in lower extremity performance (Henwood et al., 2008).

This demonstrates influential changes occur even in healthy independently living older adults as a result of high-intensity resistance training. The ability of one form of structured exercise, high-intensity resistance training, to improve a myriad of factors important for the preservation of muscular strength, body composition and functional status is incredibly important for future health recommendations.

Past studies with older adults revealed that stroke, hip fracture and diabetes are commonly associated with low scores of four to six in the short physical performance battery (SPPB) of lower extremity performance that are associated with higher BMI levels (Ferrucci et al., 2000). They are also four times more likely to have disability in activities of daily living or functional

mobility after a four year follow-up period (Guralnik et al., 1995). The SPPB is scored in the range from four to twelve, with twelve representing a higher level of function. Our study population, with a mean score of 11.5 at baseline allowed no capacity to detect any change over the intervention even if their lower extremity performance had improved significantly within all subjects, between high and low meat groups or gender.

We chose the SPPB as a simple and easy to administer test of functional status, as compared to other functionally based performance tests for older adults that are more elaborate in set-up and administration (Cress et al., 1996). Our results indicate that this test, which has been effective within other populations at revealing disability in more frail populations, and even with higher functioning older adults, may not be comprehensive for older adults on the higher end of the spectrum. The fact that our study population experienced significant improvements in leg strength over the course of the intervention which were not shown as a change in lower extremity performance, further suggests the need to investigate other modes of assessing functional performance in such populations.

It also appeared (strictly based on personal observation of the investigator) that the subjects in the study were of high socioeconomic status, which has been associated with higher functioning in an elderly cohort (Seeman et al., 1994). Furthermore, the subjects were engaged in a range of social, community and employment endeavors suggesting they were “mentally” well. Two of the subjects still maintained full time employment and many were actively involved with caring for grandchildren, teaching ballroom dancing

and structured forms of aerobic activity such as indoor soccer and “surf life saving club”, to name a few.

Regardless of this observation and the lack of change seen with these tools, personal communication between subjects and the investigator anecdotally revealed prominent lifestyle changes occurring with respect to ease of performing tasks, stamina and spontaneous levels of physical activity. A self-designed (but untested) questionnaire was devised by the investigator and given to the subjects at the end of the intervention in response to consistent reports volunteered by the subjects to the investigator regarding improved quality of life, greater ease of performing tasks and desire to do be more active throughout the day. These observations, could signify the protection that resistance training may offer older adults, as walking greater distances per day (>2 miles) has been shown to be associated with lower overall mortality rates (Hakim et al., 1998).

Because of these subjective reports of improvement within daily activities and quality of life, it would be prudent for future research to investigate strictly higher functioning older adults and devise assessment tools to further categorize this sub-population within the aging population. If health organizations are successful at promoting greater and more intense physical activity within normal everyday populations, it may become the norm that future aging populations are of higher functional status, therefore necessitating such tools.

The lack of improvement in the one-mile walk test could also be due to the higher level of function within our study population. Our subjects revealed greater average oxygen consumption (30ml/kg/min) estimated from the one-mile walk test compared to other average values (16 ml/kg/min) when this specific field test is administered to older healthy populations (Bazzano et al., 1998). As with the SPPB and PASE subjective tests of functionality, there may be little room for improvement when using this field test for higher functioning adults, as subjects may have reached a maximum comfortable walking speed, and further ability to increase physical capacity was not possible, as neither running, nor inclines exist within this field test. A maximal aerobic test that requires greater oxygen reserves and utilizes more overall functional capacity may be required to investigate changes to aerobic capacity as a result of resistance training in higher functioning older adults. The ability for resistance training to improve aerobic capacity is not clear as results have been documented that show either increases (Frontera et al., 1990) or no change (Hagberg et al., 1989; Fahlman et al., 2002;). As the informal questionnaire reported subjects being able to walk up hills easier, faster and with less fatigue, although only speculation, it is quite possible that improvements to aerobic capacity would have been seen with more direct measurement of maximal aerobic capacity in our cohort.

Gait speed is the most closely related variable to self-perceived physical function (Cress et al., 1995) and has been shown to be the most sensitive measure to predict future functional dependence in already well functioning and independently living older Japanese adults (Shinkai et al., 2000). Coupled with reports of leg press power (a function of strength and speed) representing

the largest variation in walking speed, this shows the importance of maintaining leg strength to have adequate ability to achieve and maintain higher levels of more intense activity, such as walking faster. Walking assessments (distance covered over a six minute period) have been shown to improve significantly when high-intensity resistance training, but not lower intensities, are used with frail older adults (Seynnes et al., 2004) again emphasizing the value of high-intensity resistance training.

In the act of walking the quadriceps is critical, as it is the prime mover for extension at the knee joint (which propels a person forwards in walking). Regional thigh-specific losses with age are greater in the quadriceps muscle than in the hamstring muscle (Frontera et al., 2008), suggesting the importance of attenuating the decline of lower extremity muscles, specifically the quadriceps muscle, which could indirectly influence future disability by ameliorating the act of walking.

With skeletal muscle being a large oxygen-consuming tissue (Tzankoff and Norris, 1977) and a major contributor to glucose homeostasis, attenuating the muscle bulk of the lower extremities may have significant and positive impact on metabolic factors such as overall basal metabolic rate and type 2 diabetes. As a prime site for glucose metabolism, the relationship between sarcopenia and insulin resistance that can accompany the aging process has been considered (Evans, 1997). High-intensity resistance training improves insulin action and body composition status in healthy older men (Miller et al., 1994). High-intensity resistance training interventions with patients with type 2

diabetes have shown similar improvements (Dunstan et al., 2002), whereas lower intensity resistance training does not (Dunstan et al., 2005).

The ability of older adults to adapt positively to higher intensity resistance training is well documented and reports of injury as a result of higher intensity training intervention are infrequent. Up to 19% of older men and women suffered an injury when strength tested using 1RM protocol, with five of those injuries being related to the leg extension testing and four of those five stemming from a prior knee injury (Pollock et al., 1991). Within our study, one subject dropped-out as a result of a past injury, but this did not occur during the strength testing, nor did any subjects suffer an injury as a result of our chosen 4RM strength testing. This indicates it to be a suitable mode for assessing strength gains and allows for an appropriate mode in which to adjust training loads. Five subjects terminated due to musculoskeletal discomfort, and of those five, two were related to knee pain and three due to low back discomfort as a result of the training. Although our training frequency was low (twice per week), three subjects did drop-out due to competing social or community obligations, which again could indicate the high functioning nature of this group.

Care must be taken when implementing exercise routines with older adults as the implementation of a robust exercise training regime that consisted of strength, endurance and balance training was too comprehensive for this subject population to adhere to on a regular basis (Baker et al., 2007). This suggests that in order to target older higher functioning adults and to have high

adherence to regimes, care must be taken in ensuring interventions are not over burdening nor overly complex.

The older adult poses an interesting challenge for health care providers and those attempting to set recommendations and guidelines for this specific population. Due to their heterogeneity and with strength training being “uncommon” for this generation a “one-size-fits-all” approach does not work (Chao et al., 2000). Women may sense that structured exercise regimes are “unladylike” and “unfeminine” (Chao et al., 2000), however it was clear that the women in our study were eager to learn and train, and had very little reservation with such stigmas. One factor that contributed greatly to the adherence and eagerness of both males and females was the concurrent recruitment of partners. Therefore future studies should consider adopting such recruitment procedures (husband/wife, siblings, friends etc.) to positively influence attendance and adherence outcomes. This also dictates what commonly occurs in real-life situations, as many chronic exercisers report that exercising with a partner not only encourages them to exercise but challenges their intensity level (personal observation).

Another feature of two day per week and solely high-intensity resistance training is that it appears therapeutic-based, in some ways similar to getting a dose of medicine, whereas more comprehensive programs would necessitate a major adjustment of lifestyle, which from the outset would deter not only older adults but most adults in general from even contemplating the addition of an exercise regime into their daily life. Many recommendations are vague and non-direct, and simply promoting “becoming more physically active” is

not enough to reinforce the grave importance of physical activity or even spark interest amongst the adult population. The Western world is at a point where health care systems are being overtaxed and the influx of older populations must be seriously considered and primary prevention used as a tool to truly allow our future aged to age successfully and gracefully.

This study confirmed age and gender related differences in muscle strength in an overtly healthy population. Nevertheless, neither age-related decline nor female gender represent empirical limitations to muscle strength. The twice-weekly resistance training program stimulated marked increases in strength in males and females across our entire age-range and neither age nor gender should be seen as barriers to improving strength through resistance training. Overall this study revealed that already high functioning and active older adults have the capacity to tolerate high-intensity interventions and gain significant lower body strength. The improvements as well as associations with measured thigh fat in anthropometry and CT scan analysis should be noted, confirming the usefulness of simple anthropometric measures to detect changes from intervention-based studies. This could also provide a cost-effective tool for health and fitness professionals to measure the progress of active aging adults embarking on an exercise regime. The failure to measure any increase in skeletal muscle mass could signify the need to offer periodized resistance training regimes to older adults that target muscle hypertrophy along with muscle strength and/or use more sensitive measures to detect changes. The usefulness of such regimes to increase and preserve muscle mass, and therefore strength and deter the onset of sarcopenia may significantly impact current exercise recommendations for the older adult. The

role of protein and specific sources of protein, may play a larger role when investigating interventions aimed at muscle hypertrophy, therefore the lack of findings within the high and low meat groups should not be abandoned. The inability to detect changes in our subjects lower extremity performance, physical activity levels and estimated aerobic capacity, does not deny the possibility that changes did occur that were simply not detected. Therefore other methods of assessment suited towards such cohorts are necessary and must be investigated if future investigations should study the effects of exercise and diet intervention on skeletal muscle function and functional status in higher-functioning older adults.

Conclusions

Overall, this study undeniably brought to the forefront the capacity for healthy older adults to improve and enhance physical function with high-intensity resistance training. The strength gains and favourable changes to body composition with such a specific and intense, yet relatively small actual training time, demonstrated the feasibility of such protocols for an everyday aging population. These findings are critical and must not be dismissed or treated as inconsequential as the implementation of exercise regimes similar to what this study achieved are important for health policy makers. Therefore the potential to positively impact future generations by improving the success of the World's growing aging population is promising.

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-Appendices-

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- P.** Bioelectrical impedance recording sheet and prediction equation
- Q.** Mid-thigh computed tomography scan analysis procedures

**University of Wollongong
Smart Foods Centre
SUBJECT INFORMATION SHEET**

A. PROJECT TITLE

THE POTENTIAL FOR RED MEAT TO MODIFY SKELETAL MUSCLE FUNCTION TO PROMOTE FATIGUE RESISTANCE IN OLDER ADULTS

B. FUNDING BODY FOR THE STUDY

MEAT AND LIVESTOCK AUSTRALIA

C. PROJECT OBJECTIVES

The objectives of this project are to examine the effects of red meat consumption and a resistance (strength) training program on maintenance of skeletal muscle function in older adults. Proper skeletal muscle function is crucial for maintaining personal independence and pursuing a higher quality of life into old age. Proper dietary recommendations and adequate physical activity levels are two components that contribute to the enhancement of skeletal muscle function. This multi-disciplinary study will evaluate and provide valuable information in the areas of human performance thought to be key in maintaining independent active lifestyles of older Australians.

The entire study will require your involvement for 18 weeks. Within this time you will be asked to undertake 2 weeks of familiarization protocols, 12-week resistance training program, and 4 weeks of screening and testing procedures while maintaining a controlled intake of red meat during the 10 week resistance training program. Before, and on completion of the exercise training program you will undergo a series of measurements involving blood and urine sampling, body composition measurements, questionnaire regarding diet and physical activities (both written and interview), and physical testing procedures. Some of the tests may be repeated at the midway point.

D. MEASUREMENTS

Sessions 1 and 2 will take place before and on completion of the 12 week resistance training program.

SESSION 1 - This session will take approximately 2 hours.

A blood sample of (9mls) will be taken by an experienced registered nurse and urine samples will be collected twice throughout the study (pre- and post-intervention). This will allow researchers to analyze certain elements found in your blood and urine.

Body composition measurements will be taken twice throughout the study (pre- and post-intervention) to monitor any changes in muscle size and body fat levels. Measurements to be taken will include height, weight, skinfolds and limb circumferences.

A short battery of physical performance tests will be made to evaluate your ability to balance, walk and stand. The researcher will demonstrate all tests to be performed.

The three standing balance positions will include semi-tandem, tandem and a side-to-side position. Researchers will support your arm while you position your feet to the appropriate stance. When you are ready, their support will be released and timing will begin. Timing is stopped when you move your feet or you require support, or when 10 seconds has elapsed.

You will then walk for 8 feet on a course with no obstructions at your usual walking speed.

You will then sit in a normal straight-backed chair next to a wall. You will place your arms across your chest and stand from the chair once. If you are able to perform this you will then do the same procedure for five repetitions.

SESSION 2 - This session will take approximately 1.5 hours

These testing procedures will serve to measure your lower and upper body strength. Therefore they will serve to identify any changes in your muscle strength that may occur over the 10-week training program. Prior to testing, a basic warm-up will be done which will involve brisk walking and light stretching exercises. Lower body strength testing will be done on the machines you will use for training sessions. The body movements to be tested will be a leg press and leg extension. Both tests will be performed in a supported seated position. You will be allowed a warm-up period prior to the testing procedures.

Your forearm strength will be tested using an apparatus that requires you to grip with your hand. You will remain seated during this test and you will be required to squeeze the testing apparatus as hard as possible. You will perform this three times with one hand and you will be allowed adequate rest.

A timed 1.6 km walk will be performed to measure your heart and lung capacity which is a basic measure of physical fitness. You will be required to walk a distance of 1.6 km as quickly as possible without compromising proper walking form or beginning to run. Your heart rate will be taken throughout and during the final 15 seconds of the walk with the use of a heart rate monitor. Measurements of blood pressure will also be performed before and after the walk.

E. DIETARY INTERVENTION

Before the intervention begins we will schedule a time with a dietician from the University of Wollongong. They will administer a diet history interview that will be used to establish your habitual food consumption patterns before and after you complete the study. This will allow the researchers to determine how much red meat you will be required to eat during the study. This will take approximately 1.5 hours.

Red meat will be used as the dietary intervention and you will be given weekly supplies of unfrozen red meat to prepare and consume throughout the week. You will also be given tips and recipes from the researchers on preparing the red meat.

F. EXERCISE INTERVENTION

Before the training begins you will participate in 2 familiarization sessions over 2 weeks in which time you will learn proper exercise technique. The resistance training program, along with midway strength testing will be 11 weeks in length and will be supervised by a qualified exercise specialist. You will be required to travel to the Wollongong City Council Gymnasium for these training and strength testing sessions.

Each week you will participate in two training sessions. Each class will take approximately 90 minutes in length. During this time you will undergo proper exercise instruction along with a proper warm-up, cool-down and stretching exercises.

Exercises to be performed will be leg press and leg extension exercises. The first 5 weeks of training will be progressive in nature and you will exercise at moderate (65-75% of maximum) intensities and will be asked to perform from 12-15 repetitions of each exercise three times. The final week of the first 5 weeks you will exercise at higher (80% of maximum) intensities and will be asked to perform 8 repetitions of each exercise two times with the third set being performed to volitional fatigue. The second 5 weeks of training you will exercise at higher intensities (80% of maximum) and will be asked to perform 8 repetitions of each exercise two times with the third set being performed to volitional fatigue. You will be permitted 2 minutes of rest between each exercise set. This will also be a time to determine the maximum amount of weight that you are able to lift for each of the exercises. This will allow the researchers to adjust the weight for subsequent training sessions.

APPENDIX B

INFORMED CONSENT FORM

**Project:
THE POTENTIAL FOR RED MEAT TO MODIFY SKELETAL MUSCLE FUNCTION TO
PROMOTE FATIGUE RESISTANCE IN OLDER ADULTS**

Investigators: Associate Professor Peter McLennan, Associate Professor Lee Astheimer, Associate Professor Linda Tapsell, Mr. Herbert Groeller, Dr. Alice Owen, Miss Irene Gutteridge

G. RISKS AND DISCOMFORTS

The physical testing procedures and resistance exercises which you will perform upon medical clearance from your physician will require moderate to high intensities of effort. There is a possibility that you may experience delayed muscle soreness 1-2 days after these procedures take place. This is a common adaptive physiological response to exercise. The researchers will attempt to minimize this discomfort and any risks involved in all protocols of the study by adopting a cautious approach and using protocols that have proven to be safe an involve minimal risk.

I, _____
[name]

of _____
[address]

H. PUBLICATION OF RESULTS

Findings of this study will be used to meet Irene Gutteridge's requirements to complete a Masters Degree within the Department of Biomedical Science therefore results obtained will be used for her thesis. Findings will be reported to the study sponsor, Meat and Livestock Australia and submitted to refereed scientific journals. Your anonymity will remain confidential at all times as you will be identified by code rather than your actual name throughout reports and publications. At the end of the study you will receive feedback regarding the outcomes of the study.

have read and understood the information for participants on the above named research study and have discussed it.

[signature]

I. FREEDOM OF CONSENT

Participation in this study is entirely on a voluntary basis. You are free to deny consent before or during the testing and/or intervention procedures. Your participation and/or withdrawal of consent will not influence your present and/or future involvement with the University of Wollongong. You have the right to withdraw consent at any time during the experiments, and this right shall be preserved over and above the goals of the research.

I am aware of the procedures involved in the study, including any inconvenience, risk, discomfort or side effects and of their implications.

I freely choose to participate in this study and understand that I can withdraw at any time.

I also understand that individual data in this research study is strictly confidential.

I hereby agree to participate in this research study.

J. CONFIDENTIALITY

All questions, answers and results of this study will be treated with absolute confidentiality. Your results will be grouped with others for report purposes; however you will not be individually identified.

Name:.....

Signature:.....

Date:.....

Name of witness:.....

K. ENQUIRIES

Any questions concerning the procedures and/or rationale used in this investigation are welcome at any time. Please ask if you require an explanation if you do not fully understand any of the procedures that you have just read. The initial contact person is the investigator Associate Professor Peter McLennan Department of Biomedical Science, University of Wollongong (phone # 02 4221 4183). Any enquiries regarding the conduct of this investigation may be directed to the University of Wollongong's Ethics Officer, Karen McRae on (02) 4221 4457.

Signature of witness:.....

APPENDIX C

Medical Questionnaire

Please answer the following questions with either YES or NO

- A. Do I get chest pains while at rest and/or during exertion?
YES NO
- B. If the answer to A is “yes”, is it true that I have not had a physician diagnose these pains yet?
YES NO
- C. Have I ever had a heart attack?
YES NO
- D. If the answer to C is “yes”, was my heart attack in the last year?
YES NO
- E. Do I have high blood pressure?
YES NO
- F. If you do not know the answer to question E, answer this: Was my last blood pressure reading more than 150/100?
YES NO
- G. Am I short of breath after extremely mild exertion and sometimes even at rest or at night in bed?
YES NO
- H. Do I have any ulcerated wounds or cuts on my feet that do not seem to heal?
YES NO
- I. Have I lost 10 pounds or more in the past 6 months without trying and to my surprise?
YES NO
- J. Do I get pain in my buttocks or the back of my legs, my thighs and calves, when I walk?
YES NO
- K. While at rest, do I frequently experience fast irregular heart beats or, at the other extreme, very slow beat? (Although a low HR can be a sign of an efficient and well-conditioned heart, a very low rate can also indicated a nearly complete heart block) .
YES NO
- L. Am I currently being treated for any heart or circulatory condition, such as a vascular disease, stroke, angina, hypertension, congestive heart failure, poor circulation in the legs, valvular disease, blood clots, or pulmonary disease?
YES NO
- M. As an adult, have I ever had a fracture of the hip, spine or wrist?
YES NO
- N. Did I fall more than twice in the past year (no matter what reason)?
YES NO
- O. Do I have diabetes?
YES NO

APPENDIX D

The American Heart Associations Scientific Statement Exercise Standards for Testing and Training

Absolute and Relative Contraindications to Exercise Testing

Absolute

- Acute MI (within 2 days)
- High-risk unstable angina
- Uncontrolled cardiac arrhythmias causing symptoms of hemodynamic compromise
- Active endocarditis
- Symptomatic severe aortic stenosis
- Decompensated symptomatic heart failure
- Acute pulmonary embolus or pulmonary infarction
- Acute noncardiac disorder that may affect exercise performance or be aggravated by exercise (eg., infection, renal failure, thyrotoxicosis)
- Acute myocarditis or pericarditis
- Inability to obtain consent

Relative*

- Left main coronary stenosis or its equivalent
- Moderate stenotic valvular heart disease
- Electrolyte abnormalities
- Tachyarrhythmias or bradyarrhythmias
- Atrial fibrillation with uncontrolled ventricular rate
- Hypertrophic cardiomyopathy
- Mental impairment leading to inability to cooperate
- High-degree AV block

*Relative contraindications can be superseded if benefits outweigh risks of exercise.

Fletcher, G. (2001). "Exercise standard for testing and training: A statement for healthcare professionals from the American Heart Association." *Circulation* **104**: 1694-1740.

APPENDIX E

University of Wollongong



SMART FOODS CENTRE

Diet History Interview Protocol

The purpose of the diet history is to obtain an account of a person's usual food intake. Structurally it takes the form of a description of meals consumed throughout the day, with a food frequency cross-check (Burke,1947). One way of looking at the first component is that of a story with a beginning (usually breakfast) and end (usually supper). Using the narrative approach to taking the history means that the participant is given the opportunity to finish her/his story first before she/he is asked any more questions. In this way the flow of the participant's information-giving is not interrupted and she/he is able to mention aspects which are seen as relevant in this context. Additional comments (not necessarily on food per se) made during this description may provide some insights for further questions or discussion later on. In introducing the diet history, reference is made to the notion of **'usual'**, meaning within the last couple of months, and of a **time sequence** for the description, such as the duration of the day. Participants are asked to provide a general description and then point out variations to the pattern.

Interview schedule

- Explain the purpose of the interview. Advise the participant that you are seeking a description of usual eating patterns and suggest she/he start with the beginning of the day.
- If the participant begins with the first meal of the day and uses time references or meal sequences of the day to progress with the description do not interrupt the story, merely indicate that you are listening (nod, write, say "hmm" "yes").
- If the participant stops at intervals along the way waiting for you to respond, provide narrative support to continue e.g., "was that all for breakfast", "do you have anything after that?"
 - If the participant responds with "it depends" be sure to encourage all possible variations on that topic (usually a meal description).
 - If the participant says "probably" in defining amounts of foods, use visual aids to support this estimation process.
 - If the participant goes into explanations for why/how they consume certain foods acknowledge it in a supportive non-judgemental way, but keep the account on track.
 - When the participant has reached the end of the day, look at what you have noted and identify areas that you need more detail on. This will depend on the purpose for taking the history. Ask specific strategic questions.
 - Summarize the overall pattern of the diet and ask whether there is a great deal of variation in this pattern. Note the variation.
- Proceed with a food frequency checklist and questions on food preparation.
- Ask the participant if there is anything else he/she would like to add to what she has told you and if she thinks you have a true reflection of her usual eating patterns.

Prepared by : Associate Professor Linda Tapsell APD. Updated February 1999.

Food Frequency Checklist

"How much of these foods would you consume in one day, or one week?"

Bread
Fruit
Milk
Cheese
Yoghurt
Eggs
Cakes, Biscuits
Chocolates, Nuts
Lollies
Chips, snack foods
Soft drinks, cordials
Alcohol
Vitamin supplements

Food Preparation

What kind of butter/margarine do you use?
What oil/fat do you cook in?
How do you prepare meat? vegetables?
What cuts of meat do you use? Do you eat the fat, skin ?
Do you use mayonnaise, dressings, sauces or gravies?
How often do you eat takeaway foods?
How often do you eat at restaurants?
How often do you eat at regular social gatherings?
Which kinds of ready-made foods do you consume? How often?

APPENDIX G

Name:.....

In order to assist us in providing you with cuts of meat which you are comfortable consuming, could you please fill in the following survey.

Which of the following red meat cuts would you be prepared to eat, and how frequently?

	Regularly	Occasionally	Never
Lean beef mince	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Beef rump steak	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Beef rib eye-steak	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Lamb fillets	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Lamb chops	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Lamb cutlets	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Beef sausages	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Veal schnitzel	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Roast lamb	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Roast beef	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

APPENDIX H

Summary of physical activity recommendation for older adults (Nelson et al., 2007)

Nelson, M. E., Rejeski, W. J., Blair, S. N., W.Duncan, P., Jidge, J. O., King, A. C., Macera, C. A., and Castaneda-Sceppa, C. (2007). Physical activity and public health in older adults. Recommendations from the American College of Sports Medicine and the American Heart Association. *Circulation* **116**, 1094-1105.

APPENDIX I

Ratings of Perceived Exertion (RPE)

“During the exercise test we want you to pay close attention to how hard you feel the exercise work rate it. This felling should reflect your total amount of exertion and fatigue, combining all sensation and feelings of physical stress, effort and fatigue. Don’t concern yourself with any one factor such as leg pain, shortness of breath or exercise intensity but try to concentrate on your total, inner feeling of exertion. Try not to underestimate or overestimate your feelings of exertion; be as accurate as you can”

Borg, G. (1982). "Psychophysical basis of perceived exertion." Medicine and science in

Week
 Name:.....Code:.....
 Intensity = _____
 Sets = _____
 Training Session #.....
 Date: _____
 Recorder:.....

Leg Extension	Last Week	This Week	↑ or ↓	Weight Adjustments	(+0 to +10)	RPE
Set 1						
Set 2	N/A	N/A		Mass Added:		
Set 3	N/A	N/A		FINAL MASS LIFTED:		
Problems:						

Recorder:.....

Leg Press Seat #	Last Week	This Week	↑ or ↓	Weight Adjustments	(+0 to +10)	RPE
Set 1						
Set 2	N/A	N/A		Mass Added:		
Set 3	N/A	N/A		FINAL MASS LIFTED:		
Comments:						

APPENDIX K

4 RM STRENGTH TESTING

Name:.....Code:.....

Date:.....

- Warm-up completed
- Stretching completed

Protocol:

1. Warm-up set of 10 repetitions of 50% predicted 4RM.
2. REST 2 minutes while next person does STEP 1
3. Perform predicted 4RM
4. REST 2 minutes while next person does STEP 2.
5. Perform another 4RM with increasing mass if not a 10/10 during STEP 3.
6. REST 2 minutes while next person does STEP 5.
7. Perform another 4RM with increasing mass if not at maximal 4RM during STEP 5.

Recorder:.....

LEG PRESS Seat: Predicted 4RM =	1.Warm-up: 50% of 4RM X 10 <input type="checkbox"/>	2.REST ~ 2 min.	3. 1st Attempt <input type="checkbox"/>	4.REST ~ 2 min.	5. 2nd Attempt <input type="checkbox"/>	6. REST ~ 2 min.	7. 3rd Attempt <input type="checkbox"/>	8. 4 th if necessary <input type="checkbox"/>
			Mass to Add:		Mass to Add:		Mass to Add:	
NOTES:								

Recorder:.....

LEG EXT. Predicted 4RM =	1.Warm-up: 50% of 4RM X 10 <input type="checkbox"/>	2.REST ~ 2 min.	3. 1st Attempt <input type="checkbox"/>	4.REST ~ 2 min.	5. 2nd Attempt <input type="checkbox"/>	6. REST ~ 2 min.	7. 3rd Attempt <input type="checkbox"/>	8. 4 th if necessary <input type="checkbox"/>
			Mass to Add:		Mass to Add:		Mass to Add:	
Comments:								

APPENDIX L

Kline, G. M., Porcari, J. P., Hintermeister, R., Freedson, P. S., Ward, A., McCarron, R. F., Ross, J., and Rippe, J. M. (1987). Estimation of VO₂max from a one mile track walk, gender, age, and body weight. *Medicine and Science in Sports and Exercise* **19**, 253-259.

APPENDIX M

Physical Activity Scale for the Elderly (PASE) Scoring Form

Type of Activity	Activity Weight	Activity Frequency	Weight X Frequency
2. Walk Outside Home	20	a.	
3. Light Sport/Rec Activities	21	a.	
4. Moderate Sport/Rec Activities	23	a.	
5. Strenuous Sport/Rec Activities	23	a.	
6. Muscle Strength/Endurance Exercise	30	a.	
7. Light Housework	25	b.	
8. Heavy Housework	25	b.	
9a. Home Repairs	30	b.	
9b. Lawn work or yard care	36	b.	
9c. Outdoor gardening	20	b.	
9d. Caring for another person.	35	b.	
10. Work for pay or as volunteer.	21	c.	

PASE SCORE:

Activity Time to Hours Per Day Conversion Table

Days of Activity	Hours Per Day of Activity	Hours Per Day
0. Never		0
1. Seldom	1. Less than 1 hour	0.11
	2. 1-2 hours	0.32
	3. 2-4 hours	0.64
	4. More than 4 hours	1.07
2. Sometimes	1. Less than 1 hour	0.25
	2. 1-2 hours	0.75
	3. 2-4 hours	1.50
	4. More than 4 hours	2.50
3. Often	1. Less than 1 hour	0.43
	2. 1-2 hours	1.29
	3. 2-4 hours	2.57
	4. More than 4 hours	4.29

APPENDIX N

Scoring for the Short Physical Performance Battery

Category	Side-by-side Stand (seconds)	Semi-Tandem (seconds)	Full Tandem (seconds)
0	0-9 Tried but unable Not attempted	< 10 Tried but unable Not attempted	-
1	10	0-9 Tried but unable Not attempted	-
2	-	10	0-2 Tried but unable Not attempted
3	-	10	3-9
4	-	10	10
Total			

Standing Balance Tests – See above table

8 Foot Walking Course

Greater than 8.70 seconds = 1 point

Between 6.21 to 8.70 seconds = 2 points

Between 4.82 to 6.20 seconds = 3 points

Less than 4.82 = 4 point

Chair Stands

Unable to complete 5 stands = 0 points

Greater than 16.7 seconds = 1 point

Between 13.7 to 16.6 seconds = 2 points

Between 11.2 to 13.6 seconds = 3 points

Less than 11.2 seconds = 4 points

APPENDIX O

Anthropometric Sites and Measurements

Skinfolds measured (mm) included:

Biceps

Skinfold is raised on the mid-acromiale-radiale line so that fold is parallel to axis of the upper arm.

Triceps

Skinfold is raised on the posterior mid-acromiale-radiale line so that fold is parallel to line of upper arm.

Subscapular

Skinfold is raised 2cm along a line that runs laterally and obliquely downward from the subscapulare landmark at an angle ~45° which is determined by the natural fold lines of the skin.

Iliac Crest

Skinfold is raised superior to iliac crest on the ilio-axilla line. Fingers of the tester exert pressure inward so that fingers roll over the iliac crest. The tester used the index finger to grasp the skinfold to be measured. The fold will run slightly downward medially to the body.

Supraspinale

Skinfold is found ~5-7 cm above the iliospinale and is raised where the anterior axillary border intersects at the horizontal level of the superior border of the ilium.

Abdominal

Skinfold is raised vertically 5 cm from the right hand side of the omphalion (mid-point of the navel).

Seated Mid-Thigh

Subject bends right leg to a 90° angle seated on a chair. Skinfold is taken parallel to the long axis of the femur at the midpoint between the inguinal fold and superior border of the patella.

Medial Calf

Subjects knee is flexed at 90° angle while standing and calf relaxed. Skinfold is raised vertically on the medial aspect of calf at the level of maximal circumference.

Girths measured (cm) included:

Upper Arm (relaxed)

Arm is hanging relaxed to the side of subject and girth is measured at the mid-acromiale-radiale level.

Upper Arm (flexed)

Arm is raised horizontally with forearm ~45° angle to the upper arm. The tester stands to the rear of the subject and positions tape. Subject is then asked to first partially flex bicep to position tape appropriately. Then subject is instructed to fully contract the bicep by clenching the fist and keeping the 45° angle. Measurement is taken at the maximum circumference.

Waist

Measurement is taken at the level of the trunk where the girth is most minimal. If there is no noticeable indentation, the measurement is taken midway between the lowest rib (laterally) and the ilio-cristale landmark.

Gluteal

Measurement is made at the greatest protuberance of the gluteals. Measurement is taken when subject is standing with both feet together and the tester at the side of the subject.

Thigh

Measurement is taken 1cm below the gluteal furrow. Measurement is taken when subject is standing with both feet together and the tester at the side of the subject.

Calf

Subject stands on an elevated surface with weight equally distributed on both feet. The maximal girth is measured by manipulating the position of the tape in a series of up and down measurements.

Specific Thigh Measurements

Thigh Length

Thigh length (L) measurement is taken from the superior border of the patella to the anterior superior iliac spine.

Thigh Skinfolds (mm)

Skinfold S01

Taken 10 cm above the middle point of the thigh length (L).

Skinfold S02

Taken at the middle point of the thigh length (L).

Skinfold S03

Taken 10 cm below the middle of the thigh length (L).

APPENDIX P

APPENDIX Q

INSTRUCTIONS FOR SCAN ANALYSIS.

Opening the files:

1. Open Image-Pro Plus from the desktop.
2. Go to File menu – Open – select one of the coded scan files (either .jpg or .tif)

Inverting and enlarging the scan:

1. Go to Enhance menu – select Invert Image.
2. Click the Zoom icon in the toolbar. Click once on the scan image.
3. Click the full screen button on the top right of the scan.

Calibrating:

1. Adjust the screen so that the whole 10cm rule is showing in the image.
2. Go to the Measurement menu – select Calibration – select Spatial...
3. Check that the Unit is in cm.
4. Select the Image button in the Pixels/Unit box.
5. Move the line that pops up, over to the cm rule, and adjust it so that it is equal height.
6. Type 10 in the box asking how many units the reference represents – click OK.
7. **Record the calibration factor** (should be identical for the X and Y components – just record one).

Outlining each leg:

1. Go to the Measurement menu – select Measurements – select Features.
2. Select the shape that looks like an upsidedown house from the box on the left of the screen.
3. Minimise the screen, so that the scans are showing.
4. Click on the edge of one leg to start to outline – click all the way round (many straight line). When you complete one leg, right click to end the trace.
5. Now do the other leg, right click to complete.
6. **Record the number in green for each leg.** This is the cross-sectional area (CSA).
Note: the right leg is always on the left side of the screen.
7. Bring the Measurements screen back up to full size.
8. **Record the length for each leg** (corresponds to the CSA). This is the total perimeter.
9. Close the Measurements screen.

Defining the important region:

1. Click the squiggle shape icon from the toolbar.
2. Outline around the legs (both together). Stick fairly close to the edges, but don't cross them.

Kyle, U., Genton, L., Karsegard, L., Slosman, D., and Pichard, C. (2001). Single prediction equation for bioelectrical impedance analysis in adults age 20-94 years. *Nutrition* **17**, 248-253.

Selecting tissue ranges:

1. With the area that you have just squiggled around still highlighted, go to the Measure menu – select Count/Size.
2. Click the Select Ranges button.
3. Move the box so that you can see the scan.
4. Adjust the thin red line (to the left) so that only bone is highlighted in red (be consistent in your technique for all the scans).
5. Next, click the New button, and move the thin yellow line so that only muscle is highlighted in yellow.
6. Click Close.
7. Make sure that both the Measure Objects and Apply Filter Ranges boxes are checked.

Measuring the tissues:

1. Still in the Count/Size box, select Options...
2. Check the Fill Holes box. Click OK.
3. Go to the Measure menu – select Select Measurements – scroll down the left hand menu, selecting the Area and Perimeter options (these should appear in the right hand menu – they won't need to be reset for future scans).
4. Click Count.
5. Go to the View menu – select Measurement Data.
6. Click the Sort Down option (this will arrange the areas from highest to lowest).
7. Look for the first two numbers that are red flagged – click on each one at a time to see whether they correspond to the right or left leg. **Record the area figure for each leg** – they are the Bone Area (fill) measures.
8. Go back to the Count/Size box, select Options and deselect the Fill Holes box.
9. Click Count.
10. Go back to the Measurement Data box – the numbers should have changed.
11. Look for the first two numbers that are red flagged – check which corresponds to which leg. **Record the area and length for each leg** – these are the Bone Area and Bone perimeter (no fill) measures.
12. Look for the numbers that are yellow flagged. Click on each one at a time to see which leg and region they correspond to. Decide which ones are actually measuring muscle tissue – **record them under the Muscle Area section**. Be consistent between scans in deciding what areas are muscle, versus blood vessels etc.

Enter all data into the spreadsheet. Close any windows open within the Image-program (not the main one) – now go to the File – Open menu and start on a new scan. The last column of the spreadsheet needs to be calculated using the following formula:

Total CSA = (filled bone area + muscle area).