The influence of diet-induced weight loss and aerobic exercise on skeletal muscle mass in obese older adults.

by

Peter Joseph Chomentowski 3rd

BA Physical Education and Health, University of North Carolina Wilmington, 2001

MS Exercise Physiology, University of Pittsburgh, 2005

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This dissertation was presented

by

Peter Joseph Chomentowski 3rd

It was defended on November 14th 2007

and approved by

Dr. John Jakicic, PhD, HPA Department

Dr. Elizabeth Nagle, PhD, HPA Department

Dr. Simon Watkins, PhD, Cell Biology Department

Dissertation Advisor: Dr. Bret Goodpaster, PhD, HPA Department

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Peter Joseph Chomentowski 3rd, M.S.

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While diet-induced weight loss is a common method for the reduction of excess fat-mass, there is also a concomitant reduction in lean fat-free mass. This loss of lean muscle mass could exacerbate the loss of muscle mass, i.e. sarcopenia, that normally occurs in older men and women. However, the effects of intentional diet-induced weight loss on the loss of muscle mass in older adults have not been elucidated. **PURPOSE:** The purpose of this study was to investigate the effects of diet-induced weight loss, alone and in combination with moderate aerobic exercise, on skeletal muscle mass in older adults. **METHODS:** Twenty-nine overweight(BMI=31.81±3.37kg/m^2) older(67.23±4.2years) men(n=13) and women(n=16) completed a 4-month intervention consisting of diet-induced weight loss(WL) with or without or exercise(WL/EX).The WL intervention consisted of a 10% reduction in total body weight(kg) through a caloric restriction of 500-1000kcal/day. The WL/EX group achieved the same weight loss and exercised 3-5 times a week, 35-45 minutes, at a heart rate 65-75% of maximal predicted. Whole body DEXA, thigh computed tomography(CT) and a percutaneous muscle biopsy on the left vastus lateralis were collected to assess changes in skeletal muscle mass. **RESULTS:** Mixed ANOVA demonstrated both groups had decreases in mean bodyweight (WL, -9.2%; WL/EX, -9.1%) and whole body fat mass (WL, -17%; WL/EX, -21%). However, whole body fat-free mass decreased in the WL (-4%) and didn't change in the WL/EX (-1%). Similarly, Type I muscle fiber area decreased in the WL (-19%) and remained unchanged in the WL/EX (3%). Type IIA fiber area decreased in both groups (WL, -15%; WL/EX, -8%). There was no change in Type IIX fiber area between WL and WL/EX. Thigh muscle cross-sectional area by CT decreased in both groups (WL, -5%; WL/EX, -4%). **CONCLUSION:** Diet-induced weight loss in the absence of increased physical activity significantly decreased fat-free muscle mass in older adults. Further, the addition of moderate aerobic exercise to intentional weight loss attenuated or prevented the loss of muscle mass in overweight to obese older adults.

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PREFACE

I would like to extend my deepest appreciation and gratitude to my committee members for their time, patience and guidance and most importantly support throughout my progression and completion of this manuscript.

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1.0 CHAPTER 1

1.1 INTRODUCTION

Sarcopenia is a term that was first coined from the Greek by Irwin H. Rosenberg in 1989(1); *Sarx* means flesh and *penia* means loss(130). Sarcopenia refers to the loss of skeletal muscle mass accompanied with a decline in skeletal muscle strength observed with increasing age (1). This involuntary loss of skeletal muscle mass and strength has been found to be a leading contributor to disability and frailty in older adults. Sarcopenia increases the risks for falls and susceptibility to injury and, consequently, can lead to loss of independence and physical mobility (11, 15, 42, 147, 156).

Sarcopenia has been interpreted to be both the initial amount of muscle mass and the rate at which muscle mass declines with age (131). It's estimated that muscle mass decreases approximately 3-8% per decade after the age of 30 and this rate of decline is even higher after the age of 60 (67, 106). Decreases in skeletal muscle cross-sectional area (CSA) have been reported to be up to 40% between the ages of 20 and 60 years (26, 150). This age related decline in muscle mass have direct impacts on decreases in muscular strength. Healthy men and women in their seventh and eighth decades have, on average, 20-40% less strength compared to their younger counterparts (24). This relationship between the rates of muscle mass loss and the rate of muscular strength loss raises questions concerning the relative contribution of muscle mass and strength to the loss of function, frailty, and disability in older adults.

The population above the age of 65 in the Unites States continues to grow at an unprecedented rate. In the Unites States alone there are over 39 million Americans over the age of 65 years. Within 10 years this number is expected to increase by 6 million (14). Recent estimates indicate that approximately 45% of the older U.S. population is sarcopenic (74) and that approximately 20% is functionally disabled (101). The estimated direct health care costs attributed to Sarcopenia in 2000 were $18.5 million ($10.8 million in men, $7.7 million in women) (80). It's estimated that these figures could increase two fold over the next 10 years.

Sarcopenia is a normal part of aging and is associated with a decline in muscle mass and subsequently, muscle strength. This age related decline has recently been recognized as a main contributor to the risk for injury, loss of independence and changes in quality of life in older adults (147, 156). With more of the population living beyond the age of 65, the numbers of individuals that will become sarcopenic and functionally disabled will also certainly increase. Sarcopenia occurs in most individuals to some degree as a consequence of the aging process. The adaptations of skeletal muscle with age are evident (table 1), and these have been directly linked to frailty and increased disability in older adults (11, 42, 142).

Table 1. Anatomic Changes in the Aging Muscle

Decreased muscle mass and cross sectional area
Increase in type I fiber number
Decrease in type II fiber number
Decrease in type II fiber size with no change in type I fiber size
Infiltration of fat and connective tissue
Decreased number of motor units

Obesity is a major factor confounding the influence of Sarcopenia on the loss of muscle function in older men and women. Older men and women who are overweight or obese have greater lean mass as well as greater overall fat mass. Further, these older adults have substantially greater muscle mass than their normal weight counterparts. Recent studies have indicated that older overweight adults have a reduced functional capacity that cannot be accounted for by Sarcopenia (52, 113).

The increasing age and alarming increase in the prevalence of obesity in the United States (115) implores the examination of appropriate interventions designed to maintain muscle mass while decreasing fat mass specific to older adults. On one hand, diet-induced weight loss has been examined mostly in middle-aged adults as a means of reducing excess body weight and body fat for optimal health. However, diet-induced weight loss has been associated with the loss of lean muscle mass. Diet-induced weight loss produces a change in whole body mass with about 75% from decreases in fat mass and 25% from decreases in fat free mass. This might suggest that diet-induced weight loss in older adults, who are more prone to the development of Sarcopenia, may even exhibit greater declines in muscle mass beyond the normal aging process (113).

While the exact cause of Sarcopenia and accompanying loss of function is not completely known, decreases in physical activity may be a primary factor. As persons age they have the tendency to slow down and participate in less regular physical activity (63). This lack of physical activity contributes to characteristics of disuse, such as increases in body fat, decreases in muscle mass, and decreases in muscle quality (9, 44, 49, 142). As activity increases the characteristics of disuse are slowed and may even be reversed. Prominent research over the last two decades has shown that resistance exercise (11, 16, 32, 43, 45, 46, 140, 143) and aerobic exercise (22) have the potential to slow age related changes in skeletal muscle. These findings suggest that increased physical activity levels in the elderly can have positive effects on lowering body fat, preserving muscle mass and strength, and improving functional performance. However, the ability of exercise to attenuate the loss of muscle mass with intentional, diet-induced weight loss in older adults is not known.

1.2 2008_DISSERTATION_PDF_COPY(4-17).DOCPURPOSE AND SIGNIFICANCE OF THIS STUDY

Purpose: **The overall purpose of this project is to determine the effect of calorie restriction, both with and without moderate aerobic exercise, on skeletal muscle mass in older obese adults.**

Significance: This project will be the first direct examination of the effects of intentional, diet-induced weight loss on detailed aspects of muscle mass using in vivo imaging of muscle mass and ex vivo muscle biopsy in older obese adults. This project will be accomplished by conducting this project within the existing framework of weight loss and exercise intervention programs in older adults. The effects of diet-induced weight loss will be examined, with and without concomitant moderate aerobic exercise. This will allow for the evaluation diet-induced weight loss has on skeletal muscle in older adults, while examining potential positive effects of aerobic exercise to attenuate these potentially negative effects on skeletal muscle.

1.3 AIMS AND HYPOTHESIS

Primary aims:

Research question 1:

What are the effects of diet-induced weight loss on skeletal muscle mass in obese older adults?

Hypothesis 1a:

Diet-induced weight loss, without concomitant increased physical activity, will decrease muscle mass as derived by cross-sectional area on computed tomography.

Hypothesis 2a:

Diet induced weight loss, without concomitant increased physical activity, will decrease whole body fat free muscle mass as derived by dual energy x-ray absorptiometry (DEXA).

Hypothesis 3a:

Diet-induced weight loss, without concomitant increased physical activity, will have a greater decrease of type I and type II muscle fiber areas as derived from percutaneous muscle biopsy of vastus lateralis.

Research question 2:

Can moderate aerobic exercise, consisting of mostly walking, attenuate the loss of muscle mass that may accompany diet-induced weight loss?

Hypothesis 1a:

Aerobic exercise will partially attenuate the loss of muscle mass in obese older adults who lose weight through caloric restriction as derived by cross-sectional area on computed tomography.

Hypothesis 2a:

Aerobic exercise will partially attenuate the loss of fat free muscle mass in obese older adults who lose weight through caloric restriction as derived by dual energy x-ray absorptiometry (DEXA).

Hypothesis 3a:

Aerobic exercise will partially attenuate the loss of type I and type II muscle fiber areas in obese older adults who lose weight through caloric restriction as derived from percutaneous muscle biopsy of vastus lateralis.

<u>Secondary aims:</u>

Research question 1:

What are the effects of diet-induced weight loss on skeletal muscle myofibril area in obese older adults?

Hypothesis 1a:

Diet-induced weight loss, without concomitant increased physical activity, will have a greater decrease in skeletal muscle myofibril area as derived from percutaneous muscle biopsy of vastus lateralis.

Research question 2:

Can moderate aerobic exercise, consisting of mostly walking, attenuate the loss of muscle myofibril area that may accompany diet-induced weight loss?

Hypothesis 2a:

Aerobic exercise will partially attenuate the loss of skeletal muscle myofibril area in obese older adults who lose weight through caloric restriction as derived from percutaneous muscle biopsy of vastus lateralis.

1.4 CONCLUSIONS

Much of the decline in skeletal muscle function with aging seems to be related to the progressive reduction of muscle mass. The loss of muscle mass and strength with aging represents a major cause of functional decline and disability. While the exact mechanisms of Sarcopenia are still unknown there is evidence to support the role of decreased physical activity in the acceleration of this condition. Both the loss of mass muscle and strength has been suggested as primary contributors to falls, injuries, disability and loss of independence in the elderly population. Studies have shown increased physical activity levels slow the age related changes in skeletal muscle. A majority of these studies have found that increases in muscle mass and strength with increased physical activity levels have positive effects on decreasing risk factors for individuals becoming sarcopenic.

While diet-induced weight loss has yielded positive results for reducing excess body fat, improving metabolic outcomes, and reducing cardiovascular disease risk in middle-aged adults, there is acceleration in the loss of skeletal muscle mass and strength with weight loss. This may suggest that diet-induced weight loss alone in older overweight and obese adults could result in greater decreases in fat free muscle mass compared to individuals who participate in regular physical activity combined with an induced weight loss. This leads to the proposal that increased physical activity levels could attenuate the loss of skeletal muscle mass that might occur with diet-induced weight loss in older obese adults.

1.5 DEFINITIONS

Sarcopenia

Is a term utilized to define the loss of muscle and strength that occurs with age.

Sarcopenic

Classified in older adults as skeletal muscle mass less than 2 standard deviations below a mean of a younger healthy control.

Functional disabled

Is defined as having difficultly or the complete inability to perform certain activities suitable for his or her own age (carrying out basic life activities necessary for independent living for persons aged 65 or older).

Frailty

Is a term used to signify a multidimensional syndrome of loss of reserves (energy, physical ability, cognitive, health) that gives rise to vulnerability.

Resistance exercise

Is a form strength training in which effort is performed against a specific opposing force (weight lifting).

Aerobic exercise

Exercise that increases the need for oxygen utilization (walking, bicycle, swimming).

Muscle quality

Is defined as strength per unit of muscle mass.

Skeletal muscle myofibril

Each individual muscle fiber contains several hundred to several thousand myofibrils. These are composed of the contractile myofilaments actin and myosin and are divided into smaller subunits called sarcomeres.

Type I skeletal muscle fibers

Fibers that are resistant to fatigue, with high levels of oxidatative enzyme activity and low levels of glycolytic activity. These fibers are associated with low firing frequency, high mitochondria content and slow twitch contraction.

Type IIA skeletal muscle fibers

Fibers that are relatively fatigue resistance with intermediate oxidative and glycolytic enzyme activity. These fibers are associated with high firing frequency and fast twitch contraction.

Type IIX skeletal muscle fibers

Fibers with high levels of glycolytic enzyme activity and fatigue rapidly. These fibers are associated with high firing frequency, low mitochondria and fast contraction time.

BMI Body mass Index

Is a common measurement expressing the relationship (ratio) of weight to height.

2.0 CHAPTER 2

2.1 SARCOPENIA

2.1.1 EPIDEMOLOGY OF SARCOPENIA

The aging process is associated with progressive declines in musculoskeletal health, including a decrease in muscle mass and quality, and a concomitant reduction in muscle strength (123). The declines in muscle mass, strength and function is part of the involuntary aging process, and has been termed Sarcopenia (1, 38, 94, 135). Sarcopenia is determined by two factors: the initial amount of muscle mass and the rate at which muscle mass declines with age (131). The loss of muscle mass with age is inevitable and is believed to be a major contributing factor in the decline in muscle strength (129, 135, 150). Men and women experience some degree of sarcopenia as they age, yet the degree is variable in the severity of this condition. Methods for estimating the prevalence of Sarcopenia and associated risk factors was first developed by the New Mexico Group. In the New Mexico Elder Survey, Baumgartner and associates (10) measured appendicular muscle mass by DEXA (Dual energy x-ray absorptiometry) in 883 elderly Hispanic and white men and women. Sarcopenia was defined as a muscle mass 2 SD (standard deviations) below the mean for young healthy participants (mean age = 29 years) in the Rosetta study (154), a large cross-sectional study of body composition in

New York. They found the prevalence of sarcopenia ranged from 13% to 24% in persons aged 65 to 70 years and was over 50% for those older than 80 years. A recent study by Iannuzzi-Sucich and colleagues (73) used DEXA to quantify appendicular skeletal muscle mass in 195 women and 142 men aged 64 to 93 years old. They defined sarcopenia as 2 SD below a younger reference population and found prevalence rates for sarcopenia of 23% in women and 27% in men and these rates climbed to 31% and 45% in men and women over the age of 80 years. The results yielded by both of these studies suggest that skeletal muscle loss with age is significantly increased after the 6^{th} and 7^{th} decade and continues to increase after the 8^{th} decade of life. Because of conventional wisdom sarcopenia was believed to affect more women than men because of the longer life expectance and the higher rates of disability (91). However, results from Baumgartner (8) and Iannuzzi-Sucich (73) suggest that the process of sarcopenia affects both sexes, and may have a greater effect in men. And this is believed to be attributed to the larger initial muscle mass men have compared to women.

Sarcopenia refers specifically to the loss of muscle mass and strength with aging and both of these factors have been linked as potential risk factors for disability and frailty in older adults. The New Mexico Study (8) found this was very evident with sarcopenic women having 3.6 times higher rates of disability and men 4.1 times higher rates compared with those individuals who had greater muscle mass. Janssen and colleagues (80) investigated disability in a cross sectional survey study and found that 20% of the older U.S population is functionally disabled, and that Sarcopenia is directly related to physical disability in older men and women (14, 74, 77). This suggests a clear relationship may exist between the loss of skeletal muscle mass and strength and physical functional ability of older adults.

2.1.2 MECHANISMS OF SARCOPENIA

Multiple, interrelated factors have been hypothesized in the progressive development of Sarcopenia (Figure 1). These elements in various degrees have been demonstrated to contribute to age related decreases in skeletal muscle mass, strength, MQ (muscle quality) and functional ability.

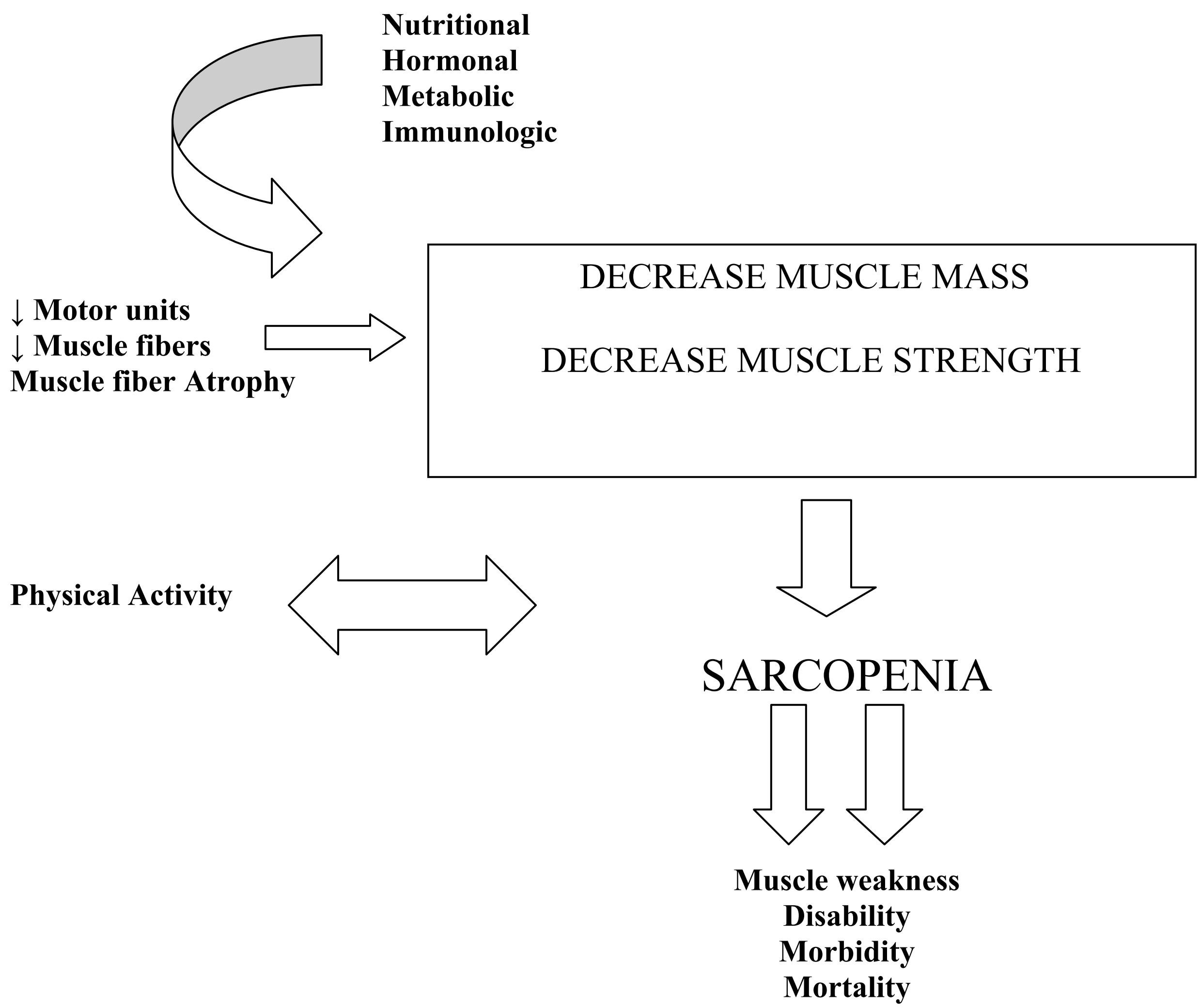


Figure 1. Factors contributing to sarcopenia

The prominent contributing factor of declines in strength and functional ability in older men and women is the age-related losses in muscle mass. Muscle mass is maintained past the 5th decade with only modest losses of 10% of whole muscle cross-sectional area (CSA) between age 24-50 years (108). But between the ages of 50-80 years the decrease in muscle CSA increases to 30%. This demonstrates that total skeletal muscle CSA decreases by ~ 40% between the ages of 24 and 80 years of age (150, 159, 160). Cross-sectional slices taken from the vastus lateralis of the quadriceps muscle have demonstrated 50% fewer type I and type II fibers by the ninth decade compared with muscles of 20 year old subjects (97). And it's estimated that on average 5% of muscle mass is lost from the fourth decade on, and this decrease may be even more rapid after the age of 65.

Based on the findings of a reduced average size of skeletal muscle CSA, it's been suggested that losses in α-motorneurons may play an important role in age-related muscle losses (13, 25-27). Studies using motor unit number estimate techniques (23, 25) have demonstrated considerable losses of whole functioning motor units in proximal and distal muscles of the upper and lower extremities (25, 141) and this appears to be greatest among the larger force production type II motor fibers. When these findings are combined with the physiological changes in skeletal muscle they could suggest that loses in motorneurons may play a role in age-related losses of muscle mass and muscle fiber number (5, 27, 28).

The observation that inadequate energy intake causes negative nitrogen balance indicates that malnutrition may be an important cause of Sarcopenia in the elderly (120). Castaneda and colleagues (18) demonstrated that eating half the recommended dietary allowance (RDA) of protein (0.8 g/kg/day) led to significant declines in strength, body cell mass, and insulin-like growth factor-1 (IGF-1) levels in postmenopausal women. Estimates taken by the

United States Dietary Association (USDA) suggest that 1 out of 3 older adults over the age of 60 years eat less than the RDA of 0.8 g/kg/day, and 1 out 5 eat less than 75% of the RDA for protein intake (131). Campbell and collogues (17) examined diets containing either meat or lacto-ovo vegetarian combined with resistance training in men aged 51-60 years. They found larger gains in fat free mass in the meat-contained diet (higher protein content) with resistance training compared to the vegetarian. These findings could suggest that low levels of protein intake may have a significant effect on decreasing skeletal muscle protein synthesis contributing to declines in muscle fiber size and number.

Both testosterone and estrogen have important anabolic effects on skeletal muscle and subsequently muscle strength. Estrogen levels decline steady during menopause while declines in testosterone haven't been clearly defined. Between the ages of 25 and 75 years, serum testosterone levels decline by 30%, free testosterone levels decline by 50%, and these declines become more advanced with age (82, 108). In the New Mexico Aging Study, Baumgartner et al. (10) found that free testosterone levels , IGF-1 and physical activity were strong predictors of muscle mass and strength. Individuals who had higher levels of muscle mass and strength also had higher levels of free testosterone. Declines in testosterone with aging have been reported in cross sectional (58) and longitudinal studies (108) suggesting lower hormone levels may contribute to age-related losses of muscle mass and strength.

Decreased physical activity can also play a primary role in age-related sarcopenia. It has been documented that older men and women who are less physical active have less skeletal muscle mass and increased prevalence of disability (36, 37, 39, 131, 150). Many numerous short term studies implementing resistance training have documented consistent increases in muscle mass and strength in older men and women (40, 43, 46, 59, 65, 96, 105, 140) and even in the frail elderly (≥ 90 yr old) (41). While published research is limited, increases in muscle mass and size have also been documented with aerobic training, (22) suggesting that aerobic training can have positive effects on the attenuation of skeletal muscle mass similar to resistance training. These findings suggest that decreases in physical activity could have a potential harmful impact in the development of Sarcopenia and that increased levels of physical activity from resistance exercise and aerobic exercise can aid in the prevention and even reversal of Sarcopenia.

All of the following factors are believed to play a role in the development of Sarcopenia. While the exact patho-physiology is still under investigation, a majority of researchers believe that the major contributing factors are the decreases in skeletal muscle mass and strength and a reduced physical activity level. Other factors play a role but the extent by which these factors contribute to the development of sarcopenia alone or in combination are still to be examined.

2.1.3 AGE RELATED LOSS OF MUSCLE MASS

With increased age there is a reduction in muscle size. One of the first studies that indicated muscle loss with aging was conducted by Allen et al. in 1960 measuring total body potassium related to age. In 1977 Tzankoff and Norris (149) measured excretion of creatinine in 959 individuals aged 20 to 97 years of age and found that age amounted to a reduction in muscle mass of one third. The introduction radiological technology now allows for muscle cross-sectional areas to be measured in different limb groups using various techniques: ultrasound, computed tomography scanning (CT), and magnetic resonance imaging (MRI). Young et al (159, 160) used ultrasound imaging and reported finding 25-35% reductions in muscle CSA (cross-sectional areas) in older men and women compared with younger counterparts. The use of CT imaging has also provided similar results in the quadriceps muscle (84, 116), biceps muscle (84, 116, 128), and triceps muscle (127).

Total muscle mass and whole muscle size peak at about the age of 24 years (108). After the second decade muscle mass levels off until about the fifth decade of life when muscle mass and strength begin to decline. This decline continues throughout the aging process with greater declines be documented after the eighth decade. Table 2 provides a summary of the changes in skeletal muscle with the normal aging process. Histological data on age-related skeletal muscle atrophy have yielded promising and consistent results from samples taken on the vastus lateralis muscle of the quadriceps: type II fiber (fast twitch) size is reduced with age and the size of the type I fiber (slow twitch) is less effected (27, 60, 90, 93, 95, 97, 129). On average the reductions in type II fiber size are 20 to 50% with age, and the reductions in type I range from 1 to 25% (24). Lexell et al. (97) found that type II fibers experience a 26% loss in CSA while type I did not differ between subjects age 20 and 80 years. Coggan et al. (21) reported that samples from aged male subjects demonstrated a 13% decline in type IIA fiber size and a 22% decline in type IIX fiber size compared to younger subjects. They also reported declines in women of 24% and 30% for type IIA and IIX muscle fiber size with minimal changes in the type I fiber size. Type I fibers are required for more low-level force production while type II fibers are capable of generating more power. This reduction in the force generating fibers is believed to be a major contributor in the development of Sarcopenia and declines in muscle strength and functional ability.

While the reductions in fiber size are related to reductions in muscle mass, other investigations have suggested that reductions in total muscle fiber number may play an important role. These decreases in muscle fiber numbers can also be fiber specific at the cellular level. Lexell et al. found that in aging muscle the percentage of type I fibers increase with age while the percentage of type II fibers decrease as a percentage of the total fiber number (table 2) (10, 141). They also noted that the decrease in fiber number with age was similar for both the type IIA and type IIX fibers. On average type II fibers fall from 60% of total muscle fibers in sedentary young men to less than 30% after the age of 80 years (87). Upon further analysis of the cross-sectional area of the vastus lateralis, it was determined that the reduction in total numbers of skeletal muscle fibers (primarily type IIA and type IIX) are significantly related to age-related declines in muscle strength. (93-95).

Research has demonstrated that skeletal muscle mass decreases with increased age. These decreases have been documented in declines in fiber size and fiber percentage. Type I fiber size changes minimally with age while the type II fibers which are capable of generating more power decrease. Muscle fiber numbers also change with age, the percentage of type I fibers increase with age while the percentage type II fibers decrease as a percentage of the total fiber number.

Table 2. Changes in the Skeletal Muscle with Age.

Variables	Aging
Muscle mass	Decrease
Type I fiber percentage	Increase
Type II fiber percentage	Decrease
Type I area	No change
Type II area	Decrease
Oxidative capacity	Decrease
Glycolytic capacity	No change
Capillary density	Decrease
Strength	Decrease

2.1.4 AGE RELATED LOSS OF MUSCLE STRENGTH AND QUALITY

The incremental loss of skeletal muscle strength has been recognized as a normal part of the aging process. Numerous studies have shown that skeletal muscle strength (the maximal amount of force exerted in a single attempt) is also decreased in aging muscle (60, 141, 159, 160). Studies have found this to be true for both genders (159-161) as well as in the upper and lower extremities.

Cross-sectional data demonstrates that muscular strength tends to increase and peak between the 2nd and 3rd decade of life and remains constant until about 45 to 50 years of age in men (89). Muscle strength begins to decrease between the 5th and 6th decade and a more rapid loss of muscle strength is evident beyond the age of 60 years (61, 89). The average age-related decrease in strength in the older adults ranges from 20-40% (110, 111, 161). With increased age the decreases in muscle strength are even higher (50% or more) past the 9th decade (110, 111) when compared to young healthy adults. Table 3 provides a review of cross-sectional studies that examined the decreases in knee extensor strength of health older adults in their 7th and 8th

decades compared to a healthy younger control. Men and women past the 7^{th} decade of life exhibit muscle strength roughly 25-45% lower than younger counterparts. These investigations illustrate that muscle strength is declined by the 7^{th} and 8^{th} decade of life in men and women.

Longitudinal studies have also provided significant evidence that muscle strength decreases with age in men and women. Aniansson et al. (4) documented decreases in muscle strength of 3.2% per year in a 7 yr. follow up of 23 men aged 73-86 years, and these declines in strength were accompanied with a 14% decrease in type IIA fiber area, 25% reduction in type IIX fiber area, and no significant change in type I fiber area. Frontera et al. (47) reevaluated muscle strength in 9 men from a previous study 12 years before and found isokinetic strength losses of 30% for knee extensors over a 12 year period.

While attempting to determine other contributing factors for the decreases in muscle strength, researchers have begun investigating the effects of muscle quality. The term muscle quality (MQ) refers to strength per unit muscle mass and is also termed specific tension. Data regarding aging and adaptations to MQ have yielded conflicting results. Some authors have found that MQ is compromised with age (98, 99), while others have reported MQ is unaffected (107, 117). Frontera et al. (47) assessed MQ using force produced relative to whole muscle CSA and tension generated by isolated muscle fibers. When the isolated fibers were examined at a cellular level they found that type I & II fibers in the aged subject's exhibited a MQ 30% less than younger subjects. This could suggest that the muscle strength per fiber may also be decreased with aging and needs to be evaluated further for its role in Sarcopenia.

Much of the strength decline that is observed with aging can be explained by the age-related loss of muscle mass that occurs in senescent muscle (45, 103, 124). Sarcopenia has been estimated to account for about 90% of the age related strength declines. This indicates that decreases in skeletal muscle mass play a large the role in strength declines with aging, while other factors such as decreased motor unit activation may also contribute.

Table 3. Age-Related Changes in Knee Extensor Strength.

Study	Gender	Age decade	Testing conditions	% of young adult strength
Larsson et al.(88)	M	7^{th}	Isometric	**75**
Murray et al.(109)	M	$8\text{-}9^{th}$	Isometric	**55**
Murray et al. (112)	F	$8\text{-}9^{th}$	Isometric	**63**
Young et al.(158)	F	$8\text{-}9^{th}$	Isometric	**65**
Young et al. (157)	F	8^{th}	Isometric	**61**
Overhend et al. (118)	M	7^{th}	Isometric	**76**
Ivey et al.(72)	M	$7\text{-}8^{th}$	Isometric	**76**
	F	$7\text{-}8^{th}$	Isometric	**75**

2.1.5 AGE RELATED CHANGES IN BODY FAT COMPOSITION

Obesity is increasing rapidly and recently more attention has been focused on the problem of obesity in the elderly because obesity has quantitatively different effects on morbidity and mortality in older adults compared to younger individuals. Studies have shown with increased age that BMI and fat mass are positively related to disability (48, 108, 153), e.g., limitation in activities of daily living (91) , waking upstairs, walking on flat surfaces (52); pulmonary disease; diabetes; and arthritis (3). Cross-sectional and longitudinal studies have shown body composition changes with age with an increase in fat mass, a decrease in muscle mass, and changes in body fat distribution. It has been observed that with increase age visceral abdominal adiposity, which is related to morbidity and mortality, increases and subcutaneous abdominal adiposity decreases with no change in (BMI) body mass index (162). A recent study by Hughes et al. evaluated comprehensive 10 year longitudinal data for limb and trunk skin fold and circumference changes in older men and women. They found that when whole body fat measures were included 39% of the study group had decreases in subcutaneous fat (-23%) while total body fat increased (71). This illustrates that with increased age abdominal fat mass increases and also can be redistributed to different adipose depots including muscle. This was recently demonstrated in skeletal muscle in a sample of subjects over the age of 70 years in the Health ABC study. Using CT scans at the mid thigh, Goodpaster et al. (52) showed that with increased age, the amount of fat stored in and around muscles also increases with age. Thus with increased age muscle mass decreases and intra-muscular adiposity increases. With aging, fat mass increases well past the 6th decade and recent research has demonstrated that with increased body fat there is also a redistribution of abdominal adipose tissues (visceral) and an increase in intra-muscular adiposity.

2.2 DIET-INDUCED WEIGHT LOSS

2.2.1 INDUCED WEIGHT LOSS AND SKELETAL MUSCLE MASS

Diet induced weight loss causes a decrease in both fat mass and fat-free mass, approximately 75% of weight loss is composed of fat and 25% of fat free mass (7). The aim of induced weight loss in obese and overweight older adults is the loss of fat mass (FM), but inevitably a proportion of weight loss is fat-free mass. Loss of fat-free mass may be undesirable if excessive as non-adipose tissues are responsible for the majority of resting metabolic rate, regulation of core body temperature, preservation of skeletal integrity, and maintenance of function and quality of life as the body ages (102). Disability occurs when the diminished muscle mass causes an inability to perform normal daily activities (33). This raises a concern whether induced weight loss without physical activity may exacerbate Sarcopenia (113, 135) (114, 136) and impair physical functioning in older adults (135).

Fat-free muscle mass has been demonstrated to decline with induced weight loss (132). Studies have documented declines in fat free mass with diet-induced weight loss in younger adults while limited information has been demonstrated in older adults. In a systematic review Chaston and colleagues (20) examined the proportion of weight lost as fat-free mass (FFM) by various weight loss interventions in different aged subjects (table 4). They concluded that the degree of caloric restriction was positively associated with percent of fat-free mass loss (%FFML) and that the degree of caloric restriction, exercise and rate of weight loss influence the proportion of weight loss as FFM.

Table 4. Summary- dietary and behavioral weight loss interventions on the %FFML.

Author	Year	Inter.	Study type	Weight loss (kg)	Percent FFML	wks	Age	Sex	Initial BMI	Method
Gower et al.(57)	2002	**LCD**	Obs	**13.1**	**5.9**	10	21-48	F	29.2	DEXA
Due et al.(31)	2004	**LCD**	RCT	**10.2**	**11.8**	52	19-55		30.8	DEXA
Leenan et al.(92)	1993	**LCD**	RCT	**11.7**	**12.0**	13	27-51	F	30.9	DEXA
Gotfredsen et al.(51)	2001	**LCD**	RCT	**10.2**	**11.8**	52	30-50		36.9	UWW
Gower et al.(57)	2002	**LCD**	Obs	**12.7**	**12.0**	10	21-48	F	28.7	DEXA
Due et al.(31)	2004	**LCD**	RCT	**15.6**	**13.5**	52	19-55		35.0	DEXA
Kockx et al.(85)	1999	**LCD**	Obs	**11.4**	**14.0**	13	26-49	F	31.3	UWW
Tchernof et al.(145)	2002	**LCD**	Obs	**14.5**	**18.6**	13.9	50-60	F	35.2	DEXA
Purnell et al.(122)	2000	**LCD**	Obs	**10.0**	**20.0**	13	60-75	M	31.0	UWW
Leehan et al.(92)	1993	**LCD**	Obs	**12.6**	**22.2**	13	27-51	M	30.5	UWW
Kockx et al.(85)	1999	**LCD**	Obs	**12.1**	**24.8**	13	26-49	M	30.5	UWW
Tchernof et al.(146)	2000	**LCD**	Obs	**14.1**	**25.5**	56	50-60	F	35.4	DEXA
Pronk et al.(121)	1992	**VLCD**	CCT	**20.8**	**23.0**	13		F	35.0	DEXA
Hoie et al.(66)	1993	**VLCD**	Obs	**11.1**	**23.4**	9	18-72	F	30.0	UWW
Hoie et al.(66)	1993	**VLCD**	Obs	**14.2**	**31.0**	9	18-72	M	30.0	UWW
Eston et al.(35)	1992	**VLCD**	Obs	**11.5**	**37.4**	6	23-57	F	35.0	UWW
Pronk et al.(121)	1992	**VLCD/ex**	CCT	**22.1**	**14.8**	13		F	35.0	DEXA
Goodpaster et al.(56)	1999	**VLCD/ex**	CCT	**12.2**	**17.2**	17	35-40	F	34.0	UWW
Pronk et al.(121)	1992	**VLCD/ex**	CCT	**20.6**	**22.5**	13		F	35.0	UWW
Pronk et al.(121)	1992	**VLCD/ex**	CCT	**21.5**	**24.1**	13		F	35.0	DEXA
Goodpaster et al.(56)	1999	**VLCD/ex**	CCT	**17.6**	**26.1**	17	35-40	M	34.5	DEXA

CCT, clinical controlled intervention; DEXA, dual energy X-ray Absorptiometry; F, female; M, male; Obs, observational study; RCT, randomized controlled trail; UWW, under water weighing; LCD low calorie diet; VLCD very low calorie diet; VLCD/ex, very low calorie diet + exercise.

2.2.2 INDUCED WEIGHT LOSS AND EXERCISE

A recent study by Villareal et al. (151) reported that when induced weight loss was combined with exercise training in older adults (age ≥ 65 years) fat free mass was maintained. These results were yielded with diet therapy and a combination of endurance and weight training 3 times a week for 26 weeks. This preservation of fat free mass was consistent with results from studies conducted in younger subjects which showed that exercise training reduces diet-induced weight loss of lean tissue (7, 50). Weiss and colleagues (155) reported data collected in the

CALERIE study (Comprehensive Assessment of Long-term Effects of Reducing Intake Energy) and found that after 12 months thigh muscle volume and average thigh muscle CSA decreased significantly in the caloric restriction group but not in the exercise group. The decreases in thigh muscle volume in the CR (calorie restriction) group was correlated with the degree of change in body weight while the EX (exercise) had no such relationship. These results indicate that 12 months of caloric restriction resulted in significant decreases in thigh muscle mass while endurance exercise preserved thigh muscle mass in older adults (mean age 57.3 years). Table 5 provides a review of selected studies that have demonstrated physical activity can have a protective effect on fat-free mass attenuation when combined with low calorie diet in younger adults. These research studies documented similar decreases in weight loss (fat mass) of about 10-14 kg over the sixteen week intervention. These findings also demonstrated that when a LCD (Low calorie diet) was combined with aerobic training there was a greater preservation of fat free mass. The evidence yielded from these studies provides information that aerobic exercise may have an attenuation effect on fat free mass with induced weight loss.

Table 5. Three randomized controlled trails comparing FFM loss on a LCD, LCD+ aerobic, LCD+ resistance exercise using MRI (Magnetic Resonance Imaging).

Author	Year	Inter.	Weight loss (kg)	%FFML	Wks	Age	Sex	Initial BMI
Janssen & Ross et al.(79)	1999	LCD	11.7	35.9	16	35-45	M	31.6
Janssen & Ross et al.(79)	1999	**LCD+ aerobic**	11.4	**17.5**	16	35-45	M	33.0
Janssen & Ross et al.(79)	1999	LCD+ resistance	12.7	18.9	16	35-45	M	33.6
Janssen & Ross et al.(79)	1999	LCD	10.7	23.4	16	35-40	F	34.5
Janssen & Ross et al.(79)	1999	**LCD+ aerobic**	11.5	**8.7**	16	35-40	F	35.5
Janssen & Ross et al.(79)	1999	LCD+ resistance	10.0	13.0	16	35-40	F	32.5
Rice et al.(126)	1999	LCD	12.1	29.7	16	40-50	M	31.9
Rice et al.(126)	1999	**LCD+ aerobic**	11.5	**15.6**	16	40-50	M	32.3
Rice et al.(126)	1999	LCD+ resistance	13.6	20.6	16	40-50	M	33.8
Janssen et al.(75)	2002	LCD	10.0	22.0	16	35-40	F	33.7
Janssen et al.(75)	2002	**LCD+ aerobic**	11.1	**10.8**	16	35-40	F	36.0
Janssen et al.(75)	2002	LCD+ resistance	10.0	14.0	16	35-40	F	31.6

* LCD – Low calorie diet, FFML – Fat free mass loss, Inter – Intervention.

2.3 AEROBIC EXERCISE IN OLDER ADULTS

2.3.1 PHYSILOIGICAL ADAPTATIONS TO AEROBIC EXERCISE

There are several marked adaptations to aerobic exercise with regular performance in younger and older adults. The most prominent has been the increase in VO2 max (64, 68), which has been demonstrated to increase 20-30% in adults 60 to 80 years of age. Capillary supply to muscles has been shown to change in response to aerobic training through an increase in capillary to muscle fiber ratio (2, 69). There is an increase in the size and number of the mitochondria of the muscle cell (68), and this is most apparent in the type I due to the higher aerobic capacity and mitochondria density. And last muscle fiber size has been found to increase

(22) and decrease (86) with aerobic training. The percentage of type I fibers has been shown to increase minimally with training in either young or older adults and type II fibers have been demonstrated to shift to the more aerobic(22, 70). The less aerobic type IIX fibers (Low oxidative capacity) have been shown to decrease with aerobic exercise training while the type IIA (Intermediate oxidative capacity) increase with training.

2.3.2 AEROBIC EXERCISE AND SKELETAL MUSCLE MASS

While muscle hypertrophy is not as prominent with aerobic training there still are muscle fiber changes at the fiber level that accompany training. These are most noticeable with a decrease in type IIX fibers (anaerobic), an increase in type IIA fibers (aerobic/glycolytic), and a minimal increase in type I fibers (aerobic). Coggan et al. (22) examined aerobic exercise training in older adults (mean age 64 years) that consisted of walking/jogging 4 days a week, 45 min/day for 9-12 months. The results demonstrated that aerobic exercise training increased VO^2 max by 23% and there was a significant decrease in number of type IIX fibers with a concomitant significant increase in the number type IIA fibers of the vastus lateralis muscle. They also observed that aerobic exercise training increased muscle cross sectional area of type I fibers by 12% and type IIA fibers by 10%. Sakkas et al. (137) examined a 6 month aerobic training program on the morphology of the gastrocnemius muscle in older adults (mean age 56 years). They reported significant increases in CSA in type I (32%), type IIA (54%), and type IIBX (36%) following the exercise intervention program. After taking into consideration the different fiber types, the aerobic exercise training demonstrated a 46% increase in total muscle fiber area compared to the pre intervention measures. Charifi and associates (19) recently examined the effects of cycling 4 day/wk, 45 min/day for 14 weeks on muscle morphology in older men (mean age 73 years). While they found there was no significant difference in whole fiber size (pre-training: 5,463 ± 971 μm^2; post training: 6,197 ± 1,708 μm^2), they did demonstrate that endurance training eliciting muscle hypertrophy, due to an significant increase in muscle fiber area of the type IIA fibers. While minimal research has been presented for aerobic exercise as a means for muscle hypertrophy in older adults, the results have shown that aerobic exercise could have positive effects on increasing muscle mass in older adults similar to younger individuals. This leads to the suggestion that aerobic exercise could attenuate the loss of muscle mass to a certain degree in older adults.

2.4 RESISTANCE EXERCISE IN OLDER ADULTS

2.4.1 MUSCLE ADAPTATIONS TO RESISTANCE TRAINING

It has been clearly defined that skeletal muscle of older adults can hypertrophy in response to resistance training and this hypertrophy is positively correlated with increases in muscle strength and increased functional ability. It has been well documented that both type I & II fibers increase with size as a result of resistance training (46, 62, 65, 148). While both type I and type II fibers increase in size in response to resistance training, it would appear that the increase in type II fiber size is more important. Type II fibers produce more force per unit area than type I fibers (12) thus any reduction in the force producing fibers may play a large role in strength declines with age.

In one of the first prominent studies Frontera and colleagues (46) reported that 12 weeks of strength training in the elderly (3 days/wk, 3 sets at 80% of 1 RM) resulted in a 9% increase in thigh muscle area as measured by CT scanning and muscle biopsy samples demonstrated a 34% increase in type I muscle fiber area and a 28% increase in type II fiber area. These results were in adult males aged 60-72 years and demonstrated skeletal muscle hypertrophies in older adults similar to younger adults. In a similar investigation that consisted of 8 weeks, Fiatarone et al. (40) reported an average increase of 11% in quadriceps area in subjects with an average age of 90 years. Since the inception of these early studies research has shown consistent results in documenting increases in muscle mass (42, 105) and strength (6, 105, 140, 143) with resistance training in older adults. And these improvements in muscle mass and strength have been positively correlated to improvements in functional performance in older adults (42, 81, 105, 138, 143).

2.5 CONCLUSION

Sarcopenia is a condition that will affect the majority of adults with age. The involuntary loss of muscle mass is part of the natural aging process, and if left unchecked can lead to weakness, disability, falls, and loss of independence. With this decrease in skeletal muscle mass it's documented that skeletal muscle strength decreases in a similar linear pattern. Research has documented that with increased age that muscle mass decreases 10-40% between the ages of 30 and 80 years. These decreases effect whole muscle CSA and are fiber specific with type II (fast twitch) fibers declining while the type I fibers remain unchanged. These decreases in muscle mass and strength are believed to play a major role in the development of decreased functional ability, morbidity, and mortality in older adults. While the exact patho-physiology of Sarcopenia has yet to be determined, research has developed a strong case for the declines of skeletal muscle mass and decreased physical activity as primary contributors.

Muscle mass decreases and fat mass increases with aging muscle. Obesity is a global epidemic that affects young and older adults similar. Weight gain steadily increases with age and this increased fat mass has been shown to have effects on functional ability. Individuals with more fat mass are reported to have lower functional abilities then leaner subjects of the same age. While induced weight loss is the most common method for reducing excess fat mass, it's been proposed that decreases in fat mass through weight loss without the inclusion of physical activity could result in larger decreases in fat free mass. This suggests that induced weight loss alone in the elderly could in fact accelerate sarcopenia. This has lead to the suggestion that physical activity (aerobic & resistance exercise) should be included with induced weight loss in older adults to slow the decreases of fat free mass.

Physical activity has been documented to be a safe prevention measure in Sarcopenia. Research has demonstrated that resistance and aerobic exercise can have positive effects on preserving lean muscle mass in older adults. When combined with weight loss research has documented decreases in excess fat mass with minimal decreases in fat free mass. This suggests that daily physical activity should be implemented in weight loss and Sarcopenia interventions in older adults.

3.0 CHAPTER 3

3.1 SUBJECTS AND SCREENING

Sedentary (Less than 2 days of physical activity weekly) and overweight or moderately obese (Body mass index, BMI = 25.0 to 38.0 $kg{\cdot}m^{-2}$) men and women (age 60 to 75 years) were recruited from local newspaper ads, mailing letters, and advertisement on local public transportation services. When subjects first contacted the study coordinator they were given an opportunity to ask initial questions and were provided with an additional description of the study. As in current studies, the study coordinator, or another member of the research team, asked and recorded some preliminary screening questions: age, height, weight, current level of physical activity, family history, and current health status. Those who meet the requirements for eligibility for pre-screening were then scheduled for an out-patient screening visit at the Clinical Translation Research Center (CTRC) located on the 8^{th} floor of Montefiore Hospital (Pittsburgh, Pa).

3.2 SCREENING VISITS

Subjects on their initial screening visit were given the information about the study and were explained what procedures will be asked of them during the intervention study. Subjects were given time to ask questions about study procedures and these were answered by the screening technicians, a study physician or the primary investigator. Following the screening subjects were asked to give written consent demonstrating they understand the risks and benefits of participated in an intervention research study. Once the subject had provided written informed consent the following procedures were performed at baseline within 2-4 visits. The OGTT test is performed as a part of the parent study but was not used for any measurement in this project.

1. A fasting blood draw was taken for the determination of lipid profiles, electrolytes, blood glucose, CBC, platelet count and HbgA1-C.
2. A medical history and general physical was performed by a cardiology fellow or a study physician. The general physical exam consists of an assessment of: Head, ears, nose, eyes, throat, heart, lungs, abdomen, genitalia/rectal, extremities, skin, neurological function, and/or other problems that may be noted by the physician.
3. Oral glucose tolerance test (OGTT) – Subjects ingested 75g glucose solution (Glucola) within a two minute period. Blood samples were taken prior to ingestion and every 30 minutes thereafter until 120 minutes. Plasma glucose was measured immediately following sampling in the GCRC using an YSI glucose analyzer using oxidase method.

Following these procedures it was determined if they have met the eligibility. Subjects were deemed eligible if they meet the inclusion criteria and are absent of all the exclusion criteria (table 6). If they meet the requirements they were scheduled for the beginning experimental procedures which will be conducted over a 2-3 week period.

Table 6. Inclusion and exclusion criteria

INCLUSION CRITERIA

60 – 75 years
Stable weight (No Gain/Loss of > 6 lbs in 6 months)
Impaired Glucose Tolerance or Newly, untreated, undiagnosed type 2 diabetes • IGT: Fasting Glucose $\geq 100 \leq 126$ 2-Hour OGTT ≥ 140 • T2D: Fasting Glucose $\geq 126 \leq 200$ 2-Hour OGTT ≥ 200
Sedentary
Non-smoker
BMI 25.0-38.0 KG/M^2
Resting Blood Pressure $\leq$ 150mmHg systolic and $\leq$ 95 mmHg diastolic
Note from PCP/Cardiologist for exercise clearance if positive stress test symptoms were observed from GXT

EXCLUSION CRITERIA

Clinically significant CVD including h/o MI
Peripheral Vascular Disease
Hepatic, renal, muscular/neuromuscular, or active hematologic/oncologic disease
Clinically diminished pulse
Presence of bruits in lower extremities
Previous history of pulmonary emboli
Peripheral Neuropathy
Currently not engaged in a regular program and have a VO2 max pre-training value > 55 ml/kg-fat free mass min., indicative of moderate fitness.
Anemia (Hematocrit <34%)
Any contraindications to moderate exercise (Please specify)
Inability and/ or unwillingness to comply with the protocol as written
Active alcohol or substance abuse (Past 5 Years)
Total cholesterol >300 mg/dL
Triglyceride >350 mg/dL
ALT >80, AST>80, Alk Phos >240
Proteinuria (defined as >1 + on routine dipstick), hypothyroidism (sTSH>8)
Therapeutic Doses of Nicotinic Acid
Oral glucocorticoids
Females currently on hormone replacement therapy (HRT) less than 6 months
Claustrophobia
Previous difficulty with lidocaine or other local anesthetic
Stress test symptoms: • Positive ECG (> 2mm ST segment depression) <u>without</u> PCP cardiologist permission to participate • Signs or symptoms of cardiovascular decomposition (hypotensive response to exercise) • Onset of angina or angina like symptoms, shortness of breath, change in heart rhythm, signs of poor perfusion (light-headedness), tightness, • Hypotension

3.3 EXPERIMENTAL PROCEDURES

Once a subject was determined to be eligible for enrollment in this investigation based on the results of the screening procedures, they were scheduled for the following experimental procedures to be conducted within 2-3 visits. The experimental procedures were performed at baseline and post test following the intervention phase. Time frame for the complete study including the experimental procedures will be 24 weeks: Baseline testing (weeks -4 to 0), intervention (weeks 1 to 16), and post test (weeks 17 to 20).

3.3.1 MAXIMAL OXYGEN CONSUMPTION TEST – VO_2 max

Subjects performed a cycle ergometer VO^2 max test at the Obesity Nutrition Research Center (ONRC) exercise physiology laboratory located in Montefiore hospital. This test was used to determine safety for physical activity, physical fitness and heart rate range for the exercise intervention sessions. ECG interpretation and blood pressure response was used to determine the safety of the subjects to be enrolled in a physical exercise intervention. All exercise tests were supervised by certified exercise physiologists and a study physician or cardiology fellow was present during the test. Exercise tests lasted about 6-12 minutes and were administered on a cycle ergometer (Sensormedics, Yorba Linda, Ca) using a protocol designed for older adults. The intensity levels for males begin at 50 watts for two minutes and were increased 25 watts every two minutes. Females begin at 25 watts for the first two minutes and were increased 25 watts every two minutes. Subjects breathed through a mouthpiece connected to a two way breathing valve (Hans Rudolph, Kansas City, MO) during the test, and expired air was collected into a mixing chamber interfaced to a computerized metabolic cart (Sensor Medics CS 2900) to measure expiratory flow and expired air for CO_2 and O_2 fractions. The metabolic cart analyzed the data for O_2 consumption (VO_2) every 30 seconds. The criteria for termination were voluntary exhaustion or one of the following: a) RER equal or greater to 1.15, b) HR max equal or greater to their age predicted maximal heart rate (220-age), or c) a plateau in the VO_2 max curve. Heart rate, ECG, RPE and blood pressure were recorded prior, during (every 2 minutes), and after the conclusion of the max test. After termination of the test each subject cooled down pedaling at 25 watts for 2 minutes and then they were asked to lay down supine until there heart rate and blood pressure return to normal resting values and subjects were then cleared to leave by the exercise physiologist.

Individuals were excluded from further participation if signs and/or symptoms occur prior to the test or during exercise testing and were then referred to their PCP for further care. The exercise test was stopped if the subject had any of the following: a) a positive ECG (> 2mm ST segment depression), b) signs or symptoms of cardiovascular decompensate (hypotensive response to exercise), c) onset of angina or angina like symptoms, d) shortness of breath, e) change in heart rhythm, f) signs of poor perfusion (light-headedness). The ONRC is equipped with cardiac emergency equipment (crash cart and Zoll Def.) and if an emergency develops a code C will be called to hospital emergency staff for immediate medical response.

3.3.2 DUAL ENERGY X-RAY ABSORPTIOMETRY (DEXA)

On the same day of the VO^2 max test subjects had a DEXA scan for body composition measurement. DEXA allows for simultaneous measuring of fat and lean components and the determination of regional quantities of these components. Dual-energy X-ray absorptiometry (DEXA) was used to explore changes in total and regional fat mass (FM) and fat free muscle mass (FFM), including appendicular (leg and arm) skeletal muscle mass (ASMM). FM, FFM and ASMM were determined using a GE Lunar prodigy scanner with EnCore software 2005 (GE Healthcare). These measurements allowed for the identification of changes in skeletal muscle mass (FFM) and fat mass (FM) in distinct regional areas of the body (trunk, legs, and arms). Whole body scans were acquired with the subject supine and aligned with scanner table as prescribed by the manufacturer and the DEXA was completed at the ONRC by a certified technician. The GE scanner is calibrated once a week by the certified technician to maintain correct values.

The amount of radiation exposure received from a DXA scan is about 0.002 rem. A rem is a unit of radiation. This amount of radiation is a small part (0.3 rem) of the average whole body radiation exposure that each member of the public receives per year from radiation exposure that is recognized as being totally free of the risk of causing genetic defects (cellular abnormalities) or cancer. The risk associated with the amount of radiation exposure received from this procedure is considered to be low and comparable to other everyday risks.

3.3.3 COMPUTED TOMOGRAPHY

Subjects had a mid-thigh (mid-point of femur) and abdominal (between L4 and L5 vertebrate) CT scan performed at Center Commons MRI and CT office-Shadyside (Pittsburgh, Pa). The CT images were used to determine thigh skeletal muscle area (cm^2) as a primary variable and abdominal adipose tissue area (cm^2) and abdominal muscle mass (Psoas muscle and erector spinae) as secondary variables for this intervention. A GE Hi-speed multiple slice scanner (GE Healthcare) was used to capture the images and these images were analyzed using SliceOmatic software version 3 (Tomovision, Montréal, QC, Canada). Pre and post measurements of muscle and fat were conducted by the same technician and the technician also analyzed 10 (test/re-test) images of previous CT technicians for a determination of reliable measures within a 5% difference. Computed tomography can differentiate tissues *in vivo* based on their attenuation characteristics, which depend on their density and the electron per unit mass. Attenuation values on CT are measured in Hounsfield Units (HU), which are based upon a linear attenuation coefficient scale using water as the reference (0 HU). Hounsfield scale is a quantitative scale for describing radiodensity. Adipose tissue is less dense then water and displays a negative attenuation value and muscle is denser then water displaying a positive attenuation value. Adipose tissue and skeletal muscle were measured by selecting the regions of interest as defined by these attenuation values: Adipose tissue is a negative attenuation (-190 to -30 HU) and muscle is a positive attenuation (0 to 100 HU); as previously described by Goodpaster et al (52). Muscle mass was also measured in normal density muscle. The attenuation values for muscle (0 to 100) can also be subdivided in low and normal density muscle (83). Low density muscle (0 to 30) is muscle at the lower end of attenuation values which is composed of less lean tissue. Normal density muscle is classified as leaner muscle mass (35 to 100). Both of these measures are related to the attenuation values of fat mass (-190 to -30). The Data was measured in mm^2 and will be converted to cm^2 to statistical analysis.

3.3.4 PERCUTANEOUS MUSCLE BIOPSY

The muscle biopsy was conducted by a study physician at the CTRC (Clinical Translation Research Center, Montefiore Hospital, Pittsburgh, Pa) before and after interventions for measurement of primary and secondary aims. Using 2% buffered lidocane for anesthetic, a small ¼ incision was made in the vastus lateralis muscle to place the needle obtaining a 100-150 mg of tissue. Following the removal of the tissue specimen a sterile bandage is applied and subjects are asked to refrain from physical activity for a period of at least two days. Specimens were trimmed of excess adipose tissue and frozen in isopetane and stored for indefinite amount of time until the immuno-histochemical analysis is performed.

3.3.5 LIGHT MICROSCOPY

Baseline and changes in skeletal muscle cross sectional area were measured from percutaneous muscle biopsy of vastus lateralis. Skeletal muscle area was measured in type I (oxidative), type IIA (aerobic/glycolytic), and type IIX (low oxidative) using immuno-histochemical staining. This procedure involves the techniques where an antibody is used to link a cellular antigen specifically to a stain that can be more readily seen with a microscope. The samples were fixed frozen samples that were cryoprotected with a stabilizer prior to freezing to maintain the structure of the tissue cell. The histologist in the ONRC prepared the samples in a hydro-stabilizing solution and then the samples were sectioned. The samples were sectioned into pieces 10-15 μm for best clarity and integrity because smaller sections tend to tear during cutting and larger sections tend to be more difficult to stain. Once the samples are prepared on slides they were photographed for analysis using a Leica DM 4000b light microscope (Leica Microsystems Gnbh, Wetzlar, Germany).

The immuno-histochemical staining produces a bright red color in type I fibers, light green color in type IIA and a dark green color in type IIX for identification. Using Northern Exposure software 10 muscle fibers were chosen for each muscle fiber type (I, IIA, IIX) and the area was computed using manual planimetry to outline of each muscle fiber. Muscle fibers were measured in microns squared (μm^2) and an average fiber area for the 10 fibers for type I, type IIA and type IIX will be calculated. Mean fiber areas were calculated for type I and type II (combining IIA & IIX), and for the whole mean muscle fiber area (combining type I, IIA, & IIX). The whole mean muscle fiber area was calculated by the following equation for each subject:

(type I mean fiber area * # of fibers measured) + (type IIA mean fiber area * # of fibers measured) + (type IIX mean fiber area * # of fibers measured) / total # of fibers measured.

3.3.6 TRANSMISSION ELECTRON MICROSCOPY (TEM)

Baseline and changes in skeletal muscle myofibrils area were examined using an electron microscope procedure. A portion of the vastus lateralis muscle obtained from the percutaneous muscle biopsy was cut into smaller pieces (1 x 1 x 2 mm), fixed in 2.5% glutaraldehyde, post fixed in 1% osmium tetroxide, dehydrated, and embedded in Epon for TEM. For each subject (pre and post) 10 random cross-sectional images were taken of inter-myofibrillar regions at x36, 000 magnifications with a JEM-1210 microscope (JEOL-Ltd., Tokyo, Japan). Myofibrillar area was calculated by manual planimetry of individual muscle myofibrils for all 10 micrographs (average 5 to 20 per image) using digital imaging software (Metamorph 6.3; Molecular Devices Corp., Sunnyvale, Ca). From the individual micrographs a mean myofibrillar area for pre and post testing was calculated as a whole mean value for all 10 micrographs (average mean of 10 micrographs).

3.4 RANDOMIZATION AND GROUPING

All of the subjects diagnosed as impaired glucose tolerant in the parent study were randomized into one of the following three groups: Weight loss (WL), Weight loss combined with Exercise (WL & EX), or Exercise alone (EX) for 4 months. All subjects diagnosed as newly type 2 diabetics were not randomized and were enrolled into the weight loss and exercise group. The completed Diabetes prevention program conducted by the American Diabetes Association found that people with pre-diabetes can prevent the development of type 2 diabetes by making changes in their diet and increasing their level of physical activity. We believed with this known research that withholding diet or exercise from these subjects would be unethical, so these individuals were placed by default in the WL/EX group to aid in reducing risk factors for the development of type 2 diabetes. The analysis of the effects of induced weight loss with and without exercise will focus on the results for the weight loss alone and the weight loss combined with exercise.

3.4.1 WEIGHT LOSS INTERVENTION

The goal of the weight loss intervention is to produce a total body weight loss of 8-10% of the pre screening body weight. A clinical nutritionist from the ONRC was in charge of the weight loss reduction. A caloric restriction of 500-1000 kcal/day (<30% of calories from fat) was implemented based on the subjects pre screening weight. Total caloric needs were determined by multiplying the subjects pre-screening weight (kg) by 25. From this the nutritionist made the required adjustments (500-100 daily) to produce a negative caloric intake resulting in a loss of 1-2 pounds of body weight per week. All subjects kept a 7 day food record for the duration of the 16 week intervention. They were responsible for recording their daily food choices for the week and calculating their daily and weekly total caloric intake and fat intake. Once a week the subjects had a behavioral meeting with the clinical nutritionist to discuss their progress or digress. They had to present their daily food records for the nutritionist to examine for caloric intake and fat intake on a day to day basis. From this the nutritionist made recommendations for changes to meet their daily personal requirements for caloric intake and fat intake. During this meeting subjects had a weekly body weight assessment of total body weight using a Scale Tronix electronic scale (Tronix Inc, Wheaton IL). The subjects were also instructed to not increase physical activity beyond what any they were participating in prior to the initial screening. Adherence to the induced-weight loss intervention was set as ≥ 5% decrease in total body weight in kilograms over the 4-month intervention for both the WL and WL/EX groups. Subjects with less than a 5% decrease in total body weight were classified as not compliant.

3.4.2 EXERCISE INTERVENTION

Subjects were asked to exercise a minimum of 4 times a week and a maximal of 6 times weekly for 45 minutes. Subjects will have 3 supervised and 2 unsupervised exercise sessions per week. The supervised exercise sessions at the ONRC and unsupervised exercise sessions were divided into 3 groupings: weeks 1-4, weeks 5-8, and weeks 9-16. The first 4 weeks subjects exercised for a minimal of 30 minutes at an intensity of 60-70% of their maximal heart rate determined from their maximal heart rate recorded during the maximal oxygen consumption test. During weeks 5-8, exercise sessions were increased in duration to 40 minutes minimal with the same intensity of 60-70% of their maximal heart rate. The last 8 weeks (9-16), exercise sessions were continued at 45 minutes and the intensity of the exercise was raised to 75% of their maximal heart rate. To maintain their heart rate all subjects were provided a F1 Polar heart rate monitor (Polar, Finland) and were instructed on how to use the watch properly.

The 3 supervised exercise sessions were conducted by exercise physiologists at the exercise training facility located in the Obesity Nutrition Research Center on the 8th floor of Montefiore hospital (Pittsburgh, Pa). There were no more than 3 subjects exercising at one time in the lab for safety and the lab was opened from 6:30am to 4:30 pm. All subjects were advised to wear clothing that is conducive for physical activity (walking shoes, shorts, T-shirt). The lab exercise sessions consisted of treadmill walking and stationary cycling. Subjects choose the modes of exercise for their sessions using the available equipment. Sessions were approximately 1 hour in length and begin with 5 minutes of light stretching, followed by 45 minutes of exercise, and 5 minutes of light stretching at the end. Subjects wore a polar heart rate monitor (Polar, Finland) for the exercise session and at the end of each session an average heart rate, duration and RPE (Rating of Perceived Exertion) were recorded. Each subject had a binder with exercise logs provided for them to record their lab exercise sessions (Time, intensity, and heart rate). These binders also include their target heart rate ranges for their unsupervised exercise sessions. These records were recorded in an electronic database in the lab by the exercise physiologist in charge. These databases were used to calculate calories burned (minute, session, weekly, and an average) based on heart rate and time, and to track exercise adherence rates (times per week and duration). Weekly the exercise physiologist recorded all activity performed outside of the lab faculty that was recorded by the subjects and checked for exercise adherence of the subjects.

The 2 unsupervised exercise sessions were performed outside the ONRC exercise faculty. The subjects are instructed in the different forms of outside activity that can be recorded and that their heart rate average must fall within the heart rate range during their physical activity session. These exercise sessions will be composed primary of moderate walking. All activity must be recorded and the heart rate monitor must be worn for exercise adherence. Subjects wore their heart rate monitor and recorded their time and average heart rate after competition.

Subjects were instructed to exercise 4-6 times a week with a preference for 5 times a week. Subjects were considered in adherence if they maintained an average of ≥ 3.5 exercise sessions per week for supervised and unsupervised exercise sessions. For the supervised exercise sessions ≥ 2.5 exercise sessions per week was classified as compliant, and for the unsupervised ≥ 1.0 exercise sessions per week was classified as compliant. If subjects fall below this cut point an exercise physiologist contacted the subjects for a behavioral meeting to discuss any difficulties they may be having with the intervention. From this meeting intervention strategies were designed to help the subjects complete the intervention.

3.5 DATA ANALYSIS

3.5.1 STATISTICAL ANAYLSIS

Statistical analyses were performed using SPSS version 15 (Chicago, IL) software, with statistical significance defined as $p \leq 0.05$. Data was initially analyzed to provide descriptive statistical information on subject characteristics: age, body weight, BMI, fat mass, fat free mass, % fat mass. Analyses were conducted to determine if the data are normally distributed before conducting additional analyses.

Repeated measures analysis (ANOVA) (group x time) were used to assess differences in relative and absolute body composition and muscle fiber composition across the different treatment groups.

3.5.2 POWER ANALYSIS

Preliminary CT data on the changes of muscle mass was used to determine the power for the groups. 10 subjects (5 diet alone, 5 combination) were analyzed for changes in mean muscle CSA for both thighs combined. The diet alone group demonstrated a 5 percent change in muscle CSA (-5.2 ± 0.04) and the combo group demonstrated a 2 percent change in muscle CSA (-1.9 ± 0.06). This small sample size of 10 subjects demonstrated a power of 1.

4.0 CHAPTER 4

4.1 RESULTS

4.1.1 SUBJECTS

12 subjects were randomized into the WL group, and 22 were entered into the WL/EX group. Unfortunately, one subject dropped out of the WL group and 4 dropped out of the WL/EX group for a total of 5 subjects (15% drop out rate). Only those who completed the interventions were included in the analyses. The WL group consisted of 11 subjects (4 male & 7 female), and the WL/EX group consisted of 18 subjects (9 male & 9 female). The WL/EX group consisted of 9 randomized subjects and 9 enrolled subjects classified as newly type 2 diabetic. The 9 subjects enrolled in the WL/EX group were newly diagnosed with type 2 diabetes and had similar baseline characteristics to those without diabetes. Thus these groups were collapsed for the remainder of the analyses. Table 7 depicts the baseline characteristics of only the subjects that completed the entire 16-week intervention. The mean age was similar in each group. The WL/EX group had a slightly higher lean body mass, and the WL group had a higher fat mass. This was probably due to a gender effect of the WL group having proportionately less men (4/7) compared to the WL/EX group (9/9). All other baseline measures (table 7) were similar between the groups and there was no significant difference between groups for baseline measures. The

WL/EX group was examined for significant differences internally between subjects diagnosed as newly type 2 diabetics and impaired glucose tolerant. It was found that all baseline measures were similar between the two groups and no significant difference was established (table 7).

Baseline characteristics were examined for a gender effect which is represented in table 8. Male subjects in both the WL and WL/EX groups demonstrated a higher initial body weight and fat free mass compared to female subjects and female subjects in both groups exhibited a higher initial BMI, fat mass and percent fat compared to the male subjects. While these differences were visible there was no significant difference between male and female subjects for baseline characteristics.

4.1.2 BASELINE CHANGES

Changes in body composition characteristics for the intervention groups are represented in table 9. Both groups (WL & WL/EX) displayed significant decreases in body weight, BMI, FM, and % FM following the 4-month intervention. For FFM a significant decrease was observed for the WL group while the WL/EX group displayed no change. A group x time interaction effect demonstrated that there were significant differences between groups ($p = 0.04$).

Changes in body composition characteristics were examined for differences between newly diagnosed type 2 diabetics (T2DM) and the impaired glucose tolerant subjects (IGT) in the WL/EX group, table 10. Both groups displayed significant decreases in body weight, BMI, FM, and % FM following the 4-month intervention. For FFM a significant decrease was observed in the IGT group while the T2DN group had no change in FFM. A significant group x time interaction effect suggested that there were significant differences between the IGT group and the T2DM groups ($p = 0.03$).

Changes in baseline characteristics were examined for gender differences and are represented in table 11. In the WL group men and women both had significant decreases in body weight, BMI, FFM, FM, and % FM following the 4-month intervention. The WL/EX group had similar decrease in body weight, BMI, FM, and % FM, but displayed no change for FFM following the intervention. No interaction effect was found for baseline measurements between male and females subjects for the WL or the WL/EX groups.

4.1.3 ADHERENCE

Subjects in both groups who completed the intervention meet with the dietician once a week for the full 4 months. The diet-induced weight loss intervention was successful in producing the intended weight loss of 8-10% of their body weight in kilograms. The WL group had a significant decrease in body weight of -9.2% over the 4-month intervention. 2 subjects had decreases of less than 5% which classified them as noncompliant (≥ 5% for compliance), but the remaining 9 subjects demonstrated a 7 to 18% decrease in body weight which met adherence standards. The WL/EX group had a decrease in body weight of -9.1% and 3 subjects had decreases of less than 5%, but the remaining 15 subjects had decreases ranging between 6 to 18% for adherence standards. The total mean adherence rate for aerobic exercise (supervised and unsupervised) was 3.79 ± 1.02 times a week for the entire 4-month intervention and this met adherence standards (≥ 3.5 exercise sessions per week). The supervised exercise sessions yielded a mean adherence rate of 2.49 times a week of aerobic exercise. The unsupervised exercise session yielded a mean adherence rate of 1.29 times a week of aerobic exercise. Exercise sessions consisted of primary of treadmill and outside walking (97%) and stationary cycling as the secondary mode of exercise (3%). The WL/EX group averaged 39.0 minutes of aerobic exercise per session for the supervised and unsupervised exercise sessions.

4.1.4 WHOLE BODY TISSUE MASS

The WL and WL/EX group both had significant decreases in total body weight in kilograms (WL - 8.3 ± 4.7 kilograms, -9.2% decrease & WL/EX - 8.4 ± 3.5 kilograms; -9.1% decrease) which is represented in table 9. The weight loss (WL) and weight loss with exercise (WL/EX) had whole body tissue mass measured using dual energy x-ray absorptiometry for four regions of interest: whole body, trunk, arms and legs. Table 9 represents the changes yielded from the 4-month diet and exercise intervention on total body fat mass (FM) and fat free mass (FFM). The WL group had a -17% decrease in total body fat and the WL/EX group demonstrated a -21% decrease in total body fat (table 9 & figure 2). The decrease in fat mass for the WL/EX group was similar to the fat mass decreases in the WL group so no significant interaction effect was present between groups ($p = 0.5$). Total body FFM also decreased by -4% in the WL group while in the WL/EX group there was no change (< -1%). There was group-time interaction effect in the loss of whole body FFM ($p = 0.04$), indicating that there were significant differences between the WL and WL/EX groups following the 4-month intervention (Table 9). The proportionate reductions in total tissue mass translate into a 77% decrease from total body FM with and a concomitant 22% decrease from total body FFM for the WL group. And the WL/EX group had proportionate reductions in total tissue mass that translate into a greater change in total body fat mass (92%) with a lesser change in fat free mass (8%) compared to the WL group.

Whole body tissue mass was examined for differences between gender following the 4-month intervention which is represented in table 13. In the WL group both men (-20%) and women (-5%) had significant decreases in body FM so no interaction effect was present ($p = 0.5$). FFM remained unchanged in men (-3%) and decreased in women in the WL group (-14%), and a group main effect was observed for the changes in FFM means suggesting there was a greater decrease in mean FFM in women compared to men (table 13). Similarly, the WL/EX group also had significant decreases in FM for both men (-20%) and women (-17%) so no interaction effect was observed ($p = 0.87$). Conversely FFM for both men (-2%) and women (-2%) in the WL/EX group displayed no change which is represent in table 13, and no significant interaction effect was found for whole body FFM ($p = 0.28$).

4.1.5 REGIONAL BODY COMPISITION CHANGES WITH DEXA

Arm appendicular data in table 12 showed modest changes in arm FM and arm FFM for the WL and WL/EX groups. The WL group had a -9% decrease in arm FM and the WL/EX group had a -19% decrease in FM (table 12). FFM displayed no significant changes for either group following the intervention. No statistical significant interaction was found between the WL and WL/EX groups related to the intervention.

Leg appendicular data yielded slightly different results then the arm data (table 12). Similar to arm FM both groups revealed a significant decrease in leg FM (WL -16%, WL/EX -17%) which is presented in table 12. Inverse of the arm FFM results both the WL group (-3%) and the WL/EX group (-2%) had significant decreases in leg FFM which table 12 illustrates. No statistical significant interaction was found between the WL and WL/EX groups related to the intervention.

4.1.1 THIGH MUSCLE MASS AREA BY CT

Thigh muscle cross-sectional area (CSA) decreased -5% in the WL group and similarly decreased by -4% in the WL/EX group following the 4-month intervention which is represented in table 14 and figure 3. Equally both groups had decreases in thigh normal density muscle (NDM) of -8% (WL) and -3% (WL/EX) following the 4-month intervention. Both groups displayed similar results in relation to decreases in thigh CSA and NDM. No significant interaction was found for CSA ($p = 0.53$) or NDM ($p = 0.09$) for between subjects, table 14.

Similar changes in thigh CSA were also observed between gender groupings in the WL group. Thigh CSA decreased by -6% in the men and by -5% in the women which is represented in table 15. A group main effect was observed suggesting that the difference in means was greater in the male subjects compared to the female (table 15). Equally NDM decreased for both genders (male -11%, female -5%) and there was group-time interaction effect in the loss of NDM ($p = 0.02$), indicating that there were significant differences between groups over the 4-month diet intervention (Table 15). The WL/EX group had mildly different results when compared to the WL group for gender differences (table 16). Thigh CSA significantly decreased by -5% in the male subjects and by -2% in the female subjects. A significant group-time interaction effect was found for the loss of CSA ($p = 0.01$), indicating that there significant differences between male and female subjects over the 4-month diet intervention. No significant decrease was found for NDM for the male (-3%) or female (-1%) subjects in the WL/EX group. A significant group main effect was observed which suggests that the difference in means was greater in the male subjects compared to the female (table 16).

4.1.2 ABDOMINAL MUSCLE AREA BY CT

Abdominal muscle area was measured in the psoas major and the erector spinae muscles which surround and stabilize the spinal column. No significant changes were found for the WL (3%) or the WL/EX (-1%) groups in psoas major muscle area following the intervention, table 14. Similarly no changes were found for erector spinae muscle area for the WL (-2%) or the WL/EX (-2%) groups. In the WL group (table 15), no changes were found between male (1%) or the female subjects (3%) for psoas muscle area, but a significant group main effect was observed suggesting that the difference in means was greater in the female subjects compared to the males ($p = 0.01$). Equally no changes were observed for erector spinae muscle mass in male (-3%) or females (-1%) subjects following intervention. In the WL/EX group (table 16), no changes were found between male (-3%) or the female subjects (0%) for psoas muscle area, but a significant group main effect was observed suggesting that the difference in means was greater in the male subjects compared to the females ($p = 0.00$). No changes were observed for male (-5%) for female (4%) subjects for the erector spinae muscles.

4.1.1 MUSCLE FIBER AREA BY PERCUTANEOUS BIOPSY

Muscle fibers were first examined by specific fiber area for any changes related to the intervention between the WL and WL/EX groups. Table 17 and figure 4 displays the changes in specific muscle fiber areas. The type I (slow twitch) muscle fibers in the WL group decreased 16% over the course of the 4 month intervention. Conversely the type I fiber area in the WL/EX group remained relatively unchanged (7%). The changes in type I muscle fiber area were different between the two groups and a statistically significant interaction effect was found between the WL and WL/EX groups ($p = 0.02$). Type IIA muscle fiber area (intermediate fast/slow) in the WL group decreased by -11% and the WL/EX also displayed a -4% change in type IIA muscle fiber area (table 17). No significant interaction effect was found between the two groups ($p = 0.46$). Interestingly the type IIX muscle fiber area (fast twitch) also changed in the WL group (-11%) and the WL/EX group (13%). While these changes were different between the two groups no significant interaction effect was found between the WL and WL/EX groups ($p = 0.09$).

Specific muscle fibers were examined for any changes related to the intervention between male and female subjects. Both male and female subjects in the WL group had significant decreases (male -20%, female -19%) in type I muscle fiber area which is presented in table 18. A significant group main effect was observed ($p = 0.05$) indicating that there was differences in means between the two groups following the intervention. No significant changes were observed for type IIA muscle fiber area for gender (male -12%, female -18%). Similarly no significant changes were found between male (-29%) and female subjects (-3%) for type IIX muscle fiber area, but a significant group main effect was observed ($p = 0.02$) indicating that the differences in group means was greater in the male subjects (table 18). The WL/EX group displayed modestly different results following the 4-month intervention. Type I muscle fibers remained unchanged in both men (-3%) and women (-15%), but a significant group main effect was observed ($p = 0.01$) indicating there were differences in the group means (table 18). Similarly type IIA muscle fibers remained unchanged (male -9%, female -5%) and a group main effect was observed ($p = 0.00$) indicating there were differences in group means. Type IIX muscle fibers equally remained unchanged for both male (7%) and female (17%) subjects following the 4-month intervention and a significant group main effect was observed indicating there was a difference in group means ($p = 0.05$).

Muscle fibers were also combined for examination of muscle fibers based on contraction speed (slow vs. fast) and the results for the WL and WL/EX groups are displayed in table 20 and figure 5. The WL group had decreases in both type I (slow) and Type II (fast) muscle fiber area. The type I decreased -16% as previous stated in the WL group and in the WL/EX remained unchanged, and there was a significant interaction effect between the two groups ($p < 0.01$) which is illustrated in figure 7. Type II muscle fiber area decreased -15% in the WL group in a similar manner to the type I fibers. The WL/EX group had no change (< 1%) in type II area. No significant interaction effect was found ($p= 0.23$) present between the WL and WL/EX group for changes in type II muscle fiber area (table 20).

Slow and fast muscle fibers were also examined for gender differences related to changes in muscle fiber area. The WL group had a significant decrease in type I muscle fiber area which is presented in table 18. Type II muscle fiber area displayed changes for male (-20%) and females (-17%), but neither of these were found to be significant (table 21). The WL/EX group displayed no changes for type I muscle fiber area following the intervention and similarly had no changes (male -8% & female -1%) in type II muscle fiber area which is represented in table 22.

A significant group main effect was observed (p = 0.00) suggesting that there were differences in group means.

Muscle fibers were also combined through a weighted calculation to determine each subjects total mean fiber area which is displayed in table 20 and figure 6 for the WL and WL/EX groups. Collectively the WL group had a decrease of -16% in total muscle fiber area over the 4 month intervention which is represented in table figure 6. Alternatively the WL/EX group no change (2%) in total muscle fiber area following the 4 month intervention. The results for the two groups are different with the WL group demonstrating substantial significant decrease in total muscle fiber area compared to the WL/EX group. These changes were evident and there was a significant interaction effect found between the two groups for total muscle fiber area (p= 0.05) which is illustrated in figure 8. In the WL group no changes were observed for differences in total mean muscle fiber area between male (-17%) and female (-19%) subjects, but a significant group main effect was observed (p = 0.00) suggesting that there were differences in group means (table 21). The WL/EX group equally had no change in total muscle fiber area (male -5% & female 5%) and a significant group main effect was observed (p = 0.00) suggesting that there are differences in group means which is represented in table 22.

Table 7. Baseline Characteristics (Group comparison)

	WL	WL/EX		WL/EX *T2DM*	WL/EX *IGT*
Subject number	11	18		9	9
Gender (M/F)	4/7	9/9		4/5	5/4
Age (years)	68.4 ± 1.5	66.4 ± 1.0		66.8 ± 1.	66.1 ± 1.4
Body weight (kg)	89.5 ± 3.1	90.8 ± 3.4		89.2 ± 5.7	91.9 ± 4.1
BMI	32.1 ± 1.0	31.6 ± 1.0		31.8 ± 1.4	31.4 ± 1.0
Whole body FFM (kg)	47.3 ± 2.5	51.3 ± 2.7		49.5 ± 3.9	53.2 ± 4.0
Whole body FM (kg)	38.4 ± 2.0	35.6 ± 1.9		36.1 ± 3.2	35.3 ± 2.2
Whole body % FM	43.6 ± 1.8	39.9 ± 1.8		40.8 ± 3.4	38.9 ± 2.8

WL = weight loss only, WL/WX = weight loss and exercise, T2DM = type 2 diabetics, IGT = impaired glucose tolerant

FFM = fat free mass, FM = fat mass

Values are means ± SEM

Table 8. Baseline Characteristics (Gender comparison)

	WL			WL/EX	
	Male	Female		Male	Female
Subject number	4	7		9	9
Body weight (kg)	97.1 ± 3.6	85.2 ± 3.7		100.6 ± 3.8	80.5 ± 3.2
BMI	32.2 ± 1.6	33.8 ± 1.1		31.9 ± 1.2	31.4 ± 1.0
Whole body FFM (kg)	56.9 ± 1.1	41.7 ± 1.5		61.6 ± 1.9	41.1 ± 1.2
Whole body FM (kg)	36.4 ± 3.1	39.6 ± 2.7		34.8 ± 2.4	36.4 ± 3.1
Whole body % FM	37.6 ± 1.9	47.1 ± 1.6		34.6 ± 1.2	45.1 ± 2.2

WL = weight loss only, WL/WX = weight loss and exercise, Values are means ± SEM

Table 9. Body Composition Changes (Group comparison)

	WL			WL/EX			Interaction effect	Main effect time	Main effect group
	Pre n = 11	Post		Pre n = 18	Post		P values		
Weight (kg)	89.5 ± 3.4	81.2 ± 2.8		90.8 ± 3.4	82.4 ± 3.3		.896	.000	.815
BMI	32.9 ± 1.0	28.8 ± 1.0		31.6 ± 1.0	28.8 ± 1.0		.438	.000	.851
FFM (kg)	47.3 ± 2.5	45.3 ± 2.5		51.3 ± 2.7	50.7 ± 2.6		.044	.000	.234
FM (kg)	38.4 ± 2.0	32.2 ± 2.1		35.6 ± 1.9	28.6 ± 2.0		.528	.000	.286
% FM	43.6 ± 1.8	40.1 ± 2.2		39.9 ± 1.8	34.7 ± 2.1		.205	.000	.128

WL = weight loss only, WL/WX = weight loss and exercise, Values are means ± SEM, $p < 0.05$

Table 10. Body Composition Changes (WL/EX - metabolic status comparison)

	IGT			T2DM			Interaction effect	Main effect time	Main effect group
	Pre n = 9	Post		Pre n = 9	Post		P values		
Weight	91.9 ± 4.1	84.1 ± 3.4		89.2 ± 80.7	80.7 ± 5.8		.731	.000	.669
BMI	31.4 ± 1.0	28.7 ± 1.0		31.8 ± 1.4	28.8 ± 1.5		.546	.000	.924
FFM (kg)	53.3 ± 4.0	51.8 ± 3.7		49.4 ± 3.8	49.5 ± 3.8		.025	.046	.580
FM (kg)	35.3 ± 2.2	29.2 ± 1.9		36.1 ± 3.2	28.1 ± 3.6		.237	.000	.969
% FM	38.9 ± 2.7	35.1 ± 2.7		40.7 ± 2.4	34.4 ± 3.4		.131	.000	.902

T2DM = type 2 diabetics, IGT = impaired glucose tolerant, Values are means ± SEM, $p < 0.05$

Table 11. Body Composition Changes (Gender comparison)

	Male			Female			Interaction effect	Main effect time	Main effect group
	Pre n = 4	Post		Pre n = 7	Post		P values		
WL GROUP									
Weight	97.1 ± 3.6	87.9 ± 3.5		85.2 ± 3.7	77.3 ± 3.7		.720	.000	.056
BMI	29.3 ± 1.0	26.8 ± 1.0		33.7 ± 1.1	30.0 ± 1.0		.213	.000	.028
FFM (kg)	56.9 ± 1.0	55.1 ± 1.0		41.8 ± 1.5	39.7 ± 1.3		.867	.014	.000
FM (kg)	36.4 ± 3.1	29.2 ± 3.8		39.6 ± 2.7	33.9 ± 2.4		.503	.000	.366
% FM	37.6 ± 1.8	33.1 ± 3.0		47.1 ± 1.6	44.1 ± 1.6		.312	.001	.004
WL/EX GROUP	n = 9			n = 9					
Weight (kg)	100 ± 3.8	90.8 ± 4.2		80.5 ± 3.2	73.9 ± 3.2		.051	.000	.002
BMI	31.8 ± 1.1	28.7 ± 1.3		31.9 ± 1.3	28.9 ± 1.2		.515	.000	.779
FFM (kg)	61.6 ± 1.9	60.6 ± 1.6		41.3 ± 1.2	40.7 ± 1.1		.396	.078	.000
FM (kg)	34.8 ± 2.4	27.1 ± 2.6		36.5 ± 3.1	30.2 ± 3.1		.275	.000	.534
% FM	34.7 ± 1.2	29.1 ± 1.8		45.1 ± 2.2	40.4 ± 2.8		.561	.000	.001

WL = weight loss only, WL/WX = weight loss and exercise, FFM = fat free mass, FM = fat mass,

Values are means ± SEM, $p < 0.05$

Table 12. DEXA results (Group comparison)

	WL			WL/EX			Interaction effect	Main effect time	Main effect group
DEXA	Pre	Post		Pre	Post		P values		
Arm tissue									
• FFM	5.0 ± .43	5.6 ± .37		5.7 ± .42	5.6 ± .38		.352	.778	.321
• FM	3.4 ± .22	3.1 ± .27		3.1 ± .23	2.5 ± .19		.077	.000	.180
Leg tissue									
• FFM	15.7 ± 1.0	15.2 ± 1.0		17.1 ± 1.0	16.8 ± 1.0		.397	.010	.309
• FM	13.3 ± 1.5	11.1 ± 1.3		11.2 ± 1.0	9.4 ± 1.0		.470	.000	.203
Trunk tissue									
• FFM	22.6 ± 1.1	21.6 ± 1.2		24.8 ± 1.25	24.5 ± 1.1		.191	.026	.157
• FM	21.0 ± 1.1	16.9 ± 1.1		20.1 ± .99	16.1 ± 1.1		.786	.000	.638

WL = weight loss only, WL/WX = weight loss and exercise,

FFM = fat free mass, FM = fat mass, Values are means ± SEM, $p < 0.05$

Table 13. DEXA results (Gender comparison)

	Male			Female			Interaction effect	Main effect time	Main effect group
Whole body tissue	Pre	Post		Pre	Post		P values		
WL									
• FFM	56.9 ± 1.0	55.1 ± 1.		39.6 ± 2.7	33.9 ±2.4		.867	.014	.000
• FM	36.4 ± 3.1	29.2 ± 3.8		41.7 ± 1.5	39.7 ± 1.3		.503	.000	.366
WL/EX									
• FFM	61.6 ± 1.9	60.1 ± 1.6		41.1 ± 1.2	40.1 ± 1.1		.396	.078	.000
• FM	34.8 ± 2.4	27.1 ± 2.6		36.4 ± 3.1	30.2 ± 3.1		.275	.000	.543

FFM = fat free mass, FM = fat mass, Values are means ± SEM, $p < 0.05$

Table 14. CT results (Group comparison)

	WL			WL/EX			Interaction effect	Main effect time	Main effect group
CT	Pre	Post		Pre	Post		P values		
Thigh									
• CSA	108.5 ± 5.5	102.8 ± 5.3		124.7 ± 7.6	120.3 ± 6.9		.525	.000	.109
• NDM	81.1 ± 5.6	74.5 ± 4.7		93.4 ± 6.5	90.9 ± 6.6		.084	.001	.111
• Mean HU	43.2 ± .87	42.4 ± 2.9		42.4 ± .79	42.7 ± .70		.194	.618	.776
Abdominal									
• Psoas major	12.0 ± 1.1	12.3 ± 1.1		14.4 ± .89	14.2 ± .81		.135	.943	.122
• Erector spinae	15.4 ± 1.1	15.1 ± .71		16.2 ± 1.3	15.9 ± 1.1		.852	.400	.632

WL = weight loss only, WL/EX = weight loss and exercise,

CSA = cross sectional area, NDM = normal density muscle, HU units = Hounsfield units

Values are means ± SE, $p < 0.05$

Table 15. CT results (WL Gender comparison)

	Male			Female			Interaction effect	Main effect time	Main effect group
CT	Pre	Post		Pre	Post		P values		
WL									
• CSA	129.1 ± 4.4	121.9 ± 5.3		96.7 ± 3.2	91.9 ± 3.8		.347	.001	.000
• NDM	98.3 ± 7.8	87.1 ± 7.9		71.3 ± 4.4	67.5 ± 4.1		.020	.000	.017
• Mean HU	43.1 ± 1.5	41.1 ± 1.8		42.5 ± 1.1	42.6 ± 1.0		.025	.052	.821
WL									
• Psoas major	15.6 ± 1.1	15.8 ± 1.0		9.95 ± 1.1	10.2 ± 1.0		.811	.437	.005
• Erector spinae	16.7 ± 2.3	16.2 ± 1.4		14.7 ± 1.1	14.5 ± 1.0		.831	.561	.317

CSA = cross sectional area, NDM = normal density muscle, HU units = Hounsfield units

Values are means ± SE, $p < 0.05$

Table 16. CT results (WL/EX Gender comparison)

	Male			**Female**			Interaction effect	Main effect time	Main effect group
CT	Pre	Post		Pre	Post		P values		
WL/EX									
• CSA	153.4 ± 4.6	145.7 ± 5.4		96.2 ± 4.5	94.9 ± 3.4		.010	.001	.000
• NDM	115.9 ± 4.7	111.7 ± 3.8		70.9 ± 5.4	70.3 ± 3.7		.235	.110	.000
• Mean HU units	43.2 ± 1.1	43.6 ± 1.0		41.9 ± 1.2	42.1 ± 1.0		.921	.536	.325
WL/EX									
• Psoas major	17.8 ± .43	17.3 ± .20		11.1 ± .59	11.1 ± .57		.135	.213	.000
• Erector spinae	18.7 ± 1.3	17.8 ± 1.2		13.6 ± 1.8	14.1 ± 1.7		.053	.530	.057

CSA = cross sectional area, NDM = normal density muscle, HU units = Hounsfield units

Values are means ± SE, $p < 0.05$

Table 17. Muscle Fiber Area & percentage (Group comparison)

	WL			WL/EX			Interaction effect	Main effect time	Main effect group
MUSCLE FIBERS	Pre	Post		Pre	Post		P values		
Fiber area									
• Type I	6913 ± 706	5584 ± 422		6430 ± 533	6648± 537		.017	.079	.708
• Type IIA	6678 ± 888	5646 ± 531		6320 ± 424	5833 ± 379		.457	.045	.902
• Type IIX	4307 ± 603	3514 ± 364		4595 ± 399	5060 ± 613		.090	.645	.164
Fiber percent									
• Type I	51.9 ± 12.2	48.6 ± 9.9		50.8 ± 11.9	50.3 ± 12.7		.641	.503	.941
• Type IIA	39.6 ± 11.2	42.6 ± 8.5		41.6 ± 10.1	44.8 ± 11.4		.958	.264	.528
• Type IIX	8.3 ± 5.9	9.9 ± 9.7		9.1 ± 8.6	6.5 ± 8.0		.245	.810	.683

Fiber area measured in microns squared μm^2, Values are means ± SEM, $p < 0.05$

Table 18. Muscle Fiber Area (Gender comparison)

	Male			**Female**			Interaction effect	Main effect time	Main effect group
FIBER AREA	Pre	Post		Pre	Post		P values		
WL GROUP									
• Type I	8525 ± 1610	6795 ± 927		5993 ± 422	4839 ± 336		.502	.012	.051
• Type IIA	7391 ± 1802	6485 ± 954		6271 ± 999	5167 ± 610		.885	.165	.407
• Type IIX	5744 ± 930	4076 ± 689		3158 ± 218	3064 ± 280		.142	.106	.022
WL/EX GROUP									
• Type I	7811 ± 702	7591 ± 806		4876 ± 314	5587 ± 516		.264	.549	.008
• Type IIA	7392 ± 488	6708 ± 525		5115 ± 422	4849 ± 290		.640	.295	.000
• Type IIX	5350 ± 340	5742 ± 945		3463 ± 439	4038 ± 667		.863	.370	.048

Fiber area measured in microns squared μm^2, Values are means ± SEM, $p < 0.05$

Table 19. Muscle Fiber Percentage (Group comparison)

	Male			**Female**			Interaction effect	Main effect time	Main effect group
FIBER PERCENT	Pre	Post		Pre	Post		P values		
WL GROUP • Type I	47.8 ± 6.5	44.8 ± 5.7		54.3 ± 4.6	50.8 ± 3.6		.960	.591	.175
• Type IIA	44.1 ± 5.6	49.1 ± 3.3		37.1 ± 4.8	38.8 ± 2.8		.742	.512	.056
• Type IIX	6.1 ± 3.3	7.2 ± 4.1		8.6 ± 2.3	11.3 ± 4.7		.686	.771	.603
WL/EX GROUP • Type I	51.9 ± 4.7	47.8 ± 3.7		49.8 ± 4.0	52.7 ± 5.2		.277	.849	.881
• Type IIA	39.6 ± 2.0	48.9 ± 3.5		43.6 ± 4.4	40.6 ± 4.2		.046	.277	.639
• Type IIX	10.6 ± 3.7	4.8 ± 1.9		10.4 ± 3.9	6.6 ± 3.2		.773	.113	.535

Values are means ± SEM, $p < 0.05$

Table 20. Total Muscle Fiber Area (Group comparison)

	WL			**WL/EX**			Interaction effect	Main effect time	Main effect group
MUSCLE FIBERS	Pre	Post		Pre	Post		P values		
Type 2 (IIA & IIX) • Fiber area	5816 ± 799	4749 ± 433		5864 ± 422	5561 ± 384		.231	.037	.513
Mean fiber area (I, IIA, IIX) • Fiber area	6421 ± 722	5217 ± 449		6162 ± 422	6077 ± 413		.056	.029	.648

Fiber area measured in microns squared μm^2, Values are means ± SEM, $p < 0.05$

Table 21. Total Muscle Fiber Area (WL Gender comparison)

	Male			**Female**			Interaction effect	Main effect time	Main effect group
MUSCLE FIERS	Pre	Post		Pre	Post		P values		
WL (IIA & IIX) • Fiber area	6567 ± 1341	5280 ± 623		5387 ± 989	4445 ± 574		.756	.069	.442
WL Mean fiber area (I, IIA, IIX) • Fiber area	7565 ± 999	6258 ± 772		5767 ± 737	4623 ± 444		.523	.474	.001

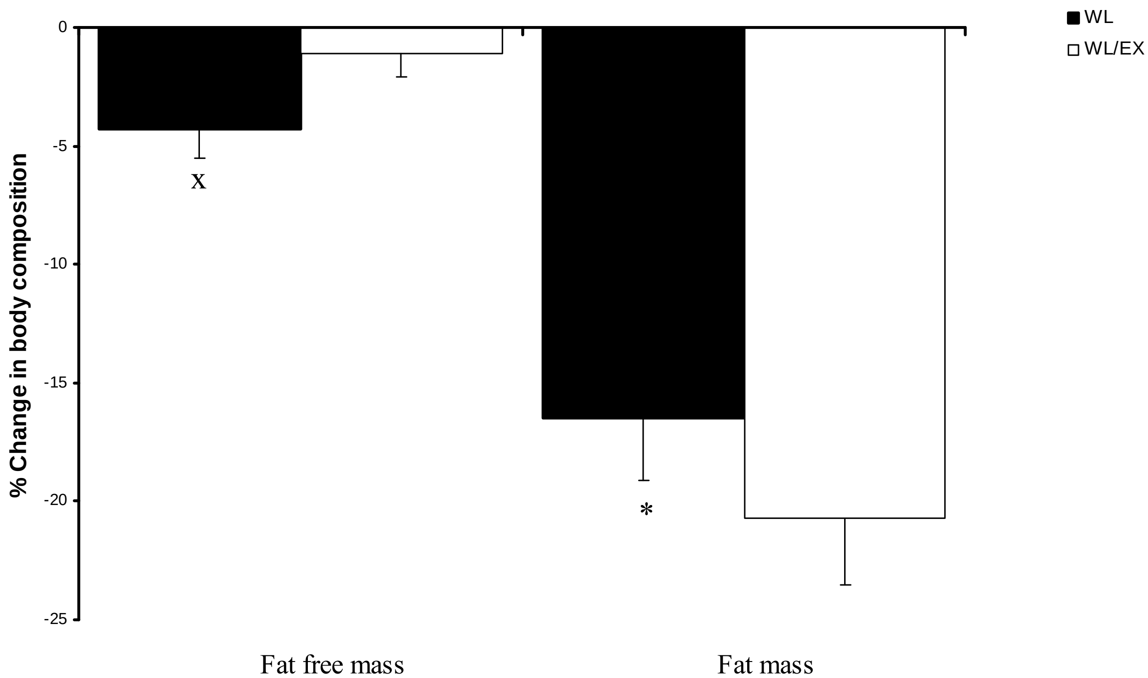
Fiber area measured in microns squared μm^2, Values are means ± SEM, $p < 0.05$

Table 22. Total Muscle Fiber Area (WL/EX Gender comparison)

	Male			**Female**			Interaction effect	Main effect time	Main effect group
MUSCLE FIERS	Pre	Post		Pre	Post		P values		
WL /EX (IIA & IIX) • Fiber area	6946 ± 515	6401 ± 561		4647 ± 351	4615 ± 286		.523	.474	.001
WL/EX Mean fiber area (I, IIA, IIX) • Fiber area	7334 ± 482	6950 ± 616		4845 ± 314	5096 ± 286		.395	.857	.001

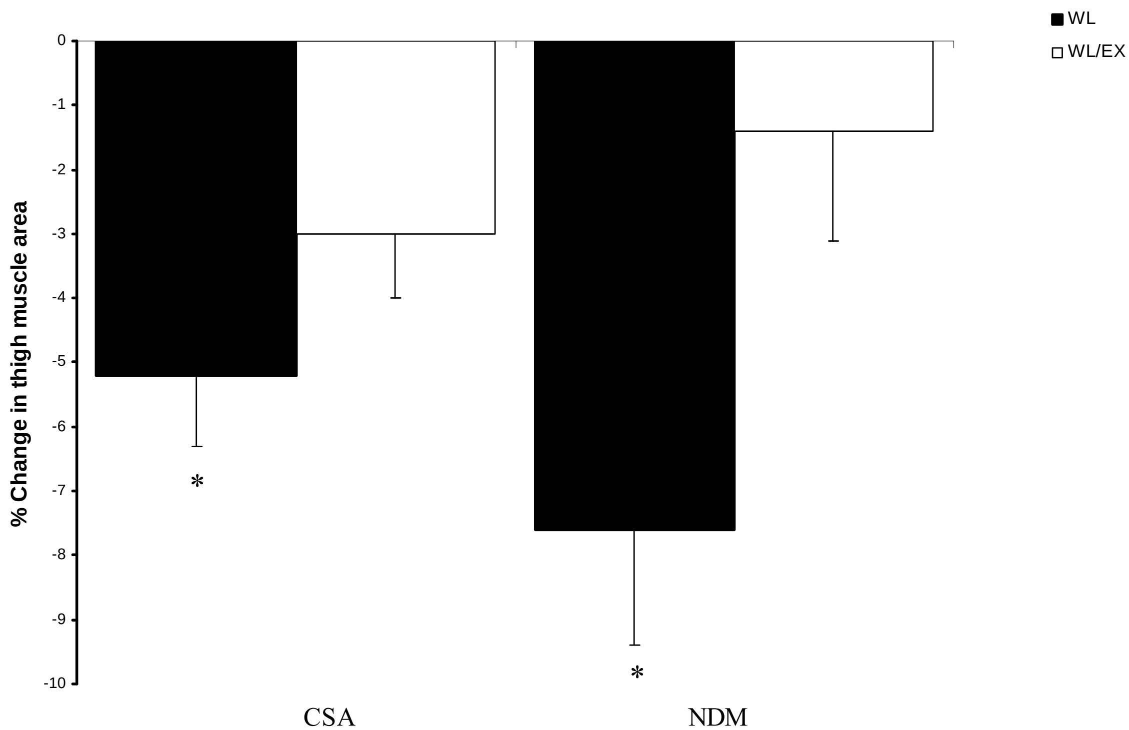
Fiber area measured in microns squared μm^2, Values are means ± SEM, $p < 0.05$

Figure 2. Whole body tissue changes



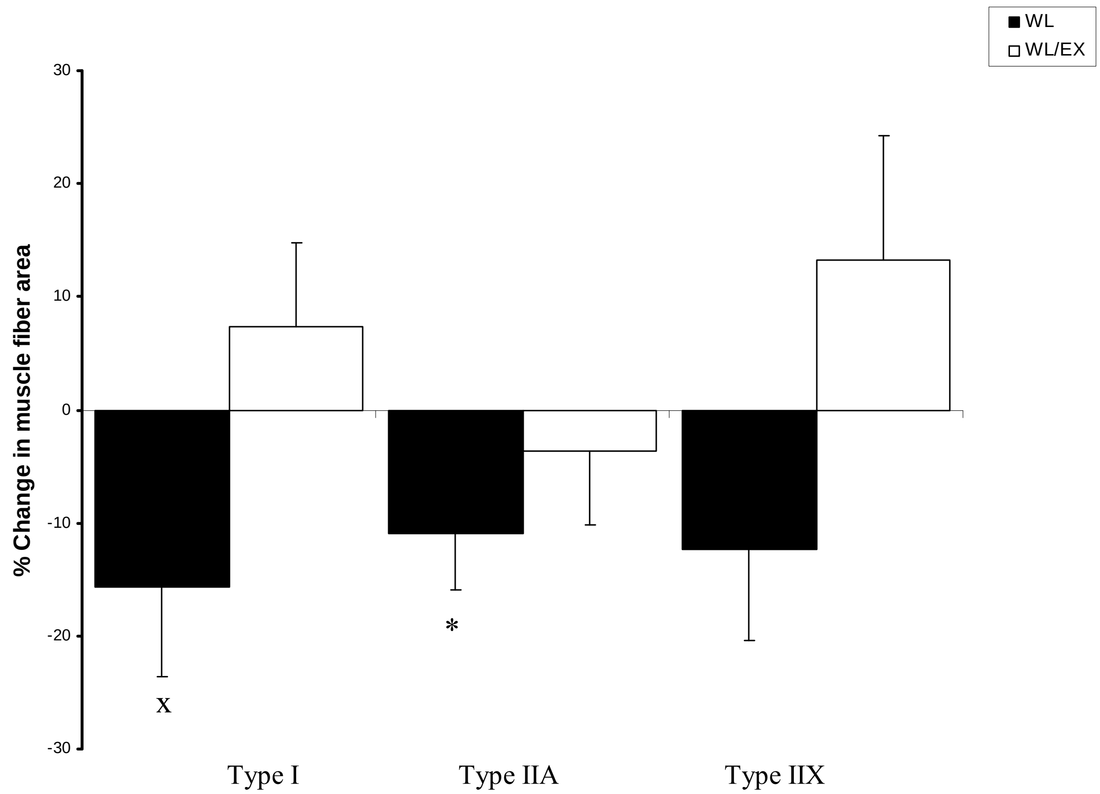
Percent change in whole body fat mass and fat-free mass from between baseline and post test.
FFM = Fat Free Mass, **FM** = Fat Mass, Values = Percent change ± SEM
x = Group x Time effect, < 0.05
* = Main effect time, < 0.05

Figure 3. Thigh lean muscle mass



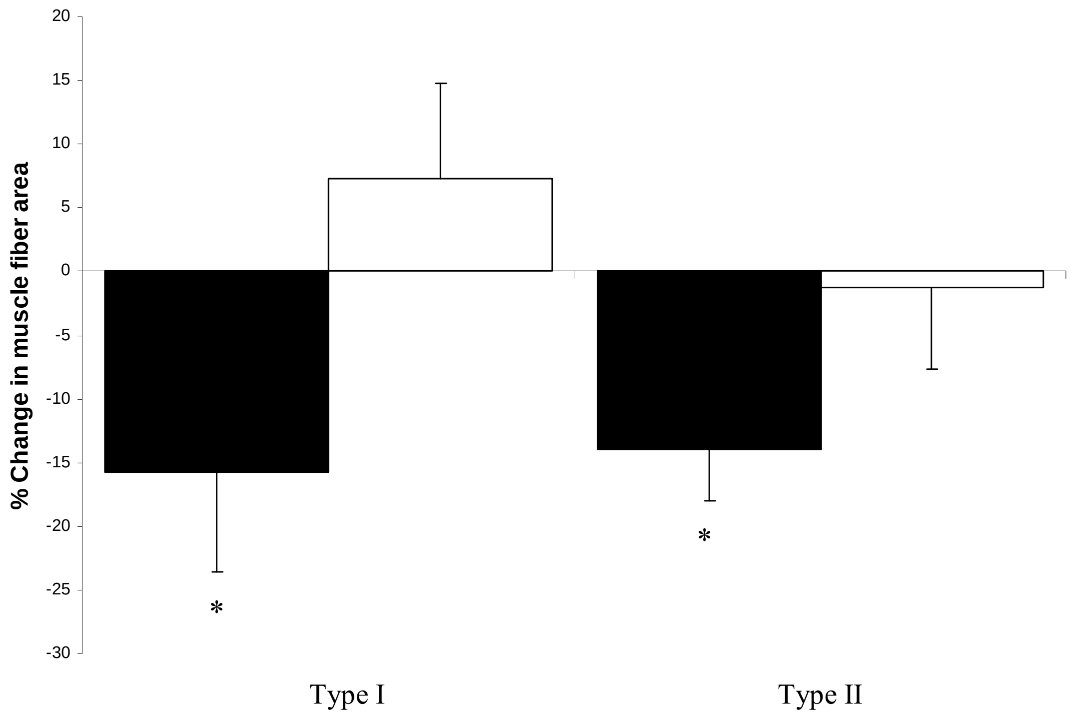
Percent change in thigh cross-sectional area (CSA) and thigh normal density muscle area (NDM). Values = Percent change ± SEM, * = Main effect time < 0.05

Figure 4. Muscle Fiber Area (I, IIA, IIX) – Percent change



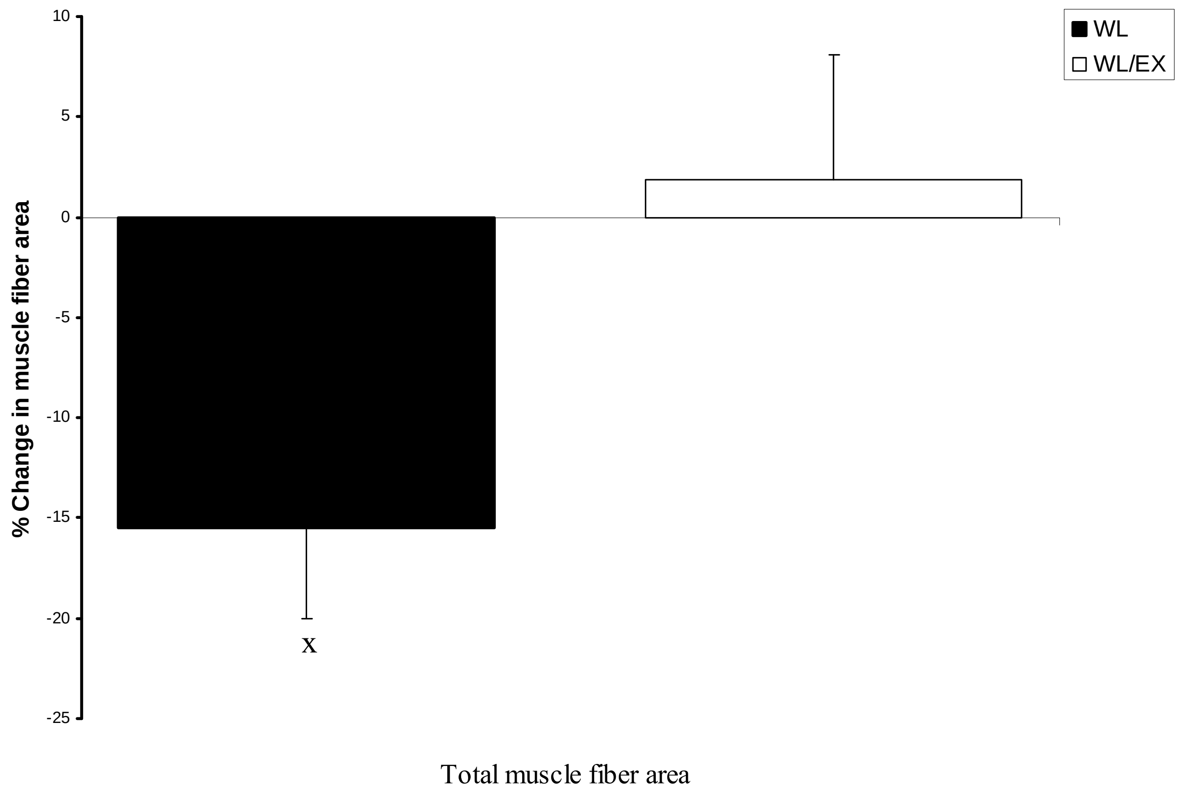
Percent change in specific muscle fiber areas, Values = Percent change ± SEM
X = Group x Time effect < 0.05, * = Main effect time < 0.05

Figure 5. Muscle Fiber Area (Type I & II) – Percent change



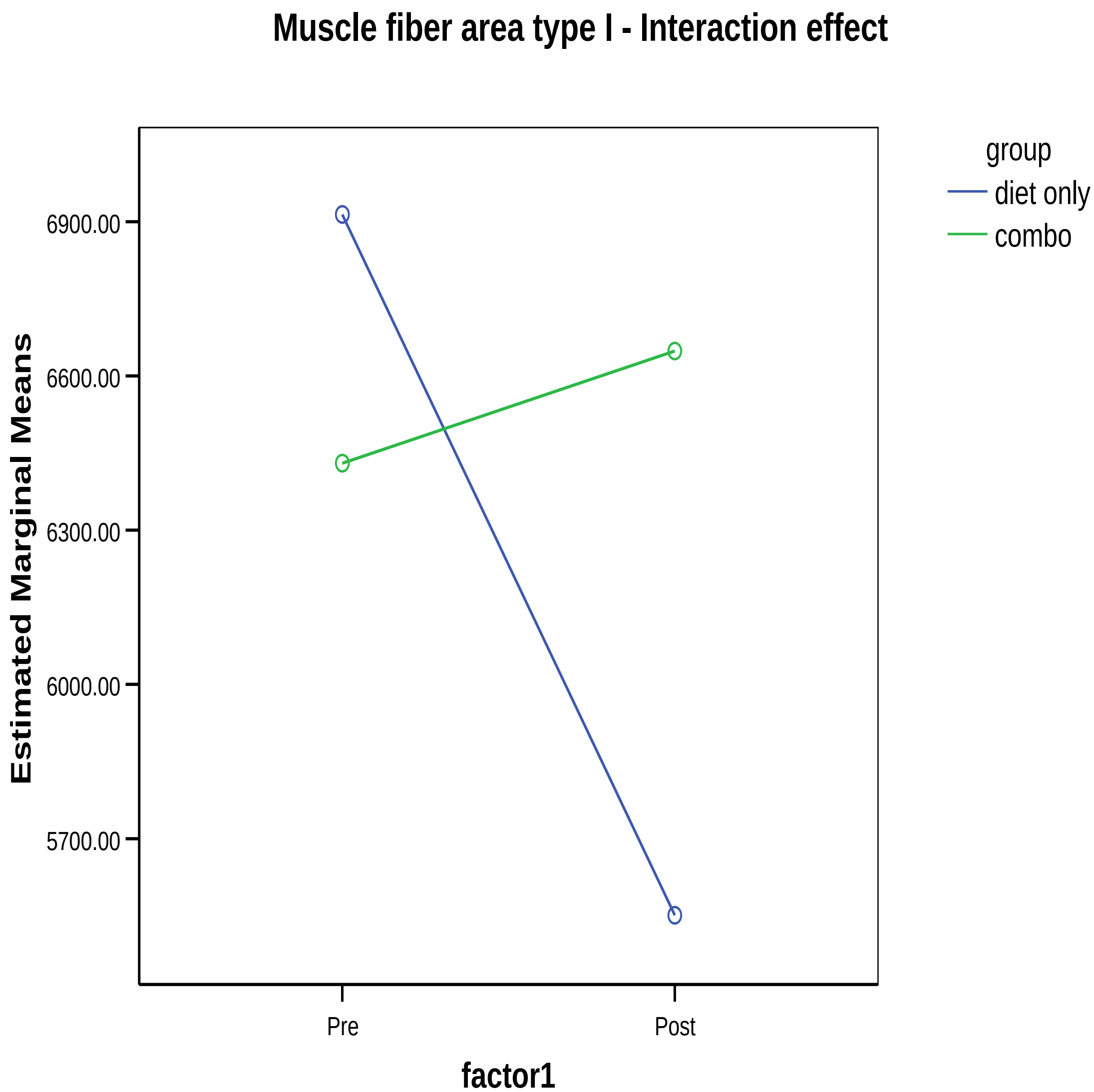
Percent change in muscle fiber areas based on contraction speed,
Values = Percent change ± SEM, * = Main effect time < 0.05

Figure 6. Total Fiber Area – Percent change



Percent change in total fiber area (I, IIA, IIX), Values = Percent change ± SEM,
X= Group x Time effect < 0.05,

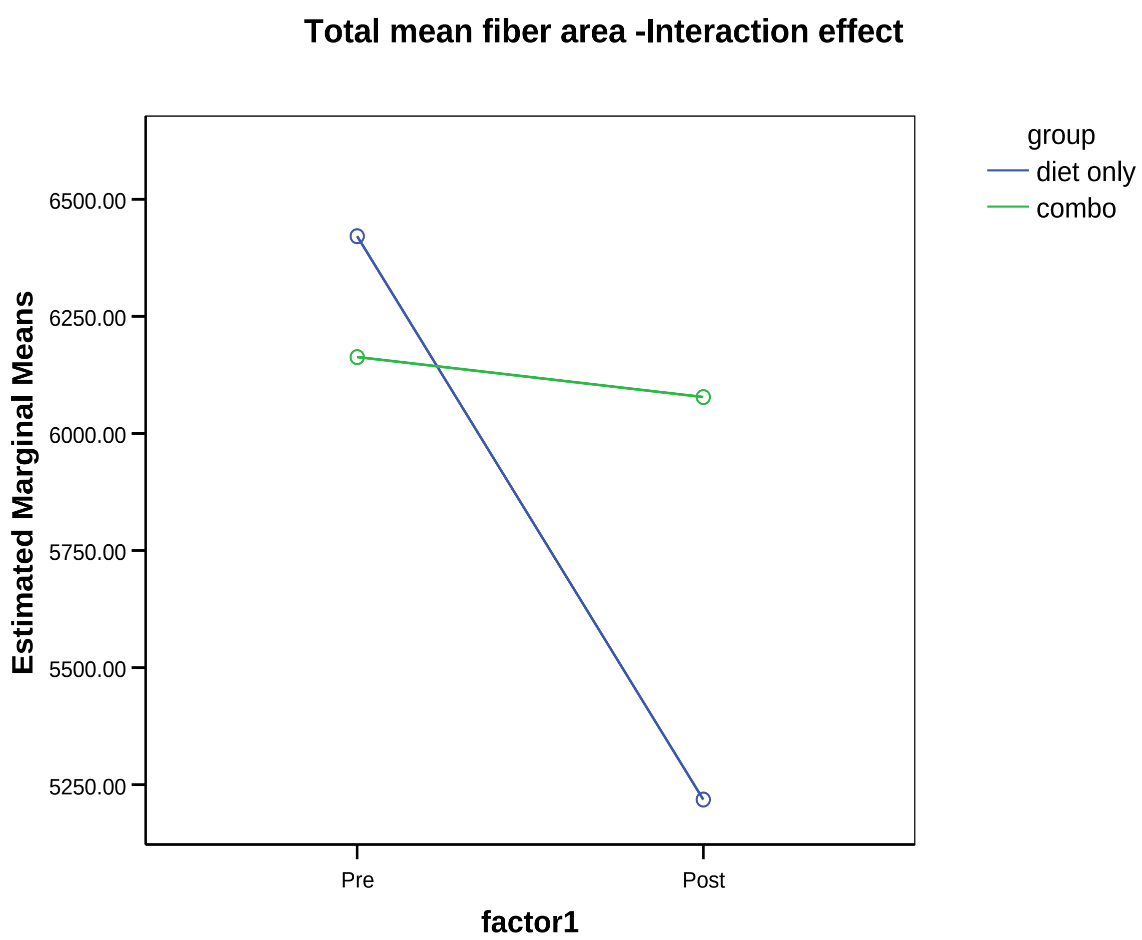
Figure 7. Type I Muscle Fiber Area – Interaction effect



	Pre	**Post**
Diet only	6113 ± 706	5584 ± 422
Combo	6430 ± 533	6648 ± 537

P = 0.02 – Group x Time interaction effect
Values = Mean fiber area ± SEM

Figure 8. Total Muscle Fiber Area – Interaction effect



	Pre	Post
Diet only	6421 ± 722	5217 ± 449
Combo	6162 ± 422	6077 ± 413

P = 0.01 – Group x Time interaction effect
Values = Mean fiber area ± SEM

5.0 CHAPTER 5

5.1.1 DISCUSSION

Aging has been associated with both a loss of muscle mass and an increase in adiposity. Although energy restriction-induced weight loss can produce desired effects to reduce cardiovascular and metabolic disease risk, it can also cause a loss of lean tissue in young and middle-age adults. The aims of this study were two-fold. The first objective was to determine whether diet-induced weight loss in older (>60 years of age) would result in a significant loss of muscle mass. The second key objective was to determine whether or not moderate aerobic exercise can attenuate the potentially adverse effects that weight loss alone might have on muscle mass. Specifically, the effects of four months of caloric restriction only or dietary restriction in combination with moderate aerobic exercise, on skeletal muscle mass in overweight and obese older men and women were examined.

A primary finding of this study was that diet-induced weight loss resulted in a significant loss of lean, fat free mass in conjunction with the loss of body fat. These findings are consistent with previous data in younger subjects demonstrating that diet-induced weight loss results in a loss of body fat but also a loss of skeletal muscle mass (44). The body weight loss induced by energy restriction over 4-6 months typically results in a substantial proportionate loss (~25%) in lean mass (76, 78, 132). Weight loss through dietary restriction is a common method for reducing excess body fat (34) and improving health risks related to increased adiposity. This might suggest that diet-induced weight loss in older adults, who are more prone to the development of sarcopenia, may accelerate declines in muscle mass beyond the normal aging process (113). To date, there has been little investigation into the effects of intentional weight loss on muscle mass in older adults. The potentially negative effects of intentional weight loss on the loss of muscle mass is particularly relevant to older adults who are becoming more obese (115), while at the same time are losing muscle mass(38, 94, 123, 135). Therefore the gain in body fat and loss of muscle is generally unique to the older population. Indeed, it has recently been suggested that "sarcopenic obesity" is a stronger risk factor for functional decline than sarcopenia, or the loss of muscle, itself (52, 113, 119, 152).

A unique aspect of this study was the use of a variety of methods to assess the intervention effects on changes in muscle mass. The loss of fat free lean mass assessed by dual energy X-ray absorptiometry (DXA) with weight loss alone was consistent with the loss of thigh muscle mass quantified directly by computed tomography (CT). However, neither the psoas major nor the erector spinae muscles were affected by weight loss in these older adults. These CT data are consistent with the regional fat free mass data assessed by DXA, which demonstrated that weight loss decreased leg lean mass but had no effect on either trunk or arm lean mass. This raises the intriguing possibility that the effects of intentional weight loss may affect specific muscle groups. Further analysis of the current CT scan images could be performed to examine whether quadriceps (knee extensors) and hamstring (knee extensors) muscle groups are affected differently by weight loss.

Changes in muscle mass were also assessed at the cellular level with percutaneous biopsies of vastus lateralis. Consistent with the changes in muscle mass determined with noninvasive imaging, weight loss also reduced the size of muscle fibers (cells). This is the first study to quantify changes in muscle fiber size with intentional weight loss. Moreover, weight loss resulted in a similar decrease (~15%) in the size of both type 1 and type 2 muscle fibers. It is widely accepted that aging is associated with a preferential loss of type 2 muscle fibers (13, 25, 60, 90, 93, 97, 129). Human skeletal muscle is comprised of different cell, or fiber types. Type 1 fibers are more oxidative, have a higher mitochondria density, and have higher intramyocellular lipid than type 2 fibers(139). Type 1 fibers also exhibit slower contraction speeds (100). It is unclear whether or not diet-induced changes in muscle glycogen, intramyocellular lipid (55) or protein content may partly explain the loss of muscle fiber size, cross-sectional area on CT or leg lean mass on DXA. Nonetheless, the effects of intentional diet-induced weight loss on specific muscle types may have important implications in determining the relative positive or negative effects on functional or metabolic outcomes in older adults. Therefore, this study provides important insight into the effects of intentional energy restriction on the loss of muscle mass in older adults.

A second key finding in this study was that moderate aerobic exercise attenuated the loss of lean muscle mass induced by energy restriction. The addition of exercise in combination with diet-induced weight loss has been examined as a means for reducing body weight and preserving lean muscle mass. These recent studies have demonstrated that exercise helped preserve lean muscle mass during weight loss in younger and older men and women (29, 104). Therefore, these results are in accord with those in the current study. The current study is unique, however, in that this is the first direct comparison of weight loss only to weight loss plus the addition of moderate exercise in older (>60 years of age) adults who may be beginning the sharp decline in age-associated muscle mass. There are several additional novel aspects of the current study that merit discussion.

The WL (weight loss only) and WL/EX (weight loss & exercise) lost similar amounts of body weight over the 4-month intervention. Examination of whole body composition changes using DXA revealed that both groups lost a similar amount of weight and body fat. The WL group lost a significant amount (-4%) of fat free mass (FFM) assessed by DXA, while the -1% loss of FFM for the WL/EX group did not reach significance. Moreover, the loss of FFM was significantly less for those who performed moderate exercise in conjunction with the diet-induced weight loss (interaction effect, p = .044). When the proportionate change in fat mass and FFM were examined, of the total tissue lost in the WL group, 77% was from fat and 22% was from FFM. With WL/EX, 92% of the total tissue was lost as fat and 8% was lost as FFM. These proportionate changes were also found to be statistically significant for an interaction effect (p = .044) between the WL and WL/EX groups. The current study confirms these earlier studies (76, 125, 133, 134) and adds to this literature by directly examining weight loss and exercise-induced changes in muscle mass using imaging and muscle biopsies.

The WL and WL/EX groups had similar decreases in thigh muscle cross-sectional area (CSA) and normal density muscle (NDM) area over the 4-month intervention. The effects of weight loss without concomitant exercise to decrease muscle mass assessed by imaging (MRI or CT) is in agreement with previous studies demonstrating decreases in absolute thigh muscle volume (50, 151, 155). Surprisingly, and in contrast to the results obtained by DXA, exercise did not significantly attenuate this loss of muscle mass assessed by CT. Normal density muscle, which represents the amount of muscle with higher attenuation values on CT, i.e. skeletal muscle with relatively less fat content (53, 54) decreased with WL (-8%) and tended to decrease (-3%) in WL/EX. The current study does not convincingly confirm earlier studies that have demonstrating preservation of thigh muscle mass with moderate exercise when combined with weight loss (155). It is possible that an effect of exercise to preserve the loss of normal density muscle would have been observed had we studied a larger number of subjects in each group. Moreover, it is quite possible that these findings may be muscle group-specific. Examination of the quadriceps and hamstrings separately for thigh muscle mass may shed light on whether there is any specific muscle group interaction with weight loss and moderate exercise on the loss of muscle mass.

This is the first study to examine the potential of moderate exercise to preserve the loss of muscle mass induced by energy restriction examined at the cellular level. A novel key finding of this study was that moderate exercise fairly dramatically prevented the loss in muscle fiber size with intentional weight loss. Studies have shown with increased age that type I muscle fiber area (slow twitch) remain relatively stable and type II (fast twitch) decrease (60, 90, 93, 97, 116, 155). Muscle fiber size was examined for changes related to weight loss and weight loss with moderate exercise in three ways: 1. Fiber specific (type I, IIA, IIX), 2. Contraction speed (slow & fast), and 3. Total mean fiber area (mean area (I, IIA, IIX) / fiber number measured). The novel aspect for evaluating fiber size in three ways was to determine how specific muscle fiber size may change with weight loss and exercise.

The WL group lost a significant decrease in the amount (-19%) of type I fiber area assessed by percutaneous biopsy of the vastus lateralis, while the WL/EX group remained relatively unchanged in type 1 fiber size. The difference in this change was different between WL and WL/EX (interaction effect, $p = 0.02$). This increase in type I fiber size is in line with previous studies that have documented increases in type I fiber size with aerobic exercise (22). Type IIA fiber size was found to change in both groups following the 4-month intervention. The

WL group demonstrated significant decreases in type IIA fiber size and in a similar manner the WL/EX group demonstrating comparable results (p= 0.04). The WL group also demonstrated a change in type IIX fiber area while the WL/Ex group had an inverse change in type IIX fiber size. While neither of these changes were significant (p = 0.64) these results demonstrate a similar pattern in changes in the WL group compared to the WL/EX. This study is the first to investigate muscle mass changes at the cellular level when combined with induced weight loss. This study confirmed that weight loss without concomitant exercise resulted in similar significant decrease in both type I (-19%) and type II (-15%) fiber size (144). The significant decrease in total muscle fiber size (-18%) in the weight loss group also confirms other studies that muscle mass is decreased with weight loss (20, 78, 113, 155) conversely exercise combined with weight loss had a preservation effect on muscle mass and this was confirmed by this study. The WL/EX group demonstrated a modest change in type I fiber size and no change (< 1%) in type II fiber size that had been previous demonstrated with aerobic exercise alone (22). Exercise combined with weight loss attenuated total muscle fiber area (< 1% change) indicating that moderate aerobic exercise can preserve the diet-induced loss of muscle mass at the cellular level (30, 76).

This study was not without limitations. The first limitation of this study was the small number of subjects who had a high variability in weight loss between the intervention groups. A relatively small number of subjects from each group could be considered not in compliance due to their lower percent change in body weight. This study also lacked enough power to determine if there were any gender effects between the WL and WL/EX groups for changes in fat free mass with weight loss and exercise. While moderate aerobic exercise was using in the intervention phase a measure of functional performance was not included in this study. The inclusion of a muscular strength test may have provided insight into changes of muscle strength that may accompany decreased muscle mass with weight loss.

5.1.2 CONCLUSION

Diet-induced weight loss by energy restriction decreases muscle mass in older adults. These results were observed using a variety of methods, including whole body composition, direct measure of muscle mass using imaging, and muscle cell size assessed by muscle biopsy. These decreases in muscle mass with weight loss has been hypothesized in the acceleration of Sarcopenia in older adults, resulting in a greater decreases in muscle mass and strength beyond the natural aging process. This study confirmed that weight loss without concomitant exercise results in greater decreases in lean muscle mass when compared to weight loss combined with exercise. These results have shown that weight loss alone and weight loss combined with exercise demonstrate similar decreases in total body fat mass, and that weight loss combined with exercise results in an attenuation of the loss of muscle mass.

The implications of these novel findings are clinically significant. This study indicates that diet-induced weight loss alone may have detrimental effects on reducing skeletal muscle in older adults at risk for the development of muscle weakness and disability. However, the addition of moderate aerobic exercise, mostly walking, has a powerful effect when combined with diet-induced weight loss to preserve muscle mass. Thus, this study suggests a program of increased physical activity in older adults who are trying to lose weight. Future studies are needed to determine whether or not these changes in muscle mass are associated with functional outcomes such as muscle strength, power or mobility.

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