ABSTRACT

Title of Document: EFFECTS OF ACUTE POSTURAL CHANGE ON MID-THIGH CROSS-SECTIONAL AREA AS MEASURED BY COMPUTED TOMOGRAPHY

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Fluid shifts resulting from postural change present a potential source of error when assessing the CSA of limb tissue using CT. In the present study mid-thigh axial scans of 13 older women were obtained at 5, 10, and 15 minutes of supine rest. Scans were analyzed for changes in CSA of subcutaneous fat(SF), low density muscle(LDM) and normal density muscle(NDM) tissue. A significant decrease was found in NDM CSA at 15 minutes (2.3 ± 0.8, 1.6%, $P<0.05$) with no change in LDM or SF CSA between any time interval The results of the current study suggest that the potential measurement error associated with fluid shifting out of the tissues can be minimized when baseline and follow-up CT-derived images of mid-thigh CSA are obtained within the first 10 minutes the subject assumes the supine position and that the CSA of NDM and LDM may be affected differently by loss of HP.

EFFECTS OF ACUTE POSTURAL CHANGE ON MID-THIGH CROSS-SECTIONAL AREA AS MEASURED BY COMPUTED TOMOGRAPHY

By

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Dedication

I would like to dedicate my thesis to all of my wonderful clients. You all encouraged me when I was frustrated, accommodated me when my schedule was crazy and, most importantly of all, unfailingly believed in my eventual success. To a person, you are kind, wise, generous, and sincerely inspirational. Thank you for being so patient, I feel deeply blessed to still have you all in my life.

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CHAPTER 1: INTRODUCTION

Introduction

The tissue composition of thigh cross-sectional area (CSA) can provide valuable information about the structural, contractile and metabolic function of human skeletal muscle tissue. Increases in thigh muscle CSA due to resistance exercise training have been associated with increases in strength, while decreases in thigh muscle CSA have been associated with age-related changes in skeletal muscle strength and have been found to be a strong predictor of mortality in subjects with wasting diseases (69). Alterations in skeletal muscle tissue density due to interstitial fat accumulation are associated with the development of insulin resistance and type 2 diabetes mellitus (36), as well as the advancement of muscle-related disease states (40; 54).

Computerized axial tomography (CT) is an *in vivo* imaging technique that employs X-ray technology to provide information about internal anatomy (49). Studies utilizing cadaver analysis have established CT as an effective measurement tool for determining thigh muscle CSA (50) and have demonstrated the ability of CT to accurately distinguish between skeletal muscle containing interstitial adipose tissue, or low density muscle tissue (LDM) and adipose-free skeletal muscle, or normal density muscle tissue (NDM) (74). Computed tomography has been shown to be a sensitive measure of changes in thigh tissue composition (16; 48; 86) and has been heavily utilized for this purpose in clinical and research settings. Through the attenuation of X-rays, CT technology distinguishes between tissue types such as bone skeletal muscle and adipose tissue based on density (49). Fluid content is a

significant contributor to tissue density and therefore, tissue fluid shifts, resulting from postural changes, present a potential source of error when assessing the composition of limb tissue using CT techniques.

The effects of long term postural alteration on fluid shifts in relation to measurement of thigh CSA via CT have been extensively explored in the literature (3-5; 55; 56; 88; 89). While the protocols in these studies used supine rest for several hours to several weeks, very few studies have been published that examined the effects of fluid shifts on skeletal muscle CSA as a result of acute postural change from the upright to supine position (9; 15). This potential effect was explored by Berg and colleagues, who used bioelectrical impedance analysis and a 2-hour supine bed-rest protocol during which time four CT scans were performed. The investigation found that there was a significant decrease in both thigh skeletal muscle and thigh subcutaneous fat (SF) CSA which correlated with changes in limb fluid volume during the first hour of supine rest (9). These findings lead Berg to recommend standardization of test procedures when employing CT techniques to assess peripheral tissue CSA (9) and some researchers have implemented a pre-CT scan protocol of from 10 to 60 minutes of supine rest based on these data (25; 45; 63; 91). Unpublished pilot data gathered in this lab found no significant changes in muscle volume when CT-generated images of single slice mid-thigh muscle were analyzed following 15 minutes of supine rest. These findings suggest the need to expand the current research regarding the potential effects postural shifts may have on the measurement of thigh tissue CSA as assessed using CT.

Since 10 to 60 minutes of supine rest prior to each CT scanning session can substantially tax limited resources when research conditions require volunteer human subjects, multiple scanning sessions and off-site scanning facilities, it would be of practical interest to determine if shorter rest periods have an effect on mid-thigh subcutaneous fat and skeletal muscle CSA as measured by CT. Further, while the Berg investigation used CT technology to assess the effects of postural shifts on thigh skeletal muscle as a whole, it is possible to use CT to distinguish between NDM and LDM. The assessment of skeletal muscle in terms of density has been shown to provide valuable information associating skeletal muscle composition with health risk and disease progression (36; 40; 54; 69). Evaluating the CSA of both types of skeletal muscle tissue may provide a more specific understanding of the manner in which postural shifts may introduce error in to the assessment of skeletal muscle composition.

Statement of Problem

The present study was designed to accomplish two goals: 1) to expand the research exploring the effects that changing from the upright to the supine posture evokes in thigh tissue CSA as measured by CT after five, ten and fifteen minutes and 2) to investigate the effect such postural shifts convey to skeletal muscle CSA when the muscular area of interest is evaluated in terms of normal density and low density skeletal muscle.

Experimental Hypothesis

No statistically significant change in cross-sectional area will be seen in mid-thigh normal density skeletal muscle, low density thigh skeletal muscle and thigh subcutaneous fat, as measured by CT, between 5, 10 or 15 minutes of supine rest.

Delimitations

1) The study is limited to a population of 13 postmenopausal women between the ages of 51-81 years.
2) All CT scans were performed at the Washington Adventist Hospital and all CT scan analyses was performed using Mipave software (NIH)
3) All subject refrained from vigorous exercise at least 6 hours before scans.
4) All subjects abstained for caffeine consumption for at least 3 hours before scans

Limitations

1) Length of time subjects spent in a standing position immediately prior to the CT scan was not uniform.
2) All subjects were volunteers and not randomly selected, thus limiting the generalizability of the results.
3) While all scans for any individual subject were performed at one time, the season of the year and time of day during which any particular subject was scanned varied.

Definitions

Acute – having a sudden onset, sharp rise, and short course

Computerized Axial Tomography (CT) - an in vivo imaging technique that employs X-ray technology to provide information about internal structures at the tissue level

Hounsfield Unit (HU) – the unit of measure assigned to a pixel reflecting the linear attenuation coefficient of the material represented by that pixel

Linear attenuation coefficient - the fraction of a beam of x-rays that is absorbed per unit thickness of the absorbing material; beam absorption is dependent on a material's density and number of electrons per unit mass

Long-term – a prolonged period of time; in the context of this study, more than two hours

Low Density Skeletal Muscle (LDM) – pixels that fall in a range of 0 to 30 Hounsfield units, thought to represent skeletal muscle infiltrated with adipose tissue

Mid-Thigh cross-sectional area – a two-dimensional axial image slice of the right and left legs located at 250mm below the most distal aspect of the ischial tuberosities, acquired using computed tomography

Normal Density Skeletal Muscle (NDM) – pixels that fall in a range of 31 to 100 Hounsfield units, thought to represent skeletal muscle with little infiltration of adipose tissue

Pixel - a two-dimensional representation of the average tissues properties in a three-dimensional section of tissue as assessed by computed tomography; pixel numbers range from -1000 to 3095 with the numerical value of a pixel, known as a Hounsfield

unit, corresponding to a specific level of gray within the image and represents the linear attenuation coefficient of the material being analyzed

Pseudohypertrophy – an enlargement of skeletal muscle due to replacement of muscle tissue with fat instead of additional muscle tissue

Scout Image – an scan of pertinent skeletal structures used to accurately establish the location of anatomical areas of interest for an individual subject

Subcutaneous Fat (SF) - pixels that fall in a range of -190 to -30 Hounsfield units, thought adipose tissue

Supine Rest – lying in the face-up position on a flat surface oriented at 90° to the floor, legs straight, arms straight at side of body

CHAPTER 2: REVIEW OF LITERATURE

A. Introduction

This literature review chapter is composed of six main sections. The first section focuses on the importance of thigh CSA assessment to the fields of exercise physiology and muscle pathology research. The second section discusses the methods used to assess thigh CSA, with the third section focusing specifically on CT, its contribution to thigh CSA research and the pros and cons of using CT technology. In the fourth section, the extraneous factors that can confound the measurement of thigh CSA are reviewed, with the fifth section devoted to an extensive description of the contribution of hydrostatic pressure to skeletal muscle fluid content and the consequences presented when hydrostatic pressure is altered with postural shifts. The remainder of the chapter reviews the research exploring the possible effects of acute postural change on the measurement of leg skeletal muscle.

B. Cross-Sectional Area

Measurement of axial thigh CSA provides a three compartment assessment of regional tissue composition including bone, adipose and skeletal muscle tissue. Assessment of thigh CSA allows adipose tissue to be further delineated into subcutaneous fat and fat deposited beneath the fascial layer of the muscle (36). When thigh CSA is analyzed with the appropriate technologies, variations in muscle tissue density due to interstitial fat infiltration are revealed allowing a quantifiable

qualitative distinction to be made between muscle tissue of normal (NDM) or low density (LDM) (32).

The ability to measure and evaluate these thigh components *in vivo* has provided a crucial advancement in understanding the complex relationship between exercise, physiology and body composition. For example, in the pursuit to understand the pathologies of muscular diseases such as Duchenne's muscular dystrophy (DMD), measurement of femur CSA can provide a useful index relating bone CSA to muscle development as an indication of normal or abnormal muscular growth (54). In another interesting example, Goodpaster and colleagues used the analysis of thigh adipose tissue CSA to determined that, unlike visceral fat deposits, subcutaneous fat deposition in the thigh region is not a marker for insulin resistance (IR) or the development of non-insulin dependent diabetes mellitus (NIDDM) and found that, in obese subjects, thigh subcutaneous fat was inversely associated with the development of the metabolic syndrome (34). Perhaps most noteworthy, the ability to gather data on thigh axial CSA has vastly increased our understanding of the influences of gender (70; 76; 95; 96) activity level (62), training state (27; 43; 67; 91), disease progression (10; 20; 39; 48; 69; 81) and aging (11; 14; 23; 26; 86; 95; 96) on skeletal muscle tissue. *In vivo* analysis of thigh CSA has allowed for the measurement of LMD resulting from the fatty infiltration of thigh skeletal muscle. A reduction in thigh muscle density has been associated with an increased risk of developing IR, NIDDM and the pseudohypertrophy of thigh skeletal muscle tissue associated with the progression of muscle wasting diseases (35; 40; 61; 84).

C. Measurement Techniques

Before the introduction of *in vivo* imaging, the relationship between muscle strength, endurance, metabolic processes and lower limb composition was estimated using anthropometric techniques such as those obtained through the measurement of skin folds and circumferences or was extrapolated from muscle biopsies and cadaver studies (18). Though often used in clinical and field settings to estimate changes in regional body composition, even under ideal conditions skinfolds and circumferences carry a 3 to 4 percent rate of error and are limited in sensitivity by equations that assume a standard proportion of subcutaneous fat to more internally deposited fat as well as a uniform density of skeletal muscle tissue (8; 69). Indeed, reports have shown that thigh circumference measurements remain similar despite changes in muscle CSA as measured using *in vivo* imaging (85; 97). The very limited number of cadaver studies involving the analysis of lower limb composition are obviously restricted by the inability to correlate composition measures to regional and systemic physiological function, actual biomechanical performance or the effects of intervention research protocols (18). While invasive and somewhat painful, muscle biopsies do provide the opportunity for direct assessment of the composition of the muscle cells sampled and can be used in longitudinal/training research, however, a muscle biopsy represents less than 1% of all cells in the quadriceps and studies combining cadaver CSA analysis with biopsy sampling found single samples to have low predictive value for over all skeletal muscle composition (28; 65; 66).

The measurement of thigh tissue CSA using i*n vivo* imaging technologies such as ultrasonography (US), magnetic resonance imaging (MRI) and CT have, to

varying degrees, been shown to be valid, reliable, non-invasive analysis techniques that lend themselves readily to the gathering of both cross-sectional and longitudinal data. The inherent advantages and disadvantages of US and MRI will be discussed in brief below followed by an extensive discussion of the biophysics, history and pros and cons of CT technology.

Ultrasound

The first in situ measurement of muscle CSA in living humans was made possible by the development of US which was used by Ikai, et al., in a study that confirmed a long hypothesized positive relationship between maximal isometric strength and muscle CSA (52). Ultrasound has subsequently been used to investigate the relationship between thigh CSA and isometric strength in both young and aging populations (95; 96), changes in thigh muscle CSA in response to strength training (43; 85) and the effects of disuse and aging on thigh muscle CSA (75; 82). This technique uses high frequency sound waves produced by a hand-held probe to create images of internal anatomy that can be viewed in motion or still. Using the probe, the sound waves are directed through the surface of the skin and are reflected off anatomical structures allowing the construction of cross-sectional images (64). The B-mode of US has been shown to allow good differentiation between connective tissue and skeletal muscle that enables an accurate distinction to be made between borders when measuring individual muscle groups (82). The B-mode US method has been shown to provide images of sufficient clarity and tissue contrast that rival MRI and have been found superior to CT for the assessment of changes in skeletal muscle

in elderly women (82; 85). Ultrasound has the advantage of being relatively inexpensive both in per machine and per examination cost, is highly mobile allowing the unit to more readily travel to the subject, and introduces no additional risk to the subjects as it is a non-ionizing technology (22). Nevertheless, US imaging can be problematic in that adipose tissue is highly reflective of sound beams, thus limiting the use of US with obese patients (22). Because US is basically a manual technique, the quality of the images obtained are critically dependent on operator skill. Scanning sites are marked superficially on the skin and, with repeated measures, great care must be taken to match scan landmarks precisely. Inconsistency in the angle at which the transducer is held relative to the surface of the skin can result in erroneous measurement of muscle size and depth and, if the pressure applied when manipulating the transducer is too great, the muscle of interest can be flattened resulting in distorted values (17). Along with a high potential for human error, US also requires a relatively long scanning time to obtain a useable CSA image, approximately 20 minutes, compared to six minutes for MRI and seconds for CT (82).

MRI

The introduction of MRI in 1984 provided a non-ionizing *in vivo* imaging method capable of producing highly detailed images of soft tissue (64). By creating a very strong magnetic field, the MRI device causes hydrogen protons, found in water and lipid molecules, to align with the field and become excited. As the protons return to the relaxed state, the energy released creates a signal detected by the MRI and the information is transformed into a visual representation of the tissues (18). Estimates of skeletal muscle CSA by MRI have been shown to be highly correlated with

cadaver measurements (74) and MRI has been shown to have a superior ability to differentiate between skeletal muscle and connective tissue that allows easy calculation of the CSA of individual muscles (19). When comparing MRI estimations of thigh skeletal muscle CSA to CSA measured directly from cadaver axial slices, Engstrom and colleagues found that the image obtained through MRI allowed for a better distinction between the muscle bellies of vastus lateralis and vastus intermedius than could be made with direct observation of the cadaver axial slice (19). The technology of MRI allows for the detection of intramuscular adipose tissue, permitting the identification and quantification of LMD (35; 74). MRI has been shown to be sensitive to changes in CSA due to resistance exercise training (76) and changes such as increases in LDM associated with the aging process (14). The ability to produce axial images that can be used to clearly distinguish between individual muscles and distinguish NDM form LDM, as well as the non-ionizing nature of MRI, are the obvious advantages of this technology. However, there are some disadvantages that limit the use of MRI in the research setting. MRI technology is very expensive and in high clinical demand which can limit access for purely research purposes (18). The strong magnetic field generated by MRI restricts the study of subjects with implanted metal objects and some MRI machines have a small diameter magnet that precludes the study of some obese subjects and those prone to claustrophobia.

D. Computerized Tomography

Biophysics of the Technology

Computerized axial tomography is an *in vivo* imaging technique that employs X-ray technology to provide information about internal structures at the tissue level (49). The image produced through CT is composed of a two-dimensional matrix of picture elements or pixels. Each pixel in the CT image has a specific number that corresponds to a specific location within the subject. Pixel numbers range from -1000 to 3095. The numerical value of a pixel within the matrix corresponds to a specific level of gray within the image and this numerical value is referred to as a Hounsfield Unit (HU) (49). The CT x-ray tube transmits photons through tissue while detectors, positioned opposite the photon transmitter, assess how many photons passed through the tissue. The difference in the number of photons transmitted compared to the number of photons detected is known as an attenuation of the x-ray beam. Different tissue types will attenuate the beam to differing amounts. Hounsfield units assigned to a pixel correspond to the average linear attenuation coefficient of the tissues represented by the pixel. Attenuation characteristics of a tissue depend on tissue density and the ratio of electrons-to-mass of the elements that make up the tissue. In this regard, water is the reference for the linear attenuation coefficient scale and is assigned a value of 0 HU. Tissues less dense than water, such as fat, are assigned a negative HU value and appear as darker gray in the CT image, while tissues more dense than water, such as muscle, are assigned a positive HU value and appear in the CT image as lighter gray (83). Adipose tissue has been shown to display attenuation

values of –190 to –30 HU, while skeletal muscle has been shown to display attenuation values of 0 to 100 HU (29; 31).

Development and History

CT technology, introduced in 1973, was originally designed for use in brain imaging (49). Up until the development of the CT technique, the technology of *in vivo* imaging was restricted to x-ray imaging procedures where an image is formed when x-rays pass through a structure placed before a photographic plate. This technique superimposes all objects from front to back. In order to clearly distinguish any one object, it must stand out from the various tissues in front and behind it. Due to the risks inherent in radiation exposure, the need to limit the number of photons that may be passed through a patient also limited the amount of information that could be obtained through these conventional x-ray techniques. Instead of simply passing the x-rays front to back, the CT machine circles the object of interest and passes the x-ray beam through the edges of the object from all directions at once, measuring the attenuation coefficients of the tissues that comprise that section of the object and then uses a computer to organize all the information in to a high-resolution image with clear boundaries between subcutaneous adipose tissue, skeletal muscle, visceral organs, brain tissue and osseous tissue. The invention of the CT scanner revolutionized the field of radiology and earned the inventors, Godfrey Newbold Hounsfield and Allan MacCloud Cormack, the 1979 Noble Prize in medicine (83). In 1974, Robert Ledley further expanded CT technology by developing the whole-body CT scanner (83) and CT quickly became recognized as a valid and reliable estimator of both total body and regional tissue volumes (46; 47; 87). Early work by

Häggmark, T and colleagues compared skeletal muscle fiber CSA, obtained by muscle biopsy, to human thigh vastus lateralis muscle CSA as measured by CT and found the mean fiber area to be highly correlated ($r = 0.91$) to the CSA and concluded that the CSA of thigh muscle can be accurately determined by CT (42). Computed tomography has since proved instrumental in revealing crucial correlations between the measurement of the composition of thigh CSA and the structural, contractile and metabolic function of human skeletal muscle tissue, as well as, the critical role muscle density plays in the development and progression of both regional and systemic disease states.

Thigh CSA and Muscle Function

In 1983 Maughan and colleagues used CT to explore the relationship between thigh skeletal muscle CSA and the maximum isometric force that could be produced by the involved muscle groups and found a positive correlation between isometric force and muscle CSA (70). The researchers determined the cross-sectional image of the thigh produced by CT to provide the "clarity necessary to easily distinguish between subcutaneous fat and muscle tissue". While the Maughan study did find a large variation in the ratio of isometric force-to-CSA between subjects, the unique ability of CT technology to distinguish, *in vivo*, low density tissue within the normal density muscle tissue, allowed the investigators to correct for this as a potential source of the variation (70). One of the first resistance training studies to employ CT technology was conducted by Horber and colleagues and reported in a 1985 issue of the European Journal of Clinical Investigation (48). In this study researchers compared measurements of mid-thigh CSA before and after a 50 day isokinetic

training protocol and found not only a significant increase in thigh muscle CSA, but were also able to take advantage of the ability of CT to distinguish between muscle tissue densities and report that the resistance exercise training protocol resulted in a significant increase in muscle tissue density as well (48). In a quest to further understand the relationship between muscular endurance and muscle morphology, Lorentzon and colleagues were able to use a combination of quadriceps muscle CSA, as measured by CT, and quadriceps muscle fiber type composition as estimated with muscle biopsy, to determine that muscular fatigue during repetitive knee extension was significantly correlated to type II fiber content of the quadriceps even after correcting for muscle CSA (67). Mid-thigh skeletal muscle CSA obtained through CT was reported by Klitgaard and colleagues in 1990 to elucidate the relationship between ageing, physical activity and the maintenance of thigh skeletal muscle mass. By comparing the CSA of quadriceps muscles of elderly men participating in regular resistance training to those of younger and age-matched controls as well as aged-matched men participating in regular swimming, the investigators found the quadriceps of the resistance training group to have significantly greater CSA than the age-matched groups, yet not statistically different from the younger group. These results lead the researchers to suggest that aging human skeletal muscle retains the ability to adapt to resistance exercise training (62). In a cross-sectional study comparing CT images of the mid-thigh CSA of untrained young and older men, Overend and colleagues reported that, while they saw no significant difference in total muscle CSA between the groups, they did find, in the elderly group, the *actual* skeletal muscle CSA to be decreased due to an increased infiltration of fat and non-

muscular tissue within the muscles (80). When elderly women were put through either an 18 week resistance exercise training or endurance exercise training regimen, Sipila and colleagues compared mid-thigh CT scans taken at baseline and at the end of the training protocol and discovered, in the resistance trained group only, a significant increase in muscle CSA as well as a significant decrease in the proportion of muscle infiltrated with fat (86).

Overall, the high resolution images produced with CT have assisted researchers in confirming the positive correlation suspected to exist between human thigh skeletal muscle force and muscle CSA and have allowed researchers to factor into this relationship the proportion of muscle CSA infiltrated with fat, thus creating the possibility for a more specific analysis of muscle function. In addition, access to CT technology has allowed researchers to recognize the capacity of resistance exercise training to increase skeletal muscle CSA as well as reduce the proportion of LDM CSA in the elderly, resulting in a reversal of age-related impairment of muscle performance.

Thigh CSA and Muscle Health

In 1983 Jones and colleagues published a study that used axial mid-thigh and mid–calf CT scans to compare the size of calf and quadriceps muscle of children with DMD with those of control subjects (54). While the study data showed the DMD group to have the expected calf muscle hypertrophy, CT technology allowed the investigators to take advantage of the information available in the mid-thigh CSA image to 1) develop a ratio of femur CSA to mid-calf muscle CSA as an index of stature, hence, giving the researchers a way to judge if the calf muscle size is, in fact,

larger than would be expected in a normal subject and 2) to identify the extent to which the *pseudohypertrophy* associated with the early stages of DMD is the result of actual muscle hypertrophy or the infiltration of fat and/or connective tissue in to the normal muscle tissue. In the past, this information regarding the nature of the DMD disease process could only be obtained via post mortem examination; plus, the repeatability of CT provided the added advantage of allowing the clinician to track the course of disease progression in a particular individual (54). That same year, CT was used to examine the condition, composition and strength (defined as force per unit CSA) of mid-thigh skeletal muscle in 50 subjects with several different forms of muscular disorder including DMD, limb girdle syndrome, Becker muscular dystrophy, fascioscapulohumeral and scapuloperoneal dystrophies as well as metabolic and inflammatory myopathies. Grindrod et al., reported that, in relation to muscular disorders, the CT image was capable of providing valuable information regarding 1) the distribution of muscles affected by the disease, 2) quantification of the disease process through comparing muscle CSA with long bone CSA, 3) the relationship between muscle CSA and the contraction force production of diseased muscles and 4) the distinction between truly hypertrophic muscle and pseudohypertrophic muscle due to replacement by non-muscle tissue (40). Nordal et al., designed a study to see if CT could be used to differentiate between primary muscular disease and muscular disease developed as a secondary consequence of neurological disease and if the analysis of diseased muscle via CT image could predict muscle function (77). While the investigation determined that examination of the skeletal muscle image provided by CT did not result in the ability to distinguish

between primary and secondary muscular disease, the researchers were surprised to find the extent to which connective tissue and fat cells had invaded the muscles affected by the neurological diseases with many of the subjects presenting a degree of infiltration comparable to advanced cases of muscular dystrophy. The researchers also reported a positive correlation between actual quadriceps CSA and maximal isometric power similar to that expected in healthy individuals, thus suggesting that the mass of the muscle that consisted of normal fibers did retain relatively normal functionality.

Thigh CSA and Low Density Muscle

While it had long been noted that a correlation exists between skeletal muscle mass and insulin resistance (20), Kelley and colleagues endeavored to gain insight in to this association by analyzing the relationship between thigh composition and obesity in individuals with and without non-insulin-dependent diabetes mellitus (NIDDM). By employing CT imaging, the investigators observed that an inverse relationship existed between decreases in thigh lean tissue density and obesity, as well as an association between NIDDM and decreases in thigh lean tissue density independent of obesity (60). Now recognized as a major factor in the complex set of metabolic conditions that result in insulin resistance and NIDDM, the contribution of skeletal muscle composition and density to this condition was brought to bear through the use of CT in the analysis of mid-thigh skeletal muscle. In 1997, Goodpaster et al, conducted a study designed to elucidate the relationship between insulin sensitivity and thigh skeletal muscle composition (32). Using CT attenuation values to assess thigh muscle density and a euglycemic glucose clamp to assess insulin sensitivity of

both lean and obese subjects, the density of thigh skeletal muscle was observed to be a good predictor of insulin sensitivity. In fact, muscle density was shown to be better than abdominal fat content, in predicting insulin resistance in obese individuals (32). When CT was used by Goodpaster and colleagues in 1999 to assess the effects of weight loss due to caloric restriction on obese individuals, they observed that the obese subjects had greater mid-thigh skeletal muscle CSA at baseline than non-obese subjects however, the skeletal muscle of the obese subjects had lower mean attenuation values indicative of fat infiltration (30). After the weight loss, the obese showed very little loss of the normal density muscle CSA and a significant decrease in the CSA of LDM. Because the image produced through CT is able to provide clearly defined borders between tissue such as fat, muscle, fascia and bone, Goodpaster et al., were able to more precisely assess the connection between thigh skeletal muscle composition and insulin resistance by distinguishing the border between thigh subcutaneous adipose tissue, subfacial tissue and skeletal muscle tissue (36). This distinction made possible the further division of adipose tissue collected in the muscular compartment in to subfacial adipose tissue, adipose tissue that has accumulated around the muscle but distinct from subcutaneous, and adipose tissue that has infiltrated the muscle groups, referred to as intermuscular adipose tissue. The investigators found that despite the fact that subcutaneous fat made up nearly 90% of total thigh adipose tissue it was not associated with insulin resistance. Surprisingly, the subfacial and intermuscular fat was most highly associated with insulin resistance in obese subjects regardless of age or gender. These findings highlight the important advantage CT technology has afforded researchers in providing a tool capable of

distinguishing, *in vivo*, thigh SF from skeletal muscle tissue as well as the capacity to identifying the presence of LDM and to quantifying the extent to which fat has accumulated within the muscle compartment.

Validation of CT as Tool for Measuring CSA

Studies utilizing cadaver analysis have established CT as an effective measurement tool for determining thigh muscle CSA (51) and have demonstrated the ability of CT to accurately distinguish between skeletal muscle containing interstitial adipose tissue, and adipose-free skeletal muscle (74) In 1985, the efficacy of CT technology in accurately assessing thigh composition CSA was explored by Hudash and colleagues. The right and left legs of a human cadaver were scanned using CT, the sites of the radiological scans were labeled and the cadaver was cut cross-sectionally at those sites and photographed. Using a semi-automatic digitized planimeter, the photos of the axial thigh slices were then measured along with the corresponding CT images. The study found that the CT image site from the mid-thigh provided the most accurate and reliable assessment of muscle CSA and that in general, the CT images accurately depicted thigh bone, fat and total thigh size. The investigators also concluded that, in combination with the accuracy of the technique, the high resolution of muscle groups provided and the non-invasive nature of the technique, CT technology was "uniquely suited" (50) for use in research settings that require repeated scanning such as the type of intervention studies commonly conducted in exercise physiology and sports medicine. Mitsiopoulos and colleagues used data gathered from two cadavers to explore the accuracy of CT in measuring fat free muscle CSA, the CSA of subcutaneous adipose tissue and the fat infiltration of

skeletal muscle. Using a protocol similar to the one reported by Hudash, axial photographs and CT images of the cadaver legs were analyzed using computer software. The investigation determined the estimates of all three tissue types acquired through CT techniques to be accurate estimates of the cadaver sections (74). Because of the association of skeletal muscle fat infiltration with the development of several diseases including NIDDM and DMD, Goodpaster and colleagues conducted an extensive investigation to validate the ability of CT attenuation values to accurately reflect lipid content and the ability of CT to reliably detect biological variability in muscle attenuation with in a given muscle and between muscle groups (33). A test-retest study was conducted and CT was found to demonstrate a muscle attenuation variance of less than 1%. Using lipid emulsion phantoms, CT was shown to assign attenuation values that reflect sensitivity to small changes in lipid content similar to differences in lipid content seen when comparing the skeletal muscle of lean subjects to that of obese subjects. In conjunction with muscle biopsies, CT attenuation values where confirmed to be significantly associated with actual skeletal muscle content thus prompting the investigators to declare CT to be a valid non-invasive means of exploring the relationship between the relative lipid content within skeletal muscle and research interventions.

Advantages and Disadvantages of CT

As evidenced above, the main advantage of CT technology is that images obtained through CT can provide an accurate, reliable and detailed *in vivo* assessment of thigh tissue CSA. CT technology also has the advantage of speed, i.e. scans for the entire length of the human thigh can be obtained in less than 2 minutes, a process that

would take possibly 45 minutes using MRI technology. And while, like MRI, CT is very expensive both per unit and per image, the speed at which images can be produced requires less monopolization of the machine, allowing researchers more access to CT units located in clinical settings. The fact that CT images are obtained by employing ionizing radiation is the most obvious disadvantage of this technology. The amount of radiation exposure from a single CT scan of the mid-thigh region is about 0.00032 Rems depending on the CT machine (72). This exposure is considered quite low and comparable to everyday risk (6). For comparison, radiation workers are permitted, by federal regulation, a maximum radiation exposure of 5 Rems per year to any single body organ, and naturally occurring radiation (cosmic radiation, radon, etc.) produces whole body radiation exposure of approximately 3.0 Rems per year (1; 2). While there is no minimum amount of radiation exposure that is recognized as being totally free of risk from causing genetic mutations or cancer, the major risk from high radiation exposure is passing on damaged genes to offspring. While this risk is typically of less concern to those beyond childbearing years, it limits the use of CT technology in research protocols involving children and reproductively viable women.

E. Confounding Factors in Thigh CSA Measurement

When using CT technology to evaluate changes in thigh CSA, the assumption has been made that any changes detected in tissue area can be attributed to the natural process or applied intervention under investigation. In order for this assumption to be valid, attempts must be made to control all extraneous factors that may confound the accuracy of the measurement. Because CT technology generates images based on

tissue density, and the protein, fat and fluid content of muscle tissue significantly contribute to the average density of each pixel in the CT image, research conditions must control for any extraneous variables that may exert an effect on these elements.

Factors Affecting Protein and Fat Content

As reported above, resistance exercise training has been shown to increase skeletal muscle density by increasing the CSA of lean tissue (27). Weight loss, as well as resistance exercise training, have been shown to decrease the CSA of SF and LDM (36; 38; 86) and natural physiological processes resulting from disease progression, sedentary lifestyle and aging have been shown to generate decreases in muscle density through increases in the CSA of muscle infiltrated with fat (62). These changes in the fat and protein content of muscle take place over an extended period of time (greater than 2 hours, see Definitions p.5 Chapter 1), and can be controlled for by assembling subject groups homogeneous for age and training state as well as creating research protocols designed to screen for a medical history of muscle related diseases and monitor dietary and physical activity habits.

Factors Affecting Fluid Content

Because of the low water content, approximately 14 percent, subcutaneous fat CSA would be expected to be little affected by changes in fluid content (92). However, approximately 75 percent of human skeletal muscle is composed of water (12) and conditions that increase or reduce skeletal muscle water content would be more likely to result in changes in the measurement of skeletal muscle CSA. The intracellular fluid of the muscle cell maintains an isotonic relationship with the fluid

in the interstitial spaces. Water moving out of the interstitial space will result in a compensatory movement of water from the intracellular fluid to the interstitial fluid, re-establishing the isotonic state. When this isotonic relationship is disrupted, the cells will temporally swell or shrink in size depending on the direction of water movement. The composition of the interstitial fluid is dependent on the pressure and solute gradient of the plasma circulating through the capillaries, hence any mechanism that alters the pressure of the capillaries or composition of the plasma, potentially requires adjustments in both the interstitial and intracellular fluids. Several conditions, for example exercise, glycogen content, and changes in hydrostatic pressure, can alter muscle water content and therefore must be taken in to account when assessing CSA of skeletal muscle.

The Effects of Exercise and Muscle Glycogen Content

At the start of exercise, blood flow to the working muscle significantly increases in response to the increased metabolic demand and a rise in temperature (78). This change in blood flow resulting in an increase in muscle fluid content is referred to as exercise induced hyperemia. Hyperemia produces a swelling of the muscle cells that has been shown to persist, and even increase, during the post-exercise recovery phase and results in increases in muscle CSA (24; 73; 78).

Glucose, the major fuel for energy production in humans, is stored in the form of glycogen, with skeletal muscle being the body's major site for glycogen storage. Glycogen stored in the skeletal muscle tissue is bound with water, with one gram of glycogen binding approximately three grams of water (12). It has been observed that increases or decreases in the glycogen content of muscle will produce the attendant

variation in muscle water content resulting in muscle density alterations (79). Just as a high fluid content can cause muscle tissue to swell, increasing CSA, muscle glycogen depletion, due to endurance exercise or low carbohydrate dieting, may reduce muscle's water content, resulting in decreased muscle CSA.

To reduce the probability that measured changes in the muscle CSA are due to exercise-induced or muscle glycogen related changes in muscle water, subjects should be instructed to refrain from engaging in exercise for several hours before measurements are taken and should be requested to keep a 24 hour food record before the first measurement and replicate that diet prior to any subsequent measurements (13).

F. The Effects Hydrostatic Pressure

Because we are composed of mostly water, in the standing position the human body is like a column of water with the surface of the water at the level of the heart and the base of the column at the level of the feet. Hydrostatic pressure (HP) is the pressure the weight of a fluid exerts on the layers of fluid below it and has been elegantly expressed by Pascal's law:

$$\mathbf{P = \rho\, h\, g}$$

where **P** is the pressure, **ρ** is the density of the fluid, **h** is the height of the fluid column and **g** is the acceleration due to gravity (21). The difference in HP for any point along the column can be calculated by measuring the height difference between that point and the surface of the column, in this case, the level of the heart. With the **ρ** and **g** remaining constant, it can be seen from the equation that the greatest pressure would exist at the base of the column, in this case the feet, as the difference in **h**

would be maximal. It can also be seen from Pascal's law that a negative HP will exist at points above the heart, for example the neck and head. When the human body assumes the supine position, the column of fluid is reduced to the depth of the body with very little difference in HP between body regions, as the vast majority of the "column" is at the level of the heart.

Hydrostatic pressure is accounted for in the circulatory system's efforts to maintain consistent cardiovascular pressure and changes in HP are eventually accommodated through changes in heart rate and peripheral resistance (53). As mentioned above, the exchange of fluid between the capillaries and the interstitial space is influenced, in part, by capillary pressure, which is adjusted to account for HP (7). A reduction in HP due to a posture change, for example going from standing to supine, would bring about a reduction in capillary pressure, causing fluid to move from the interstitial space to the plasma (71). In order to re-establish an isotonic relationship with the interstitial fluid, this reduction in HP may also trigger the movement of intracellular water to the interstitial space, causing the muscle cells to shrink.

The effects a change in HP can potentially exert on the measurement of thigh tissue CSA is delimited by the time interval between pressure changes, the systemic return of homeostasis and the time of measurement. The efflux of fluid from the interstitial space to circulating plasma has been shown to stabilized after about 25 minutes (41; 94) and, while some evidence exists that, with supine rest, decreases in leg volume due to fluid shifts persist for up to 40 minutes (94), some investigators

have found that 90 percent or more of the changes in leg volume occur within the first 30 seconds of postural change (93).

Computed tomography images are obtained with the subject in the supine position (see Figure 1). As the subject changes from the standing to the supine position, the loss of HP may elicit a significant change in skeletal muscle cell size, thus, potentially confounding the measurement of muscle CSA. While CT technology allows for the expeditious procurement of images, the question remains as to whether the significant biophysical changes resulting from a loss of HP occur rapidly enough to confound measurement of thigh tissue CSA.

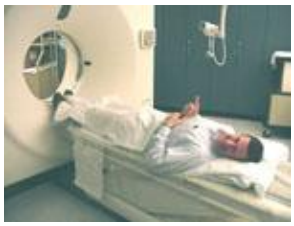

Figure. 1 CT machine with subject prepared for measurement.

The Acute Effects of Supine Posture on Lower Limb CSA

Interest in the effects of weightlessness on astronauts has generated a large body of research focused on the long-term influence of HP loss on skeletal muscle CSA. However, to date very few studies have examined the short term, or acute effects of HP loss, due to postural change, on the skeletal muscle of the leg, fewer still have focused on thigh CSA, and only one paper in the published literature has explored these effects using CT technology.

In 1931, Waterfield investigated the suggestion that loss of plasma from the circulation when standing is due to the "leakage of plasma in to the tissues of the lower extremities" (94). Waterfield hypothesized that if this were true, than it would be evidenced by an increase in leg volume upon standing and a decrease in leg volume upon assuming the supine position. Whole leg volume was measured using a water displacement method with the subject placing the leg in a tank of water and the

measure of leg volume change was the change in the water level, from baseline, after a period of standing or lying down. The study reported a shrinkage in leg volume of approximately 60 to 80cc during the first 40 minutes in the supine position that continued, although more slowly, over a longer unspecified period of time (94). While the physics of HP would predict that the supine position would exert a greater fluid loss on calf muscle than thigh muscle, ergo a greater contribution of calf volume change to whole leg volume change than would the thigh, the method of leg volume measurement used in this experiment excludes the possibility of identifying the contribution to the change made by different regions of the leg. Furthermore, measuring whole leg volume with the water displacement technique can not provide direct measures of the effects of fluid loss on specific tissues types such as NDM and LDM. While the findings of this study do suggest an acute fluid shift away from the leg when assuming the supine position, the investigator used an *n* of 1, himself (94), and reports only the absolute volume changes. These limitations make it difficult to assess the degree of error postural change may convey to thigh muscle measurement and restricts the possibility of extrapolating the findings of this research to a larger population.

When investigating the short term changes in transcapillary pressures and calf muscle function in humans subjected to 8 hours of 5° head-down tilt, Hargens et al, measured calf circumference using a tape measure (44). The investigation detected no significant change in calf circumference when comparing measures taken standing, in the horizontal position, and after 30 minutes in the head-down tilt position, with mean ± SD circumferences of 36.9 ± 0.5, 36.7 ± 0.5, and 36.7 ± 0.6cm

respectively (44). A significant decrease in calf circumference was recorded only after the subjects had been in the head-down tilt position for 4 hours. Information on the amount of time the subjects may have been in the standing or horizontal position before measures were taken was not provided, the number of subjects was low ($n = 6$) and are not homogeneous with respect to age (22 – 52 yrs.) (44). Furthermore, the measurement technique was unable to provide information on specific tissue compartments and the study offers no insight into how thigh tissue might be affected by postural change. However, the results suggested that any significant effect changing from the standing to the supine position exerts on measurement of lower limb tissue area may not occur until after at least 30 minutes.

A study conducted by Thornton and colleagues, examining leg volume changes during three commonly used microgravity stimulation techniques, reported a decrease of approximately 1% in thigh volume after 30 minutes in the supine position (90). Horizontal baseline measures were made within five minutes of adopting the supine position and thigh volume was calculated using girth measurements and a truncated cone formula. The researchers used a "stocking jig" (90), an elastic fabric stocking covering the length of the leg, with non-extensible tapes attached longitudinally at eight points, with the upper four used to calculate thigh volume. Ideally, this should eliminate some of the human error involved in girth measurements. The researchers also took steps to reduce other confounding factors by using subjects homogeneous for sex, age and fitness level and by controlling exercise and diet for 24 hours before testing (90). While the results indicate an acute decrease in thigh volume within 30 minutes of supine posture, the measurement technique used

can provide no information as to how different tissue compartments of the thigh are being affected by the decrease or how this decrease might introduce error in the measurement of thigh CSA.

Watenpaugh and associates used a motorized tilt table and a digitized strain gauge to monitor the effects of posture change on calf volume and neck volume as indicators of acute fluid distribution (93). The strain gauge continuously recorded changes in volume as the table moved from 90° to 54° to 30° to 12° to 0°, the supine position. The table remained at each tilt for 30 seconds with a 10 second interval as the table moved to the next angle. While calf volume decreased approximately 1.7% from 90° to 0°, changes were found to take place almost instantaneously, leveling off by the end of each 30 second interval (93). The results of this study shed no light on acute posture change and fluid shifts as they affect the measurement of thigh CSA in general or the CSA of specific tissue compartments. However, based on these data an argument could be made that any fluid shift resulting from postural change would be negligible after 5 minutes supine. When the time intervals are summed, the decrease in calf volume, from 90º to 0º, took place in a little over two and a half minutes. When a CT protocol requires that a subject move immediately from the upright to the supine position, these data suggest that a change in calf volume of approximately 1.7%, could be expected to confound the measurement of calf CSA during the first three minutes of horizontal posture, with the significant fluid shift complete with in five minutes. If CT images were obtained after 5 minutes in the supine position, tissue fluid content could be considered stable, thus reducing measurement error.

In 1996, Conley and colleagues conducted an investigation to examine the extent and time course of changes in lower limb skeletal muscle CSA resulting from postural shifts. MRI technology and upright, horizontal, as well as 6° head-down tilt postures were used in the evaluation of the effects of fluid redistribution on calf muscle CSA (15). Upright measures made from images obtained in the evening served as the baseline to be compared with measures made from images obtained after 12 hours of horizontal rest, and at 30 minutes, 2 hours and 6 hours after returning to standing. The authors reported a decrease in calf CSA of 8 ± 2% after 12 hours in the horizontal position and that calf CSA returned to baseline levels with in 30 minutes of resuming the upright posture (15). While no data are reported in the study regarding the acute effects on thigh CSA nor does it examine the effects on measurement of lower limb CSA of changing from the upright to the horizontal position, the rapid return to baseline measures upon resuming the upright posture after 12 hours supine, does suggest that muscle fluid redistribution brought on by postural change, stabilizes in approximately 30 minutes. It should be noted that, while not specified in the Conley paper, all MRI scans used in the study, even those termed "upright" would have been obtained in the supine position as upright MRI technology was not introduced until 1996 (http://www.fonar.com/history.htm). The Conley study does report that a time interval of four minutes and forty seconds was required to obtain the images. Based on the increase seen after 30 minutes, the findings of the Conley study are in disagreement with the findings of Watenpaugh, that fluid redistribution with posture change is "almost instantaneous" with fluid shifts leveling off with in 30 seconds of the posture change (93). It is also of interest to note that the

investigators found the changes in calf muscle CSA not to be correlated with changes in proton transverse relaxation time (T_2), contradicting the expectation that water moving into the muscle, hence increasing the hydrogen atoms, would result in increased T_2 (15).

The only published study, to this author's knowledge, using CT technology to explore the acute effects of fluid redistribution resulting from postural shifts on the measurement of thigh tissue CSA, was conducted by Berg et al., in 1993 (9). Single-slice axial thigh scans were obtained from 7 male subjects similar in age and physical activity levels. The subjects were restricted from vigorous activity and alcohol intake 24 hours prior to afternoon testing when CT scans were taken. The site of the scans was 220 mm below the proximal aspect of the head of the femur, at 1, 20, 60 and 120 minutes supine. The CSA of individual thigh tissue compartments were calculated from measurements of bone, muscle and bone and total thigh area. The results showed a significant decrease in both thigh subcutaneous fat and thigh skeletal muscle of 4.1 ± 3.7 and 1.9 ± 1.1%, respectively, during 2 hours of supine rest, the majority of the decrease taking place between minutes 1 and 20, with virtually no change in thigh muscle or subcutaneous fat between minutes 20, 60, and 120 (9). The study also looked at changes in calf tissue, finding significant decreases the CSA of subcutaneous fat and muscle of 4.4 ± 3.7 and 5.5 ± 2.7%, respectively, with these changes taking place continuously through out the 2 hours (9). The investigators used bioelectrical impedance analysis data to confirm that the decreases in thigh tissue CSA corresponded with decreases in tissue fluid content (9). The study also examined the affects of fluid loss on the radiological density of thigh skeletal muscle tissue and

found that, despite significant decreases in muscle fluid content and CSA, there was no significant difference seen in thigh muscle radiological density after 2 hours supine as determined by CT. This finding suggested that acute loss of fluid content may not impede the ability of CT to accurately distinguish between thigh NDM and LDM and that decreases in thigh muscle CSA due to fluid loss are the result of muscle shrinkage. The data gathered in the study lead Berg and colleagues to recommend that a protocol of 1 hour supine before obtaining CT scans of the lower limbs, may reduce the chance of postural induced fluid loss confounding the measurement of tissue CSA.

A pilot study conducted in our lab found no significant difference in thigh muscle volume when single slice scans were taken at 5, 10 and 15 minutes in the supine position (unpublished data). Thigh skeletal muscle was identified and analyzed as NDM and LDM, and total muscle, with total muscle calculated by summing NDM and LDM. The pilot study was not with out limitations, the subject number was low (n = 4), there was no data gathered on the subcutaneous fat compartment and an image analysis protocol was used that required muscle tissue be distinguished from other thigh tissue using a manual technique greatly dependent on the skill of the technician outlining the tissue, possibly introducing error into the measurement. Even so, the results of our pilot study appear to contradict the findings of Berg and Conley (9; 15).

H. Summary

Research has demonstrated the value of information gathered from the assessment of thigh skeletal muscle tissue CSA to both exercise and medical sciences. CT technology has been used extensively as an accurate, valid and convenient tool for acquiring data on thigh tissue CSA with the capacity to distinguish healthy normal density skeletal muscle tissue from the more pathological low density skeletal muscle tissue. The ability to rapidly acquire images is an important practical advantage CT technology has over other *in vivo* imaging techniques. As with all instruments of measurement, extraneous factors can introduce error in to the data and therefore the conditions under which CT images are obtained must control for these variables. Because of skeletal muscle is composed mostly of water, variations in muscle water content when images are acquired may be a source of measurement error. While many factors that could cause an alteration in muscle fluid content require chronic exposure to the condition, changes in HP could impose an immediate influence on muscle fluid content. The loss of HP that occurs as a body moves form the standing to supine position causes a fluid redistribution that may confound the measurement of thigh CSA if data is obtained while tissue fluid content is in flux. The published literature investigating the acute effects of fluid shifts induced by postural change on measurement of thigh CSA is limited, with only one study using CT technology, and contradictory, with research reporting time intervals from as little as 30 seconds to as long as 1 hour need for fluid stabilization after a change in posture.

CHAPTER 3: METHODS

A. Subjects

Thirteen female subjects, ranging in age from 51 to 81 years, were recruited from the general local population through mass mailing, group emailing services and word of mouth. Subjects were in the sedentary state, having self-reported not participating in regular vigorous exercise for at least 6 months. After all methods, procedures and risks were explained, subjects read and signed a written consent form, which was approved by the Institutional Review Board of the University of Maryland, College Park.

B. Imaging Procedures

Subjects arrived at the CT scanning site, Washington Adventist Hospital, having abstained from exercise for at least 6 hours and consumption of caffeine at least 3 hours prior to scan. Each subject was instructed to remove any metal objects from clothing found between the hips and knees and, fully clothed, was positioned on the mobile CT table. The subject entered the scanner feet first and a scout image was obtained to establish orientation of the skeletal landmarks. CT scans were obtained using a model GE QX/I LightSpeed CT scanner. The images were obtained at 120 kVp with the scanning time set at 1s at 40 mA. A 48-cm field of view and a 512x512 matrix was used to obtain a pixel resolution of 0.94 mm.

In order to assess time related changes in CSA of SF, NDM and LDM due to posture change, scans were performed at three time points during 15 minutes of supine rest, at minutes 5(CT5), 10(CT10), and 15(CT15), with subject remaining

continuously supine. Each scan was taken at the same anatomical location, 250mm below the most distal aspect of the ischial tuberosities with a slice thickness of 10mm. In order to ensure accuracy of repeated slice location, the imaging site at 5 minutes was stored in the scanner's computer memory and automatically repeated at each pre-determined time interval. All procedures involved in obtaining the CT images, from positioning the subjects to operation of the CT equipment, was performed by the radiology staff of the Washington Adventist Hospital under instruction and supervision of the research investigators.

C. Image Analysis

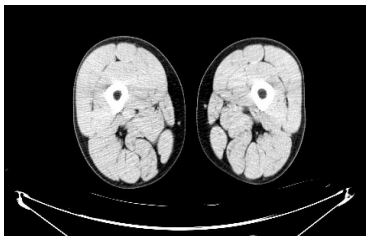

Figure 2. A two-dimensional CT image of mid-thigh axial slice. Note that as tissue density increases, image lightens.

The image produced through CT is composed of a two-dimensional matrix of pixels. Hounsfield units are assigned to a pixel and correspond to the linear attenuation coefficient of the tissue represented by the pixel, as well as a specific level of gray with in the image. Attenuation characteristics of a tissue depend on tissue density (83). Water is the reference for the linear attenuation coefficient scale and is assigned a value of 0HU. As can be seen in Figure 2, tissues less dense than water, such as fat, are assigned a negative HU value and appear as darker gray in the CT image, while tissues more dense than water, such as muscle, are assigned a positive HU value and appear in the CT image as lighter gray (83). Adipose tissue has been shown to display attenuation values of –190 to –30 HU, while skeletal muscle has been shown to display attenuation values of 0 to 100 HU (32; 37). Research has suggested that lower skeletal muscle attenuation values reflect lower density muscle tissue resulting from an increase in skeletal

muscle lipid content (30; 32; 54; 59; 68; 77) and can therefore be useful in assessing disease risk (36; 40; 54). In accordance with previous research (30; 36), this study used the following range of linear attenuation coefficients to distinguish tissue types: SF is set at –190 to –30 HU, LDM set at 0 to 30 HU, and NDM set at 31 to 100 HU.

The CT scans were blinded for time and identity and a single technician performed all image analysis using MIPAV software (NIH, Bethesda). The software uses an automatic highlighting technique to define areas of interest. This feature of the software was used to establish the outermost edge of the thigh subcutaneous region for both right and left thighs. By programming the range of Hounsfield units for each of the three tissues types, the same software was used to measure the axial image for total tissue area (cm^2) with respect to LDM, NDM, and SF with both thighs assessed together as a single unit (see Figure 3).

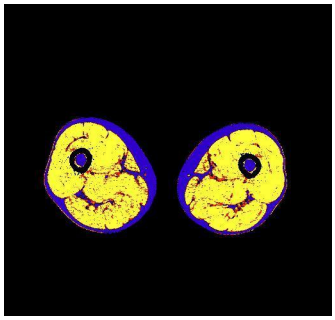

Figure 3. Mid-thigh CT image using Mipave plug-in. SF is represented in blue, HDM is highlighted in yellow and LDM is highlighted in red.

A pilot study was conducted to verify the reliability of this technique. Mid-thigh CT scans from 25 subjects were blinded and, using Mipav software, analyzed on three separate occasions for CSA of SF, NDM and LDM. The coefficient of variance from this data showed the technique to be highly reproducible (cv = 0.005).

D. Statistical Analysis

All statistical analysis was performed using SAS software. The length of time, in minutes, 5, 10 or 15 minutes, the subject was supine when the CT scan was

taken was used as the independent variable. The dependent variables were the cross sectional area, in centimeters squared (cm^2) of SF, LDM and NDM of the mid thigh image.

The data for each tissue type are presented as mean $\pm$ SE and were analyzed using a one-way (Time x CSA of tissue type) analysis of variance (ANOVA) with repeated measures over time. All tissue data sets met the ANOVA assumption of normality with the exception of SF which required a log transformation. Means and differences are reported with out the log correction.

Multiple means comparisons were run with a Tukey adjustment to identify the time interval, 5 and 10 minutes, 5 and 15 minutes and 10 and 15 minutes, during which any significant difference in tissue CSA occurred. The level of significance was established at $P < 0.05$.

CHAPTER 4: RESULTS AND DISCUSSION

Results

The physical characteristics of the subjects are shown in Table 1. The subjects had a mean age of 65.4 ± 9.9 years, a mean height of 161.3 ± 5.7 cm and a mean body weight of 72.8 ± 16.4 kg. The mean BMI for the subjects was 27 ± 6.1 kg/m², with a mean percent body fat of 39.2 ± 5.9.

Table 1. Physical Characteristics of the Subjects

n	Age, yrs	Height, cm	Weight, kg	BMI kg/m²	% Body Fat
13	65.4 ± 9.9	161.3 ± 5.7	72.8 ± 16.4	27.0 ± 6.1	39.2 ± 5.9

Values are means ± SD

The mean CSAs at each time interval for each tissue type are listed in Table 2. Time supine was found to exert a significant effect on the CSA of NDM ($P < 0.05$) with no significant effect found on the CSA of LDM or SF (Table 2).

Table 2. Mean CSA of Tissue Type at Each Time Point

Tissue Type	CSA (cm²) of Thigh Tissue Type		
	CT5	CT10	CT15
SF	215.7± 20.5	216.3 ± 20.5	216.6 ± 20.5
NDM*	143.3 ± 5.8	142.0 ± 5.8	141.0 ± 5.8
LDM	38.1 ± 4.1	38.8 ± 4.1	39.0 ± 4.1

Data is mean ± SE cm²; SF = subcutaneous fat; NDM = normal density muscle; LDM = low density muscle; CT5 = scan taken at 5 min, CT10 = scans taken at 10 min., CT15 = scans taken at 15 min. supine; * denotes significant change occurred in tissue type CSA, $P < 0.05$.

The results of multiple means comparisons, run to identify any specific time interval that conveyed a significant change in CSA, revealed that a significant decrease in the CSA of NDM of 1.6 %, 2.3 ± 0.8cm² (Table 3), occurred between CT5 and CT15 ($P < 0.05$). There was no significant difference found in NDM CSA between CT5 and CT10, or between CT10 and CT15.

All tissue data sets met the ANOVA assumption of normality with the exception of SF which required a log transformation. Means and differences are reported with out log correction.

Table 3. Absolute Difference and Percent Change in CSA with Time

Tissue Type	Differences in CSA					
	CT5 – CT10		CT5 – CT15		CT10 – CT15	
	Diff.	%Change	Diff.	% Change	Diff.	%Change
SF	0.6 ± 0.5	0.3	0.9 ± 0.5	0.4	0.3 ± 0.5	0.1
NDM	1.2 ± 0.8	-1.0	2.3 ± 0.8	-1.6*	1.0 ± 0.8	-0.7
LDM	0.7 ± 0.4	1.9	0.8 ± 0.4	2.0	0.1 ± 0.4	0.1

* denotes a significant difference at the $P < 0.05$ level; Diff = difference between the means in cm². Differences are mean ± SE of the difference in cm².

Discussion

The loss of hydrostatic pressure (HP) that occurs as a person moves from the standing to the supine position causes a fluid redistribution that may confound the measurement of thigh CSA if data is obtained while tissue fluid content is in flux. The published literature investigating the acute effects of fluid shifts induced by postural change on measurement of thigh CSA is limited and contradictory, with research reporting time intervals from as little as 30 seconds to as long as 1 hour needed for

fluid stabilization after a change in posture (9; 15; 44; 90; 93; 94). The only published study to this author's knowledge using CT technology to examine the acute effects of fluid redistribution resulting from postural shifts on the measurement of thigh tissue CSA, was conducted by Berg et al., in 1993 (9). Berg et al. analyzed CT axial thigh scans of the left leg of 7 young, physically active men obtained at 1, 20, 60 and 120 minutes of supine rest. Individual thigh tissue compartments were determined using a manually manipulated tracking ball to define the tissue areas and the CSA of subcutaneous fat and skeletal muscle was then calculated from measurements of bone, muscle and bone and total thigh area. A significant decrease in total muscle CSA as well as the CSA of subcutaneous fat was observed at 60 minutes in the supine position with the majority of the decrease in thigh tissue taking place in the first 20 minutes (9). These findings lead Berg et al. to recommend that subjects remain supine for 60 minutes before obtaining scans when employing CT techniques to assess peripheral tissue CSA (9; 15). Other researchers have implemented a pre-CT scan protocol of from 10 to 60 minutes of supine rest based on these data (25; 45; 58; 91). Since 10 to 60 minutes of supine rest prior to each CT scanning session can substantially tax limited resources when research conditions require volunteer human subjects, multiple scanning sessions and off-site scanning facilities, it is of practical interest to determine if shorter rest periods have an effect on mid-thigh subcutaneous fat and skeletal muscle CSA as measured by CT.

The present study was conducted in an effort to establish a more definitive understanding of the effect that changing from the standing to the supine position may have on CT-derived measurements of thigh tissue CSA during the first 15

minutes supine. In addition, it was the intent to investigate the effect such postural shifts convey to skeletal muscle CSA when the muscular area of interest is evaluated in terms of normal density (NDM) and low density (LDM) skeletal muscle. Using CT technology, mid-thigh axial images were obtained at 5, 10 and 15 minutes in the supine position and analyzed for changes in SF, NDM, and LDM cross sectional area. Based on unpublished pilot study data from our research group showing no significant change in thigh tissue volume between 5 and 15 minutes supine, it was hypothesized that no significant change in the CSA of any tissue type examined would be found.

The current study resulted in three major findings. First, a significant decrease in NDM cross-sectional area between 5 and 15 minutes supine was observed. Secondly, no significant difference in the CSA of SF was found for any time interval and finally, the measurement of NDM and LDM may be affected differently by loss of HP.

Contrary to expectation, a significant mean decrease of 2.3 ± 0.8 cm² or 1.6% was observed in NDM cross sectional area when CT scans taken at 5 minutes were compared with scans taken at 15 minutes. No significant difference was seen in the CSA of NDM between scans taken at 5 minutes and scans taken at 10 minutes or between scans taken at 10 minutes and 15 minutes. Hydrostatic pressure is known to be an important factor in the circulatory system's efforts to maintain consistent cardiovascular pressure and changes in HP are eventually accounted for through changes in peripheral resistance (53). A postural change, for example going from standing to supine, could result in a reduction in HP. A decrease in HP could bring

about a reduction in capillary pressure, causing intracellular water to move out of the muscle cells in to the interstitial space and finally into the capillaries and the circulating plasma (7; 71). Because skeletal muscle tissue is approximately 75% water (12), this loss of intracellular water can cause muscle cells to shrink, potentially decreasing muscle CSA. The reduction in NDM seen in the present study is similar to the findings of Berg and colleagues, where a decrease in total mid-thigh muscle CSA of ~2% after subjects remained supine for 120 minutes was observed (9). While statistically significant, the practical significance of a decrease in muscle CSA the magnitude of that seen in the present study may be open to question, however, CT is often used to measure changes in thigh muscle CSA in response to resistance training and these changes can vary widely depending on the type of training program and the age and sex of the subjects. Increases ranging from 1.5 to 11% have been reported in the literature, with an average reported increase of ~7% (23; 25; 27; 76; 86; 91). For example, Sipila et al. reported an increase of 1.5% when elderly women completed 16 weeks of resistance training and Frontera et al. reported an 11% increase in thigh muscle CSA after older men underwent 12 weeks of resistance training (27; 86). If an increase in muscle CSA of 1.5% is found to be statistically significant, it is then all the more important to ensure that a consistent protocol was followed regarding time spent in the supine position prior to the recording of the CT images. Without a standardized procedure regarding pre-scanning time supine, it is feasible for testing conditions to allow baseline scans to be taken after a subject has been supine for 15 minutes and a post-intervention follow-up scan to be taken within the first 5 minutes potentially resulting in a statistically significant difference in thigh muscle CSA

possibly due strictly to the difference in muscle fluid content at the moment of scanning and not as a result of any applied intervention

While in the current study SF cross-sectional area increased by ~0.5% over 15 minutes supine, this increase was not found to be statistically significant. This finding is in sharp contrast to the findings of the Berg study where a ~4% decrease in thigh SF CSA was seen after 20 minutes in the supine position (9). It is difficult to explain this apparent contradiction. One possibility is the difference in the techniques used to define tissue areas; the Berg study used a manual technique to define boarders between areas of interest and while the present study used an automated technique, where the measurement of the CSA of the three tissues examined in the current study was based on a preprogrammed range of HU. An automated computerized technique potentially reduces subjectivity while a manual technique can be more open to experimenter error. Another possibility is to speculate that, due to the low water content of subcutaneous adipose tissue (92), fluid efflux is relatively slow-moving, not resulting in significant decreases in SF CSA until some time interval greater than 15 minutes.

The current investigation is the first to examine the acute effects of postural shifts on the CSA of both normal and low density thigh skeletal muscle. Low density skeletal muscle is characterized by the infiltration of fat, resulting in a reduced CT attenuation value, relative to skeletal muscle of normal density. A reduction in thigh muscle density is associated with the aging process and obesity (11) and has been found to be a marker for increased risk of developing IR, NIDDM and the pseudohypertrophy of thigh skeletal muscle tissue associated with muscle wasting

diseases (35; 40; 57; 84). After 15 minutes in the supine position there was no significant change in the CSA of LDM. This is in contrast to a significant decrease of 1.6% seen in the CSA of NDM at 15 minutes. The mechanism responsible for this apparent difference in fluid fluctuation in response to postural change between NDM and LDM is not known. It may be of interest for future studies to investigate the water content of skeletal muscle infiltrated with fat relative to skeletal muscle of normal density.

The current study found no change in the CSA of SF or LDM and a significant decrease in NDM CSA at 15 minutes in the supine position. As no significant change in NDM CSA was found between 5 and 10 minutes of supine rest, the results of this study suggest that any fluid efflux from NDM potentially resulting from a decrease in HP, may not introduce significant error into the measurement of mid-thigh NDM CSA until after at least 10 minutes in the supine position and may have no significant effect on the CT-derived measurement of mid-thigh SF or LDM up to the first 15 minutes. As a practical matter, the ability to rapidly acquire images is an important advantage CT technology has over other *in vivo* imaging techniques as multiple axial scans for the entire length of the human thigh can be obtained in less than 5 minutes. In light of the findings of the present study, requiring subjects to lie supine for 10 to 60 minutes prior to CT scanning may actually introduce error into to measurement of the CSA of mid-thigh tissues and may impose an unnecessary time burden on to what is otherwise an accurate, reliable and expeditious procedure. The results of the current study suggest that the potential measurement error associated with fluid shifting out of the tissues can be minimized when baseline and follow-up

CT-derived images of mid-thigh tissue CSA are obtained with in the first 10 minutes the subject assumes the supine position and that the CSA of NDM and LDM may be affected differently by loss of HP.

CHAPTER 5: SUMMARY, FINDINGS, CONCLUSIONS AND RECOMMENDATIONS

A. Summary

This study was designed to expand the research exploring what effect changing from the upright to the supine posture may exert on the computed tomography-derived measurement of mid-thigh SF, NDM and LDM. Based on pilot data, it was hypothesized that the CSA of mid-thigh SF, NDM and LDM, as measured by CT, would show no significant change at CT5, CT10, and CT15 minutes in the supine position.

The 13 sedentary post-menopausal women between the ages of 51 and 81 years who participated in the study, had mid-thigh axial images obtained using a model GE QX/I LightSpeed CT scanner. The CSA of each tissue type was measured using MIPAVE software (NIH, Bethesda) with the range of Hounsfield units (HU) for each tissue type set at between –190 to –30 HU for SF, between 0 to 30 HU for LDM, and between 31 to 100 HU for NDM. While there was no significant difference found in the CSA of SF or LDM between CT5, CT10 or CT15, time supine was found to exert a significant effect on the CSA of NDM ($P = 0.03$). The results of the multiple means comparisons revealed that a significant decrease in the CSA of NDM of $2.3 \pm 0.6 cm^2$ (1.6%), occurred between CT5 and CT15 with no significant difference found in NDM CSA between CT5 and CT10, or between CT10 and CT15.

B. Findings

1. The CSA of mid-thigh SF, LDM and NDM did not significantly change between 5 and 10 minutes in the supine position.
2. The CSA of mid-thigh SF, LDM and NDM did not significantly change between 10 and 15 minutes in the supine position.
3. The CSA of mid-thigh SF and LDM showed no significant change between 5 and 15 minutes in the supine position.
4. The CSA of mid-thigh NDM decreased significantly between 5 and 15 minutes in the supine position.

C. Conclusions

It can be concluded that 10 min. in the supine position does not result in a significant change in the mid-thigh CSA of SF, LDM or NDM as measured by CT. In agreement with previous research, the current study found that remaining supine longer than 10 minutes can cause a significant reduction in the CSA of mid-thigh NDM. The outcome of this study also demonstrated that skeletal muscle of the mid-thigh that has a reduced density due to the infiltration of fat, is affected differently by postural change than skeletal muscle of normal density. This difference may possibly be due to a difference in tissue fluid content. Further investigations into the effects of postural shifts on the CSA of mid-thigh tissue need to be done to confirm the findings of the current study.

D. Recommendations

1. When utilizing longitudinally gather data on the CSA of mid-thigh tissues, the potential for fluid shifting out of skeletal muscle to confound the measurements can be minimized if computed tomography images are consistently obtained within the first 10 minutes the subject assumes the supine position.
2. Further investigations need to be done on a variety of subject groups including younger women, men both younger and older, as well as physically active or exercise trained subjects.
3. The results of the current study should be confirmed using MRI techniques.
4. The fluid content of LDM in comparison to NDM needs further exploration.

APPENDIX I: IRB APPROVAL MEMO

UNIVERSITY OF MARYLAND
INSTITUTIONAL REVIEW BOARD

2100 Lee Building
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MEMORANDUM

Approval of Request to Add Investigator

TO: Dr. Bernard Hurley
Department of Kinesiology

FROM: Drs. Marc Rogers and J. Dennis O'Connor
Co-Chairpersons, Institutional Review Board

DATE: December 2, 2004

IRB PROTOCOL IDENTIFICATION NUMBER AND PROJECT TITLE:
00830; "Effects of Gene Variations on Age- and Strength Training-Induced Changes in Muscular Strength, Body Composition, Glucose Metabolism, Lipoprotein-lipid Profiles"

Please note: IRB approval of this project expires at the end of *December 2005.*

This is to notify you that we have updated our records to indicate that Ms. Linda Cerniglia has been added to the research team of the project indicated above, that her data analysis activities have been added to the approved protocol, and that the analysis she performs will form a part of her Master's thesis. Thank you.

APPENDIX II: RAW DATA

Subject Characteristics

Age	Height cm	Weight kg	BMI	% Body Fat
51	165.1	82.6	30	34
65	158.5	62.8	24	40
64	156.2	84.4	34	44
66	165.1	84.8	31	43
65	170.2	60.3	20	42
81	151.4	55.3	24	32
78	163.5	75.4	28	44
57	161.2	61.69	23	40
54	165.4	110.53	40	48
54	168.5	55.54	19	29
75	159.9	63.19	24	37
77	153.2	62	26	32
63	158.9	87.89	34	45

Subcutaneous Fat CSA (cm^2)

CT5	CT10	CT15
3900.41	3945.41	3938.03
2425.96	2453.56	2473.51
1621.23	1620.53	1605.59
2709.84	2717.67	2719.25
1336.64	1341.21	1333.92
2288.32	2287.44	2279.62
1601.45	1599.52	1589.68
1603.65	1585.28	1617.54
1667.73	1658.94	1645.49
2722.94	2729.18	2736.04
2416.55	2421.65	2426.21
1311.94	1335.23	1346.48
2432.55	2421.29	2443.01

Normal Density Skeletal Muscle CSA (cm²)

CT5	CT10	CT15
1518.39	1510.84	1460.48
1623.69	1652.69	1613.15
1695.41	1641.36	1687.41
1500.29	1431.12	1433.85
1571.75	1556.28	1562.96
1265.71	1250.07	1243.48
1155.49	1159.89	1173.43
1453.54	1464.43	1428.84
1130.54	1121.31	1121.66
1106.02	1093.98	1046.25
1392.89	1390.25	1343.41
1476.12	1493.17	1483.24
1735.93	1698.14	1730.65

Low Density Skeletal Muscle CSA (cm²)

CT5	CT10	CT15
646.265	671.66	645.29
521.54	521.98	533.145
486.65	518.73	489.99
476.46	476.28	477.51
141.86	145.89	147.74
292.85	299.268	293.64
279.76	277.47	267.09
291.18	276.68	310.08
308.49	313.59	308.94
422.31	424.34	448.42
332.58	339.35	354.90
212.61	225.26	233.09
543.08	558.11	545.98

APPENDIX III: STATISTICS

ANOVA with repeated measures

NDM

Effect	Num DF	Den DF	F value	Significance
Time	2	24	4.08	0.0298

LDM

Effect	Num DF	Den DF	F value	Significance
Time	2	24	2.39	0.113

SF (w/Log Transformation)

Effect	Num DF	Den DF	F value	Significance
Time	2	24	0.89	0.4243

Multiple Means Comparisons

NDM

Effect	Time (min)	Time (min)	Estimated Diff	SE	DF	t value	Significance	Adjustment	Adj. P
Time	5	10	124.8	80.062	24	1.56	0.1321	Tukey-Kramer	0.2825
Time	5	15	228.45	80.062	24	2.85	0.0088	Tukey-Kramer	0.023
Time	10	15	103.64	80.062	24	1.29	0.2078	Tukey-Kramer	0.4118

LDM

Effect	Time (min)	Time (min)	Estimated Diff	SE	DF	t value	Significance	Adjustment	Adj. P
Time	5	10	-71.5294	39.3146	24	1.56	0.1321	Tukey-Kramer	0.2825
Time	5	15	-77.0733	39.3146	24	2.85	0.0088	Tukey-Kramer	0.023
Time	10	15	-5.5439	39.3146	24	1.29	0.2078	Tukey-Kramer	0.4118

SF

Effect	Time (min)	Time (min)	Estimated Diff	SE	DF	t value	Significance	Adjustment	Adj. P
Time	5	10	-0.00092	0.00111	24	-0.83	0.4143	Tukey-Kramer	0.4143
Time	5	15	-0.00146	0.00111	24	-1.32	0.1998	Tukey-Kramer	0.1998
Time	10	15	-0.00054	0.00111	24	-0.49	0.6302	Tukey-Kramer	0.6302

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