DETERMINATION OF EFFECTS OF PHYSICAL ACTIVITY AND ADIPOSITY ON HUMAN THIGH COMPOSITION USING MAGNETIC RESONANCE TECHNIQUES

BY

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THESIS

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This thesis project developed a magnetic resonance imaging and spectroscopy (MRI/MRS) protocol to quickly and non-invasively assess the fat composition of the thigh in three groups of older women varying in adiposity and habitual physical activity levels. The primary objective was the development of a methodology to quantify gross adipose tissue depots and intramuscular lipid concentrations towards developing and substantiating the use of MRI/MRS to measure muscle quality. Secondary objectives were to quantify the differences between the three distinct groups: obese (O); lean and sedentary (LS), and lean and active (LA) and to determine the effect of two different four-month interventions on the thigh adipose composition of the obese women: weight-loss diet (DI) and weight-stable exercise (EX).

In the cross-sectional portion of the study, no significant differences in relative adipose tissue volumes or intramuscular lipid concentrations were found between the groups O and LS, revealing that the lipid distribution and storage were similar between obese and lean, sedentary subjects. However, group LA had 10% less relative fat volume and double the relative amount of lipids stored in a metabolically active state than O and LS (both p<0.05). The favorable impact of physical activity on adipose composition was further confirmed empirically in the longitudinal portion of the study. Group DI increased lipid concentrations without a change in distribution, due to loss of lean mass, whereas group EX had a favorable redistribution of intramuscular lipids without a loss of overall lipid concentration. The results of this project add to the growing literature regarding the importance of physical activity for the management of intramuscular lipids, even in individuals who are normal weight.
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CHAPTER 1

Introduction

1.1 Significance

An aging population in conjunction with an increase in the incidence of obesity [1] is a disastrous combination leading to potential, related consequences such as mobility limitation [2] and metabolic syndrome [3]. Mobility, and especially locomotion in healthy individuals, is related to muscle strength which is correlated with skeletal muscle mass and quality. Aging is a primary risk factor for sarcopenia, defined as low skeletal muscle mass relative to body weight or height, but studies have shown that obese elderly have relatively less skeletal muscle [2,4] and poorer muscle quality [5-7] when compared to non-obese elderly. Metabolic syndrome comprises many abnormalities, including central (abdominal) obesity and insulin resistance, and increases the risk of metabolic diseases such as type 2 diabetes and cardiovascular disease. While aging and obesity have been regarded as risk factors for metabolic syndrome, recent studies have found evidence that fat distribution [3] and physical activity level [8] are also important.

The quantification of muscle quality is intimately related to measuring “fitness.” As a first approximation, muscle quality can be assessed in terms of the spatial distribution of lean and fat mass. The “fit-or-fat” debate has long been a source of debate for physiologists and plays a key role in the determination of clinical interventions to prevent or ameliorate disability resulting from disordered body composition in the elderly. Repeatedly monitoring local muscle quality changes in the same location with standard techniques, such as muscle biopsies, is not feasible due to destruction of the tissue. For this reason, non-invasive techniques to identify health risk at
the earliest possible stages are crucial. Proton magnetic resonance imaging (MRI) provides a viable alternative method to biopsy and can probe any part of the body with multiple contrast methods. MRI has been used to quantify fat depots within the body, ostensibly the defining characteristic of obesity. The MRI techniques used include $T_1$-weighted imaging [9,10], single-voxel spectroscopy [11], and fat-selective RF pulse methods [12,13]. Additionally, diffusion tensor imaging (DTI) in conjunction with fiber tractography has been used to visualize the structure of skeletal muscle [14-16].

As a second approximation, the particular form of fat depot can be used to assess health risk, but this requires more sophisticated MRI techniques. The two-point Dixon technique [17] has been recently implemented to study subcutaneous, intermuscular, and intramuscular fat content in the thigh [18]. The use of a water and fat separation imaging technique grants the ability to reliably segment the fat depots in any region of interest. The relative quantities of gross adipose tissue provide information regarding the health state of the muscle. However, proton magnetic spectroscopy ($^1$H-MRS) [19] is required to distinguish the lipids from which intramuscular fat is comprised: intramyocellular lipids (IMCL) and extramyocellular lipids (EMCL) [12,20-23]. The absolute lipid content of skeletal muscle, particularly the concentration of IMCL, is known to change with muscle function and health state and has been proposed as a indicator of muscle and metabolic disease or dysfunction [12,24], such as that associated with type 2 diabetes [12,20,21]. While EMCL is relatively metabolically inert, IMCL can be utilized by the mitochondria making it the preferred form of lipid storage in a healthy muscle. Thus, the relative concentrations of IMCL and EMCL are important when assessing muscular lipid storage [25]. However, the specific muscle is an important factor, as Hwang et al. [25] reported concentrations of IMCL and EMCL varying between various lower leg muscles (soleus, tibialis
posterior, and tibialis anterior). Goodpaster [20] has reported IMCL concentrations in the vastus lateralis ranging from 0.5% to 4% in subjects of various health states and body compositions, including lean and sedentary, obese, type 2 diabetic, and exercise-trained. In healthy adults, IMCL and EMCL concentrations of 1.5% and 2.1% in the tibialis anterior were reported by Torriani et al. [26].

Comparisons are often made between obese and lean subjects, but this takes only adiposity into account; a successful intervention must effect fundamental, rather than superficial changes. In older adults, dieting for weight-loss often induces a proportionally greater loss in fat-free mass than fat mass compared to younger adults [27], which is undesirable. On the other hand, physical activity, particularly in the non-obese [3], has been found to significantly alter lipid distribution [28] without negatively affecting lean mass. Therefore, the goal of this study is to measure and compare the fat content and distribution in older women varying in adiposity and habitual physical activity and to determine the relative effectiveness of weight loss or exercise to benefit thigh adipose composition of obese women.

1.2 Specific Aims

1.2.1 Primary Aim One

The first task was to design an MRI protocol for quantifying gross adipose tissue and concentrations of IMCL and EMCL that would be repeatable after an intervention that conferred physical changes in the subjects. This was performed using two fat-sensitive techniques: Dixon’s method and spectroscopy. Diffusion tensor imaging (DTI) was also performed to obtain structural information providing another metric for muscle quality.
1.2.2 Primary Aim Two

A cross-sectional study of three groups of women with different fitness levels (obese; lean and sedentary; and lean and active) was conducted to quantify their muscle quality in terms of muscle fat composition using the Dixon and spectroscopy data to assess the relative impact of “fitness vs. fatness” on muscle adipose composition.

1.2.3 Primary Aim Three

The quantification of muscle quality in aim 2 was evaluated using a longitudinal study to assess the relative effects of either a weight-loss diet or a weight-stable exercise program on muscle adipose composition.

1.2.4 Hypotheses

Regarding the cross-sectional aim two, it was anticipated that the adipose tissue will be in the following order: O>LS>LA; O will have the greatest amount of adipose tissue, LA the least, and LS being between the O and LA. LA will have relatively greater IMCL than O and LS. Regarding the longitudinal aim three, it was hypothesized that DI will not significantly alter relative lipid proportions, but will reduce absolute fat volume, whereby EX would reduce relative fat proportions and increase IMCL concentration. The challenge addressed in this project is to prove these hypotheses with a subject sample that is large enough to establish statistical significance.
CHAPTER 2
Design of a Magnetic Resonance Imaging Experimental Protocol

This study was a joint effort involving two groups in the Department of Kinesiology and Community Health and the Department of Mechanical Science and Engineering of the University of Illinois at Urbana-Champaign. The Kinesiology group was responsible for providing the intervention and taking physical metrics such as leg strength, metabolic rate, balance and gait information, and insulin sensitivity, while the Mechanical Engineering group was responsible for the MRI/MRS experiments and analysis of the MRI/MRS results. The study involved both cross-sectional and longitudinal components, with the second set of measurements to be taken after four to six months.

Design of the MRI protocol was subject to several limitations, such as patient size and comfort. In addition, the longitudinal portion of the study required that the measurements be repeatable in the same location after the four to six month intervention period. The combination of these factors resulted in the following constraints: restriction of patient motion; repeatable placement of slices; and short scan duration. The methods that were developed to resolve the first two issues and the MRI sequences chosen and modified to provide high image quality, while keeping scan duration short are explained later in this chapter. Performing these scans required two separate, but interrelated protocols for dealing with the subject before and after the scan and for operation of the MRI scanner itself.

The human aspect of the protocol involved situating the subject in the scanner and properly immobilizing them, while maintaining comfort for the hour-long scan session during which they must remain immobile. Due to the large size of some of the subjects and the
confined space of the bore of the magnet, there were challenges in positioning the leg in the center of the magnet for optimum image quality.

The operational aspect of the protocol involved either developing or modifying an existing sequence to image the thigh. Some standard sequences, such as localizers, could be used without any modifications, but others such as 3-D, DTI, and spectroscopy scans required a large number of parameters to be changed in order to obtain useful data in a reasonable time frame. Developing the methodology for localization of the slices required a great deal of repetition to ensure the robustness of the system, as well as to reduce the downtime during subject scanning. Due to the complex and time-consuming nature of designing an MRI sequence and testing it, development of a custom DTI sequence was abandoned in favor of optimizing the parameters of an existing DTI sequence, such as diffusion-weighting and number of averages. Experiments were required to determine the proper voxel size, voxel placement, number of averages per spectrum, and number of spectra to take when performing spectroscopy. A significant portion of each scan was devoted to “shimming” the magnetic field to correct for gradient inhomogeneity, requiring a large time investment to understand how it works and gain the experience to perform it rapidly in order to avoid delays when scanning the subjects.

2.1 Landmarking

This study involved human subjects and included both cross-sectional and longitudinal components, which made it necessary to have robust methods for positioning the subjects and determining the imaging slices. These were accomplished by first immobilizing the thigh and then developing a technique that could be repeated for each subject in order to image the same location each time. All techniques were designed for use with and data was collected on a
Siemens Magnetom Trio 3T whole-body scanner (Siemens AG Medical Solutions, Erlangen, Germany) with the subjects oriented in a feet-first supine position. Imaging was performed using a combination of an eight-channel spine coil and a flexible body matrix surface coil centered over the midpoint of the left thigh.

2.1.1 Immobilization of the Thigh

The subjects for this study included lean and obese older women who were required to stay motionless during the entire MRI/MRS scan session that lasted approximately one hour. Perfect immobilization is a nearly impossible feat without some form of sedation. Given the limited space within the bore of the magnet and the material constraints due to the nature of the MRI scanner, it was decided that rather than full immobilization, it would be best to establish an equilibrium position to which the subjects would naturally return to should there be any movement.

This positioning was accomplished by employing the use of the EP-216 vacuum splint (Emergency Products + Research, Kent, Ohio, USA). A vacuum splint is a large pouch containing many small plastic beads which are free to move until the air is removed from the splint. The splint is highly conformable to the body part and a hard shell can be formed very rapidly, usually within ten seconds. After positioning a subject on the bed of the MRI scanner, the vacuum splint was wrapped around the subject’s leg and strapped down to the bed to prevent subject movement. The air was then extracted from the splint to further immobilize the leg and the straps retightened. Subjects reported an improvement in comfort due to the use of the vacuum splint.
2.1.2 3-D Localization by Anatomical Scanning

In order to make accurate comparisons, it was necessary to minimize inter- and intra-subject variability. Thus, the goal was to design a procedure to locate a central imaging slice on the thigh for each subject without relying on structural landmarks that could change within a span of six months. As such, superficial markers were eliminated as a choice. Instead, a high-resolution 3-D turbo spin echo sequence was incorporated into the MRI protocol to find bony landmarks on the femur, because bone changes slowly relative to other tissues. The sequence parameters were: one coronal slab; phase-encoding in the right-to-left direction; TR/TE=2500/230ms; FOV=500x500mm$^2$; matrix size=320x320; 192 slices per slab; and slice thickness=1.4mm. 3-D visualization software available on the MRI scanner was used to manipulate the images (Fig. 2.1). One bony landmark near either end of the femur was selected: the proximal landmark was the center of the femoral head and the distal landmark was the center of the frontal protrusion of the patella. An axis was constructed from these two points and the central imaging slice was oriented orthogonally to this axis at its midpoint. The repeatability of the localization procedure is within 1mm in the axial direction.

The anatomical scanning time was approximately six minutes per 3-D scan. Taller subjects required two scans of the upper and lower thigh in order to find both landmarks. The image sets were combined using the in-line composer on the scanner. After scanning, the manual determination of the location of the center slice by the operator took approximately three minutes.
2.2 Diffusion Tensor Imaging

2.2.1 Background

Muscle has a hierarchical structure that can be simplified as tubes within tubes. The fibers within a muscle (myocytes) are generally aligned and grouped into bundles called fascicles. Due to this structure, there is an inherent anisotropy in diffusivity in the axial and transverse directions \[29\] which may be altered by pathological conditions. Diffusion-weighted (DW) MRI can be used to calculate the diffusion coefficient of water molecules in a specified direction. The MRI signal, \( S \), is related to the diffusion coefficient by the formula

\[
S = S_0 e^{-bD}
\]  \hspace{1cm} (2.1)

where \( S_0 \) is the signal intensity in the absence of diffusion-weighting, the \( b \)-value is the strength of the diffusion-weighting, and \( D \) is the diffusion coefficient. An increase in diffusivity or the diffusion-weighting causes increased dephasing in the spins of the protons, resulting in a decreased MRI signal.

DTI uses DW-MRI data measured in at least six non-collinear directions in order to construct a symmetric diffusion tensor for each voxel. Through singular value decomposition, the tensor can be represented as three eigenvalues, each with an associated eigenvector. The diffusion in the voxel can then be visualized as an ellipsoid with radii equal to the eigenvalues in the directions specified by the eigenvectors. It is commonly agreed that the primary eigenvector (greatest eigenvalue) is aligned with the structure, but there is not yet a standard interpretation for the secondary and tertiary eigenvectors. The primary eigenvectors can be used with fiber tracking algorithms to model the structure.
2.2.2 DTI Sequence

A twice-refocused spin echo DTI sequence with slice interleaving was selected for this study. The sequence was run with the following parameters: $b=550$ s/mm$^2$; thirty diffusion-encoding directions; TR/TE=3000/71 ms; FOV=250x250 mm$^2$; matrix size=76x76; ten averages; seven slices; and slice thickness=10 mm. The DTI scan time was approximately sixteen minutes per subject.

2.2.3 Reconstruction and Post-processing

DTI analysis was performed using custom scripts written in MATLAB (The Mathworks, Inc., Natick, MA, USA). Fiber tracking was performed using the Trackvis software package [30].

2.2.4 Phantom and Leg Experiments

Verification of the DTI sequence and reconstruction scripts was performed using a custom phantom. The phantom contained water, soybean oil, and thin fibers (0.1 mm inner diameter) filled with 20% Intralipid, as shown in Figure 2.2. The fiber direction as determined by the primary eigenvector was found to agree with the physical structure of the phantom.

DTI was also performed on a human calf and the fibers tracked. Seven axial slices were taken centered at the thickest cross-section of the calf. Figure 2.3 shows that there is good agreement between the calf of a cadaver and the in-vivo DTI results. The tibialis anterior and posterior are straight muscles that are aligned with the leg. This is reflected in the blue color of the fibers. The gastrocnemii are bipennate and have a pennation angle that injects a red tint into the reconstructed fibers. The soleus is a complex muscle with fibers running in many different directions and the reconstructed fibers can be observed in many orientations with different colors.
In-vivo DTI of the thigh was performed in seven slices centered at the location determined by the previously described localization method.

2.3 Two-Point Dixon Technique

2.3.1 Background

The measured signal in proton MRI comes from a variety of sources, in particular water and lipids. However, the protons in water and lipid do not behave identically due to differences in chemical bonding. This can be utilized to separate water and fat from combined images using Dixon’s method [31]. When using the two-point Dixon technique, two sets of magnitude and phase data are taken where the lipid signal is either in-phase ($\phi = 0$) or out-of-phase ($\phi = \pi$). By recognizing that the image (complex) has contributions from protons in both the water and lipids, the two sets of images are

$$m_{in} = m_w + m_f$$  \hspace{1cm} (2.2) \\
and \\
$$m_{out} = m_w - m_f$$  \hspace{1cm} (2.3) \\

where $m_w$ is the contribution from water protons and $m_f$ is the contribution from lipid protons.

Rewriting Equations 2.2 and 2.3 to solve for $m_w$ and $m_f$ gives the separated images

$$m_w = \frac{1}{2}(m_{in} + m_{out})$$  \hspace{1cm} (2.4) \\
and \\
$$m_f = \frac{1}{2}(m_{in} - m_{out})$$  \hspace{1cm} (2.5) \\

which are useful for identifying tissue and characterizing voxels.

2.3.2 Dixon Sequence

A clinical abdominal two-point Dixon imaging sequence was modified with the following parameters: TR/TE1/TE2=241/2.45/3.99ms, FOV=25x25cm$^2$, matrix size=256x256,
seven slices, and slice thickness=10cm. The two-point Dixon scan time was thirty-four seconds per subject.

2.3.3 Reconstruction and Post-processing

Fat-water separation was performed using custom scripts written in MATLAB. Segmentation of the fat- and water-separated images was performed using Amira (Visage Imaging GmbH, Berlin, Germany). Regions of interest (ROI) for subcutaneous fat (SUBQ), intermuscular fat (INTER), and muscle (MUSC) were drawn using intensity thresholds in the center slice of the fat- and water-separated images as shown in Figure 2.4C. Relative volumes for these depots were determined against the total volume of the thigh cross-section minus the bone (TOTAL). Intramuscular fat concentration ([INTRA]) was calculated from the intensity of the fat-separated image in the quadriceps muscle relative to the non-separated image intensity.

2.3.4 Phantom and Leg Experiments

The sequence and water-fat separation algorithm was verified using a phantom consisting of two concentric cylinders. The inner cylinder was filled with soybean oil and the outer cylinder was filled with water. The water- and fat-separated images shown in Figure 2.5 verified the contents of each compartment. A calculation of [INTRA] showed that the oil voxels contained approximately 85% lipid, while the water voxels contained over 99% water.

In-vivo measurements of the thigh were performed in the same seven slices as the DTI.

2.4 Magnetic Resonance Spectroscopy

2.4.1 Background

The two-point Dixon technique is sufficient to obtain gross values for fat depots, but is unable to differentiate between IMCL and EMCL. MRS identifies molecules by their chemical
shift in ppm from the reference molecule. The chemical shift of a molecule corresponds to its resonance frequency, which is dependent on its chemical bonds. Electron clouds shield the hydrogen nuclei from the magnetic field and this is a function of chemical bonds as well as orientation with respect to the magnetic field in some cases. While IMCL and EMCL are both lipids, the organization is different and coupling between neighboring chemical groups creates differences in chemical shift. IMCL is present within the muscle fibers as small, spherical droplets that can be metabolized, while the metabolically inert EMCL is stored within adipocytes and has a bulk structure roughly consistent with the myofiber geometry. The best differentiation between the IMCL and EMCL peaks requires that the voxel be placed outside of intermuscular fat depots (primarily EMCL) and that the myofiber direction is aligned with the main scanner magnetic field, $B_0$.

Like all in vivo MR measurements, MRS data are typically noisy so multiple averages are required in order to derive reliable spectra. In order to determine the required level of averaging, the signal-to-noise ratio (SNR) for a spectrum can be calculated as follows:

$$SNR_{db} = 10 \times \log_{10} \left( \frac{P_{signal}}{P_{noise}} \right)$$  \hspace{1cm} (2.6)$$

where $SNR_{db}$ is the SNR in decibels, $P_{signal}$ is the total power (area) of the signal [32], and $P_{noise}$ is the total power (area) of the noise. The ratio of $P_{signal}$ and $P_{noise}$ is also reported as SNR when the dynamic range of the signal is not high. Due to differences in phase, the SNR does not vary with the square root of the number of averages in some cases [33].

### 2.4.2 Single-Voxel Spectroscopy sequence

A PRESS sequence was selected for its higher SNR relative to STEAM. In addition, CHESS was employed for water saturation. The sequence parameters were: TR/TE=2000/30ms; eight averages per acquisition; and single voxel size=15x15x10mm$^3$. Water-suppressed spectra
were collected thirteen times for peak alignment and averaging ($\text{SNR}_{\text{dB}} = 14\text{dB}$). This number was confirmed to be sufficient ($\text{SNR} > 8$ at a voxel volume of $2\text{cm}^3$) by a comparison of SNR with the number of spectra taken (Fig. 2.6). The location of the peaks was also found to be consistent within 0.001ppm; this result did not vary with number of spectra. Two non-water-suppressed spectra were collected to allow for averaging and normalization of individual lipid signals to the total signal ($\text{SNR}_{\text{dB}} = 23\text{dB}$). At twenty-four seconds per scan, the total spectroscopy scan time was six minutes per subject.

2.4.3 Reconstruction and Post-processing

Processing was performed using custom MATLAB scripts provided by Dr. Hernando. Similarly acquired spectra were combined, and the water and lipid peaks were fitted using Lorentzian functions with Gaussian priors. The water (4.7ppm) and combined lipid (1.6ppm) peaks were fitted in the non-water-suppressed spectra, as shown in Figure 2.7. In the water-suppressed spectra (Fig. 2.8), five peaks including the water (4.7ppm), main IMCL (1.4ppm), and main EMCL (1.6ppm) peaks were fitted. The other three peaks corresponded to creatine and other metabolites which were not the focus of this study, but were still a significant portion of the signal.

In the non-water-suppressed spectra, areas were calculated for the water and lipid components and the total area approximated as the sum of these two areas. Total lipid concentration ([LPD]) was calculated as given in Equation 2.7.

$$[\text{LPD}] = \frac{A_{\text{lipid}}}{A_{\text{water}} + A_{\text{lipid}}}$$

Equation 2.7

For the water suppressed spectra, total area was determined using all five fitted peaks. The IMCL fraction was calculated as
\[ IMCLfraction = \frac{A_{IMCL}}{A_{IMCL} + A_{EMCL}} \] (2.8)

and is a measure of lipid distribution. This was then used with the total lipid concentration to calculate the absolute IMCL ([IMCL]) and EMCL ([EMCL]) concentrations.

\[ [IMCL] = IMCLfraction \times [LPD] \] (2.9)

\[ [EMCL] = [LPD] - [IMCL] \] (2.10)

2.4.4 Phantom and Leg Experiments

The single-voxel spectroscopy sequence was tested and the reconstruction method verified using a bottle of 20% Intralipid (Sigma-Aldrich, St. Louis, MO, USA). The voxel placement can be found in Figure 2.9. [LPD] was calculated to be 20.95%, which is close to the nominal value of 20%. The distribution of lipid molecules in the Intralipid is more similar to IMCL than EMCL, as it is an emulsion rather than a solid. This is confirmed with an IMCL fraction of 70.60%.

For in-vivo measurements, the voxel was placed adjacent to the femur in the vastus medialis as shown in Figure 2.10.
2.5 Figures

**Figure 2.1.** Screenshot of MRI scanner monitor during the determination of the center slice of the thigh using the 3-D rotation and alignment tools available on the MRI scanner. The intersection of the sagittal (top left) and coronal (top right) slices defines the axis that helps determine the central slice orientation (bottom left).
Figure 2.2. Cross-sectional view in MRI scan of the custom phantom used to test the DTI sequence. The Intralipid-filled fibers form a cross in the center. Three cylindrical chambers were filled with soybean oil (bright spots) and the rest was filled with water. The location of the oil is shifted due to the chemical shift artifact.
Figure 2.3. Cadaver calf (left, Visible Human Project) and reconstructed calf with fibers (right, image courtesy Mr. Gharibans). Muscle groups are clearly delineated. MG and LG are the gastrocnemius medialis and lateralis, SOL is the soleus, TA and TP are the tibialis anterior and posterior, and PL is the peroneus longus. The color of each fiber is dependent on its local orientation using the common scheme (blue is head-foot, red is right-left, and green is front-back).
Figure 2.4. Dixon method outputs showing sample fat separated images for (A) obese, (B) lean and sedentary, and (D) lean and active. Regions of interest are shown in (C) for fat depots SUBQ (yellow), INTER (blue), and MUSC (pink).
Figure 2.5. Top: MRI spin density image of a phantom comprising an oil-filled inner cylinder enclosed by an outer water-filled cylinder. Bottom: Water- (left) and fat-separated (right) MRI images. Sloshing motions due to shaking of the scanner bed create intensity variations in the water, but do not affect the separation.
Figure 2.6. Variation of SNR and SNR_{dB} with number of water-suppressed MR spectra taken.
Figure 2.7. Reconstructed non-water-saturated spectrum from single-voxel MRS output displaying two distinct peaks.

Figure 2.8. Reconstructed water-saturated spectrum from single-voxel MRS output with three distinct peaks.
Figure 2.9. Placement of voxel for spectroscopy in a bottle of Intralipid.

Figure 2.10. Placement of voxel for single-voxel MRS in the vastus medialis.
3.1 Subjects

In this study, a total of fifty-two postmenopausal, elderly women (age ≥ 59) were recruited into three distinct categories: obese (O), lean/sedentary (LS), and lean/active (LA). Women with a body mass index (BMI) greater than 28 were assigned to group O (n=29). Women with a BMI between 20 and 24.9 were further divided into groups LS (n=11) and LA (n=12) based on their daily activity level as determined by a pedometer. The obese subjects were then randomized into two interventions consisting of a) calorically restricted diet without physical activity (DI) intended to elicit approximately 10% weight loss and b) exercise training without caloric restriction (EX) consisting of 3 days per week of combined endurance and strength training exercise. Each exercise session was supervised and lasted 90 minutes broken down as follows: warm-up (15min), aerobic exercise (primarily walking, 30min), resistance training (30min), and balance and flexibility exercises during the cool-down period (15min). Exercise intensity was increased gradually from 65-75% of VO$_{2\text{peak}}$ to between 70 and 85% of VO$_{2\text{peak}}$ over the four month intervention period. The division of subject groups for the cross-sectional and longitudinal groups is contained in Figure 3.1. The study was approved by the University of Illinois at Urbana-Champaign Institutional Review Board and written consent was obtained from each volunteer.
3.2 Lipid Storage and Distribution

3.2.1 Statistical Analysis

Consideration was given to the sample size necessary to achieve results of adequate statistical significance. Based on work by Torriani et al. [26], it was determined that a minimum change of 15% could be statistically detected in IMCL using proton MRS with a sample size of 20 subjects (an effect size of ~1.0, power of approximately 80%). In a sample, rather than a population, the effect size is a measure of strength in an apparent relationship. It can be used in conjunction with a p-value to assess statistical significance, but does not determine the confidence interval, or vice versa. As long as the effect size is not zero, any statistical comparison will show significant differences with a sufficiently large sample size. Work by Janssen et al. [34] indicates that the relative effect of caloric restriction compared to caloric restriction and exercise for weight loss produced an effect size of 0.60 in premenopausal women who experienced a 10% weight loss over 4 months. We anticipated that our effect would be stronger as our groups differed completely in intervention mode (one has caloric restriction, one exercise, and neither is a combined treatment).

The unpaired Student's t-test was performed for the calculated Dixon and MRS parameters in Table 3.1 using unequal variances and the null hypotheses: $\mu_O - \mu_{LS} \leq 0$, $\mu_O - \mu_{LA} \leq 0$, and $\mu_{LS} - \mu_{LA} \leq 0$.

3.2.2 Dixon Technique

While the volume of the thighs of obese subjects were found to be larger than those of lean subjects, no significant differences were found in relative volumes of subcutaneous and intermuscular fat depots between groups O and LS; nor was there a significant difference in the intramuscular fat concentration between the groups. In contrast, group O had higher relative fat
volumes than group LA (SUBQ, p<0.01; INTER, p<0.05) and a higher intramuscular fat concentration ([INTRA], p<0.05). Despite having similar total volumes, the compositions of thighs in groups LS and LA were significantly different in fat content and distribution. Relative volumes for SUBQ (p<0.05) and INTER (p<0.1) were greater in group LS while MUSC was greater in group LA (p<0.05). Group LS also had a greater [INTRA] (p<0.1).

3.2.3 Single-Voxel Spectroscopy

No significant differences were found in lipid content or distribution between groups O and LS. While the absolute concentrations of lipids were found to be greater in group O than in group LA ([LPD] and [EMCL], p<0.005; [IMCL], p<0.1), the IMCL fraction was greater in group LA than in group O (p<0.1). The same results were found when comparing group LS to group LA; however, [IMCL] was not found to be significantly different between the two lean groups.

The majority of the lipid signal comes from the EMCL which manifested as similar results for the statistical analyses between groups for [LPD] and [EMCL]. Due to the relatively lower contribution of IMCL to the total lipid signal, variations in [IMCL] do not necessarily reflect the comparisons for [LPD]. In fact, IMCL fraction is higher in the more active lean subjects, despite having a lower concentration of lipids present in the muscle. This difference in lipid storage is consistent with the current understanding of lipid metabolism [20]. [LPD] and IMCL fraction are relevant quantities in differentiating the muscle adipose composition of subjects.

3.2.4 Importance of Physical Activity on Fitness

The Dixon and spectroscopy findings are largely consistent with each other and with previous work done in muscle [20,25,28]. A graphical summary is offered in Figure 3.2. Obese
subjects (unfit) typically have more adipose tissue and higher concentrations of lipids within the skeletal muscle structure than lean subjects (ostensibly fit). However, for lean subjects the distribution of lipids appears to depend on the subject’s physical activity level.

Significant differences were found between groups LS and LA using both MRI techniques, while groups O and LS were found to be more similar. This suggests that fitness is not a function of maintaining a normal body weight, but that physical activity is the catalyst for the body to remodel itself in a way that would be considered fit. This is a classic example of form following function; lean subjects have less total lipids to be stored as adipose tissue or within the muscles, but the muscles of sedentary subjects do not require that the lipids be stored in a metabolically active form. This results in an increase in adipose tissue and lipid distributions that are similar to those of the obese subjects, rather than the active subjects. Thus, sedentary subjects should also be classified as unfit, rather than fit, as their muscles already show metabolic changes that belie their outward appearance.

3.3 Post-Intervention MRS

3.3.1 Statistical Analysis

The Student’s t-test was performed for the calculated MRS parameters in Table 3.2 using unequal variances. For DI pre- and post-intervention, a paired test was used with the null hypothesis $\mu_{DI,post} - \mu_{DI,pre} = 0$. A paired test was used for the group EX (pre and post) with the null hypothesis $\mu_{EX,post} - \mu_{EX,pre} \leq 0$. The DI and EX were compared to each other using an unpaired test with the null hypothesis: $\mu_{EX,post} - \mu_{DI,post} \leq 0$. Unpaired tests were used to compare the diet and exercise groups to the lean, sedentary group to control for the effect of no intervention with the null hypotheses $\mu_{DI,post} - \mu_{LS} = 0$ and $\mu_{EX,post} - \mu_{LS} \leq 0$. 
3.3.2 Effect of Interventions on Intramuscular Lipids

The effects of diet and exercise interventions can be seen in Figure 3.3. The diet produced statistically significant increases in [LPD] (p=0.0667), [IMCL] (p=0.0160), and [EMCL] (p=0.0932), but not in IMCL fraction (p=0.617). While not statistically significant, the trend was a decrease in IMCL fraction. The overall increase in [LPD] is consistent with research that has found a greater loss of fat-free mass than fat mass during weight-loss in the elderly [27]. However, the lack of a stimulus for the body to change the way that it stores the lipids results in the absence of change in IMCL fraction. In the exercise group, no change in [LPD] was detected (p=0.3333). However, statistically significant changes were found in [IMCL] (increased, p=0.0989) and [EMCL] (decreased, p=0.0137), resulting in a borderline increase in IMCL fraction (p=0.1138). While the overall composition of the muscle did not change, the intramuscular lipids were redistributed due to the demands of the exercise. This effect has been documented in several studies [35].

A direct comparison between the two intervention groups was also performed, but no differences were statistically significant in [LPD], [EMCL], or IMCL fraction. [IMCL] in group EX was elevated (borderline, p=0.1040) relative to group DI. Inconsistencies between the two intervention groups and the relative changes they experienced resulted from the small sample sizes and differences in effect size and statistical power between the paired and unpaired tests. When compared to group LS, the group DI had insignificant increases in [LPD] (p=0.3325), [EMCL] (p=0.4842), and IMCL fraction (p=0.2920), but an increase in [IMCL] that may be significant with larger sample sizes (p=0.1088). Group EX fared slightly better when compared to group LS. [LPD] (p=0.1111) and [EMCL] (p=0.2799) increased, but not significantly. On the other hand, increases in [IMCL] (p=0.0406) and IMCL fraction (p=0.0600) were statistically
significant. These results are represented in Figure 3.4. The difficulty of finding statistical significance due to the large variances suggests that either the groups were too small, compliance was not uniform, the intervention was not long enough to produce the desired changes, or that the MRI measures were unreliable.

The MRS results show that a weight-loss diet does not promote a healthy distribution of intramuscular lipids. In fact, for elderly individuals it may be detrimental as a result of increasing the relative portion of lipid stored as EMCL. On the other hand, a weight-stable exercise regimen promotes a beneficial redistribution of intramuscular lipids from EMCL to IMCL, despite not significantly reducing overall lipid levels. Differences in individuals may also affect the results. Without knowing the previous state of the muscle, it is not possible to properly assess the changes induced by diet or exercise. The reduced variance of the paired statistics made it possible to detect differences that were not as notable when comparing populations, especially with smaller samples.
### Table 3.1. Summary of absolute and relative fat volumes and concentrations for all three groups.

<table>
<thead>
<tr>
<th></th>
<th>Obese (O)</th>
<th>Lean, sedentary (LS) / active (LA)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\muO \pm \sigmaO$</td>
<td>$\muLS \pm \sigmaLS$</td>
</tr>
<tr>
<td><strong>TOTAL (cm$^3$)</strong></td>
<td>280.03 ± 51.92</td>
<td>208.01 ± 29.20</td>
</tr>
<tr>
<td><strong>SUBQ (cm$^3$)</strong></td>
<td>163.24 ± 48.85</td>
<td>115.19 ± 23.92</td>
</tr>
<tr>
<td><strong>INTER (cm$^3$)</strong></td>
<td>31.06 ± 8.87</td>
<td>22.17 ± 3.40</td>
</tr>
<tr>
<td><strong>MUSC (cm$^3$)</strong></td>
<td>85.73 ± 13.32</td>
<td>70.66 ± 6.69</td>
</tr>
<tr>
<td><strong>SUBQ (%)</strong></td>
<td>57.32 ± 8.18</td>
<td>54.88 ± 5.04</td>
</tr>
<tr>
<td><strong>INTER (%)</strong></td>
<td>11.23 ± 3.12</td>
<td>10.74 ± 1.48</td>
</tr>
<tr>
<td><strong>MUSC (%)</strong></td>
<td>31.45 ± 6.88</td>
<td>34.38 ± 4.44</td>
</tr>
<tr>
<td><strong>[INTRA] (%)</strong></td>
<td>9.74 ± 1.82</td>
<td>9.41 ± 0.89</td>
</tr>
<tr>
<td><strong>[LPD] (%)</strong></td>
<td>6.31 ± 2.02</td>
<td>5.39 ± 1.82</td>
</tr>
<tr>
<td><strong>IMCL fraction</strong></td>
<td>0.14 ± 0.11</td>
<td>0.10 ± 0.12</td>
</tr>
<tr>
<td><strong>[IMCL] (%)</strong></td>
<td>0.75 ± 0.47</td>
<td>0.47 ± 0.54</td>
</tr>
<tr>
<td><strong>[EMCL] (%)</strong></td>
<td>5.56 ± 2.16</td>
<td>4.92 ± 1.96</td>
</tr>
</tbody>
</table>
Table 3.2. Post-intervention MRS results. Pre-intervention group means are not identical.

<table>
<thead>
<tr>
<th></th>
<th>Diet (DI)</th>
<th>Exercise (EX)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>[lipid] (%)</td>
<td>5.08 ± 2.67</td>
<td>6.80 ± 3.72</td>
</tr>
<tr>
<td>IMCL fraction</td>
<td>0.17 ± 0.12</td>
<td>0.16 ± 0.09</td>
</tr>
<tr>
<td>[IMCL] (%)</td>
<td>0.64 ± 0.30</td>
<td>0.84 ± 0.26</td>
</tr>
<tr>
<td>[EMCL] (%)</td>
<td>4.45 ± 2.85</td>
<td>5.96 ± 3.80</td>
</tr>
</tbody>
</table>
Figure 3.1. Stratification of the fifty-two subjects into three groups for the cross-sectional study. The obese group was then split in half for the intervention, but not all subjects completed the study. The lean, sedentary group was used as a time-control (no intervention).
<table>
<thead>
<tr>
<th></th>
<th>Absolute Volume</th>
<th>Relative Volume</th>
<th>Lipid Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total</strong></td>
<td>O</td>
<td>O</td>
<td>O</td>
</tr>
<tr>
<td></td>
<td>( \leq ) ( \leq )</td>
<td>( \leq ) ( \leq )</td>
<td>( \leq ) ( \leq )</td>
</tr>
<tr>
<td>LS</td>
<td>LA</td>
<td>LS</td>
<td>LS</td>
</tr>
<tr>
<td><strong>Fat</strong></td>
<td>O</td>
<td>O</td>
<td>O</td>
</tr>
<tr>
<td></td>
<td>( \leq ) ( \leq )</td>
<td>( \leq ) ( \leq )</td>
<td>( \leq ) ( \leq )</td>
</tr>
<tr>
<td>LS</td>
<td>LA</td>
<td>LS</td>
<td>LS</td>
</tr>
<tr>
<td><strong>Muscle</strong></td>
<td>O</td>
<td>O</td>
<td>O</td>
</tr>
<tr>
<td></td>
<td>( \leq ) ( \leq )</td>
<td>( \leq ) ( \leq )</td>
<td>( \leq ) ( \leq )</td>
</tr>
<tr>
<td>LS</td>
<td>LA</td>
<td>LS</td>
<td>LS</td>
</tr>
</tbody>
</table>

**Figure 3.2.** Relationships in cross-sectional parameters with respect to adiposity and physical activity level.
## Lipid Concentration

<table>
<thead>
<tr>
<th>Total Pre</th>
<th>IMCL fraction Pre</th>
</tr>
</thead>
<tbody>
<tr>
<td>DI EX</td>
<td>DI EX</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>[IMCL] Pre</th>
<th>[EMCL] Pre</th>
</tr>
</thead>
<tbody>
<tr>
<td>DI EX</td>
<td>DI EX</td>
</tr>
</tbody>
</table>

**Figure 3.3.** Changes in the diet and exercise groups relative to their pre-intervention MRS parameters.
<table>
<thead>
<tr>
<th>Lipid Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total</strong></td>
</tr>
<tr>
<td>LS</td>
</tr>
<tr>
<td>↓ ↓</td>
</tr>
<tr>
<td>DI = EX</td>
</tr>
<tr>
<td>[IMCL]</td>
</tr>
<tr>
<td>LS</td>
</tr>
<tr>
<td>↑ ↑</td>
</tr>
<tr>
<td>DI &lt; EX</td>
</tr>
</tbody>
</table>

**Figure 3.4.** Relationships between MRS parameters for the diet and exercise intervention groups and the lean, sedentary group.
CHAPTER 4

Summary and Conclusions

This thesis spanned the successful development of an MRI protocol capable of rapidly imaging obese subjects and the interpretation of the MRI and MRS results as they pertain to the storage of lipids in the thighs of older women. As a non-invasive imaging technique, MRI is very useful to researchers and physicians in diagnosing and treating disease. We set out to prove that MRI can also be used to assess the effect of various interventions on obese, elderly women. The challenges in the application of MRI to obesity studies are limited space within the bore of the magnet, relative slowness and indirect nature of the imaging method, and imaging artifacts introduced by fat. The work done dealt with issues of patient comfort, patient motion, repeatability after physical change, scan duration, and post-processing of data.

Fifty-two older female subjects were scanned, some twice, necessitating a robust protocol to remove sources of error. The subjects were recruited to vary in weight status and physical activity with three distinct groups: obese, lean and sedentary, and lean and active. Obese subjects were further divided into a weight-loss diet group and an exercise group with an intervention period of four months. In order to minimize patient motion during the scans, a vacuum splint was utilized. A high-resolution 3-D anatomical scan was employed along with tools available on the MRI scanner to develop a method for positioning the center imaging slice in the middle of the thigh each time. An axis was constructed from the center of the femoral head and the front protrusion of the patella and the slice oriented normal to the axis in its center. The bony landmarks were selected for their permanence over the four month intervention period.
Diffusion tensor imaging was included in the protocol as an avenue for further work regarding changes in the structure and connectivity of skeletal muscle with regard to adiposity and physical activity levels. As the single longest scan in the protocol, a great deal of work was put into obtaining a sufficient SNR while keeping the scan duration at a minimum. Post-processing of the DTI analysis was not included in this work.

A two-point Dixon method and proton magnetic resonance spectroscopy were employed to quantify gross adipose tissue depots and intramuscular lipid concentrations, respectively. In the cross-sectional portion of the study, the Dixon and MRS results revealed no difference in relative fat volumes and intramuscular lipid concentrations between the obese and lean, sedentary groups despite the difference in absolute fat mass. However, physical activity in the lean, active group was shown to significantly reduce relative fat volume and intramuscular lipid concentration. A closer look at the distribution of intramuscular lipid informed us that in the active subjects, there was a greater fraction of intramyocellular lipids which can be metabolized by the mitochondria and are a preferable form of lipid storage. Thus, it was found that being lean does not suggest physical fitness, but physical activity level is a determinant of fitness.

The longitudinal aspect of the study involved two interventions, a weight-loss diet and a weight-stable exercise, that attempted to make the obese subjects more similar to the subjects of the two lean groups. This was done to explore to effectiveness of each treatment at improving physical fitness. The diet was found to not be metabolically beneficial. Due to the loss of lean mass, lipid concentrations increased and there were indications of [EMCL] increasing more than [IMCL]. However, exercise was beneficial, despite not decreasing fat mass. [IMCL] increased and [EMCL] decreased as a result of physical activity, which is in line with the fitter, active subjects.
While the MRI/MRS techniques employed are not new, the work done employs a novel blend of such methods to non-invasively investigate the structure of the human thigh. The results reveal that risk for metabolic syndrome and disease can be detected quickly and painlessly in subjects that outwardly appear to be healthy. An increase in physical activity level seems to promote a better, relative to mitochondrial access to the lipid for metabolism, lipid distribution in lean individuals, which hints at a possible method for prevention. This work leaves behind a wealth of data that can be used to gain insight on muscle structure and the effectiveness of interventions of weight loss and physical activity to change lipid distribution.
REFERENCES


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