INVESTIGATION OF HUMAN MUSCLE VARIABILITY AND ITS EFFECT ON MUSCULOSKELETAL MODELS

A Dissertation in
Kinesiology
by
Benjamin W. Infantolino

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The dissertation of Benjamin W. Infantolino was reviewed and approved* by the following:

John H. Challis  
Professor of Kinesiology; Graduate Program Director  
Dissertation Adviser  
Chair of Committee

Stephan J. Piazza  
Associate Professor of Kinesiology, Mechanical Engineering, and Orthopaedics and Rehabilitation

Robert B. Eckhardt  
Professor of Developmental Genetics and Evolutionary Morphology

Andris Freivalds  
Professor of Industrial and Manufacturing Engineering

*Signatures are on file in the Graduate School.
ABSTRACT

Variability in the musculoskeletal system is apparent at the human body level in terms of body height and shape. However, daily activities such as walking demonstrate stereotypical movement patterns across individuals. The literature suggests that although muscles produce stereotypical movements, at various levels of muscle variability exists. For example, whole muscles demonstrate differences in the portion of the force-length curve on which they act on. This has been demonstrated between different muscles as well as between the same muscles in different individuals. Evidence has also shown that sarcomere lengths differ along the length of muscle fibers. The First Dorsal Interosseous (FDI) muscle is the only muscle to abduct the second metacarpophalangeal joint and is therefore of interest to study its variability in light of its singular action. The purpose of the four studies in this dissertation was to investigate variability in the musculoskeletal system at various levels for the FDI muscle. Specifically, the purpose of the first study was to investigate how the output of a FDI musculoskeletal model changes with changes in model parameters. The purpose of the second study was to investigate how individual sarcomere lengths varied along single FDI muscle fibers. The purpose of the third study was to measure subject-specific FDI muscle model parameters \textit{in vivo}. The purpose of the final study was to investigate, using magnetic resonance imaging, the arrangement of muscle fascicles within the FDI muscle. The studies showed that: 1. that accurate model output requires specimen-specific model parameters; 2. individual sarcomere lengths along muscle fibers exhibit long-range correlations, which has implications for determining the properties of the whole muscle from a sample of its fibers; 3. full characterization of a model of the FDI \textit{in vivo} is feasible, using ultrasound imaging and a custom-made dynamometer; and 4. muscle fascicles demonstrate a complex architecture with some fascicles arranged in series. In study 1 model predicted joint moments were sensitive to the FDI model parameter set used; joint moments differed between parameter sets by up to 884.3%. In study 3 muscle model parameters were determined for three subjects, these parameters showed variability between the subjects for example the maximum velocity of fiber shortening ranged from 4.7 to 9.7 optimum fiber lengths per second. These findings demonstrate variability at multiple levels of muscle in the FDI muscle, which suggests that other muscles may also exhibit this variability which could be investigated using the methods presented in this dissertation.
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CHAPTER 1

Introduction

1.1 General Introduction

Musculoskeletal models have many applications in biomechanics; for example, the investigation of orthopedic procedures such as tendon transfer and lengthening, the examination of the effects of strength training on muscle, and as an aid in the basic understanding of muscle function. Eykhoff (1974) described a model as “a representation of the essential aspects of an existing system (or a system to be constructed) which presents knowledge of that system in usable form”. Many of these musculoskeletal models use a Hill-type muscle model combined with parameters (e.g., muscle pennation angle, muscle moment arm, tendon stiffness) of the musculoskeletal system of interest to simulate the in vivo actions of muscles. Typically, assumptions are made about some or all of these parameters for the purpose of expediency, cost, or because their determination is prohibitive. These assumptions can influence the model output but the degree to which they affect the output has not been quantified thoroughly.

The force generated by muscle exhibits both a length (Gordon et al., 1966) and a velocity (Hill, 1938) dependent characteristic. In many musculoskeletal models, both of these general properties are assumed to be invariant between subjects and muscles and are scaled to subjects using geometric scaling (e.g., Zajac, 1989). For example, muscles are often assumed to work at or near the plateau of the force-length curve, producing near maximum force (assuming maximal activation) throughout their operating range. There is some evidence to suggest that this does not occur in all muscles and that different portions of the curve are used by different individuals or different muscles (e.g., Herzog et al., 1991; Lieber et al., 1994).

Individual sarcomeres exhibit force-length properties and an average sarcomere length is therefore often used to calculate the optimal length of whole muscle. Many models use measured sarcomere lengths to scale measured muscle length to optimum muscle length. The assumption in the model is that the muscle acts as a single scaled sarcomere with the optimal muscle length corresponding to optimal sarcomere length. The current methods of measuring sarcomere length are based on one of the two
following assumptions: sarcomere lengths along a muscle fiber are either equal in length, or exhibit a Gaussian distribution. The equation used to calculate sarcomere length from a diffraction pattern of a laser assumes the sarcomere’s spacing are of equal size (e.g., Lieber et al., 1990). Other methods involve counting a number of sarcomeres, measuring the distance that encompasses that number of sarcomeres and using those values to calculate an average sarcomere length (e.g., Wickiewicz et al., 1983). In this case, the average calculation implies a Gaussian distribution. However, neither technique may be accurate enough in light of the evidence of Huxley and Peachey (1961) that sarcomere lengths within a fiber are inhomogeneous. Some researchers have proposed that a non-uniform distribution of sarcomere lengths can explain muscle potentiation (Morgan, 1990). Muscle potentiation is defined as a larger than expected force, with the expected force based on the current state of the muscle (i.e. muscle length, velocity, and activation level). An alternative to current techniques for estimating optimum fiber length that incorporates sarcomere length inhomogeneity is counting the number of sarcomeres comprising a muscle fiber. This has, to date, not been reported in the literature, yet is a necessary component in understanding in vivo muscle mechanics. Finally, optimal muscle length is important when considering the maximum velocity of shortening. A muscle’s maximum velocity of shortening is based on the number of serial sarcomeres and the optimal length of a muscle is an indication of the number of serial sarcomeres (Faulkner et al., 1986).

The particular arrangement of muscle fascicles in a whole muscle would appear to have a functional significance (e.g., Gans and Bock, 1965). This arrangement, termed muscle architecture, has been measured predominantly in cadavers (e.g., Wickiewicz et al., 1983; Friederich and Brand, 1990) and is used in musculoskeletal models to determine the force the muscle exerts on the tendon. Unfortunately, there is a dearth of muscle architecture data in the literature and this is a limitation for musculoskeletal models. In addition to the lack of cadaveric measurements, there appears to be a large amount of variability in muscle architecture among individuals (e.g., Infantolino and Challis, 2010), making it difficult to generalize a muscle model based on a limited number of cadaver specimens or subjects imaged. A better solution is to perform in vivo imaging of subjects to create subject-specific musculoskeletal models. Finally, many models assume fascicles run from the proximal to distal tendon in a muscle (Koryak, 2008).
However, detailed fascicle dissection has indicated this may not be the case for some muscles (e.g., Heron and Richmond, 1993). This lack of information is detrimental because without knowledge of fascicle length and distribution, fascicles cannot be accurately modeled in musculoskeletal models. Muscle tissue has a force-length property and if a particular division of muscle (i.e. fascicles) has varying lengths then the force-length properties of the whole muscle may be affected by this.

A muscle transmits its force to bone via its tendon. The tendon is sometimes considered to be inextensible while other times tendon stiffness is included in musculoskeletal models (e.g., Zajac, 1989). Tendon stiffness has been shown to differ between genders and individuals (e.g., Kubo et al., 2003; Arampatzis et al., 2007) and by assuming a generic tendon stiffness a musculoskeletal model may be unable to accurately represent the in vivo muscle action of any particular subject.

Whole muscle contraction in vivo can cause joint rotation. The moment produced at a joint is the cross product of the force transmitted to the bone and the moment arm of the muscle. In musculoskeletal models, many times the moment arm of the muscle is based on skeletal architecture determined for an assumed generalizable sample (e.g., Delp et al., 1990). The skeletal architecture is often based on cadaveric measurements and there is evidence that muscle attachment locations vary among individuals even when allowing for variations in specimen size (e.g., Duda et al., 1996). Therefore, the ability to generalize muscle moment arms to individuals may be limited with this method of moment arm determination.

Many assumptions exist within current musculoskeletal modeling practice. In some instances the assumptions may be valid while in others the assumptions could produce inappropriate results. Many of the model inputs described by Zajac (1989) are easily measureable in vivo and therefore could be used to create subject-specific models that should be more accurate for a given subject than generic musculoskeletal models. The moment arm of a muscle can be calculated in vivo using the tendon excursion method (Lee and Piazza, 2008). Using the moment arm and measuring the moment-angle relationship of a joint, the force-length relationship can be determined for a bi-articular muscle (e.g., Winter and Challis, 2008). Tendon stiffness, which is the ratio of muscle
force on the tendon and the tendon stretch which occurs due to that force, can be measured using a quick stretch method (Cook and McDonagh, 1996) or by measuring the tendon stretch using ultrasound during muscle contraction (Arampatzis et al., 2005). As multiple muscles may insert into one tendon or multiple muscles may cross a single joint, the tendon stiffness for individual muscles may be difficult to measure. Muscle architectural measures such as muscle thickness, pennation angle, and volume can be measured in vivo using ultrasound (e.g., Narici, 1999; Infantolino et al., 2007).

Zajac (1989) identified four key measurements that heavily influence muscle model output: tendon slack length, the optimal length of a muscle fiber, the maximum whole muscle force, and a time constant used to describe muscle fiber shortening velocity. Pennation angle was identified as important but Zajac demonstrated that pennation angle only affected his model output when the pennation angle was larger than 15°. Tendon slack length, and maximal whole muscle force (estimated from imaged muscle cross-sectional area) are relatively easily measurable in vivo (e.g., Kawakami et al., 1995; Narici, 1999). In addition, the tendon stiffness can be estimated in vivo, for certain muscles, as well (Arampatzis et al., 2005). The time constant is used to scale the force-velocity property of muscle but a subject-specific force-velocity property can be determined (e.g., Camilleri and Hull, 2005). However, optimal muscle length is difficult to measure in vivo. Laser diffraction has been proposed for this purpose in vivo (e.g., Lieber et al., 1994) however a few problems arise with this technique. The first problem is that the laser diffraction technique is an invasive technique. This is in contrast to all of the other techniques mentioned above for measuring model parameters in vivo. The second problem is a question of accuracy. The equation traditionally used to calculate sarcomere length from a diffraction pattern assumes that the sarcomeres are equally spaced but there is evidence that this is not the case (e.g., Huxley and Peachey, 1961; Joumaa and Herzog, 2010). The inhomogeneity of sarcomeres must be examined to allow researchers to determine if it could affect the model parameters and therefore model output.

To determine the effect of subject-specific musculoskeletal parameters on musculoskeletal models a suitable muscle must be used such that motor redundancy is not a factor and thus, an indeterminate system is avoided. One such muscle in the
human body is the First Dorsal Interosseous (FDI) muscle (Eyler and Markee, 1954; Lauer et al., 1999). The gross musculoskeletal architecture has been described previously (Infantolino and Challis, 2010) which would allow for a musculoskeletal model to be constructed. Using cadaver-based data, the difference in model output can be assessed when using cadaver-specific inputs versus the input from the average of cadavers. In addition, subject-specific parameters can be measured in vivo using the methods previously described. By measuring kinetics and controlling the kinematics of the second metacarpophalangeal joint during abduction through the use of a finger dynamometer, additional muscle parameters can be estimated.

1.2 Purpose of Study
The purpose of these studies was to elucidate various aspects of muscle to aid in the production of more accurate musculoskeletal models. The studies in this dissertation focused on measuring subject-specific parameters, identifying individual sarcomere length variations, and investigating the distribution of fascicles within muscle.

1.3 Specific Aims
The specific aims of this study were,
1. To develop and validate a musculoskeletal model of the FDI based on cadaveric data. The purpose of this aim was to investigate the changes in model output based on different cadaver-specific model inputs.

2. To measure the length of each sarcomere along complete muscle fibers removed from cadaveric muscle. The purpose of this aim was to assess the distribution of individual sarcomere lengths in muscle fibers and its influence on estimates of optimum fiber length.

3. To develop and use a custom finger dynamometer that can control the kinematics of second metacarpophalangeal joint during abduction and measure kinetics and model inputs (muscle force-length and force-velocity characteristics, and tendon stiffness) on live subjects to produce subject-specific models. The dynamometer assisted in the measurement of dynamic muscle architecture and moment arm. The purpose of this aim was to investigate the feasibility of determining subject specific model parameters.
4. To investigate the fascicles of whole muscles using both dissection and imaging techniques. The purpose of this aim was to determine the distribution of fascicle lengths within the FDI and to investigate the arrangement of the fascicles in whole muscle, potentially providing insights into how fascicle orientation influences whole muscle function.

1.4 Study Overview
A musculoskeletal model of the FDI was developed based on cadaveric data. Using the model, and previously measured cadaveric data, average cadaver and cadaver-specific models were created to determine how cadaver-specific model outputs differ from average cadaver model outputs. Computer software was developed to automatically measure sarcomere lengths along muscle fiber images. The nature of the distribution of sarcomere lengths in cadaveric muscle fibers was assessed using detrended fluctuation analysis. A finger dynamometer was developed and used to measure some of the parameters for a musculoskeletal model of the FDI. The finger dynamometer controlled finger kinematics, which allowed for the measurement of the kinetics of the joint, muscle force-length and force-velocity characteristics, and tendon stiffness using the dynamometer and muscle architecture and moment arm when combined with ultrasound. These measures were used to characterize the FDI muscle in vivo so that a subject-specific model could be constructed for each subject. Connective tissue arrangement in intact FDI muscles was measured using three-dimensional image reconstruction of MR images. The lengths of all individual fascicles comprising whole muscles were measured, to compare fascicle length with whole muscle length.

1.5 Dissertation Structure
Chapter two contains the review of literature. Chapter three describes the musculoskeletal model of the FDI and the cadaver-specific model analysis (specific aim 1). Chapter four focuses on the measurement of individual sarcomere lengths along a muscle fiber, using automated sarcomere length measurement software (specific aim 2). Chapter five describes the finger dynamometer and the determination of subject specific musculoskeletal parameters (specific aim 3). Chapter six describes the results measuring connective tissue arrangement in intact FDI muscles using MR imaging and
the measurement of all individual fascicle lengths comprising whole muscle (specific aim 4). Chapter seven contains the discussion and conclusions of the dissertation.
References


CHAPTER 2

Review of Literature

2.1 Introduction
This chapter contains the review of literature pertaining to the construction of musculoskeletal models and the in vivo determination of parameters for those models. Section 2.2 discusses Hill-type muscle models. Section 2.3 describes the force-length property of muscle, how this can be modeled, and how model parameters can be determined in vivo. Section 2.4 describes the force-velocity property in muscle, how this can be modeled, and how model parameters can be determined in vivo. Section 2.5 describes the effect of fascicle arrangement on the force output of the muscle, how this can be modeled, and how fascicle arrangement can be determined in vivo. Section 2.6 describes tendon properties, how this can be modeled, and how model parameters can be determined in vivo. Section 2.7 describes moment arms of muscles, how these can be modeled, and how the model parameters can be determined in vivo. Section 2.8 is a summary of this chapter.

2.2 Hill-Type Muscle Models
One method of classifying models is to describe a model as either phenomenological or reductionist. Phenomenological models describe muscle using a black-box approach. With a black-box approach, the actual mechanism of muscle contraction is not important. The goal is to have the model adequately predict the action of a muscle given appropriate inputs. Reductionist models use the mechanisms of muscle contraction (e.g., cross-bridge formation rates) to predict muscle action. These models can be used to determine how the mechanics of muscle contraction change given a change in muscle action.

In 1957, A. F. Huxley proposed a reductionist model of the sliding filament theory of muscle contraction that was proposed in 1954 (Huxley and Niedergerke, 1954; Huxley and Hanson, 1954). In this description, the forward (cross-bridge attachment) rate was modeled as a function of the axial distance between an active actin binding site and a myosin head. The reverse (cross-bridge separation) rate was modeled as a function of the axial distance between actin and myosin; as well as the availability of a high-energy
phosphate (ATP). The model predicts the energy liberated as heat as a consequence of cross-bridge cycling. Using the data from A. V. Hill’s experiments on frog muscle, (Hill, 1938) the equations were fit based on the amount of energy liberated as a function of concentric contraction velocity (Huxley, 1957). This model has the advantage of incorporating the experimental observations of the sliding filament theory and the ability to explain the experimental results of Hill. Being a reductionist model that is focused on the single muscle fiber level, this model would be difficult to use at the whole muscle level. This difficulty arises as many other factors (i.e. muscle geometry and tendon stiffness) influence whole muscle function and these factors are not accounted for in the model. These factors can be accounted for, but would add a large amount of computation time. In addition, the parameters for cross-bridge mechanics have not been investigated nearly as much as the parameters for force-length and force-velocity properties as modeled in phenomenological models.

Zahalak (1981) proposed a lumped parameter model using three first-order differential equations that were a modification of Huxley’s 1957 model. The goal of this model was to simplify the cross-bridge kinetic models of muscle contraction so that they were computationally more efficient. This permitted modeling of muscle mechanics at the whole muscle level and not just at the single fiber level. The proposed model fit experimental data nearly as well as the Huxley model. The advantages of the model by Zahalak were that it could describe whole muscle function, and it proved to be computationally efficient.

Reductionist models are useful when attempting to elucidate the mechanisms by which muscle functions. Reductionist models are difficult to use for two reasons, long computation time and the parameters used in the models are not as well researched or familiar as those parameters used in phenomenological models. Due to these considerations, the rest of this section will focus on phenomenological models.

In 1938, A.V. Hill investigated how the velocity of muscle fiber shortening, length of muscle fiber, and activation level affect the heat liberated by a muscle fiber. Hill performed experiments allowing muscle fibers to shorten against varying masses while the muscle fiber was stimulated at varying intensities. During these experiments, the
heat liberated from the muscle was measured using a custom built thermopile. In addition, the heat liberated during isometric contractions was also measured for varying lengths of muscle fiber. It was found that the amount of heat liberated was dependent on the activation level, as well as the length at which the muscle was acting. A later study confirmed Hill’s assumption that heat liberated from muscle was also dependent on the contraction velocity of muscle (Hill, 1964). The muscle fiber length where maximal heat liberation occurs was described as the optimum length of the muscle. Hill described a model of muscle using elastic and dampening elements. The contractile element is an elastic element in series with a dashpot, and the contractile element is in parallel with a second elastic element. A muscle model that incorporates a velocity, length, and activation dependent contractile element in series with an elastic element has been called a Hill-type muscle model in reference to this work by Hill (Winters, 1990).

Bahler (1968) developed a phenomenological model of muscle contraction based on experiments performed on rat gracilis anticus muscle. A two component model of muscle was proposed with a contractile and series elastic element. The series elastic element was modeled with one equation describing the extensibility of the tendon when a force was applied to it. The contractile element force was described the following two equations: a force generator equation and an internal load equation. The force generator was length dependent and the internal load was velocity and length dependent.

The parallel elastic element was not included in the model; as the author stated that the series elastic element was much stiffer than the parallel component. The deformation in the series element will not allow the parallel element to reach a length where an appreciable amount of force can be generated. The rat muscle was placed in a set-up and subjected to isometric, isotonic, and quick release experiments to elucidate the properties of the series elastic and contractile elements. The quick release experiment results provided data on the tendon length change based on the force applied to the system. These values were used in an equation that did not have a direct physical representation of tendon but fit the experimental results. The isometric and isotonic experimental results allowed the researchers to fit equations that described the force-
length and force-velocity properties of the muscle. Again, the equations did not have any physical representation other than the equation fit the experimental results. One limitation was that the rest length of the muscle was used to normalize the developed equations. Muscle rest length may not be the same as optimal length in some muscles and therefore normalized results should be used with caution as the rest length of one muscle may not be in the same state of filament overlap as the same muscle in another subject (e.g. Lieber et al., 1994).

Zajac (1989) developed a phenomenological model to describe musculotendon function. Specifically, the model incorporated the force-length and force-velocity properties of muscle, and activation dynamics. The framework for the model was a Hill-type muscle model with a series elastic element (tendon) as well as a contractile element (Figure 2.1).

![Figure 2.1](image)

**Figure 2.1.** Schematic of muscle model developed by Zajac (1989). $F^T$ is the force applied to the tendon, $F^M$ is the force of the muscle, $w$ is the muscle thickness, $\alpha$ is the pennation angle, $\ell^T$ is the tendon length, $\ell^M$ is the length of a muscle fiber, $\ell^M_\alpha$ is the length of muscle belly, and $\ell^{MT}$ is the length of the musculotendon complex.

Dimensionless force-length and force-velocity curves were used to describe the contractile element. The series elastic element was a representation of the external and internal tendons of the muscle. This was modeled using a linear force-strain curve. The force-strain curve was scaled using maximal isometric muscle force and the slack length of the tendon. Tendon strain and stress were predefined based on material properties of...
tendons. Normalized tendon force is equal to normalized tendon stress. Tendon length under load is calculated from tendon strain and the unloaded length of the tendon. Muscle fiber pennation affects the amount of force that is transmitted to the tendon (Gans and Bock, 1965). Zajac demonstrated that only muscles which are pennated over 15° will result in a significant decrease in the force applied to the tendon. The parameters used to tune the model were tendon slack length, maximal muscle force, optimum muscle fiber length, and a time constant to describe the maximal shortening velocity of muscle. These properties were used to scale the dimensionless force-length, force-velocity, and activation dynamics equations. Tendon slack length and optimal fiber length were used to scale the contractile element dynamic equation. A limitation to this model is that the muscle is assumed to be a scaled version of a single sarcomere. This model, or slight variants, has been used extensively in a variety of models of human movement (e.g., Delp et al., 1990; Redl et al., 2007).

2.3 Force-Length Properties of Muscle
Ramsey and Street (1940) investigated the length-tension relationship of muscle in isolated frog muscle fibers. The fiber was mounted in an apparatus that allowed the investigators to control the length of the fiber and measure the tension it produced. At different lengths, the muscle was maximally stimulated and the tension produced was measured. Force was seen to increase as fiber length increased from a shortened state, then plateau. As the fiber was lengthened beyond the point of force plateau, the force was seen to diminish with increasing length. The length at which the force plateau occurred was called the optimum length of the muscle fiber. During passive tests, force increased significantly after the muscle fiber was lengthened past optimum length. No permanent damage was noted in the muscle fiber for contractions between 60 and 200% of optimum fiber length.

Gordon et al. (1966) verified the findings of Ramsey and Street (1940) using frog muscles. They used the same type of apparatus as Ramsey and Street but added a component that could image the muscle fiber. The authors placed two small pieces of gold leaf onto the muscle fiber and used a spot follower to control the distance between the two pieces of gold leaf. The distance between gold leaf pieces was fed to a servo motor which maintained a fixed distance between the gold leaf pieces. This allowed for
the development of an isometric contraction for the length of muscle between the gold leaf pieces. The force plateau was found to occur between 2.0 and 2.2 µm, with tension produced between lengths of 1.3 and 3.65 µm. Gordon et al. measured the tension developed by the muscle at various speeds and noted their results were similar to that of Hill (1938). The change in force seen during length changes was attributed to the varying overlap between actin and myosin. At long lengths, the overlap was slight, not allowing many cross-bridges to form. In a shortened state, actin filaments begin to interfere with each other and therefore formation of cross-bridges. At the optimum length, a maximal number of cross-bridges can be formed, producing maximal force (Figure 2.2).

To incorporate the force-length property of whole muscle into a whole muscle model, the optimal length of whole muscle must be known. One method of estimating optimal whole muscle length is to measure the length of sarcomeres in the muscle, and use that measurement to scale measured whole muscle length to optimal whole muscle length. Lieber et al. (1994) measured the length of sarcomeres in the wrist extensors through a range of wrist joint angles. Sarcomere lengths were measured using a laser diffraction device. The measurement of sarcomere length using laser diffraction is based on the fact that light will diffract when passed through a grating size equal in order of magnitude to the wavelength of light. If the light passing through the object is of a single wavelength (monochromatic) and coherent, then the diffraction pattern will be visible on a screen placed on the far side of the object. The measured sarcomere lengths were shown to change with wrist angle, demonstrating that laser diffraction could be used in vivo to measure sarcomere lengths. Two limitations to this method exist. The first limitation is that the procedure is invasive, making it difficult to perform in most situations. The second limitation is that implicit in the diffraction equation used is the assumption that the space between gratings is equal.
Huxley and Peachey (1961) used frog muscle to investigate the tension creep that is seen in isolated muscle fibers. Their hypothesis was that the creep was due to a non-uniform distribution of sarcomeres along the muscle fiber. An apparatus was constructed such that the length of a single fiber could be controlled, the tension produced by the fiber measured, and the fiber stimulated to produce tension. The apparatus held a section of the fiber stationary while the rest of the fiber could move freely. Photographs of the striation spacing along the muscle were taken via microscope. It was found that the striation spacing varied along the length of a muscle. As a general rule, the striation spacing at both ends of the muscle fiber was shorter than the spacing in the middle. The change in spacing was quite rapid and occurred only at
the extreme ends of the muscle fiber. This variation was consistent for different fiber lengths. A limitation to this technique is that only sections of the muscle fiber were imaged, and the sarcomere lengths were only an average of a group of sarcomeres so the individual variation in sarcomere lengths could not be determined.

Winter and Challis (2008) described a method for the determination of the force-length properties of biarticular muscles in vivo. The force-length relationship for the gastrocnemius muscle of twenty eight subjects was determined using a Biodex dynamometer. Subjects were asked to produce maximal voluntary plantarflexion contractions at varying knee and ankle angles. Muscle force was calculated based on the moment arm of the Achilles tendon and the moment output of the Biodex. The force produced at varying knee angles for each ankle angle was used to estimate the force produced by the gastrocnemius for each ankle angle. A change in knee angle produces a change in gastrocnemius length, but not soleus length, so by varying the knee angle at each ankle angle the force of the gastrocnemius could be estimated. The gastrocnemius force-length curves were defined as ascending, plateau, or descending based on where on the curve the peak force occurred. It was found that most subjects operated on the ascending limb while three subjects worked on the descending limb and one worked on the plateau. Two limitations to this study were the use of moment arm data from the literature and the assumption that the Achilles tendon was inextensible.

The force-length property observed in single muscle fibers is due to the overlap between the proteins actin and myosin. Sarcomeres can typically produce force between 50% below and 50% above its optimal length Gordon et al. (1966). Researchers have used measured sarcomere lengths to scale measured whole muscle lengths to optimal whole muscle lengths (e.g., Wickiewicz et al., 1983). This method using measured sarcomere lengths has limitations and methods exist for measuring the force-length property of whole muscles in vivo. The use of in vivo force-length property estimation for whole muscles allows for the incorporation of this property into subject-specific muscle models.

2.4 Force-Velocity Properties of Muscle

Hill (1938) performed experiments on isolated frog muscle at varying activation levels to measure the heat that is liberated during shortening of muscle. Hill found that when a
muscle shortens under varying loads, the heat produced by the muscle is constant for fixed lengths of shortening. The mechanical work done by the muscle is different in each condition, yet the heat produced is the same for each load. From these observations, Hill produced his now famous equation:

\[(P + a)v = b(P_0 - P)\]  

[2.1]

Where \(P\) is the tension in the muscle, \(P_0\) is the maximal isometric tension in the muscle, \(a\) is the shortening heat per centimeter for a muscle, \(b\) is a constant describing the absolute rate of energy liberation, and \(v\) is the velocity of shortening muscle. Hill (1938) reported that when \(a\) is multiplied by the distance the muscle shortens the resulting value is the amount of heat liberated during shortening. Hill was unable to accurately measure heat of lengthening and therefore only discussed the heat of shortening. Hill’s equation of the force-velocity properties of muscle is used extensively in models to describe the concentric force-velocity relationship of muscle (Figure 2.3).

Otten (1987) developed a model of the rat jaw musculature using morphological data to investigate the neural control of the jaw function of the rat. The force-velocity curve in the dynamic model is based on the equation described by Hill (1938). The model was used to predict the kinematics of the rat jaw and the results were compared with known jaw kinematics. The model was optimized with the objective to minimize the activation of the muscles. Once optimized, various parameters in the model were changed to determine what effect they have on the kinematics of the rat jaw. Otten found that when the mass of the jaw was reduced to zero or if the activation of the muscles were doubled the velocity of jaw closing remained fairly constant. This effect was attributed to the force-velocity curve in the model, which was in effect keeping the model stable. Otten concluded that the force-velocity curve is necessary in dynamic models for the preservation of stability. One assumption in this model is that the recruitment of muscles was homogeneous throughout the muscle. This assumption means that unlike live muscle which is recruited in motor units, the muscle model recruits the whole muscle but only at a fraction of its total capacity which is controlled by the activation level. This assumption is common in musculoskeletal modeling.
Camilleri and Hull (2005) examined the dependency of maximal shortening velocity and a shape parameter used in the equation developed by Hill in 1938 (equation 2.1) on the activation of muscle. Subjects were strapped to an ergometer with their shoulder at zero degrees of flexion, elbow at 95 degrees of flexion, and wrist at zero degrees of flexion. Subjects were required to contract their triceps brachii to produce 25, 50, 75, or 100% of their maximal isometric elbow extension moment for each trial. Once the required moment was reached and was maintained for one second, the restraint holding the elbow was removed and the forearm was allowed to move free. A forward dynamic simulation was used to predict forearm motion. The simulation used Hill's equation (Hill, 1938) as the force-velocity component of the model and the simulation was repeated for differing shape parameters and maximal shortening velocities. The agreement between the simulation result and the experimental result were used to determine if the shape parameter and the maximal shortening velocity parameter varied depending on the activation. Maximal shortening velocity was found to vary with activation, but the shape parameter did not. One limitation to this study was the use of values from the literature for muscle length and moment arm values.

**Figure 2.3** Force velocity relationship of muscle.
The force-velocity property in muscle is an important component in muscle models predicting dynamic actions. The equation developed by Hill in 1938 is still used in models today. The use of this equation indicates that modelers typically do not attempt to use subject-specific force-velocity parameters. The force-velocity property of muscles can be estimated in vivo and therefore this should be investigated to determine if subject-specific force-velocity properties need to be used in musculoskeletal models.

### 2.5 Geometrical Arrangement of Muscle

Gans and Bock (1965) wrote an article regarding the role of a muscle’s geometrical arrangement with respect to its function. This work is significant as it is one of the earliest works on muscle geometry in the modern literature. Gans and Bock began by describing the structure of muscle fibers, the force-length and force velocity properties of muscle fibers, fiber pennation angle, and contraction of the musculotendon complex. According to the authors, each fiber arrangement in a muscle allows that muscle to perform a specific task. For example, the fascicles of a sphincter muscle are arranged in a circular fashion; which allow the muscle to close a passageway. Pennation angle allows for more muscle fibers to attach to a fixed area of tendon, thus increasing the force applied to the tendon. The trade-off of pennation is a decrease in excursion of the muscle fibers. When muscle is pennated, the lengths of muscle fibers in the muscle decrease to maintain the thickness of the muscle. Muscle can only shorten to 50% below its optimal length and still produce force, thereby limiting the excursion of the muscle.

Many muscle models use the geometrical arrangement of muscle to guide the function of the muscle in the model (e.g. Pierrynowski and Morrison, 1985; Delp et al., 1990). Typically, the models rely on cadaver based measurements to provide the information about muscle geometry. The data from two studies are routinely used in musculoskeletal models (Wickiewicz et al., 1983; Friederich and Brand, 1990).

Wickiewicz et al. (1983) reported muscle physiological cross-sectional area (PCSA), fascicle length, sarcomere length, and pennation angle for three human cadaver lower limbs. The authors calculated optimal fascicle length using the measured fascicle and sarcomere lengths and the optimal sarcomere length of 2.2 µm (Gordon et al., 1966).
The authors then describe some observations they made while dissecting the legs, such as the fact that the PCSA of the muscles that oppose gravity was twice that of their antagonists. Numerous limitations exist with this study. First, the lack of cadaver data (age, sex, height, mass) removes any ability to match the measured cadaver data with live subjects or even to scale the measurements to live subjects. The number of cadavers ("three hemipelvectomy sections") also limits the conclusions that can be drawn from the measurements as it is difficult to obtain a normally distributed estimation of population statistics with only three samples. Finally, the optimum sarcomere length used (2.2 µm) was based on frog muscle. The authors should have used the value of 2.7 µm from Walker and Schrodt (1974) which was measured from human sarcomeres. The incorrect sarcomere length produces an 18.5% error. Unfortunately, the data presented by Wickiewicz et al. (1983) are not sufficient to construct a complete musculoskeletal model of the leg.

Freiderich and Brand (1990) reported muscle length, mean fiber length, muscle volume, PCSA, and pennation angle for the muscles in the leg of two human cadavers. The authors note a large amount of variability in the PCSA data and state that this variability would make PCSA difficult to calculate in a live subject based on their data. Freiderich and Brand did report cadaver age, height, mass, and sex. This study was limited by the number of specimens used. The specimens in the study covered a wide range of age (37 to 63 years old) and size (168 to 183 cm height and 59 to 91 kg mass). As with Wickiewicz et al. (1983) the data presented by Freiderich and Brand is not sufficient to construct a complete model of the leg.

Delp et al. (1990) presented a graphics based musculoskeletal model of the lower extremity using the muscle model described by Zajac in 1989. The muscle data from Wickiewicz et al. (1983) and Freiderich and Brand (1990) were used to define the muscle geometrical arrangement for the model with Wickiewicz et. al. (1983) being the primary source. Tendon slack lengths were calculated as the difference between the length of the muscle tendon complex based on the bone geometry and the muscle lengths reported in the literature. The bone geometry based muscle tendon complex length estimates were calculated as the distance between the origin and insertion of the
muscle at peak joint moment production \textit{in vivo}. A limitation to this study is the use of a mixed set of cadaver data to produce a single model.

Narici (1999) describes the ability to use Magnetic Resonance Imaging (MRI) and ultrasound to measure muscle geometry \textit{in vivo}. MRI can be used to measure muscle volume by measuring serial cross-sections of muscle on successive MRI images. Two serial cross-sections and the distance between the cross-sections can be used to calculate a section volume and the section volumes can be summed to produce the total volume of muscle. Ultrasound can be used to measure fascicle length and pennation angle non-invasively. Narici states that placing the ultrasound probe along the longitudinal axis of a muscle will allow the user to image the fascicles, pennation angle, and muscle thickness on a single image. Using these non-invasive techniques, the muscle geometry of most muscles in the human body can be determined for individual subjects, allowing for the creation of subject-specific models. Some limitations are due to the use of MRI for the estimation of muscle volume. The limitations associated with MRI include cost, small bore size, inability to image with metal implants, and lack of portability. Ultrasound is a relatively low-cost imaging device that is portable, can be used with any size subject, and is not contraindicated with metal implants, making it a viable option for researchers to determine muscle geometry \textit{in vivo}. A limitation of ultrasound is the requirement to place the probe in multiple positions to image all of each muscle.

Infantolino et al. (2007) described the use of ultrasound to estimate the volume of the vastus lateralis muscle. In this study, seven vastus lateralis muscles were scanned, \textit{in situ}, with ultrasound prior to dissection. The volume of the muscles were estimated using the method similar to that described by Narici (1999). Each muscle was then dissected out of the cadaver and its volume was determined directly using hydrostatic weighing. The authors found that the ultrasound method accurately estimated the volume of the vastus lateralis muscles. This technique allows for non-invasive determination of muscle volume on live subjects. Muscle volume can be used in conjunction with pennation angle to calculate PCSA, which is a parameter used in many muscle models. One limitation to this technique is that only superficial muscles can be effectively imaged using ultrasound.
One final assumption about the geometrical arrangement of muscle that should be addressed is the idea that muscle fascicles run from the proximal to the distal tendon and are the same length (e.g. Otten, 1988; Koryak, 2008). If this assumption is true, then the fascicles can be modeled together as one contractile element as the force-length and force-velocity properties of each fascicle would be equal to the force-length and force-velocity property of the whole muscle. However, if the fascicles are of differing lengths, then the force-length and force-velocity properties of the individual fascicles would differ, causing the whole muscle properties to be a more complex summation of the individual fascicle properties.

The geometrical arrangement of muscle is an important parameter in muscle models. Typically, this parameter has been based upon cadaveric values from the literature. Measurement of the geometrical arrangement of muscle in vivo is possible using MRI or ultrasound imaging techniques. Ultrasound has some advantages over MRI including cost, portability, and fewer contraindications.

2.6 Tendon Properties

Benedict et al. (1968) examined the stress-strain characteristics of unembalmed human tendons. Tendons were acquired from amputated lower limbs and were refrigerated for up to four hours before the tendons were dissected from the limbs. The cross-sectional areas of the tendons were measured then the ends of the tendons were wound with copper wire and clamped in a tensioning machine. The force on the tendon was measured using strain gauges. The tendon was stretched at a fixed rate until a predetermined load was reached. Once this load was reached, the tendon stretch stopped and the length of a section of tendon was measured and compared to its known, unloaded length to calculate tendon strain. After this measurement the tendon loading continued until the next predetermined load was reached. The results showed a large variation in the breaking stress (51 to 144 MPa) with flexor tendons slightly weaker than extensor tendons. The breaking strain was found to be between 7 and 10 percent. For unembalmed extensor tendons, tested prior to 12 hours after limb amputation, the stress-strain curve was non-linear and concave downward. Unembalmed flexor tendons exhibited a toe region at the beginning of the stress-strain curve and failed at a strain of 4%. Both flexor and extensor tendons demonstrated linear stress-strain curves when
tested 12 hours after limb amputation. One limitation to the study was the amount of
tendon slip that may have occurred between the tendon and the apparatus during the
loading process. The authors state that very little tendon slip did occur and conclude
this limitation is not significant compared with the results of the tests.

Pierrynowski and Morrison (1985) developed a musculoskeletal model of a human leg.
The muscles were modeled with a Hill-type muscle model comprised of a contractile
element in series with an elastic element. The values reported by Benedict et al. (1968)
were used in the formulation of the equation used to describe the length change in a
tendon based on the force applied to it and its cross-sectional area. The equation for
tendon deformation was:

\[
L_t = L_{to} \left( 1.0 + \frac{F_m}{E \cdot A_t} \right)
\]

Where \( L_t \) is the final tendon length under load \( F_m \), \( L_{to} \) is the slack length of the tendon, \( F_m \)
is the force the muscle exerts on the tendon, \( E \) is Young’s Modulus, and \( A_t \) is the cross-
sectional area of the tendon. The Young’s Modulus of 1400 N/mm^2 was calculated for
4% strain based on the stress-strain data of Benedict et al. (1968).

Arampatzis et al. (2005) measured, \textit{in vivo}, the strain of the human gastrocnemius
tendon and aponeurosis during a plantarflexion task. The left gastrocnemius was
investigated in twelve subjects. The subjects were placed in a Biodex dynamometer,
with the ankle in neutral (90 degrees), knee flexed at zero degrees, and hip flexed to 90
degrees. The subjects were asked to plantarflex at various increments of their maximal
voluntary isometric contraction level with a slow force buildup and decay. Kinematic
data were measured using a Vicon motion analysis system. Two ultrasound probes
were used to image the gastrocnemius tendon and aponeurosis. The kinematic data
was used to correct for any foot motion that may have occurred due to the compliance in
the dynamometer and therefore caused an error in the strain measurements. The slack
length of the tendon and aponeurosis was measured using ultrasound. It was found that
the aponeurosis elongated more than the tendon at higher contraction intensities and
this difference was statistically significant at contractions producing above 50% of the
total moment. Variability between subjects was also seen for both tendon and aponeurosis. In some subjects, during submaximal activation, the tendon experienced more strain than the aponeurosis and in other subjects, the aponeurosis experienced more strain. In addition, at maximal joint moment, tendon and aponeurosis strain varied between 3 and 9% in the subjects. At maximal activation the strains measured in the tendon and aponeurosis were equal. One limitation to this study, that was stated by the authors, was that the ankle axis and the axis of the dynamometer were not necessarily in line with each other.

Morgan (1977) described a technique to measure the active and passive components of muscle stiffness. The soleus muscle was isolated in anesthetized cats and the distal tendinous insertion was clamped to a load cell in series with a motor that controlled musculotendon length. The muscle was stimulated at a fixed stimulus rate to produce a tension on the tendon then the musculotendon complex was stretched by one millimeter at a rate of 50 millimeters per second using the motor. This stretch was performed at varying muscle lengths as well at varying stimulus rates. Force at the tendon clamp was measured. Change in tendon length is described by the equation:

\[
\Delta x = \Delta P \left( C_T + \frac{\alpha_0}{P} \right)
\]

[2.3]

Where \(\Delta x\) is the change in musculotendon length, \(\Delta P\) is the small deviation in measured tension after the stretch from isometric tension prior to the stretch, \(C_T\) is the elastic compliance of the tendon, \(\alpha_0\) is the stiffness dependant on the cross-bridges in a muscle, and \(P\) is the isometric tension prior to stretch. \(\alpha_0\) was regressed on \(P\) where \(C_T\) will be the slope of the regression line. Tendon compliance is represented by the value \(C_T\) and can thus be separated from the compliance of the cross-bridges, represented by \(\alpha_0\). By assuming the stiffness of the cross-bridges do not change with changing muscle tension then the y-intercept of the \(\alpha_0\) versus \(P\) graph \((\alpha_0)\) will be the stiffness of the cross-bridges. Cook and McDonagh (1996) used a modified version of this procedure to determine the stiffness of the first dorsal interosseous tendon in vivo.
The elastic properties of tendons are an important component of Hill-type muscle models for slow motions. Studies of isolated tendons indicate variability between tendons of different muscles (Benedict et al., 1968). In vivo studies demonstrate variability among subjects and between the tendon and aponeurosis. The determination of tendon stiffness is possible in vivo, allowing for the integration of tendon stiffness values into subject-specific muscle models. However, this tendon stiffness cannot be muscle specific if multiple muscles insert into a single tendon.

2.7 Moment Arms

A moment arm is the perpendicular distance between the line of force application and the center of rotation. Moment arms may be a fixed value or may vary as the object rotates. Muscles produce force, yet joints rotate, therefore to fully characterize a muscle’s function about a joint the moment arm of the muscle must be determined.

Two commonly used methods for determining moment arms are the geometric method and the tendon excursion method. For the geometric method, the joint center of rotation is estimated and the perpendicular distance between the line of action of the muscle and the joint center of rotation is considered to be the moment arm of the muscle (e.g. Rugg et al., 1990). In the tendon excursion method, the length change of the muscle is measured and regressed against joint angle changes. The moment arm is calculated by taking the derivative of the function fitted to the tendon excursion versus joint angle data (e.g. Brand et al., 1975; An et al., 1983). Pandy (1999) described the limitations of these methods and the complications associated with joints with multiple degrees of freedom and joints that translate as opposed to, or in addition to, rotate. The limitation to the geometrical method is the assumption that the joint acts as a single degree of freedom joint, which is not typical of most joints in the human body.

Brand et al. (1975) measured the moment arm of the finger flexors and extensors. Two fresh frozen cadaveric hands were used to measure the moment arm of the finger flexors and extensors. Tendon excursion was measured by determining the displacement of a marker on a suture attached to the tendon. Joint angle was measured via a video monitor. Tendon excursion and joint angle were used to determine the moment arm of the tendon. It was found that the moment arm of the finger flexors and
extensors remained relatively constant through the mid-range of motion but changed when the joint was moved in a range beyond 90 degrees of flexion.

Delp et al. (1990) presented a graphics based model of the lower extremity and to incorporate moment arms into the model, the following procedure was used. Bone surface geometry was collected from a human skeleton and the origin and insertions of each muscle in the lower extremity were defined. Muscles were assumed to move in a straight line from origin to insertion unless the muscle obviously wrapped around a bone or was constrained by a retinaculum. Muscles such as these were constrained to follow those paths using “via points” which did not allow a muscle to pass through a bone or “bowstring” off of a bone. Using this information, the bones were “moved” in the computer program to recreate joint motion and the change of the muscle length was recorded. With the musculotendon complex excursion data and the change in joint angle, the moment arm of the muscle could be calculated using the tendon excursion method. One limitation to this study was that the muscle paths do not explicitly take into account the volume of the muscles as the muscles are treated as line segments. This implies that all muscle fibers within a muscle have the same moment arm and this assumption has been shown to create errors in musculoskeletal models (e.g. Blemker and Delp, 2005). Another limitation is that the variability of muscle attachments on the femur (Duda et al., 1996) was not take into account. If there is variability between individuals in muscle attachment sites on the femur then there is a possibility that the moment arms for those individuals will be different by virtue of their attachment points.

Ito et al. (2000) described a method for measuring moment arms in vivo using ultrasound. The method uses the tendon excursion method to calculate the moment arm of a particular muscle. Seven subjects had their right foot strapped to a dynamometer such that the axis of the dynamometer was in line with the axis of ankle rotation. An ultrasound probe was aligned over the tibialis anterior muscle such that the insertion of a fascicle into the deep aponeurosis could be imaged throughout a set range of motion of the ankle. The rotation of the ankle was controlled using the dynamometer and the tendon excursion was manually measured from the ultrasound output every 5 degrees. A limitation to this study was that the fatigue level could have varied between successive images. Automated tracking software has been described which
automatically measures tendon excursion from a series of ultrasound images (Lee et al., 2008). This software uses a Lucas-Kanade algorithm to track the myotendinous junction of a muscle as a limb is moved through a known range of motion. This enables researchers to calculate the moment arm of muscle \textit{in vivo} rapidly.

MRI can also be used for the determination of moment arms. Rugg et al. (1990) used MRI images to determine the moment arm of muscles crossing the ankle joint using the geometrical method. Blemker et al. (2007) discuss the feasibility of either using a series of MRI images or using real-time MRI to measure tendon excursion \textit{in vivo}, allowing for the determination of muscle moment arms using the tendon excursion method.

The moment arm of a muscle is the way in which a muscle’s force is translated to a joint moment. Various methods exist for determining the moment arm of a muscle. Some musculoskeletal models estimate the moment arm based on the location of a muscle origin and insertion digitized from a single skeleton. Using ultrasound or MRI, the moment arm of a muscle may be determined non-invasively \textit{in vivo}.

2.8 Summary

The parameters used to construct a Hill-type muscle model are the force-length and force-velocity muscle components, geometrical arrangement of muscle, tendon stiffness, tendon slack length, muscle activation, and moment arm. Current muscle models use some or all of these parameters with varying degrees of subject specificity. However, most of the parameters can be determined non-invasively. These non-invasive methods can be used to produce subject-specific Hill-type muscle models which may be more accurate than generalized Hill-type muscle models. The sensitivity of Hill-type muscle model output to variations in muscle parameters needs to be investigated to assess which parameters are most important to determine for each individual subject.
References


CHAPTER 3

The Influence of Model Parameter Source on Muscle Model Output

3.1 Abstract
Many musculoskeletal models use cadaver data to provide some of the model inputs. However, there is a dearth of cadaver measurements in the literature and rarely do the sources provide enough information to allow for a complete musculoskeletal model to be constructed from a single cadaver. This lack of information leads to the question: how important are the model input parameters for the musculoskeletal model output? The purpose of this study was to investigate how the maximal moments generated from a cadaver-specific musculoskeletal model differed from the maximal moment generated from a model based on the average of the cadavers. This study focused on the First Dorsal Interosseous muscle due to its unique functional role as the sole second metacarpophalangeal joint abductor. The muscle model was constructed using architectural parameters measured from eight First Dorsal Interosseous muscles removed from cadavers. The parameters measured on the cadavers were, optimum fiber length, tendon slack length, physiological cross-sectional area, pennation angle, and muscle moment arm. For simulated static conditions differences between generated moments varied with tendon strain, cadaver-specific parameters, and moment arm. These differences in output are important as it indicates a need to use subject-specific muscle parameters when creating musculoskeletal models.

3.2 Introduction
Models of the musculoskeletal system are being used for providing insights into various aspects of human movement. For example, such models have been used to examine healthy gait (Anderson and Pandy, 2001), gait with cerebral palsy (Fox et al., 2009), jumping (Van Soest et al., 1993), and cycling (Neptune et al., 2000). The models can be considered to have three components: a model component representing the muscles, a model component representing system mechanics, and a model component representing the control of the system. With any model its performance is dependent on appropriate model formulation and having appropriate parameters for the various model components.
In models of the musculoskeletal system the model component representing the muscles requires parameters relating to each of the modeled muscles. These model components vary with the sophistication of the model, but irrespective of the model some reliance on cadaver data is inevitable. The key four parameters obtained from cadaver derived morphological data are tendon slack length, muscle cross-sectional area (from which peak isometric force can be derived), optimal fiber length, and fiber pennation angle (Zajac, 1989; Out et al., 1996). Imaging techniques do provide the opportunity for measuring some or all of these parameters (e.g., Blemker et al., 2007), but the use of cadaver based estimates is still the most common approach.

Although muscle models have proven a powerful tool for the analysis of human movement, there is, unfortunately, a dearth of data on which to parameterize these models. If models of the lower limb are used as an example the most common sources of data are muscle parameters from the studies of Wickiewicz et al. (1983) and Friederich and Brand (1990), yet these two studies dissected only three and two cadavers respectively. In fact neither study provides sufficient information to fully parameterize a muscle model so data from the two studies is often combined (e.g., Hoy et al., 1990). These studies raise the question of the appropriateness of combining data from different studies to specify the parameters required for a muscle model. There are some other studies which are more comprehensive in nature, for example Klein Horsman et al. (2007) provided a set of parameters for the major muscles of the lower limb from the detailed dissection of only one limb.

Given the value of musculoskeletal models but the corresponding lack of large databases of muscle model parameters it is of interest to examine how model parameters from different sources influence model performance. Therefore the purpose of this study was to examine the sensitivity of a model of muscle under static conditions to the parameters describing the model. Specifically muscle model parameters were determined from the dissection and analysis of eight First Dorsal Interosseous (FDI) muscles, these model parameters were then systematically input into a model of the muscle to examine the influence on model output of the source of the data.
3.3 Methods
In overview, data were collected from eight cadaver hands which provided inputs to a muscle model. Various permutations of the muscle model data set were input into the model to examine the sensitivity of its performance to these parameters. The FDI was selected for this study because in humans it is a rare instance of only one muscle being responsible for a particular joint motion: it abducts the index finger metacarpophalangeal joint (Masquelet et al., 1986).

3.3.1 Muscle Model Formulation
The muscle model used here was a Hill-type model similar in formulation to other models presented in the literature (e.g., Zajac, 1989; Slager et al., 1997; Lichtwark and Wilson, 2008; Miller and Hamill, 2009). The model consisted of a contractile element, and a series elastic element (Figure 3.1), with the force output of the model described by the following equation,

\[ F_m = q \cdot \cos(\theta) \cdot F_{\text{max}} \cdot F_L(L_F) \]  

[3.1]

Where,

- \( F_m \) - muscle force applied to the tendon,
- \( q \) - current active state of muscle model \((0 \leq q \leq 1)\),
- \( \theta \) – fiber pennation angle at current fiber length
- \( F_{\text{max}} \) - maximum isometric force possible by muscle model,
- \( F_L(L_F) \) - fraction of the normalized force-length curve that the model can produce at its current fiber length \( L_F \).
Figure 3.1. Schematic representation of the muscle model, showing how muscle forces related to pennation angle. All nomenclature are described in the main text.

The normalized force-length properties of the muscle model were represented by,

$$F_L(L_F) = 1 - \left(\frac{L_F - L_{F,OPT}}{wL_{F,OPT}}\right)^2$$  \hspace{1cm} [3.2]

Where,

$L_{F,OPT}$ - optimum length of muscle fiber, which is the length at which the fibers can produce their maximum force,

and $w$ is a parameter indicating the width of force-length curve.

The force-extension curve of the series elastic element is represented by,

$$L_T = L_{TR} + \frac{F_m c}{F_{max}} L_{TR}$$  \hspace{1cm} [3.3]

Where,

$L_T$ - the current length of the tendon,

$L_{TR}$ - the slack length of the tendon,
and $c$ is the extension of tendon under maximum isometric force as a fraction of tendon resting length.

It was assumed that the tendon had a linear stress-strain curve (Arampatzis et al., 2005).

Under the isometric conditions examined the muscle forces were determined using the following procedure. From the cadaver data for a given joint angle the length of the muscle-tendon complex was known. Initially muscle fiber length was computed assuming the tendon was at its slack length and the fibers comprised the remainder of the length of the muscle-tendon complex length. Muscle force was computed for this length. Given this estimate of muscle force, the tendon stretch was computed and muscle fiber length recomputed. The muscle fiber length was computed for this revised fiber length, and once again muscle force and tendon length computed. This procedure was repeated iteratively until no changes in fiber or tendon lengths were recorded.

### 3.3.2 Model Parameters from Cadavers

The necessary muscle model parameters were obtained from the dissection of eight cadaver hands. These data represent a sub-set of the data presented in Infantolino and Challis (2010). In the following the methods for data collection will be outlined, for more detail consult Infantolino and Challis (2010).

Eight hands from embalmed cadavers were dissected with the FDI muscle removed using blunt dissection. Pennation angles were measured in both heads using a standard goniometer. As the length of the fascicles varied between samples all pennation angles were referenced to optimum fiber length, this was achieved using a planimetric model of muscle geometry (Otten, 1988). Muscle thickness was assumed to remain constant irrespective of fascicle length and therefore other aspects of muscle geometry could be computed (Figure 3.1). The mass of each muscle was measured to the nearest 0.01 g immediately after dissection, and then used in combination with fiber length and pennation angle to compute muscle physiological cross-sectional area (PCSA; Narici, 1999). External tendon length ($L_T$) and muscle belly length were measured to the nearest 0.5 mm using a standard rule and a stereo dissecting microscope at 5x magnification.
From each FDI two muscle fascicles were removed from each head of the FDI and placed in 20% nitric acid to digest the connective tissue surrounding the muscle fibers (Close, 1964). Following acid digestion forceps were used to remove individual fibers portions of approximately 5 mm in length. Digital images of the fiber sections were taken from a light microscope and in these the number of sarcomeres counted. Given the length of the muscle belly, the length of these samples, the number of sarcomeres in each sample, and the optimal sarcomere length for human muscle fibers (2.7 µm; Walker and Schrodt, 1974) the optimum muscle belly length was computed. Optimal muscle belly length was computed since muscle fascicles were much shorter than the muscle belly and the entire muscle belly was acting on the tendon.

The moment arm of these FDI was estimated using the tendon-excision method (An et al., 1983). The moment arm was determined for the typical in vivo range of motion for the abduction of the second metacarpal-phalangeal joint of 20° (Kendall et al., 2005). Angles were defined so that 0° of abduction described the position in which the second distal phalanx was in-line with the second metacarpal (Figure 3.2).

![Figure 3.2. Joint angle definition for the second metacarpal-phalangeal joint. The center of the joint is the center of rotation for the angle measurement.](image)

The cadaver hands were placed in a specially designed rig which fixed all skeletal components while the second metacarpal-phalangeal joint was moved through a physiologic range of motion. During this motion both tendon excursion (via a linear cable extensometer), and joint angle (via a goniometer) were recorded. Varying order
polynomials were fit to the joint angle tendon excursion data, for each cadaver’s data polynomials of higher than a first order polynomial had residuals to the fit which were smaller than the noise level in the measurements, therefore the first order polynomials were used for subsequent analysis. As first derivative order polynomials were used the moment arm of the muscles were constant for the joint range of motion studied. Table 3.1 presents the individual and average cadaver basic anthropometry and muscle architecture parameters.

Table 3.1: For each of the cadaver basic anthropometry and muscle specific parameters.

<table>
<thead>
<tr>
<th>Hand #</th>
<th>Cadaver Height (cm)</th>
<th>Metacarpal Two Length (mm)</th>
<th>Optimum Belly Length (mm)</th>
<th>Tendon Length (mm)</th>
<th>PCSA (cm²)</th>
<th>Pennation Angle (Degree)</th>
<th>Moment Arm (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>161</td>
<td>62.4</td>
<td>70.1</td>
<td>3.0</td>
<td>1.67</td>
<td>22.5</td>
<td>1.8</td>
</tr>
<tr>
<td>2</td>
<td>161</td>
<td>55.1</td>
<td>84.6</td>
<td>2.0</td>
<td>0.85</td>
<td>20</td>
<td>2.6</td>
</tr>
<tr>
<td>3</td>
<td>180</td>
<td>55.8</td>
<td>64.8</td>
<td>5.0</td>
<td>2.01</td>
<td>17.5</td>
<td>2.2</td>
</tr>
<tr>
<td>4</td>
<td>180</td>
<td>48.6</td>
<td>66.9</td>
<td>7.0</td>
<td>1.82</td>
<td>14.5</td>
<td>2.5</td>
</tr>
<tr>
<td>5</td>
<td>174</td>
<td>68.1</td>
<td>73.1</td>
<td>0.0</td>
<td>2.61</td>
<td>15.5</td>
<td>1.9</td>
</tr>
<tr>
<td>6</td>
<td>174</td>
<td>48.4</td>
<td>135.1</td>
<td>4.0</td>
<td>0.93</td>
<td>19.5</td>
<td>1.6</td>
</tr>
<tr>
<td>7</td>
<td>169</td>
<td>51.0</td>
<td>65.3</td>
<td>2.0</td>
<td>2.25</td>
<td>17.5</td>
<td>2.3</td>
</tr>
<tr>
<td>8</td>
<td>169</td>
<td>45.4</td>
<td>64.2</td>
<td>2.0</td>
<td>2.63</td>
<td>22.5</td>
<td>1.9</td>
</tr>
<tr>
<td>Mean</td>
<td>171</td>
<td>54.3</td>
<td>78.0</td>
<td>3.1</td>
<td>1.85</td>
<td>18.7</td>
<td>2.1</td>
</tr>
</tbody>
</table>

3.3.3 Other Model Parameters
There are model parameters which were not determined from the dissection of the cadavers. Many muscle models treat these model parameters as generic, that is they are general across all muscles (e.g., Zajac, 1989). The specification of the following will be outlined: width of the force-length curve, tendon strain under maximum isometric force, and the active state of the muscle.

Isolated muscle fibers muscles can exert force for a length approximately 50% shorter than the optimum length and 50% longer that that length (Gordon et al., 1966). But the
spread of force-length curve is influenced by variation in optimum fiber lengths of the fibers comprising a muscle (Huijing, 1985). To account for this variation the parameter $w$ in equation 3.2 indicating the spread of the force-length curve was set to 0.7 (Challis, 2000).

Various sources have reported that tendon strain under maximum isometric force is 0.04 (e.g., Morgan et al., 1978; Woittiez et al., 1984; Bobbert et al., 1986), although lower values are sometimes used (e.g., 0.03 in Zajac, 1989). In vivo for the human gastrocnemius Arampatzis et al. (2005) found a range from 0.03 to 0.09 for 12 subjects performing maximum isometric efforts. Therefore in this study tendon strain under maximum isometric force ($\varepsilon$ in equation 3.3) was systematically varied from 0.0 to 0.08.

In equation 3.1 the force produced by the muscle is a function of the active state, which is assumed to reflect the recruitment as well as the firing rate, or rate coding, of the $\alpha$-motorneurons. In the static simulations performed the active state was assumed to be one.

### 3.3.4 Model Sensitivity

Simulations were performed to assess the effect of different parameters on the model output for simulations of the action of the FDI. Three simulation groups were used to investigate the effect of various parameters on model output. In the first simulation group, the focus was to determine the effect that tendon strain has on model output since tendon strain has been observed to vary in live subjects (e.g., Arampatzis et al., 2005) but models use single tendon strain values (e.g., Delp et al., 1990). The second simulation group focused on the ability of an average cadaver to represent a specific cadaver since many models use averaged cadaver values for many of their inputs (e.g., Delp et al., 1990). The third simulation group focused on the effect of using subject-specific moment arms with all other model parameters being cadaver-based since subject-specific moment arms are readily measured in vivo (e.g., Lee and Piazza, 2008).

There were three groups of simulations each investigating various aspects of muscle model sensitivity:
1. Effect of \( c \) on model output – the value of the tendon strain, \( c \), was varied for each of the cadaver parameter sets. Tendon strain values examined were 0, 0.04, and 0.08.

2. Effect of cadaver-specific model input – for each cadaver the moments it could produce were predicted for its specific model parameters. Then, for example, the accuracy of cadaver #1 being simulated using the data set for cadaver #2 was evaluated, and then for cadaver #3, and so on for all eight cadavers. This process was repeated for each cadaver, and the other cadaver parameter sets. Two other model parameter sets were also generated. The first was the mean of all of the cadaver data. The second set was based around the variability in the study of Wickiewicz et al. (1983). The Wickiewicz et al. (1983) data set was based on the dissection of three cadavers, and has been the basis of other models (e.g., Delp et al., 1990). Therefore the data from three cadavers were selected from the eight cadavers in this study so that the mean data obtained from three cadavers gave a coefficient of variation of 16% for the optimum fiber length. This coefficient of variation reflects the variability in Wickiewicz et al. (1983). For these simulations based on the FDI specific results of Cook and McDonagh (1996) \( c \) was set to a value of 0.04.

3. Effect of moment arm on model output – it is feasible to estimate moment arms \textit{in vivo} (e.g., Lee and Piazza, 2008) therefore the simulations in 2, were repeated but this time assuming that the cadaver specific moment arm were known. This reflects a situation which may occur, you can determine the muscle moment arms, but require cadaver data for the muscle model parameters. For these simulations based on the FDI specific results of Cook and McDonagh (1996) \( c \) was set to a value of 0.04.

To assess model performance, the moments were estimated at 101 angles throughout the range of motion for the static simulation performed using the cadaver specific data sets, the average of cadaver parameters, and the Wickiewicz et al. mean data sets. Maximum muscle force was calculated using the PCSA of the muscle multiplied by the specific tension of human muscle (410 kPa – Marx et al., 2006). The series of predicted
moments were compared by computing the percent root mean square difference, between the cadaver specific values and a data set used to approximate that cadaver. The percent room mean square difference (%RMSE) was calculated using the formula:

\[
\%RMSE = 100 \frac{\sqrt[n]{\sum_{i=1}^{n} (crit_i - est_i)^2}}{\sqrt[n]{\sum_{i=1}^{n} crit_i}}
\]

Where,
- \(crit_i\) – the \(i\)th criterion value
- \(est_i\) – the \(i\)th estimated value
and \(n\) is the number of data points

3.4 Results
For all simulations as the joint angle increased from 0 degrees to 20 degrees the moment produced decreased. With increasing angle the length of the FDI became shorter, which as the muscle worked on the ascending limb of its force-length curve for all simulations meant there was a corresponding decrease in muscle force, and therefore muscle moment. Therefore in all cases the peak moment occurred at 0 degrees.

The percent root mean square difference between no tendon strain and 4% strain and no strain and 8% was calculated using the model output for each cadaver muscle. As tendon strain increased, the percent root mean square difference between the no strain condition increased (Table 3.2). Figure 3.3 demonstrates the variation between tendon strain values for a representative muscle.

Table 3.2. The root mean square difference between model output of joint moments for no tendon strain, 4%, and 8% tendon strain. Moments were produced during isometric simulations throughout a 20 degree range of motion.
Figure 3.3. Joint moment produced using the full parameter set from muscle #3 during isometric simulation throughout a 20 degree range of motion for three different tendon maximum tendon strain parameters. Moments were normalized with respect to the maximum moment generated by muscle #3 at $c = 0.04$.

Different cadaver-specific models produced different outputs, the average model input as well as the Wickiewicz et al. average did not approximate the cadaver-specific muscles well, except in a few cases (Table 3.3). For all simulations, the tendon strain was fixed at 4%. Figure 3.4 is a representative graph of the muscles in the simulation attempting to approximate the output of muscle #3, while Figure 3.5 illustrates the maximum muscle force for each muscle, during the simulations.
Table 3.3. Percent root mean square differences between the model outputs of joint moments for eight cadaver-specific muscles, the average of the muscles, and the Wickiewicz et al. average. Moments were produced during isometric simulations throughout a 20 degree range of motion.

<table>
<thead>
<tr>
<th>Muscle</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>Average</th>
<th>Wickiewicz</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>46.3</td>
<td>87.8</td>
<td>77.8</td>
<td>120.5</td>
<td>77.7</td>
<td>92.9</td>
<td>104.4</td>
<td>31.3</td>
<td>101.8</td>
</tr>
<tr>
<td>2</td>
<td>84.8</td>
<td>-</td>
<td>245.5</td>
<td>227.0</td>
<td>305.5</td>
<td>59.2</td>
<td>254.4</td>
<td>276.0</td>
<td>141.9</td>
<td>271.1</td>
</tr>
<tr>
<td>3</td>
<td>46.8</td>
<td>71.5</td>
<td>-</td>
<td>6.0</td>
<td>17.6</td>
<td>88.1</td>
<td>6.3</td>
<td>8.9</td>
<td>30.2</td>
<td>7.6</td>
</tr>
<tr>
<td>4</td>
<td>43.8</td>
<td>69.7</td>
<td>6.3</td>
<td>-</td>
<td>24.7</td>
<td>87.5</td>
<td>9.0</td>
<td>15.2</td>
<td>26.2</td>
<td>13.6</td>
</tr>
<tr>
<td>5</td>
<td>54.8</td>
<td>75.9</td>
<td>15.0</td>
<td>20.0</td>
<td>-</td>
<td>89.9</td>
<td>14.8</td>
<td>7.8</td>
<td>40.7</td>
<td>9.3</td>
</tr>
<tr>
<td>6</td>
<td>347.4</td>
<td>144.5</td>
<td>739.1</td>
<td>695.2</td>
<td>884.3</td>
<td>-</td>
<td>763.0</td>
<td>813.7</td>
<td>487.4</td>
<td>802.1</td>
</tr>
<tr>
<td>7</td>
<td>48.2</td>
<td>72.0</td>
<td>6.2</td>
<td>8.3</td>
<td>16.9</td>
<td>88.4</td>
<td>-</td>
<td>8.2</td>
<td>32.0</td>
<td>6.5</td>
</tr>
<tr>
<td>8</td>
<td>51.1</td>
<td>73.8</td>
<td>8.2</td>
<td>13.3</td>
<td>8.4</td>
<td>89.1</td>
<td>7.7</td>
<td>-</td>
<td>35.9</td>
<td>1.6</td>
</tr>
<tr>
<td>Average</td>
<td>23.8</td>
<td>59.0</td>
<td>43.2</td>
<td>35.4</td>
<td>68.2</td>
<td>83.0</td>
<td>47.0</td>
<td>55.8</td>
<td>-</td>
<td>53.7</td>
</tr>
<tr>
<td>Wickiewicz</td>
<td>50.5</td>
<td>73.4</td>
<td>7.1</td>
<td>12.0</td>
<td>10.1</td>
<td>89.0</td>
<td>6.2</td>
<td>1.6</td>
<td>35.0</td>
<td>-</td>
</tr>
</tbody>
</table>

Figure 3.4. Graph illustrating joint moments for five muscles, including the average muscle, and a Wickiewicz et al. average muscle. The moments were generated during simulations of isometric contractions throughout a 20 degree range of motion and normalized with respect to the maximum moment generated by muscle #3.
Figure 3.5. Graph illustrating the maximum muscle force achieved for eight cadaver-specific muscles, average of muscles (#9), and Wickiewicz et al. average (#10). Forces were produced during simulations of isometric contractions throughout a 20 degree range of motion.

As different moment arms were used, some were able to approximate the output of a muscle better than others (Table 3.4). In addition, while one moment arm may have approximated one cadaver-specific output well, it may have not approximated another as well. Figure 3.6 is a representative graph of the model output of one muscle using various moment arms in the model parameters.
Table 3.4. Percent root mean square differences between cadaver-specific model outputs of joint moments and the model outputs of the model using different moment arms. Moments were produced during isometric simulations throughout a 20 degree range of motion.

<table>
<thead>
<tr>
<th>Cadaver Source of Muscle of Moment Arm</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle 1</td>
<td>-</td>
<td>17.2</td>
<td>9.2</td>
<td>14.8</td>
<td>1.7</td>
<td>6.3</td>
<td>11.7</td>
<td>2.2</td>
</tr>
<tr>
<td>Muscle 2</td>
<td>19.8</td>
<td>-</td>
<td>9.2</td>
<td>2.8</td>
<td>17.9</td>
<td>27.2</td>
<td>6.3</td>
<td>17.2</td>
</tr>
<tr>
<td>Muscle 3</td>
<td>5.1</td>
<td>4.7</td>
<td>-</td>
<td>3.3</td>
<td>4.2</td>
<td>8.4</td>
<td>1.4</td>
<td>3.9</td>
</tr>
<tr>
<td>Muscle 4</td>
<td>9.2</td>
<td>1.6</td>
<td>3.6</td>
<td>-</td>
<td>8.2</td>
<td>12.9</td>
<td>2.0</td>
<td>7.9</td>
</tr>
<tr>
<td>Muscle 5</td>
<td>0.7</td>
<td>7.2</td>
<td>3.4</td>
<td>6.1</td>
<td>-</td>
<td>3.5</td>
<td>4.6</td>
<td>0.3</td>
</tr>
<tr>
<td>Muscle 6</td>
<td>9.4</td>
<td>33.5</td>
<td>22.5</td>
<td>30.3</td>
<td>11.8</td>
<td>-</td>
<td>26.0</td>
<td>12.6</td>
</tr>
<tr>
<td>Muscle 7</td>
<td>9.2</td>
<td>4.5</td>
<td>2.0</td>
<td>2.5</td>
<td>7.9</td>
<td>14.1</td>
<td>-</td>
<td>7.5</td>
</tr>
<tr>
<td>Muscle 8</td>
<td>1.5</td>
<td>10.3</td>
<td>4.7</td>
<td>8.6</td>
<td>0.4</td>
<td>5.5</td>
<td>6.4</td>
<td>-</td>
</tr>
</tbody>
</table>

Figure 3.6. Representative graph of the effect of varying moment arm on model output. The muscle model parameters from muscle #3 were used for the simulations, but with the moment arms of cadavers 2, 4, 6, and 8. Moments were produced during isometric simulations throughout a 20 degree range of motion and normalized with respect to the maximum moment generated by muscle #3.

3.5 Discussion
The purpose of this study was to examine the sensitivity of a model of muscle under static conditions to the parameters describing the model. Model output for muscles were
compared for varying tendon compliance. The cadaver-specific outputs were compared to the model outputs from parameter sets of the average of the cadavers and the average of three cadavers that best represented the variance in the cadavers of Wickiewicz et al., 1983). Model outputs were compared for muscles with fixed moment arms while the other cadaver parameters were varied. The differences in moment production has implications for the use of cadaveric musculoskeletal parameters in musculoskeletal models as many models use one or more cadaveric sources for inputs to predict live subject motion. The simulations demonstrated that muscle strain does not have as large of an effect on the output of a static muscle model as using an average set of model parameters or variations in moment arm. The effect of these changes in parameters ranged from 0.3 to 884.3 percent root mean square error, indicating that some of the variations have little effect on the overall model output while other variations can have large effects on model outputs.

It was observed that as the tendon strain parameter increased, little change occurred in model output for the static simulations. However, the changes that were noted demonstrated that as tendon strain increased, the difference between the tendon strain condition and the no tendon strain condition increased. Neither the mean or Wickiewicz et al. mean data set were any more accurate at predicting the force for a specific muscle data set than any other muscle data set. Finally, by fixing the moment arm and changing the rest of the cadaver input parameters the results show that simply using cadaver-specific moment arms do not produce accurate cadaver-specific model outputs.

These results are specific to the FDI and may not generalize to other muscles. Of course the FDI, and its modeling, is of interest in its own right because the FDI due to its unique role in joint motion has been frequently investigated. For example, Milner-Brown et al. (1973) and Kornatz et al. (2005) used the FDI to examine motor unit recruitment, Davies et al. (1985) used it to examine strength training, and Cook and McDonagh (1996) to examine tendon stiffness.

Imaging methods allow for the in vivo estimation of some musculoskeletal parameters for the input into musculoskeletal models. Lee et al. (2008) demonstrated that ultrasound methods can be used to estimate the moment arm of a muscle in vivo. Blemker et al. (2007) described how Magnetic Resonance Imaging can be used to
obtain muscle architecture (such as muscle volume and pennation angle), joint motion, muscle moment arms, and muscle tissue deformation. By using these methods, subject-specific musculoskeletal model parameters can be obtained in order to produce potentially more accurate model output. However, even if the key parameters for a model can be determined from Magnetic Resonance Imaging, it would still be likely that parameters will be determined for a small number of samples and then extrapolated to other subjects. This option should probably be avoided because, as this study demonstrates, specific parameters in musculoskeletal models produce differing results. Conceptually it is a good idea that muscle model parameters obtained from a cadaver or cadavers can be scaled to live subjects. Pierrynowski (1995) presented a detailed procedure outlining how, based on geometric scaling principles, cadaver measured muscle parameters could be scaled to a live subjects. Given that databases of cadaver derived model parameters are available (e.g., Yamaguchi and Sawa, 1990; Van der Helm and Yamaguchi, 2000), such a scaling approach is attractive. For the FDI data in this study the correlation between cadaver height or the length of the second metacarpal, the available factors to use in scaling, and the muscle model parameters was generally low, and at best could express less than 50% of the variance in the data.

This study has shown that cadaver-specific musculoskeletal model parameters can produce different moment patterns under static conditions than the moments produced from average cadaver model parameters. This demonstrates that, at least for the FDI muscle, cadaver-specific model parameters are necessary for producing accurate static model outputs.
References


CHAPTER 4

Individual Sarcomere Lengths in Whole Muscle Fibers, and Optimal Fiber Length Computation.

4.1 Abstract

Estimation of muscle fiber optimum length is typically accomplished using either laser diffraction or by counting the number of sarcomeres in a portion of the muscle fiber, measuring the distance that encompasses those sarcomeres and dividing by the number of sarcomeres to obtain an average sarcomere length. If the sarcomeres are not uniformly distributed either of these techniques could produce errors when estimating optimum lengths. The purposes of this study were: to describe new software that automatically analyzes digital images of skeletal muscle fibers to measure individual sarcomere lengths; and to use this software to measure individual sarcomere lengths along complete muscle fibers to examine the influence of computing whole muscle fiber properties from portions of the fiber. Six complete muscle fibers were imaged using a digital camera attached to a microscope. The images were then processed to achieve the best resolution possible, individual sarcomeres along the image were detected, and each individual sarcomere length was measured. The software accuracy was compared to that of manual measurement and was found to be as accurate. In addition, the time to measure individual sarcomere lengths was greatly reduced using the software compared with manual measurement. The arrangement of individual sarcomere lengths demonstrated long-range correlations, which indicates problems in assuming only a portion of a fiber can be used to determine whole fiber properties. This study has provided evidence on the number of sarcomeres which must be analyzed to infer the properties of whole muscles.

4.2 Introduction

Muscle fibers have a characteristic relationship between their length and the force they produce. This force-length curve is determined by the proteins in the sarcomeres comprising a fiber. Force is produced when one protein, myosin, interacts with another protein, actin (Huxley and Niedergerke, 1954; Huxley and Hanson, 1954). The force produced varies according to the amount of overlap between these proteins. The relationship between sarcomere length and force output of the sarcomere is approximately parabolic in shape (e.g. Gordon et al., 1966). There is some evidence to
suggest that an inhomogeneous sarcomere distribution exists along a muscle fiber. Huxley and Peachy (1961) described a sarcomere distribution along a fiber where sarcomere lengths were shorter at the ends of a fiber and longer in the middle.

One parameter that has been used extensively to characterize the force-length curve of muscle is the muscle optimum length (e.g., Zajac, 1989). The muscle optimum length is the length of the muscle at which the muscle fibers can generate the maximum force isometrically. This length also provides important information about the function of the muscle. For example, Lieber (1993) used the ratio of fiber length to moment arm as an indicator of normalized fiber excursion in vivo. Alexander and Bennet-Clark (1977) used the ratio of fiber to tendon length to indicate the ability of the muscle-tendon system to store elastic energy. The optimum length of muscle as well as being a key parameter in models of muscle (Zajac, 1989), is a key indicator of muscle function in vivo.

In cadaver muscles, sarcomere lengths have been measured two different ways, either by viewing the sarcomeres with a light microscope (e.g., Sacks and Roy, 1982; Wickiewicz et al., 1983) or by laser diffraction (e.g., Sandow, 1936; Klein Horsman et al., 2007). Laser diffraction purportedly produces an average length value based on all of the sarcomeres that are illuminated with the laser beam. Inherent in the equation used to determine sarcomere lengths from a diffraction pattern is the assumption that the diffraction grating caused by the sarcomeres is of equal spacing. This assumption makes it impossible to study the variation in individual sarcomere lengths along a single muscle fiber. For the light microscope method, either a small number of sarcomeres among the total sarcomeres comprising a fiber are measured individually, or a large number of sarcomeres are counted along a known length and the average sarcomere length in that region is calculated. Measuring individual sarcomere lengths allows for the investigation of sarcomere length variability yet is extremely time consuming considering the number of serial sarcomeres in muscle fibers (between 2000 and 2500 sarcomeres per 10 millimeters of fiber; Williams and Goldspink, 1971). Counting sarcomeres over a measured distance only produces an average sarcomere length and does not evaluate the variance in individual sarcomere lengths.
Digital photography has made it possible to capture digital images from microscopes. This digital format can be exploited to aid in the rapid measurement and analysis of microscope images. The striation pattern of skeletal muscle fibers makes it an ideal image that can be analyzed automatically, speeding up sarcomere length measurement dramatically, compared with manual measurement. It therefore permits the determination of the length of all sarcomeres along the length of a fiber. The first purpose of this study was to describe a technique that uses computer software to analyze digital images of skeletal muscle fibers to measure individual sarcomere lengths automatically. The second purpose was to use the software to measure individual sarcomere lengths along complete muscle fibers to examine the influence of computing whole muscle fiber properties from portions of the fiber.

4.3 Methods

The following sections will first briefly describe the preparation of the samples used in this study. They will then provide a detailed description of the software used to process the sarcomere images, identify the sarcomeres, measure their lengths, evaluate the software performance, and conclude with a description of the use of the software to measure individual sarcomere lengths along single, complete muscle fibers.

4.3.1 Sample Extraction, Preparation, and Imaging

One complete First Dorsal Interosseous (FDI) muscle was removed from a cadaver using blunt dissection. On inspection the muscle appeared to be of typical dimensions for a FDI muscle (Infantolino and Challis, 2010). A single fascicle was removed from the muscle and placed in a 20% nitric acid solution for eight hours to digest the connective tissue holding the individual muscle fibers together. With the aid of a dissecting microscope at 25x magnification, six single muscle fibers were teased out and mounted on a glass slide using saline and a glass cover slide. Digital images were obtained using a digital camera (Cannon EOS 350D, 8 megapixels, Cannon, USA) attached to a light microscope (Nikon Microphot – FXA, Nikon, Japan) to produce an image with a field of view of 305 µm at 200x magnification, which were stored on a personal computer. To ensure that no portions of the fiber were missed or imaged twice, a motor driven scan stage was used (Nikon SD-100). This stage was accurate to 0.1 µm, enabling the user
to accurately move the stage while photographing the entire muscle fiber. This also allowed two sequential images to be spliced together, speeding analysis time.

4.3.2 Overview of the Software

The digital images were initially in RGB format and were converted to a black and white image using a two step process. Subsequent analysis of this black and white image permitted determination of the location of sarcomeres. Once the locations of all sarcomeres were known, the length of each sarcomere was measured using the Euclidean distance between sarcomere locations. The software was implemented in MATLAB (version 7.4.0) and used the Image Processing toolbox (version 5.4).

4.3.3 Image Processing

The image from the digital camera was in RGB format and was processed to a black and white image for sarcomere detection. Initially, the RGB image was converted to grayscale (Figure 4.1a) by using a weighted sum of the red, green, and blue values in the original image according to the formula,

$$BGR_{\text{Gray}} = 0.2989R + 0.5870G + 0.1140B$$

Where,

- $Gray$ – grayscale value of the pixel
- $R$ – red pixel value from the original image
- $G$ – green pixel value from the original image
- $B$ – blue pixel value from the original image

Next, the image was filtered using a 2-D median filter (Lim, 1990) to reduce the noise in the image but preserve the edges of the sarcomeres. Non-uniform illumination was corrected for by using the background of the image as a mask and then subtracting the mask from the original image (Gonzalez and Woods, 2002). The noise in the background also affects the object of interest. Subtracting the background from the entire image, removes the background noise on the object of interest. Once the background is subtracted, the intensity values of the image were adjusted so that 1% of the data is saturated at low and high intensities; which increases the contrast of the image. Therefore, by subtracting the background from the image and adjusting the intensity values, the object of interest in the image is enhanced.
On the resulting image a 2-D median filter was used again to remove any additional noise. Finally, the grayscale image was converted to a pure black and white image (Figure 4.1b) using a thresholding algorithm (Otsu, 1979). This algorithm seeks to maximize the separability between the background and the object. The digital record of the black and white image contained only values of zero or one in a matrix, the binary image matrix.

4.3.4 Region of Interest Selection

Using this binary image matrix, knots were placed along the image by the user to define the region of interest in the muscle fiber. The placement of the knots allows the software to avoid areas of the fiber that may not be conducive to measuring due to fiber damage or poor image quality. It also obviates the need to image the fiber only when it is straight. Placement of knots was done so that the string stayed as perpendicular to the sarcomeres as possible. For a whole fiber approximately 100 knots are placed along its
length. The knots were then used to create a string representing the pixels along the direct line between adjacent knots (Figure 4.2). A straight line was fitted between adjacent knots to predict points along the string. Based on this string, a region of interest was defined by including the twenty pixels above and below the string at every column location. Along the string, a window 41 pixels high and the length of the string was created by the user’s knots (Figure 4.2). This region of interest window was filtered using an average filter (Gonzalez and Woods, 2002) to reduce any remaining noise that would impede the sarcomere detection algorithm (Figure 4.1c). Based upon the irregularity seen in the sarcomere borders, (Figure 4.1c) a window of 41 pixels high was chosen to ensure that the irregularity in sarcomere borders was accounted for and did not bias the sarcomere length measurement. Alternative window sizes were explored, and 41 proved to produce the best compromise between capturing sarcomere border irregularities and minimizing time for computation.

![Figure 4.2](image.png)

**Figure 4.2.** Image of the user-defined region of interest on sarcomere images. The circles are the knots that the user selected on the image. The dark thin line is the string connecting the knots and the grey line is the area containing 20 pixels above and below the string, which produced the region of interest window. (The image is in grayscale to facilitate viewing the image, the string, and the region of interest in one image. In reality the software processes a black and white image.)

### 4.3.5. Sarcomere Detection

Using as an example a small region of interest (Figure 4.3), the rows were analyzed to determine the location and length of each individual sarcomere along the whole region of interest. A moving window of three rows constituted the analysis window. Averages
down each column were calculated using a moving window of three pixels tall. This produced an array, called here the average array, one row tall by the length of the region of interest window (Table 4.1). The average array could only contain values of 0, $\frac{1}{3}$, $\frac{2}{3}$, and 1.

Moving along an average array, a moving sum was computed using a window of three values (Table 4.1) that was compared with a predetermined edge detection threshold for the detection of the edge of a potential sarcomere. The edge of a sarcomere was defined as the transition from black pixels to white pixels (section A to B in Figure 4.3). The edge detection threshold was selected by examining different known sarcomere edges and calculating the moving sum for each edge. In addition, sections that could have been mistaken as edges were also examined. Moving sum values were calculated for these sections as well and the edge detection threshold was selected so that false edges were eliminated but true edges were detected. This edge detection threshold was 0.6. Once the moving sum was above this value, the edge of a sarcomere was considered detected.

**Figure 4.3.** Illustrative example of a region of interest window 42 pixels wide. Black squares represent a value of 0, white squares represent a value of 1. Only 19 of the 41 rows are shown here. Regions have been labeled A, B, C, D, and E to aid descriptions in the text.

Once a leading edge was detected, the moving sum was computed for three sequential average array values until the sum was above the sarcomere detection threshold value, which was 2.2. Once a sarcomere was confirmed (section B in Figure 4.3), the location
(column and row values) at which it was detected was recorded. After detection, the trailing edge of the white portion of the sarcomere was found using a trailing edge detection process based on the same algorithm as the leading edge detection routine (section B to C in Figure 4.3). Once the trailing edge was found, the edge detection routine was used to cross the black space (section C in Figure 4.3) after the white space; (section B in Figure 4.3) and to find the next potential sarcomere (section C to D in Figure 4.3). This process was repeated until the end of the average array. Once the end of the average array was reached, the average array was recomputed for the next set of three rows (i.e. rows 2, 3, and 4) and the sarcomere detection process began again. Due to this process, 39 average arrays could be produced from the 41 rows in the region of interest window. Once the sarcomere detection routine had run through all the rows of the region of interest window, the locations of all of the sarcomeres for each row in the region of interest window were stored. Other threshold values were investigated, but failed to be as accurate as the threshold values that were selected.

4.3.6 Sarcomere Length Measurement
The length of each sarcomere was determined by calculating the Euclidean distance between successive sarcomere edges. The string between knots was used to identify the sarcomeres; the parallel edges of each sarcomere were used as the reference for determining sarcomere length. Using this technique, individual sarcomere lengths were measured 39 times and by taking the average, the accuracy of the individual sarcomere length measurements was increased.

4.3.7 Evaluation
An image of a portion of a muscle fiber ~0.05 mm in length was used to validate the accuracy of the software. A red dot was placed on the processed muscle fiber image to indicate where the software detected each sarcomere. Two independent evaluators examined this image to determine if the software could accurately identify individual sarcomeres. One evaluator also measured sarcomere lengths manually by viewing the binary image matrix in the array editor of MATLAB. The distance in pixels between sarcomere edges was counted then converted to micrometers to measure the length of each sarcomere. The software was used to measure the same sarcomere lengths and the results were compared.
Table 4.1. Example of average array, and moving sum values based the first three rows of Figure 3, for the first 19 columns.

<table>
<thead>
<tr>
<th>Column</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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<th>7</th>
<th>8</th>
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<th>10</th>
<th>11</th>
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<th>19</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average Array</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>½</td>
<td>½</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Moving Sum</td>
<td>-</td>
<td>-</td>
<td>0</td>
<td>0</td>
<td>½</td>
<td>1</td>
<td>2</td>
<td>2½</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>
While the evaluator was measuring sarcomere lengths, the time to complete the measures was measured. In addition, the time to complete sarcomere length measurement using an Intel Pentium Core 2 Duo, 2.33 GHz, 1.96 GB RAM, computer was determined. These two measures were compared to determine how much time the computer software could save compared with manual digitization.

4.3.8 Sarcomere Length Analysis
To investigate the variability in individual sarcomere lengths, six complete muscle fibers from a FDI muscle were imaged and the software measured the individual sarcomere lengths along each entire fiber. For these six muscle fibers, the number of sarcomere along each fiber were known; using this information and the knowledge that optimal sarcomere length for human muscle is 2.7 µm (Walker and Schrodt, 1974), the criterion optimum length of each muscle fiber was calculated. Walker and Schrodt (1974) determined human fiber optimum sarcomere length from electron micrographs. From this information, the appropriate fraction of the total number of sarcomeres which must be measured to permit estimation of optimum fiber length was determined. In this study, it will be assumed that estimations of optimal fiber length within 5% of the criterion value are acceptable. The procedure selected for estimating optimum fiber length was based on the method used in a laboratory environment (e.g. Meijer et al., 1998). This method consists of counting the number of sarcomeres in a known length of muscle fiber. Using this value the total number of sarcomeres in the fiber is estimated (from which optimum fiber length can be computed). Samples of the entire fiber were selected to estimate whole fiber optimum length. The sample sizes were 10, 20, 40, 60, 80, 100, 200, 300, 400, 500, 600, 700, 800, 900, and 1000 sarcomeres, approximately between 0.3% and 45% of the total number of sarcomeres in a fiber. Each sample began at a randomly selected location along the muscle fiber to emulate the procedure of Meijer et al. (1998). Five hundred random locations were used for each fiber. The optimum fiber length was calculated for each individual sample size at each location. The mean and standard deviation of the optimal fiber length from each of the 500 random samples for each sample size were calculated.

In addition, the detrended fluctuation analysis (DFA; Peng et al., 1994) was used to determine the nature of the distribution in the sarcomere lengths along a single fiber.
The output of the DFA is the parameter alpha, which gives information about the analyzed data. For uncorrelated data (Gaussian distribution) alpha = 0.5. When 0 < alpha < 0.5, power law anti-correlations are present in the data and when 0.5 < alpha < 1 power law correlations (long-range correlations) are present. The DFA does not distinguish between signal components due to deterministic factors, and that due to random noise in the measurement process. Therefore, a surrogate data test was used to confirm that the results were reflecting the properties of the subject signal and not measurement system noise (Schreiber and Schmitz, 2000). To generate the surrogate data, an original data set is randomized by computing the fast Fourier transform (FFT) of the original data set, and then computing the inverse FFT of the signal with the phase of the FFT randomized (Theiler et al., 1992). For each data set 25 surrogate data sets were computed and the alpha values determined. These DFA values were then compared with the alpha values of the original data.

4.4 Results
Two investigators viewed the locations where the software had identified 20 sarcomeres. Both investigators determined that the software could accurately identify 100% of the sarcomere locations when compared with the investigator’s identified sarcomeres using visual inspection. One investigator manually measured the sarcomere lengths of 20 sarcomeres by counting the number of pixels between sarcomere locations. The accuracy was determined by comparing the range of computer measured values with the sarcomere values measured by the investigator. All investigator measured values which were within the software measured sarcomere lengths (out of 39 total measures per sarcomere) for each corresponding sarcomere along the fiber (Figure 4.4). Therefore, the software can accurately measure the length of each sarcomere.

The manual sarcomere measurement of 20 sarcomeres took approximately four minutes while the computer sarcomere length measurement took approximately 16 seconds with approximately 37.5% of the time for knot specification by the user. Writing sarcomere data to computer memory took 15.6% of the time, and processing the images took 9.4% of the time.
The remaining 37.5% of total time was devoted to image display, data retrieval, and other software processes. In addition, the manual measurement only made one sarcomere length measurement per sarcomere compared with 39 measurements made by the software. To measure lengths of all sarcomeres one time in a single muscle fiber (~2000 sarcomeres) manually it would take over six hours, while it would take only 47 minutes of computer time to measure that same number of sarcomeres 39 times per sarcomere using the software described here.

The software measured each sarcomere length along six whole muscle fibers. Figure 4.5 illustrates the length of each individual sarcomere plotted against the position of that sarcomere along the length of a single muscle fiber. This analysis shows sarcomere length inhomogeneity with sarcomere position. The DFA produced an average alpha value of 0.80 ± 0.08, indicating that long-range correlations existed in the distribution of the sarcomeres along each of the analyzed fibers. Surrogate analysis using the DFA indicated that the long-range correlations were due to the particular arrangement of the sarcomeres.
sarcomeres in each fiber, not measurement noise. Therefore, the sarcomeres lengths in these muscle fibers were not normally distributed.

**Figure 4.5.** Sarcomere length versus sarcomere position along a single representative muscle fiber.

As the number of sarcomeres sampled increased, two trends in the data were observed (Figure 4.6). First, as sarcomere sample number increased, the estimated optimal fiber length became closer to the actual optimal fiber length. Secondly, as the sample size increased, the standard deviation of the estimated optimal fiber length decreased. To illustrate the error that can occur in optimal fiber length estimation, the optimum length of each of the six fibers was calculated based on the shortest and longest continuous 60 sarcomeres (Table 4.2). These values are in contrast to the criterion optimum fiber length. Figure 4.6 illustrates that by using a limited number of sarcomeres, the estimated optimal fiber length is consistently higher than that of the criterion. Estimates of optimal fiber length tend to be overestimates when lower numbers of sarcomeres are used, this is because the distribution of sarcomere lengths is skewed.
Figure 4.6. Estimated optimal fiber length (Lfopt) versus percent of total sarcomeres for one fiber. The error bars represent the standard deviation of the 500 estimated optimal fiber lengths.

Table 4.2. Optimal fascicle length based on all the sarcomeres in a muscle fiber, the shortest sarcomere, and the longest sarcomere. Lfopt-min is the optimum fiber length based on the shortest 60 continuous sarcomeres. Lfopt-max is the optimum fiber length based on the longest 60 continuous sarcomeres.

<table>
<thead>
<tr>
<th>Fiber Number</th>
<th>Criterion Lfopt (mm)</th>
<th>Lfopt-min (mm)</th>
<th>Lfopt-max (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8.9</td>
<td>21.2</td>
<td>5.6</td>
</tr>
<tr>
<td>2</td>
<td>5.9</td>
<td>14.4</td>
<td>3.8</td>
</tr>
<tr>
<td>3</td>
<td>6.4</td>
<td>15.0</td>
<td>4.0</td>
</tr>
<tr>
<td>4</td>
<td>3.6</td>
<td>9.2</td>
<td>2.4</td>
</tr>
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<td>5</td>
<td>4.2</td>
<td>10.2</td>
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<tr>
<td>6</td>
<td>3.8</td>
<td>8.8</td>
<td>2.4</td>
</tr>
</tbody>
</table>

4.5 Discussion
The sarcomere length measurement software accurately detected sarcomeres and measured their lengths. The images produced from the software indicated that all sarcomeres were correctly identified. One investigator manually measured 20 sarcomere lengths and the software accurately measured the same sarcomere lengths. This indicates that the length measurement software is an accurate way to measure individual sarcomere lengths along muscle fibers. By automating this measurement...
process, the time to measure large numbers of sarcomeres was decreased dramatically compared with the human based manual method. Automation of the measurement process also reduced measurement error in two important ways. First, the software made 39 estimates of each sarcomere length and reported the average of those measurements, which reduces, by averaging, random errors. Secondly, manual measurement is subject to strain on the investigator (e.g., eye strain and boredom) which increases the likelihood of measurement error.

It is necessary to know the number of sarcomeres comprising a fiber within a muscle in order to assess the operating range of a muscle. For example, in many Hill-type models, a normalized force-length curve is used to describe the force-length property in muscle (e.g., Zajac, 1989). These force-length properties are dictated by the number of sarcomeres comprising a muscle fiber. Attempts to describe these force-length properties have counted sarcomere numbers in only a small portion of cadaver muscle fiber (e.g., Wickiewicz et al., 1983). The present study, for the analysis of whole fibers, has shown that sarcomeres are not homogenously distributed along the length of a fiber. Huxley and Peachey (1961) and Joumaa and Herzog (2010) have shown that such a non-homogeneous distribution is also present in live tissue. Figure 4.5 illustrates this sarcomere length inhomogeneity. It is feasible that by only manually counting sarcomeres for a portion of the fiber that this inhomogeneity is causing mis-interpretation of the force-length properties of muscle. This automated technique presented here has distinct advantages in terms of completeness and speed of analysis.

For the FDI at least, 35% of the total number of sarcomeres must be measured before an accurate estimate of optimum fiber length can be calculated (Figure 4.6). For the FDI, 35% of its total length represents approximately 700 sarcomeres. This value is in contrast to the 60 sarcomeres that Langenderfer et al. (2004) proposed as a target number required for the estimation of fiber optimum length. That value was not based on analysis of complete muscle fibers. Thirty five percent of total muscle fiber length is larger than what is typically measured in one laser diffraction measurement. Laser diffraction typically uses a laser beam of 1 mm in width (Lieber et al., 1984). With muscle belly lengths in the FDI muscle ranging from 45.0 to 80.0 mm (Infantolino and
Challis, 2010) the minimum number of diffraction measurements that are needed to accurately estimate optimum length would be 16 measurements.

Sarcomere length measurements are used to scale measured muscle fascicle lengths to optimal fascicle lengths, thereby determining in vivo where a muscle acts on its force-length curve (Winter and Challis, 2010). By incorrectly estimating a sarcomere length, the scaled fascicle length will also be incorrect. Muscle fibers can lengthen to 50% above their optimal length and shorten to 50% below their optimal length and still produce force (Gordon et al., 1966). The force-length curve of a single muscle fiber was calculated using the shortest and longest average of 60 continuous sarcomeres, as suggested by Langenderfer et al. (2004). This sarcomere average was used to scale the measured fiber length to optimal length. Figure 4.7 demonstrates the difference in the muscle fiber force-length curves.

**Figure 4.7.** Force-length curve for a muscle fiber based on longest (dashed green line) and shortest (dotted blue line) sarcomere lengths in the fiber. The solid black line is the force-length curve for the criterion optimum length fiber.
Long-range correlations were indicated by the DFA analysis. Measurement of many individual sarcomeres is the only way to elucidate the variability in individual sarcomere lengths. The equation used to estimate sarcomere length via laser diffraction assumes that the diffraction grating caused by the sarcomeres is composed of equally spaced gratings (Lieber et al., 1994). Since the sarcomere lengths are not equally spaced along a muscle fiber nor are they distributed in a Gaussian manner, laser diffraction must be used with caution to determine individual sarcomere lengths or optimum fiber lengths.

In summary, automated software was developed to detect and measure individual sarcomeres along the length of a muscle fiber image. The software was evaluated against manual detection and measurement, and was found to be as accurate as manual measurement. The software took considerably less time to measure the sarcomeres along a section of muscle fiber compared with an individual manually measuring the same sarcomeres. This software can be used in the investigation of sarcomere length distribution along a whole muscle fiber, as well as in the meat industry to examine the tenderness of meat (e.g., Smulders et al., 1990). The data from six complete muscle fibers indicate that approximately 35% of the total sarcomeres in a fiber must be measured to produce a estimate of optimum fiber length within 5% of the known optimum fiber length. DFA results indicate long-range correlations exist with respect to the individual sarcomere lengths along a muscle fiber. Laser diffraction cannot capture this subtlety. This study has provided evidence on the number of sarcomeres which must be analyzed to infer the properties of whole muscles.
References


CHAPTER 5
Measuring Subject-Specific Muscle Model Parameters of the First Dorsal Interosseous In Vivo

5.1 Abstract
Musculoskeletal models typically base some or all of their parameters on a data source other than the subject being modeled. There is evidence that cadaveric measurements do not scale appropriately to every subject. However, measurement of musculoskeletal model parameters in vivo has been described for many of the parameters necessary to construct a musculoskeletal model. The purpose of this study was to fully characterize a muscle so that an entirely subject-specific model may be constructed. The study focused on the First Dorsal Interosseous due to its unique anatomical arrangement and function as the sole abductor of the second metacarpophalangeal joint. The right First Dorsal Interosseous muscle of three subjects was examined in this study. Parameters were determined using ultrasound imaging and a custom-built finger dynamometer. Some parameters were measured directly (muscle and tendon lengths) while other parameters had to be estimated, for example by recording force and angle data and fitting the data to a force-length equation to estimate the parameters of the force-length curve. It was shown that full characterization of the muscle was possible and that variability existed in the musculoskeletal model parameters among the three subjects. This variability demonstrates the need for increased use of in vivo measurement to produce subject-specific musculoskeletal models.

5.2 Introduction
Eykhoff (1974) described a model as “a representation of the essential aspects of an existing system (or a system to be constructed) which presents knowledge of that system in usable form”. Models that represent the musculoskeletal system typically rely (directly or indirectly) on cadaveric measures for the architectural parameters necessary for the completion of the model (e.g., Pierrynowski and Morrison, 1985; Delp et al., 1990). When using cadaver-based measures to model a live subject, the inherent assumption is that the cadaver is similar to the live subject. In many cases, the anthropometric measures of the cadavers are not known, making it impossible to determine if the cadaver and live subject were similar in size and shape. In addition, the
force-length and force-velocity curves for muscle are typically scaled from generic force-length and force-velocity curves, not measured directly on the subject that is being represented in the model (e.g., Zajac, 1989). The assumption that the parameters required to describe the force-length and force-velocity curves do not vary between subjects is not consistent with some reports in the literature that indicate variability between subjects (e.g., Herzog et al., 1991; Camilleri and Hull, 2005).

Key components used in many musculoskeletal models include, the parameters used to describe the force-length and force-velocity curves, muscle architecture, tendon stiffness, and the moment arm of the muscle. The force-length and force-velocity parameters describe the force a muscle can produce at varying fiber lengths and velocities. Muscle architecture includes muscle cross-sectional area, pennation angle, and tendon resting length. Cross-sectional area is used to estimate the maximum isometric force that can be produced by the muscle (e.g., Narici, 1999). Pennation angle is used to determine the component of muscle force that is applied to the tendon. Tendon resting length can be used with a tendon stretch parameter to describe the length a tendon will be when a given amount of force is applied to it, assuming a linear force-length response of the tendon. Finally, the moment arm of a muscle determines the moment a muscle can produce about a joint given its force, and indicates the muscle-tendon complex length change for a given change in joint angle.

Variability in the key components of musculoskeletal models has been reported in the literature. Duda et al. (1996) found that variability existed in the attachment sites of muscles onto the femur and that this variability could not be accounted for by osteometric scaling between specimens. This indicates that osteometric scaling of cadaveric data may not produce subject-specific locations of muscle attachment sites. Infantolino and Challis (2010) reported variability among the muscle architectural parameters of the First Dorsal Interosseous (FDI) muscle. In addition, functional ratios for the FDI, such as physiological cross-sectional area to tendon cross-sectional area, did not reduce the variability, indicating that based on form, the function of each muscle differed among individuals (Infantolino and Challis, 2010). Herzog et al. (1991) demonstrated that the region of the force-length curve of the rectus femoris used by runners and speed skaters or cyclists differed. All of these examples serve to
demonstrate that the key components of musculoskeletal models can vary to an extent that a “one size fits all” approach may not be accurate enough for subject-specific musculoskeletal modeling.

Variability between individuals and the inability to reduce variability with scaling or normalization creates a barrier to the development of truly subject-specific musculoskeletal models. However, there are techniques that can be used in vivo to estimate the necessary parameters for musculoskeletal models. Narici (1999) has used magnetic resonance imaging (MRI) to measure aspects of muscle architecture and Infantolino et al. (2007) has used ultrasound to measure muscle volumes. Herzog et al. (1991) described a method in which the force-length relationship of a bi-articular muscle can be determined, while Camilleri and Hull (2005) described a method to estimate a shape parameter for the force-velocity curve of a group of muscles. The moment arm of a muscle can be determined using the tendon excursion method (An et al., 1983), using ultrasound and an automated tracking algorithm to track the tendon excursion (Lee et al., 2008). Arampatzis et al. (2005) used ultrasound to measure the length of the tendon, both at rest and during muscle contractions in order to estimate tendon properties.

The FDI muscle is a unique muscle in that it is the only muscle responsible for abduction of the index finger. This unique attribute makes it an ideal muscle to fully characterize using in vivo techniques to produce a subject-specific musculoskeletal model. There were two purposes to this study. 1. To describe a finger dynamometer that can be used to determine some of the properties of the FDI. 2. To fully characterize FDI muscles in vivo so that a subject-specific musculoskeletal model can be created and in vivo variability can be assessed.

5.3 Methods
The following sections will describe the model used to describe the actions of the FDI during index finger abduction at the index finger metacarpophalangeal joint, the development of the finger dynamometer, and the methods used to estimate the parameters necessary for the construction of a subject-specific musculoskeletal model.
5.3.1 Model of the FDI

The moment produced by the FDI at the index finger metacarpophalangeal joint during index finger abduction can be represented in equation form,

\[ M_J = r_{\text{muscle}}(\phi)F_m = r(\phi)q F_{\text{MAX}} \cdot \cos(\theta) \cdot F_L(L_F)F_V(V_F) \] [5.1]

Where,

- \( M_J \) - moment at the joint caused by the FDI,
- \( r_{\text{muscle}} \) - moment arm of the muscle at the index finger metacarpophalangeal joint at joint angle \( \phi \),
- \( F_m \) - muscle force,
- \( q \) - current active state of muscle model \((0 \leq q \leq 1)\),
- \( F_{\text{MAX}} \) - maximum isometric force possible by muscle model,
- \( \theta \) - pennation angle of the muscle
- \( F_L(L_F) \) - fraction of the normalized force-length curve that the model can produce at its current fiber length \( L_F \),
- \( F_V(V_F) \) is the fraction of normalized force-velocity curve that the model can produce at its current fiber velocity \( V_F \).

The details of each of the model components are described in the following sections. For each model component there are a set of parameters which must be determined. As far as feasible the model parameters were determined from experimental data. In these cases the parameters were determined so that the fit of the model to the available data minimized the sum of the squares of the differences between model output and subject output. For example, if for the force-velocity properties the task was to find the two model parameters \((V_{F,\text{MAX}}, k)\) by minimizing the difference between the experimentally determined muscle forces \(F_{\text{Expt}}\) and the model estimated forces \(F_{\text{Model}}\),

\[ \min \sum (F_{\text{Expt}} - F_{\text{Model}})^2 \] [5.2]

The model coefficients were computed using the Levenberg-Marquardt non-linear least squares algorithm (More, 1977).
To determine some of the model parameters other components of the model were required. For example, the force-length model parameters had to be determined before the force-velocity parameters because the maximal velocity of muscle fiber shortening (measured in optimum fiber lengths per second) is dependent upon the optimum length of the muscle fiber. Therefore the model parameters were determined in a fixed sequence (see Table 5.1).

5.3.2 Finger dynamometer construction
Commercially available dynamometers do not have attachments that can be used to control the second metacarpophalangeal joint. Therefore, a finger dynamometer was constructed in order to fulfill the following requirements. The dynamometer was required to control both finger angle and angular velocity while measuring the moment generated about the index metacarpophalangeal joint. By controlling both finger angle and angular velocity, some properties of the FDI can be assessed with the finger dynamometer as described below.

The finger dynamometer was built using a linear actuator (IAI Corporation, Torrance, CA, model: ERC-RA54-1-PM-12-100-WO1, motion range: 100mm, force range: 245 N; Figure 5.1). The actuator pushes the finger sled. Between the actuator and the sled is a three axis force sensor. A potentiometer is in parallel with the actuator. The force sensor (Kistler, Amherst, NY, model 9317B) has a range from 0 to 2000 N in the Z (axial) direction and 0 to 600 N in the X and Y directions with an accuracy of 0.01 N. The potentiometer (Maurey Instrument Company, Alsip, Il, model: M2326) has a range from 0 to 104 mm. The force sensor provides data on how much force is applied by the finger sled and the position sensor provides information on the location of the actuator and thus the finger angle. A computer controls the position, velocity, and acceleration of the linear actuator using a control program (PC Interface Software for RC, ver. 6.00.04.00-E, IAI Corporation, Torrance, CA) supplied by the actuator manufacturer. The overall accuracy of the system was tested and was found to be within 0.02 mm for controlling the actuator position and 0.2 mm for measuring the position of the actuator which lead to 0.01° and 0.1° error in joint angle respectively.
<table>
<thead>
<tr>
<th>Parameters</th>
<th>Measurement</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>A Muscle-tendon length (section 5.3.3)</td>
<td>Ultrasound imaging</td>
<td>Isometric finger abduction at a joint angle of 0°</td>
</tr>
<tr>
<td>B Moment Arm (section 5.3.4)</td>
<td>Ultrasound imaging, dynamometer</td>
<td>Isokinetic finger abduction 5°, range of motion 20°</td>
</tr>
<tr>
<td>C Tendon properties (section 5.3.5)</td>
<td>Dynamometer</td>
<td>Quick stretch protocol</td>
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<tr>
<td>D Force-Length (section 5.3.6)</td>
<td>Ultrasound imaging, dynamometer</td>
<td>Maximum isometric finger abduction at 2° intervals through the range of motion</td>
</tr>
<tr>
<td>E Force-Velocity (section 5.3.7)</td>
<td>Dynamometer</td>
<td>Isokinetic finger abduction at approximately (5°, 30°, 60°, 90°) through the range of motion</td>
</tr>
</tbody>
</table>

Note: please see text for definitions of the abbreviations for the parameters.
The force applied by the finger sled is converted into a moment by using the moment arm of the finger sled. The moment arm of the finger sled was calculated by measuring fixed lengths on the dynamometer which allowed for the calculation of the moment arm for incremental actuator length changes. The linear actuator is not rigidly fixed to the finger sled, and therefore the moment arm of the finger sled varies throughout the range of motion of the sled. The function (Figure 5.2) used to calculate the moment arm from the linear actuator length was,

$$r_{sled} = \sqrt{L_{act}^2 + L_{fix}^2 - 2 \cdot L_{act} \cdot L_{fix} \cdot \cos(\theta_{fix})}$$  \[5.3\]

Where,

- $r_{sled}$ – moment arm of the sled (Figure 5.3),
- $L_{act}$ – current length of the linear actuator,
- $L_{fix}$ – fixed length on the dynamometer (Figure 5.3),
- $\theta_{fix}$ a fixed angle on the dynamometer.

The lengths are based on the dynamometer, with $L_{fix}$ being a constant value, and $L_{act}$ being variable with one end coexistent with one end of $L_{fix}$ and the other is the contact point on the finger sled (Figure 5.3). Due to the variability in finger sled moment arm the angular velocities were approximate. The linear velocity of the actuator selected was that which produced the desired angular velocity at the mid-range of the finger sled. The maximum error in desired velocities throughout the range of motion was 7%.
Figure 5.2. Graph of moment arm of the finger sled versus linear actuator length from its origin, see equation 5.3 for definitions of moment arm and actuator position.

Figure 5.3. The fixed measurements on the finger dynamometer.
By positioning the second metacarpophalangeal joint center over the finger sled axis, the moment generated by the FDI will be equal to that measured by the finger dynamometer.

The finger dynamometer has three safety features. The computer, which controlled the linear actuator range, velocity, and acceleration, can be set so that the actuator does not go beyond a specified range for each of these. This range can be set for each subject. The mechanical stop can be set to any end range and was set for each individual. The mechanical stop was tested and the linear actuator cannot move beyond the position set by the mechanical stop. The comfort stop interrupts power to the linear actuator, removing all force on the finger immediately. Each subject placed their hand over the comfort stop during all trials.

5.3.3 Measurement of the Muscle-Tendon Complex Length of the FDI
The muscle-tendon complex length of the FDI was measured at zero degrees of abduction of the index finger metacarpophalangeal joint (Figure 5.4).

![Figure 5.4. Joint angle definition for the second metacarpophalangeal joint. The center of the joint is the center of rotation for the angle measurement.](image)

The finger was placed at zero degrees of abduction using the finger dynamometer and a 7.5 MHz ultrasound probe (SSD-1000, Aloka, Japan) in B-mode was used to image the entire FDI muscle-tendon complex. Subjects were asked to produce a slight isometric contraction to eliminate any slack in the muscle-tendon complex. A single image from
the ultrasound was captured using Scion Image software (NIH Image, version Beta 4.0.2, National Institutes of Health, Bethesda, MD). Scion Image was also used to measure the muscle-tendon length. The muscle-tendon length at 0 degrees of finger abduction was the reference muscle-tendon length \(L_{MT, REF}\).

### 5.3.4 Measurement of the Moment Arm of the FDI

The moment arm of the FDI muscle was measured using the tendon excursion method (e.g. An et al., 1983). The motion of the index finger was controlled using the finger dynamometer and the tendon excursion was measured from ultrasound which was recorded at 30 Hz. The subject was asked to perform a maximal voluntary contraction throughout the entire range of motion while the dynamometer moved at 5°/s. Tendon excursion was measured manually from the ultrasound images using Scion Image by one experienced investigator by digitizing the same recognizable point of the tendon for each frame (Figure 5.5).

**Figure 5.5.** Ultrasound image of FDI with tracked point on the tendon circled.

Moment arm, as calculated by the tendon excursion method, is the derivative of the excursion of the tendon with respect to joint angle.
\[ r_{\text{muscle}} = \frac{dL_T}{d\phi} \]  

[5.4]

Where,

\( dL_T \) – change in tendon length,

and \( d\phi \) is the corresponding change in joint angle.

A first order polynomial was fit to the tendon excursion data with respect to joint angle as higher order polynomials fitted the measurement noise. Given tendon excursion as a function of joint angle, differentiation of this function with respect to angle gives moment arm, which as a first order polynomial was used, produces a fixed value. Using \( L_{MT,REF} \), the actual length of the muscle-tendon complex \( (L_{MT}) \) can be determined for each joint angle,

\[ L_{MT} = L_{MT,REF} - r_{\text{muscle}} \cdot \phi \]  

[5.5]

5.3.5 Measurement of the Tendon Properties of the FDI

The stiffness of the tendon was measured using the procedure described by Cook and McDonagh (1996). The stiffness of muscle will vary with its activation level while the stiffness of the tendon will be invariant of muscle activation levels assuming a linear stress-strain curve for the tendon. Therefore, the tendon stiffness can be extracted from the muscle-tendon complex stiffness by varying the muscle activation level.

Muscle-tendon complex stiffness is the force applied to the complex divided by the deformation that the complex experiences. The index finger was placed in the finger dynamometer at 17 degrees of abduction to allow for an appropriate range for the finger to travel through during the trial. For each trial the subject was instructed to perform an isometric contraction and the contraction intensities varied from 30 to 100% of maximal volitional control in 10% increments. Once the subject achieved a steady force at the desired intensity, the actuator forcefully adducted the finger at 372 °/s over a range of 17 degrees. During each trial the force on the actuator was recorded. Using the moment arm of the finger sled, the moment produced by the muscle-tendon complex was
calculated throughout each trial. Then, the force of the muscle-tendon complex was
calculated using the moment and moment arm of the muscle-tendon complex. The
steady state force \((F_0)\) and the force halfway through the forced range of motion \((F_1)\)
were extracted from the force recordings for each trial. The subject-specific change in
muscle-tendon length \((L_{MT})\) halfway through the forced range of motion was calculated
\((\Delta L_{MT})\). The stiffness of the muscle-tendon complex \((K_{MT})\) was calculated using the
following formula,

\[
K_{MT} = \frac{F_1 - F_0}{\Delta L_{MT}}
\]  

[5.6]

Morgan (1977) described the equation that is used to separate the tendon stiffness from
the muscle fiber stiffness,

\[
\frac{F_0}{K_{MT}} = \frac{F_0}{K_T} \cdot a
\]  

[5.7]

Where,

\(K_T\) – tendon stiffness
and \(a\) is a constant related to muscle fiber stiffness

A single-order polynomial was fit to the \(F_0/K_{MT}\) versus \(F_0\) data with the slope of the line
equal to tendon compliance \((1/K_T)\) and the intercept equal to \(a\). The RMS error of the
linear fit was less than measurement error, therefore the first order fit was chosen.

5.3.6 Measurement of Force-Length FDI Properties
Measurement of the force-length property of the FDI was accomplished by placing the
finger sled of the dynamometer at two degree intervals throughout the joint range of
motion and having the subject perform a maximal voluntary muscle contraction at each
angle. Three contractions were performed at each angle to reduce the influence of
random error. The subject rested for one minute between each contraction to reduce
the likelihood of fatigue of the FDI. During each contraction, ultrasound was used to
capture an image of the pennation angle and thickness of the FDI (Figure 5.6).
Pennation angle and thickness were measured in Scion Image. The force generated by the FDI was calculated using the moment arm of the FDI and the measured moment of the FDI at the second metacarpophalangeal joint. The length of the muscle-tendon complex was calculated using the moment arm of the muscle, $L_{MT,REF}$, and joint angle. Using the force generated by the muscle, tendon slack length, and the tendon stretch parameter, the length of the tendon was calculated, and thus the effective length of the muscle belly was calculated. Thickness varied less than 1 mm over the full range of motion. Therefore, given this approximately constant thickness a planimetric model of muscle pennation angle can be used (Figure 5.7, Otten, 1988). Using the measured pennation angle and the muscle thickness ($t$) the muscle fascicle length ($L_{fac}$) was calculated (Figure 5.7; Reeves and Narici, 2003),

$$ L_{fac} = \frac{t}{\sin(\theta)} $$  \hspace{1cm} [5.8]
Figure 5.7. Planimetric model of one head of the FDI muscle used to calculate the muscle fascicle length ($L_{fac}$).

The muscle length data was adjusted by the measured pennation angle to account for the angle the fiber made with the line of action of the muscle. From the muscle force and length data, the force-length curve width parameter, $w$, the skewness parameter $s$, the roundness parameter $R$, the maximal isometric muscle force $F_{MAX}$, and the optimal muscle fiber length, $L_{F,\text{OPT}}$, were determined. The equation, under these isometric conditions describing muscle force was,

$$F_m = F_{MAX} \cdot \exp \left[ - \left( \frac{L_{F}}{L_{F,\text{OPT}}} \right)^{s} - 1 \right]^{R} \right]$$

[5.9]

5.3.7 Measurement of Force-Velocity FDI Properties
The force-velocity property was determined by measuring the moment generated by the finger during constant angular velocity motion of the finger sled. Subjects were asked to perform maximal voluntary efforts both concentrically and eccentrically. The effective muscle length ($L_{MT}$) was calculated using equation 5.5 and was used to account for the effect the force-length property of the FDI had on the force produced at each velocity. Changes in tendon length due to its compliance were accounted for in the same manner as for the force-length curve determination. Three trials were performed at each angular
velocity with a concentric and eccentric component to each trial. Eleven angular velocities were used (±15°/s, ±30°/s, ±60°/s, ±90°/s,…, and ±300°/s) for both the concentric and eccentric conditions. FDI force was calculated using the moment generated by the FDI at the second metacarpophalangeal joint and the moment arm of the muscle. Muscle-tendon complex velocity was determined using moment arm and the joint angular velocity. Muscle force, tendon slack length, and the tendon stretch parameter were used to calculate tendon length at each time step. Tendon velocity was subtracted from muscle-tendon complex velocity to obtain muscle velocity. Muscle fiber velocity was adjusted by pennation angle to account for the angle the muscle fiber made with the line of action of the muscle. From the muscle force and muscle fiber velocity the force-velocity shape parameter, \( k \), was determined. The equation, under these dynamic conditions describing muscle force was,

\[
F_m = F_{MAX} \cdot F_L(L_F) \cdot \left[\frac{(V_{F,MAX} - V_F)}{(V_{F,MAX} + k \cdot V_F)}\right] \quad \text{for } V_F \geq 0 \quad [5.10]
\]

The previously determined force-length parameters were placed into the equation and were allowed to vary by 1% of their previously determined value. This helped the fit converge to a physiologically reasonable solution. From the muscle force and muscle fiber velocity the parameter \( F_{SAT} \) was determined. The equation, under these dynamic conditions describing muscle force was,

\[
F_m = F_{MAX} \cdot F_L(L_F) \cdot \left[F_{SAT} - 0.5 \left[\frac{(V_{F,MAX} + V_F)}{(V_{F,MAX} - 2 \cdot k \cdot V_F)}\right]\right] \quad \text{for } V_F < 0 \quad [5.11]
\]

The previously determined force-length and force-velocity parameters were allowed to vary by 1% of their previously determined values which helped the algorithm converge to a physiologically reasonable value for \( F_{SAT} \).
5.4 Results

The model parameters for the FDI were determined for three subjects. Their characteristics were: Subject 1: 26 years old, 65.8 kg, 1.72 m, 9.1 cm index finger length; Subject 2: age 22 years old, 63.5 kg, 1.70 m, 7.8 cm index finger length; Subject 3: 26 years old, 72.6 kg, 1.63 m, 7.8 index finger length. The parameters describing their FDI are shown in Table 5.2. The right hand of all subjects were used as the dynamometer was designed to examine the right FDI muscle.

Variability existed in many of the parameters measured except for $R$ and $k$. The roundness parameter assumes only integer values which increases the likelihood that all individuals would have the same value. The force-velocity shape parameter did not vary as it did not seem to have a large effect on the fit of the equation compared with the $V_{F,\text{MAX}}$ parameter. This was determined by systematic variation of $k$ and $V_{F,\text{MAX}}$ and observation of the resulting fit of the equation.

The force-length curve for all three subjects was plotted on the same axes for comparison (Figure 5.8). Muscle force was normalized with respect to the largest maximal isometric muscle force (subject 1). The curves demonstrated that there were differences between the subjects in the optimal fiber length, the skewness, width, and the maximal muscle force. Due to variations in the skewness and optimum fiber length parameters, the curves are of differing lengths. The coefficient of variation of the optimum length (13.1%) was greater than the coefficient of variation of the subjects’ height (2.8%) or index finger length (9.1%).

The force-velocity curve for all three subjects was plotted on the same axes for comparison (Figure 5.9). Muscle force was normalized to each individual subject’s maximal isometric muscle force. The curve demonstrated the differences between the subjects in the $F_{\text{SAT}}$ and $V_{F,\text{MAX}}$ parameters, even though $V_{F,\text{MAX}}$ is normalized with respect to optimum fiber length.
Table 5.2. Subject-specific parameters determined \textit{in vivo} for the FDI muscle.

<table>
<thead>
<tr>
<th>Subject</th>
<th>$L_{MT, REF}$ (mm)</th>
<th>$R$ (mm)</th>
<th>$L_{TR}$ (mm)</th>
<th>$V/K_T$ (mm/N)</th>
<th>$\theta$ (deg)</th>
<th>$F_{MAX}$ (N)</th>
<th>$L_{F, OPT}$ (mm)</th>
<th>$w$ (-)</th>
<th>$R$ (-)</th>
<th>$S$ (-)</th>
<th>$V_{T, MAX}$ ($L_{F, OPT}$)</th>
<th>$k$ (-)</th>
<th>$F_{SAT}$ (% of $F_{MAX}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>68.4</td>
<td>14.8</td>
<td>21.8</td>
<td>0.0012</td>
<td>12.0</td>
<td>100.58</td>
<td>53.44</td>
<td>0.31</td>
<td>2</td>
<td>-1.20</td>
<td>9.69</td>
<td>4</td>
<td>1.73</td>
</tr>
<tr>
<td>2</td>
<td>55.7</td>
<td>8.9</td>
<td>12.9</td>
<td>0.0066</td>
<td>9.3</td>
<td>85.35</td>
<td>46.29</td>
<td>0.20</td>
<td>2</td>
<td>-0.67</td>
<td>4.70</td>
<td>4</td>
<td>1.5</td>
</tr>
<tr>
<td>3</td>
<td>49.5</td>
<td>12.1</td>
<td>17.5</td>
<td>0.0073</td>
<td>14.5</td>
<td>92.22</td>
<td>41.18</td>
<td>0.45</td>
<td>2</td>
<td>-0.71</td>
<td>5.36</td>
<td>4</td>
<td>1.5</td>
</tr>
</tbody>
</table>

\textbf{Note} – please see text for definitions of the abbreviations for the parameters.
Figure 5.8. Force-normalized muscle fiber force-length curves for three subjects. Subject 1 is the dashed line, subject 2 is the dash-dot line, and subject 3 is the dotted line. Force is normalized with respect to the greatest isometric muscle force (subject 1).

Figure 5.9. Force-normalized muscle fiber force-velocity curves for three subjects. Subject 1 is the dashed line, subject 2 is the dash-dot line, and subject 3 is the dotted line. Force is normalized with respect to each subject's maximal isometric muscle force and fiber velocity with respect to each subject's optimal fiber length.
5.5 Discussion

This study aimed to demonstrate the feasibility of fully characterizing the First Dorsal Interosseous muscle \textit{in vivo}. The results demonstrate that it is possible to do so and furthermore, the results demonstrate variability in the musculoskeletal model parameters between subjects. This variability has implications for the construction of musculoskeletal models that are used to describe a specific individual.

This variability is important for the construction of subject-specific musculoskeletal models for two reasons, first pertaining to the origin of the model inputs and second to the output of the model. In many musculoskeletal models the model input parameters are derived from cadaver measurements in the literature (e.g., Pierrynowski and Morrison, 1985; Delp et al., 1990) and some of the parameters used to describe the force-length and force-velocity curves of the muscles are assumed to be invariant between subjects (e.g., Zajac, 1989). Model validation by comparing the functional output of models (e.g., knee angular velocity throughout the gait cycle) for different subjects does not check the underlying parameters of the subject-specific muscles which may result in incorrect subject-specific parameters being adopted. While the incorrect parameters produce accurate recreations of known kinematics in one situation, the same parameters may not produce accurate recreations of a novel situation where no comparator exists and may provide misleading assessments of muscle function.

The subject-specific parameters determined \textit{in vivo} were compared with reported values in the literature to determine if they were realistic. $L_{MT,REF}$, $\theta$, and $L_{F,OPT}$ were within the cadaver values reported in Infantolino and Challis (2010). Both $r$ and $L_{TR}$ were higher than the reported values in Infantolino and Challis (2010) and An et al. (1983). The increased $L_{TR}$ value could be explained by the difference in measurement techniques as ultrasound may have picked up more tendinous material inside the muscle than the dissection technique. The difference in $r$ values may be due to cadaveric and live-subject values are being compared. By dividing $F_{MAX}$ by the specific tension in muscle (25N/cm²; Fukunaga et al., 1996), and multiplying by the cosine of the pennation angle, the physiological cross-sectional area of the muscle can be calculated. The calculated physiological cross-sectional areas for the three subjects were slightly larger than the range of values reported in Infantolino and Challis (2010). This is understandable,
however, since the values in the literature were cadaver based and not based on blood engorged muscles in live subjects. The tendon compliance \((1/K_T)\) for subjects 2 and 3 were within the values reported from Cook and McDonagh (1996) for live subject human FDI tendon, while subject 1 was smaller than the reported values. All values were smaller than those reported by Morgan (1977) for cat muscle. Hatze (1981) reported a \(w\) parameter of 0.28 based on the data from Gordon et al. (1966). The values in this study were higher than this value, however, Hatze’s parameter value is based on a single sarcomere, whereas the values in this study were based upon a whole muscle. Variability in fascicle lengths (as seen in chapter 6) would cause the \(w\) parameter to increase. The roundness parameter describes the roundness of the force-length curve and this roundness is expected to increase with increasing variability in the optimal length of muscle fibers (seen in chapter 3) and fascicles (seen in chapter 6). Skewness of the force-length curve is expected when the muscle is comprised of some fascicles that are arranged in series with one another and some fascicles that are not arranged in series. The skew parameters determined in this study reflect this serial arrangement, which was described in chapter 6. The skewness parameter value was negative, which indicated that the force-length curve would be shifted to the left. This shift makes sense when considering the serial arrangement of some fascicles. Only a few will have that arrangement, which would tend to extend the tail of the force-length curve on the descending limb. Faulkner et al. (1986) reported maximal muscle shortening velocity as six times the optimal muscle fiber length per second. Two of the \(V_{F,MAX}\) parameters determined in this study were below this value. The one value which was above could be explained by having a greater number of serially arranged muscle fascicles, which was demonstrated as possible in the FDI in chapter 6. The \(V_{F,MAX}\) values reflect the \(L_{F,OPT}\) values, which were higher than the average \(L_{F,OPT}\) reported in Infantolino and Challis (2010). Faulkner et al. (1986) reported a force-velocity shape parameter of 4, which is in agreement with the values determined in this study. Finally, Katz (1939) described the maximal eccentric force in isolated muscle to be around 1.6 times of maximal isometric force. The results in this study are in agreement with the values described by Katz (1939). Other studies have found maximal eccentric force \(in vivo\) to be about 1.2 times maximal isometric force (Doss and Karpovic, 1965; Westing et al., 1988). However, these studies examined more complex systems where multiple muscles contribute to a single joint action.
Some musculoskeletal models use a structural approach where each part of the model has a direct physiological representation (e.g. Huxley, 1957). Other musculoskeletal models use a phenomenological approach where the parts of the model do not necessarily have a direct physiological representation, but the model does produce a correct output for a given input (e.g. Bahler, 1968). This study combined both approaches to construct the final model. Some aspects of the model ($r$, $L_{TR}$, and $L_{F, OPT}$) have direct physiological meaning. However, other parameters ($w$, $R$, $s$, and $k$) do not have a direct of a physiological meaning. These parameters represent a general concept in the model (e.g., force-length curve shape) but do not relate directly to a component of a muscle.

A limitation to this study was the inability of the dynamometer to produce high velocities that would be near the maximum velocity of shortening for the FDI muscle. This limitation was due to the linear velocity limit of the actuator. Using a different actuator with a higher linear velocity would allow for the production of muscle velocities closer to the maximum velocity of shortening. This would help with the estimate of $V_{f, MAX}$ as less of the force-velocity curve would have to be extrapolated. With an increase in velocity though, the muscle activation parameter would be less likely to reach maximum, which was assumed in this study.

This study has described a series of methods used to fully characterize the FDI muscle for the purpose of constructing a musculoskeletal model of the muscle. It was shown that full characterization is possible for the FDI with parameters that are physiologically reasonable, which will allow for more detailed analysis of the function of the FDI in the future.
References


CHAPTER 6

The Arrangement of Fascicles in Whole Muscle

6.1 Abstract

Connective tissue is important in muscle both for the transmission of forces as well as dictating the arrangement of the contractile material in the muscle. It was hypothesized that the FDI will demonstrate a complex architecture indicated by a large range of fascicle lengths, and by the series arrangement of some of the fascicles. The fascicle level of muscle is typically ignored for either the muscle fiber level or the whole muscle level. However, the fascicle level of muscle does contribute to muscle function and therefore should be studied. Blunt dissection and Magnetic Resonance Imaging were used to investigate the number and length of all individual fascicles in whole muscle and the arrangement of the contractile tissue in the First Dorsal Interosseous muscle. The results indicate that fascicles are not the same length, and that the connective tissue is arranged in a complex manner within the muscle. Serially arranged fascicles were observed, and fascicle paths were not linear. These arrangements of the fascicles will influence the force-length and force-velocity properties of the whole muscle.

6.2 Introduction

In many models of human muscle, the structure of muscle is represented so that the muscle is assumed to behave as if it is a single myofibril (e.g., Koryak, 2008), albeit with greater ability to produce force. Data to parameterize such a model often comes from the dissection and analysis of muscle fibers (e.g., Wickiewicz et al., 1983). Of course, muscle structure is much more complex although a parsimonious representation of muscle has advantages as a simple model is more tractable than a more complex representation. A number of studies hint at the complex structure of muscle having a strong influence on its function and suggest a more complex representation of muscle is warranted in muscle models.

Loeb et al. (1987) found, in cat muscle, that many of the fibers are shorter than the muscle belly, they do not terminate at the tendon, and appear to be arranged in series with other muscle fibers. Fibers in series will influence the working range over which a
muscle can produce force, and increase the maximum velocity at which the muscle can shorten. In adult human sartorius and gracilis muscles Heron and Richmond (1993) found that these long muscles were composed of relatively short fibers arranged in series. Trotter (1993) has proposed that the connective tissue internal to the muscle can assist in the transmission of the force produced by the contractile material to the external tendon, removing the need for all of the fibers of a muscle to terminate at the external tendon.

Variations in muscle fiber lengths and arrangement can influence the function of whole muscle. For example, the force-length properties of a muscle composed of varying fiber lengths may not be the same as the force-length properties of a muscle with all fibers the same length (Challis, 2000). A muscle fiber can produce force between 50% above and below its optimal length (Gordon et al., 1966). It is conceivable that at a given whole muscle length, some fibers could be at a length where they could not produce force while others could be at a length where they produce maximal force. The velocity at which a muscle can shorten is a function of the maximum velocity of shortening of the fibers comprising the muscle belly (Zajac, 1989). This maximum velocity of fiber shortening is proportional of the length of the fibers (Faulkner et al., 1986), if some of the fibers are arranged in series then these fibers can be considered to act as a single fiber, effectively doubling their maximum velocity of shortening and concomitantly changing the maximum velocity of shortening of the whole muscle. Clearly both the length of the muscle fibers and the arrangement of these fibers can influence the function of the muscle they comprise.

Muscle fibers are bundled together to form fascicles. Simple blunt dissection of all the fascicles comprising a muscle should provide insight into the lengths of the individual fascicles comprising a muscle. The muscle fascicles are surrounded by connective tissue which can be identified via magnetic resonance imaging (MRI). Therefore by tracking the connective tissue surrounding the fascicles it should be possible to examine the arrangement of the fascicles within a muscle. Therefore there were two purposes to this study. 1. To assess, via blunt dissection, the number and the length of all the fascicles and fascicle tendons comprising the First Dorsal Interosseous (FDI) muscle. 2. To visually identify, via MRI, the path of the fascicles comprising the FDI. It was
hypothesized that the FDI will demonstrate a complex architecture indicated by a large range of fascicle lengths, and by the series arrangement of some of the fascicles.

6.3 Methods

The following sections describe the blunt dissection procedure for the analysis of fascicles comprising FDI muscles, the procedures necessary to prepare the FDI specimen for MRI analysis, and the software used to analyze the images from the MRI.

6.3.1 Fascicle Dissection Procedure

Four different FDI muscles were removed from two cadavers using blunt dissection. The cadaver characteristics were: Cadaver 1: male, 92 years old, 178 cm in height, cause of death – atherosclerotic heart disease; Cadaver 2: female, 56 years old, 174 cm in height, cause of death - hypothermia. Once the muscles were removed, they were soaked in a 20% nitric acid solution for approximately one half hour to facilitate the dissection process by digesting some of the connective tissue. Fascicles were dissected using blunt dissection with the aid of a Cannon RE-350 video visualizer (Cannon, New York, USA). Each fascicle was placed on a flat graduated surface and was imaged using the video visualizer and the dissection was recorded with a DVD recorder using the output of the video visualizer. Still images of fascicles were taken from the dissection videos. Subsequent analysis of the fascicle and fascicle tendon dimensions were performed on the recorded fascicle images.

The length of the whole muscle’s externally visible tendon was also measured, as was the length of the muscle belly using the same program with an image of the whole muscle. Once the muscle was fully dissected, the fascicle muscle belly length and the fascicle tendon lengths were measured using a custom MATLAB program (The MathWorks Natick, USA). A fascicle tendon length was defined as the length of connective tissue connected to either end of a fascicle muscle belly (Figure 6.1). These fascicle tendons were portions of the internal tendon that came free of the main aponeurosis with gentle teasing with forceps. The program had previously been validated for accuracy and repeatability using objects of known length. The accuracy of the measurement procedure was assessed to ±0.5 mm.
6.3.2 MRI Procedure

Two additional embalmed cadavers each had one FDI muscle removed for a total of two FDI muscles. The muscle was removed with the tendon intact using blunt dissection techniques described previously (Infantolino and Challis, 2010). To ensure the entire tendon was removed, the muscle was freed from its distal origins then reflected to allow a scalpel to ride along the bony, distal insertion until the entire tendon was separated from the bone. The cadaver characteristics were: Cadaver 3: female, 76 years old, 152 cm in height, 67.37 kg in mass, cause of death – end stage congestive heart failure; Cadaver 4: male, 72 years old, 160 cm in height, 72.60 kg in mass, cause of death – esophageal carcinoma. To reduce the MR scanning time the tissue was immersed in a 1.5% Magnevist (Bayer Health Care, Wayne, NJ) phosphor-buffered solution for 7 days. The achieved short T1 (33 ms) and T2 (7 ms) times allowed for fast imaging with a high contrast-to-noise ratio. To prevent the tissue from drying out and to minimize magnetic susceptibility artifacts (when a diamagnetic substance such as water distorts the linear magnetic field gradients) during scanning the specimens were surrounded by a flourinert liquid FD-43 (3M, St. Paul, MN). All experiments were conducted in a vertical 14.1 tesla Varian MRI unit (Varian Inc., Palo Alto, CA) with direct drive technology. A millipede resonator (Varian) with an inner diameter of 40 mm was used to acquire three-dimensional spin echo images of the muscle tissue. Typical muscle fascicles contain between 10 and 100 muscle fibers, with muscle fiber diameters between 50 and 100 µm (Jones and Round, 1990). A standard imaging experiment with an isotropic resolution of 75 µm comprised a field of view of 45 x 20 x 20 mm³ and a matrix size of 600 x 268 (75% partial Fourier: 201) x 268. With 12 averages and a repetition time of 75 ms (echo time 11.7 ms) the total scan time was 13.5 hours. MATLAB (The MathWorks, Inc., Natick, MA) was used for post-processing which converted raw data (in k space) to
visible images. By zero-filling each direction with a factor of two the pixel resolution of the standard imaging experiment was 37.5 μm³.

SPIERS software was used to analyze the MRI images (Sutton et al., 2001; Sutton et al., 2002). Connective tissue was identified using a thresholding algorithm. The thresholding algorithm assigns pixels, between user defined intensity values, to a segment. Each segment represents a different type of tissue. Intensity values varied between 0 and 255. Each object of interest was assigned to a mask by the operator. The boundaries of the muscle were assigned to a mask by one trained operator which allowed the entire muscle to be identified without including any noise in the image outside of the muscle. Three-dimensional objects were generated by creating triangles from the pixels that were simultaneously assigned to a specific segment and mask. This allowed for rapid and accurate identification of objects as only the pixels in the defined mask and connective tissue segment were considered to be part of that particular connective tissue object. Pixels in the mask that were in the muscle segment were not included in the connective tissue object, drastically reducing time to track individual connective tissue objects.

6.4 Results
The results from the dissection are presented initially, and then the imaging results.

6.4.1 Dissection Results
The number of fascicles in a muscle ranged from 61 to 101 fascicles per whole muscle. The dissection results demonstrated that the fascicles did not run from one end of the muscle to the other and are not the same length as the whole muscle belly. In addition, the tendon lengths measured on the fascicles were not similar to the external tendon lengths measured on the whole muscles (Figures 6.2 and 6.3).
6.4.2 MRI Results

In both muscles, the connective tissue was arranged in a spiraling fashion (Figure 6.4). The connective tissue made an approximately one quarter turn along the length of the muscle (Figure 6.5).
Figure 6.4. Connective tissue in the FDI muscle from an oblique view for cadaver #4. Arrows indicate the muscle line of action.

Figure 6.5. Two MRI derived images showing the connective tissue (teal) surround a fascicle spiraling around the FDI aponeurosis (blue), for cadaver # 4.

For only a portion of the approximately 80 fascicles comprising the FDI could the connective tissue surrounding each fascicle be tracked. Of the ten tracked fascicles in each FDI, these fascicles did not appear to extend from one end of the muscle to the other as the connective tissue did not run from one end to the other. In addition,
connective tissue was seen to be arranged serially, indicating that muscle fascicles were arranged in a serial fashion (Figure 6.6). Notice section V, VI, and VII are all arranged in series. Some sections of connective tissue were long (III) while others were much shorter (IV), which agreed with the dissected fascicle length results. For cadaver #4 of the ten tracked fascicles only four terminated at the aponeurosis; Figure 6.6 does not display two of these fascicles because they are located on the other side of the aponeurosis. For cadaver #3, three of the fascicles terminated at the aponeurosis, two were in series with each other.

![Figure 6.6](image)

**Figure 6.6.** The central aponeurosis (large blue structure), and the fascicles tracked in the MR image from their surrounding connective tissue, for Cadaver #4. Note the red (V), olive (VI), and African violet (VII) colored connective tissue strands arranged in serial fashion in the center of the image.

### 6.5 Discussion

The results of the dissections and MRI permit acceptance of the study hypothesis that the FDI will demonstrate a complex architecture indicated by a large range of fascicle lengths, and by the series arrangement of some of the fascicles.

This study aimed to examine the architecture of the FDI. Blunt dissection of the muscle fascicles comprising the FDI allowed analysis of their lengths. There was variability in the lengths of the fascicles comprising all analyzed FDI muscles. This variability has implications for the force-length properties of muscle. Clearly the properties of one
fascicle cannot be used to infer the properties of all the fascicles comprising a muscle, and the range of lengths will have impact on the force-length properties of muscle (Figure 6.7). Note from the figure that a variety of arrangements of the contractile structures in the muscle can produce different force-length properties for the combined structure.

**Figure 6.7.** Various arrangements of contractile elements seen in muscle, and the corresponding change in isometric force production, and in the maximum shortening velocity. The maximum velocity of shortening is represented by n optimum fiber lengths per second (lfopt/s), where n will vary with muscle fiber type (Faulkner et al., 1986).
While the analysis of fascicle lengths provided evidence of lack of uniformity of fascicle lengths the MRI analysis revealed a complex arrangement of these fascicles. The fascicles appeared to spiral along the length of the FDI, with some of the fascicles being arranged in series. Previously in long human muscle Heron and Richmond (1993) demonstrated some fascicles were serially arranged. This serial arrangement has implications for both the force-length and force-velocity properties of the whole muscle. These series arrangement will increase the working range of the muscle, and increase the maximum velocity of shortening (Figure 6.7).

While this study has been able to identify important aspects of the architecture of a specific muscle, it only provides a static snapshot of the muscle. In vivo there will be interacting forces which will be dictated by its dynamically changing architecture (Van Leeuwen and Spoor, 1993). In vivo the changing architecture of a muscle, the rat plantaris, has been measured but technical restrictions mean only superficial fibers can be monitored (Savelberg et al., 2001). Their study demonstrated helical deformation of the muscle caused by transverse forces between the fibers, presumably by the interdigitating connective tissues. Three-dimensional finite element models (e.g., Yucesoy et al., 2002), offer the potential to examine the complex architectural interactions in muscle with the static architecture from MRI providing model parameters and initial conditions.

A limitation to this study is the ability to generalize the findings to other FDI muscles and other muscles in the body. Currently, the MRI techniques used in this study can only be used to investigate isolated cadaveric tissues as the bore of the MRI is too small (40 mm) and the scan time too long (13.5 hours) to be feasible to study live subjects. This study used two muscles for the MRI data and four for the dissection data. Improved tracking software may be able to automatically identify and track the path of the fascicles comprising a muscle, further enhancing our knowledge of muscle structure and therefore function.

This study has offered insight into the complex architecture of the FDI muscle. Using blunt dissection, it was found that individual fascicles are not representative of the whole
muscle. MRI demonstrated that some of the fascicles were connected in series, which has important implications for muscle function.
References


CHAPTER 7

Discussion

7.1 Introduction
This chapter contains the discussion of the dissertation. Section 7.2 reviews the findings of the dissertation. Section 7.3 discusses the implications of the findings of the dissertation. Section 7.4 describes the limitations of the studies in the dissertation. Section 7.5 describes some future studies that arise from the studies in the dissertation. The final section, section 7.6, contains the conclusions of the dissertation.

7.2 Review of Findings
The preceding studies, Chapter 3, 4, 5 and 6, produced a number of findings regarding the biomechanics of the First Dorsal Interosseous (FDI) muscle.

The specific aim of chapter 3 was to use a musculoskeletal model to investigate how cadaver-specific model inputs affect model output compared to the model output from mean cadaver data. The purpose of this aim was to investigate the changes in model output based on different cadaver-specific model inputs. The parameters from eight cadaver muscles were used as inputs into a musculoskeletal model of the FDI as was the average of the eight cadaver parameters. The model demonstrated that cadaver-specific parameters produced different model outputs than an average cadaver musculoskeletal model.

The specific aim of chapter 4 was to measure the length of each sarcomere along complete muscle fibers removed from cadaveric muscle. This aim permitted assessment of the distribution of individual sarcomere lengths in muscle fibers, and the influence of this distribution on estimates of optimum fiber length. Chapter 4 described a computer algorithm that automatically measured the length of individual sarcomeres along a segment of isolated muscle fiber. Six complete muscle fibers were removed from a FDI muscle and imaged. Analysis of the individual sarcomere lengths demonstrated a non-uniform distribution along a whole muscle fiber, and long-range correlations of the sarcomere lengths with respect to their location along the muscle fiber.
The specific aim of chapter 5 was to develop and use a custom finger dynamometer that can control the kinematics of the second metacarpophalangeal joint during abduction and measure kinetics of the joint. The dynamometer combined with ultrasound imaging allowed for the determination of force-length and force-velocity characteristics, tendon stiffness, and muscle moment arm of subjects to produce subject-specific models. The purpose of this aim was to investigate the feasibility of determining subject specific model parameters. Chapter 5 described \textit{in vivo} methods used to estimate the parameters necessary to create a subject-specific musculoskeletal model of the FDI muscle. These parameters included those required to fully describe the mechanical properties of the FDI muscle were measured in three subjects. It was shown that full quantification of FDI for a musculoskeletal model is possible \textit{in vivo}.

The specific aim of chapter 6 was to examine the fascicles of whole muscles using both dissection and imaging techniques. The purpose of this aim was to determine the distribution of fascicle lengths within the FDI, and to investigate the arrangement of the fascicles in whole muscle, potentially providing insights into how fascicle orientation influences whole muscle function. Chapter 6 described the methods used to investigate the fascicle arrangement in the FDI muscle using both dissection and Magnetic Resonance Imaging (MRI). Four FDI muscles were dissected fascicle by fascicle and a further two muscles were imaged using MRI. The MRI images were used to construct a three-dimensional image of the connective tissue in the muscle. The connective tissue was used to track the path of the fascicles comprising the FDI. Both the dissection and MRI demonstrated that the contractile material in the FDI is arranged in a complex fashion. Varying fascicle lengths that are not representative of the whole muscle (dissection), and serially arranged and spiraling fascicles (MRI) are characteristics that contribute to the complex arrangement of fascicles within the FDI muscle.

These studies as a whole demonstrated that subject-specific parameters are important for accurate musculoskeletal model output, and muscle contractile tissue is arranged in a complex fashion. Many musculoskeletal models ignore these findings which is potentially detrimental to these models producing accurate output.
7.3 Implications of Findings

The findings from the studies comprising this dissertation have implications for the construction of musculoskeletal models. The implications of these findings will be described with the caveat that these results are pertinent to the FDI but similar results would be expected for at least other human muscles.

Chapter 3 demonstrated that cadaver-specific musculoskeletal model parameters produce different results than musculoskeletal models parameters based on the average of the individual cadavers. The findings from chapter 3 imply generic models may not produce subject-specific results. This is important for models that use multiple and/or incomplete cadaveric sources for their model input (e.g., Pierrynowski and Morrison, 1985; Delp et al., 1990) as these models may not even be able to accurately represent the cadaver that the model was predominately based upon. Furthermore, the use of these models to represent multiple living subjects will potentially lead to incorrect model output for a given subject.

Chapter 4 demonstrated that individual sarcomere lengths exhibited long-range correlations with respect to the length of the fiber. This implies that each individual sarcomere length influences other sarcomere lengths in the fiber. In addition, the sarcomere lengths are non-uniformly distributed along the muscle fiber length. The findings from chapter 4 imply that the laser diffraction technique used to estimate sarcomere lengths (e.g., Klein Horsman et al., 2007) cannot capture the variations in individual sarcomere lengths. This is because implicit in the equation used to estimate sarcomere length via laser diffraction is the assumption that all the sarcomere illuminated by the laser are of equal lengths. Therefore, average sarcomere length measurements made in cadaver tissue using laser diffraction should be interpreted with caution in light of the findings in chapter 4.

Chapter 5 demonstrated that non-invasive full characterization of the FDI muscle is possible for the construction of a musculoskeletal model. The findings from chapter 5 imply that subject-specific model parameter determination is possible for the FDI muscle in order to create a subject-specific musculoskeletal model. By creating a subject-specific model, the mechanics of the FDI muscle can be better understood. This increased understanding can be used to examine, for example, the effects of muscle
training on muscle properties. It also demonstrates that producing subject-specific models is possible and should be undertaken more often as opposed to using piece-meal cadaver data to construct a model. However, some of the methods are not as feasible for other joints where multiple muscles are responsible for a joint action.

Chapter 6 demonstrated a more complex arrangement of contractile tissue than is typically expressed in musculoskeletal models. The findings from chapter 6 imply that the ability to visualize complex architecture provides guidance for appropriate model formulation (e.g., Yucesoy et al., 2002). This increased guidance helps explain observations that may be considered erroneous otherwise. For example, the serial arrangement of muscle fascicles can explain the observation in chapter 5 of a maximum shortening velocity of a whole muscle to be higher than the 6 $L_{F,OPT}/s$ maximum reported in Faulkner et al. (1986). Each individual fascicle can maximally shorten at a fixed rate but if the fascicles are serially linked, the composite two fascicles will have a maximum shortening twice that of the individual fascicles.

The studies in this dissertation focused on various aspects of the FDI muscle. Variability was demonstrated at multiple levels of the muscle. At the sarcomere level, the lengths of individual sarcomere lengths along single, complete, muscle fibers demonstrated long-range correlations. At the fascicle level of muscle, the lengths of fascicles were seen to vary and not be representative of the whole muscle that they were dissected from. At the whole muscle level, both cadavers and live subjects demonstrated variability in the musculoskeletal model parameters that were measured.

The range of observed variability in the studies of this dissertation on multiple muscle levels implies that simple geometric scaling of cadaveric parameters may not be enough to produce accurate, subject-specific musculoskeletal models. However, with the findings of the studies in this dissertation the interaction between the different levels of muscle may begin to be investigated. For example, the sarcomere and fascicle length variability may help explain the skew and roundness parameters of the $in vivo$ force-length curve demonstrated in chapter 5.
7.4 Limitations of Studies

In chapter 3 the major limitation was the data on which the model was formulated was cadaveric, and therefore not all parameters were able to be measured \((c, w, q,\) and specific tension). This limitation reflects the limitation that many musculoskeletal models have. However, by assuming that the values that were not measured were invariant between cadavers the influence of the variability in the cadaver based parameters was highlighted. All of the cadavers measurements used chapter 3 to parameterize a muscle model followed well-established practices for the in vitro measurement of muscle architecture. The muscle architecture values are combined and used extensively in musculoskeletal modeling (e.g., Delp et al., 1990). The results from chapter 3 should be considered in light of the results of chapters 4, 5, and 6. In chapter 4 it was shown that, using 60 continuous sarcomeres does not necessarily produce an accurate measure of optimum muscle fiber length, suggesting the optimum fascicle lengths were poorly estimated. The results from chapter 3 do not agree with the results in chapter 5 in an absolute sense, however, this does not detract from the findings in chapter 3 as it was designed to explore the influence of cadaver based parameters on model output. The results in chapter 3 suggest that different cadaveric values cannot be combined and used to approximate specific muscles. Chapter 6 shows that some fascicles are arranged in series, effectively making them one long fiber, this would indicate that muscle belly optimum length was probably under estimated in chapter 3.

In chapter 4 the limitation was that embalmed tissue was used instead of fresh or live muscle tissue. The use of embalmed tissue is common in the biomechanics literature (e.g., Wickiewicz et al., 1983; Friederich and Brand, 1990). Cutts (1988) reported that fixation did not affect sarcomere lengths when compared with fresh tissue. Finally, even if fixation causes changes to sarcomere lengths, it would not be likely to cause long-range correlations to appear if the correlations did not exist in the fresh tissue.

In chapter 5 there was a limitation in sample size and muscle velocity range. An increase in muscle velocity through an increase in actuator velocity would have increased the number of measurements that could have been used to fit the force-velocity parameters, however, this would have probably only resulted in a more speedy convergence of the Levenberg-Marquardt algorithm, and not have drastically different
parameters. Finally, the subjects were all roughly the same size, so investigating different subjects may increase the variability.

Finally, the limitations to chapter 6 include the bore size of the MRI machine, acquisition time, and sample size. The bore size and acquisition time of the MRI are constraints based on the desired measurement resolution which restricted measurements to cadaver tissue only. The small sample size does not change the complexity of muscle fascicle arrangement seen in the FDI muscles that were analyzed. By increasing the sample size the frequency of this complexity in a larger sample of muscles may be described.

7.5 Future Studies
A number of potential studies could be conducted based on the methods or findings in the current group of studies.

Based on the findings in chapter 3 a future study would be to test more complex systems (knee, ankle) to determine if variability is still evident in muscles surrounding those joints. One questions if increased daily functional demand will lead to a decrease in variability in the muscle model parameters. Perhaps the lack of demand on the FDI contributes to the variability seen in chapter 3.

From the methods in chapter 4, a future study would be to capture a video of an isometrically contracting muscle fiber section. The muscle section would be long enough to be imaged in its entirety in a single field of view. The video would encompass the entire muscle section in its field of view, thus allowing for the length of each sarcomere to be measured. Through multiple isometric contractions, the optimal fiber section length can be determined. The individual sarcomere lengths can be measured at the optimal fiber section length and it can be determined what percentage of the sarcomeres are actually acting at their optimal length.

Using the finger dynamometer from chapter 5 the effect of different methods of training on the parameters used to describe the force-length and force-velocity curve could be examined. Subjects would be tested before entering into a muscle training program. Subjects would be randomly assigned to a high force, low repetitions program (arguably
for muscle hypertrophy), a low force, high repetitions program (arguably for muscle endurance), or a control program. Subjects would be retested every other week for 12 weeks to determine if muscle parameters change with muscle training and if different changes are observed based upon the different training programs.

Using the contractile tissue tracking methods from chapter 6, the connective tissue in other muscles could be examined. One likely source of high-resolution MRI data would be the Visible Human Project which could be used as pilot data to explore the possibility of extending the methods to live subjects as the cadaver scanned in the Visible Human Project was placed in a scanner that could be used for a live subject. This would provide the ability to observe the three-dimensional arrangement of connective tissue in live subjects. By observing connective tissue in live subjects, muscle models could be tailored even more specifically to subjects.

7.6 Final Conclusions

The studies showed:

1. Cadaver-specific musculoskeletal models produce different model outputs than the output of a model based on average cadaver parameters.
2. Individual sarcomere lengths along a complete muscle fiber exhibit a variable distribution.
3. Full characterization of the FDI is possible using non-invasive in vivo methods.
4. Serial arrangement of muscle fascicles in the FDI muscle.
References


APPENDIX A

Informed Consent Form for Social Science Research

Title of Project:  
Musculoskeletal Parameters of the First Dorsal Interosseous Muscle

Principal Investigator:  
John H. Challis, Ph.D.  
Biomechanics Laboratory  
29 Recreation Building  
The Pennsylvania State University  
University Park, PA 16802  
Phone  +(814) 863-3675  
Fax  +(814) 863-4755  
Email  jhc10@psu.edu  

Other Investigator(s):  
Benjamin Infantolino

1. Purpose of the Study: The purpose of this research is to measure the muscle parameters of the first dorsal interosseous muscle. The muscle is in the first web space of your hand. The influence of muscle properties on the output of musculoskeletal models is not fully understood. We believe that individual parameters greatly influence the output of the model. Through this research, we hope to better understand the relationship between individual muscle parameters and model output.

2. Procedures to be followed:

a) Collection of data from ultrasound machine – You will sit in front of a device that can test the strength of your index finger. Your hand will be placed onto the machine and set in place so that the machine can move your
index finger. The ultrasound probe as well as your muscle will be covered in gel to help create clearer images on the ultrasound machine. The gel will not cause an allergic reaction. The ultrasound will be secured to your hand with medical tape.

b) Performance/Collection of data at different angles and velocities-
You will be asked to perform a series of maximal voluntary finger contractions on the finger machine. In some trials the machine will not move while in others the machine will move at a fixed speed. During the speed trials, the machine may push your finger. During all trials you will be asked to perform a maximal voluntary contraction.

3. Discomforts and Risks: The risks in participating in this research are few. You may feel some discomfort as you push against the machine. Also you may feel some discomfort from the medical tape that is holding the ultrasound probe. If this happens you may tell the person conducting the study to move it for you. Also you may experience some soreness the next day if this is a muscle you do not use a lot.

4. Benefits: While there are no direct benefits to you for participating in this research, your data will give us a greater understanding of how individual muscle parameters effect the output of models which can prove to be useful in future research and/ or serve clinical uses.

5. Duration/Time:

The structure of data collection for each subject will be as follows:
- Potential subjects will be shown the Biomechanics Laboratory and the data collection equipment.
- Addressing of any potential subject questions.
- Collecting informed consent.
- Measurements using the ultrasound equipment.
- Collection of data from the finger machine
• Collection of finger and hand size data.

Your time commitment for this research will be one session, lasting no more than two hours.

6. **Statement of Confidentiality:** Your participation in this research is confidential. The data will be stored and secured in the Biomechanics Laboratory in a password-protected file. Only the Investigator and his assistants will have access to these files and the data will be kept for the length of time required by federal regulations (3 years) and then destroyed. Penn State’s Office for Research Protections, the Institutional Review Board and the Office for Human Research Protections in the Department of Health and Human Services may review records related to this research study. In the event of a publication or presentation resulting from the research, no personally identifiable information will be shared.

7. **Right to Ask Questions** Please contact John Challis at (814) 863-3675 with questions, complaints or concerns about this research. You can also call this number if you feel this study has harmed you. If you have any questions, concerns, problems about your rights as a research participant or would like to offer input, please contact The Pennsylvania State University’s Office for Research Protections (ORP) at (814) 865-1775. The ORP cannot answer questions about research procedures. All questions about research procedures can only be answered by the research team.

8. **Voluntary Participation:** Your decision to be in this research is voluntary. You can stop at any time. You do not have to answer any questions you do not want to answer. Refusal to take part in or withdrawing from this study will involve no penalty or loss of benefits you would receive otherwise. Refusal to take part will not affect your grade in Dr. Challis’ course.

9. **Injury Clause:** In the unlikely event you become injured as a result of your participation in this research, medical care is available but neither financial compensation nor free medical treatment is provided. By signing this document,
you are not waiving any rights that you have against The Pennsylvania State University for injury resulting from negligence of the University or its investigators.

You must be 18 years of age or older to consent to take part in this research. If you agree to take part in this research and the information outlined above, please sign your name and indicate the date below.

You will be given a copy of this consent form for your records.

_____________________________________________  _____________________
Research Participant Signature                           Date

_____________________________________________  _____________________
Person Obtaining Consent                                Date
Connective Tissue Volume in Human Muscle

Introduction

In an early study, Hermann (1888) measured that the connective tissue contributed 14.5% of total muscle volume in frog sartorius muscle. Using a planimeter, a device used to measure the area of an irregular two-dimensional shape, Fenn (1936) obtained a value of 17.5% for connective tissue space in frog muscle. Mobley and Eisenberg (1975) measured the cross-sectional areas of frog sartorius muscle fibers and estimated the connective tissue occupied 17% of the whole muscle volume. There is no apparent report of the portion of the total muscle volume occupied by connective tissue in human muscle.

The specific tension of muscle is the maximum isometric force a muscle can produce per unit of cross-sectional area. Therefore, if the cross-sectional area of the whole muscle is known, the maximum isometric force that muscle can generate can be calculated. In muscle models, the specific tension used is typically based upon isolated muscle fiber preparations because the muscle fibers can be reliably fully activated (Zajac, 1989). This creates problems when using the specific tension values in muscle models as the muscles to be modeled potentially contain substantial amounts of connective tissue, and therefore the cross-sectional area of the muscle will include both contractile and connective tissues. This will lead to an over estimation of the maximum isometric force generating capabilities of a muscle. Magnetic Resonance Imaging (MRI) of muscle can be used to quantify the percentage of connective tissue in a muscle.

The purpose of this study was to assess the percentage of connective tissue in whole muscle. This percentage can indicate how much material in muscle does not directly contribute to muscle force production, which would help more accurately describe a muscle’s potential for force production. The percentage of connective tissue was assessed using MRI to image the FDI muscle and computer software to identify connective and contractile tissue. The hypothesis was that the percent of connective tissue in human muscle will be similar to that found in the muscles of other species (e.g., Mobley and Eisenberg, 1975).
Methods
Two embalmed cadavers each had one FDI muscle removed for a total of two FDI muscles. The cadaver characteristics were: Cadaver 1: female, 76 years old, 152 cm in height, 67.37 kg in mass, cause of death – end stage congestive heart failure; Cadaver 2: male, 72 years old, 160 cm in height, 72.60 kg in mass, cause of death – esophageal carcinoma. The muscles were removed with the tendon intact using blunt dissection techniques described previously (Infantolino and Challis, 2010). To ensure the entire tendon was removed, the muscle was freed from its distal origins then reflected to allow a scalpel to ride along the bony, distal insertion until the entire tendon was separated from the bone. To reduce the Magnetic Resonance scanning time the tissue was immersed in a 1.5% Magnevist (Bayer Health Care, Wayne, NJ) phosphor-buffered solution for 7 days. The achieved short T1 (33 ms) and T2 (7 ms) times allowed for fast imaging with a high contrast-to-noise ratio. To prevent the tissue from drying out and to minimize magnetic susceptibility artifacts (when a diamagnetic substance such as water distorts the linear magnetic field gradients) during scanning the specimens were surrounded by a flourinert liquid FD-43 (3M, St. Paul, MN). All experiments were conducted in a vertical 14.1 tesla Varian MRI unit (Varian Inc., Palo Alto, CA) with direct drive technology. A millipede resonator (Varian) with an inner diameter of 40 mm was used to acquire three-dimensional spin echo images of the muscle tissue. Images up to an isotropic resolution of 50 µm were acquired. Typical muscle fascicle diameters are between 0.5 and 10 mm (Jones and Round, 1990). A standard imaging experiment with an isotropic resolution of 75 µm comprised a field of view of 45 x 20 x 20 mm³ and a matrix size of 600 x 268 (75% partial Fourier: 201) x 268. With 12 averages and a repetition time of 75 ms (echo time 11.7 ms) the total scan time was 13.5 hours. MATLAB (The MathWorks, Inc., Natick, MA) was used for post-processing which converted raw data (in k space) to visible images. By zero-filling each direction with a factor of two the pixel resolution of the standard imaging experiment was 37.5 µm³.

Mimics (Materialise, Leuven, Belgium) software was used to analyze the MRI images. Connective tissue was identified by a thresholding algorithm in Mimics. The thresholding algorithm assigns pixels, between user defined intensity values, to a mask. For this muscle, the thresholding was performed on the axial images. The walls of vascular tissue were identified as connective tissue while the lumen was identified as muscle.
Each mask represents a different tissue. Intensity values for the images varied between -1023 and 3056, allowing for accurate discrimination between connective and contractile tissue. The boundaries of whole FDI muscles were identified in the axial plane, by one trained operator, in the software which allowed the thresholding to be constrained to the whole muscle and not due to any noise in the image outside of the muscle.

Volumes were generated for both the outlined whole muscle and the connective tissue using triangles to enclose either the thresholded pixels or following the user defined muscle boundaries. For the whole muscle, approximately 270,000 triangles were used while between 8 and 15 million triangles were used to create the fascia volumes. Volumes were computed by determining the number of voxels contained by the triangles. Distance between pixels was known from the MRI scan and input into Mimics, which allowed for the calculation of the voxel volume. When creating a volume using thresholded pixels, the boundaries of the volume were defined by the full-width at half-maximum for the intensity values of the pixels.

Results and Discussion
The connective tissue volume was divided by the whole muscle volume for each muscle to determine the percent of connective tissue in the muscles (Figure 1). The connective tissue comprised 13.9% and 8.5% of total muscle volume for the two muscles.
Figure B1. Cross-sectional view of FDI muscle with connective tissue thresholded. The cross-section is midway along the longitudinal axis of the muscle. Pink material is the connective tissue in the muscle.

The amount of connective tissue in whole muscle is important in muscle modeling so that corrections can be made when scaling the specific tension measured in isolated muscle preparations to whole muscles. Since only one particular muscle was used in this study so it is difficult to generalize these findings to other muscles in the human body. However, this methodology may be applied to other human muscles. The hypothesis was supported by the MRI data, that is in the FDI connective tissue occupied between 8.3 and 13.9% of total whole muscle volume which is in agreement with the values noted for frog tissue (Hermann, 1888; Fenn, 1936; Mobley and Eisenberg, 1975).

References


APPENDIX C

Pennation Angle Variability in Human Whole Muscle

Introduction
Traditionally muscle pennation angles are either measured on the surface of a dissected muscle using a goniometer (e.g., Infantolino and Challis, 2010) or using an imaging technique such as ultrasound (e.g. Narici, 1999) which displays a single cross-section of muscle. Magnetic Resonance Imaging (MRI) produces images throughout the muscle in the three anatomical planes and in two of the planes (frontal and sagittal), pennation angles can be measured. By measuring the pennation angle in both planes for all images, the variability of pennation angle can be quantified. The purpose of this study was to describe the variability of pennation angle observed throughout the First Dorsal Interosseous (FDI) muscle. Pennation angles were measured using MRI of the FDI muscle.

Methods
One FDI muscle was removed from each of two embalmed cadavers for a total of two FDI muscles. Each muscle was removed using the methods described in Infantolino and Challis (2010) to ensure complete removal of the muscle and the tendon. The cadaver characteristics were: Cadaver 1: female, 76 years old, 152 cm in height, 67.37 kg in mass, cause of death – end stage congestive heart failure; Cadaver 2: male, 72 years old, 160 cm in height, 72.60 kg in mass, cause of death – esophageal carcinoma. The tissue was immersed in a 1.5% Magnevist (Bayer Health Care, Wayne, NJ) phosphor-buffered solution for 7 days to reduce the MR scanning time. The achieved short T1 (33 ms) and T2 (7 ms) times allowed for fast imaging with a high contrast-to-noise ratio. Specimens were surrounded by a flourinert liquid FD-43 (3M, St. Paul, MN) to prevent the tissue from drying out and to minimize magnetic susceptibility artifacts (when a diamagnetic substance such as water distorts the linear magnetic field gradients) during scanning. All experiments were conducted in a vertical 14.1 tesla Varian MRI unit (Varian Inc., Palo Alto, CA) with direct drive technology. Three-dimensional spin echo images of the muscle tissue were acquired using a millipede resonator (Varian) with an inner diameter of 40 mm. Images up to an isotropic resolution of 50 µm were acquired. Typical muscle fascicles contain between 10 and 100 muscle
fibers with muscle fiber diameters between 50 and 100 µm (Jones and Round, 1990). A standard imaging experiment with an isotropic resolution of 75 µm comprised a field of view of 45 x 20 x 20 mm³ and a matrix size of 600 x 268 (75% partial Fourier: 201) x 268. With 12 averages and a repetition time of 75 ms (echo time 11.7 ms) the total scan time was 13.5 hours. Converted raw data (in k space) to visible images was completed in MATLAB (The MathWorks, Inc., Natick, MA). A pixel resolution of the standard imaging experiment was 37.5 µm³ was achieved by zero-filling each direction with a factor of two.

The software Mimics (Materialise, Leuven, Belgium) was used to measure the pennation in each image for the medial-lateral and anterior posterior image planes (37.5 µm thick slices). One angle measurement was taken along the medial-lateral axis (Figure C1) while two measurements were taken along the anterior-posterior axis (Figure C2) because of the two headed nature of the muscle. Measures of pennation were made with an attempt to use the same location in the muscle throughout the different slices. In addition, for one image in each imaging plane all the pennation angles that could be measured were. This was to demonstrate the variability in pennation angles that could be measured within the same image. In this case the measures were made in a plane which approximates the superficial surface used when making measures on cadavers using a goniometer.

**Results and Discussion**

Along the medial-lateral axis the first cadaver muscle was 16.8 mm long (448 slices), while in the second it was 10.8 mm long (287 slices). In the anterior-posterior axis the first muscle was 11.4 mm long (305 slices) and the second cadaver muscle was 8.9 mm long (237 slices).

Nearly all the pennation angles throughout the muscle demonstrated non-normal distributions assessed using an Anderson-Darling statistic ($p < 0.05$ for all measures but one). Pennation angle was regressed against the percent of total muscle length for the axis along which the pennation angle was measured (Figures C3-5).
Figure C1. Pennation angle measured in an image along the medial-lateral axis of cadaver one, where the dashed line represents the aponeurosis while the solid line represents the line of action of the fiber.

Figure C2. Pennation angles measured in an image along the anterior-posterior axis of cadaver one, where the dashed line represents the aponeurosis while the solid line represents the line of action of the fiber.
Figure C3. FDI pennation angles in the medial-lateral axis with linear regression line, fitted to the data from both cadaver muscles indicate data trend.

Figure C4. FDI first metacarpal pennation angles in the anterior-posterior axis with linear regression line fitted to the data from both cadaver muscles indicate data trend.
Figure C5. FDI second metacarpal pennation angles in the anterior-posterior axis with linear regression line fitted to the data from both cadaver muscles indicate data trend.

The multiple pennation angle measures in one image demonstrated a coefficient of variation of 57% for the medial-lateral axis, 33% for the first metacarpal head of the FDI, and 19% for the second metacarpal head of the FDI for the first cadaver. All of these measures demonstrated normal distributions. This variability in pennation angle has functional significance as pennation angles were measured above 15° which is the angle at which Zajac (1989) stated that pennation angle makes a contribution to muscle force production.

The non-normal distribution of pennation angles along an axis hints at a more complex distribution of fascicles than assumed when a single pennation angle is used to represent an entire muscle. Therefore, this distribution indicates that a single pennation angle may not be an appropriately accurate measure to describe the arrangement of muscle fascicles in a whole muscle. The multiple measures of the pennation angles within one image demonstrate that many pennation angles can be observed and these pennation angles can vary by an amount that can have functional significance.
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Vita

Benjamin W. Infantolino

Education

The Pennsylvania State University
University Park, PA 16802

Doctor of Philosophy: Kinesiology/ Biomechanics
   May 2011

Master of Science: Kinesiology/ Biomechanics
   December 2007
   Masters Thesis: Architecture of the First Dorsal Interosseous
   Muscle

Bachelor of Science: Kinesiology/ Athletic Training with Honors
   May 2006
   Senior Thesis: Determining the Validity of Ultrasound for the
   Estimation of Muscle Volumes In Vivo

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