Muscular force production during non-isometric contractions: Towards numerical muscle modeling

by

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Muscular force production during non-isometric contractions: Towards numerical muscle modeling

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Abstract

The main objective of the study was to investigate skeletal muscle force production during isometric contractions, active muscle stretches and shortenings. The motivation behind this work is to improve the dominant model of muscle contraction force generation based on the theories of Hill. The effect of force modification was observed after concentric and eccentric contractions and also stretch-shortening cycles. It has been shown that this force modification is not related to lengthening/shortening velocity, and the steady-state force after non-isometric contractions can be well described by initial isometric force and mechanical work performed by and on the muscle during length variations. The time constants calculated for isometric force redevelopment appeared to be in certain relations with those for initial isometric force development, an observation which extended our basis for ongoing muscle modeling.

The main method of the project consists in two extensive series of experiments on mouse skeletal muscles. Analysis of the first series of experiments, concentric contractions, with an emphasis on the force depression has been presented in Paper 1. Paper 2 is based on contractions with various stretches and shortenings as well as their combination, force modification and its predictor are the quantities of interest. The third part of the project is also based on the second series of experiments. Timing aspects of the force production were calculated there.

Descriptors: Mouse skeletal muscle; Transient-length contractions; Muscular force; Force modification; History effect; Muscle modelling

Preface

This thesis studies skeletal muscle force production under non-isometric contractions. In the first part an introduction to the field, a short description of the experimental part and analysis of the timing aspects of the force production are presented. The second part consists of the following papers:

Paper 1. KOSTERINA N., WESTERBLAD H., LÄNNERGREN J. AND ERIKSSON A., 2008
"Muscular force production after concentric contraction", Journal of Biomechanics 44(11), 2422–2429

Paper 2. KOSTERINA N., WESTERBLAD H. AND ERIKSSON A., 2009
"Mechanical work as predictor of force enhancement and force depression", Accepted for the Journal of Biomechanics

Division of work between authors

The research project was initiated by Anders Eriksson (AE) who also acted as supervisor. AE, Håkan Westerblad (HW) and Natalia Kosterina (NK) have continuously discussed the progress of the project during the course of the work.

Paper 1 and Paper 2

The experimental paradigm was developed by AE, NK and HW. The experiments were performed by NK and AE under supervision of HW, with a technical support from Jan Lännergren and Shi-Jin Zhang at Karolinska Institutet. The analysis of the results was done by NK with feedback from AE and HW. The papers were written by NK with inputs from AE and HW.

Timing of force production in transient-length muscle contractions

The experimental paradigm was developed by AE and NK. The experimental results were taken from the experiments performed for the Paper 1 and Paper 2. The analysis of the results was done by NK with feedback from AE and HW.

Contents

Part I

CHAPTER 1

Introduction

The importance of skeletal muscles can not be overestimated. The basic muscle function is force production, which in turn causes movements through muscle contraction. All creatures locomote owing to muscles ability, and human would be feeble without properly functioning muscles.

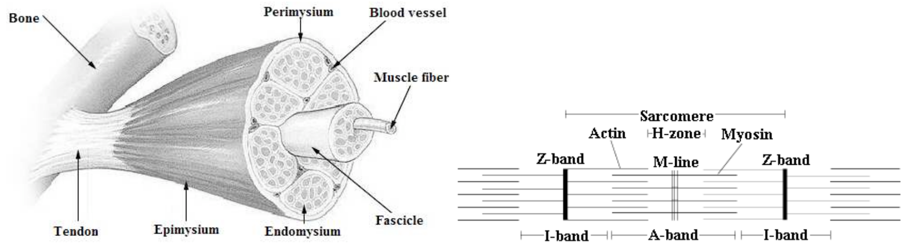
The first description of a contracting and expanding organ providing movement belongs to Aristotle (384-322 B.C., De Motu Animalium). Galen (129-201 A.D., De Tremore) characterised muscle as a true organ, therefore he can be considered as founder of a science of muscles, or myology. Vesalius (1543, De Numano Corporis Fabrica) described muscle components and discovered that muscle implicates the contractile power. Swammerdam (1663, c.f. Needham (1971)) demonstrated consistency of muscular volume with an elementary experiment. Croone (1664, De Ratione Motus Musculorum) first found a cause of muscle contraction, which is a brain signal. In the past century, possibilities to perform advanced experiments led to revolutionary discoveries, some of them will be discussed below in detail. New techniques of the last two decades allow measurements at the molecular level and they are used regularly by many groups (Nigg and Herzog 1999). However, there are some unexplained phenomena, giving a basis for future studies.

1.1. Anatomy and physiology of skeletal muscle

Muscle is a highly organized and structured organ. Each muscle component is related with a specific function. There are three muscle types: skeletal, muscular, and smooth, or non-striated. They have significant differences in structure and functioning. This study is based on skeletal muscles. These are controlled voluntarily but not by the autonomic nervous system. Skeletal muscle is attached by tendons to bones at the origin (proximal end) and insertion (distal end) points. Each organ is made up of skeletal muscle tissue, connective tissue, nerve tissue, and blood or vascular tissue. The entire muscle is covered by a connective tissue sheath called the epimysium (Fig. 1.1a). Muscle is divided into compartments, fascicles, surrounded by the perimysium. These bundles consist of a number of cells called muscle fibres (Nigg and Herzog 1999; Herzog 2000; Hamill and Knutzen 2008).

The connective tissue covering muscle fibres, i.e. endomysium, protects the delicate cells and provides pathways for the passage of blood vessels and nerves. Muscle fibre consists of myofibrils arranged in a parallel way, making skeletal muscle striated. Myofibrils are made of repetitive contractile units called sarcomeres (Fig. 1.1b).

Sarcomeres are separated by the Z-band and composed of three filament systems. These play a major role in muscle contraction. The thick filaments are located in the middle of sarcomere, they are composed of myosin protein connected with Z-disc by titin to give stability and structure to the sarcomere. The thin filaments consist of actin filaments, they lie parallel to thick filaments. Each myosin molecule is surrounded at both ends by six thin actin filaments.



(a) (b)

FIGURE 1.1. Schematic illustration of (a) skeletal muscle and (b) sarcomere structures. Re-drawn and modified from Hamill and Knutzen (2008).

Sliding filament theory

The thick and thin filaments within the sarcomere slide past one another, shortening the entire length of the sarcomere. This sliding filament theory explaining muscle force production was suggested by Huxley and Niedergerke (1954) and Huxley and Hanson (1954). Later on, Huxley (1957) developed the cross-bridge theory, which has became a basic concept for muscular force production and has not been changed significantly since. The idea lies in sliding of thin and thick filaments along one another. The myosin heads interact with the actin filaments and bend to pull the actin using ATP (Adenosine triphosphate), thus shortening sarcomeres while the length of filaments does not change (Nigg and Herzog 1999). The chemical bonds formed during contraction are called cross-bridges.

A lengthened sarcomere is able to form a limited number of cross-bridges in an active state, while a shortened sarcomere has interference of thin filaments as they start to overlap. As a results, muscle fibres produce forces dependent on sarcomere lengths (Gordon et al. 1966; Hamill and Knutzen 2008). A muscle length giving maximum tetanic isometric force is called the optimal length. It is essential to consider the maximum force, the optimal length, and the range of muscle lengths when talking about the contractile properties of the muscle. These parameters are shown on the length-tension curves (Fig. 1.3), each curve actually represents the results of many experiments plotted on the same graph (Lieber and Fridén 2000).

Pennation angle

As was described above, force production is a result of protein-filament interactionhappening in sarcomeres, but it also depends on fibre arrangement within the muscle. A fusiform muscle contains fibres lying essentially parallel to the line connecting distal and proximal tendons. These muscles are more flexible than pennate ones. In pennate muscle types, fibres run at a certain (pennation) angle to the line of action, making them suitable for stabilization and force production, Fig. 1.2, Nigg and Herzog (1999); Hamill and Knutzen (2008).

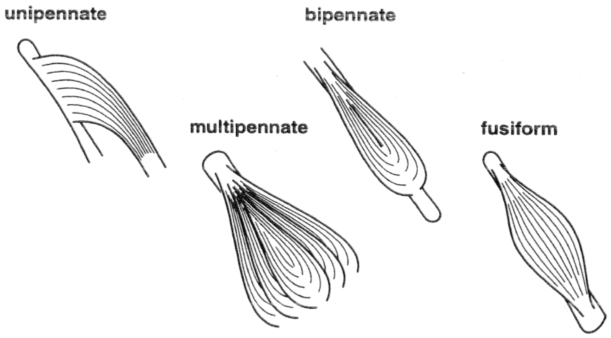


FIGURE 1.2. Classification of muscles depending on the arrangement of fibres within a muscle. Re-drawn and modified from Nigg and Herzog (1999).

Cross sectional area and fibre length

The contractile properties of a muscle are also dependent on optimal fibre length and physiological cross sectional area ('PCSA'). The muscles with a larger PCSA promote a larger force production (Nigg and Herzog 1999; Lieber and Fridén 2000), Fig. 1.3a. Practically, PCSA can be obtained as the ratio between the muscle volume and fibre length:

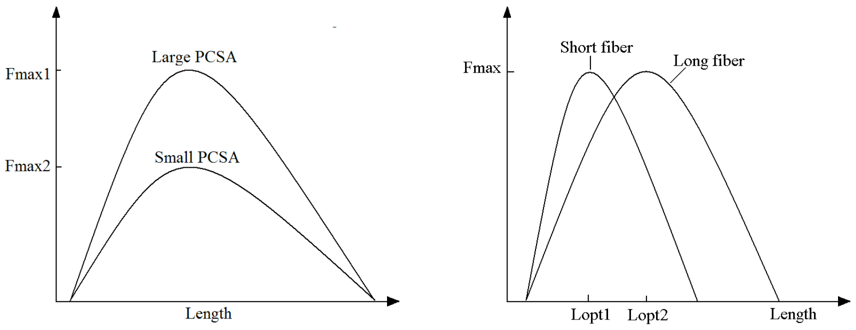
$$PCSA = m\,\cos\alpha/(\rho\, l_f) \tag{1.1}$$

where m is the muscle weight, α is the pennation angle, ρ is the muscle density, equal to $1056\,\mathrm{kg/m^3}$, and l_f is the fibre length (Hamill and Knutzen 2008).

When comparing two fibres with different optimal lengths but the same PCSA and pennation angle, the short-fibred muscle produces the same force as long-fibred, but the range of possible muscle lengths is smaller (Lieber and Fridén 2000), Fig. 1.3b. This is essential in movements, because body segments move when a muscle connecting them shortens or lengthenings, changing the angle of the joint.

Fiber types

Another criterion influencing muscle strength is ratio of different fibre types composing the muscle (Hill 1970). Skeletal muscle fibres can be divided into 3 types: slow twitch oxidative (Type I), fast twitch oxidative glycolytic (Type IIA) and fast twitch glycolitic (Type IIB) (Peter et al. 1972). The discriminating factor is the maximum shortening velocity of the fibres (Nigg and Herzog 1999). Fast twitch muscle fibres demand more energy to perform contraction than the slow twitch fibres (Hill 1938). In case of repeated contractions, the fast twitch fibres lose their ability to produce maximum isometric force rapidly, while the slow twitch fibres can stay feasible for a



(a) (b)

FIGURE 1.3. Schematic illustration of force-length relations of skeletal muscles with identical pennation angle and (a) fibre length or (b) PCSA. Muscles with a large PCSA produce higher force. Longer muscles have a wider range of possible lengths. Re-drawn from Lieber and Fridén (2000).

longer period of time. The cause is that the extensor digitorum longus ('EDL') muscles are more susceptible to fatigue (Burke and Edgerton 1975). All skeletal muscles include fibres of different types. The soleus muscle is almost exclusively composed of slow twitch fibres, while the EDL and extraocular muscles dominantly consist of fast twitch type fibres. The ratio of the fibre types changes with age: the number of slow fibres increases, and that of fast fibres decreases (Hirofuji et al. 1992). The ratio of fibres of different types can also be changed over time for an individual due to muscle activity (Wernig et al. 1990). The fibre composition in each muscle is optimised for its function, e.g. the soleus plays an important role in standing, the EDL is meant for extension of toes and ankle (Palastanga et al. 2002).

1.2. Muscle contraction

As mentioned above, skeletal muscle is controlled by the central nervous system, which includes the brain and the spinal cord. Voluntary muscle contractions are initiated in the brain, the signals in the form of action potentials pass through the nervous system to the specialized cell, motor neuron, that causes a series of chemical reactions and an ion exchange through the fibre membrane in a specialised junction, the synapse (Fig. 1.4). The transient change in membrane potential is the action potential. This condition promotes the release of Ca^{2+} ions, which invoke cross-bridge formation and innervate the muscle fibres. The cross-bridge cycle repeats itself as long as the action potential exists. When the stimulation stops, the Ca^{2+} ions return back into the sarcoplasmic reticulum, preventing cross-bridge formation in the relaxed state (Nigg and Herzog 1999; Hamill and Knutzen 2008).

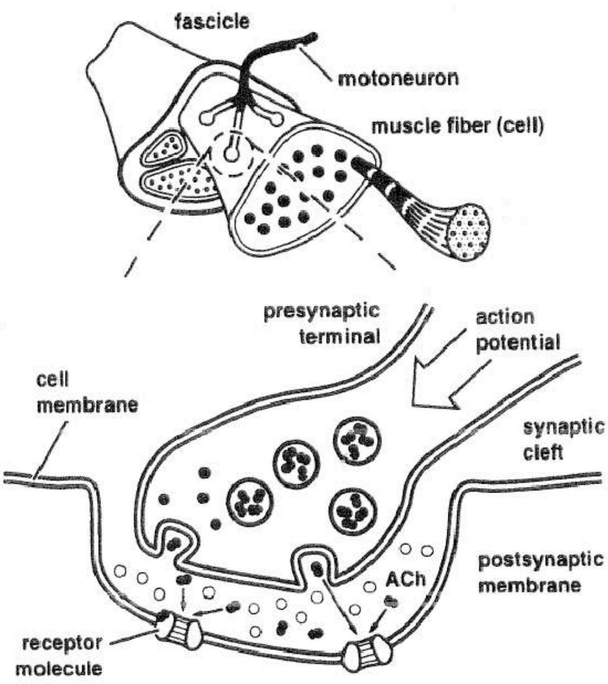


FIGURE 1.4. Schematic illustration of transferring action potential to the muscle fibre. To perform any movement the brain sends a signal through the nervous system towards the muscle. When the signal reaches the muscles, it divides into small branches and delivers the action potential to the fibres. This causes a series of chemical reactions and an ion exchange through the fibre membrane in the synapse. Re-drawn and modified from Nigg and Herzog (1999).

1.2.1. *Muscle stimulation*

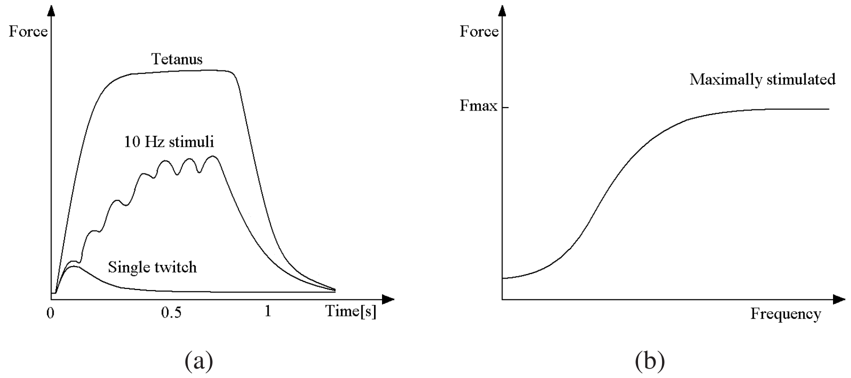
Skeletal muscles are able to produce varying levels of contractile force. Contractile responses from the muscle should be provoked to perform experiments on skeletal muscle in vitro. To deliver electrical charge, single pulse or pulse trains are provided by specialised devices. A muscle response to a stimulus is subject to some parameters such as the strength and the frequency of the pulses, the muscle length, the velocity of the muscle contraction, and the fibre composition of the muscle (Chaffin and Andersson 1991).

Multiple fibre summation

When a signal is weak, only some of the fibres in the muscle are recruited. With increasing voltage, additional units are supplied and the force becomes stronger, until a total contraction happens. Therefore, via multiple fibre summation, the muscle attains a maximally stimulated state and the twitch amplitude reaches its maximum (Hamill and Knutzen 2008).

Frequency summation

A single twitch invokes a muscle contraction and a subsequent relaxation. If another twitch is delivered before the muscle relaxes, a greater force will be produced. Increasing the frequency at which action potentials are sent to muscle fibres is called frequency summation phenomenon (Chaffin and Andersson 1991), Fig. 1.5. A muscle is maximally stimulated if the signals are maximum in strength and frequency, therefore increasing of these parameters beyond these levels will not lead to the muscular force growth (Hamill and Knutzen 2008).



(a) (b)

FIGURE 1.5. (a) Schematic illustration of force-time traces for a muscle when a single twitch, 10 Hz stimuli and a tetanus were applied. The muscle force rises after each stimulus until it reaches a so called tetanic plateau when the force does not grow with following twitches. The plateau becomes smoother and the tetanic force increases with increasing frequency until the force reaches a limit. (b) Schematic illustration of force-frequency relationship. The tetanic force increases with frequency until it reaches a limit, the muscle becomes maximally stimulated. Figure (a) is re-drawn from Nurhussen (2006), (b) is based on experimental results on soleus muscle.

Active and Passive forces

Understanding of force-length relations is essential in performing tests on skeletal muscles (Fig. 1.6). When the muscle is not stimulated, it produces a passive force, which is close to zero if the length is below a certain length, slack length, which in general is very close to the optimal length, i.e., the length at which the fully activated muscle can produce a maximal isometric force. Stretching of the muscle over the optimal length leads to a rapid force increase induced by connective tissue resistance to the stretch. Scientists suggest titin and myofibrils as the origin of the passive muscle tension (Yoshioka et al. 1986; Horowits and Podolsky 1987; Funatsu et al. 1990).

The force produced during activation due to cross-bridges formation is called an active muscle force. The maximum active force is produced at the optimal length

(Nigg and Herzog 1999; Hamill and Knutzen 2008), this has been described above (Section 1.1). Active force is obtained under isometric contractions and maximum activation. Combined together, the passive and active forces make a strong resistance to lengthening, far beyond the peak of active force (Nigg and Herzog 1999). Though the active force component is the most important in the studies, it is possible to measure only the total and passive muscle forces during experiments. The active force is defined as the difference between these components.

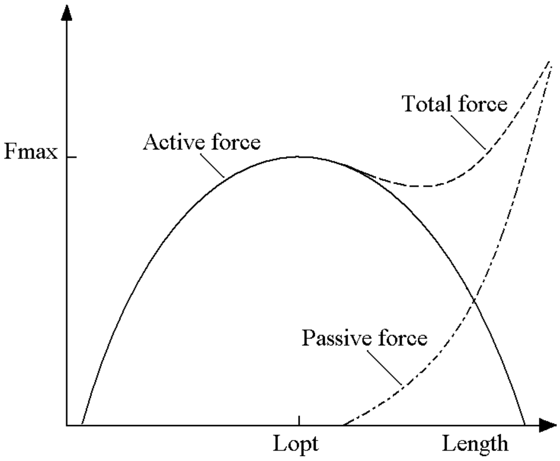


FIGURE 1.6. Schematic illustration of force-length relations. The dash-dotted line shows the passive force, dashed line - total force, and solid line - active muscle force. Re-drawn and modified from Hamill and Knutzen (2008).

Tension-Length relations

To define the dynamic properties of the cross-bridges which cycle during muscle contraction, the force-velocity relationship has been used. The muscle shortens at a certain velocity against a constant load. The general form of the force-velocity relation is shown in Fig. 1.7. The resistive force decreases in a hyperbolic fashion down to zero when the shortening velocity increases (Nigg and Herzog 1999; Lieber and Fridén 2000; Hamill and Knutzen 2008). This is intuitively obvious as when lifting two unequal loads, the light load can be moved much more quickly.

An important characteristic is the mechanical work performed by the muscle during contractions (Fig. 1.7). The mechanical work is defined as the product of force and velocity integrated over a specific time period. The muscle does not produce work at zero load and very high load, while the optimal muscle shortening occurs at approximately one-third of maximum shortening velocity (Hill 1938; Nigg and Herzog 1999; Lieber and Fridén 2000).

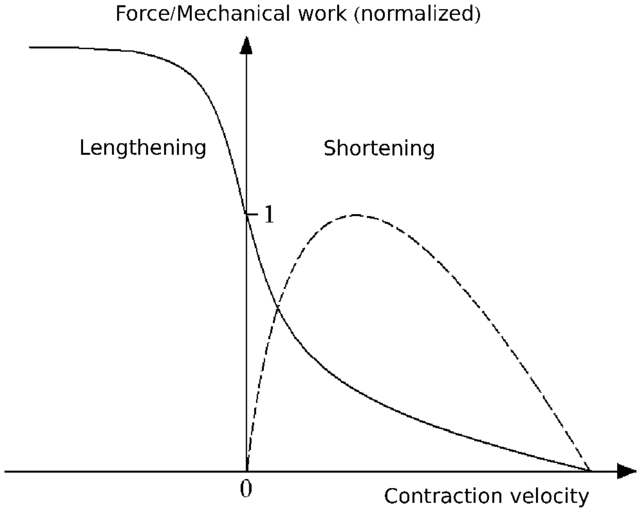


FIGURE 1.7. Schematic illustration of force - velocity (solid line) and mechanical work - velocity (dashed line) relations. The mark ‘1‘ correspond to the isometric contraction at optimal length. Redrawn and modified from Nigg and Herzog (1999) and Hamill and Knutzen (2008).

1.2.2. *Types of contractions*

The term ’muscle contraction’ can be misleading, as muscles can generate force while shortening, lengthening, or at a constant length. There are many ways when the muscle can generate the force and the type is governed by the interaction of the muscle itself with the compliance of the surrounding system (Hamill and Knutzen 2008).

Isometric contractions

An isometric contraction of a muscle happens without changing muscle length. Most of experiments, providing data that were presented above, are performed under isometric conditions. These contractions can be represented by a static position with a load, the muscles are in tension and do not change their lengths, Fig. 1.8b. This kind of contractions are used in the optimal length finding. Examples of force-time traces for isometric contractions are shown in Fig. 1.9.

Concentric contraction

During a concentric contraction the muscle shortens while generating force and therefore changes the angle of the joint. In relation to the elbow, a concentric contraction of the biceps would cause the arm to bend at the elbow (Fig. 1.8c).

Eccentric contraction

Eccentric contraction occurs when the muscle lengthens in an active state. This happens while an opposing force is greater than the force generated by the muscle. During an eccentric contraction of the biceps muscle, the elbow starts the movement

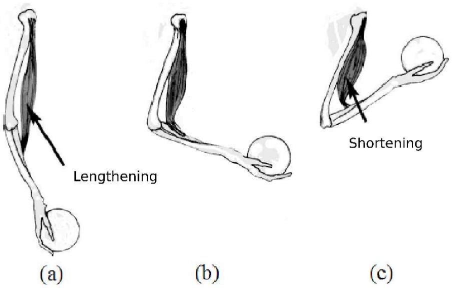


FIGURE 1.8. Examples of different types of contractions that occur in biceps during exercises. (a) Eccentric contraction, lengthening with a constant load; (b) isometric contraction, static load; (c) concentric contraction, shortening with a constant load. Re-drawn from Hamill and Knutzen (2008).

while bent and then extends as the hand moves away from the shoulder due to lengthening of the biceps muscle (Fig. 1.8a).

History effect

It has been shown experimentally than the steady-state force following concentric contraction does not reach the maximum isometric force associated with the final length (Abbott and Aubert 1952; Edman et al. 1978; Marechal and Plaghki 1979; Herzog and Leonard 1997), Fig. 1.10a. There is a similar force modification following active lengthening of the muscle, as the redeveloped force is higher than the isometric force: force enhancement, (Schachar et al. 2002; Rassier and Herzog 2002; Bagni et al. 2005; Herzog 2005; Herzog et al. 2006; Morgan 2007), Fig. 1.10b. Moreover, there is a force modification following lengthening-shortening and shortening-lengthening cycles (Epstein and Herzog 1998). These phenomena are usually referred to as history dependence of active force production, and were firstly described by Abbott and Aubert (1952). The history effect is observed in experiments but is not accurately explained until now (Herzog and Leonard 2005; Morgan 2007; Herzog et al. 2008). Muscular force consists of active and passive components, and both active and passive force modifications occur with active shortening or lengthening, (Bagni et al. 2002; Rassier and Herzog 2002; Joumaa et al. 2008; Herzog et al. 2008). An interesting observation was made by Julian and Morgan (1979); Morgan et al. (2000). They have noticed that a short stimulation interruption eliminates the history effect after non-isometric contractions.

Possible mechanisms of force modification

There have been many attempts to explain the mechanism of force modification, some of them were disproved by later investigations, others led to new questions. Sarcomere length non-uniformities were proposed by Marechal and Plaghki (1979) as a possible mechanism of force depression after shortening. The results by Edman et al. (1978, 1993) support the structural non-uniformities as the underlying mechanism of force depression, but Granzier and Pollack (1989) disproved this idea by specific

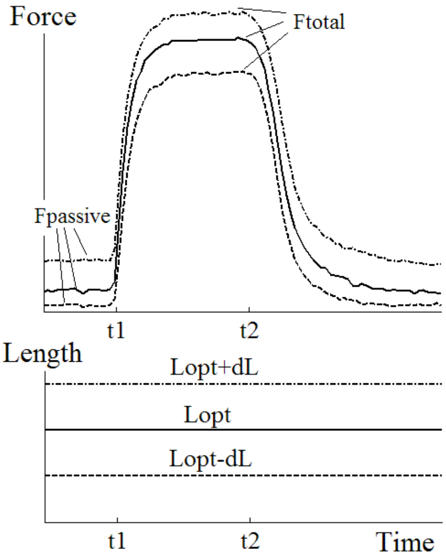
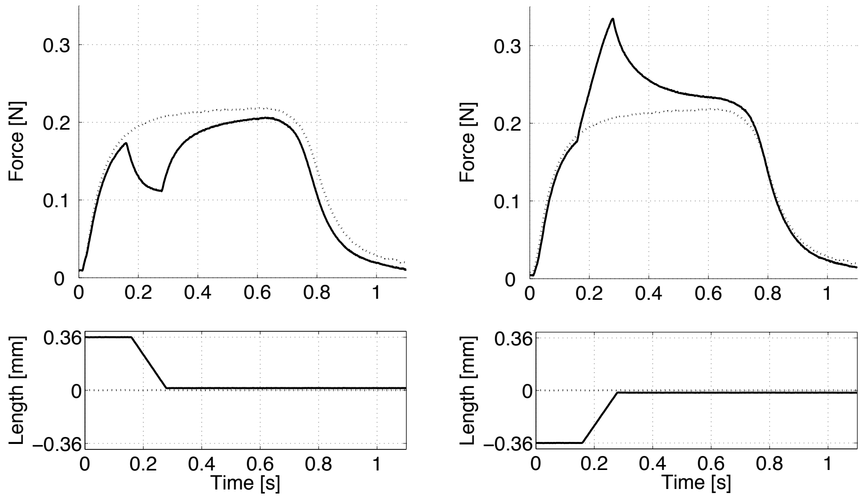


FIGURE 1.9. Examples of force-time traces for a mouse soleus muscle electrically stimulated at different muscle lengths. The stimulation was applied between times t_1 and t_2. When the stimulation stops, force drops down to the initial passive force, i.e. related to the muscle length. Active force is maximum at optimal length (solid line). Forces are lower at length below the optimal (dashed line, Length= $L_{opt} - dL$). Passive force increases with stretch, this can be seen on the trace for the muscle at $L_{opt} + dL$ length (dash-dotted line), but the active force decreases.

experiments. The force depression is associated with a decrease in the muscle stiffness or cross-bridge cycling, (Sugi and Tsuchiya 1988; Razumova et al. 1999; Lee and Herzog 2003). For many decades, it has been generally accepted that the active force depression is influenced by shortening magnitude, (Abbott and Aubert 1952; Herzog and Leonard 1997; Lou et al. 1998; Schachar et al. 2004; Bullimore et al. 2007) and speed of shortening (Marechal and Plaghki 1979; Sugi and Tsuchiya 1988; Herzog and Leonard 1997; Morgan et al. 2000; Lee and Herzog 2003). The study, presented in Paper 1, has shown that these descriptions are true, but only for a certain experimental paradigm.

The mechanism of force enhancement invoked by eccentric contractions is poorly understood in comparison to force depression. In a recent review, Morgan (2007) stated that these observations might be explained by non-uniformity of half-sarcomere lengthening, but the muscle experiments suffer from various problems. At the same time this theory was not certified by Herzog et al. (2008), but they suggested a calcium-dependent increase in titin stiffness as a cause of the passive force component. Herzog et al. (2006) suspect that the active component of force enhancement is associated with



(a) (b)

FIGURE 1.10. Examples of force production during (a) concentric and (b) eccentric contractions (solid lines), performed on soleus skeletal muscle. Isometric contraction at the optimal length is shown as dotted lines. Length is expressed as difference to optimal length. Force grows when the stimulation starts; after the force almost reached its maximum value, (a) shortening or (b) lengthening were applied, this led to a rapid force fall/rise until the end of the ramp. While the length was held at the optimal value, the force redeveloped to a new steady-state value, different from the initial isometric force value. This difference is called the force modification.

changes in the cross-bridge kinetics that might be reflected in decreased detachment rates following active muscle lengthening. Edman et al. (1978) proposed that the tetanic force enhancement after a lengthening can be described as a parallel elastic element that is formed, reorganized or re-aligned during activation.

The present results show that the mechanical work is a good predictor of force modification following active lengthening, shortening, and lengthening-shortening cycle despite the different mechanisms underlying these phenomena, Paper 1 and Paper 2. This observation might provoke debate in the explanation of physiological aspects, but it serves as source for improving of skeletal muscle model.

1.3. Skeletal muscle modeling

The current work does not include muscle modeling, but the motivation of the ongoing project is to create a numerical muscle model that can be used for solution of optimization problem. The new model should consider the memory effect described above. The series of experiments have been performed to provide a basis for the modeling.

Hill type muscle model

Theoretical models of skeletal muscle differ considerably depending on the structural level and the problem to be solved. The main interest in biomechanics is to estimate and predict the forces of entire muscle. The first significant findings were made by Hill (1938), when he described an experimental relation between muscular force and contraction velocity:

$$(V + b) \cdot (F + a) = b \cdot (F_0 + a), \tag{1.2}$$

where F is the transient muscle force, F_0 is the maximum muscle force, V is the contraction velocity, a and b are specific constants. The Hill-type muscle models have been dominant for a long time and play a major role in musculo-skeletal simulations of varying complexities. The original Hill-type muscle element consisted of a contractile element that has force-velocity properties according to the Eq. 1.2. In order to improve the muscle model, a parallel or series elastic elements, viscous dampers, and other passive elements were considered in addition to the contractile element (Zajac et al. (1986); van Soest and Bobbert (1993) and many others), Fig. 1.11. These components represent the connective tissue surrounding the entire muscle and muscle fibres, tendons connecting muscle with bones. A rheological model by Günther et al. (2007) is also based on Hill element, and connects the activation level and the length-velocity values. Even though, it does not consider the history effect after non-isometric contractions. Forcinito et al. (1998) developed an additional component for a standard muscle model, with an 'elastic rack', or a step-wise parallel elasticity, engaged upon muscle activation. This addition seems to give a simple muscle memory, and could be part of a transient model.

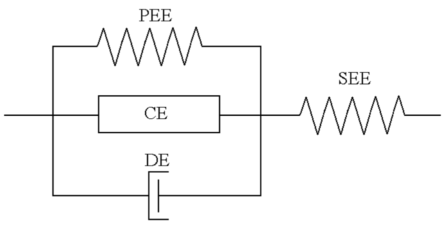


FIGURE 1.11. Typical Hill type muscle element consisting of contractile element (CE), parallel elastic element (PEE), series elastic element (SEE), and damping element (DE).

Numerical description of force production

For the active isometric force immediately continuing a transient-length contraction, the description of a force modification focussed on an asymptotic, i.e., theoretical long-term steady-state force value. This was compared to the asymptotic force for an initial isometric contraction, where length was held constant from the start of stimulation. Such values were evaluated from the force-time trace by curve-fitting. With experimental length variations according to Fig. 1.10, the curve fitting considered the isometric phases before and after a length variation. A good numerical fit was generally obtained with exponential functions of the form:

$$F(t) = F_\infty + (F_a - F_\infty) \cdot e^{-(t-t_a)/\tau} \tag{1.3}$$

where the force F at a time t goes from a value F_a at time t_a to a steady-state asymptotic value F_∞ through an exponential function with a time constant τ. The conclusion in Paper 1 was that the time constant τ_r for an isometric phase following shortening was –for practical purposes– well predicted by the one, τ_o, for an initial isometric contraction.

The results show that the transient force production in an isometric phase of a contraction following a length variation can be well predicted by the introduction of a force modification, considering the history through the work quantity, and the initial time constant, being a typical parameter for a muscle individual. The force modification, positive or negative, is here seen as a difference to the isometric force obtainable at an initial isometric contraction at the corresponding length.

The experiments performed also allow analysis of the transient-length phases of contraction. The hypothesis in the present work is that these phases can, on a macroscopic level, be described by similar expressions. The shortening and lengthening phases of experiments performed have been mathematically analyzed.

The mathematical analysis of force-time traces takes the experience from fitting Eq. (1.3) to isometric phases as inspiration. From a mechanical viewpoint, the contents of the equation would indicate the presence of a viscous damper in series with the force generator in the muscle. This can be seen by identifying the equation as an evolution process, where the time differential of the force is described by:

$$\dot{F}(t) \equiv \frac{dF}{dt} = \frac{1}{\tau}\left(F_\infty - F(t)\right) \tag{1.4}$$

where F_∞ is the asymptotic force, and the superposed dot denotes a time differential, i.e., the slope of the force-time trace.

A common way to consider an evolution expression like the one in Eq. (1.4) is through the state-space, where $F(t)$ and $\dot{F}(t)$ are seen as the axes in a plane diagram, and where an expression of the form in Eq. (1.4) will come out as a straight line, with a slope of $-\frac{1}{\tau}$, and coming to $\dot{F} = 0$ for $F = F_\infty$.

Such a state-space visualization of experimental time-data will thus reveal if the measured quantity, here muscular force, is realistically described by a function like the one in Eq. (1.3). Straight lines in the diagram will confirm this assumption for a phase of the interval, whereas curved lines will correspond to phases, where either the behaviour is not exponential, or the asymptotic value is not a constant attractor. It is already here noted, that the non-isometric parts of the force-trace should not be straight lines, as the changing length will in itself implicate differences in isometric force.

1.4. Objectives

The objective of the present study was to investigate the effect from a preceding transient contraction on the isometric force production and to define parameters required for skeletal muscle modeling. Predicting transient force production under various length regimes is a necessity for improved description of muscular action in numerical simulations of movement, (Kaphle and Eriksson 2008; Yamane and Nakamura 2007). The experiments have been developed to provide a foundation of an improved formulation of the dynamic muscular force production. In particular, the subjects of interest are the isometric force development in the beginning of activation, the force behaviour during active isovelocity lengthening and shortening, the isometric force redeveloping after non-isometric contraction, and muscle deactivation. These aspects should consider the force change in relation to time. Knowledge of these descriptions will give a base to consider the history effect after non-isometric contraction in a rheological muscle model. The findings of this study may also shed light on the mechanisms of force modification though no experiments were performed to directly investigate mechanisms underlying the active force modifications after length changes.

1.5. Thesis outline

The thesis is composed of three studies. The first one implies a series of concentric contraction experiments and investigation of the force depression. The second study consists in an expanded series of experiments for the purpose of force modification examination after various combinations of active lengthenings and shortenings. The third part deals with analysis of the second series experiments, by means of the state-space view, aiming at a macroscopic interpretation of the force production in transient-length contractions.

The results from the first two studies have been published in two separate papers. These are presented in the Part II of the thesis: 'Paper 1', titled as 'Muscular force production after concentric contraction', and 'Paper 2', titled as 'Mechanical work as predictor of force enhancement and force depression'. The third part of the study is planned to be submitted during summer 2009 as an independent article titled ‘Timing of force production in transient-length muscle contractions‘. This study is represented along the Part I of the thesis.

A review of the experiments is given in Section 2.1. Section 2.2 describes the mathematical analysis procedure, for both the force modification and the timing of force production. Results for the third part of the study, the timing aspects of the force production, are given in Chapter 3, and discussed in Chapter 4.

CHAPTER 2

Experiments on mouse soleus and EDL muscles

2.1. Materials and methods

The main method of the project consists in two extensive series of experiments on mouse skeletal muscles. Analysis of the first series of experiments has been presented in Paper 1, it was based on the force depression following concentric contractions. Paper 2 is based on contractions with various lengthenings and shortenings as well as their combination, force modification and its predictor are the quantities of interest. The third part of the project is also based on the second series of experiments. Timing aspects of the force production were calculated there [1].

2.1.1. *Muscle preparation*

Muscles of adult male NMRI strain mice were used. The white mice were housed at room temperature with 12/12-hours light/dark cycle for at least 8 weeks. Animals were given food and water ad libitum. Their weight was $\sim$30 g. Animals were euthanized by rapid cervical dislocation and thereafter EDL and soleus muscles were isolated. The Stockholm North local ethical committee approved the experimental procedures.

The soleus is a powerful flat muscle in the back part of the lower leg. It runs from the upperposterior surfaces of the tibia and fibula to the calcaneus, Fig. 2.1a. The soleus muscle is responsible for plantarflexion of the ankle joint. The EDL is a pennate muscle, situated at the lateral part of the front of the leg, it originates at the upper anterior surface of the fibula, passes through the front of the ankle joint, and divides into four tendons which run to the lateral four toes, Fig. 2.1b. The EDL provides dorsal extension of toes and ankle, Palastanga et al. (2002); Hamill and Knutzen (2008).

Soleus muscle contains about 80% slow type fibers (Asmussen and Maréchal 1989; Wernig et al. 1990). EDL muscle has at least 95% of fast twitch muscles, both type IIA and IIB (Wernig et al. 1990). The mixture of fibers depends on age and activity of the animal (Hirofuji et al. 1992; Wernig et al. 1990). The soleus muscle reaches a steady state in 0.3 s after the activation begins. For EDL this time is about 0.15, this is relatively fast. EDL is very sensitive to lengthening, therefore experiments on EDL muscles usually take more time and efforts (Burke and Edgerton 1975).

Muscle preparation and solution, supporting ability of the muscle to contract, are described in detail in Paper 1, Section 2.2 and Paper 2, Section 2.2.

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[1] A manuscript based on timing aspects of the force production will be submitted during summer 2009 with a tentative title 'Timing of force production in transient-length muscle contractions'.

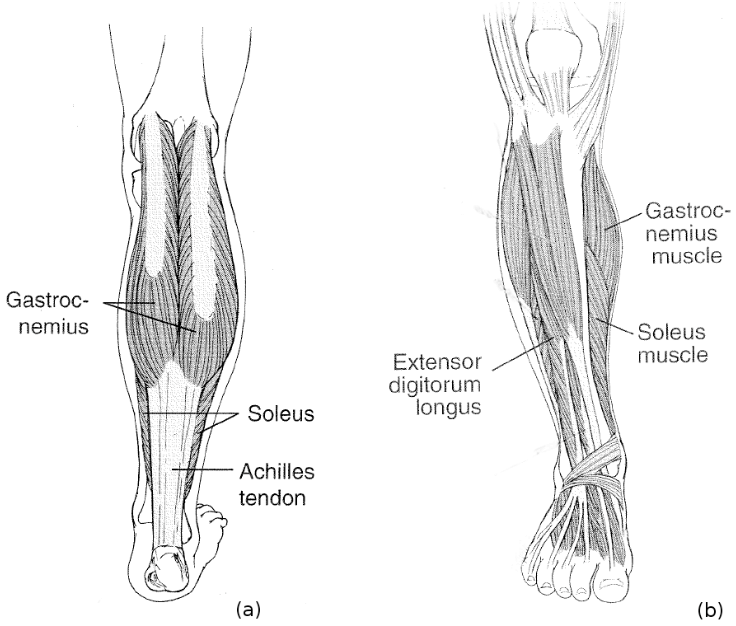

FIGURE 2.1. Schematic illustration of soleus and EDL muscles in human body. Reproduced from Hamill and Knutzen (2008)

2.1.2. *Experimental design*

Description of the experimental setup is presented in Paper 1, Section 2.3, therefore only main aspects will be stated in this section. The muscle was mounted between an adjustable holder from one side and a length controlling system and a force transducer from another side. The stimulation was applied through two plate electrodes placed along the muscle (Fig. 2.2). Stimulation, length and muscle force were controlled and recorded via computer and the Spike2 software (CED, Cambridge, UK).

2.1.3. *Testing paradigm*

The thesis is based on two series of experiments. The main part of these series consists in non-isometric experiments. Isometric contractions have been performed to define the individual optimal muscle length and also to verify the ability of the muscle to produce the maximum isometric force.

The first series of experiments consists of concentric contractions with various shortenings and ramp times, performed on soleus and EDL muscles. The experiments can be divided into three groups depending on the final muscle length, i.e. the muscle length after active shortening. The isometric force redevelopment at optimal ($l_{\text{fin}} = l_o$), suboptimal ($l_{\text{fin}} = l_0 - 0.72\text{mm}$) and superoptimal ($l_{\text{fin}} = l_0 + 0.72\text{mm}$) muscle lengths was preceded by the same set of ramps [2].

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[2] The extensive description of the concentric contraction experiments can be found in Paper 1, Section 2.4.

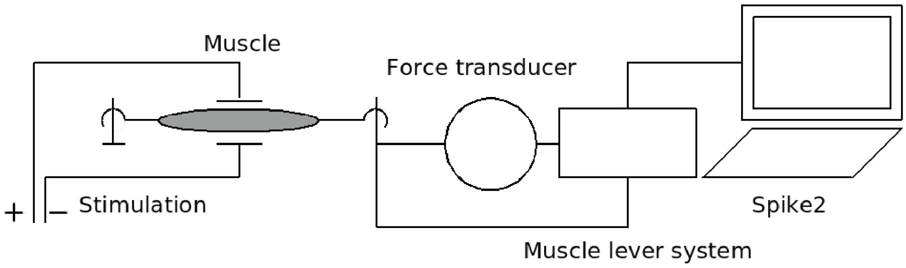

FIGURE 2.2. Schema of the experimental apparatus. The muscle was mounted between an adjustable holder from one side (left on the figure) and a length controlling system and a force transducer from another side. The stimulation was applied through two plate electrodes placed along the muscle. Stimulation, length and muscle force were controlled and recorded via computer and the Spike2 software (CED, Cambridge, UK).

The second stage of the project consisted in expanded set of ramps applied on soleus and EDL muscles. The final muscle length, l_{fin}, was equal to the optimal, l_o in most of tests. All the experiments can be divided into four groups depending on the character of the ramps. These are 'Shortening', 'Lengthening', 'Lengthening-Shortening' and 'Long Shortening'. Examples of force-time traces are presented in Fig. 2.3 [3]

2.2. Theory

The experimental results were analyzed to test two aspects of force production: the force level reachable during various regimes and the timing when the force reaches a steady-state condition.

2.2.1. *Isometric force evaluation*

To test the steady-state muscular forces, the force-time histories of isometric force development phases have been fitted by exponential functions. The exponential fitting of the force-time traces is generally used and shows a good agreement with the experimental data (Hancock et al. 2004; Corr and Herzog 2005). The mechanical work produced by and on the muscle during lengthening and shortening ramps was calculated by integrating the force multiplied by ramp velocity, over the ramp period [4].

2.2.2. *Time constant evaluation*

Force and length data were recorded at 500 Hz during experiments. Noise in the data was low, hardly visible in the time traces of the quantities. As, however, for the

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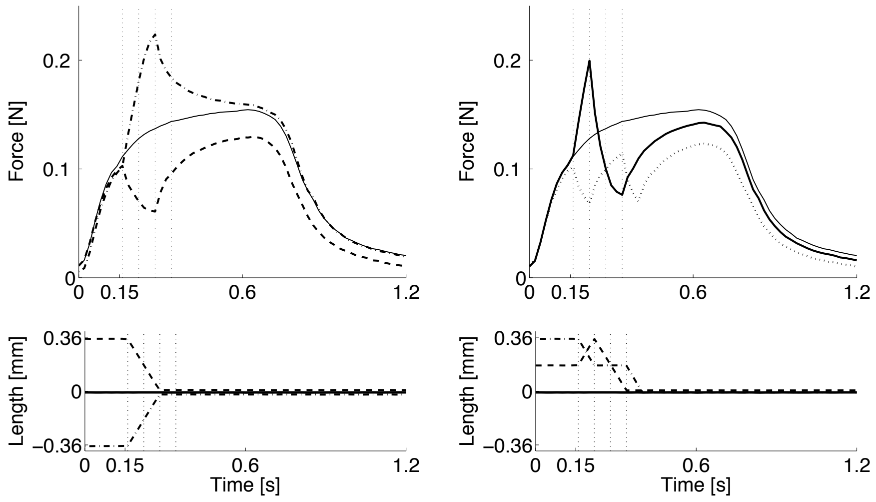
[3] The extensive description of the second series of experiments can be found in Paper 2, Section 2.4.

[4] The extensive description of the evaluation procedure for the isometric steady-state force, active and passive force modification, and the mechanical work performed by or on the muscle can be found in Paper 1, Section 2.5 and Paper 2, Section 2.5.



(a) (b)

FIGURE 2.3. Examples of force-time traces on mouse soleus muscles. Thin solid lines correspond to isometric contractions. Transient-length contractions: (a) dash-dot line - lengthening and dashed line - shortening on 0.36 mm in 0.12 s, (b) dotted line - two-step shortening on 0.18 mm in 0.06 s each with 0.12 s delay between them, thick solid line - lengthening-shortening cycle. All experiments end at the optimal length of the muscle. Time t=0 denotes start of stimulation, Length=0 the individual optimal length.

present purposes the time differentials of the force data were needed, a filtering of force data was performed. This filtering was defined by second-order Butterworth lowpass filter with a cutoff frequency of 60 Hz, performed in Matlab (version R2006a, The MathWorks, Inc., Natick, MA, USA). The force differential was evaluated in the midpoints of the time steps recorded, through a central difference approximation; corresponding force values were the averages between the neighboring points:

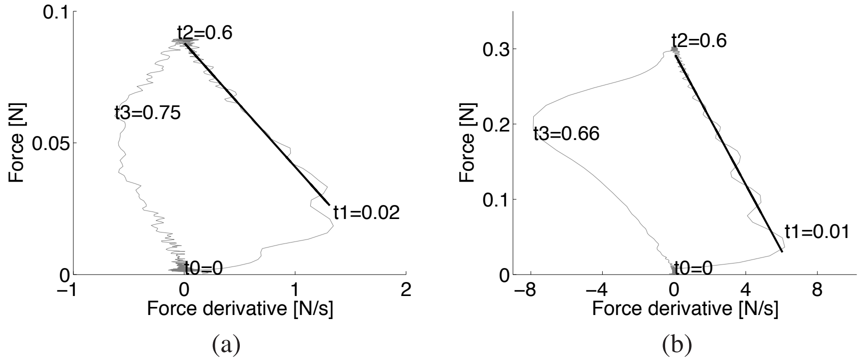
$$t_{i+1/2} = \frac{1}{2}\left(t_i + t_{i+1}\right) \tag{2.1}$$

$$F_{i+1/2} = \frac{1}{2}\left(F_i + F_{i+1}\right) \tag{2.2}$$

$$\dot{F}_{i+1/2} = \frac{1}{\Delta t}\left(F_{i+1} - F_i\right) \tag{2.3}$$

where the indices refer to experimental time $t_i = i \cdot \Delta t = i \cdot 0.002\,[\mathrm{s}]$ since start of stimulation.

A state-space visualization of the experimental dynamic parameters has been used to evaluate the timing constants of the muscular force production. All experiments were plotted in state-space diagrams, with $F(t)$ as ordinate and $\dot{F}(t)$ as abscissa. The borderlines between the different iso-kinetic parts of the curves were marked, and each such segment treated separately. As examples, the state-space diagrams of some of the experiments are given in Figs. 2.4–2.7, with the time marks at interesting points. The filtered data are presented on state-space diagrams.

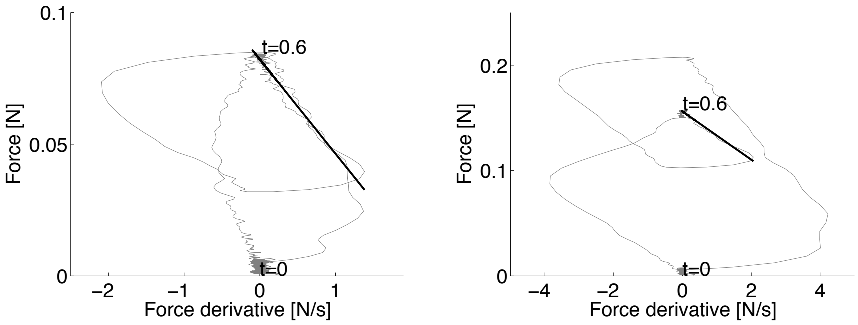


(a) (b)

FIGURE 2.4. State-space plot of example isometric test in Fig. 2.3, showing relation between current force and force time differential values over experimental time. Time $t_0 = 0$ denotes start of stimulation, stimulation is ended at $t_2 = 0.6\,\text{s}$. Time references on curve indicate different phases of experiment. The force time differential, $\dot{F}(t)$, increases with the muscle activation until they reach their maximum values at time t_1, $100\,\%$ activation. The force value, $F(t)$, rises from the beginning of stimulation and reaches a steady-state value when the $\dot{F}(t)$ drops down to zero (time t_2). After the stimulation terminates, $F(t)$, $\dot{F}(t)$, and muscle activation start to decrease. The muscle becomes deactivated when $\dot{F}(t)$ drops to its minimum (time t_3, $0\,\%$ activation).

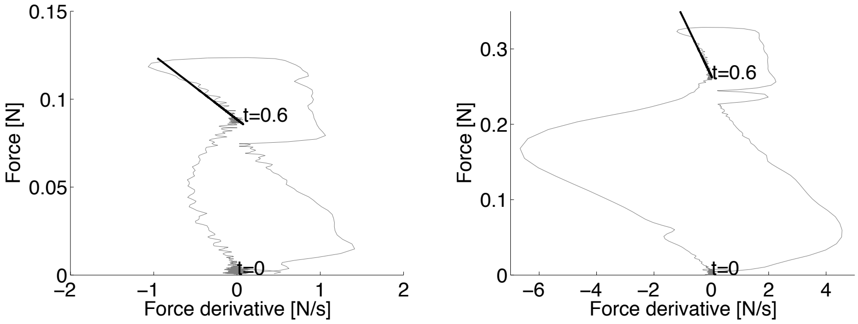
It is obvious from Figs. 2.4–2.7 that starting and ending stimulation causes non-straight lines corresponding to a less than full muscle activation. In Fig. 2.4, time stamps are marked where the assumed constant straight lines start, which were assumed to correspond to times when $100\,\%$ and $0\,\%$ activation was reached from start and end of stimulation. These points were defined as a maximum and minimum values of the force time differential, $\dot{F}(t)$, on a relevant time interval, on the ground that the maximum concentration of free Ca^{++} is nearly correlated with the maximum rate of rice of force (Stein et al. 1982). Times t_+ and t_- for each experiment denote the intervals of this rise and drop of activation between 0 and $100\,\%$.

Having defined these time instances, the time constants for the straight lines of all isometric phases can be evaluated from a straight-line fitting. This method is illustrative to ascertain that isometric force development is similar to an exponential function. The time constants were also evaluated by exponential fitting of the force-time traces on the same interval. These values can be assumed to be valid for isometric, fully



(a) (b)

FIGURE 2.5. State-space plot of example shortening test, showing relation between current force and force time differential values over experimental time. Time $t = 0$ denotes start of stimulation, stimulation is ended at $t = 0.6$ s. The straight lines are a regression fit of the isometric force redevelopment after the shortening ramp.

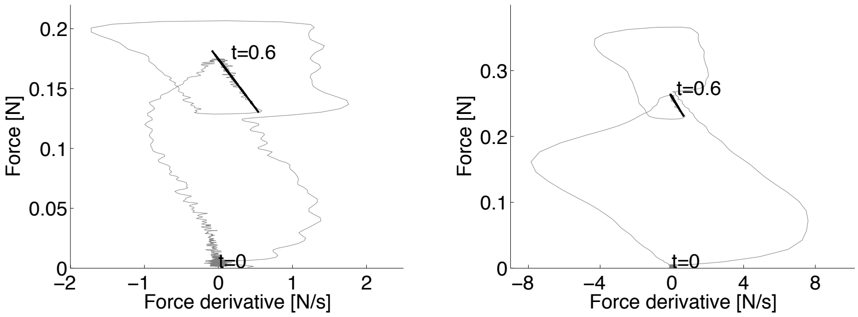


(a) (b)

FIGURE 2.6. State-space plot of example lengthening test, showing relation between current force and force time differential values over experimental time. Time $t = 0$ denotes start of stimulation, stimulation is ended at $t = 0.6$ s. The straight lines are a regression fit of the isometric force redevelopment after the lengthening ramp.

active or fully passive phases. We denote by τ_1 the time constant for an active initial isometric case, by τ_r^-, τ_r^+ and $\tau_r^\pm$ the one for active recovery after shortening, lengthening and lengthening-shortening cycle, and by τ_0 the one for passive force loss.

As will be shown in Chapter 3, the time constants τ_r^-, τ_r^+ and $\tau_r^\pm$ for active phases of the experiments are in a certain relations with the initial time constant, τ_1, for a specific muscle individual. This fact is used for the investigation of the non-isometric phases of experiments. Assuming that the force is always striving towards an asymptotic value at a specific exponential rate, but noting that this can not be constant



(a) (b)

FIGURE 2.7. State-space plot of example lengthening-shortening test, showing relation between current force and force time differential values over experimental time. Time $t = 0$ denotes start of stimulation, stimulation is ended at $t = 0.6\,\mathrm{s}$. The straight lines are a regression fit of the isometric force redevelopment after the lengthening-shortening ramp.

while the length changes, an 'instantaneous asymptotic' force value was evaluated at all time instances recorded, from Eq. (1.4), as:

$$F^*_\infty(t) = F(t) + \tau \cdot \dot{F}(t) \qquad (2.4)$$

where F^*_∞ is the force value, attracting the force evolution at time t, $\tau = \tau_1$ is used from the beginning of stimulation until the end of the ramp, $\tau = k \cdot \tau_1$ is used for isometric force redevelopment after the ramp, k is the coefficient corresponding to the type of length regime. The 'instantaneous asymptotic' force was plotted as a function of time for all experiments. Examples corresponding to the cases in Figs. 2.4–2.7 are given in Fig. 2.8.

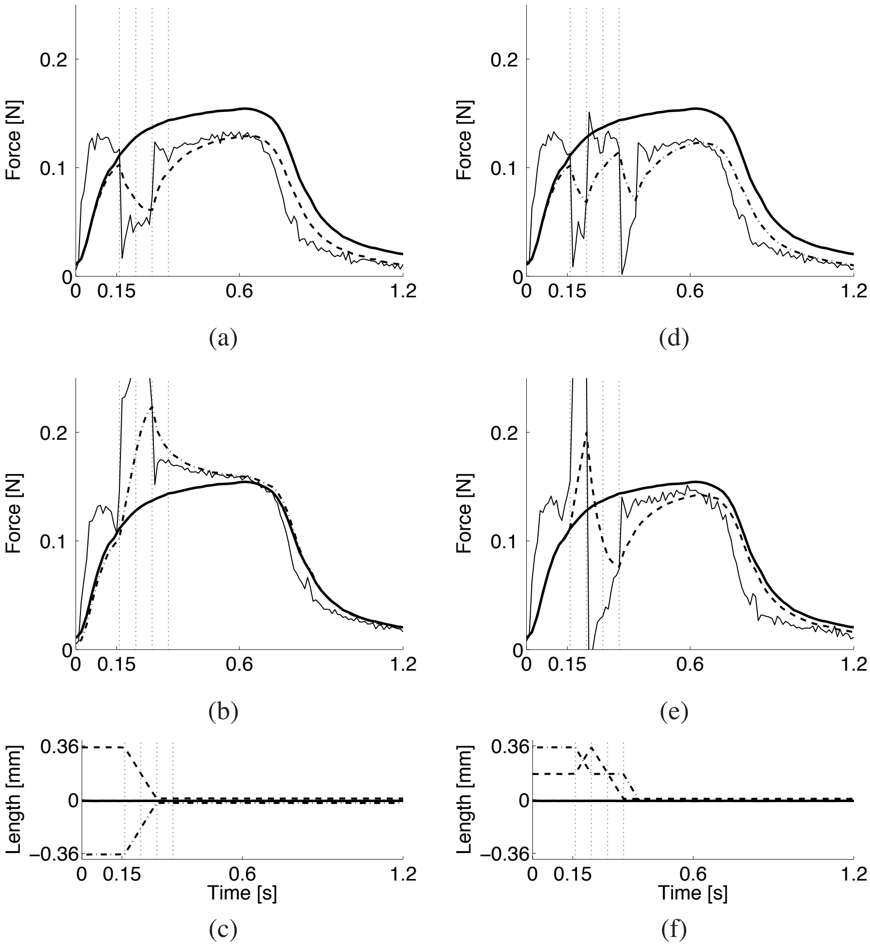


FIGURE 2.8. Instantaneous asymptotic force values F^*_∞ (thin solid lines) as functions of time for the example experiments in Fig. 2.3. Thick solid line - isometric contraction at an optimal length, dashed line (a) - shortening and dash-dot line (b) - lengthening on 0.36 mm in 0.12 s, dashed line (d) - two-step shortening on 0.18 mm in 0.06 s each with 0.12 s delay between them, dash-dot line (e) - lengthening-shortening cycle. All experiments end at the optimal length of the muscle. Time t=0 denotes start of stimulation, Length=0 the individual optimal length.

CHAPTER 3

Results

3.1. Force depression

The results of the series of concentric contractions show a positive correlation between the force depression and the mechanical work, whereas the force depression was not correlated to the velocity of shortening. Depression of the passive force component was also observed following all stimulations. Experiments show that the fully stimulated redevelopment of isometric force following concentric contraction follows a time function similar to the creation of force when isometric muscle is initially stimulated [1].

3.2. Force modification after length variation

The second series of experiments shows a negative linear correlation between the force modification and the mechanical work produced on or by the muscle. The linear fits show that this correlation exists within each set of experiments (shortening, long shortening, lengthening, lengthening-shortening). A corresponding modification of the passive force component following each stimulation was also observed [2].

3.3. Timing of force production

Time constants of isometric force redevelopment after non-isometric contractions are given in Tab. 3.1. The time constants of isometric force redeveloping, τ_r, are different from the initial timings, τ_1. But these time constants are similar inside sets of the experiments, this can be seen from the standard deviations for τ_r^-, τ_r^+ and $\tau_r^\pm$. A coefficient characterizing the type of non-isometric contraction, k, can be used to define the time constant, τ_r, of isometric force redevelopment as follows:

$$\tau_r = k \cdot \tau_1. \tag{3.1}$$

The multiplier k is presented in Tab. 3.1.

For isometric contractions, times of activation rise, t_+, were 0.037 ± 0.012 s for soleus and 0.012 ± 0.005 s for EDL muscles, times of activation drop, t_-, were 0.174 ± 0.019 s for soleus and 0.059 ± 0.011 s for EDL muscles. We can see that EDL muscles are about three times faster then soleus in activation but two times slower in deactivation.

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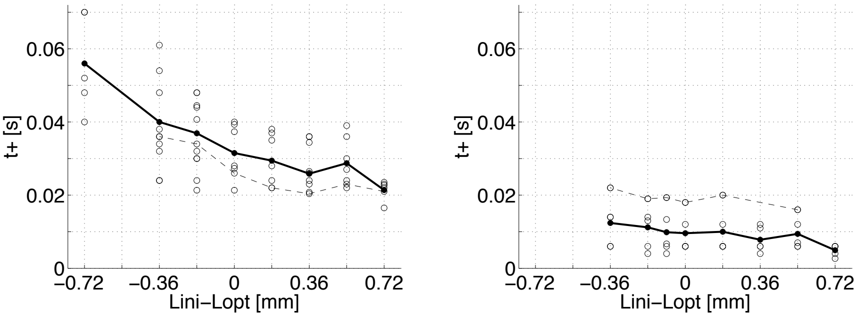
[1] The extended results supported by figures are presented in Paper 1, Section 3

[2] The extended results supported by figures are presented in Paper 2, Section 3

TABLE 3.1. Time constants of isometric force redevelopment after non-isometric contractions and their ratios with initial time constant. The time constant were calculated from linear approximation of $F(t) - \dot{F}(t)$ curve on isometric phases. Results from n $= 5 - 6$ soleus muscles and n $= 5$ EDL muscles are given as mean ± standard deviation, [s].

Experiments	Soleus		EDL	
Isometric	τ_1 0.078 ± 0.019	$-$	τ_1 0.036 ± 0.008	$-$
Shortening	τ_r^- 0.045 ± 0.001	τ_r^-/τ_1 0.703 ± 0.020	τ_r^- 0.026 ± 0.001	τ_r^-/τ_1 0.832 ± 0.019
Lengthening (negative)	τ_r^+ 0.039 ± 0.002	τ_r^+/τ_1 0.532 ± 0.024	τ_r^+ 0.079 ± 0.007	τ_r^+/τ_1 2.186 ± 0.218
Lengthening-Shortening	$\tau_r^\pm$ 0.064 ± 0.004	$\tau_r^\pm/\tau_1$ 0.818 ± 0.065	$\tau_r^\pm$ 0.026 ± 0.003	$\tau_r^\pm/\tau_1$ 0.761 ± 0.179

The lengthening and shortening ramps were applied after isometric force preactivation, therefore it was possible to measure the activation time, t_+, for different initial muscle lengths. It has been noticed that the activation time, t_+, depends on the muscle length, Fig. 3.1, a lengthened muscle reaches a full activation state faster than a shortened one.



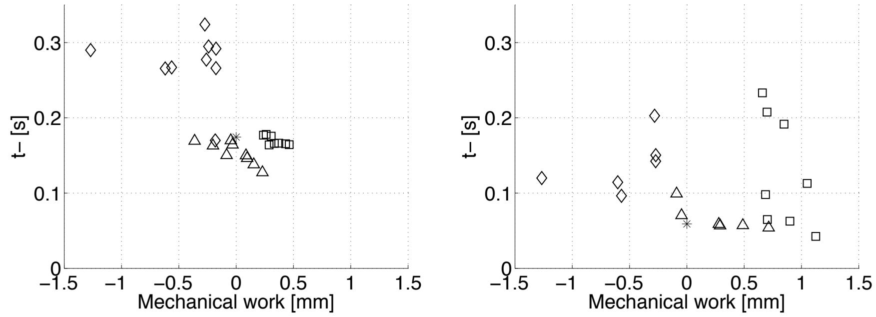
(a) (b)

FIGURE 3.1. Times of activation rise, t_+, at different muscle lengths. The nodes of the thick solid line relate to a mean activation time value of all experiments started at a corresponding length, l_{ini}, for n= $5..6$ soleus (a) and EDL (b) muscles. Circles correspond to individual muscles (mean values for isometric force development at each length), the dashed line connects individual values for a specific muscle.

TABLE 3.2. Times of muscle deactivation after stimulation termination, t_-, following isometric and non-isometric contractions. Results from n $= 5 - 6$ soleus muscles and n $= 5$ EDL muscles are given as mean ± standard deviation, [s].

Experiments	Soleus	EDL
Isometric	0.174 ± 0.019	0.059 ± 0.011
Shortening	0.171 ± 0.032	0.186 ± 0.065
Lengthening	0.282 ± 0.102	0.138 ± 0.065
Lengthening-Shortening	0.153 ± 0.025	0.066 ± 0.026

The deactivation time, t_-, depends on length history, Fig. 3.2, Tab. 3.2. For soleus muscles, the deactivation after active shortening and lengthening-shortening cycle takes almost the same time as after an isometric contraction, while deactivation after active lengthening takes two times longer period. For EDL muscles, the deactivation after an isometric contractions happens up to three times faster than after a non-isometric contraction. This observation is not induced by fatigue, because t_- for isometric verification tests remained stable, the standard variation for individuals was about 10 $\%$ for soleus and 5 $\%$ for EDL muscles.



(a) (b)

FIGURE 3.2. Times of activation drop, t_-, as a function of performed mechanical work. Results from n $= 5 - 6$ soleus muscles and n $= 5$ EDL muscles presented as a mean values: Shortening - squares, Lengthening - diamonds, Lengthening-Shortening - triangles. Average value of deactivation time after an isometric contraction is marked as asterisk.

CHAPTER 4

Discussion and conclusions

4.1. Discussion

The study was motivated by an intention to improve the predictive capacity of available macroscopic transient muscle model such as the common Hill-type models. The main objective was to evaluate an accurate description of the muscular force during various regimes.

The series of concentric contraction experiments has shown that a memory effect exists in muscular force production. It has been proved that the muscle history does not depend on the velocity of shortening as was assumed before (Sugi and Tsuchiya 1988; Josephson and Stokes 1999; Herzog et al. 2000). Mechanical work produced during active shortening was suggested as a predictive parameter [1].

The first results induced a wider investigation. A variety of lengthening and shortening ramps and combinations of those certified the mechanical work produced by and on the muscle to be a good predictor for the force modification. This measure considers both magnitude and velocity of the changing length. Moreover, the finding is important in explaining the mechanism underlying history dependent muscle behaviour [2].

To create a basis for a numerical muscle model, the timing aspects of force production should be described. An exponential fitting of the force-time traces is generally used (Hancock et al. 2004; Corr and Herzog 2005). The state-space diagrams show linear relations between the muscular force and the force time differential during isometric force development. This observation confirms the admissibility of the exponential nature of the muscular force development (Stein et al. 1982).

The time constants characterizing the force development at a constant muscle length were calculated from the state-space diagrams. These constants appeared approximately two times larger for soleus than for EDL muscles (Tab. 3.1). This is expected due to the different natures of the fibres composing these muscles. The soleus muscle mainly consists of Type I, slow fibres, and the EDL fibres relate to the Type II A and B, or fast fibres. The obtained results are consistant with previous work (Luff 1981; Ranatunga 1982; Stein et al. 1982; Brooks and Faulkner 1988).

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[1] The full discussion of the history effect in concentric contractions can be found in Paper 1, Section 4.

[2] The full discussion of the history effect observed in the second series of experiments can be found in Paper 2, Section 4.

It has been noticed that the force after non-isometric contractions also follows an exponential function, Tab. 3.1. The time constants related to isometric force redevelopment are specific for the type of preceding length variation. This finding gives a more accurate description of the transient muscular force variation, and can be applied in the skeletal muscle model.

The state-space description allows an evaluation of activation and de-activation times of the muscle. Here, these are defined as the times between a change in stimulation (0/100%) and the time when muscular force enter an exponential development curve, i.e., the straight lines in the diagram.

The activation times appeared approximately three times larger for soleus than for EDL muscles due to difference between the slow and fast muscles. Stephenson and Williams (1982) indicated the maximum Ca-activation when the force reaches 50% of the steady-state level. This activation time was slightly longer at shorter carcomere lengths for each type, what agrees with our observation, Fig. 3.1.

The deactivation after isometric contraction in an EDL muscle takes a shorter time than after applied ramps. An interesting observation is that the change in deactivation time is not correlated with muscle fatigue, because the muscle deactivation after isometric verification tests remained stable through the whole series of experiments. Moreover, Fitts (1994); Vøllestad et al. (1997) associate fatigue with a slowing of relaxation time, which was not observed here. Stephenson and Williams (1981, 1982) reported that the fast-twitch fibres relax much faster than slow fibers. Our results are in agreement with this statement in cases of relaxation after isometric and non-isometric contractions.

It can be noticed from the Figs. 2.4–2.7 that the linear relations between the force and force time differential after destimulation become linear after a drop of force from the steady value by 50%. This agrees with Stein et al. (1982), as they showed that the force decays exponentially after falling to a force value one-half to two-thirds of the peak. Half-relaxation time is the subject of interest for many scientists. This corresponds to the deactivation time calculated according to our method, because the force derivative reaches its minimum when the force falls to one-half of the steady-state value.

At the cellular level, the relaxation process is regulated by enzymes controling the rate of Ca^{++} re-uptake in the sarcoplasmic reticulum and, possibly, also the rate of cross-bridge detachment (Edwards et al. 1975; Fitts 1994). Vøllestad et al. (1997) suggest that the faster relaxation is caused by an increased turnover rate of the sarcoplasmic reticulum Ca^{++}-ATPase or the myosin ATPase. This can increase the energy cost of contraction. Our results support this idea because muscles require more energy to perform mechanical work during non-isometric contractions than during isometric stimulations.

4.2. Conclusion

The study confirms the presence of active and passive force modification following contraction with various length changes. The results show that muscular force does not only depend on length and length time differential. The steady-state force also takes into account the accumulated length history, as shown in transient-length contractions. Our results indicate that the mechanical work produced by or on the muscle during length variation is a good general predictor for the steady-state force modification induced by transient-length contractions. The experimental analysis allowed us to specify the time constants of the exponential functions describing isometric force redevelopment after non-isometric contractions. The muscle activation times calculated from the state-space diagrams were in agreement with the generally accepted muscle properties, thereby demonstrating the reliability of the method.

Summarizing the results, it can be stated that the physiological experiments have given a basis for an improved description of the muscular force production, with respect to both reachable force level and the timing aspects. This force formulation can consider the history effect following non-isometric contractions and can be applied in skeletal muscle modelling.

4.3. Outlook

The currently available numerical muscular models do not take several important aspects into account. The more accurate formulation of the muscle force production considers the force level and the timing of force development in different regimes. The force modification after non-isometric contractions and the force development during isometric force development can be introduced in a new muscle model. It is the objective for the further work to develop such a numerical muscle model for inclusion in general human movement simulations.

Summary of Papers

Paper 1

In paper 1 a series of concentric contraction experiments was performed. The results have shown that the steady-state force following active shortening does not reach the maximum isometric force associated with the final length. Isolated extensor digitorum longus and soleus muscles from mice were used to investigate the force produced by a muscle. The muscles were pre-stimulated at fixed length, shortened and then held isometrically to give maximum post-shortening forces, before de-stimulation. The mechanical work during active shortening was evaluated by integrating the product of force and shortening velocity over the shortening period. The main finding is a positive relation between the force depression and the mechanical work, whereas the force depression was not correlated to the velocity of shortening. Depression of the passive force component was also observed following all stimulations. Experiments show that the fully stimulated redevelopment of isometric force following concentric contraction follows a time function similar to the creation of force when isometric muscle is initially stimulated. The conclusion is that the isometric force development after active shortening can be well described by an asymptotic force which is decided by the produced work, and the initial isometric time constant.

Paper 2

In paper 2 a transient force production during various non-isometric contractions was investigated. Isolated extensor digitorum longus and soleus muscles from mice (NMRI strain) were used to investigate the force produced by a muscle, and some parameters hypothetically influencing this history-dependent force modification. The muscles were pre-stimulated at a fixed length, then different lengthening/shortening episodes were introduced, whereafter changes of the active force were recorded while the muscles were held isometrically to approach a steady-state force before de-stimulation. The mechanical work during active lengthening and shortening was evaluated by integrating the product of force and ramp velocity over the length-varying period. The results show a negative linear correlation between the force modification and the mechanical work produced on or by the muscle, continuous between shortening and lengthening. A corresponding modification of the passive force component following each stimulation was also observed. The conclusion is that the isometric force attained after lengthening or shortening is well described by an asymptotic force which is determined by the mechanical work.

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Part II

Papers

Paper 1

Muscular force production after concentric contraction

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The steady-state force following active shortening does not reach the maximum isometric force associated with the final length. Isolated extensor digitorum longus and soleus muscles from mice (NMRI strain) were used to investigate the force produced by a muscle, and some parameters hypothetically influencing this shortening-induced force depression. The muscles were pre-stimulated at fixed length, shortened and then held isometrically to give maximum post-shortening forces, before de-stimulation. The shortening magnitude was 0.18 mm, 0.36 mm or 0.72 mm (about 2-7% of optimal length), time of shortening was chosen as 0.03 s, 0.06 s and 0.12 s, and final length as +0.72, 0 and -0.72 mm, related to optimal length. The mechanical work during active shortening was evaluated by integrating the product of force and shortening velocity over the shortening period. The results show a positive correlation between the force depression and the mechanical work, whereas the force depression was not correlated to the velocity of shortening. Depression of the passive force component was also observed following all stimulations. Experiments show that the fully stimulated redevelopment of isometric force following concentric contraction follows a time function similar to the creation of force when isometric muscle is initially stimulated. The conclusion is that the isometric force development after active shortening can be well described by an asymptotic force which is decided by the produced work, and the initial isometric time constant.

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1. Introduction

The main function of the skeletal muscle is to provide force during walking, running and other everyday actions. Muscles generate work during activities and produce movement, and it is important to know the mechanisms underlying such processes as muscle shortening and stretching. In particular, the understanding of transient force production under various length regimes is a necessity for improved description of muscular action in numerical simulations of movement, (Eriksson 2008; Kaphle and Eriksson 2008).

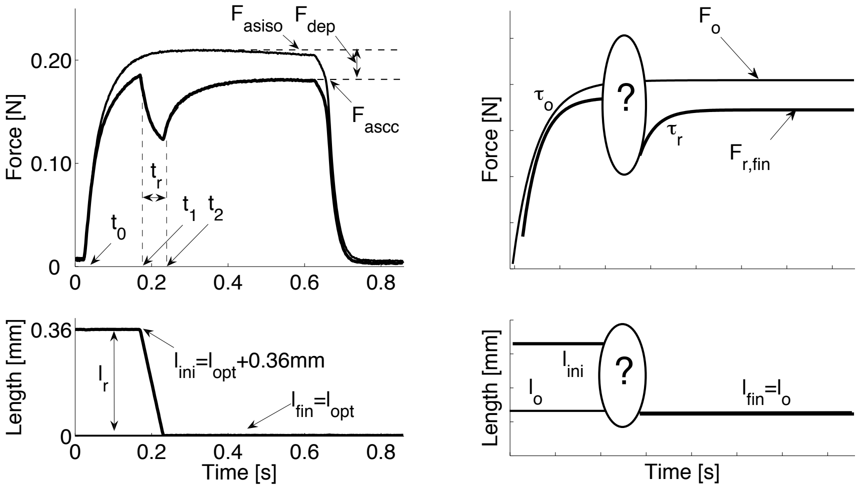
One important aspect is the history dependence in force production. For instance, the steady-state force following concentric contraction does not reach the maximum isometric force associated with the final length. This property is typically referred to as shortening-induced force depression, (Abbott and Aubert 1952). It has been generally accepted that this depression is increasing with increasing shortening magnitude, (Abbott and Aubert 1952; Herzog and Leonard 1997; Lou et al. 1998; Schachar et al. 2004; Bullimore et al. 2007). Furthermore, force depression is long lasting, (Herzog et al. 1998), and is associated with a decrease in the muscle stiffness, (Sugi and Tsuchiya 1988; Razumova et al. 1999; Lee and Herzog 2003). Moreover, it is argued that force depression is directly influenced by the speed of shortening (Marechal and Plaghki 1979; Sugi and Tsuchiya 1988; Herzog and Leonard 1997; Morgan et al. 2000; Lee and Herzog 2003). The latter opinion is based on experiments in which a muscle was shortened by a given magnitude and with a given stimulation, but at different speeds. There is a similar force modification following active stretch of the muscle, as the redeveloped force is higher than the isometric force: force enhancement, (Schachar et al. 2002; Rassier and Herzog 2002; Bagni et al. 2005; Herzog 2005). Muscular force consists of active and passive components, and both active and passive force modification occurs with shortening or stretch.

The present work was motivated by efforts towards numerical modeling of muscular force production, as discussed by Eriksson (2008). In this context, a general description of transient macroscopic muscle force production was seen as:

$$F(t) = \overline{F}(l, \dot{l}, a; t) + dF(h; t), \tag{1}$$

where 'transient' indicates a time-dependence, and 'macroscopic' that the force is seen in an external context, without un-necessary internal phenomenological details. The first term in the equation is thereby the part predicted by, e.g., a Hill-type model. Many such models have been developed, e.g., Günther et al. (2007), but they all lack memory for previous contractions, Forcinito et al. (1998). The aim of the present investigation was to find a basis for creation of the second term, which is a symbolic description of a correction to the force, due to history effects. In Eq. (1), the force F at a time t is related to a force value $\overline{F}$ dependent on the current muscle length l, the current time differential of the length $\dot{l}$, and a stimulation measure a, which is essential but not investigated here. In the force modification dF, the history parameter h reflects parameters for the individual muscle (optimal muscle length, maximum isometric force, etc.) and the context (experimental or live-state description). In the post-shortening isometric situation, the only transient effects in a Hill-type numerical model, which are related to the length variation of the contractile component of the model, disappear, and only the differences shown by the second term remain. After concentric contractions, this is what is commonly denoted as the force depression F_{dep}. In Fig. 1a force depression is seen as the difference between the redeveloped force following active shortening and the force of purely isometric contraction at the same length, both seen in a hypothetical long-term state, neglecting that fatigue effects will reduce the force.

Modeling post-shortening force is, however, not only a question of a steady-state value, as force produced by a muscle will show a time variation essential in a numerical simulation of movement. In the present context, this is visible between the reduced force at the end of a concentric contraction and the steady-state isometric force at the



(a)

(b)

FIGURE 1. Schematic illustration of studied contractions. (a) example of force trace for stimulated mouse EDL muscle held isometrically for 0.6 s; stimulated isometrically for $t_1 - t_0 = 0.15$ s, shortened by $l_\mathrm{r} = 0.36$ mm during $t_\mathrm{r} = t_2 - t_1 = 0.06$ s and then held isometrically for 0.39 s. $\mathrm{F}_\mathrm{asiso}$ and F_ascc are asymptotic values for the muscular force production after isometric and concentric contractions, respectively. (b) evaluation model for force production in initial and post-shortening isometric phases (shortening phase is not evaluated). F_o and $\mathrm{F}_\mathrm{r,fin}$ are values for the muscular force production after isometric and concentric contractions, respectively. τ_o and τ_r are time constants describing force-time relations at length l_o during isometric and post-shortening isometric contractions, respectively. Length expressed as difference to optimal length.

final length. The assumption here was that the time variation can be approximately represented by an asymptotic force and a time evolution function. In the interpretation of experiments, the time function was assumed to be an exponential, described by a time constant, Fig. 1b and Eq. (2).

The objective of the present study was to investigate asymptotic value and time constant for the muscular force production after concentric contractions of different parameters. Representative values for a description of the post-shortening force variation were sought through systematic shortening experiments on mouse muscles, in different muscular length regions.

2. Materials and methods

2.1. *Animals*

Adult male mice (NMRI strain) were housed at room temperature and fed ad libitum. The weight of the mice was about 30 g. Animals were killed by rapid neck disarticulation. The experiments were approved by the Stockholm North local ethical committee.

2.2. *Muscle preparation and mounting*

Experiments were performed on isolated extensor digitorum longus (EDL) and soleus (SOL) muscles. Only one EDL and one SOL muscle was used from each mouse in order to avoid statistical dependence effects, but also due to constraints on experimental time. Small stainless steel hooks were tied, using a thin nylon thread, to the tendons very close (~ 0.1 mm) to the muscle belly. The muscle was then mounted between a force transducer, Dual-Mode Muscle Lever System (Aurora Scientic, Ontario, Canada), and an adjustable holder in a lab-built muscle bath. The muscle was manually stretched by separating the mountings until it began to produce a passive force. This muscle length was thereby close to the optimal length allowing maximum active isometric force.

The weight of the muscle was measured after the experiments. A nominal cross-sectional area was evaluated from tissue density ρ $=1056\,\mathrm{kg/m^3}$ and the individual optimal muscle length.

The final results are based on results from n $= 4$ EDL muscles and n $= 5$ SOL muscles, and are given as mean ± standard deviation. Optimal forces were $F_{\mathrm{opt}} = 0.237 \pm 0.072\,\mathrm{N}$ for EDL and $F_{\mathrm{opt}} = 0.215 \pm 0.028\,\mathrm{N}$ for SOL muscles. Optimal lengths were $11.73 \pm 1.14\,\mathrm{mm}$ and $11.18 \pm 1.12\,\mathrm{mm}$, and cross-sectional areas were $1.11 \pm 0.10\,\mathrm{mm}^2$ and $1.37 \pm 0.44\,\mathrm{mm}^2$, respectively.

During the experiment, the muscle was bathed in a continuously stirred Tyrode solution of the following composition (all in mM): 121 NaCl, 5 KCl, 0.5 $MgCl_2$, 1.8 $CaCl_2$, 0.4 NaH_2PO_4, 0.1 NaEDTA, 24 $NaHCO_3$, 5.5 glucose. Fetal calf serum (0.2%) was added to the solution. The solution was bubbled with $95\%O_2/5\%CO_2$ (pH 7.4). Experiments were performed at room temperature ($\sim 24°C$); at this temperature the contraction timing of mouse muscles is similar to human muscles at body temperature ($\sim 37°C$).

Testing of each muscle was initiated following at least 30 minutes of rest after mounting, and took about 3 hours, with at least one minute of rest between stimulations.

2.3. *Experimental design, stimulation and registration*

A prescribed length variation scheme was applied to the muscle; the precision in a desired length was ~ 0.01 mm. Stimulations with trains of supramaximal square current pulses (0.5 ms) were applied via platinum plate electrodes at specified frequencies (100 Hz for EDL and 70 Hz for SOL), previously found to give tetanic contraction.

Stimulation patterns and length variations were controlled via computer and the Spike2 software (CED, Cambridge, UK). Records of stimulation, force and muscle length were stored for further analysis in a computer, with sampling frequency 500 Hz.

Isometric tests were first performed to find the optimal muscle length for each specimen. This was done through stretching the muscles by small steps, stimulating it, and noting the maximum force produced and the passive force (1 s after stimulation) at every length, the difference being the active force. The highest active force, F_{opt}, defined an optimum length, l_{opt}, which was used as basis for the length variations in subsequent experiments. It was verified that the isometric force varied approximately parabolically around the optimal length. Lengths l were defined as a difference to individual optimal length.

2.4. *Testing paradigm*

The testing sequence was designed to see force as function of length, rather than vice versa. Force produced by the muscle was therefore measured as function of time, for prescribed variations in specimen length. By choosing suitable final muscle length, l_{fin}, ramp size, l_r, and ramp time, t_r, (Fig. 1a), different combinations of length and velocity were obtained. Isometric contractions are special cases with $l_r = 0$.

The central experiments in the series were the concentric contractions. The muscles were fist passively lengthened to $l_{\mathrm{ini}} = l_{\mathrm{fin}} + l_r$. The EDL and SOL muscles were isometrically stimulated for $t_1 - t_0$ =150 ms and 500 ms, respectively, before shortening, in order to give an almost maximum pre-shortening isometric force. Total stimulation times were 600 ms and 1500 ms, respectively, limited in order to avoid fatigue effects. Passive forces were measured 1 s after stimulation at length l_{fin}. A pure shortening ramp without sudden initial steps was used, in order to show time evolution of force in different length regimes, cf. Barclay and Lichtwark (2007).

Three sets of experiments were performed on all muscles. In the first series, final muscle length was equal to the optimum length $l_{\mathrm{fin}} = 0$, the others used $l_{\mathrm{fin}} = +0.72$ mm and $l_{\mathrm{fin}} = -0.72$ mm, respectively.

Ramp size l_r and ramp times t_r were chosen systematically to allow comparisons of steady-state active force modifications. Thus, l_r was 0.18 mm, 0.36 mm and 0.72 mm, and t_r was 0.03 s, 0.06 s and 0.12 s; all nine combinations were tested creating shortening velocities $1.5 \leq l_r/t_r \leq 24$ mm/s (corresponding to between ~ 0.15 and $\sim 2.4\ l_{\mathrm{opt}}/\mathrm{s}$). For each muscle, 27 contractions were therefore recorded, being the combinations of three ramp sizes, three ramp times and three final lengths. Isometric tests were done at optimal length after every nine shortening experiments, to verify that the force output remained stable.

2.5. *Evaluation procedure*

The force-time histories recorded were fitted by exponential functions, through an inhouse equal weights least-squares algorithm in Matlab (version R2006a, The MathWorks, Inc., Natick, MA, USA), with a view on the experiments as in Fig. 1b, giving expressions of the form:

$$F(t) = F_{\text{r,fin}} + (F_{\text{a}} - F_{\text{r,fin}}) \cdot \exp(-1/\tau_r \cdot (t - t_2)), \tag{2}$$

where F_{a}, $F_{\text{r,fin}}$, and $1/\tau_r$ were results from fitting for a phase starting at time t_2. The equation defines an exponential curve for F, starting at $F = F_a$ at time $t = t_2$, and with $F = F_{\text{r,fin}}$ as the long term asymptote as $t \to \infty$. The redeveloped isometric force-time trace between end of ramp and end of stimulation was considered.

A similar evaluation of force evolution was performed for isometric cases at lengths l_{fin}, until maximum force was reached. Similarly as in Eq. (2), isometric asymptotic forces F_{o}, $F_{\text{o,+}}$ and $F_{\text{o,-}}$ were obtained for the three final lengths, with $F_{\text{o}} \approx F_{\text{opt}}$. Isometric force development gave exponential time constants τ_{o}, $\tau_{\text{o,+}}$, $\tau_{\text{o,-}}$.

From the obtained values, the steady-state force depression was evaluated. In each series of experiments, the force depression F_{dep} was obtained as the difference between the asymptotic redeveloped isometric force following shortening and the asymptotic isometric force for the current final length, Fig 1b, as:

$$F_{\text{dep}} = -(F_{\text{r,fin}} - F_{\text{o}}) - F_{\text{dep,p}}. \tag{3}$$

The active depression of the muscular force was obtained by subtracting the passive parts, measured at the post-stimulation phases at l_{fin}, from $F_{\text{r,fin}}$ and F_{o}. A depression in passive force was evaluated as the reduction in passive force 1 s after stimulation, according to:

$$F_{\text{dep,p}} = -(F_{\text{fin,p}} - F_{\text{o,p}}) \tag{4}$$

where $F_{\text{fin,p}}$ is the passive force 1 s after the concentric contraction, and $F_{\text{o,p}}$ the passive force after isometric contraction.

Mechanical work produced by the muscle during shortening was evaluated by integrating the force multiplied by shortening velocity, over the ramp period:

$$W = \int_{l_{\text{ini}}}^{l_{\text{fin}}} F \, dl \equiv -\int_{t_1}^{t_1+t_r} F(t) \frac{dl}{dt} dt \tag{5}$$

where the second of the equivalent forms was used for evaluation. The integral was evaluated numerically through all recorded force values in the considered interval. Negative work from passive force during initial stretching to l_{ini} was not considered.

3. Results

Examples of total force and length traces in EDL and SOL muscles, for optimal, superoptimal and suboptimal final muscle lengths are given in Fig. 2. The force representing the maximal sustained output was reached or almost reached before shortening was introduced. During active shortening, force rapidly decreased, and once shortening was completed, force recovered and attained a new steady-state value. This new steady-state force was consistently smaller than the corresponding isometric reference force, regardless of the ramp conditions.

The exponential fitting showed good agreement with experimental data for isometric force development and force redevelopment after active shortening ($R^2 > 0.99$) (Hancock et al. 2004; Corr and Herzog 2005).

Figure 3 shows average active force depressions after shortening as functions of shortening velocity. Lines are drawn in the figures to connect experiments with either the same ramp time t_r (a), or the same ramp size l_r, (b). Average force depression is similarly plotted as function of average mechanical work in Fig. 4, showing good correlation in all situations.

The experiments at superoptimal and suboptimal final lengths gave similar relations between force depression, mechanical work and rise time constants as the cases with optimal final length.

Passive force was consistently slightly lower after both isometric and concentric contractions, but redeveloped during the rest between experiments. For concentric cases, the difference was more pronounced, indicating a passive force depression. The average passive force depression, $F_{\mathrm{dep,p}}$, is plotted in Fig. 5 for EDL and SOL muscles with $l_{\mathrm{fin}} = 0$ mm, $l_{\mathrm{fin}} = -0.72$ mm and $l_{\mathrm{fin}} = +0.72$ mm.

4. Discussion

The main objective of this work was to evaluate the reliability of some proposed descriptions for the steady-state force depression, i.e., a modification following concentric contraction. This force depression shows that muscular force is not — even asymptotically — described by only the first term of Eq. (1).

The results show that the shortening velocity is not a good predictor for this depression. This was verified by sets of experiments, where the velocities were produced through different combinations of ramp size and time, but with the same final isometric length. Figures 3a show that the force depression F_{dep} is positively correlated with the shortening velocity l_r/t_r when varying the ramp size, with a constant ramp time, as suggested by Sugi and Tsuchiya (1988); Herzog and Leonard (1997); Josephson and Stokes (1999); Herzog et al. (2000); Leonard and Herzog (2005); Herzog and Leonard (2007). This hypothesis is, however, contradicted by the experiments where different velocities were introduced by varying the ramp time at a constant ramp size. This is consistent with results observed in a variety of other preparations. The conclusion is that not only the shortening velocity but also the shortening magnitude must be an important parameter for the force depression, and the ramp size is not, in itself, a good predictor, as was suggested by Bullimore et al. (2007) with a stress-induced inhibition of crossbridges as explanation.

A measure which considers both magnitude and velocity of shortening is the mechanical work, W, produced by the muscular force during shortening. This measure gives positive correlation with the force depression for performed experiments, as shown by Fig. 4. This relation was also suggested by Josephson and Stokes (1999), and tentatively by Herzog et al. (2000), although the hypothesis in the latter seems to have been later discarded by Bullimore et al. (2007). Although clear for the present experiments, further experiments with other length and stimulation paradigms are needed in order to verify the generality of this correlation.

The motivation for the tentative statement in Herzog et al. (2000), that force-depression was predicted by mechanical work, was related to the amount of work needed in actin filament deformation during shortening. It can also be dependent on the metabolic changes. For instance, the rate of cross-bridge cycling, and hence the rate of ATP consumption, is higher during shortening than under isometric conditions. This leads to a more rapid breakdown of phosphocreatine during shortening, resulting in an increased concentration of phosphate ions, which tends to decrease isometric force production (Allen et al. 2008).

It is seen from Figures 3 and 4 that the EDL muscles, for all ramp parameters, give a force depression which is considerably larger then the corresponding one for SOL, although the length and force scales are very similar. Introducing a proportional function for the relation between the force depression measure F_{dep}/F_o and the mechanical work, it is seen that the proportionality constant is about 2.5 times higher for EDL than for SOL. This difference fits with the idea that the force depression is caused by metabolic factors, because the rate of ATP consumption during contractions is markedly faster in EDL than in SOL muscles (Barclay et al. 1993).

Figure 5 shows that the passive force depression is essentially independent of the parameters and the mechanical work performed in the concentric contraction experiments. This is clearly different to the active force depression, which depends on the mechanical work. As discussed above, the active force depression is likely to depend on metabolic factors and these factors would then not affect the passive force depression. Titin is a molecular spring that runs from the Z lines of sarcomeres to the M band, and it is the structure responsible for most of the passive force in muscle cells (Joumaa et al. 2008). There is an increase in passive force when contracting muscles are being stretched, i.e. the opposite to what is observed here. Titin has a central role in the stretched-induced enhancement of passive force (Joumaa et al. 2008) and it is possible that titin is also involved in the present passive force depression. Thus, our concentric contractions resulted in a reversible decrease in passive force that seems unrelated to energy metabolism, but the underlying mechanisms remain uncertain.

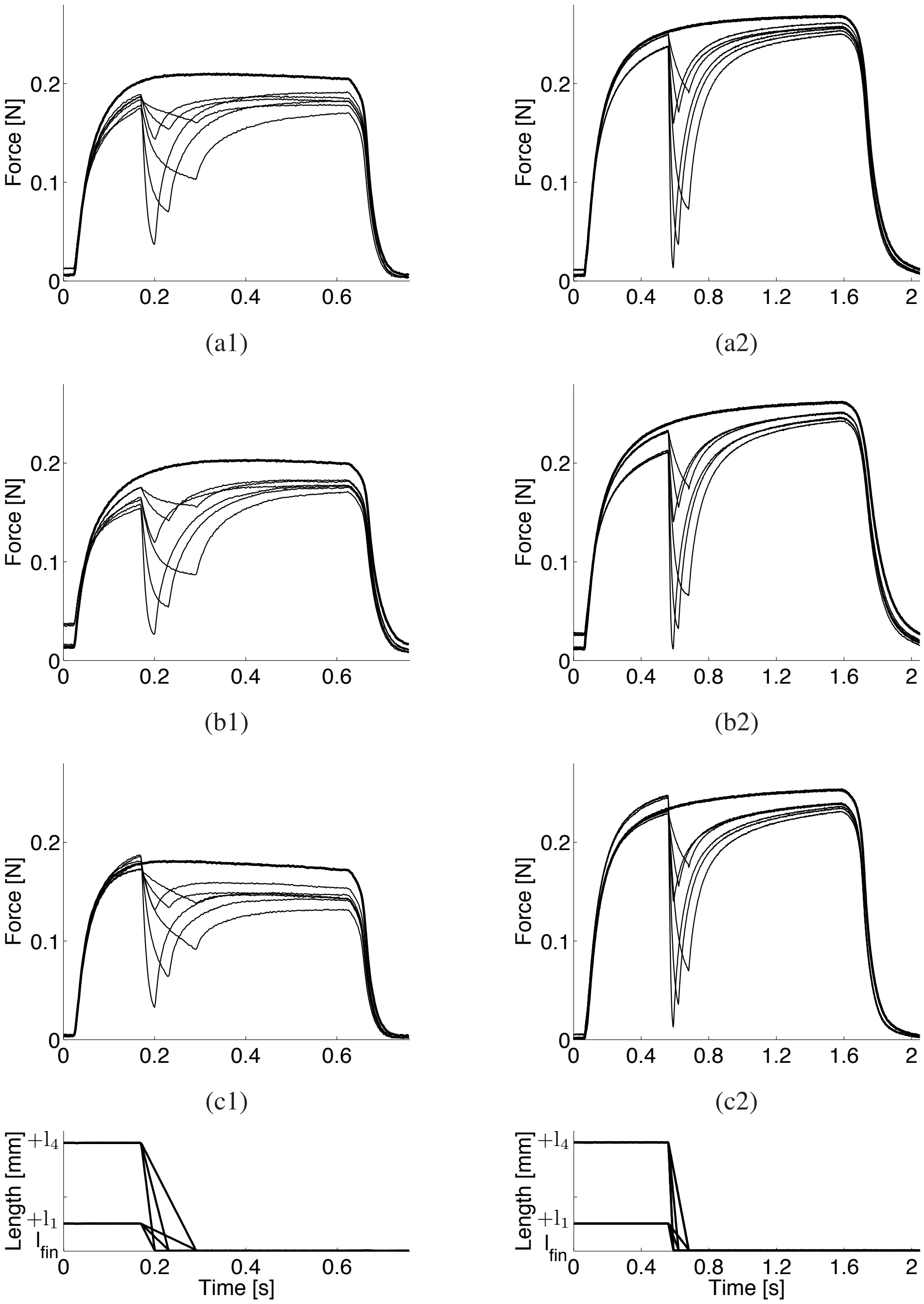
The rise time constant of redeveloped muscle force, τ_r, is shown to be, for practical simulation purposes, similar to the time constant for initial isometric contractions at optimal length, τ_{o} (Fig 6). This indicates that when the muscles are stimulated with a high intensity, the rising of the Ca^{2+} level and subsequent activation of the actin filament during the force development phase is a considerably faster process than the cross-bridge cycling leading to increased force. Once fully activated, the increase in force production occurs at the same rates, regardless of whether a shortening has just ended. This statement seems to be verified by both muscle types, but the EDL muscles show a time constant about one-third of that for SOL muscles. Time constants are in the experiments almost independent of isometric length.

The experiments clearly show that a macro expression for transient muscular force such as Eq. (1) must not only take length l and length differential $\dot{l}$ into account. Following concentric contractions, it is obvious that steady-state force also shows a history dependence, which can symbolically be seen as the second term in Eq. (1). With this indication, the functional parameter and dependence remain to be investigated. Whether a common description of the force modification is possible, valid for all contraction histories and expressed in basic muscular macro-parameters such as optimal length, maximum isometric force and inherent time scales, is an open question.

5. Conclusions

Experiments show that shortening velocity is not a good general predictor for the force modification following concentric contraction. They indicate that steady-state force depression is better predicted by the mechanical work produced by the muscle during active shortening.

The isometric force development after active shortening can be well described by an asymptotic force which is decided by the produced work, and the initial isometric time constant.



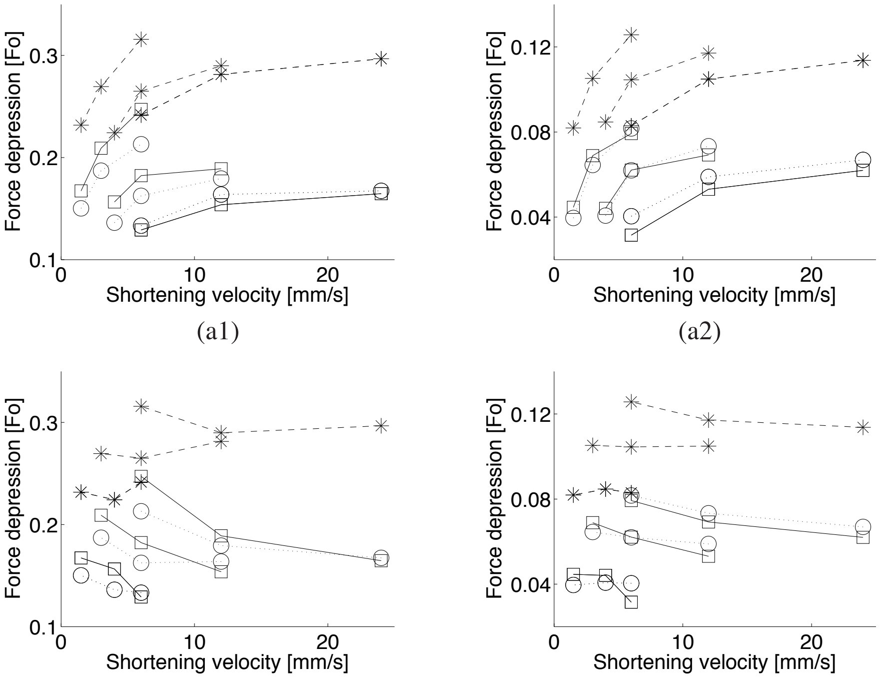
(a1) (a2)

(b1) (b2)

(c1) (c2)

(d1) (d2)

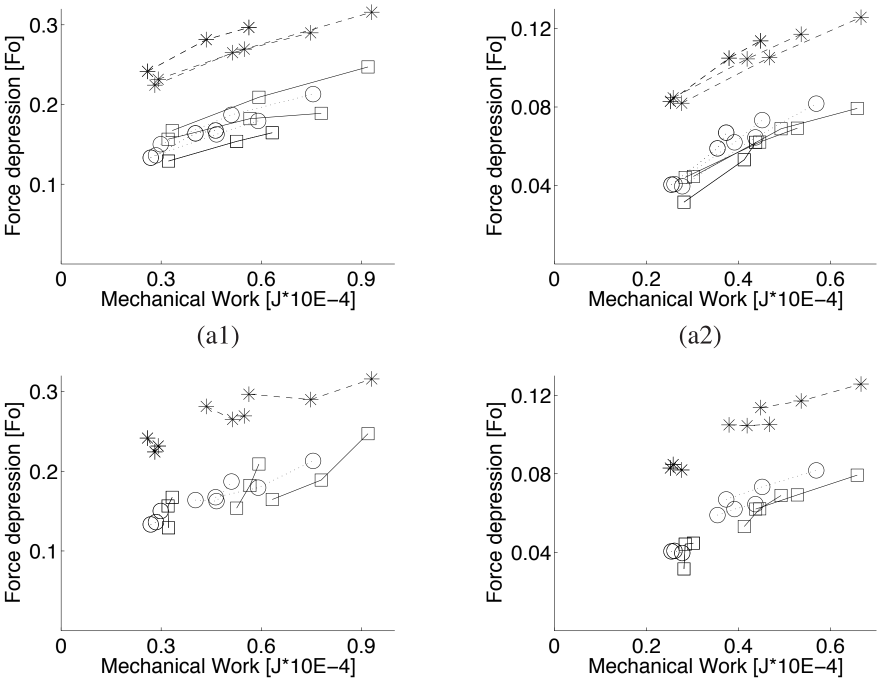
FIGURE 2. Examples of active shortening of mouse EDL (1) and SOL (2) muscles with a) optimal, b) superoptimal and c) suboptimal final muscle length: $l_{\text{fin}} = 0, +0.72, -0.72$ mm, respectively. Shortening lengths $l_{\text{r}} = 0.18, 0.72$ mm, shortening times $t_{\text{r}} = 0.03, 0.06, 0.12\,\text{s}$.



(a1) (a2)

(b1) (b2)

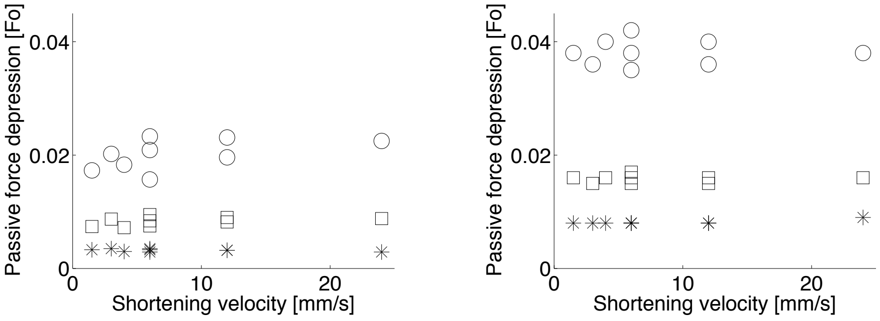
FIGURE 3. Steady-state force depression as a function of shortening velocity of mouse EDL (1) and SOL (2) muscles. Lines connect groups of experiments with the same a) shortening time, t_r, or b) shortening length, l_r. Square, circle and star markers correspond to experiments with optimal, superoptimal and suboptimal final muscle lengths: $l_{\text{fin}} = 0, +0.72, -0.72$ mm, respectively. Average value for group (n_{EDL}=4, n_{SOL}=5).



(a1) (a2)

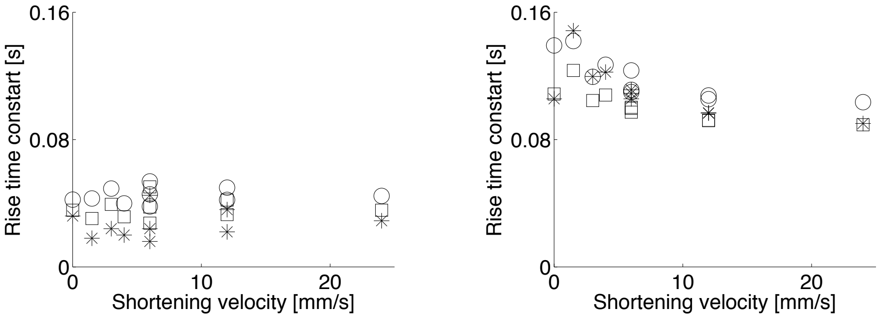
(b1) (b2)

FIGURE 4. Steady-state force depression as a function of mechanical work of mouse EDL (1) and SOL (2) muscles. Lines connect groups of experiments with the same a) t_r, b) l_r. Square, circle and star markers correspond to experiments with optimal, superoptimal and suboptimal final muscle lengths:$l_{\text{fin}} = 0, +0.72, -0.72$ mm, respectively. Average value for group (n_{EDL}=4, n_{SOL}=5).



(1) (2)

FIGURE 5. Passive force depression $F_{\text{dep,p}}$, $F_{\text{dep,p},+}$ and $F_{\text{dep,p},-}$, normalized with respect to active isometric force at a corresponding length, as a function of shortening velocity of mouse EDL (1) and SOL (2) muscles. Square, circle and star markers correspond to experiments with optimal, superoptimal and suboptimal final muscle lengths: $l_{\text{fin}} = 0, +0.72, -0.72$ mm, respectively. Average value for group (n_{EDL}=4, n_{SOL}=5).



(1) (2)

FIGURE 6. Rise time constants for isometric force development, τ_{o}, $\tau_{\text{o},+}$, $\tau_{\text{o,-}}$ (shortening velocity $= 0$), and post-shortening isometric force redevelopment, τ_r, $\tau_{r,+}$, $\tau_{r,-}$, as a function of shortening velocity of mouse EDL (1) and SOL (2) muscles. Square, circle and star markers correspond to experiments with optimal, superoptimal and suboptimal final muscle lengths: $l_{\text{fin}} = 0, +0.72, -0.72$ mm, respectively. Average value for group (n_{EDL}=4, n_{SOL}=5).

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2

Paper 2

Mechanical work as predictor of force enhancement and force depression

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The steady-state force following active muscle shortening or stretch differs from the maximum isometric force associated with the final length. This phenomenon proves that the isometric force production is not only dependent on current muscle length and length time derivative, but depends on the preceding contraction history. Isolated extensor digitorum longus and soleus muscles from mice (NMRI strain) were used to investigate the force produced by a muscle, and some parameters hypothetically influencing this history-dependent force modification. The muscles were pre-stimulated at a fixed length, then different stretch/shortening episodes were introduced, whereafter changes of the active force were recorded while the muscles were held isometrically to approach a steady-state force before de-stimulation. The mechanical work during active stretch and shortening was evaluated by integrating the product of force and ramp velocity over the length-varying period. The results show a negative linear correlation between the force modification and the mechanical work produced on or by the muscle, continuous between shortening and stretch. A corresponding modification of the passive force component following each stimulation was also observed. The conclusion is that the isometric force attained after stretch or shortening is well described by an asymptotic force which is determined by the mechanical work.

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1. Introduction

Skeletal muscles generate work during activities and produce movement, and it is important to know the mechanisms underlying such processes as muscle shortening and stretch. In particular, the understanding of transient force production under various length regimes is a necessity for improved description of muscular action in numerical simulations of movement, (Kaphle and Eriksson 2008; Yamane and Nakamura 2007).

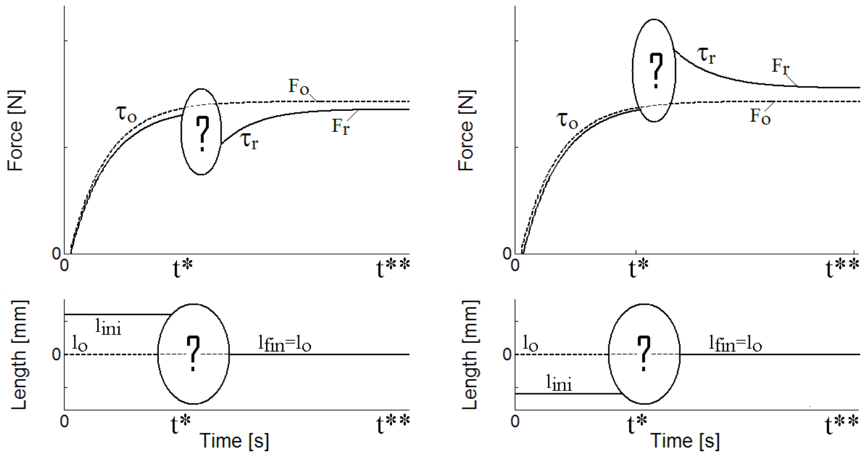
One important aspect is the history dependence of force production. For instance, the steady-state force following concentric contraction does not reach the maximum isometric force associated with the final length. This property is typically referred to as shortening-induced force depression, (Abbott and Aubert 1952). It has been generally accepted that this depression is influenced by shortening magnitude, (Abbott and Aubert 1952; Herzog and Leonard 1997; Lou et al. 1998; Schachar et al. 2004; Bullimore et al. 2007) and speed of shortening (Marechal and Plaghki 1979; Sugi and Tsuchiya 1988; Herzog and Leonard 1997; Morgan et al. 2000; Lee and Herzog 2003). A previous paper by the authors (Kosterina et al. 2008) has shown that these descriptions are true, but only for a certain experimental paradigm. A more robust predictor of force depression was shown to be the mechanical work produced by the muscle during shortening.

There is a similar force modification following active stretch of the muscle, as the redeveloped force is higher than the isometric force: force enhancement, (Schachar et al. 2002; Rassier and Herzog 2002; Bagni et al. 2005; Herzog 2005; Morgan 2007). Muscular force consists of active and passive components, and both active and passive force modifications occur with shortening or stretch, (Bagni et al. 2002; Rassier and Herzog 2002; Joumaa et al. 2008; Herzog et al. 2008).

The present work was motivated by efforts towards numerical modeling of transient macroscopic muscular force production, Eriksson (2008). 'Transient' here indicates a time-dependence, and 'macroscopic' that the force is seen in an external context, without internal phenomenological details. The history dependence is thereby to be introduced as an additional component in, e.g., a Hill-type model. Many such models have been developed, e.g., Günther et al. (2007), but they normally lack memory for previous contractions, Forcinito et al. (1998). The aim of the present investigation was to find a basis for creation of a numerical description of a correction to the force, due to history effects. This will give a total expression for the force F at a time t, related to the Hill force value $\overline{F}$, which is dependent on the current muscle length l, the current time differential of the length $\dot{l}$, and a stimulation measure a, the latter essential but not investigated here. In the post-shortening and post-stretch isometric situations, the only transient effect in a Hill-type numerical model, which is related to the length variation of the contractile component of the model, disappears. In Fig. 1, force modification is seen as the difference between the redeveloped force following active non-isometric contraction and the force of purely isometric contraction at the same length, both seen in a hypothetical long-term state, neglecting that fatigue effects will reduce the force.

Modeling redeveloped force is, however, not only a question of a steady-state value, as force produced by a muscle will show a time variation. Consideration of this effect is essential in a numerical simulation of movement. In the present context, it is visible between the redeveloped force at the end of a length-modifying contraction and the steady-state isometric force at the final length. The assumption here was that the time variation can be approximately represented by an asymptotic force and a time evolution function. In the interpretation of experiments, the time function was assumed to be an exponential, described by a time constant.

In the present study, we hypothesize that force modification, both depression and enhancement, can be related to the amount of mechanical work performed by or on the muscle during the change of length. This statement is based on previous observations,



(a) (b)

FIGURE 1. Schematic illustration of contractions studied. Evaluation model for force production in initial and (a) post-shortening or (b) post-stretch isometric phases (ramp phase is not evaluated). F_o and F_r are asymptotic values for the muscular force after isometric and transient-length contractions, respectively. τ_o and τ_r are time constants describing force-time relations at length l_o during isometric and post-ramp isometric contractions, respectively. Length expressed as difference to optimal length. The total force modification is the difference between F_r and F_o, consisting of active and passive parts.

Kosterina et al. (2008), based on investigations of force depression following concentric contractions. The objective of the present study was to investigate asymptotic values for the muscular force production after more general transient-length contractions of different parameters.

2. Materials and methods

2.1. *Animals*

Adult male mice (NMRI strain) were housed at room temperature and fed ad libitum. The weight of the mice was about 30 g. Animals were killed by rapid neck disarticulation. The experiments were approved by the Stockholm North local ethical committee.

2.2. *Muscle preparation and mounting*

Experiments were performed on isolated extensor digitorum longus (EDL) and soleus (SOL) muscles. Small stainless steel hooks were tied, using a thin nylon thread, to the tendons very close (~ 0.1 mm) to the muscle belly. The muscle was then mounted between a force transducer, Dual-Mode Muscle Lever System (Aurora Scientic, Ontario, Canada), and an adjustable holder in a lab-built muscle bath. The muscle was manually stretched until it began to produce passive force. This muscle length was thereby close to the optimal length giving maximum tetanic isometric force.

During the experiment, the muscle was bathed in a continuously stirred Tyrode solution of the following composition (mM): 121 NaCl, 5 KCl, 0.5 $MgCl_2$, 1.8 $CaCl_2$, 0.4 NaH_2PO_4, 0.1 NaEDTA, 24 $NaHCO_3$, 5.5 glucose. Fetal calf serum (0.2%) was added to the solution. The solution was bubbled with $95\%O_2/5\%CO_2$ (pH 7.4). Experiments were performed at room temperature ($\sim 24°C$); at this temperature the contraction timing of mouse muscles is similar to human muscles at body temperature ($\sim 37°C$).

Testing of each muscle was initiated following at least 30 minutes of rest after mounting, the muscles were rested at least one minute between stimulations. Each muscle was subjected to a number of the length regimes discussed below; a set of experiments contained all parametric variations for that type of length regime. The sets were introduced in different orders for the specimen.

Isometric tests were done at optimal length after every set of experiments, to verify that the force output remained stable, otherwise data from the last set were discarded. The maximum admissible decrease in optimal force was specified as 10%, and mean final force drop averaged 4% for SOL and 8% for EDL muscles. A total of 10 SOL and 14 EDL muscles were tested, but final results for each set of experiments, as further discussed below, are based on results from n $= 4 - 6$ EDL muscles and n $=$ $5 - 6$ SOL muscles. The results are given as mean ± standard deviation. Maximum active isometric forces were 0.27 ± 0.073 N for EDL and 0.143 ± 0.054 N for SOL muscles. Optimal lengths were 11.77 ± 1.00 mm and 10.41 ± 0.48 mm, and cross-sectional areas were $1.14 \pm 0.14\,\mathrm{mm}^2$ and $1.17 \pm 0.12\,\mathrm{mm}^2$, respectively.

The weight of the muscle was measured after the experiments. A nominal cross-sectional area was evaluated from tissue density $\rho = 1056\,\mathrm{kg/m}^3$ and the individual optimal muscle length.

2.3. *Experimental design, stimulation and registration*

A prescribed length variation scheme was applied to the muscle; the precision in a desired length was ~ 0.01 mm. Stimulations with trains of supramaximal square current pulses (0.5 ms) were applied via platinum plate electrodes at specified frequencies: 100 Hz for EDL and 70 Hz for SOL.

Stimulation patterns and length variations were controlled via computer and the Spike2 software (CED, Cambridge, UK). Records of stimulation, force and muscle length were stored for further analysis in a computer, with a sampling frequency of 500 Hz.

Isometric tests were first performed to find the optimal muscle length for each specimen. This was done through stretching the muscles by small steps, stimulating it, and noting the maximum force produced and the passive force (1 s after stimulation) at every length, the difference being the active force. The highest active force defined an optimum length, l_{opt}, which was used as basis for the length variations in subsequent experiments. It was verified that the active isometric force varied approximately parabolically around the optimal length. Lengths (l) were defined as a difference to individual optimal length l_{opt}.

2.4. *Testing paradigm*

The testing sequence was designed to study force as a function of length, rather than vice versa. Force produced by the muscle was therefore measured as a function of time, for prescribed variations in specimen length. By choosing suitable ramp parameters, different combinations of transient-length contractions were obtained (Table 1). Isometric contractions are special cases with no ramps.

Firstly, the muscle length was passively set as l_{ini}. After a certain time, at least 10 s, the muscle was isometrically stimulated for 150 ms before any length change, in order to give an almost maximum isometric force. Total stimulation time was 600 ms, during which the length regime was introduced; the time was limited in order to avoid fatigue effects. Passive forces were measured 1 s after the end of stimulation at the final length (l_{fin}), which was most often equal to the optimal length in present experiments.

The series of experiments can be divided into four sets according to the length variation in the stimulated state. In the 'Shortening' and 'Stretch' experiment sets, muscles were shortened or stretched from a certain length value with a certain velocity. For some of these experiments, the ramp was divided into two equal parts, and these parts were performed with a delay between them. 'Stretch-Shortening' experiments consist of active muscle stretch, followed by an active shortening, without a delay between the ramps. 'Long Shortening' experiments consisted of concentric contractions with a final length lower than optimal, $l_{\text{fin}} = -0.72\text{mm}$.

Ramp sizes (l_r) and ramp times (t_r) were chosen systematically to allow comparisons of steady-state active force modifications. These parameters are introduced in Table 1; all tested combinations were creating ramp velocities $6 \leq |l_r/t_r| \leq 24$ mm/s (corresponding to between ~ 0.6 and $\sim 2.4\ l_{\text{opt}}/\text{s}$). Contractions for each muscle were therefore recorded, being the combinations of stretch and shortening ramps.

2.5. *Evaluation procedure*

The force-time histories for the isometric force development phases were fitted by exponential functions, through an in-house equal weights least-squares algorithm in Matlab (version R2006a, The MathWorks, Inc., Natick, MA, USA), with a view on the experiments as in Fig. 1, giving expressions of the form:

$$F(t) = F_r + (F_a - F_r) \cdot \exp(-1/\tau_r \cdot (t - t_0)), \tag{1}$$

where F_a, F_r, and $1/\tau_r$ were results from fitting for a phase starting at time t_0. The equation defines an exponential curve for F, starting at $F = F_a$ at time $t = t_0$, and with $F = F_r$ as the long term asymptote as $t \to \infty$. The redeveloped isometric force-time trace between end of last ramp and end of stimulation was considered.

TABLE 1. Parameters used in 'Shortening', 'Long Shortening', 'Stretch' and 'Stretch-Shortening' experiments, where l_r, t_r and v are ramp-size, ramp-time and ramp velocity, t_d is a delay time between two ramps, l_{fin} is a final muscle length, defined as a difference to individual optimal length. Experiments marked with asterisk were performed for SOL muscles only.

Experiment name	l_{r1}, [mm]	t_{r1}, [s]	$v_1=l_{r1}/t_{r1}$, [mm/s]	t_d [s]	l_{r2}, [mm]	t_{r2}, [s]	$v_2=l_{r2}/t_{r2}$, [mm/s]	l_{fin}, [mm]
ShoOne1	−0.36	0.06	−6	0	0	0	0	0
ShoOne2	−0.72	0.06	−12	0	0	0	0	0
ShoOne3	−0.36	0.12	−3	0	0	0	0	0
ShoOne4	−0.72	0.12	−6	0	0	0	0	0
ShoTwo1	−0.18	0.03	−6	0.12	−0.18	0.03	−6	0
ShoTwo2	−0.36	0.03	−12	0.12	−0.36	0.03	−12	0
ShoTwo3	−0.18	0.06	−3	0.12	−0.18	0.06	−3	0
ShoTwo4	−0.36	0.06	−6	0.12	−0.36	0.06	−6	0
ShoLong1	−2.16	0.36	−6	0	0	0	0	−0.72
ShoLong2	−1.98	0.33	−6	0	0	0	0	−0.72
ShoLong3	−1.80	0.30	−6	0	0	0	0	−0.72
ShoLong4	−1.62	0.27	−6	0	0	0	0	−0.72
ShoLong5	−1.44	0.24	−6	0	0	0	0	−0.72
ShoLong6	−1.26	0.21	−6	0	0	0	0	−0.72
StrOne1	0.18	0.06	3	0	0	0	0	0
StrOne2	0.18	0.12	1.5	0	0	0	0	0
StrOne3	0.36	0.12	3	0	0	0	0	0
StrOne4	0.09	0.03	3	0	0	0	0	0
StrOne5	0.09	0.06	1.5	0	0	0	0	0
StrOne6	0.09	0.12	0.75	0	0	0	0	0
StrTwo*	0.18	0.06	3	0.12	0.18	0.06	3	0
StrSho1	0.18	0.12	1.5	0	−0.18	0.06	−3	0
StrSho2	0.18	0.12	1.5	0	−0.36	0.06	−6	0
StrSho3	0.18	0.12	1.5	0	−0.72	0.06	−12	0
StrSho4*	0.36	0.12	3	0	−0.18	0.06	−3	0
StrSho5*	0.36	0.12	3	0	−0.36	0.06	−6	0
StrSho6*	0.36	0.12	3	0	−0.72	0.06	−12	0
StrSho7	0.18	0.06	3	0	−0.18	0.12	−1.5	0
StrSho8	0.18	0.06	3	0	−0.36	0.12	−3	0
StrSho9	0.18	0.06	3	0	−0.72	0.12	−6	0

A similar evaluation of force evolution was performed for initial isometric cases at lengths l_{fin}. Similarly as in Eq. (1), an isometric asymptotic force (F_o) and an exponential time constant (τ_o) were obtained.

The exponential fitting showed good agreement with experimental data for isometric force development and force redevelopment after ramps ($R^2 > 0.99$) (Hancock et al. 2004; Corr and Herzog 2005).

From the obtained values, the steady-state force modification was evaluated. In each experiment, the total force modification $F_{\text{mod,total}}$ was obtained as the difference between the asymptotic redeveloped isometric force following transient-length contractions and the asymptotic isometric force, Fig. 1, as:

$$F_{\text{mod,total}} = F_r - F_o = F_{\text{mod}} + F_{\text{mod,p}}. \tag{2}$$

The active modification of the muscular force F_{mod} was obtained by subtracting the change in passive force, $F_{\text{mod,p}}$, from the total force modification, $F_{\text{mod,total}}$. The passive forces were always measured 1 s after stimulation.

Mechanical work produced by the muscle during ramps was evaluated by integrating the force multiplied by ramp velocity, over the ramp period:

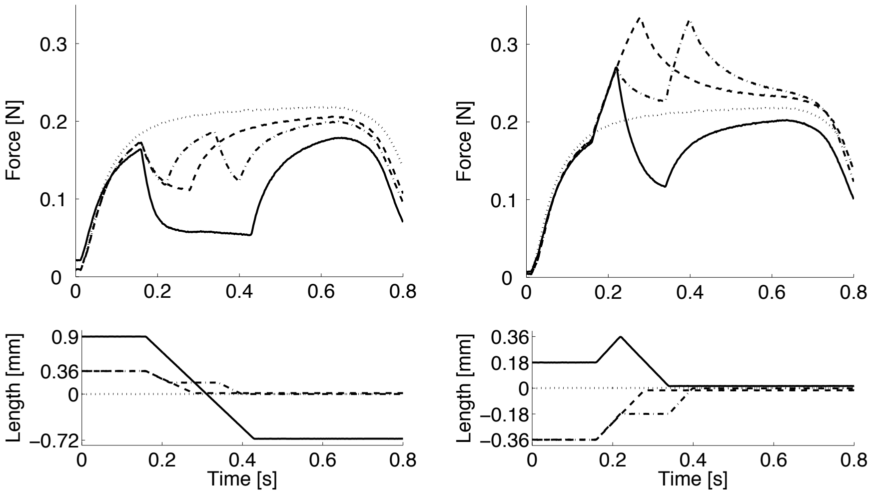
$$W = -\int_{l_{\text{ini}}}^{l_{\text{fin}}} F\,dl \equiv -\int_{t_{\text{ini}}}^{t_{\text{fin}}} F(t)\frac{dl}{dt}dt \tag{3}$$

giving positive work for shortening, negative for stretch. The integral was evaluated numerically through all recorded force values in the considered ramp intervals. Work from passive force during initial length setting to l_{ini} was not considered. In multi-ramp cases, the work was evaluated as the net value, considering positive and negative contributions.

3. Results

Examples of total force and length traces in SOL muscles, for stretch, shortening and stretch-shortening variations are shown in Fig. 2. The force was almost 90% of the maximal sustained output at initial length l_{ini} before ramps were introduced. During active shortening and stretch, force was rapidly decreasing or increasing, correspondingly. In tests with stretch and shortening in two steps, force reached an intermediate value during the delay between ramps. When ramps were completed, force approached a new steady-state value.

Figure 3 shows mean data of normalized active force modification after transient-length contractions of SOL (a) and EDL (b) muscles as functions of normalized mechanical work performed during the different ramps described in Table 1. Force modification, F_{mod}, was normalized by optimal force, F_{o}, to get a dimensionless value; mechanical work calculated as in Eq. (3) was divided by $F_{\text{o}}l_{\text{o}}$ to discard the unit. The positive and negative values of work relate to active shortening and stretch, respectively. Overall, the data points show a clear correlation between decreasing force and increasing mechanical work. The linear fits show that this correlation also exists within each set of experiments (shortening, long shortening, stretch, stretch shortening).



(a) (b)

FIGURE 2. Examples of force-time traces of studied transient-length contractions on mouse SOL muscle. Dotted lines correspond to isometric contractions at optimal muscle length. Transient-length contractions: (a) ShoLong4 - solid line, ShoOne3 - dashed line, ShoTwo3 - dash-dot line. (b) StrSho8 - solid line, StrOne3 - dashed line, StrTwo2 - dash-dot line. Parameters of mentioned experiments are described in Table. 1.

As the EDL muscle is more vulnerable to stretch, active force was decreasing by almost 1% for each active stretching. Although we do not observe a significant force enhancement for EDL muscles, the relations between force modification and mechanical work are similar with the ones for SOL muscles, when the experimental loss is considered.

In Fig. 4 we specifically address a subset of shortening experiments. In these, shortening was induced with the same velocity and the same total length change, but either in one step or in two steps with a short isometric period in between. This isometric phase increased the force produced during the second half of shortening. Therefore, the mechanical work performed was always larger with two steps than with one step of shortening, and this was associated with markedly larger force depressions both in SOL (Fig. 4a) and EDL (Fig. 4b) muscles.

The modification in passive force show a pattern similar to that for active force; that is, a correlation between decreasing passive force and increasing mechanical work during the contraction both in SOL (Fig. 5a) and EDL (Fig. 5b) muscles. It should be noted, however, that the magnitude of passive force modification was only about one tenth of that for active force.

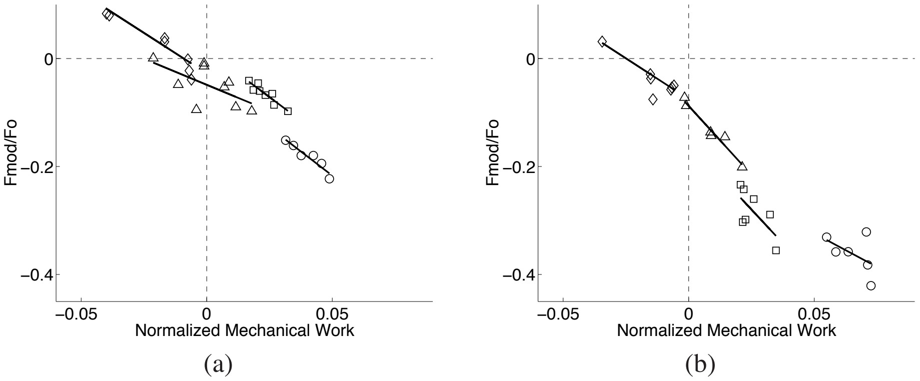


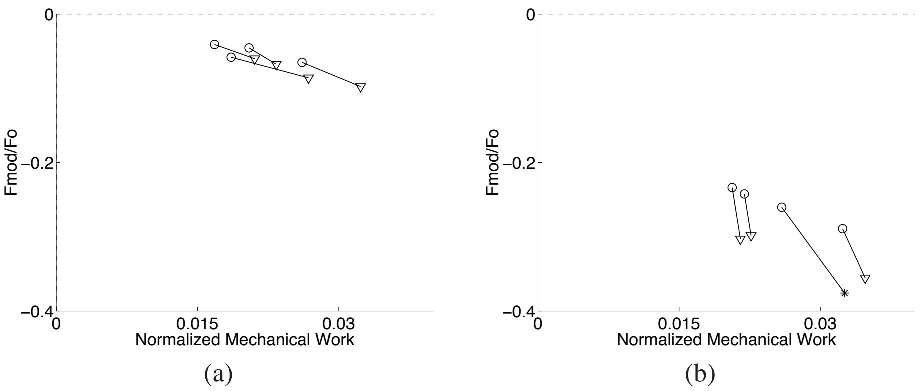
FIGURE 3. Steady-state force modification as a function of normalized mechanical work of mouse (a) SOL and (b) EDL muscles. Data points represent mean values for the groups presented in Table 1: Shortening - squares, Stretch - diamonds, Long Shortening - circles, Stretch-Shortening - triangles. Average value for group, maximum standard deviations for mechanical work are about 3%, for force modification are about 9% for both SOL and EDL.

4. Discussion

The motivation behind this study was to improve the predictive capacity of common Hill muscle models, where muscular force is described by only the current length after the transients in contractile component length have disappeared. The basic finding of the work is that a memory effect is present in muscular force production, as the steady-state isometric force is consistently modified by a preceding active shortening or stretch. This effect can be introduced in a Hill model. The results from the experiments show that the mechanical work produced by or on the muscle during the transient phase can be a good predictor for this force modification, valid for different length regimes.

Mechanical work, produced on or by the muscles during stretch and shortening, is a parameter which considers both direction magnitude and velocity of the ramp. The present results show that the mechanical work is negatively correlated with the force modification in all sets of experiments including shortening and stretches (Fig. 3). This suggests that the force depression with concentric contraction and the force enhancement with eccentric contraction are at least partially affected by a common mechanism. This is in contrast to the general idea that the force modification following shortening and stretches involves different mechanisms (Bullimore et al. 2007; Morgan 2007; Herzog et al. 2008). However it should be noted that the force enhancement observed with the present modest stretches was relatively small and that other mechanisms might become evident with more severe stretches.

Concentric contractions were performed in two different regimes. The muscles produced larger mechanical work after introducing a delay during the shortening ramp, as the force value was raised during the delay (see Fig. 2(a) and Fig. 4). The higher



(a) (b)

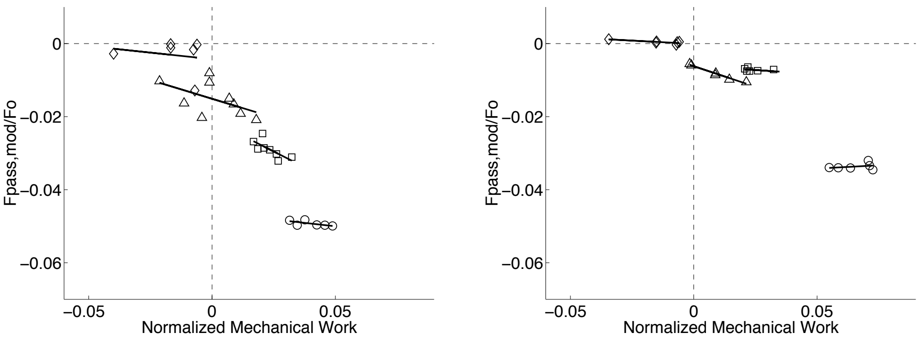
FIGURE 4. Steady-state force modification as a function of normalized mechanical work of mouse (a) SOL and (b) EDL muscles. Groups of experiments with one step Shortening - circles, two steps - triangles. Lines connect results from experiments with the same velocity and total length change. Average value for group, asterisk corresponds to group of just 3 specimens. Maximum standard deviations for mechanical work are about 1%, for force modification are about 3% and 9% for SOL and EDL, respectively.

force value at the start of the second half of the ramp makes the work quantity higher. Consistent with our hypothesis, this resulted in a larger force depression than for a single step shortening.

As stated above, the objective of the present study was to investigate the effect from a preceding transient contraction on the isometric force production. Focussing on the macroscopic transient behaviour, no experiments were thereby performed to directly investigate mechanisms underlying the active force modifications after length changes. However, some tentative mechanisms might be suggested. Cross-bridge kinetics was proposed by Marechal and Plaghki (1979) as a possible mechanism of force depression after shortening. They suggested that certain actin filaments were strained during shortening contractions and therefore unable to make normal cross-bridges. Results of our study and those from Leonard and Herzog (2005) support this theory, because work performed during shortening was a strong predictor of force depression but shortening velocity was not.

Edman et al. (1978) suggested that the tetanic force enhancement after a stretch is due to a parallel elastic element that is formed, reorganized or re-aligned during activation. This kind of mechanism might explain our finding of a similar relation between mechanical work and changes in passive and active force, albeit the changes were about ten times larger for active force (cf. Fig. 3 and Fig. 5).

That slope of the relation between the force modification, $F_{\mathrm{mod}}/F_{\mathrm{o}}$, and the normalized mechanical work, $W/(l_{\mathrm{o}} * F_{\mathrm{o}})$, is about 2 times steeper for EDL than for SOL



(a) (b)

FIGURE 5. Passive force modification following contractions as a function of normalized mechanical work of mouse (a) SOL and (b) EDL muscles. Data points represent mean values for the groups presented in Table 1: Shortening - squares, Stretch - diamonds, Long Shortening - circles, Stretch-Shortening - triangles. Average value for group, maximum standard deviations for mechanical work are about 3%, for force modification are about 5% for both SOL and EDL.

(Fig. 3). This fits with the idea that the force modification is related to metabolic factors, because the rate of ATP consumption during contractions is markedly faster in EDL than in SOL muscles (Barclay et al. 1993) and the rate of cross-bridge cycling, and hence the rate of ATP consumption, is higher during shortening than under isometric conditions (Herzog et al. 2000). This would, for instance, lead to a more rapid breakdown of phosphocreatine during shortening, resulting in an increased concentration of phosphate ions, which tends to decrease isometric force production (Allen et al. 2008). Such an explanation is difficult to reconcile with the observation by Julian and Morgan (1979) and Abbott and Aubert (1952), that force depression can be decreased and even abolished by briefly interrupting the stimulation. Such behaviour is more easily explained by a theory based on shortening-induced non-uniform sarcomere lengths (Julian and Morgan 1979; Morgan 2007), where sarcomeres are re-organized by the brief interruption of stimulation. It is, however, difficult to see how this theory could explain the continuity in modification between stretch and shortening situations.

5. Conclusions

The study confirms presence of the active and passive force modification following contraction with various length changes. The results show that muscular force does not only depend on length l and length time differential $\dot{l}$. The steady-state force also takes into account the accumulated length history, as shown in transient-length contractions. Our results indicate that the mechanical work produced by or on the muscle during length variation is a good general predictor for the steady-state force modification induced by transient-length contractions.

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