Standardization of maximal voluntary isometric contraction (MVIC) measurements from the elbow flexors

MSc Thesis

by

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and that any and all revisions required by the final
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_____

Prof. Dr. İsmail Lazoğlu

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Doç. Dr. Barış İşak

Date: _____

I would like to dedicate this thesis with gratitude to all my family, friends, professors and colleagues who have supported me on my academic journey to this date

ABSTRACT: Despite the routine measurement of maximal voluntary isometric contraction (MVIC) in research and in the clinic, no reference protocol for collecting MVIC measurements exists in the literature, specifically, with regards to the number of MVIC attempts to be performed and the duration of the rest period between contractions. The aim of this study was to develop a non-fatiguing MVIC protocol for the elbow flexors of healthy subjects. A total of 51 subjects, ages 18-34, participated in the study. Subjects were randomly assigned to complete 1 of 4 MVIC protocols with different rest periods (i.e. 10, 15, 30, and 60 seconds) between each maximal contraction. Maximum force applied to a force sensor, surface EMG of the biceps brachii muscle, subjective pain and subjective fatigue self-report scores were recorded for each attempt. Nonparametric statistical tests were used to determine differences between attempts but were inconclusive. As the development of fatigue is gradual, cumulative sum (CUSUM) charts were also constructed to explore subtle but persistent shifts in the data from the average of the first 10 attempts. An error box method, similar to what has previously been employed to detect reflex onset from electromyography records was used to determine whether continuous positive or negative deflections in the CUSUM charts were significant. CUSUM charts of the force data revealed significant negative deflections in force in the 60s, 15s and 10s groups, and in median frequency in the 15s and 10s groups. Significant positive deflections in the CUSUM charts of pain and fatigue self-report data were evident in all groups. For both pain and fatigue self-report scores, the deflections with the earliest onset were seen in the 10s group. It was concluded that the protocol with 15s of rest resulted in peripheral fatigue starting after attempt 5, but no central fatigue even after 25 successive attempts. The protocol with 15s of rest seems to have been conducive in maintaining the subject's attention to the task and motivation to do well. For the rapid assessment of elbow flexion MVIC, we therefore suggest a protocol consisting of 5 attempts, with a rest period of 15s between each attempt. We also suggest that the research team provides live encouragement during the contractions and averages the force values from the 5 attempts, given normal fluctuations in human performance. Finally, we propose a method for detecting fatigue from maximal force and median frequency data using CUSUM charts with error boxes.

ÖZETÇE: Araştırmalarda ve klinik ortamda sıkça ölçülmesine rağmen, maksimal istemli izometrik kasılmanın ölçümü için literatürde, bilhassa denek olan kişinin veya hastanın tamamlaması gereken *deneme sayısı* ve de maksimal istemli denemelerin arasındaki *dinlenme süresini* belirten standardize edilmiş bir referans protokol bulunmamaktadır. Bu çalışmanın amacı, sağlıklı deneklerde maksimal istemli izometrik kasılma ölçümü için kullanılmak üzere yorgunluğa sebep olmayan bir protokol geliştirmek idi. 18-34 yaş arası toplam 51 denek bu çalışmaya katılmıştır. Denekler, maksimal kasılmaların arasındaki dinlenme süresine göre (yani 10, 15, 30, veya 60 saniye) farklılık gösteren 4 farklı protokolden 1'ini tamamlamaya rastgele şekilde atandı. Denekler, maksimal dirsek fleksiyon kasılmasını güç ölçen bir sensöre uygularken bipolar yüzeyel EMG elektrodlar aracılığıyla biseps kasından kayıt alındı. Denekler toplam 26 tane maksimal izometrik kasılma tamamladı. Her kasılmanın süresi 5 saniye idi. Her kasılma için kasılma kuvveti, yüzeyel EMG, sübjektif ağrı ve sübjektif yorgunluk ölçüldü. Denemeler arasındaki farklılıkları karşılaştırmak için önce parametre dışı istatistiksel testler yapıldı. Daha sonra, yorgunluğun kademeli olarak geliştiği göz önünde bulundurularak verilerin, ilk 10 denemenin ortalamasına göre oluşan değişiklikleri incelemek için, birikmeli toplam grafikleri çizildi. EMG kaydından reflekslerin başlangıç noktasını saptamak için kullanılan hata kutusu metodu, birikmeli toplam grafiklerinde görülen devamlı artış veya düşüşlerin anlamlı olup olmadığına karar vermek için kullanıldı. Kuvvet verilerinden oluşturulan birikmeli toplam grafikleri, 60, 15 ve 10 saniye dinlenmeli protokollerde anlamlı düşüşler belli etti. Yüzeyel EMG kaydından hesaplanan mediyan frekans değerlerinin birikmeli toplam grafiklerinde sadece 15 ve 10 saniye dinlenmeli protokollerde anlamlı düşüş tespit etildi. Bütün protokollerde, sübjektif ağrı ve sübjektif yorgunluk verilerinin birikmeli toplam grafiklerinde anlamlı artışlar görüldü. Sübjektif ağrı ve sübjektif yorgunluk grafiklerinde, en erkenden ortaya çıkan anlamlı artışlar 10 saniye dinlenmeli protokolü tamamlayan grupta görüldü. 15 saniye dinlenmeli protokolde, 5. kasılmadan sonra periferal yorgunluğun ortaya çıktığı ama 25 deneme sonrasında bile bu protokolün merkezi yorgunluğa sebep olmadığı sonucuna varıldı. Öyle görünüyor ki, 15 saniye dinlenmeli protokol, denek olan kişinin deneye olan dikkatini ve motivasyonunu yüksek tutmasına vesile oldu. Dolayısıyla, dirsek fleksiyon kaslarının maksimal istemli izometrik kasılma seviyesinin seri tespiti için her biri arasında 15 saniye dinlenme süresi olan 5 tane kasılmadan oluşan bir protokol önermekteyiz. Ayrıca, kasılma sırasında deneklere teşvik edici yüksek sesli tezahürat yapılmasını ve insan performansının doğal değişkenliği nedeniyle 5 denemenin ortalamasının alınmasını öneriyoruz. Son olarak, kasılma kuvveti ve mediyan frekans verileri kullanılarak yorgunluk tespiti yapılabilmesi için birikmeli toplam grafikleri ve hata kutusundan oluşan yeni bir metot sunulmaktadır.

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POTENTIALLY USEFUL NOMENCLATURE

Adenosine triphosphate (ATP): a molecule which acts as the "energy currency" of the cell. It has 3 high energy phosphate bonds, which may be broken to release energy to meet the metabolic demands of the cell. When a single phosphate bond is hydrolyzed, adenosine diphosphate (ADP) and an inorganic phosphate molecule (Pi) are formed.

Between-subjects study design: a study design in which each level of the independent variable is tested in a different group of subjects

Brodmann area 4: the cortical area of the brain corresponding to the primary motor cortex

Central nervous system: the division of the nervous system consisting of the brain and spinal cord

Cerebral cortex: The outer layer of the neural tissue that makes up the brain. It is composed of gray matter (i.e. mostly cell bodies) and is 2-4 mm in thickness. It has a key role in many of the higher-level functions that are unique to humans, such as language and consciousness. In the context of the central nervous system, "cortical" refers to the cerebral cortex, which is sometimes also referred to simply as the "cortex."

Electromyography (EMG): a technique for recording the electrical activity of muscles. The two main types of EMG are *intramuscular EMG*, in which the recording is done with electrodes inserted into the muscle, and *surface EMG*, in which non-invasive electrodes placed on the skin record the electrical activity of the muscle(s) below.

Isometric contraction: a type of muscle contraction in which tension is developed in the muscle while the muscle stays the same length

Maximal voluntary contraction (MVC): a type of contraction during which a subject produces his/her maximum possible force without any physical assistance

Median frequency (of power spectrum): the frequency at which 50% of the total power within the signal's power spectrum is reached. Determination of median frequency involves integration of the power spectrum.

Mitochondria: one of the membrane-bound organelles in the eukaryotic cell. This organelle is frequently referred to as the "power-house" of the cell, since it is the location of aerobic respiration, which results in large amounts of ATP synthesis.

Motor unit: this is a functional unit which consists of a motor neuron and all the muscle fibers which it innervates.

Motor neuron: broadly, these are the neurons which control muscle movement. They are divided into two main groups of *lower motor neurons* (i.e. with somas in the brainstem or spinal cord) and *upper motor neurons* (i.e. with somas mainly in the cerebral cortex).

Muscle fatigue: there are many definitions of muscle fatigue, but for the purposes of this paper, muscle fatigue is defined as an exercise-induced decrease in the capacity of the muscle to generate its maximal force. There are two types of fatigue which may influence muscle output, specifically, *central fatigue* (i.e. occurring at the level of the spinal cord or brain) and *peripheral fatigue* (i.e. occurring at the level of the skeletal muscle).

Muscle fiber: the term for a skeletal muscle cell

Myofibril: cylindrical bundles of repeating sarcomere units

Flexion: movement of the skeleton which decreases the angle between two connected bones. In the case of elbow flexion, it refers to the decrease of the angle between the humerus and ulna bones.

Power spectrum: this term may apply to any signal; in this paper, it is used in the context of the myoelectric signal recorded with surface EMG. The power spectrum of a signal shows the distribution

of the total power in the signal as a function of the individual frequencies that make up the signal (as determined by Fourier Transform). The term is interchangeable with "power spectral density."

Sarcolemma: the cell membrane of the muscle fiber

Sarcomere: the basic contractile unit of skeletal muscle fibers. It is primarily composed of actin and myosin filaments. The length of the sarcomere shortens during contraction.

Sarcoplasm: the cytoplasm of the muscle fiber

Sarcoplasmic reticulum: a membrane-bound organelle located in skeletal muscle fibers. It is a specialized type of smooth endoplasmic reticulum which stores calcium. The calcium released from the sarcoplasmic reticulum makes muscle contraction possible.

Tetanic stimulation: high-frequency electrical stimulation with a rate of 50-200 Hz [1]

Transverse tubule ("T-tubule") network: sarcolemma membrane invaginations that penetrate into the center of the skeletal muscle fiber and which are rich with ion channels. They play a critical role in enabling action potentials to reach the center of the muscle fiber and orchestrating the increase in intracellular calcium concentration necessary for contraction to occur.

Single twitch stimulation: stimulation with a single supramaximal stimulus which typically has a frequency of 0.1 Hz and lasts 0.2 ms [1]

Twitch interpolation: a technique used to calculate voluntary activation level during a maximal voluntary contraction, typically to determine the extent of central fatigue. The electrical stimulus is delivered to the muscle or nerve during maximal contraction and any additional increases in force output are assumed to be representative of a force reserve that is inaccessible due to central fatigue.

LIST OF ABBREVIATIONS

ATP: adenosine triphosphate
ADP: adenosine diphosphate
BMI: body mass index
CNS: central nervous system
CUSUM: cumulative sum
DC: direct current
EMG: electromyography
k: average force, average median frequency, average subjective pain score or average subjective fatigue score computed from the first 10 MVIC attempts
MUAP: motor unit action potential
MVC: maximal voluntary contraction
MVIC: maximal voluntary isometric contraction
MVIC(n): n^{th} maximal voluntary isometric contraction, where n = 1→26
pH: potential hydrogen
Pi: inorganic phosphate molecule
SD or stdev: standard deviation
sEMG: surface electromyography
S(n): n^{th} point on the CUSUM chart, where n = 0→25 or 0→26.

"In physical science a first essential step in the direction of learning any subject is to find principles of numerical reckoning and methods for practicably measuring some quality connected with it. I often say that when you can measure what you are speaking about, and express it in numbers, you know something about it; but when you cannot measure it, when you cannot express it in numbers, your knowledge is of a meagre and unsatisfactory kind: it may be the beginning of knowledge, but you have scarcely, in your thoughts, advanced to the stage of *science*, whatever that may be."

> Lord Kelvin (1824 - 1907), British mathematician and physicist who developed the Kelvin scale for measuring temperature

Reproduced from: Robert K. Merton, David L. Sills, Stephen M. Stigler, "The Kelvin Dictum and Social Science: An Excursion into the History of an Idea," *Journal of the history of the behavioral sciences* 20(1984): p. 327

INTRODUCTION AND BACKGROUND

Maximal voluntary isometric contraction

Maximal voluntary isometric contraction (MVIC) is an exercise during which a person contracts a specific muscle isometrically in order to produce the greatest possible force from that muscle. MVIC force measurements are commonly used in the clinic to diagnose, assess the progression of and evaluate treatment interventions for neuromuscular diseases [2], [3], such as amyotrophic lateral sclerosis [4] and Duchenne muscular dystrophy [5]. In addition, MVIC protocols have been used in research to study fatigue [6], [7] and to investigate the effect of various factors on force production, such as caffeine [8], positive and negative feedback [9] and whole-body vibration [10].

In the recent decades, efforts to generate reference MVIC values have contributed to making the measurements of an individual more meaningful and interpretable [2], [11]. Studies have also been done to improve the protocols for measuring MVIC, for example, by describing specific movements for obtaining optimized MVIC electromyography (EMG) measurements from the shoulder muscles [12], assessing intrasubject and interexaminer reproducibility of measurements taken using a particular setup for strength measurement [13], and determining variability of MVIC measurements in healthy subjects and patients with peripheral neuromuscular disorders [3]. Despite calls for standardization of the procedures for determining MVIC [13], [14], no internationally agreed-upon set of muscle-specific MVIC protocols exists, specifically, with regards to the number of MVIC attempts to be performed and the duration of the rest period between each contraction. It is our belief that only with a scientifically tested and unanimously accepted MVIC protocol can the most reproducible, comparable and meaningful measure of a person's force producing capacity be recorded for a muscle or muscles of interest.

Given the relative ease of recording MVIC of the elbow flexors and the decline in arm strength that occurs as a result of aging [15] and many neuromuscular diseases (e.g. see Allen et al. [16]), the ability to accurately measure the force producing capability of the elbow flexors is useful in the clinic and in research. A review of studies in which subjects performed MVIC of the elbow flexors reveals that different research groups had varying methods for obtaining this measurement (Table 1), making it potentially unsound for results to be compared across studies. The aim of the present study was to test 4 different elbow flexion MVIC protocols in healthy subjects. The difference between each protocol was the duration of the rest period permitted between the 26 successive MVIC attempts (i.e. 10, 15, 30 or 60 seconds of rest). In this way, we aimed to determine the optimal rest period between successive MVIC attempts and the optimal number of attempts that the subject should perform so that the clinician or scientist can obtain the best estimate of the subject's elbow flexion MVIC before onset of fatigue. Our hypothesis was that there would be a range of MVIC attempts in one of the protocols at which on average the greatest MVIC forces were observed. First, some background information about elbow flexion anatomy, skeletal muscle physiology, the nervous system control of movement, neuromuscular fatigue and types of muscle contraction is given, after which the study and its results are presented.

Table 1: Protocols for measuring MVIC of elbow flexion from a selection of studies. Some of the details listed below pertain specifically to the study's method of determining the pre-test reference MVIC. 'Number of attempts' is per condition or for calculation of the reference MVIC. Visual feedback and verbal encouragement were assumed to have been absent from the protocol unless explicitly stated. In studies in which subjects were given a few seconds to build up force before the isometric maximal contraction, only the time during which the maximal contraction was held is included in the table.

Reference	Duration of isometric contraction	Duration of rest between attempts	Number of attempts	Visual feedback	Verbal exhortation
[17]	5 sec	3 min	3	No	No
[9]	6 sec	3 min	2	No	No
[18]	5 sec	2 min	3	No	Yes
[19]	5 sec	3 min	3	No	Yes
[20]	3 sec	≥1 min	3	Yes	Yes
[21]	2-3 sec	1 min	≥3*	Yes	Yes
[22]	6 sec	2 min	2	No	No
[15]	3 sec	2 min	2-3	Yes	Yes
[23]	3 sec	≥2 min	3-4	Yes	Yes
[24]	2-3 sec	Not specified	3	Yes	No
[25]	5 sec	1 min	3	No	Yes/No†
[26]	2-3 sec	2 min	≥12‡	Yes	Yes
[16]	2-3 sec	1 min	≥10‡	Yes	Yes

* Additional trials were performed until the peak force was achieved twice with maximum 5% variation
† Independent variable was the presence of verbal exhortation during the contraction
‡ Subjects were able to repeat any attempts they considered not to be maximal

Anatomy of elbow flexion

Essential for the movement of the skeleton are mobile joints, which establish flexible connections between the bones in order to make possible the movement of these bones relative to one another. 5 major components of the musculoskeletal system are needed to form functional joints; namely, the muscles, tendons, ligaments, cartilage and bones [27]. At the junction of the forearm and the upper arm, is one of the most stable joints in the body: the elbow joint [28]. The elbow joint is often simplified as a hinge joint between the distal end of the humerus and the proximal ends of the ulnar and radius bones [29]. Hinge joints allow movement in one plane only [30]; however, it is important to note that in fact *three* separate joints are encompassed by the single joint capsule of the elbow [29]. While the **1)** humeroulnar and **2)** humeroradial joints act as a hinge joint during flexion and extension, the **3)** proximal radioulnar joint between the radius and the ulna enables the rotation of the head of the radius during pronation and supination of the forearm [31]. The elbow joint allows for a range of extension (~0º), flexion (~140º), pronation (~80º) and supination (~80º) movements (Fig. 1). Part of what allows for the great range of motion across the elbow joint is its character as a synovial, or in other words, diarthroidal, joint [30] (see general structure in Fig. 2). This type of joint is the most mobile and common

joint type in the musculoskeletal system [27]. The joint capsule that surrounds synovial joints is lined by a synovial membrane, which secretes synovial fluid into the joint capsule [32]. Synovial fluid is a colorless or straw-colored fluid [30] with a viscosity similar to that of egg white [27]. During movement, it acts as a lubricant to reduce the friction between the articular cartilage-covered bone ends that come into contact at the joint [32], so much so that the resulting friction between the articular cartilage is less than that between an ice skate as it glides over the ice [27].

Fig. 1: Range of movements permitted by the elbow joint [29]

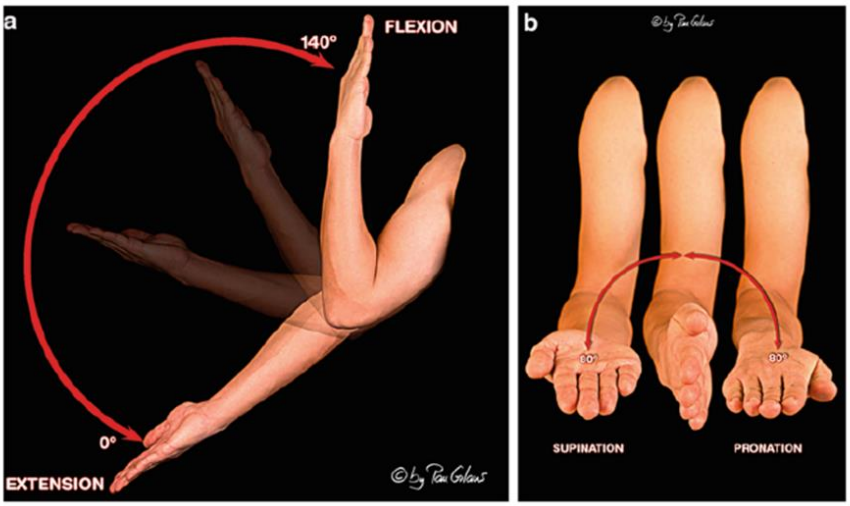
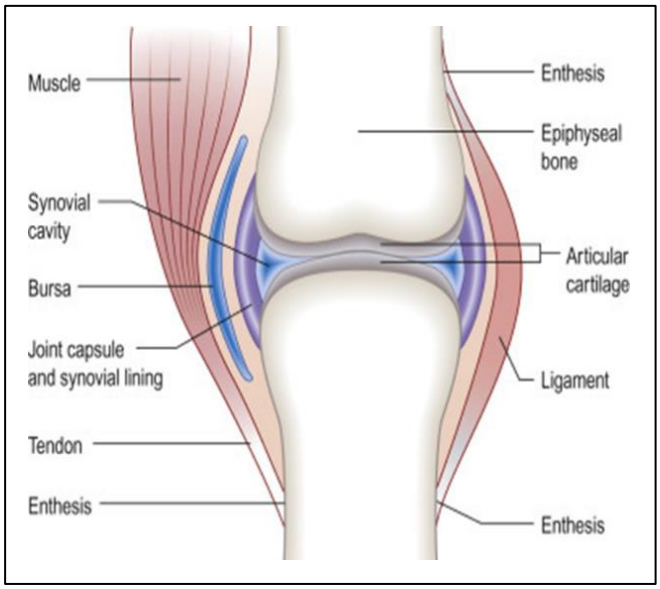


Fig. 2: Anatomy of a synovial (i.e. diarthroidal) joint [33]



There are 24 muscles that cross the elbow joint [34]. These muscles are listed in Table 2. The muscles that cross the elbow joint can be categorized into six groups depending on their function, these groups being the elbow **1)** flexors, **2)** extensors, **3)** flexors-pronators, **4)** extensor-supinators, **5)** primary pronators, and **6)** primary supinators [28]. The muscles that flex the elbow are the biceps brachii, brachialis, brachioradialis and pronator teres [35]. Each of these muscles is shown in isolation in Figure 3. Details regarding the location along the arm, blood supply, and neural innervation of, and the movement via the elbow joint produced by each flexor muscle are summarized in Table 3. Of note, when the forearm is in the supinated position, the biceps brachii contributes the greatest amount of elbow flexion force among the four elbow flexors [28]. The torque produced by the biceps brachii is at its highest when the degree of elbow flexion is 80-100º [28].

Table 2: List of the 24 muscles that cross the elbow joint [34]

1	Long head of biceps	13	Flexor carpi ulnaris
2	Short head of biceps	14	Extensor carpi ulnaris
3	Brachialis	15	Supinator
4	Medial head of triceps	16	Extensor digitorum communis, index
5	Lateral head of triceps	17	Extensor digitorum communis, long
6	Long head of triceps	18	Extensor digitorum communis, ring
7	Brachioradialis	19	Extensor digitorum communis, little
8	Anconeus	20	Palmaris longus
9	Pronator teres	21	Flexor digotorum superficialis, index
10	Flexor carpi radialis	22	Flexor digotorum superficialis, long
11	Extensor carpi radialis longus	23	Flexor digotorum superficialis, ring
12	Extensor carpi radialis brevis	24	Flexor digotorum superficialis, little

Figure 3: The elbow flexor muscles (screenshots from BioDigital [36])

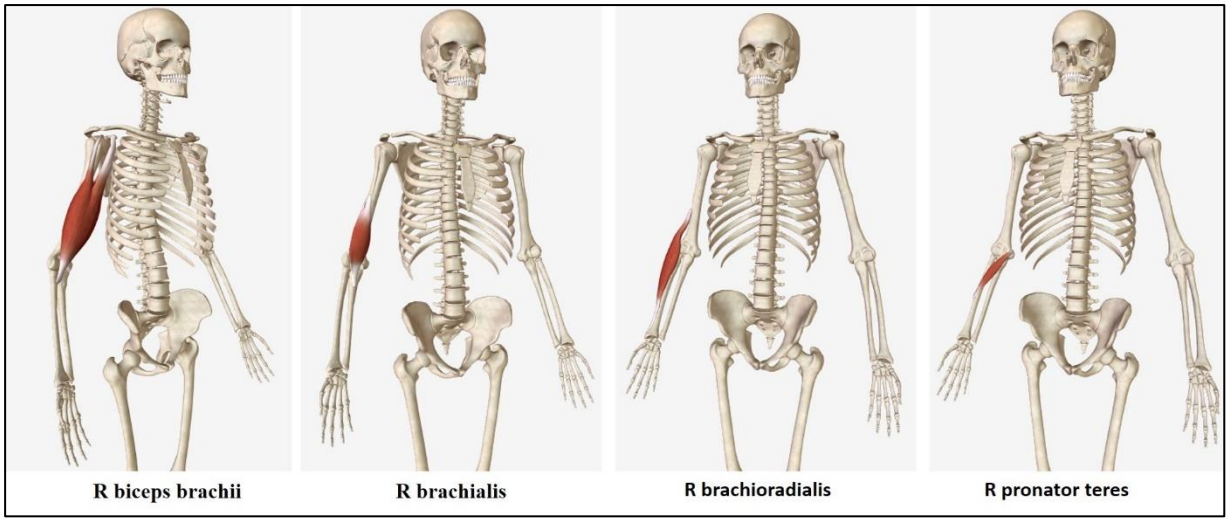


Table 3: Details regarding the location along the arm, blood supply, and neural innervation of, and the movement via the elbow joint produced by, each of the elbow flexor muscles. Compiled from *Atlas of Clinical Gross Anatomy* [37]–[39]

Elbow flexor muscle	Location	Blood supply	Neural innervation	Movement produced across elbow joint
Biceps brachii	Upper arm, anterior compartment	Brachial artery	Musculocutaneous nerve	Forearm flexion and supination
Brachialis	Upper arm, anterior compartment	Brachial artery	Musculocutaneous nerve	Forearm flexion
Brachioradialis	Posterior forearm	Radial recurrent artery	Radial nerve	Forearm flexion
Pronator teres	Anterior forearm	Anterior ulnar recurrent artery	Median nerve	Forearm flexion and pronation

Skeletal muscle physiology and contraction

Considering the 3 types of muscle in the body (i.e. skeletal, cardiac and smooth), gross examination and observation makes it immediately apparent that the elbow flexors are classified as skeletal muscle, given that they are attached to the bones of the skeletal system, are under voluntary control and produce movement of the limbs relative to each other [40]. Closer examination of skeletal muscle tissue by microscopy reveals muscle fibers, which are large, multinuclear cells with a diameter of about 50 μm and a length of up to several centimeters [41], [42]. The nuclei of these cells are located along the periphery, as evident in the skeletal muscle longitudinal sections shown in Figure 4. These muscle fibers are formed from the fusion of individual, uninuclear myoblast cells during development [41], [42]. Bound by the muscle fiber's sarcolemma is the sarcoplasm, into which the membrane invaginates to form the ion-channel rich transverse tubule (T-tubule) network and which contains the contractile machinery, sarcoplasmic reticulum, mitochondria, multiple nuclei (Fig. 5) and fuel reserves in the form of glycogen granules and fat droplets [43]. The contractile unit is comprised primarily of actin (i.e. the "thin" filament, with a diameter of 7 nm) and myosin (i.e. the "thick" filament, with a diameter of 15 nm) proteins that are organized into repeating units called sarcomeres (length of about 2.3 μm) (Fig. 6), which on a larger scale form cylindrical bundles [42]. Myosin is a molecular motor protein, which has ATPase enzymatic activity at its globular head region that can hydrolyze ATP to generate the mechanical energy necessary for the molecular movements underlying muscle contraction [41], [42]. The length of the actin and myosin filaments do not change during contraction; rather, as these proteins slide past each other during contraction, it is the sarcomere that shortens. Therefore, the smallest contractile unit of the muscle is the sarcomere [42]. Skeletal muscle fibers are classified into 3 types depending on their contractile and metabolic characteristics [41]. The 3 types of muscle fibers are compared according to certain characteristics in Table 4 on page 18. A given skeletal muscle is actually a mosaic of these 3 fiber types, with the proportions of the fiber types reflecting the function and history of use of the muscle [41]. It should also be emphasized here that the classification of skeletal muscle

fibers is not actually so clear-cut and that in fact the phenotype of skeletal muscle fibers lies more so along a continuum [44].

Fig. 4: Longitudinal section of skeletal muscle fibers [45]

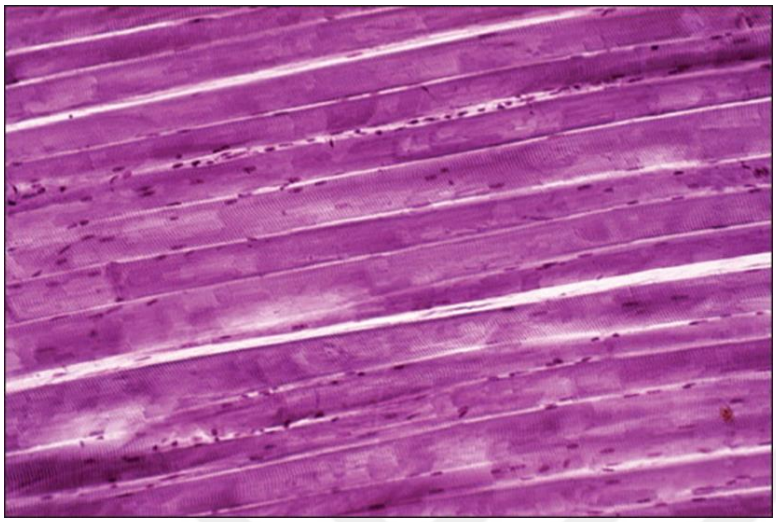


Fig. 5: General structure of the mammalian skeletal muscle fiber [46]

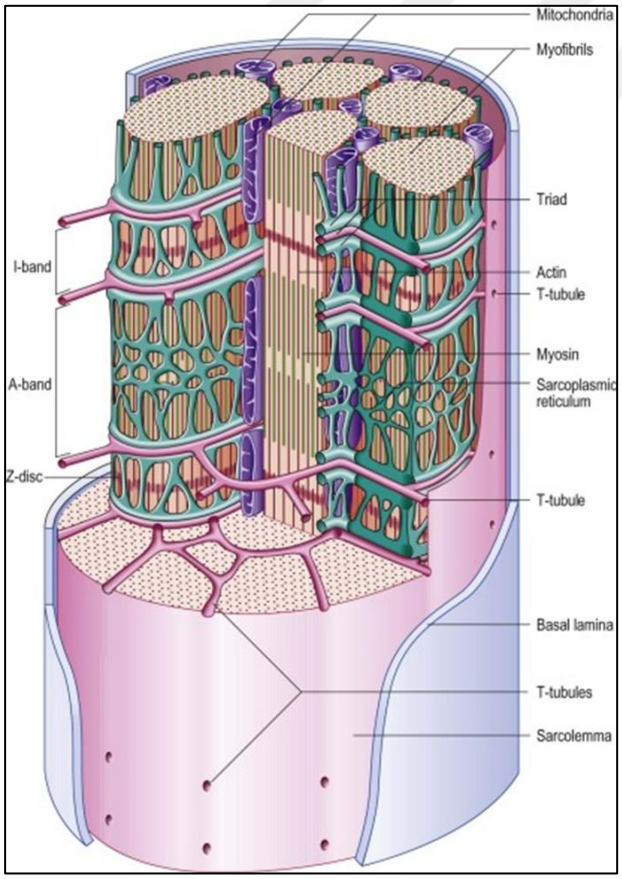


Fig. 6: Structure of the sarcomere, the basic contractile unit of skeletal muscle (slightly modified from figure 12.3 in the chapter by Koeppen & Stanton [47])

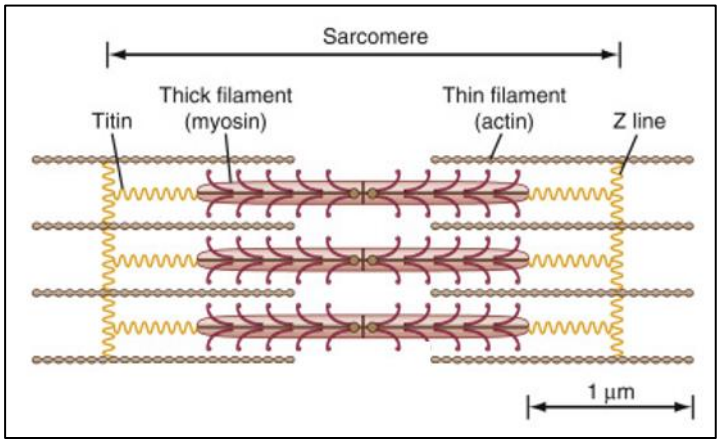


Table 4: Comparison of the 3 types of muscle fibers found in skeletal muscle. (Information is from the chapter by Mentis [41] unless otherwise specified.)

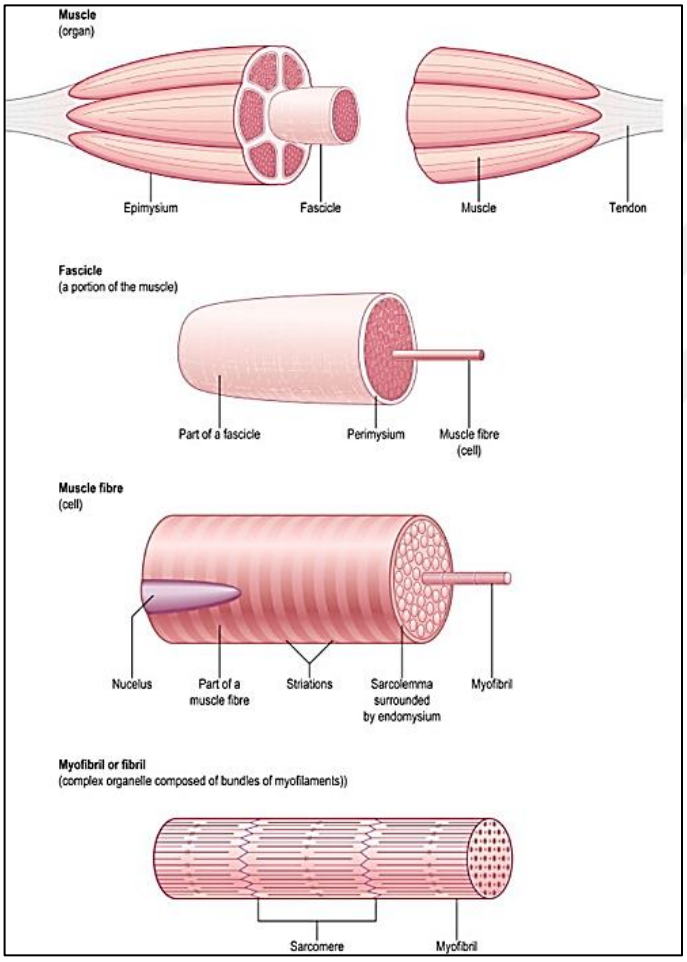
	Type I	**Type IIA**	**Type IIB**
Speed of contraction	Slow	Fast	Fast
Resistance to fatigue	High [48]	Intermediate	Low [48]
Metabolism	Aerobic	Aerobic, anaerobic	anaerobic
Mitochondrial density	High [48]	Intermediate [48]	Low [48]
Type of activity it enables	Long term, endurance activities [49] Ex: postural control, marathon running [50]	High-force contractions [49], endurance activities Ex: marathon running [50]	High-force, explosive contractions [49] Ex: powerlifting [50]

Travelling from the sarcolemma of the muscle fiber towards the outer surface of the muscle, is first a layer of connective tissue called the *endomysium* which covers each muscle fiber and harbors a delicate network of blood vessels and nerves [51]. About 20-80 muscle fibers are grouped in a parallel arrangement into fascicles [52], which are covered by another layer of connective tissue called the *perimysium*. Finally, the whole muscle is ensheathed by a final layer of tough connective tissue called the *epimysium*. These 3 connective tissue layers extend beyond the fleshy body of the muscle to form the insertions and tendons that attach the muscle to the bones [43]. The overall organization of skeletal muscle tissue is summarized in Figure 7 on the next page.

The sliding filament theory first proposed by A.F. Huxley and R. Niedergerke in 1954, describes the most widely-accepted and built-on mechanism of how the actin and myosin filaments slide past each other to shorten the sarcomere and generate tension during muscle contraction [53]. According to this theory, when the muscle is relaxed a complex made up by troponin and tropomyosin proteins blocks the sites where the myosin head attaches and forms cross-bridges to the actin filament [42]. In this relaxed state, ADP and a molecule of inorganic phosphate (Pi) left over from a previous contraction are attached to the myosin head [54]. When a nerve impulse arrives at the neuromuscular junction, acetylcholine is released from the motor neuron, which binds to nicotinic acetylcholine receptors on the muscle endplate

resulting in a flux of cations into the muscle fiber [55]. The sarcolemma becomes progressively depolarized, until an action potential is elicited which reaches the depths of the sarcoplasm via the T-tubule network, leading to the opening of voltage-gated sodium channels and the subsequent opening of L-type calcium channels that line the T-tubule network [55]. These voltage-gated L-type calcium channels are mechanically coupled to and trigger opening of the ryanodine receptor calcium channels on the sarcoplasmic reticulum, thus leading to release of calcium from stores in the sarcoplasmic reticulum [55]. As a result, the concentration of calcium in the sarcoplasm increases from about 10^{-7} M to 10^{-5} M [42].

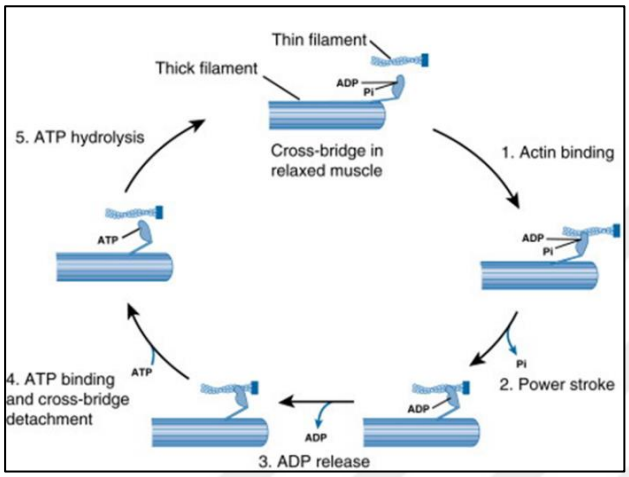
Figure 7: Organization of skeletal muscle tissue [56]



At the myofilaments, calcium binds to troponin, which causes the troponin-tropomyosin complex to shift and free the myosin binding site on the actin filament [42]. The myosin head then attaches to the exposed binding site on the actin filament and forms a cross-bridge, a process during which the Pi molecule is released [54]. During an event termed the *power stroke*, the myosin head releases the remaining ADP molecule in order to complete a movement in which it pulls itself about the actin filament in the direction of the midline of the sarcomere [57]. Myosin remains attached to the same point on the actin filament, until a new ATP molecule comes and frees it from actin [57]. This ATP

molecule is then hydrolyzed into ADP and Pi, releasing the energy necessary for the myosin head to bind to a more proximal cross-bridge site along the actin filament (i.e. with respect to the midline of the sarcomere) and repeat the entire process summarized in Figure 8, thus "inching" itself along the actin filament towards the midline of the sarcomere and shortening the sarcomere [57]. There is in fact an "optimal" sarcomere length, and thus muscle length, at which the greatest number of myosin heads are bound to actin and, in turn, the greatest tension can be produced from the muscle. This optimal sarcomere length is about 2.0-2.25 μm [58].

Figure 8: The process of cross-bridge cycling during skeletal muscle contraction [59]



Nervous system control of voluntary muscle movement

Of the 4 lobes that comprise the human cerebral cortex, the frontal lobe harbors the brain centers that are most directly involved in the planning and execution of voluntary movement. In the 1930s, neurosurgeon Wilder Penfield performed surgeries on more than 400 patients, during which he electrically stimulated different points on the surface of the motor cortex and observed the resulting contractions of the limb musculature [60]. He then created the famous homunculus, a map in the form of the human body superimposed on the precentral gyrus (i.e. primary motor cortex, Brodmann area 4). In this map, the limbs lie above the points of the cortex that control them and the size of the limbs are scaled according to the amount of the cortical surface area devoted to their movement (Fig. 9). Sculptural renditions of the homunculus make it immediately apparent that the homunculus has a disproportionally large tongue, lips and hands, representing the large amount of cortical space that enables the fine-control of these body parts [60] (Fig. 10). Although Penfield's map has provided useful insight into the topographic organization of the primary motor cortex, experiments conducted in the more recent decades have cast doubt on the existence of a one-to-one relationship between areas on the cortex and specific muscles. Rather, a more complex and mosaic map is emerging in which the movement of individual muscles may be linked to multiple sets of neurons and a specific cortical area may control *combinations* of muscles or even specific *movements* [60], [61]. Further, there is now an increased understanding of

the role in the control of movement of several non-primary motor areas in the frontal lobe that lie outside of Brodmann area 4. While these non-primary motor areas exert only a weak direct effect on motor neurons in the spinal cord, they can dramatically change the output from the primary motor cortex [62]. For example, studies done with monkeys have shown that the ventral premotor area (i.e. Brodmann area 6) is involved in preshaping the hand before picking up an object and applying a level of force that is appropriate to the expected weight of an object [62]. Experiments done in monkeys and humans suggest that the dorsal premotor area (i.e. Brodmann area 6; also called the "supplementary motor cortex" [63]) is involved in making the decision to execute a motor response based on external cues [62], [64]. It is also believed that visual and auditory information from the environment influences movement first by being processed in higher-order sensory and associational areas in the cortex and then being relayed to the primary motor cortex through the non-primary motor areas [62]. Further, there are several non-cortical regions of the brain, such as the basal ganglia and the cerebellum, which have extensive connections to the cortical motor areas [63]. The importance of these non-cortical areas in the orchestration of voluntary movement is clear from patients with diseases that disrupt the normal functions of these brain regions, such as Parkinson's disease and cerebellar ataxia.

Fig. 9: Topographic organization of the primary motor cortex (i.e. the motor homunculus) [65]

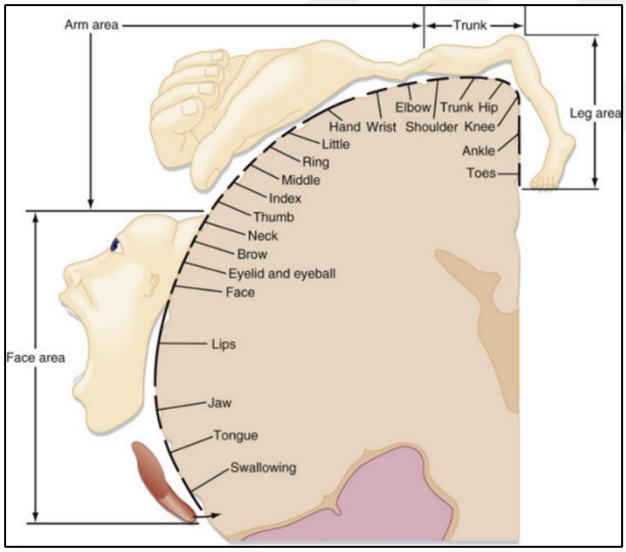
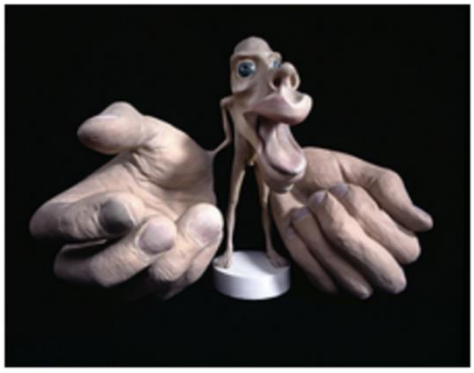


Fig. 10: 3D rendering of the motor homunculus [66]



There are 4 main pathways that descend from the brain to the spinal cord in order to control movement of the skeletal muscles. These are the **1)** vestibulospinal, **2)** reticulospinal, **3)** corticospinal and **4)** rubrospinal tracts [64]. The vestibulospinal and reticulospinal tracts form a medial system, which is primarily important for postural control. The lateral system, on the other hand, consists of the corticospinal and rubrospinal tracts which are responsible for movement of the limb muscles [64]. The corticospinal tract (i.e. the pyramidal tract) is the most direct pathway from the brain to the spinal cord [64]. In this pathway, upper motor neurons originating in layer V of the motor cortex [63] travel by way of the internal capsule, midbrain and medulla until synapsing with lower motor neurons in the spinal cord [67] (Fig. 11). The lateral corticospinal tract, shown in Figure 11, moves the muscles of the limbs and digits, while the ventral corticospinal tract (not shown), which is made up of the minority of axons that do not decussate at the caudal medulla, moves the muscles of the trunk [63]. The other tract comprising the lateral system – the rubrospinal tract – is an indirect pathway to the motor neurons in the spinal cord which is important in the voluntary movement of the arms, hands and fingers [64]. In this pathway, corticorubral neurons in layer V of Brodmann areas 4 and 6 project to the spinal cord via the red nucleus in the brainstem [64]. Both the corticospinal and rubrospinal tracts receive considerable input from the cerebellum and basal ganglia [64]. The four major descending pathways and other brain regions involved in voluntary movement are summarized in Figure 12.

Lower motor neurons that innervate the muscles are located in the ventral horn of the spinal cord. Motor neurons in the spinal cord have very vast dendritic arbors, onto which thousands of excitatory and inhibitory synapses can be made [41]. These motor neurons are classified as alpha, gamma or beta motor neurons [41]. Primarily alpha motor neurons, and also beta motor neurons, are involved in innervation of the skeletal muscles, while the gamma motor neurons only innervate the muscle spindles [41], [43]. Alpha motor neurons are also the largest neurons found in the spinal cord [41]. The synapse between the motor neuron and the muscle cell membrane is the neuromuscular junction (NMJ). In mammals, the neurotransmitter released from the motor neuron is acetylcholine [41], [63]. On the postsynaptic side of the NMJ is the motor end plate of the muscle fiber which contains a high density of receptors where the acetylcholine neurotransmitter binds [55]. The term “motor unit” refers to a motor neuron and all the muscle fibers that it innervates. In mammals, a motor neuron may innervate multiple muscle fibers, but the opposite is not true. In other words, a muscle fiber may only be innervated by a single motor neuron. Further, the muscle fibers that a single motor neuron innervates are all of the same type [41]. Motor units are categorized according to their speed of contraction (i.e. twitch speed), level of force production and fatiguability [41]. The 3 types of motor units are summarized in Table 5. Depending on their function, muscles are composed of different ratios of these 3 motor units, although typically Type S motor units account for the greatest majority of the motor units comprising a muscle [41].

Fig. 11: The (lateral) corticospinal tract [67]

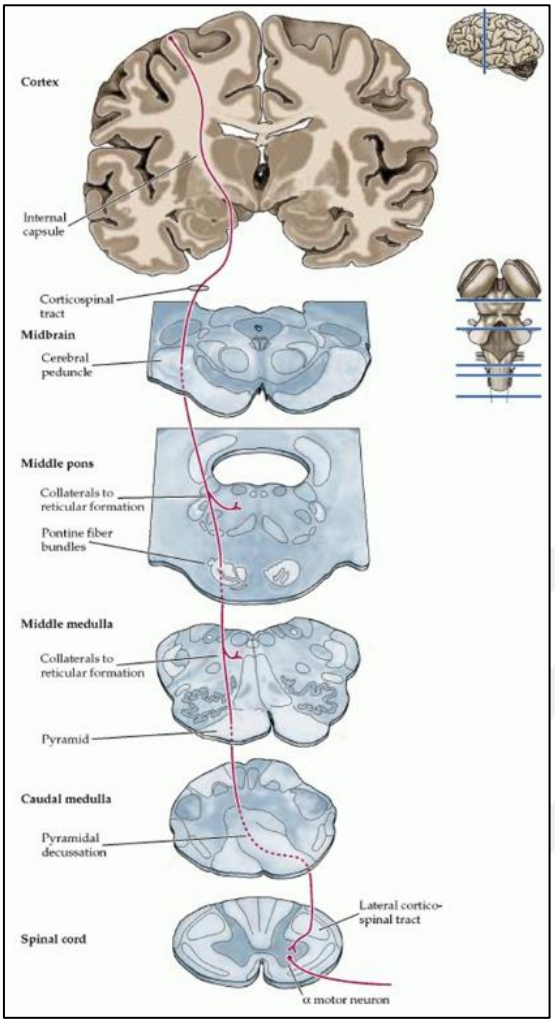


Figure 12: Summary of the major brain regions involved in movement and the 4 pathways that descend from the brain to the spinal cord for this purpose; *CS: corticospinal; RbS: rubrospinal; VS: vestibulospinal; RS: reticulospinal* [68].

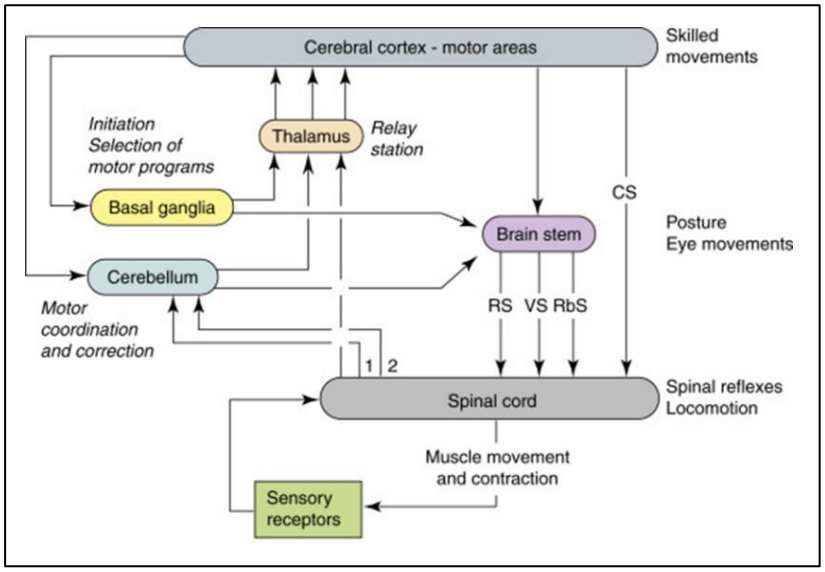


Table 5: Comparison of the 3 types of motor units. (Compiled from the chapter by Mentis [41])

	Type S	**Type FR**	**Type FF**
Speed of contraction	Slow	Fast	Fast
Level of force produced	Small	Moderate	High
Resistance to fatigue	High	Moderate	Low
Metabolism	Aerobic	Aerobic, anaerobic	Anaerobic
Type of muscle fibers innervated	Type I	Type IIA	Type IIB
Type of movements for which it is used	Postural movements Tonic movements	Fast and powerful movements	Brief bursts of strength

Skeletal muscle fatigue: etiology and measurement

Although skeletal muscle fatigue is a phenomenon which all humans are familiar with and experience on a daily basis, the scientific study of muscle fatigue is not as straightforward or simple as one would expect. The first difficulty is in defining muscle fatigue and determining how it should be measured. Traditionally, fatigue in the muscle has been thought of as a metabolic endpoint at which there are dramatic changes in the levels of glucose and free fatty acids in the bloodstream, and of glycogen in the muscle [69]. Muscle fatigue has also been described as an inability to maintain a particular level of tension [43], a reversible decline in performance [49], and as a specifically *exercise-induced* reduction in the capacity to generate maximal force [44]. The latter definition is useful because it makes a differentiation between exercise-induced muscle fatigue and a non-exercise related reduction in strength, for example, that which is caused by loss of muscle mass with increasing age [44]. In some definitions of muscle fatigue, there is also a reference to the subjective sense of fatigue, such as in the definition by Enoka and Stuart of fatigue as "…an acute impairment of performance that includes both an increase in the perceived effort necessary to exert a desired force and an eventual inability to produce this force" [70]. The quote below has been reproduced from De Luca's oft-cited 1984 review [71] because it effectively illustrates the varying perspectives with which different disciplines tend to view fatigue:

> ….far too many health specialists and life scientists appear to have accepted the concept of fatigue as being associated with, demonstrated by, or represented by an event occurring at or over an identifiable period of time. For example, it is common to think of when an individual fatigues or to indicate that an individual is fatigued when a particular task cannot be performed or maintained at a specific time. Such a notion of fatigue is inconsistent with that which has been successfully employed by engineers and physical scientists, who have considered the concept of fatigue as a time-dependent process. For example, consider a steel girder that supports the main structure of a bridge. It may well remain in place with no apparent, externally visible structural modification for over 50 years; then suddenly, in one instant a fracture develops, the girder fails and the bridge collapses. If one, observing from a distance, were to look at the main structure of the bridge for a sign of fatigue, none would be easily noted during the 50-year period. What would be noted in this mode of observation would be the failure point. All the while, however, the crystalline structure of the steel girder was undergoing an alteration caused by chemical and physical processes. In order to monitor the progression of this alteration, specimens of data from within the girder itself or externally observable modifications related to the internal alteration are required [71].

In agreement with De Luca's description, many definitions of muscle fatigue refer to a reduction in force output, rather than a point of exhaustion or task failure, emphasizing that the development of fatigue is gradual [72]. Adding to the complexity of studying muscle fatigue is that there is also no single method or accepted protocol for measuring and quantifying muscle fatigue [72]. Change in force output during MVC or during electrical stimulation of the muscle or nerves, change in time to exhaustion (also referred to as "endurance time") [44] and change in the amplitude and power spectrum of the EMG signal during sustained contractions [14] are some examples of measurements that are commonly used as indices of fatigue. Further, it is argued that the current methods for measuring muscle fatigue are "doubly subjective", since they rely both on the motivation of the subject or patient performing the task, as well as the careful and unbiased assessment of the observer [71].

The uncertainty surrounding the most appropriate description and way of measuring muscle fatigue likely stems from the fact that muscle fatigue is not caused by a single, global mechanism. Rather, it is increasingly accepted that many different mechanisms may lead to the broadly-defined state of neuromuscular fatigue, with the dominant mechanism(s) involved being determined by the specific nature of the task performed [70], [72]. Two types of neuromuscular fatigue are commonly discussed in the literature and textbooks, depending on the level at which the fatigue originates. Fatigue originating at the level of the skeletal muscle is referred to as *peripheral fatigue*, while fatigue originating within the central nervous system is referred to as *central fatigue* [49]. Given that both central and peripheral systems become stressed upon the disruption of homeostasis that occurs with rigorous exercise [69], it may not be appropriate to think of the two types of neuromuscular fatigue in isolation. Although they have been examined separately in the following paragraphs for organization and clarity, it will be clear to the reader that channels of communication exist between the peripheral and central systems, making it at times difficult to describe a particular mechanism underlying neuromuscular fatigue as having a purely central or peripheral origin.

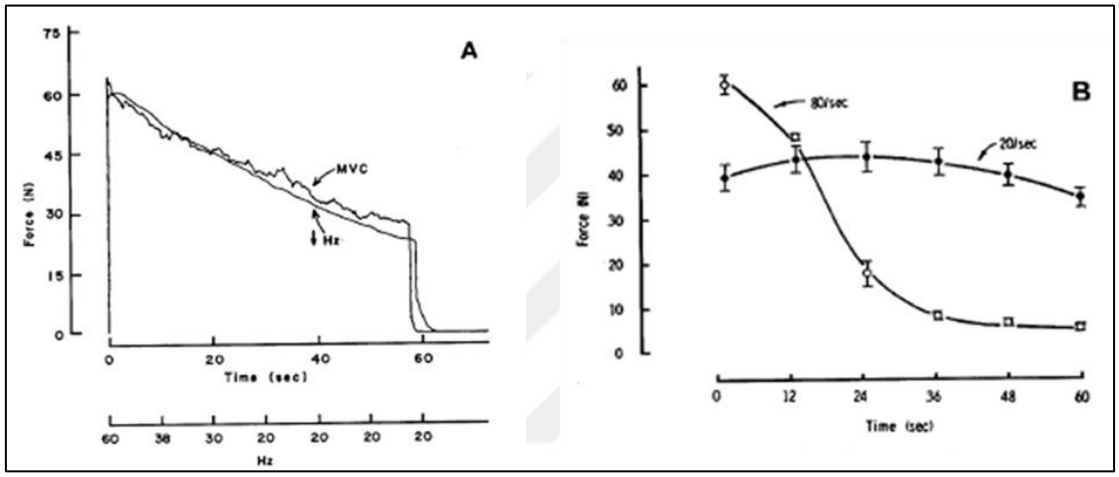
Central to the discussion about the fatigue originating at the level of the skeletal muscle is ATP, since muscle contraction can only take place in the presence of ATP, as explained earlier (i.e. see section titled, "Skeletal muscle physiology and contraction"). It is accepted that much of peripheral fatigue results from inadequate fuel supply to the muscle's contractile machinery [43]. In fact, fatigue has been suggested to be a protective mechanism against dangerously low levels of ATP, given that the absence of ATP is what causes the state of rigor mortis after death [43]. During exercise, there is an increased demand for ATP, which in turn requires rapid ATP regeneration for as long as the exercise continues [49]. The non-mitochondrial anaerobic channels for ATP synthesis can respond immediately to the increased ATP demand that occurs with short, explosive maximal efforts or with sustained, intense but submaximal efforts [49]. These anaerobic systems include the production of ATP from creatine phosphate stores as well as the ATP synthesis which occurs during the conversion of muscle glycogen and blood glucose to pyruvate [49]. Anaerobic systems are limited in their capacity to generate ATP and cannot sustain the muscle for long, however. For instance, about 16 kJ of energy can be produced from

the creatine phosphate and ATP stores in the resting muscle, which is enough to sustain about 1 minute of brisk walking only [43]. ATP synthesis via mitochondrial aerobic respiration must occur for the muscle to continue to function. While this oxygen-dependent mode of ATP synthesis cannot respond to the initial high energy demands resulting from sudden powerful exercises, it can support the muscle during lower-intensity exercises for a long duration [49]. Certain physiological and behavioral changes during exercise, such as increased blood flow to the muscles, increased heart rate and stroke volume, and increased rate and depth of breathing, enable more oxygen to be transported to the muscle for aerobic ATP synthesis [43]. Given that aerobic and anaerobic pathways of ATP synthesis respond differently to sudden increases in ATP demand, the ease with which a muscle fatigues during exercise is significantly dependent on its metabolic capacities, which in turn is determined by its muscle fiber makeup [44] (refer to Table 4).

Depletion of ATP is not the only cause of peripheral fatigue during exercise. Like ATP, calcium plays a critical role in the process of muscle contraction. Although controversial, studies have shown that the M-wave elicited from fatigued muscle is depressed, indicating a disruption in neuromuscular propagation [70]. Other sources also state that peripheral fatigue results in a disturbance of action potential propagation in the muscle fiber [44], [49], [69]. It is proposed that this disruption is caused by the exercise-induced change of potassium and sodium ion concentrations to which the sarcolemma and T-tubule network membranes are exposed [44], [72]. Impairment in neuromuscular propagation would then prevent the rise in intracellular calcium levels needed to expose myosin binding sites on actin. Control of calcium levels in the muscle fiber is altered during fatigue also because the pump which returns calcium to the sarcoplasmic reticulum and restores the calcium stores is dependent on ATP, for which there is increased demand during exercise [49]. Perhaps due to these disruptions in calcium intake and release, the force generated by actin-myosin cross-bridges is demonstrably decreased in the early phases of fatigue, with a decline evident in the actual *number* of cross-bridges as fatigue develops [49]. The accumulation of metabolites in the muscle fiber is another known cause of peripheral fatigue [72]. One such metabolite is hydrogen ions, which form when the lactic acid by-product of anaerobic respiration is converted into lactate. The accumulation of hydrogen ions results in a decrease in pH which has been demonstrated to correspond in time with low force production [49]. The metabolites that accumulate after muscle contraction have also been proposed to activate muscle afferents which evoke a reflex that signals to the motor neurons to decrease the motor unit discharge rates [72]. This decrease in motor unit firing rates that is observed in fatigued muscle is explored by the muscle wisdom hypothesis, which posits that the decrease in the discharge rate functions to minimize fatigue by matching the rate of motor unit discharge to the altered contractile properties of the fatigued muscle [73], [74]. Combined with the slowing of the relaxation rate that is seen in fatigued skeletal muscle [75], the decrease in the motor unit discharge rate accomplishes the production of levels of force that are actually higher than what would be produced if the motor unit discharge rate stayed the same [73]. This phenomenon is illustrated nicely in the study by Jones et al. [76] (Fig. 13, reproduced from Garland &

Gossen [73]). Briefly, they found that in the adductor pollicis muscle, the decline in force during sustained MVIC could only be reproduced when a high frequency of stimulation (60 Hz), was progressively lowered to 20 Hz [73], [76]. The demonstrability of this phenomenon, however, seems to be limited to certain muscle groups during MVIC [73]. Further, these changes in motor unit firing rate are considered to have a central origin by some [77]. Finally, it should be mentioned that the loss of body fluids that occurs during strenuous exercise and which stresses the cardiovascular, metabolic and thermoregulatory systems is thought to alter the microenvironment of skeletal muscle and play a role in the development of peripheral fatigue as well [69].

Fig. 13: Demonstration of the "muscle wisdom" phenomenon with electrical stimuli by Jones et al. [76]. (Figure is reproduced from the review article by Garland & Gossen [73]).



The decline in force output that occurs during a sustained MVC is best mimicked by electrical stimulation of the muscle with a gradually decreasing frequency (i.e. from 60 Hz to 20 Hz), as shown in **A**. Stimulating with a single frequency (i.e. 80 Hz or 20 Hz) as shown in **B** cannot reproduce the normal decrease in MVC.

The previously-mentioned accumulation of hydrogen ions and decreasing pH in the exercising muscle is an important physiological event for researchers studying fatigue, due to its proposed effects on the frequency makeup of the EMG signal. Some basic background information on EMG and EMG signal analysis will be provided prior to explaining the effect of fatigue on the frequency makeup of the EMG signal. Primarily, when intramuscular EMG is applied to a muscle that is minimally contracting such that only 1 motor unit is active, the extracellular activity from the muscle fibers of this single active motor unit is recorded by the intramuscular electrode as a compound electric potential, referred to as the motor unit action potential (MUAP). The MUAP is essentially the algebraic sum of action potentials from the muscle fibers of the active motor unit, and is most influenced by the muscle fibers located closest to the recording electrode [78], [79] (Fig. 14). What is recorded by surface EMG with non-invasive electrodes placed on the surface of the skin above the muscle is the sum of MUAPs from all the motor units that are active during the muscle contraction and which are in the area of detection of the surface electrodes. Thus, the surface EMG record is a more "global" signal than the intramuscular EMG record. One of the ways of analyzing the surface EMG signal is by Fast Fourier Transform (FFT).

This method of analysis is based on the mathematical property that every signal – regardless of its shape – can be decomposed into an infinite number of sine waves [80], [81]. Whereas EMG is recorded in the time domain (i.e. voltage given as a function of time), FFT transforms the signal into a histogram with frequency (Hz) and amplitude (mV) of the individual sine waves on the x- and y-axis, respectively [81]. This histogram is referred to as the *power spectrum* or *frequency spectrum* of the signal [81]. There is a clear leftward shift of the power spectrum towards the lower frequencies during muscle fatigue [71], [74], [77] (Fig. 15). Median frequency, which is the frequency at which 50% of the total power of the spectrum is reached [82], is one of the parameters of the power spectrum that is used as an index of fatigue, since it is consistent [71], not sensitive to noise and is believed to best-reflect the physiological and biochemical changes that occur in the fatiguing muscle [14]. The principal reason for the shift of the EMG power spectrum to lower frequencies seen during constant-force fatiguing contractions is proposed to be the decrease in intramuscular pH, which results in decreased membrane excitability, decreased action potential conduction velocity of the muscle fibers, and ultimately, an increased duration of and thus change in the shape of the MUAPs [14]. This argument clearly accepts that the shift of the power spectrum during fatigue is predominantly caused by peripheral factors. Nonetheless, a highly-influential and comprehensive review on the spinal and supraspinal mechanisms at play in muscle fatigue states that the increase in the myoelectric signal's low frequency content during fatigue is not definitely reflective of fatigue at the peripheral versus central level [74]. Another review has concluded that the reasons for the shift are still unresolved and that spectral shifts should be used with caution [44]. Further, it should be noted that there is disagreement between those who think that the decrease in motor unit firing during fatigue does not have an effect on the EMG power spectrum [14], [71], [83], and those who think that changes in motor unit firing and recruitment do have a role in causing the leftward shift [84], [85].

Fig. 14: Diagram of a motor unit action potential (MUAP) recorded with intramuscular EMG [79]

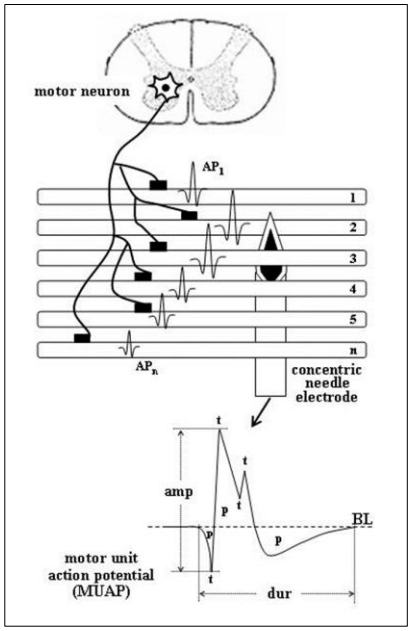
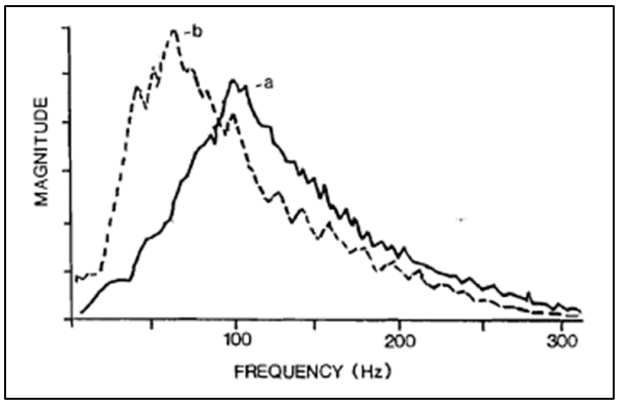


Fig. 15: Leftward shift of the EMG power spectrum of the first dorsal interosseous muscle (a) at the beginning of and (b) after about 50 seconds of maintaining a contraction of 50% MVC [71]



In contrast to peripheral fatigue, central fatigue is broadly defined as a loss in neural drive from the central nervous system. This loss in neural drive may reduce the recruitment of motor units, and alter mood, perception of effort and sensitivity to pain and discomfort [69]. The central fatigue hypothesis is based on the idea that exercise changes the levels of neurotransmitters in the central nervous system [69]. While the original hypothesis on central fatigue proposed by E.A. Newsholme focused on serotonin, this hypothesis has been revised to emphasize the role of both serotonin and dopamine. Specifically, it is thought that the ratio of serotonin to dopamine might be important, with a high ratio inducing feelings of lethargy, tiredness, low arousal and decreased motivation [69]. Serotonin has been demonstrated to alter pain perception [69] and has long been at the center of sleep research, although conclusions on its role in the sleep-wake cycle have shifted several times during the past 60 years [86]. The opposing role of dopamine in the development of central fatigue, on the other hand, is clear from the abuse of central nervous system stimulants, such as amphetamine, for improved performance in sports [87], especially endurance sports like cycling [69], together with the fact that amphetamine uses multiple mechanisms to increase *dopamine* levels in the synapse [88]. Further, caffeine, which is found in so many stimulating beverages and which readily crosses the blood brain barrier, achieves its effect on the brain by acting as an antagonist of adenosine, which otherwise inhibits the release of several excitatory neurotransmitters, including dopamine [69]. It is unlikely that only dopamine and serotonin are involved in the orchestration of central fatigue, however, and there is evidence that other neurotransmitters and mechanisms are at play. Central levels of noradrenaline, glutamate, GABA, acetylcholine, endogenous opioids and ammonia, as well as exercise-induced hypoglycemia, depletion of glycogen stores in the brain and increased permeability of the blood brain barrier have been implicated in exercise-induced central fatigue [69]. Of note, during exercise, skeletal muscle has been found to produce and release into the bloodstream significant amounts of interleukin-6 (IL-6), which is of the cytokines that are released from cells of the immune system in response to injury or infection [89], [90]. 100-fold increases in plasma IL-6 levels have been detected in runners after a marathon. This level is

similar to what is seen in a person suffering from a severe infection [89]. Given that several cytokines have already been linked to mood and fatigue [90], it might be that the release of IL-6 from exercising skeletal muscle induces the feelings of lethargy similar to what is experienced during illness [69], thus communicating peripheral fatigue to the central nervous system. Another channel of fatigue-related communication between the peripheral and central divisions is thought to exist via the group III and group IV muscle afferents, or so-called "ergoreceptors" [91]. It is proposed that these thin nerve fibers discharge in response to exercise-induced stimuli and exert an inhibitory effect on the neural drive from the CNS. These afferents are also very important for adjusting cardiovascular and ventilatory output during exercise [91]. The motor cortex and cortical areas upstream of the motor cortex are suggested to be the brain regions where the signals from these muscle afferents are processed [91].

The interpolated twitch technique is a method which should be explained here since it may be used to show that loss of MVIC force output is due to central fatigue [49]. This technique assumes that an electrical stimulus can be used to activate motor units that otherwise cannot be activated by the central nervous system during MVIC. The electrical stimulus is delivered during the maximal contraction and any increases in force are accepted to be indicative of a force reserve that the central nervous system has failed to mobilize, presumably due to central fatigue [44]. For a given MVIC attempt, a *% voluntary activation level* can be calculated by using the $(1\text{-}T_{interpolated}/T_{control}) \times 100$ formula, in which $T_{interpolated}$ is the additional force produced when the electrical pulse is delivered during the MVIC, and $T_{control}$ is the force produced when an identical electrical pulse is delivered to the resting muscle [74]. *Interpolated twitch* is the term used to define the artificial increase in force achieved with the electric stimulus. The magnitude of this increase decreases with the strength of contraction [92]. Twitch interpolation has been applied to a very wide variety of muscles and has revealed that muscles differ in terms of the level to which they can be voluntarily activated [74]. The fact that "maximal" voluntary contraction is in fact typically less than maximal has been revealed by many studies involving twitch interpolation [74]. For instance, Yue et al. has shown that their healthy subjects (n=28), both young and old, could never activate their biceps brachii muscle fully, with the average voluntary activation level being 94% and 97% for the old and young subjects, respectively [15]. Allen et al. had their subjects (n=5) come on 5 separate days and perform 10 contractions on each day. In contrast to the findings of Yue et al., they found that all subjects were able to achieve an activation level of 100% at least once, but that overall, 75% of the attempts completed during the study were not maximal. The subject with the best performance achieved 100% activation in 30/50 attempts, while the subject with the worst performance achieved this level only once [26]. In the 2001 review by Gandevia, 6 methodological considerations are put forth which should be incorporated into MVC protocols to minimize the development of central fatigue. Of note, live standardized verbal encouragement to motivate the subject is suggested [74]. The benefit of this too, is debatable. In the between-subjects study by McNair (n=20), verbal encouragement was indeed found to produce an average 5% increase in elbow flexion MVIC [25]. These results are contrasted with those of Rube & Secher, who found that there were no significant differences in initial

force during a series of 150 leg extension MVIC attempts when verbal encouragement was provided before and during the contraction versus when it was not provided at all. Further, the decline in force was *greater* when verbal encouragement was provided, pointing to what they describe as a "paradoxical influence" of cheer on performance [93]. Vøllestad has also stated in agreement that verbal encouragement and loud exhortation is not always enough to overcome the decreased drive of the fatigued central nervous system [44].

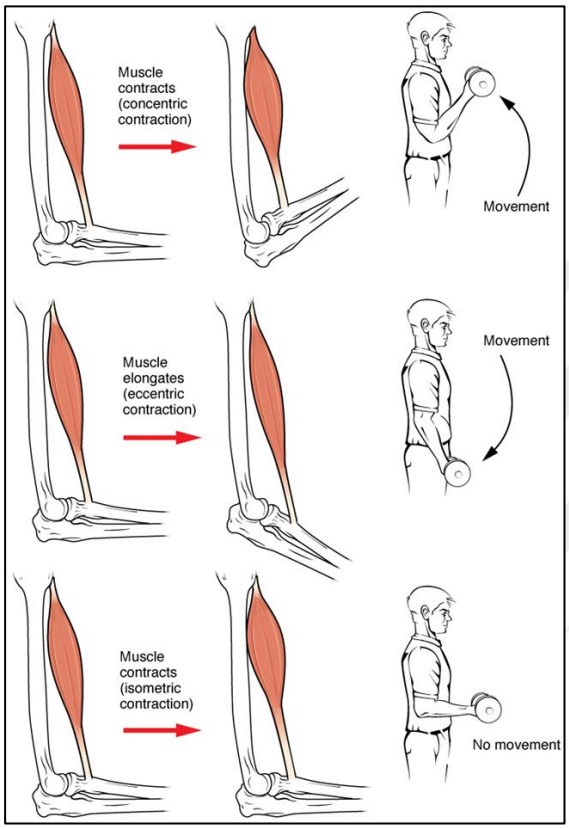
While exercise is typically thought of as fatigue-inducing, it should be noted that muscle contraction does not always lead to fatigue and may even lead to potentiation of subsequent contractions. Postactivation potentiation (PAP) is used to describe the acute phenomenon by which muscle force and rate of force development is enhanced due to a previous contraction, or in other words, due to the muscle's contractile history [94]–[96]. PAP is commonly revealed as an increase in twitch force evoked after **1)** MVC or **2)** an isometric contraction evoked with tetanic stimulation [96]. While there is consensus that PAP exists, questions about its mechanisms remain [95]. It is thought that PAP acts via the phosphorylation of myosin light regulatory chains, which results in increased sensitivity of the actin and myosin filaments to calcium [94]. This physiological change is stated to be most marked immediately following muscle activity [96] and to have high interindividual variability [95]. There is also a suggestion that increased synaptic excitation in the spinal cord may be the underlying cause of PAP [94], although evidence for this claim seems to be lacking. It has been proposed that performance of intense resistance exercises may be completed to enhance subsequent explosive and powerful movements; however, PAP seems to develop simultaneously with fatigue. Therefore, it would be the *relative* magnitude of fatigue versus PAP induced by such an exercise that determines whether subsequent force output is attenuated or potentiated [95].

Types of muscle contraction

There are several different types of contractions that muscles can perform to produce force. It perhaps makes the most sense to classify muscle contractions based on the effect that the contraction has on muscle length, since the force that a muscle produces depends on the number of cross-bridges between the actin and myosin filaments, which in turn depends on the length of the sarcomere and thus the length of the muscle [97]. In *isometric* contractions, tension develops in the muscle as the length of the muscle stays the same. The maximal amount of force is generated if the muscle is held at the length corresponding to the optimal sarcomere length during the contraction [98]. These types of contractions do not result in movement, but energy expenditure occurs as the tension in the muscle increases [56]. During *isotonic* contractions, on the other hand, limb motion is produced and the muscle changes length while the load on the muscle remains constant [49], [99]. Isotonic contractions that cause the muscle to shorten are called *concentric* contractions, whereas those that cause the muscle to lengthen are called *eccentric* contractions [99]. Isometric, concentric and eccentric contractions and summarized in Figure

16. Muscle contractions may also be described as *isokinetic* if the contraction is carried out at a constant velocity. These contractions are not observed naturally, however, and require the use of an external device to be achieved [99]. An important parameter in concentric contractions is the velocity of contraction. There is an inverse relationship between velocity of contraction and the force produced, since greater velocities provide less time for cross-bridges to form between actin and myosin [97].

Figure 16: Illustration of isometric, concentric and eccentric contractions [100]



Metabolically, isometric and concentric contractions are the most demanding contractions [101]. Concentric contractions are noted to be metabolically-demanding because they require a *fast* rate of ATP synthesis [44]. The development of fatigue during maximal isometric contractions, on the other hand, is particularly quick because during such contractions the pressure inside the muscle becomes very high, leading to a reduction or complete blockage of circulation through the muscle. In the biceps brachii and muscles of the arm, peak blood flow has been found to occur around 25% MVIC, whereas blood flow decreases below that at rest during contractions greater than 50% MVIC [71]. Fatigue does not develop so rapidly during rhythmic contractions, since the periods of relaxation enable reperfusion and oxygen delivery to the muscle fibers [43]. Compared to isometric and concentric contractions, the metabolic cost of eccentric contractions is very low, even though this type of contraction can produce large forces and promote muscle hypertrophy [49], [102]. This said, repeated eccentric contractions may lead to muscle damage, possibly because such a contraction results in the myosin heads being teared

off from the actin filament to which they are attached [56]. The mechanism underlying eccentric contractions is still an area of debate, since the cross-bridge cycling mechanism which explains concentric contractions cannot so easily be applied to eccentric contractions [98].

With regards to measuring fatigue, it has been suggested that using reductions in MVC output is the gold-standard of fatigue indexes, since fatigue originating at any point along the path connecting cortex to muscle will be reflected in the MVC output, unlike, for instance, force output from tetanic electrical stimulation which develops independently of the CNS [44]. As stated effectively in the review by Vøllestad, the force output during MVC is the "integrated result of the total chain of events" [44]. As an additional methodological consideration, surface EMG measurements are more trustworthy during isometric contractions, in the sense that the location of muscle from which the signal is detected is stable. Since the muscle does not change length during isometric contractions, there is minimal movement of the electrode on the skin surface with respect to the underlying muscle [14].

METHODS

Subjects

The protocol for these experiments was approved by the local human ethics committee in accordance with the Declaration of Helsinki. All subjects signed an informed consent form prior to participation. A total of 51 subjects participated in the study (mean age ± SD = 22.5 ± 4.0 years; mean body mass index (BMI) ± SD = 23.3 ± 3.6 kg/m^2; male/female ratio = 27/24). The majority of subjects were undergraduate or graduate students at the university where the study took place. 7 subjects were part of the research team; nevertheless, all subjects who participated in the study were naïve and untrained with regards to the experimental protocol. Subjects were apparently healthy and did not report medication use or a medical condition that would be considered to interfere with performance during the experiment.

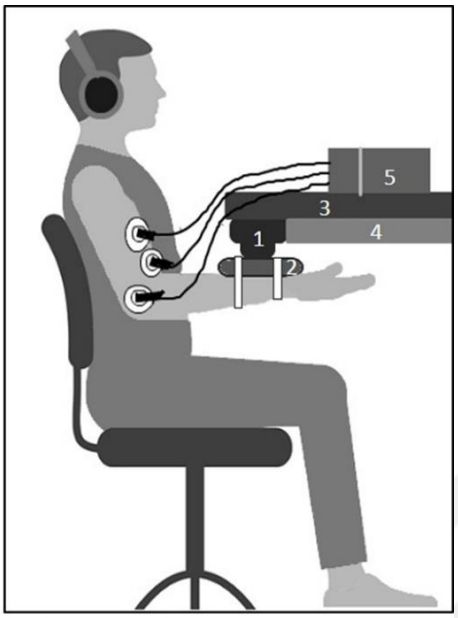
MVIC measurements

During the experimental session, subjects were seated at a table under which a BIOPAC hand dynamometer (SS25LA) had been secured. The dynamometer was connected to a four-channel BIOPAC MP36 data acquisition unit from which force information in the units of Newton was delivered in real time to a desktop computer. The sampling rate was set to 10,000 samples per second. The dominant arm (based on writing preference) of the subject was used for force measurements. A foam pad was secured to the wrist area of the forearm with surgical tape to ensure comfort during the contractions. The height and proximity of the seat with reference to the dynamometer was adjusted so that the subject could position their fully-supinated and padded forearm right below the dynamometer with the shoulder slightly abducted and the angle of elbow flexion approximately ~ 100°. Subjects were instructed to take this position during the MVIC attempts and to relax their non-dominant arm throughout the experiment (Fig. 17).

Each subject was randomly assigned to 1 of 4 MVIC protocols differing only by the duration of rest between successive maximal contractions (i.e. 10, 15, 30 and 60 seconds). For the sake of concise writing, the groups that completed the protocol with 60, 30, 15 and 10 seconds of rest between each attempt will be hereinafter referred to as the "60s", "30s", "15s", and "10s" groups, respectively. A between-subjects design was chosen in order to avoid potential effects of training and familiarity with the protocol on performance. Each MVIC lasted 5 seconds. Subjects were instructed to apply their maximum possible force to the dynamometer as if they were trying to lift the table. Throughout the experiment, subjects wore headphones through which a standardized voice recording saying "Contract, contract, contract!" in their native language indicated to them when to clench and relax their elbow flexors. After each maximal contraction, subjects were asked to rate their pain and fatigue on a scale of 0-10. During the rest periods, subjects were instructed to relax their dominant arm and place it on their lap. A total of 26 MVIC attempts were performed per subject. The last MVIC attempt (i.e. the 26th attempt; MVIC26) was termed the "cheer" trial, since the headphones were removed and the subject

was motivated with live cheer and applause from the study team. Subjects were not provided with visual feedback during the experiment.

Fig. 17: Experimental setup for measuring MVIC of the elbow flexors [103]



1. Force transducer
2. Forearm pad
3. Table
4. Styrofoam panel (to support transducer)
5. Data acquisition device

Surface EMG

Surface EMG (sEMG) recordings were obtained from the biceps brachii of the arm performing the maximal contractions. First, the skin above the muscle was cleaned using sandpaper and alcohol wipes, after which ultrasound gel was applied. Single-use pre-gelled Ag/AgCl electrodes (REDLINE® EKG 42x57) were placed on the belly of the muscle and at the point of insertion of the tendon, with ~4 cm [104] existing between the centers of the two electrodes. A third ground electrode was placed on the lateral epicondyle. The BIOPAC lead set (SS2LB) was used to connect the electrodes to the same four-channel MP36 data acquisition unit used for collecting force data. The raw sEMG data was recorded with a sampling rate of 10,000 Hz and a cutoff frequency of 5-500 Hz. Force and sEMG were recorded simultaneously throughout the experiment.

Data processing

Processing of the force data was done manually in Biopac Student Lab 3.7.7 (BIOPAC Systems, Inc. 2010). The absolute maximum force achieved during each MVIC attempt was determined by taking the highest force value observed during the 5 second contraction and subtracting from it any offset that existed in the force trace during the preceding rest phase. For normalization of maximal force values,

the force produced during the first MVIC attempt was considered as 100%. The force produced during subsequent MVIC attempts was calculated as percentages of this initial value.

The power spectral density analysis of the unfiltered and unrectified sEMG signal was computed with MATLAB R2017b software. The first step of the code removed any DC offset existing in the signal. Median frequency occurring during a 3 second epoch of the maximal contraction was then determined using Welch's Method for MVIC attempts 1 through 25 (total band power: 0-250 Hz). As explained previously, this is the frequency at which 50% of the total power within the power spectrum of the selected epoch of the signal is reached [82]. Given a sampling rate of 10,000 Hz, the window length of the Hamming window was set such that a frequency resolution of 0.6 Hz was achieved. Data normalization was carried out by setting each subject's median frequency at MVIC1 as 100%. The median frequency values associated with successive MVIC attempts were then expressed as percentages of this initial value.

Statistical analysis

All statistical analyses were carried out using IBM SPSS Statistics Version 24. For all analyses, the statistical significance was set at $p < 0.05$. The Shapiro-Wilks test was used to determine if data was normally distributed. The presence of outliers was assessed visually with boxplots. The nonparametric Friedman and Kruskal-Wallis H tests were used if outlier data and/or a non-normal distribution of data was detected. Subjective pain and fatigue scores were treated as continuous variables since subjects were able to rate their pain or fatigue up to as many decimal points as they wanted, within a range of 0-10. The data of 10 subjects were completely excluded from analysis, the most frequent reason for complete exclusion being the occurrence of a rest period at any point which exceeded the assigned rest period by more than 50%.

Cumulative sum (CUSUM) charts

Cumulative sum (CUSUM) charts (see Stapenhurst [105] for review) were constructed from normalized force data to explore subtle but persistent changes in force from the early MVIC attempts. We reasoned that fluctuations in the force producing capacity of a subject were bound to occur even before the onset of fatigue. Unlike normal fluctuations in force performance, however, we expected fatigue to cause a persistent downward deflection of the force CUSUM line. In the force CUSUM charts, we therefore considered fatigue to be a phenomenon that could carry over to the next MVIC attempt and accumulate, rather than think of it as an individual event starting and ending at a particular MVIC attempt. Indeed, there are exercise-induced changes at the peripheral level which are known to persist beyond transience, for example, the depletion of creatine phosphate stores which may take 5-15 minutes to recover [49]. As another example, muscle biopsies taken from the quadriceps femoris after 66% MVC of the knee extensors sustained until task failure have revealed an exponential decay curve with a half-time of almost 2.5 minutes for lactate [106]. The consideration of fatigue as a state which persists and

thus may accumulate and develop gradually is also consistent with De Luca's falling bridge analogy [71]. In essence, we used the CUSUM charts to evaluate whether the rest period between successive attempts was long enough to interrupt the subtle but persistent accumulation of fatigue in the periphery and/or central nervous system.

As the first step of constructing the charts, we set an arbitrary limit that by MVIC10 we would have observed the greatest deflection in force caused by non-fatigue related factors. Next, the early average force value of each subject was calculated by averaging the subject's normalized forces from the first 10 attempts (i.e. MVIC1 through MVIC10). This early average value was abbreviated as "k". For each subject, the "k" value was subtracted from normalized force values MVIC1 through the cheer trial. Finally, differences obtained from these subtraction operations were cumulatively added to the subject's "k" value. Therefore, the subject's "k" value is the starting point ("S0") of his or her CUSUM chart, and positive and negative deflections between two points on the chart are in reference to "k". The points on the CUSUM chart were abbreviated as S(n) where n = 0→25 or n = 0→26, depending on whether or not the cheer trial was included in the CUSUM analysis. The mathematical operations done to compute each point of the CUSUM charts are summarized with the equations below:

$$k = (MVIC1 + \ldots\ldots + MVIC10) / 10$$

$$S0 = k$$

$$S1 = k + (MVIC1 - k)$$

$$S2 = k + (MVIC1 - k) + (MVIC2 - k)$$

$$S(n) = k + (MVIC1 - k) + (MVIC2 - k) + \ldots\ldots + (MVICn - k)$$

Due to the presence of outliers and non-normally distributed data, the CUSUM charts of individual subjects were combined for each group by taking the *median* CUSUM value at each point. To determine whether persistent positive or negative deflections in the CUSUM charts were significant, we employed an error box approach similar to what has previously been used for the determination of reflex onset from EMG signals [107], [108]. We emphasize here that our error box method is not a modification of or in any way related to the more traditional graphical representations of variability, such as confidence intervals or error bars showing standard error of the mean. The upper and lower limits of the error box were set by calculating the maximum deflection in the negative or positive direction from "k" that occurred by completion of MVIC10 (i.e. by, and including, point S10 on the CUSUM chart). The limits of the error box were then set as "k" ± the magnitude of this maximal deflection. Significance was determined by comparing the vertical magnitude of the overall deflection with the width of the error box. Positive or negative deflections that were greater in vertical magnitude than the width of the error box were defined as being "significant" deflections. Small deflections occurring between two successive points on the CUSUM chart and which interrupted an otherwise significant deflection in the opposite direction were ignored.

The same framework was used to construct CUSUM charts of subjective pain scores, subjective fatigue scores and median frequency data. CUSUM charts were similarly evaluated using the error box method to determine if the given rest period was long enough to interrupt subtle but persistent changes in these variables. Total neuromuscular fatigue was defined as persistent decreases in maximal force which resulted in an overall deflection greater in vertical magnitude than the width of the error box. Central fatigue was defined as an increase in maximal force caused by the cheer trial which resulted in an overall deflection greater in vertical magnitude than the width of the error box. Peripheral fatigue, on the other hand, was defined as persistent decreases in maximal force that were not accounted for by central fatigue and which resulted in an overall deflection greater in vertical magnitude than the width of the error box. It was accepted that the presence of peripheral fatigue could be supported by a significant negative deflection (with respect to the error box) in the median frequency CUSUM chart. Finally, significant positive deflections (with respect to the error box) in the CUSUM charts of pain and/or fatigue self-report scores were accepted to be an indication that the subject may not have been performing at his or her maximal level across the corresponding range of attempts. For the construction of all the CUSUM charts, “k” was always calculated by averaging data from MVIC1-MVIC10. Subjects with missing data were excluded listwise.

RESULTS

Sample characteristics

Table 6 summarizes the demographic characteristics of the subjects whose data was included in the analyses. The Shapiro-Wilk test revealed that BMI was normally distributed in all groups with some outliers in the 10s group, but that age was not normally distributed in the 15s and 30s groups. The percentage of males and females was equal only in the 30s group. The majority of subjects were right-handed (92.7%, n=38).

Table 6: Summary of subject characteristics (not including excluded subjects)

	Overall	Duration of rest period			
		60 s	30 s	15 s	10 s
Number of subjects	41	11	10	11	9
% Female (N)	51.2 (21)	72.7 (8)	50.0 (5)	36.4 (4)	44.4 (4)
% Right-handed (N)	92.7 (38)	72.7 (8)	100 (10)	100 (11)	100 (9)
Average age (± stdev)	22.56 ± 4.14	26.91 ± 3.39	21.30 ± 3.16	21.09 ± 4.10	20.44 ± 1.81
Average BMI (± stdev) (kg/m^2)	23.10 ± 3.66	22.84 ± 3.78	22.47 ± 4.01	22.99 ± 2.80	24.24 ± 4.36

Normalized maximal force

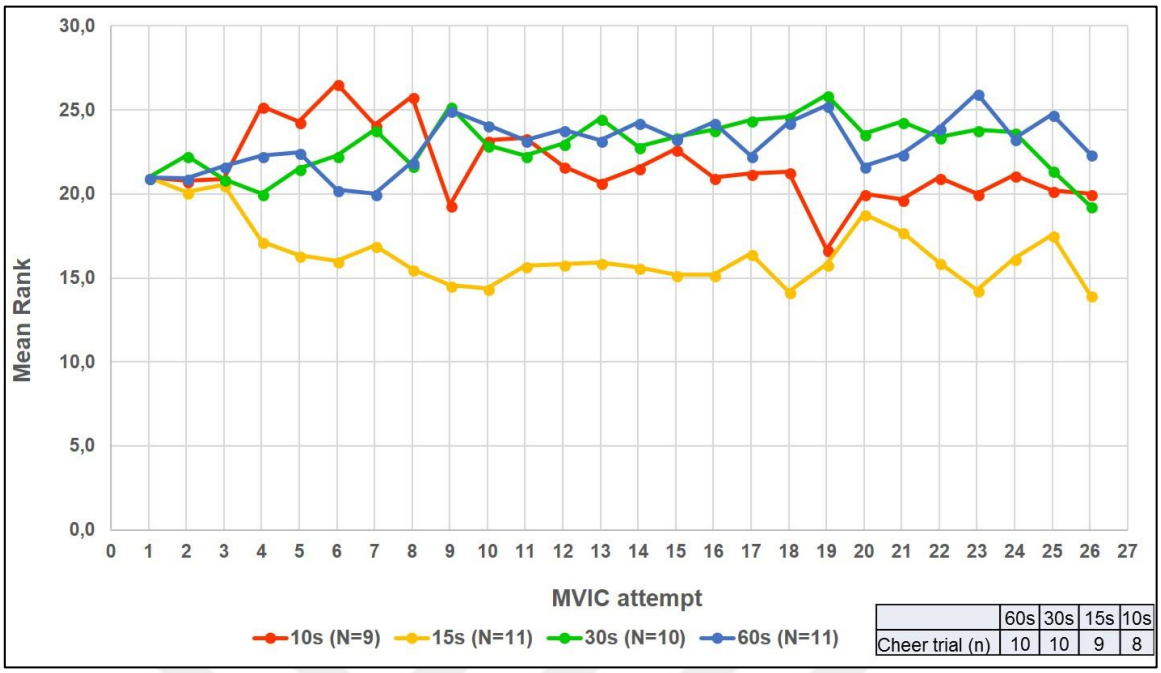
Sample raw force data from the 15s group are given in Table 7. The Kruskal-Wallis H test revealed no between-group differences in mean rank (Fig. 18). The Friedman test was then used to investigate within-group differences. Pairwise comparisons with a Bonferroni correction for multiple comparisons revealed a statistically significant difference between the normalized maximal force during MVC19 (Mdn = 85.26%) and the cheer trial (Mdn = 119.95%) in the 10s group (p=0.009) (Fig. 19). Significant negative deflections were detected in the force CUSUM charts of the 60s, 15s and 10s groups (Fig. 20). The negative deflection with the earliest onset was observed in the 15s group. This deflection started after attempt 5 and persisted through attempt 24. The negative deflections in the 60s and 10s groups started after attempts 16 and 10, respectively. The cheer trial resulted in significant positive deflections in all groups except for the 15s group.

Table 7: Sample raw force data from the subjects (S1-S11) who completed the protocol with 15 seconds of rest between each MVIC attempt. The unit of all measurements is Newton.

Attempt	*S1*	*S2*	*S3*	*S4*	*S5*	*S6*	*S7*	*S8*	*S9*	*S10*	*S11*
MVIC 1	79.7	167.5	61.1	162.0	272.0	248.7	117.4	126.4	268.1	90.0	72.7
MVIC 2	97.5	143.7	56.5	137.7	282.6	237.6	127.1	127.6	268.1	86.4	82.3
MVIC 3	111.9	132.4	38.5	144.4	249.9	234.0	155.0	126.6	267.6	97.0	88.1
MVIC 4	106.1	152.6	44.5	143.2	246.3	169.2	91.7	136.9	229.2	98.4	88.8
MVIC 5	116.5	147.5	40.7	130.9	206.8	221.4	151.4	127.1	250.8	87.3	72.5
MVIC 6	88.6	111.7	46.0	143.2	238.3	204.6	102.1	124.0	255.9	115.5	85.4
MVIC 7	97.2	134.1	43.1	142.0	241.0	209.9	97.7	119.6	154.1	98.0	92.4
MVIC 8	110.5	132.2	61.6	145.1	226.0	214.9	76.1	121.5	192.5	110.5	69.6
MVIC 9	114.1	143.5	45.3	119.4	219.8	215.2	93.9	118.2	263.1	120.6	74.4
MVIC 10	114.5	121.1	40.5	113.3	228.6	216.9	127.3	111.4	208.9	107.1	59.4
MVIC 11	95.6	113.9	42.8	104.0	215.9	226.3	167.8	111.5	184.4	100.8	87.8
MVIC 12	103.3	108.5	39.0	105.9	227.5	238.8	111.2	119.6	118.6	111.0	67.7
MVIC 13	97.0	124.0	42.9	116.8	245.5	214.2	124.9	116.2	212.0	128.5	65.5
MVIC 14	99.6	140.6	43.6	98.2	213.7	205.1	130.0	116.2	204.8	98.4	67.6
MVIC 15	108.0	129.0	42.6	131.7	210.8	208.4	91.7	116.5	202.2	111.2	69.1
MVIC 16	116.0	152.6	52.9	98.7	207.2	195.4	100.3	112.6	199.3	124.7	36.3
MVIC 17	132.1	128.5	41.2	128.5	237.5	200.0	75.1	97.2	300.1	113.9	63.8
MVIC 18	137.2	124.2	42.8	128.3	218.5	157.6	72.9	99.9	157.7	110.0	67.9
MVIC 19	127.6	120.1	41.1	141.8	203.1	176.0	72.0	96.3	223.6	111.9	70.1
MVIC 20	111.2	128.8	47.2	192.8	202.9	191.6	136.4	98.2	191.8	97.3	76.8
MVIC 21	89.3	129.2	48.4	183.4	213.3	198.6	100.4	106.4	170.2	112.4	67.2
MVIC 22	113.1	131.2	40.7	152.1	222.7	159.4	105.7	111.0	155.7	111.5	55.8
MVIC 23	129.3	118.2	35.9	159.1	202.2	161.7	81.6	109.1	110.7	108.5	50.8
MVIC 24	115.8	142.5	37.6	155.2	203.6	123.5	100.9	93.6	134.5	98.9	69.3
MVIC 25	118.7	117.9	57.3	199.1	199.5	120.6	159.3	87.9	171.1	109.1	61.4
Cheer	141.1	162.0	91.9	227.5	---*	201.2	154.8	116.2	210.4	---**	52.5

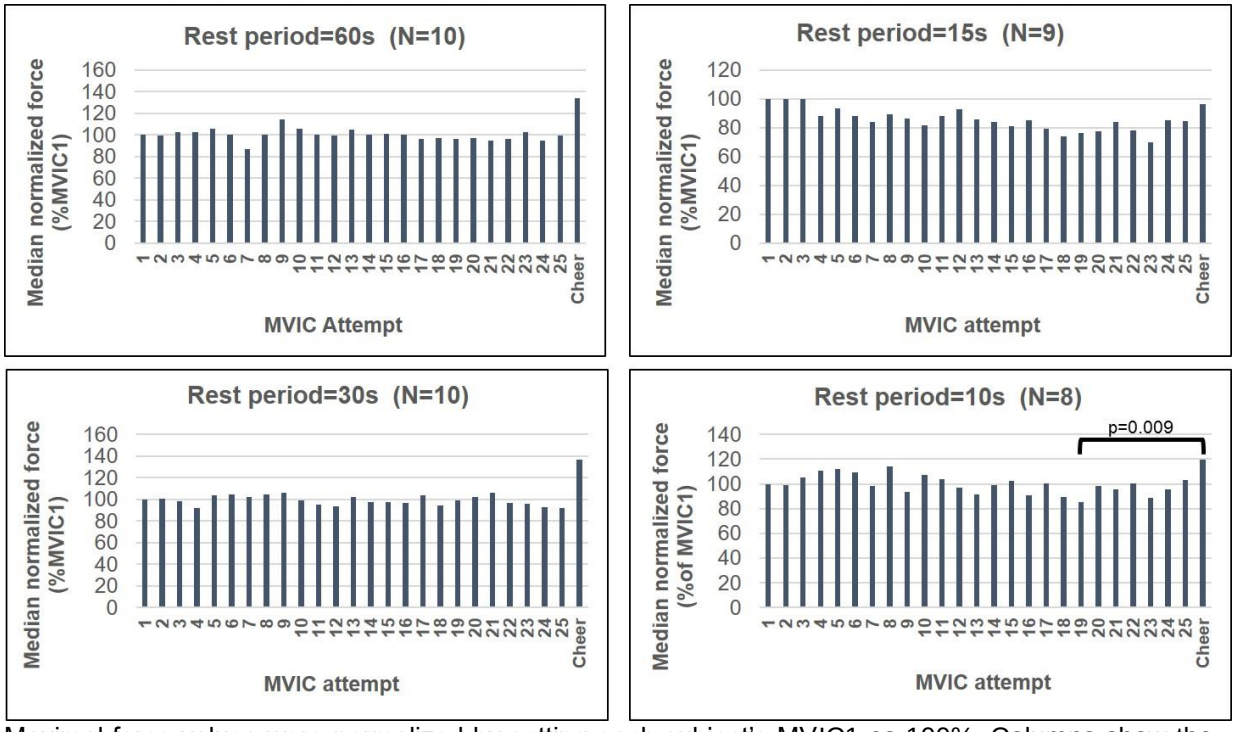
*Cheer trial data excluded because subject performed 26 MVIC attempts before the cheer trial.
**Cheer trial data excluded because preceding rest period exceeded rest period by >100%.

Fig. 18: Between-group analysis of normalized maximal force



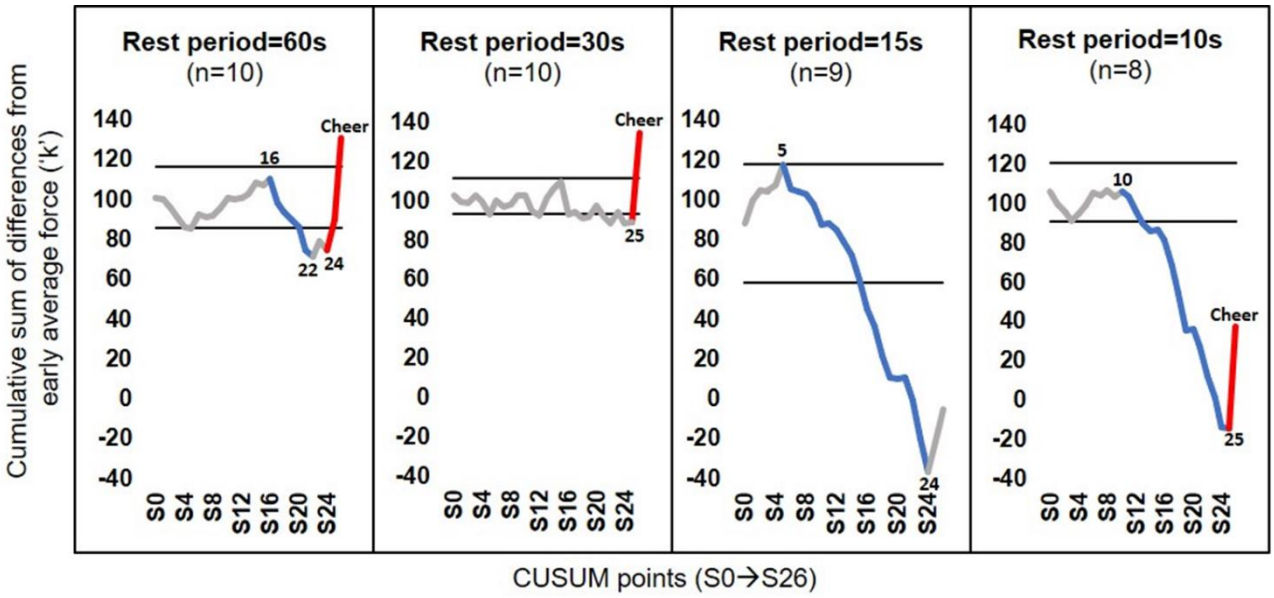
Maximal force values were normalized by setting each subject's MVIC1 as 100%. Data points in the figure show the mean rank of normalized maximal forces, as calculated by SPSS. The Kruskal-Wallis H test did not detect any significant differences in mean rank between groups. The number of subjects whose cheer trial data was included in the analysis is specified in the table on the bottom right-hand corner. The 26th MVIC attempt is the cheer trial.

Fig. 19: Within-group analysis of normalized maximal force



Maximal force values were normalized by setting each subject's MVIC1 as 100%. Columns show the median normalized force observed at each MVIC attempt. The Friedman test followed by pairwise comparisons with a Bonferroni correction revealed a statistically significant difference between the median normalized maximal force of MVIC19 (85.26%) and the cheer trial (119.95%) in the 10s group (p=0.009).

Fig. 20: Normalized maximal force CUSUM charts

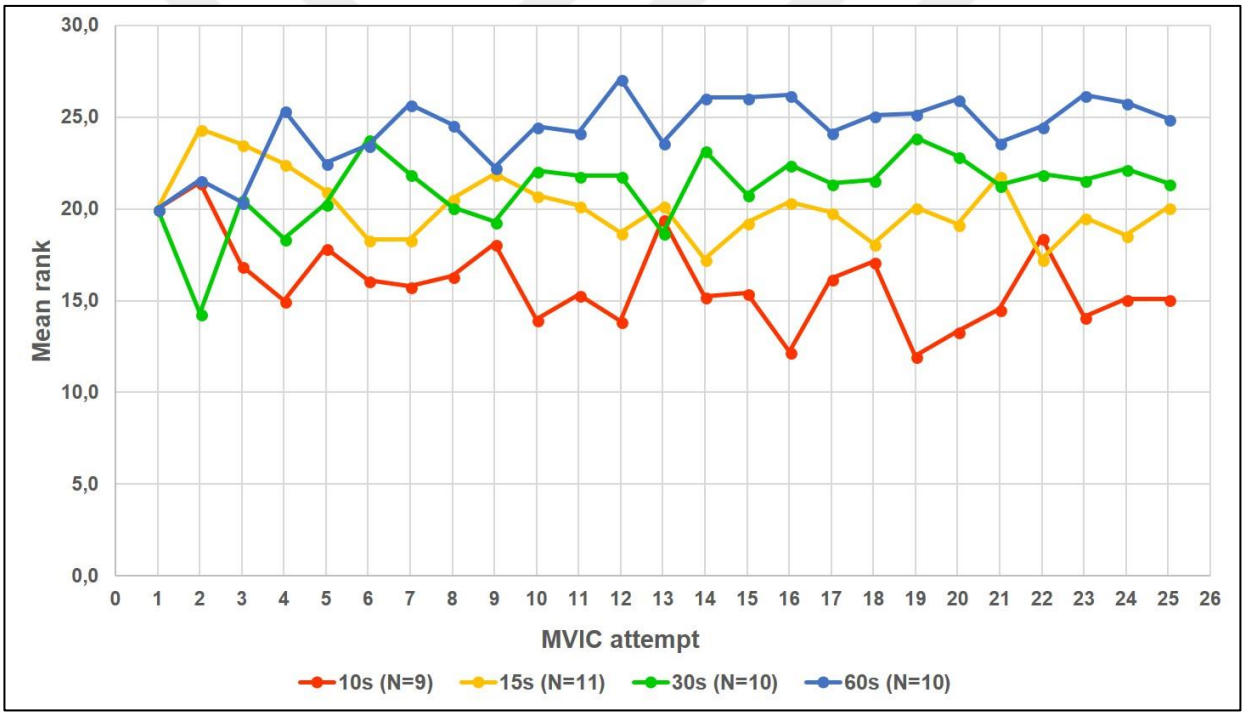


The force of each MVIC attempt was defined as the peak force value that occurred during the 5 second isometric contraction. The 'k' value was calculated for each subject by averaging the normalized force values from the first 10 attempts and was set as the starting point (S0) of the charts. Individual subject CUSUM charts were combined by taking the median values of the group at each point along the chart. Positive and negative deflections reveal whether the force observed during a particular attempt was greater or less than 'k'. Thin black horizontal lines comprise the error box. Significant positive and negative deflections are colored in red and blue, respectively, and the MVIC attempts corresponding to CUSUM points where significant deflections started or ended are indicated near the turning point. Fatigue induced by the experimental protocol was defined as persistent negative deflections with an overall vertical magnitude greater than the width of the error box. Accordingly, fatigue was detected in the protocols with 60s, 15s and 10s of rest. The lack of a significant positive deflection occurring with the cheer trial in the 15s group suggests that subjects did not experience central fatigue during the experimental session.

Normalized median frequency

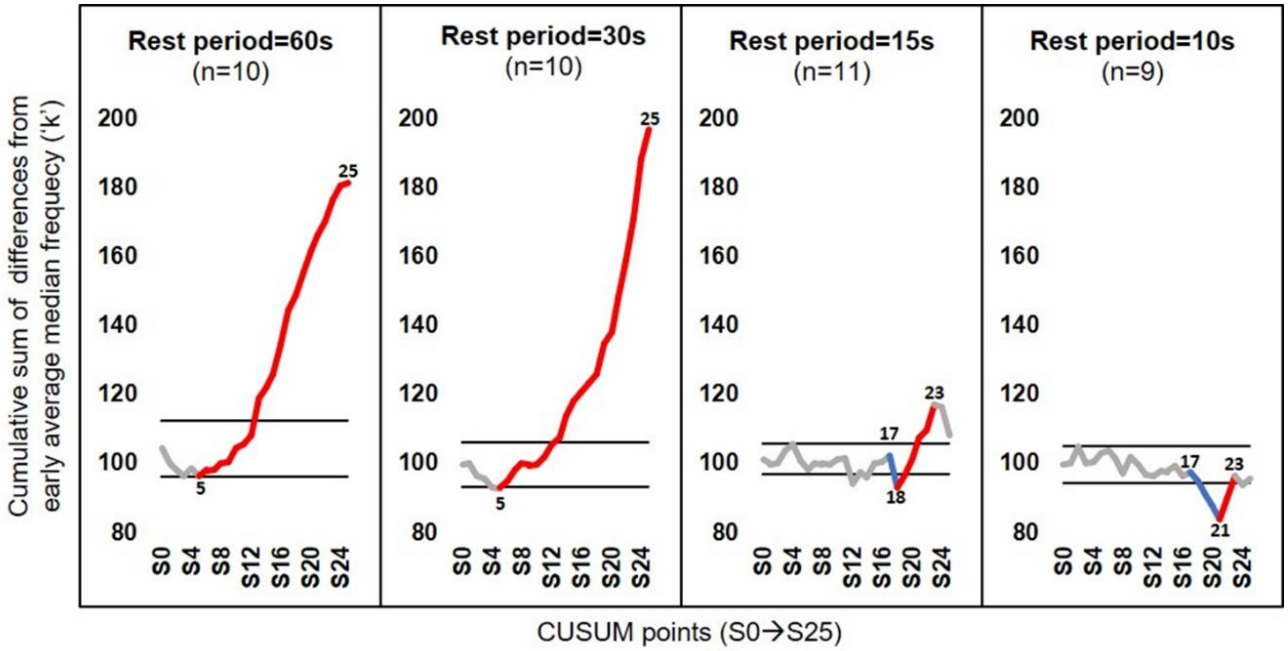
The Kruskal-Wallis H test revealed no significant differences in the mean rank between any of the 4 groups (Fig. 21). The Friedman test revealed significant within-group differences in the 60s group ($\chi 2(24) = 49.396$, $p = 0.002$) and the 30s group ($\chi 2(24) = 38.027$, $p = 0.034$); however, no significant within-group differences could be detected following pairwise comparisons with Bonferroni correction. Significant negative deflections were detected in the median frequency CUSUM charts of the 15s and 10s groups (Fig. 22). The negative deflection in the 10s group started with a small negative deflection that became evident with MVIC18 and persisted through MVIC21, whereas the negative deflection in the 15s group was caused by a drop in median frequency (compared to 'k') that occurred between MVIC17 and MVIC18.

Fig. 21: Between-group analysis of normalized median frequency data



Median frequency data was extracted from 3 second epochs within the surface EMG record of each MVIC attempt using Welch's Method in MATLAB. The median frequency of the 0-250 Hz portion of the power spectral density curve was determined. Data points show the mean rank of normalized median frequency of each attempt across the 4 groups. The Kruskal-Wallis H test did not reveal any statistically significant differences between groups. One subject in the 60s group was excluded from analysis because the sEMG signal was deemed to be too noisy. The 26th MVIC attempt (i.e. the cheer trial) was not included in this analysis.

Fig. 22: Normalized median frequency CUSUM charts

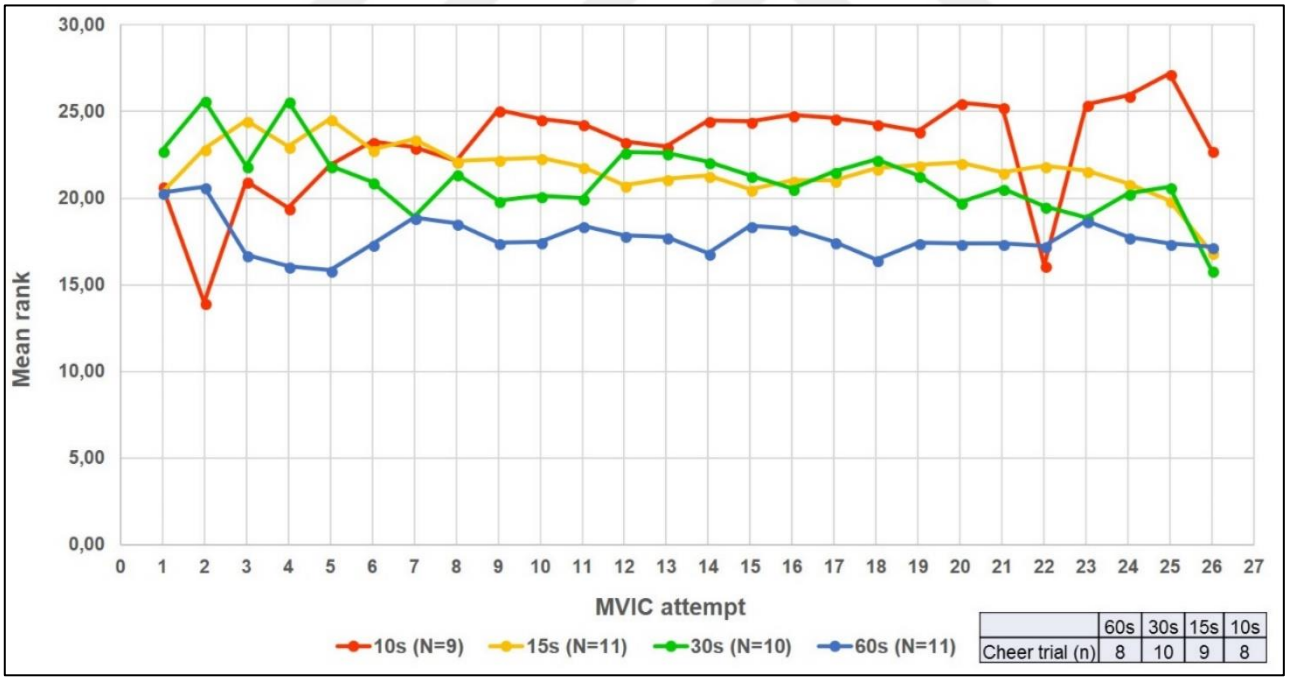


Median frequency data was extracted from 3 second epochs of the biceps brachii sEMG record of each isometric contraction. For each subject, the 'k' value was calculated by averaging the normalized median frequency of the first 10 attempts and was set as the starting point (S0) of the charts. Individual subject CUSUM charts were combined by taking the median values of the group at each point along the chart. Positive and negative deflections reveal whether the median frequency observed during a particular attempt was greater or less than 'k'. The thin black horizontal lines comprise the error box. Significant positive and negative deflections are colored in red and blue, respectively, and the MVIC attempts corresponding to CUSUM points where significant deflections started or ended are indicated near the turning point. Fatigue induced during the experimental protocol was assumed to reveal itself as persistent decreases in median frequency. The only negative deflection that persisted across multiple MVIC attempts and was deemed to be significant in comparison to the width of the error box was detected in the protocol with 10s of rest (the significant deflection in the 15s group existed only between attempts 17 and 18).

Pain self-report score

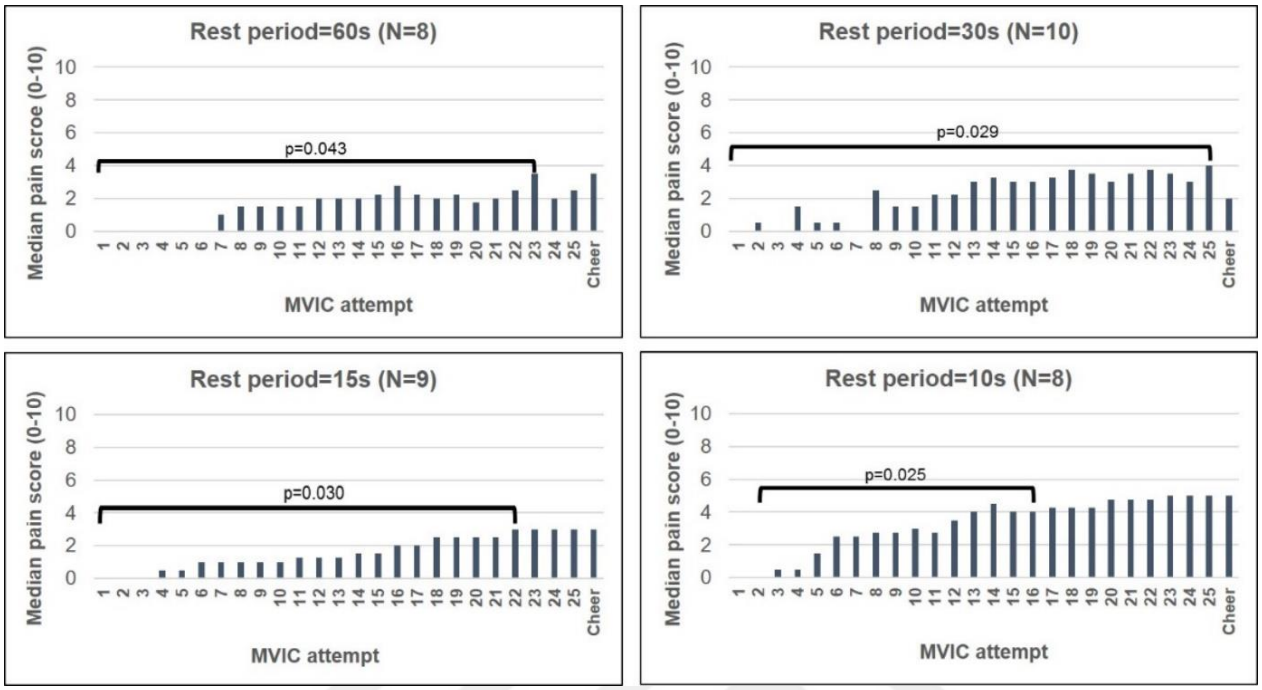
The Kruskal-Wallis H test did not reveal any statistically significant differences between groups (Fig. 23). The Friedman test, on the other hand, revealed that there were significant within-group differences in all groups (10s group: $\chi 2(25) = 175.723$, $p < 0.0005$; 15s group: $\chi 2(25) = 130.367$, $p < 0.0005$; 30s group: $\chi 2(25) = 91.625$, $p < 0.0005$; 60s group: $\chi 2(25) = 96.809$, $p < 0.0005$). Significant within-group differences persisted in all groups after pairwise comparisons with Bonferroni correction. Timewise, the first MVIC attempt with a pain score significantly greater than the pain score of one of the preceding attempts was MVIC16 in the 10s group, MVIC22 in the 15s group, MVIC25 in the 30s group, and MVIC23 in the 60s group. These comparisons are shown in Fig. 24 and the details are listed in Table 8. Persistent positive deflections that fit our criteria of significance were evident in the pain CUSUM charts of all groups (Fig. 25). The earliest significant positive deflection occurred in the 10s group and started after MVIC4. The significant positive deflections in the 60s, 30s and 15s groups started after MVIC9, MVIC9 and MVIC5, respectively. There were no significant negative deflections in any of the pain CUSUM charts.

Fig. 23: Between-group analysis of pain self-report scores



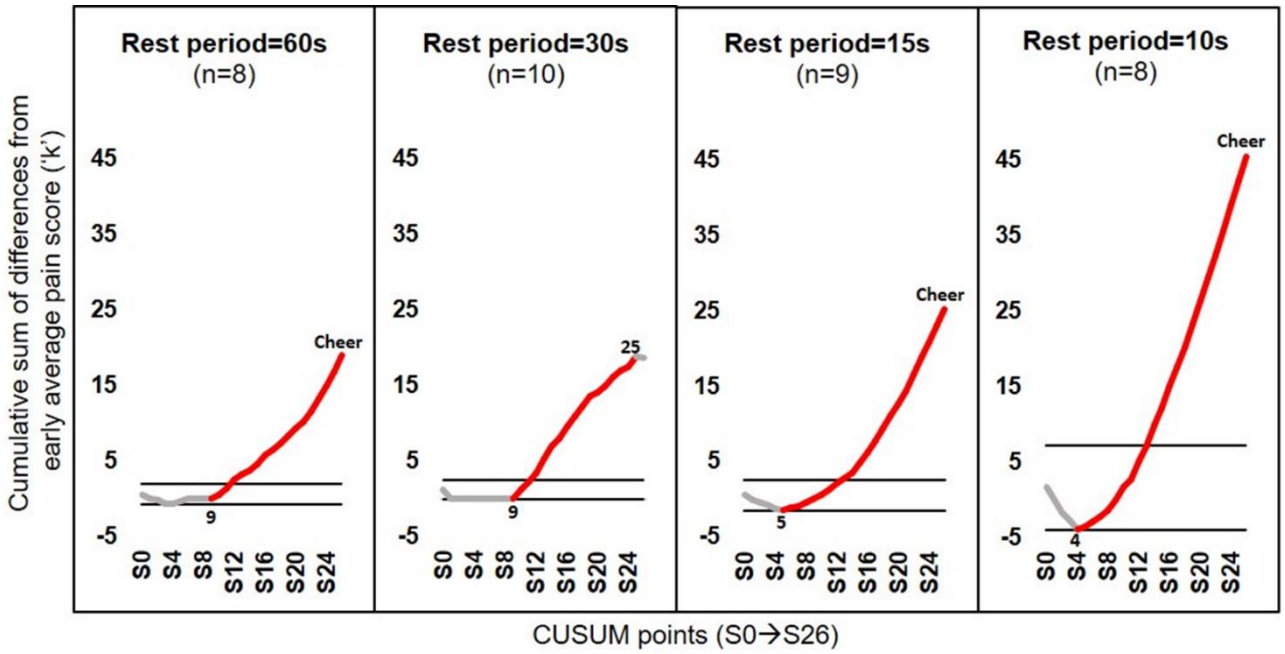
Pain self-report scores were treated as continuous variables since subjects were able to report their score to as many decimal points as they wanted, within a range of 0-10. Data points show the mean rank of pain scores reported after each attempt in the 4 groups. The Kruskal-Wallis H test did not reveal any statistically significant differences between groups. The number of subjects whose cheer trial data was included in the analysis is specified in the blue table on the bottom right-hand corner. The 26th MVIC attempt is the cheer trial.

Fig. 24: Within-group analysis of pain self-report scores (on a scale of 0-10). Significance bars and significance values are given only for the pairwise comparison which includes the first attempt to have a significantly greater score than that of a preceding attempt



Pain self-report scores were treated as continuous variables since subjects were able to report their score to as many decimal points as they wanted, from 0-10. Columns show the median pain score reported after each MVIC attempt. The Friedman test detected significant within-group differences in all groups (details are in the text). Significant differences persisted after pairwise comparisons followed by Bonferroni correction for multiple comparisons. Since each group had many pairs of MVIC attempts with significantly different median pain scores, only the comparison that included the first MVIC attempt with a pain score significantly greater than that of one of the preceding MVIC attempts is shown. More details about the comparisons specified in the figure can be found in Table 8. The reported p-value is the SPSS Bonferroni-adjusted p-value.

Fig. 25: Pain self-report score CUSUM charts

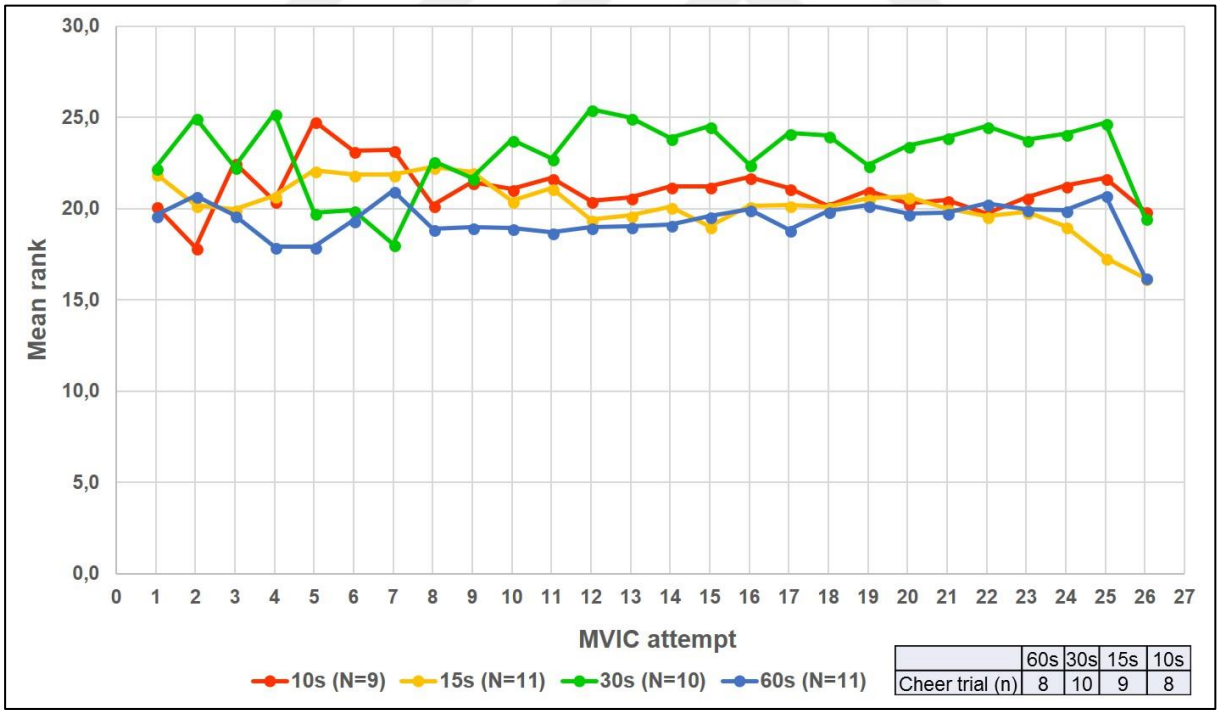


Subjects verbally reported their pain level on a scale of 0-10 immediately after each MVIC attempt and with up to as many decimal points as they wished. For each subject, the 'k' value was calculated by averaging the pain self-report score of the first 10 attempts. 'k' was set as the starting point (S0) of the CUSUM charts. Individual subject CUSUM charts were combined by taking the median values of the group at each point along the chart. Positive and negative deflections reveal whether the pain score reported after a particular attempt was greater or less than the 'k' value. The thin black horizontal lines comprise the error box. Significant positive and negative deflections are colored in red and blue, respectively, and the MVIC attempts corresponding to CUSUM points where significant deflections started or ended are indicated below the starting point of the deflection. Persistent increases in the pain self-report score that were significant based on comparison with the error box were observed in all groups. Overall, the first pain score that contributed to a significant positive deflection was reported for attempt 5 in the 10s protocol.

Fatigue self-report score

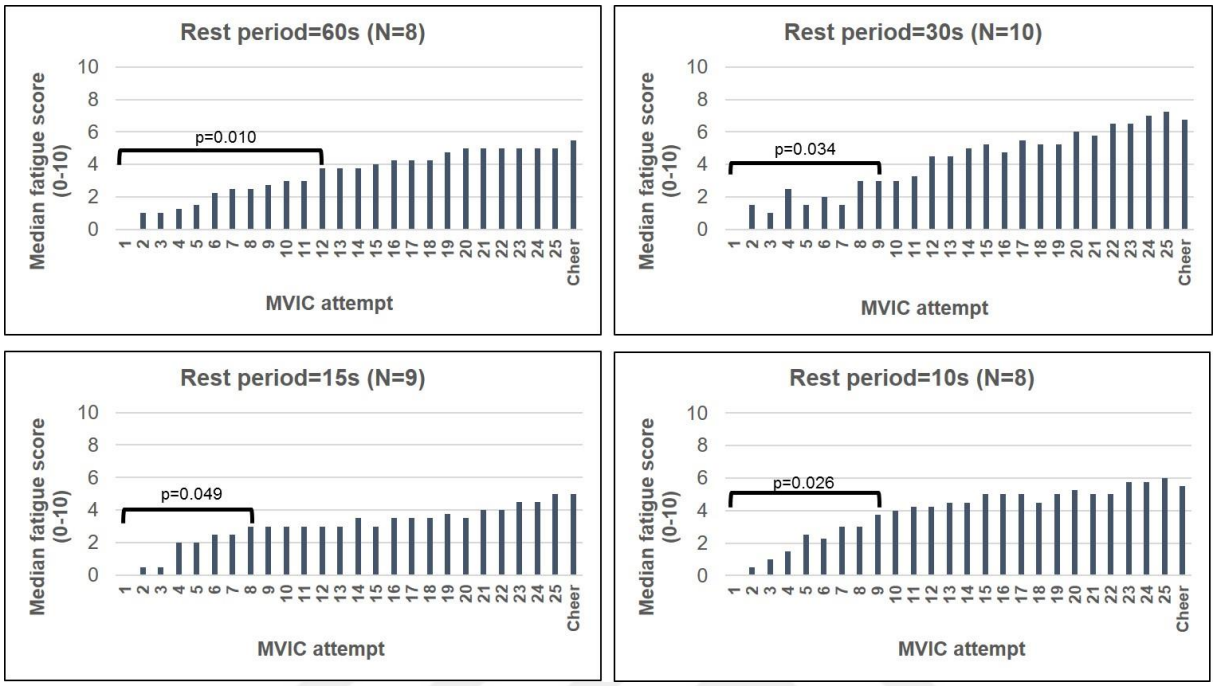
The Kruskal-Wallis H test did not reveal statistically significant differences in mean rank between any of the 4 groups (Fig. 26). The Friedman test revealed significant within-group differences in all 4 groups (10s group: $\chi2(25) = 152.206$, $p < 0.0005$; 15s group: $\chi2(25) = 173.381$, $p < 0.0005$; 30s group: $\chi2(25) = 195.930$, $p < 0.0005$; 60s group: $\chi2(25) = 172.947$, $p < 0.0005$). Significant within-group differences persisted after pairwise comparisons with Bonferroni correction. Timewise, the first MVIC attempt at which the reported fatigue score was significantly greater than the fatigue score of one of the preceding MVIC attempts was MVIC9 in the 10s group, MVIC8 in the 15s group, MVIC9 in the 30s group and MVIC12 in the 60s group. These comparisons are shown in Fig. 27 and the details are listed in Table 8. Persistent positive deflections that satisfied the criteria of significance were evident in all of the CUSUM charts constructed from fatigue self-report data (Fig. 28). Comparison of these CUSUM charts revealed that overall the earliest significant positive deflection started after MVIC5, in both the 10s and 15s groups. The significant positive deflections in the 60s and 30s groups started after MVIC6 and MVIC7, respectively. There were no significant negative deflections in any of the CUSUM charts.

Fig. 26: Between-group analysis of fatigue self-report scores



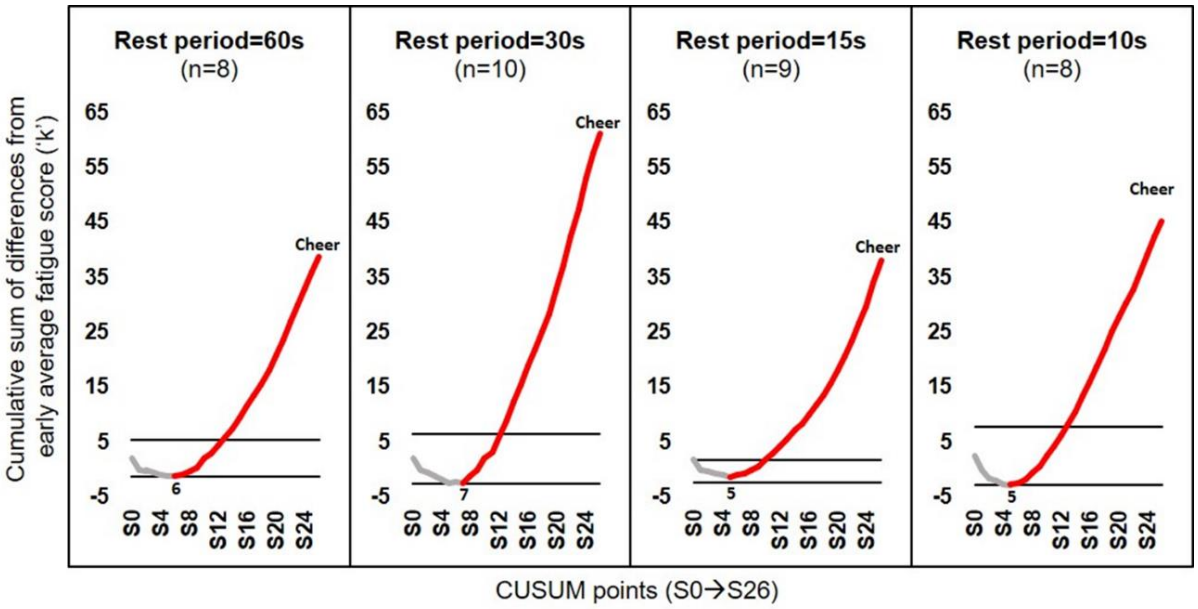
Fatigue self-report scores were treated as continuous variables since subjects were able to report their fatigue level to as many decimal points as they wanted, within a range of 0-10. Data points show the mean rank of fatigue scores reported with each attempt across the 4 groups. The Kruskal-Wallis H test did not reveal any statistically significant differences between groups. The number of subjects whose cheer trial data was included in the analysis is specified in the blue table on the bottom right-hand corner. The 26th MVIC attempt is the cheer trial.

Fig. 27: Within-group analysis of fatigue self-report scores (on a scale of 0-10). Significance bars and values are given only for the pairwise comparison that includes the first attempt to have a significantly greater score than that of a preceding attempt.



Fatigue self-report scores were treated as continuous variables since subjects were able to report their score to as many decimal points as they wanted, from 0-10. Columns show the median fatigue score reported after each MVIC attempt. The Friedman test detected significant within-group differences in all groups (details are in the text). Significant differences persisted after pairwise comparisons followed by Bonferroni correction. Since each group had many pairs of MVIC attempts with significantly different median pain scores, only the comparison that included the first MVIC attempt with a pain score significantly greater than that of one of the preceding MVIC attempts is shown. More details about the comparisons specified in the figure can be found in Table 8. The reported p-value is the SPSS Bonferroni-adjusted p-value.

Fig. 28: Fatigue self-report CUSUM charts



Subjects verbally reported their fatigue level on a scale of 0-10 immediately after each MVIC attempt and with up to as many decimal points as they wished. For each subject, the 'k' value was calculated by averaging the fatigue self-report score of the first 10 attempts. 'k' was set as the starting point (S0) of the CUSUM charts. Individual subject CUSUM charts were combined by taking the median values of the group at each point along the chart. Positive and negative deflections reveal whether the fatigue score reported after a particular attempt was greater or less than the 'k' value. The thin black horizontal lines comprise the error box. Significant positive and negative deflections are colored in red and blue, respectively, and the MVIC attempts corresponding to CUSUM points where significant deflections started or ended are indicated below the starting point of the deflection. Persistent increases in the fatigue self-report score that were significant based on comparison with the error box were observed in all groups. Overall, the first fatigue score that contributed to a significant positive deflection was reported for attempt 6 in the 10s and 15s protocols.

Table 8: Summary of the results of the within-group analysis of pain and fatigue self-report scores. The comparisons given show the first MVIC attempt with a pain/fatigue score significantly greater than that of one of the preceding MVIC attempts.

	Duration of rest period (N subjects)	**10s (8)**	**15s (9)**	**30s (10)**	**60s (8)**
Pain	Attempts compared (median pain score in parenthesis)	MVIC2 (0.00) MVIC16 (4.00)	MVIC1 (0.00) MVIC22 (3.00)	MVIC1 (0.00) MVIC25 (4.00)	MVIC1 (0.00) MVIC23 (3.50)
	SPSS Bonferroni adjusted p-value	0.025	0.030	0.029	0.043
Fatigue	Attempts compared (median pain score in parenthesis)	MVIC1 (0.00) MVIC9 (3.75)	MVIC1 (0.00) MVIC8 (3.00)	MVIC1 (0.00) MVIC9 (3.00)	MVIC1 (0.00) MVIC12 (3.75)
	SPSS Bonferroni adjusted p-value	0.026	0.049	0.034	0.010

DISCUSSION

The present study was conducted as part of an effort to optimize and standardize the protocol for obtaining MVIC measurements from the elbow flexors of healthy subjects. We aimed to determine the ideal rest period between contractions and the number of contractions that must be performed in order to practically obtain a reliable estimate of a person's capacity to produce force from their dominant elbow flexors. Nonparametric tests did not detect any statistically significant between-group or within-group differences in force measurements at any MVIC attempts (1 through cheer trial), save for a singular finding in which the median force at MVIC19 was significantly smaller than the median force at the cheer trial in the 10s group (Fig. 19).

With regards to the analysis of the force data, we next reasoned that performance-attenuating factors such as central and peripheral fatigue could manifest as subtle but persistent decreases in force. Our aim in constructing the CUSUM charts was to determine if the given rest period could interrupt these persistent decreases. By employing the error box method, we established a means of assessing whether persistent deflections in the maximal force CUSUM charts were significant, given that some degree of fluctuation in MVIC force is to be expected even before the onset of fatigue. When drawing the error box, we arbitrarily set a limit that the greatest non-fatigue related fluctuation in force would occur by MVIC10. Other groups that wish to be less conservative in their application of the error box method may decide to choose an earlier attempt as the attempt by which the greatest normal fluctuation in force is assumed to occur.

In our maximal force CUSUM charts, total neuromuscular fatigue – which we defined as persistent decreases in maximal force resulting in an overall deflection greater in vertical magnitude than the width of the error box – was detected in the 60s, 15s and 10s groups (Fig. 20). Total neuromuscular fatigue induced by the experimental protocol was not detected in the 30s group, according to the criteria set forth in the Methods section. We ascribe the significant negative deflection observed in the 60s group to central fatigue, since the vertical magnitude of this deflection is almost the same as that of the positive deflection that occurred with the cheer trial. Similar significant positive deflections due to the cheer trial were observed in the 30s and 10s groups. We conclude that both peripheral and central fatigue occurred in the 10s group, but that only central fatigue occurred in the 60s group. The force CUSUM chart of the 30s group is confounding, given the lack of a significant negative deflection and yet the striking positive deflection that occurred with the cheer trial. It is likely that the subjects in this group had an elevated level of central fatigue *before* commencing the experiment. A pre-experiment interview or questionnaire to gauge to subject's baseline mood and fatigue level would have been useful for confirming this hypothesis. If it can be definitively shown that the frequency shift of the EMG signal that is seen when a muscle fatigues indeed has a predominantly peripheral and not central origin, as argued particularly vehemently by De Luca [14], [71], then our conclusion from the force CUSUM charts regarding the lack of peripheral fatigue in the 60s and 30s groups would be supported

by the median frequency CUSUM charts as well, which do not show any significant negative deflections in the 60s and 30s groups, but do reveal significant negative deflections in the 10s and 15s groups, starting after MVIC17 in both groups (Fig. 22). With regards to the 15s group, peripheral fatigue became apparent after MVIC5 (Fig. 20) but given the lack of a significant positive deflection with the cheer trial, we concluded that the level of central fatigue was not significant, perhaps because the duration of rest was conducive in maintaining the subject's attention to the task and motivation to do well. Conversely, the 60- and 30-second-long waiting period between each attempt, and the repetition of the task every 10 seconds might have decreased the arousal and motivation of the subject to perform their best on the task.

Based on our findings and considering the pressure to complete the assessment of a patient or subject rapidly in the clinic or during an experimental session, we suggest using an MVIC protocol that involves having the subject complete 5 successive MVIC attempts with a rest period of 15 seconds between each attempt. From our maximal force CUSUM charts, we conclude that in our sample this rest period did not result in significant central fatigue even after 25 successive attempts were completed and that the accumulation of peripheral fatigue began after MVIC5 (Fig. 20); however, it will be important to confirm these conclusions about central and peripheral fatigue with the interpolated twitch technique and evaluate the percent voluntary activation level of subjects at each MVIC attempt as done in the studies by De Serres & Enoka [23], Allen, Gandevia, & McKenzie [26] and Allen et al. [16]. Although non-parametric statistical analyses of the self-report data indicated that significant increases in self-report scores in the 15s group were not seen until MVIC8 and MVIC22 for fatigue (Fig. 27) and pain (Fig. 24), respectively, the CUSUM charts of pain and fatigue self-report data from the 15s group reveal significant positive deflections that started after MVIC5 (Figs. 25 and 28, respectively). Therefore, in addition to preventing onset of peripheral fatigue and minimizing testing time, cutting the protocol at 5 attempts concludes the assessment before the onset of a subtle but gradually developing sense of pain and fatigue, which is likely to affect the subject's performance. Further, we suggest taking the *average* of the 5 MVIC attempts for a proper estimate of MVIC force given the expectation of normal fluctuations in a person's MVIC force even before the onset of fatigue. It has been suggested that it may be impossible to limit this variation in performance in human subjects to less than 5% [4]. Finally, we suggest motivating the subject to perform maximally with live verbal encouragement, instead of with a recording delivered via headphones. In all groups, the cheer trial either resulted in or contributed to a positive deflection of the force CUSUM chart, although this positive deflection was significant with comparison to the error box only in the 60, 30 and 10s groups (Fig. 20). While it could be inferred from our results that motivation by a *live* audience is more effective at helping the subject overcome central fatigue, it is also possible that subjects performed well during the cheer trial because they knew the cheer trial would be the final attempt they had to complete.

The current study tested 4 MVIC protocols that were standardized in terms of the number of MVIC attempts, subject position, subject preparation, auditory commands and lack of visual feedback. Evidence from the literature suggests that standardizing the time of day of the experimental sessions

[109] and the respiratory maneuvers performed during each MVIC attempt [17] might be important as well. Research teams or clinicians planning to build on our results might also consider, **1)** having the subject perform the MVIC attempts while lying in the supine position in order to avoid the effects of gravity on the elbow flexors [13], **2)** having subjects perform a warm-up exercise before the actual testing, as done in several studies [8], [18], [22], [25], [26], potentially to promote postactivation potentiation, **3)** providing visual feedback to the subject throughout the study, as done in several studies [15], [20], [21], [23], [24] and as suggested by Gandevia [74], **4)** recording EMG activity from the triceps brachii to ensure lack of antagonistic muscle activity during the maximal contractions, as done by Allen, Gandevia & McKenzie [26], **5)** employing a between-subjects design, given evidence of lack of a consistent training effect in protocols involving repeated MVIC attempts completed on separate days [26], [110], **6)** using a setup for strength measurement that has been proven to have high measurement reproducibility, such as the setup that was tested in the study by Colombo et al. [13], **7)** setting the *duration* of the maximal contraction as the independent variable and **8)** recording the myoelectric signal from the brachialis – rather than the biceps brachii – to investigate change in median frequency, given that this muscle is the primary flexor of the elbow [111]. For the last suggestion, it would be necessary to use intramuscular EMG, since the brachialis lies below the biceps brachii [111]. A further observation we think noteworthy of sharing with those planning to carry out a similar experiment is about recruiting subjects who are athletes. In our sample, we observed that subjects who reported practicing a team sport were particularly motivated to do well on the task and were more likely to present with objective signs of physical exertion during the session, such as sweating, muscle tremor, flushed face and being out of breath. In Gandevia's review, A.V. Hill, who received the 1922 Nobel prize in Medicine or Physiology for his work on heat production in the muscle [112], is quoted as having written, "…with young athletic people one may be sure that they really have gone 'all out'…" and "…it requires an athlete to know how to exhaust himself" [74]. While it has been suggested elsewhere that athletes are more motivated to consistently perform at their highest level [8], at the same time it should be considered that athletes are likely not representative of the average subject that will be tested in the clinical or research setting, in terms of endurance, strength and motivation. A good middle ground might be to have a subject sample that is diverse in terms of athletic status, but to exclude subjects who are not able to perform a set minimum number of attempts within the range of their predicted strength, based on their age, sex and BMI. Regression equations for predicting the strength of 20 muscle groups based on these subject characteristics can be found in the 1996 publication by the National Isometric Muscle Strength (NIMS) Database Consortium (n=493 healthy subjects) [11].

In conclusion, for rapid determination of MVIC from the elbow flexors of a healthy subject, we suggest using a protocol in which 5 attempts each lasting 5 seconds are performed with 15 seconds of rest permitted between attempts. We also suggest that the research or clinical team provides live encouragement to the subject during each attempt and averages the results from the 5 attempts to produce a single value representative of the subject's MVIC level. Given the specified durations of contraction

and rest period, our results suggest that the level of central fatigue is not significant even after 25 such contractions are performed in succession; however, a follow-up study using the interpolated twitch technique is needed to directly confirm this conclusion. Further, we introduce a method based on CUSUM charts with error boxes for detecting the subtle but persistent effect of fatigue in successive maximal force and median frequency data, and for detecting persistent increases in self-report scores that are indicative of subject discomfort and inability to perform at the maximal level. Considering that the physiological and psychological states underlying fatigue and pain may persist for longer than the duration of an MVIC attempt and the subsequent rest period, we argue that in studies with a similar design it may be more appropriate and informative to transform data collected from discrete contractions to a continuous scale – for instance by constructing CUSUM charts – rather than use the traditional statistical tests to determine whether there are significant differences between pairs of contractions.

REFERENCES

[1] "Monitoring of Neuromuscular Function," *Anesthesia Central*, 2000. [Online]. Available: https://anesth.unboundmedicine.com/anesthesia/view/ClinicalAnesthesiaProcedures/728199/all/Monitoring_of_Neuromuscular_Function. [Accessed: 21-May-2018].

[2] D. Meldrum, E. Cahalane, R. Conroy, D. Fitzgerald, and O. Hardiman, "Maximum voluntary isometric contraction: Reference values and clinical application," *Amyotroph. Lateral Scler. Off. Publ. World Fed. Neurol. Res. Group Mot. Neuron Dis.*, vol. 8, pp. 47–55, Mar. 2007.

[3] C. M. Wiles and Y. Karni, "The measurement of muscle strength in patients with peripheral neuromuscular disorders.," *J. Neurol. Neurosurg. Psychiatry*, vol. 46, no. 11, pp. 1006–1013, Nov. 1983.

[4] P. L. Andres, W. Hedlund, L. Finison, T. Conlon, M. Felmus, and T. L. Munsat, "Quantitative motor assessment in amyotrophic lateral sclerosis," *Neurology*, vol. 36, no. 7, pp. 937–941, Jul. 1986.

[5] O. M. Scott, S. A. Hyde, C. Goddard, and V. Dubowitz, "Quantitation of muscle function in children: a prospective study in Duchenne muscular dystrophy," *Muscle Nerve*, vol. 5, no. 4, pp. 291–301, Apr. 1982.

[6] B. Bigland-Ritchie, R. Johansson, O. C. Lippold, and J. J. Woods, "Contractile speed and EMG changes during fatigue of sustained maximal voluntary contractions," *J. Neurophysiol.*, vol. 50, no. 1, pp. 313–324, Jul. 1983.

[7] C. S. Fulco *et al.*, "Muscle fatigue and exhaustion during dynamic leg exercise in normoxia and hypobaric hypoxia," *J. Appl. Physiol. Bethesda Md 1985*, vol. 81, no. 5, pp. 1891–1900, Nov. 1996.

[8] T. D. Timmins and D. H. Saunders, "Effect of caffeine ingestion on maximal voluntary contraction strength in upper- and lower-body muscle groups," *J. Strength Cond. Res.*, vol. 28, no. 11, pp. 3239–3244, Nov. 2014.

[9] M. Sarıkabak, Ç. Yaman, S. Tok, and E. Binboga, "The Effects of Positive and Negative Feedback on Maximal Voluntary Contraction Level of the Biceps Brachii Muscle: Moderating Roles of Gender and Conscientiousness," *Percept. Mot. Skills*, Nov. 2016.

[10] C. J. de Ruiter, R. M. van der Linden, M. J. A. van der Zijden, A. P. Hollander, and A. de Haan, "Short-term effects of whole-body vibration on maximal voluntary isometric knee extensor force and rate of force rise," *Eur. J. Appl. Physiol.*, vol. 88, no. 4–5, pp. 472–475, Jan. 2003.

[11] The National Isometric Muscle Strength (NIMS) Database Consortium, "Muscular weakness assessment: use of normal isometric strength data.," *Arch. Phys. Med. Rehabil.*, vol. 77, no. 12, pp. 1251–1255, Dec. 1996.

[12] C. E. Boettcher, K. A. Ginn, and I. Cathers, "Standard maximum isometric voluntary contraction tests for normalizing shoulder muscle EMG," *J. Orthop. Res.*, vol. 26, no. 12, pp. 1591–1597, Dec. 2008.

[13] R. Colombo *et al.*, "Measurement of isometric muscle strength: a reproducibility study of maximal voluntary contraction in normal subjects and amyotrophic lateral sclerosis patients," *Med. Eng. Phys.*, vol. 22, no. 3, pp. 167–174, Apr. 2000.

[14] C. J. De Luca, "The Use of Surface Electromyography in Biomechanics," *J. Appl. Biomech.*, vol. 13, no. 2, pp. 135–163, May 1997.

[15] G. H. Yue, V. K. Ranganathan, V. Siemionow, J. Z. Liu, and V. Sahgal, "Older Adults Exhibit a Reduced Ability to Fully Activate Their Biceps Brachii Muscle," *J. Gerontol. Ser. A*, vol. 54, no. 5, pp. M249–M253, May 1999.

[16] G. M. Allen, S. C. Gandevia, I. R. Neering, I. Hickie, R. Jones, and J. Middleton, "Muscle performance, voluntary activation and perceived effort in normal subjects and patients with prior poliomyelitis," *Brain J. Neurol.*, vol. 117 (Pt 4), pp. 661–670, Aug. 1994.

[17] S.-Y. Lee and M.-E. Jo, “Comparison of maximum voluntary isometric contraction of the biceps on various posture and respiration conditions for normalization of electromyography data,” *J. Phys. Ther. Sci.*, vol. 28, no. 11, pp. 3007–3010, Nov. 2016.
[18] I. Halperin, S. J. Aboodarda, and D. G. Behm, “Knee extension fatigue attenuates repeated force production of the elbow flexors,” *Eur. J. Sport Sci.*, vol. 14, no. 8, pp. 823–829, 2014.
[19] S. S. Pinto, G. V. Liedtke, C. L. Alberton, E. M. da Silva, E. L. Cadore, and L. F. M. Kruel, “Electromyographic signal and force comparisons during maximal voluntary isometric contraction in water and on dry land,” *Eur. J. Appl. Physiol.*, vol. 110, no. 5, pp. 1075–1082, Nov. 2010.
[20] T. J. Dartnall, M. A. Nordstrom, and J. G. Semmler, “Motor unit synchronization is increased in biceps brachii after exercise-induced damage to elbow flexor muscles,” *J. Neurophysiol.*, vol. 99, no. 2, pp. 1008–1019, Feb. 2008.
[21] T. Yoon, B. S. Delap, E. E. Griffith, and S. K. Hunter, “Mechanisms of fatigue differ after low- and high-force fatiguing contractions in men and women,” *Muscle Nerve*, vol. 36, no. 4, pp. 515–524, Oct. 2007.
[22] T. W. Beck *et al.*, “The effects of interelectrode distance on electromyographic amplitude and mean power frequency during isokinetic and isometric muscle actions of the biceps brachii,” *J. Electromyogr. Kinesiol. Off. J. Int. Soc. Electrophysiol. Kinesiol.*, vol. 15, no. 5, pp. 482–495, Oct. 2005.
[23] S. J. De Serres and R. M. Enoka, “Older adults can maximally activate the biceps brachii muscle by voluntary command,” *J. Appl. Physiol.*, vol. 84, no. 1, pp. 284–291, Jan. 1998.
[24] D. Farina, R. Merletti, A. Rainoldi, M. Buonocore, and R. Casale, “Two methods for the measurement of voluntary contraction torque in the biceps brachii muscle,” *Med. Eng. Phys.*, vol. 21, no. 8, pp. 533–540, Oct. 1999.
[25] P. J. McNair, J. Depledge, M. Brettkelly, and S. N. Stanley, “Verbal encouragement: effects on maximum effort voluntary muscle action,” *Br. J. Sports Med.*, vol. 30, no. 3, pp. 243–245, Sep. 1996.
[26] G. M. Allen, S. C. Gandevia, and D. K. McKenzie, “Reliability of measurements of muscle strength and voluntary activation using twitch interpolation,” *Muscle Nerve*, vol. 18, no. 6, pp. 593–600, Jun. 1995.
[27] S. G. West, *Rheumatology Secrets*, 3rd ed. Philadelphia, PA: Mosby, 2014.
[28] K. E. Wilk, C. Arrigo, A. J. Yenchak, and J. R. Andrews, “Rehabilitation of Elbow Injuries,” in *Physical Rehabilitation of the Injured Athlete*, 4th ed., Philadelphia: W.B. Saunders, 2012, pp. 232–258.
[29] F. Malagelada, M. Dalmau-Pastor, J. Vega, and P. Golanó, “Elbow Anatomy,” in *Sports Injuries*, Berlin, Heidelberg: Springer-Verlag GmbH, 2014, pp. 1–30.
[30] M. Connolly, P. Eggleton, E. Kavanagh, J. Last, E. Moran, and B. Whelan, “Basic Science for Rheumatology,” in *The Rheumatology Handbook*, S. Donnelly, Ed. London: Imperial College Press, 2012, pp. 6–11.
[31] K. P. Moses, J. C. Banks, P. B. Nava, and D. K. Petersen, “Cubital Fossa and Elbow Joint,” in *Atlas of Clinical Gross Anatomy*, 2nd ed., Philadelphia, PA: Elsevier/Saunders, 2013, pp. 232–247.
[32] S. Doan-Johnson, “Synovial fluid,” *OrthopaedicsOne*, 22-Mar-2012. [Online]. Available: https://www.orthopaedicsone.com/display/Main/Synovial%20fluid. [Accessed: 01-May-2018].
[33] A. Rahman and I. Giles, “Rheumatic disease,” in *Kumar and Clark’s Clinical Medicine*, 9th ed., Philadelphia, PA: Elsevier, 2017, pp. 645–706.
[34] K. N. An, F. C. Hui, B. F. Morrey, R. L. Linscheid, and E. Y. Chao, “Muscles across the elbow joint: a biomechanical analysis,” *J. Biomech.*, vol. 14, no. 10, pp. 659–669, 1981.
[35] S. Kishner, P. Mahaney, and T. R. Gest, “Elbow Joint Anatomy: Overview, Gross Anatomy, Other Considerations,” *Medscape*, 09-Nov-2017. [Online]. Available: https://emedicine.medscape.com/article/1898896-overview.

[36] BioDigital, “Upper limb,” *BioDigital: 3D Human Visualization Platform for Anatomy and Disease*, 2018. [Online]. Available: https://human.biodigital.com/. [Accessed: 21-May-2018].
[37] K. P. Moses, J. C. Banks, P. B. Nava, and D. K. Petersen, “Arm,” in *Atlas of Clinical Gross Anatomy*, 2nd ed., Philadelphia, PA: Elsevier/Saunders, 2013, pp. 218–231.
[38] K. P. Moses, J. C. Banks, P. B. Nava, and D. K. Petersen, “Anterior Forearm,” in *Atlas of Clinical Gross Anatomy*, 2nd ed., Philadelphia, PA: Elsevier/Saunders, 2013, pp. 248–257.
[39] K. P. Moses, J. C. Banks, P. B. Nava, and D. K. Petersen, “Posterior Forearm,” in *Atlas of Clinical Gross Anatomy*, 2nd ed., Philadelphia, PA: Elsevier/Saunders, 2013, pp. 258–267.
[40] The Editors of Encyclopaedia Britannica, “Skeletal muscle,” *Encyclopedia Britannica*, 05-Jan-2018. [Online]. Available: https://www.britannica.com/science/skeletal-muscle. [Accessed: 02-May-2018].
[41] G. Z. Mentis, “The Spinal and Peripheral Motor System,” in *Fundamental Neuroscience*, 4th ed., Philadelphia, PA: Academic Press Elsevier, 2013, pp. 613–630.
[42] G. M. Cooper, “Actin, Myosin, and Cell Movement,” in *The Cell: A Molecular Approach*, 2nd ed., Sunderland (MA): Sinauer Associates, 2000.
[43] R. D. Keynes and D. J. Aidley, *Nerve and Muscle*, 3rd ed. Cambridge: Cambridge University Press, 2001.
[44] N. K. Vøllestad, “Measurement of human muscle fatigue,” *J. Neurosci. Methods*, vol. 74, no. 2, pp. 219–227, Jun. 1997.
[45] R. B. Tallitsch and R. S. Guastaferri, “Muscle tissue,” in *Histology: An Identification Manual*, Philadelphia, PA: Mosby, 2009, pp. 75–87.
[46] S. Standring, “Functional anatomy of the musculoskeletal system,” in *Gray’s Anatomy*, Philadelphia, PA: Elsevier, 2016, p. 81–122.e1.
[47] B. M. Koeppen and B. A. Stanton, “Skeletal Muscle Physiology,” in *Berne and Levy Physiology*, 7th ed., Philadelphia, PA: Elsevier, 2018, pp. 242–267.
[48] B. R. Berridge, J. F. V. Vleet, and E. Herman, “Cardiac, Vascular, and Skeletal Muscle Systems,” in *Haschek and Rousseaux’s Handbook of Toxicologic Pathology*, 3rd ed., Philadelphia, PA: Academic Press, Elsevier Inc., 2013, pp. 1567–1665.
[49] T. M. Best and C. A. Asplund, “Exercise Physiology,” in *DeLee & Drez’s Orthopaedic Sports Medicine*, 4th ed., Philadelphia, PA: Saunders, 2014, p. 72–83.e3.
[50] W. K. Ovalle and P. C. Nahirney, “Muscle Tissue,” in *Netter’s Essential Histology*, 2nd ed., Philadelphia, PA: Elsevier/Saunders, 2013, pp. 71–100.
[51] W. M. Haschek, C. G. Rousseaux, and M. A. Wallig, “Cardiovascular and Skeletal Muscle Systems,” in *Fundamentals of Toxicologic Pathology*, 2nd ed., San Diego (CA): Academic Press, 2010, pp. 319–376.
[52] R. J. Korthuis, *Anatomy of Skeletal Muscle and Its Vascular Supply*. San Rafael (CA): Morgan & Claypool Life Sciences, 2011.
[53] J. L. Krans, “The Sliding Filament Theory of Muscle Contraction,” *Nat. Educ.*, vol. 3, no. 9, p. 66, 2010.
[54] McGraw-Hill Animations, “Breakdown of ATP and Cross Bridge Movement during Muscle Contraction,” *YouTube*, 09-Jun-2017. [Online]. Available: https://www.youtube.com/watch?v=p8iKzWqUU2s. [Accessed: 07-May-2018].
[55] I. Y. Kuo and B. E. Ehrlich, “Signaling in Muscle Contraction,” *Cold Spring Harb. Perspect. Biol.*, vol. 7, no. 2, Feb. 2015.
[56] L. Robson and D. S. Court, “Bone, muscle, skin and connective tissue,” in *Medical Sciences*, 2nd ed., Philadelphia, PA, 2015, pp. 403–449.
[57] McGraw-Hill Animations, “Muscle Contraction Process,” *YouTube*, 09-Jun-2017. [Online]. Available: https://www.youtube.com/watch?v=ousflrOzQHc. [Accessed: 07-May-2018].
[58] J. A. Chromiak and J. Antonio, “Skeletal Muscle Plasticity,” in *Essentials of Sports Nutrition and Supplements*, Humana Press, 2008, pp. 21–52.

[59] M. P. Blaustein, J. P. Y. Kao, and D. R. Matteson, “Molecular motors and the mechanism of muscle contraction,” in *Cellular Physiology and Neurophysiology*, 2nd ed., Philadelphia, PA: Mosby, 2012, pp. 211–228.
[60] D. Purves *et al.*, “Functional Organization of the Primary Motor Cortex,” in *Neuroscience*, 2nd ed., Sunderland (MA): Sinauer Associates, 2001.
[61] M. Barinaga, “Remapping the motor cortex,” *Science*, vol. 268, no. 5218, pp. 1696–1698, Jun. 1995.
[62] P. A. Chouinard and T. Paus, “The Primary Motor and Premotor Areas of the Human Cerebral Cortex,” *The Neuroscientist*, vol. 12, no. 2, pp. 143–152, Apr. 2006.
[63] B. Kolb and I. Q. Whishaw, “Chapter 10: How Does the Brain Produce Movement?,” in *Introduction to Brain and Behavior*, 1st ed., Worth Publishers, 2000, pp. 354–397.
[64] M. H. Schieber and J. F. Baker, “Chapter 29: Control of Movement,” in *Fundamental Neuroscience*, 4th ed., Philadelphia, PA: Elsevier Inc., 2013, pp. 631–651.
[65] B. M. Koeppen and B. A. Stanton, “Organization of Motor Function,” in *Berne and Levy Physiology*, 7th ed., Philadelphia, PA: Elsevier, 2018, pp. 161–207.
[66] Natural History Museum (London), “Motor homunculus model,” *Science Photo Library*. [Online]. Available: http://www.sciencephoto.com/media/429991/view. [Accessed: 20-May-2018].
[67] D. Purves *et al.*, “The Primary Motor Cortex: Upper Motor Neurons That Initiate Complex Voluntary Movements,” in *Neuroscience*, 2nd ed., Sunderland (MA): Sinauer Associates, 2001.
[68] S. Grillner, “Fundamentals of Motor Systems,” in *Fundamental Neuroscience*, 4th ed., Philadelphia, PA: Academic Press Elsevier, 2013, pp. 599–611.
[69] R. Meeusen, P. Watson, H. Hasegawa, B. Roelands, and M. F. Piacentini, “Central fatigue: the serotonin hypothesis and beyond,” *Sports Med. Auckl. NZ*, vol. 36, no. 10, pp. 881–909, 2006.
[70] R. M. Enoka and D. G. Stuart, “Neurobiology of muscle fatigue,” *J. Appl. Physiol.*, vol. 72, no. 5, pp. 1631–1648, May 1992.
[71] C. J. De Luca, “Myoelectrical manifestations of localized muscular fatigue in humans,” *Crit. Rev. Biomed. Eng.*, vol. 11, no. 4, pp. 251–279, 1984.
[72] R. M. Enoka and J. Duchateau, “Muscle fatigue: what, why and how it influences muscle function,” *J. Physiol.*, vol. 586, no. 1, pp. 11–23, Jan. 2008.
[73] S. J. Garland and E. R. Gossen, “The muscular wisdom hypothesis in human muscle fatigue,” *Exerc. Sport Sci. Rev.*, vol. 30, no. 1, pp. 45–49, Jan. 2002.
[74] S. C. Gandevia, “Spinal and Supraspinal Factors in Human Muscle Fatigue,” *Physiol. Rev.*, vol. 81, no. 4, pp. 1725–1789, Jan. 2001.
[75] E. B. Cady, H. Elshove, D. A. Jones, and A. Moll, “The metabolic causes of slow relaxation in fatigued human skeletal muscle.,” *J. Physiol.*, vol. 418, pp. 327–337, Nov. 1989.
[76] D. A. Jones, B. Bigland-Ritchie, and R. H. T. Edwards, “Excitation frequency and muscle fatigue: Mechanical responses during voluntary and stimulated contractions,” *Exp. Neurol.*, vol. 64, no. 2, pp. 401–413, May 1979.
[77] H. Kranz, A. M. Williams, J. Cassell, D. J. Caddy, and R. B. Silberstein, “Factors determining the frequency content of the electromyogram,” *J. Appl. Physiol.*, vol. 55, no. 2, pp. 392–399, Aug. 1983.
[78] D. C. Preston and B. E. Shapiro, “Basic Electromyography: Analysis of Motor Unit Action Potentials,” in *Electromyography and Neuromuscular Disorders*, 3rd ed., Philadelphia, PA, 2013, pp. 235–248.
[79] I. Rodríguez-Carreño, L. Gila-Useros, and A. Malanda-Trigueros, “Motor Unit Action Potential Duration: Measurement and Significance,” in *Advances in Clinical Neurophysiology*, IntechOpen, 2012, pp. 133–160.
[80] Tektronix, “FFT Tutorial,” *YouTube*, 17-May-2012. [Online]. Available: https://www.youtube.com/watch?v=zKKGA30bHG0. [Accessed: 19-May-2018].

[81] G. De Luca, “Fundamental Concepts in EMG Signal Acquisition,” *DelSys Inc*, Mar. 2003.
[82] BIOPAC Systems, Inc., “Application Note 118: EMG Frequency Signal Analysis,” *BIOPAC Systems, Inc.*, May-2010. [Online]. Available: https://www.biopac.com/wp-content/uploads/app118.pdf. [Accessed: 22-May-2018].
[83] K. R. Mills, “Power spectral analysis of electromyogram and compound muscle action potential during muscle fatigue and recovery,” *J. Physiol.*, vol. 326, pp. 401–409, May 1982.
[84] C. Krogh-Lund and K. Jørgensen, “Modification of myo-electric power spectrum in fatigue from 15% maximal voluntary contraction of human elbow flexor muscles, to limit of endurance: reflection of conduction velocity variation and/or centrally mediated mechanisms?,” *Eur. J. Appl. Physiol.*, vol. 64, no. 4, pp. 359–370, Jul. 1992.
[85] D. B. Chaffin, “Localized muscle fatigue -definition and measurement,” *J. Occup. Med. Off. Publ. Ind. Med. Assoc.*, vol. 15, no. 4, pp. 346–354, Apr. 1973.
[86] M. Jouvet, “Sleep and Serotonin: An Unfinished Story,” *Neuropsychopharmacology*, vol. 21, no. S1, p. 24S–27S, Aug. 1999.
[87] C. L. Reardon and S. Creado, “Drug abuse in athletes,” *Subst. Abuse Rehabil.*, vol. 5, pp. 95–105, Aug. 2014.
[88] D. J. Heal, S. L. Smith, J. Gosden, and D. J. Nutt, “Amphetamine, past and present – a pharmacological and clinical perspective,” *J. Psychopharmacol. Oxf. Engl.*, vol. 27, no. 6, pp. 479–496, Jun. 2013.
[89] A. Steensberg, G. van Hall, T. Osada, M. Sacchetti, B. Saltin, and B. K. Pedersen, “Production of interleukin-6 in contracting human skeletal muscles can account for the exercise-induced increase in plasma interleukin-6,” *J. Physiol.*, vol. 529, no. Pt 1, pp. 237–242, Nov. 2000.
[90] M. Gleeson, “Interleukins and exercise,” *J. Physiol.*, vol. 529, no. Pt 1, p. 1, Nov. 2000.
[91] M. Amann, “Significance of group III and IV muscle afferents for the endurance exercising human,” *Clin. Exp. Pharmacol. Physiol.*, vol. 39, no. 9, pp. 831–835, Sep. 2012.
[92] R. D. Herbert and S. C. Gandevia, “Twitch Interpolation in Human Muscles: Mechanisms and Implications for Measurement of Voluntary Activation,” *J. Neurophysiol.*, vol. 82, no. 5, pp. 2271–2283, Nov. 1999.
[93] N. Rube and N. H. Secher, “Paradoxical influence of encouragement on muscle fatigue,” *Eur. J. Appl. Physiol.*, vol. 46, no. 1, pp. 1–7, Apr. 1981.
[94] D. Lorenz, “Postactivation potentiation: an introduction,” *Int. J. Sports Phys. Ther.*, vol. 6, no. 3, pp. 234–240, Sep. 2011.
[95] D. W. Robbins, “Postactivation potentiation and its practical applicability,” *J. Strength Cond. Res.*, vol. 19, no. 2, p. 453, May 2005.
[96] C. J. Mitchell and D. G. Sale, “Enhancement of jump performance after a 5-RM squat is associated with postactivation potentiation,” *Eur. J. Appl. Physiol.*, vol. 111, no. 8, pp. 1957–1963, Aug. 2011.
[97] The UCSD Muscle Physiology Laboratory, “Fundamental Functional Properties of Skeletal Muscle,” *Muscle Physiology*, 02-Dec-2008. [Online]. Available: http://muscle.ucsd.edu/musintro/props.shtml#LT. [Accessed: 07-May-2018].
[98] The UCSD Muscle Physiology Laboratory, “Types of Contractions,” *Muscle Physiology*, 31-May-2006. [Online]. Available: http://muscle.ucsd.edu/musintro/contractions.shtml. [Accessed: 07-May-2018].
[99] R. P. Wilder, J. G. Jenkins, P. Panchang, and S. Statuta, “Therapeutic Exercise,” in *Braddom’s Physical Medicine and Rehabilitation*, 5th ed., Philadelphia, PA: Elsevier, 2016, p. 321–346.e3.
[100] J. G. Betts *et al.*, “Nervous System Control of Muscle Tension,” in *Anatomy & Physiology*, Houston (TX): OpenStax, 2013.
[101] G. L. Close, A. Kayani, A. Vasilaki, and A. McArdle, “Skeletal muscle damage with exercise and aging,” *Sports Med. Auckl. NZ*, vol. 35, no. 5, pp. 413–427, 2005.

[102]K. Marri and R. Swaminathan, “Analysis of concentric and eccentric contractions in biceps brachii muscles using surface electromyography signals and multifractal analysis,” *Proc. Inst. Mech. Eng. [H]*, vol. 230, no. 9, pp. 829–839, Sep. 2016.
[103]N. R. Şenocak, *Experimental setup for measuring MVIC of the elbow flexors*. 2018.
[104]K. J. Tucker and K. S. Türker, “A new method to estimate signal cancellation in the human maximal M-wave,” *J. Neurosci. Methods*, vol. 149, no. 1, pp. 31–41, Nov. 2005.
[105]T. Stapenhurst, “An introduction to cusum (cumulative sum) charts,” in *Mastering Statistical Process Control*, Oxford (UK): Elsevier Butterworth-Heinemann, 2005, pp. 307–320.
[106]R. C. Harris, E. Hultman, and K. Sahlin, “Glycolytic intermediates in human muscle after isometric contraction,” *Pflüg. Arch.*, vol. 389, no. 3, pp. 277–282, Mar. 1981.
[107]K. S. Türker, J. Yang, and P. Brodin, “Conditions for excitatory or inhibitory masseteric reflexes elicited by tooth pressure in man,” *Arch. Oral Biol.*, vol. 42, no. 2, pp. 121–128, Feb. 1997.
[108]R. S. A. Brinkworth and K. S. Türker, “A method for quantifying reflex responses from intra-muscular and surface electromyogram,” *J. Neurosci. Methods*, vol. 122, no. 2, pp. 179–193, Jan. 2003.
[109]A. Gauthier, D. Davenne, A. Martin, G. Cometti, and J. Van Hoecke, “Diurnal rhythm of the muscular performance of elbow flexors during isometric contractions,” *Chronobiol. Int.*, vol. 13, no. 2, pp. 135–146, Jul. 1996.
[110]A. Rainoldi, G. Galardi, L. Maderna, G. Comi, L. Lo Conte, and R. Merletti, “Repeatability of surface EMG variables during voluntary isometric contractions of the biceps brachii muscle,” *J. Electromyogr. Kinesiol.*, vol. 9, no. 2, pp. 105–119, Apr. 1999.
[111]R. G. Eston, “Commentary,” *Br. J. Sports Med.*, vol. 30, no. 3, p. 245, Sep. 1996.
[112]“The Nobel Prize in Physiology or Medicine 1922,” *Nobelprize.org*. [Online]. Available: https://www.nobelprize.org/nobel_prizes/medicine/laureates/1922/. [Accessed: 20-May-2018].