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**The influence of knee angle on
human quadriceps femoris
performance**

Ronald D. Kooistra

The work in this thesis was carried out at the research group '(Patho-) physiology and mechanics in human performance' of the Institute for Fundamental and Clinical Human Movement Sciences, Vrije Universiteit Amsterdam.

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VRIJE UNIVERSITEIT

**The influence of knee angle on human quadriceps femoris
performance**

ACADEMISCH PROEFSCHRIFT

ter verkrijging van de graad Doctor aan
de Vrije Universiteit Amsterdam,
op gezag van de rector magnificus
prof.dr. L.M. Bouter,
in het openbaar te verdedigen
ten overstaan van de promotiecommissie
van de faculteit der Bewegingswetenschappen
op vrijdag 16 maart 2007 om 13.45 uur
in de aula van de universiteit,
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door

Ronald David Kooistra

geboren te Rotterdam

promotor: prof.dr. A. de Haan

copromotor: dr. C.J. de Rooter

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Introduction and outline

In everyday life movement of the human body is made possible by the capacity of our muscles to produce force over a considerable range of muscle lengths. By the voluntary contraction of our muscles, force is exerted and transmitted through the tendon to the bone. Consequently, bone can be held in position, (or) accelerated or decelerated across joints to bring about movement of the bony skeleton. Virtually all skeletal muscle that brings about movement is under conscious control of the brain. The brain in combination with the central nervous system determines the extent to which skeletal muscle is activated. The performance of daily activities is made possible by the ability of the central nervous system to attune the muscle's force production across different muscle lengths to the needs of the body. This represents the basis of our movement in daily life.

In order to gain a better understanding of the execution of movement, knowledge is required about the relationship between the contractile properties of muscles and their neural input from the central nervous system at different muscle lengths. In this thesis the influence of muscle length on the performance of the m. quadriceps is investigated.

In the first section of this introductory chapter the activation of muscle through brain and central nervous system is described. The second section will discuss the structure and force production of skeletal muscle at different muscle lengths. Thereafter, the measurement techniques electromyography (EMG) and near - infrared spectroscopy (NIRS) that have been used in this thesis will be described. Finally, a brief overview and outline of the following chapters is given.

Activation of the muscle

The muscle fibers that make up skeletal muscle are activated through signals (action potentials) sent by the brain and central nervous system to the motoneuron situated in the spinal cord or brain stem. Each motoneuron innervates several muscle fibers and this functional unit of movement is known as the motor unit (Fig. 1). The group of motor units that operate together to coordinate the contractions of a single muscle are considered a motor unit pool. Note that the number of muscle fibers within each motor unit can vary immensely: the eye muscles, on the one hand, may innervate

only ten fibers, whereas, on the other hand, in the quadriceps muscle a motor unit may innervate several thousand fibers. When a motor unit is activated, the fibers innervated by this motor unit are stimulated and will contract. The activation of this single motoneuron will result in a weak contraction. A stronger muscle contraction is elicited when more motor units are activated that stimulate more muscle fibers. Motor units are generally recruited in order of smallest to largest (fewest fibers to most fibers) as force of the contraction increases, this principle is known as Henneman's Size Principle (19). Another mechanism by which skeletal muscle force is regulated is called rate coding. By the control of the firing rate of each motor unit, skeletal muscle force can also be controlled (25). The combination of the size principle and rate coding allows for a considerable control of skeletal muscle force.

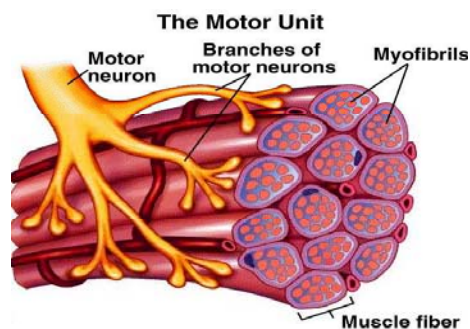


Fig. 1. Diagrammatic representation of the relationship between the motor neuron, muscle fibers, myofibrils and motor unit.

<http://academic.wsc.edu/faculty/jatodd1/351ch6outline.html>

The ability of individuals to achieve maximal voluntary activation of their muscles was first investigated in 1954 (29), and has received a lot of attention since (1, 2, 5, 12, 20, 33, 39). To be able to achieve maximal voluntary activation of a muscle one must be able to not only recruit all the motor units of the muscle, but also to drive them to maximal firing frequencies during a maximal voluntary isometric contraction. The extent to which a subject is able to voluntarily maximally activate his muscle can be assessed by the superimposition of electrical stimulation on maximal voluntary effort (1, 7).

The neural activation of the knee extensors and the effect of knee angle (muscle length) on activation are of interest. For instance, the extent to which individuals are able to maximally activate their muscles, may depend on knee angle

(3, 39), although at present there is no consensus (2, 10, 31). Accurately determining the level of maximal voluntary activation of the knee extensors at different knee angles is important in clinical practice (e.g. the detection of central vs. peripheral strength deficits in the elderly and patient groups), and in rehabilitation after for example knee injury (e.g. assessing knee angle - dependent differences in activation after anterior cruciate ligament rupture).

When investigating fatigue, knowledge of the maximal voluntary activation is also important. For instance, it is known that muscle fatigue is muscle length - dependent (21, 28, 32, 37) and also depends on the relative force level during muscle contractions (14). Therefore, equal relative contraction intensities, derived by normalizing to the maximal voluntary contraction (MVC) at each knee angle, are often used to investigate and compare muscle fatigue (endurance) at different knee angles (21, 32). Consequently, any differences in maximal voluntary activation during the MVC between knee angles will erroneously create differences in the relative contraction intensities when muscle fatigue at different knee angles is investigated. Everything else being equal, inadvertent differences between the relative contraction intensities between knee angles would make it relatively easier to contract at one knee angle compared to another.

In addition, there is evidence that activation of the muscle during fatiguing contractions changes in a length dependent manner. For example, muscle fibers may be more susceptible to activation failure at short vs. long muscle lengths (11, 16, 37). Furthermore, fatigue induced changes in surface EMG have also been shown to be length dependent (24). Finally, the voluntary drive from brain to muscle has been found to be suboptimal during fatigue (15, 26, 40). However, it is not clear whether this voluntary drive varies at different muscle lengths during fatigue.

Muscle structure and force production

Muscle structure and muscle length

Human skeletal muscle consists of muscle fibers that have a striated appearance when seen under a light microscope. This is the result of a series of light and dark bands that run perpendicular to the muscle fiber. Muscle fibers themselves are made

up of thick (myosin) and thin (actin) myofilaments organized into cylindrical bundles known as myofibrils (Fig. 1). It is the geometrical arrangement of the myofibrils that is responsible for the light and dark bands. The actin and myosin filaments are organized into repeated subunits along the length of the myofibril called sarcomeres, the active force - generating units within the muscle fiber. Sarcomeres generate force by the interaction of actin and myosin and are able to contract by the sliding of the actin and myosin filaments (22). The force generated by the sarcomeres is a function of the overlap of myosin and actin (17). As force of maximally active sarcomeres is determined by the degree of overlap between actin and myosin, sarcomere isometric force increases with an increase in length up to a plateau and then decreases at higher lengths (Fig. 2). By measurement of the force exerted by fully active sarcomeres at different known fixed lengths, the relationship between sarcomere force and length can be determined. As a consequence of the serial arrangement of sarcomeres in the myofibrils, muscle length and length range of active force production are in proportion to the number of sarcomeres arranged in series.

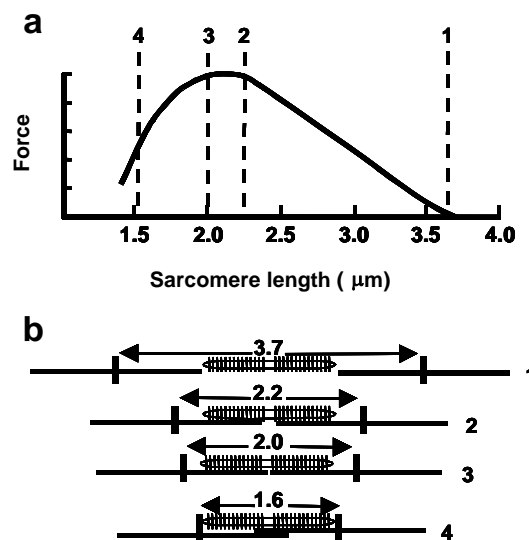


Fig. 2. Isometric force at different sarcomere lengths. a) force generated; b) arrangement of filaments at different lengths. Values are for frog muscle (redrawn Gordon et al. (17)). (Adapted from and reprinted from Jones et al. (23), with permission from Elsevier).

Force - generation

The excitation of a muscle, will lead to the shortening of the sarcomeres until active force exerted by cross - bridges connecting the actin and myosin filaments transmits this force through the tendon to the bone. But how does this force generation come about? A stimulus that is initiated in the brain can be transmitted through the central nervous system and, once the electrical signal reaches a motor neuron, the impulse is propagated along the motor neuron to the skeletal muscle fibers innervated by that particular motor neuron. To initiate the contraction of the muscle fiber, a stimulus must first enter the muscle fiber and excite the myofilaments (actin and myosin). This cause and effect relationship is called the excitation - contraction coupling. The membrane of the muscle fiber is an excitable membrane capable of generating and propagating an electrical signal (or action potential) from the motoneuron. Following an action potential, large amounts of stored calcium (Ca^{2+}) are released into the cytoplasm of the fiber. The released Ca^{2+} subsequently binds with a protein located on actin called troponin. By this binding action the troponin molecule changes its shape, thereby pulling another molecule called tropomyosin off actin. Normally the tropomyosin obstructs the binding sites for myosin on the actin filament; once calcium binds to troponin allowing tropomyosin to move, the binding sites are unblocked. Myosin binds to the newly uncovered binding sites on the actin filament forming the so - called cross bridge (Fig. 3). Subsequently, the myosin head releases inorganic phosphate allowing the head to rotate to a new angle so that the actin filament to which it is attached slides over the myosin filament toward the center of the sarcomere. Directly after this action, the myosin head is uncoupled from the actin filament and once more binds with ATP. Hydrolysis of ATP then enables the rotation of the myosin head back to its former position, this makes it ready to bind to actin again for reformation of the cross bridge. This sequence of events, from the formation of the cross bridge to the original position of the myosin head, is called a cross bridge cycle. General information concerning muscle contraction was obtained from Jones, Round and de Haan (23). The chemical and physical events occurring during the course of this cross bridge cycle are illustrated in figure 3.

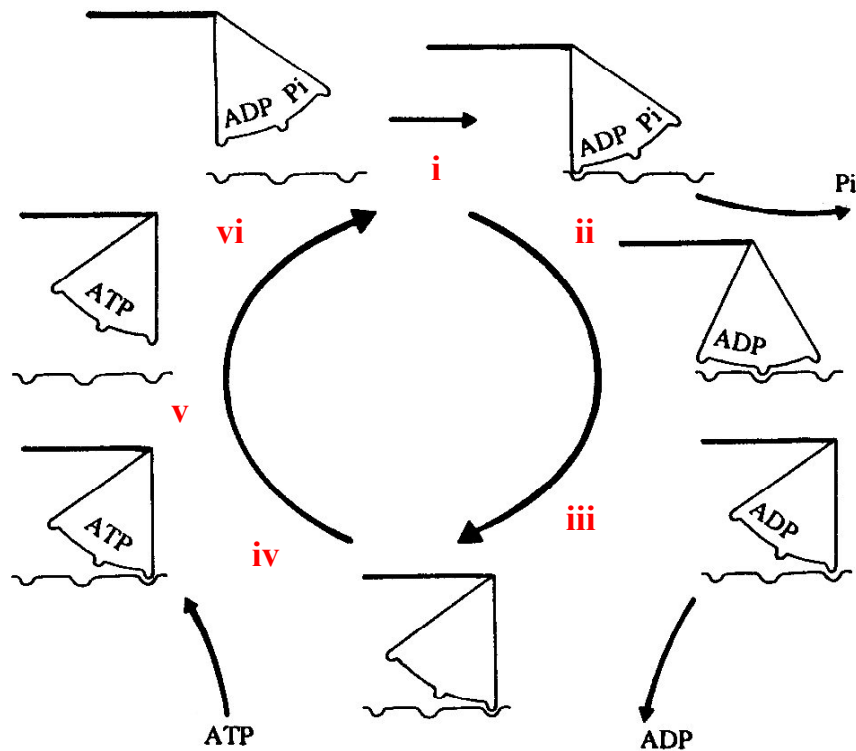


Fig. 3. Schematic overview of the cross - bridge cycle.

The first step in the cross bridge cycle consists of the attachment of actin and myosin (i). Subsequently, ATP is hydrolyzed into ADP and inorganic phosphate (P_i) that is released (ii) and this allows the myosin head to swivel to a new angle. After this event, ADP is released (iii) and ATP can bind to myosin (iv), resulting in the uncoupling of the myosin from the actin filament (v). Finally, hydrolysis of ATP results in the rotation back to the original position (vi).

(Adapted from and reprinted from Jones et al. (23), with permission from Elsevier).

Electromyography

Electromyography (EMG) is defined as the process of graphically recording the electrical activity of muscle (Encyclopedia Britannica). After the excitation of the muscle fiber by an action potential coming from the α - motorneuron, action potentials will travel in both directions along the membrane of the muscle fiber to the tendon, where they terminate. These action potentials introduce an electric current in the surrounding tissue. The electric potential, set up by this current field can be detected by the use of surface electrodes placed on the skin near the activated muscle fiber. The waveform that is picked up is called a muscle fiber action potential. The summation of the individual muscle fiber action potentials simultaneously excited by

the same α - motoneuron is called the motor unit action potential. After electrical stimulation of the nerve the sum of these motor unit action potentials can be recorded and is called the M - wave (Fig. 4). Since many muscle fibers from numerous motor units are located within a given recording area of the surface electrode, the EMG signal is a summation of all the motor unit potentials detected. The EMG signal is complicated as the summed action potentials vary in shape, size, and duration. With voluntary force increase more motor units are recruited and can be picked up by the surface electrodes. It is common practice to normalize the force (or torque) with respect to maximal isometric force that a subject can generate at the monitored joint (8). This is also common practice for EMG, where the EMG signal generated during a submaximal contraction is normalized to that obtained during an MVC. An example of a raw EMG signal during an MVC measurement is shown in figure 5.

In this thesis the measurement of surface EMG of the muscle will be used to study muscle activation during voluntary effort at different muscle lengths. Using EMG, information about muscle activation can be obtained in relation to changes in contractile properties of the human knee extensors operating at different lengths.

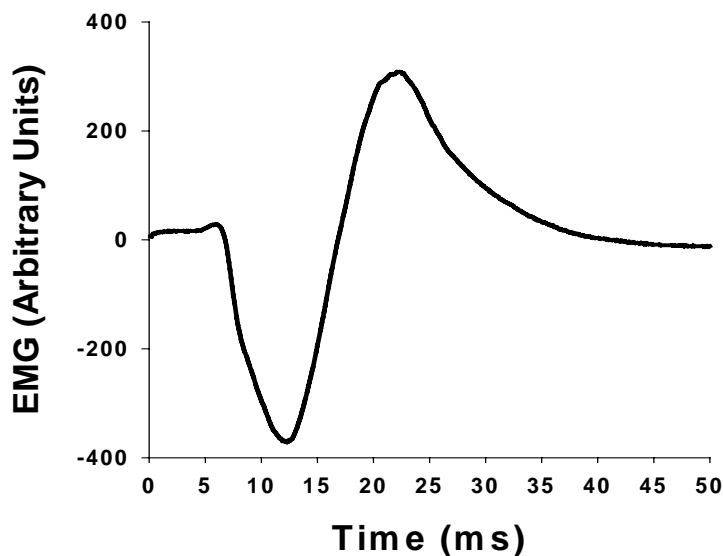


Fig. 4. Example of an M - wave.

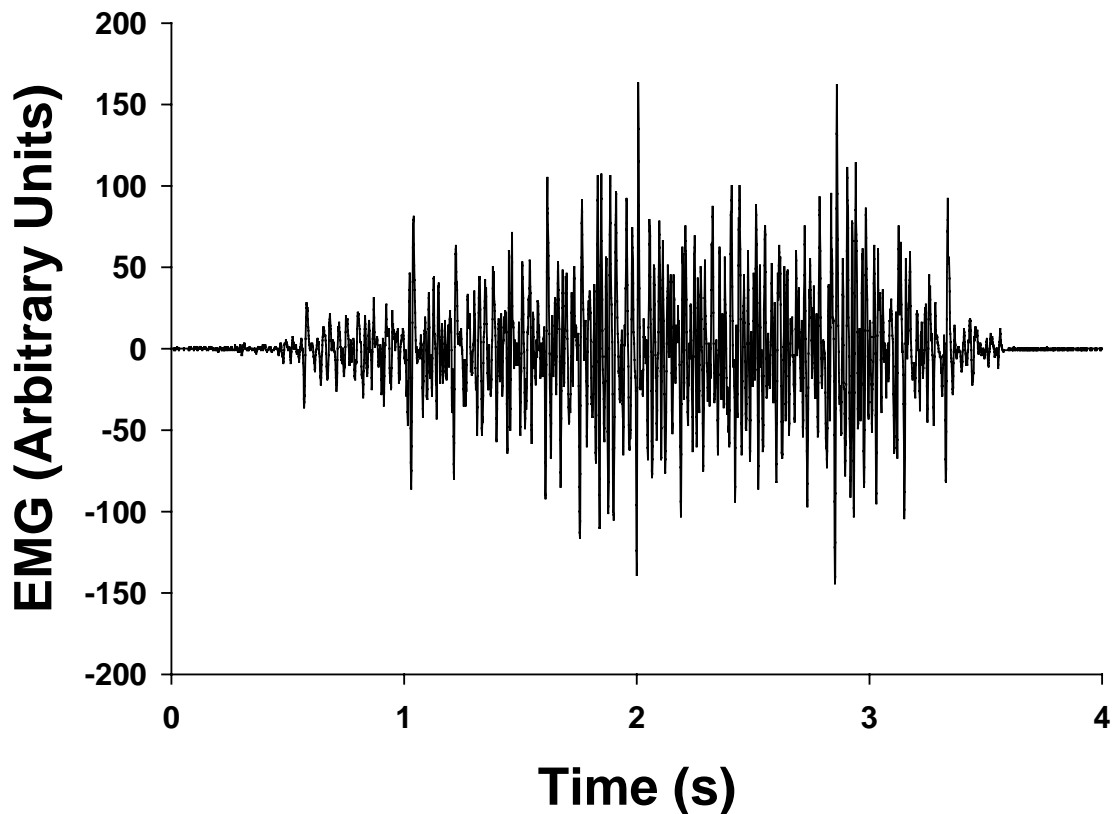


Fig. 5. Raw EMG signal obtained from the vastus lateralis muscle during a maximal voluntary contraction of the knee extensors.

Near - infrared spectroscopy

Near - infrared spectroscopy (NIRS) is an optical method that can be used for the non - invasive measurement of tissue oxygenation. It is based on the fact that near - infrared light can travel through human tissue. Near - infrared light that travels at different wavelengths is absorbed to a different degree by different chromophores. Haemoglobin, myoglobin, and to a lesser extent cytochrome oxidase are the most important chromophores absorbing near - infrared light in muscle tissue. The near - infrared spectrophotometer, as used in this thesis, generates light at two wavelengths, which travels through an optical fiber bundle called an optode. The near - infrared light penetrates the skin and is either scattered or absorbed within the tissue. Part of this scattered light is believed to describe a banana shape (6) and can be detected by a second optode also placed on the skin. By making use of the

different characteristics of near - infrared light at different wavelengths, it is possible to follow the concentration changes of the different chromophores beneath the skin. Tracking the changes in the chromophores of oxygenated and deoxygenated forms of myoglobin and haemoglobin allows us to gain information about tissue oxygenation (27).

Using NIRS, muscle oxygen consumption (mVO_2) measured under conditions of complete arterial occlusion, can be taken as a measure for energy consumption (9). In addition, it has previously been reported (18, 35) that mVO_2 increased significantly with force level (under occluded conditions) in each of the muscles investigated. Since it may be expected that for more force, more energy is required and mVO_2 has been shown to be a measure of energy consumption, this strongly suggests that mVO_2 is a good indicator of the number of force - generating cross bridges.

As mentioned previously, fatigue of the knee extensors is muscle length - dependent, and time to torque failure during submaximal isometric contractions to exhaustion is greater at extended vs. flexed knee angles (21, 32). Apparently, submaximal isometric contractions are less fatiguing at extended vs. flexed knee angles. By measuring mVO_2 at equal relative contraction intensities at different knee angles, insight may be gained in whether or not the metabolic cost of submaximal isometric contractions is less at extended compared to flexed knee angles. Using NIRS will therefore provide important extra information, and in addition the relationship between activation and energy consumption of skeletal muscle can be investigated by the simultaneous application of NIRS and EMG techniques at different muscle lengths.

Overview

During the dynamic contractions we perform daily, muscle length will change. Therefore, as an initial step, the effect of muscle length in relation to the activation of the muscle during isometric contractions will be studied. Furthermore, in every day life skeletal muscles usually operate under conditions of sub - maximal activation. Therefore, at first the emphasis in this thesis will be on submaximal isometric contractions.

The human m. quadriceps was chosen as a model in this thesis for several reasons. It is an important muscle group used for locomotor activity and its function has been extensively studied in relation to many research areas such as fatigue (32, 34), training (4, 36), age (38), and disuse (30). Furthermore, voluntary contractions allow full use of synergist muscles and those responsible for stabilization of proximal joints. These muscles are not stimulated when using artificial stimulation to investigate maximal voluntary activation. Since the m. quadriceps operates across the knee - joint with minimal influence from synergistic muscles, this muscle is a good model for the investigation of maximal voluntary activation with the use of superimposed electrical stimulation.

In this thesis the extent to which the central nervous system is able to adapt activation of the knee extensors to length - and fatigue induced changes of the muscles' contractile properties is investigated. Neural activation of the muscle will be studied with superimposed electrical stimulation and surface EMG; in addition the energy consumption of the superficial knee extensors will be measured using NIRS. The effects of muscle length and fatigue on the contractile properties of the muscle will be linked to (changes in) neural activation. Using the combination of methods described above insight can be gained concerning the underlying mechanisms that relate motor control during sub - maximal isometric contractions at different muscle lengths. The following specific questions will be addressed:

- What is the effect of knee angle (muscle length) on m. quadriceps femoris neural activation during submaximal fatiguing isometric contractions?
- How does the neural activation of the m. quadriceps femoris relate to the knee angle - dependent metabolic demand during isometric contractions?
- How tightly is neural activation coupled to the relative voluntary torque of the knee extensors?
- To what degree are differences in metabolic demand at different knee angles a consequence of differences in neural activation?

Aim and Outline

The general aim of this thesis was to study the influence of knee angle on the performance of the m. quadriceps. In **chapter 2** experiments were performed in which blood flow of the m. quadriceps was occluded and muscle activation was investigated using superimposed electrical stimulation during isometric contractions at different knee angles. This allowed insight into whether or not differences in muscle perfusion and central activation contribute to the greater endurance found at extended compared to flexed knee angles.

Previously, fatigability and $m\dot{V}O_2$ during sustained isometric contractions were found to be less at extended vs. flexed knee angles and, at low torques, less in the rectus femoris muscle than in the vastus lateralis and medialis (9). In **chapter 3** therefore $m\dot{V}O_2$ (measured using NIRS) and muscle activation (measured using surface EMG) of the superficial muscle heads of the m. quadriceps were examined at different knee angles, at several submaximal contraction intensities. The contribution of any knee angle - dependent difference in muscle activation to the knee angle - dependent difference in $m\dot{V}O_2$ could be determined. At the same time, at lower contraction intensities the contribution of any muscle - dependent differences in muscle activation to muscle - dependent differences in $m\dot{V}O_2$ could also be evaluated.

It is known that muscle fatigue depends on contraction intensity (13). Usually equal relative contraction intensities are used when investigating fatigue at different muscle lengths (21, 32). These relative intensities are generally obtained from an MVC that is dependent on the ability for maximal voluntary activation. Therefore, in **chapter 4** the relationship between voluntary activation and relative voluntary torque is evaluated at high contraction intensities ($> 70\%MVC$) in subjects capable of reaching very high levels of voluntary activation ($\sim 97\%$).

In **chapter 5** $m\dot{V}O_2$ was measured during maximal voluntary and electrically evoked quadriceps contractions at different knee angles. By using this approach, we were able to tease out to which degree $m\dot{V}O_2$ is lower at extended compared to flexed knee angles as a result of maximal voluntary activation and other processes

(e.g. potentiation) that may affect neural activation, and those which originate within the muscle. Finally, in **chapter 6** the main results are summarized and discussed.

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Muscle activation and blood flow do not explain the muscle - length dependent variation in quadriceps isometric endurance

Abstract

We investigated the role of central activation in muscle - length dependent endurance. Central activation ratio (CAR) and rectified surface EMG (rsEMG) were studied during fatigue of isometric contractions of the knee extensors at 30° and 90° knee angles (full extension = 0°). Subjects (n = 8) were tested on a custom - built ergometer. Maximal voluntary isometric knee extension with supramaximal superimposed burst stimulation (three 100 μ s pulses; 300 Hz), was performed to assess CAR and maximal torque capacity (MTC). Surface EMG signals were obtained from vastus lateralis (VL) and rectus femoris (RF) muscles. At each angle intermittent (15s on 6 off) isometric exercise at 50%MTC with superimposed stimulation was performed to exhaustion. During the fatigue task a sphygmomanometer cuff around the upper thigh ensured full occlusion (400 mmHg) of the blood supply to the knee extensors. At least two days separated fatigue tests. MTC was not different between knee angles (30°: 229.6 \pm 39.3 vs. 90°: 215.7 \pm 13.2Nm). Endurance times however, were significantly longer ($P < 0.05$) at 30° vs. 90° (87.8 \pm 18.7 vs. 54.9 \pm 12.1s, respectively) despite the CAR not differing between angles at torque failure (30°: 0.95 \pm 0.05 vs. 90°: 0.96 \pm 0.03) and full occlusion of blood supply to the knee extensors. Furthermore, rsEMG values of the VL (normalized to pre-fatigue maximum) were also similar at torque failure (30°: 56.5 \pm 12.5 vs. 90°: 58.3 \pm 15.2%), while RF EMG activity was lower at 30° (44.3 \pm 12.4%) vs. 90° (69.5 \pm 25.3%). We conclude that differences in endurance at different knee angles do not find their origin in differences in central activation and blood flow but may be a consequence of muscle - length related differences in metabolic cost.

Introduction

It has been established that knee extensor endurance during a single sustained isometric contraction to exhaustion, decreases with increasing muscle length, even at the same relative intensity of contraction (14, 22). A muscle - length dependent endurance is very relevant to everyday life because a whole range of muscle lengths are used during daily activities. The reason for this length dependent endurance remains unclear. In the past, this difference in endurance has been attributed to reduced energy requirements at shorter muscle length; because at shorter muscle length there would be a lower number of energy consuming cross - bridges and, hence, decreased metabolic product inhibition of force generation (11). However, other studies (5, 28) disproved this finding and stated that the metabolic cost of activity at different muscle lengths is the same. This would indicate that differences in energy requirement cannot explain the differences in muscle endurance.

Another explanation for the reduced endurance at greater muscle length could be a difference in the contribution of oxidative metabolism as a result of differences in muscle perfusion. Muscle perfusion can change with intramuscular pressure (29), which in turn is dependent on internal muscle force. Even though the same external torque is produced at different knee angles, each external torque can correspond to different internal muscle forces because knee moment arm changes with joint angle, as has been shown in the frog (21). In humans, there is no consensus concerning the degree of knee joint angle dependent changes in moment arm (13, 31) vs. (6, 19). This makes it difficult to investigate the potential effect of internal pressure related differences in muscle perfusion at different knee angles. However, by occluding the blood supply of the knee extensors the potential effect of knee angle dependent differences in muscle perfusion would be excluded as a possible explanation for differences in endurance. Obviously, if blood flow is fully occluded no differences in muscle perfusion will occur at different knee angles. Hisaeda et al. (14) applied arterial occlusion during sustained (1 - 2 min) isometric knee extensions yet still found significantly longer endurance times at shorter muscle lengths. However, it has been suggested by Quaresima and Ferrari (26) that occlusion in the study of

Hisaeda et al. (14) may have been incomplete due to insufficient cuff pressure (270 mmHg).

Muscle endurance may further be affected by muscle activation. Firstly, during brief maximal contractions, maximal muscle activation is seldom 100% (3); consequently the full muscle potential is not used. This should be considered during an endurance task where the exerted torque is a percentage of maximal torque capacity. Particularly, since maximal muscle activation is dependent on knee joint angle (33) and equal relative contraction intensities at different knee angles are compared. Secondly, potential knee angle dependent differences in voluntary drive, which may occur close to the point of exhaustion, may affect muscle endurance. Hunter and Enoka (15) for example conclude that muscle activation can limit the duration of submaximal fatiguing contractions of the elbow flexor muscles. However, although a large variation in knee extensor muscle activation at exhaustion between subjects (9, 18) has been found, the variation was unrelated to endurance time at the 90° knee angle (9). Nevertheless, differences in muscle activation could contribute to the knee angle dependent differences in endurance time and should be taken into consideration.

The main aim of this study was therefore to investigate the influence of neural activation on knee extensor endurance at different knee angles. The potential effect of knee angle dependent differences in muscle perfusion was eliminated by full occlusion of the blood supply during the submaximal isometric contraction. Because endurance time was expected to be longer at 30°, we hypothesized that muscle activation at torque failure would be closer to 100% at the 30° than at the 90° knee angle.

Methods

Subjects

Ten healthy male subjects (mean \pm SD age of 25.0 ± 3.7 years) volunteered to be subjects for this investigation. Before participation, each subject was thoroughly informed about the procedures and signed an informed consent. Two subjects were excluded since they did not meet the criteria for entry into our study (see

Experimental Procedures). The study was performed according to the Declaration of Helsinki and approved by the local ethics committee. Subjects did not perform any fatiguing exercise 48 hours prior to measurements.

Force recordings

Isometric force recordings were made for voluntary and electrically evoked contractions of the knee extensor muscles of the right leg. Subjects were seated in a custom - built ergometer with their hips at 70°; 0° = full extension. Shoulders, hips and lower thigh were strapped to the ergometer. The distal part of the shank was strapped to a force transducer, which was attached to the lever arm of the ergometer. A shinguard ensured subjects could exert maximal forces without discomfort at the shin. The backrest, force transducer height and its medio - lateral position were adjusted to enable precise alignment of the knee axis with the axis of rotation of the ergometer lever arm. A crank enabled changing of the knee angle of the subject. Despite fixation some change in knee angle during contraction was unavoidable. Knee angles were therefore measured with subjects delivering approximately 50% of maximal force; 0° = full knee extension. Pilot studies had shown that an increase from 50% to maximal force led to a change in knee angle of less than 2°. The distance from the knee axis to the center of the force transducer was determined for each individual to enable torque calculation. Real - time force applied to the force transducer was displayed on line on a computer monitor and digitally stored (1 kHz) on computer disc. The force signals were automatically corrected for gravity at each angle: the average force applied by the weight of the limb to the transducer during the first 50 ms after the start of a recording, with the subject seated in a relaxed manner, was set to zero force by the computer program.

Electrical stimulation

A cathode (self - adhesive stimulation electrode, 5 × 5 cm, Schwa - Medico, The Netherlands) was placed over the femoral nerve. The anode (13 × 8 cm) was placed over the gluteal fold. The quadriceps femoris muscle was stimulated transcutaneously with rectangular pulses of 100 μs using a computer - controlled constant current stimulator (Digitimer DS7H, Digitimer Ltd., Welwyn Garden City, UK). Stimulation current was increased until torque measured in response to a triplet

(three 100 μ s pulses applied at 300 Hz) leveled off at a 60° knee angle. The current was then increased by a further 50 mA to ensure supramaximal stimulation. In all subjects it was verified that supramaximal current at a 60° knee angle was supramaximal at 30° and 90° knee angles as well. The same stimulation current was used during twitch (single pulse) stimulation.

Experimental procedures

A priori it was considered possible that differences in absolute endurance time itself might contribute to potential differences in voluntary muscle activation during long lasting isometric contractions performed at different knee angles. Therefore, in the present study, subjects had to perform an additional task during which the relative load was increased in the more extended knee position in order to decrease the endurance time and to make it similar to the endurance time found with a more flexed knee.

The subjects visited our laboratory on five occasions with at least 2 days in between each session. During the three experimental sessions maximal torque capacity (MTC, see below) and central activation ratio (CAR, see below) were determined for each subject before, immediately after, and 10 min after the fatigue task (see below). The three different fatigue tasks were randomly assigned over the three experimental days. The first session was to familiarize the subjects with the set-up, the electrical stimulation and to determine the CAR and MTC of the knee extensors with superimposed electrical stimulation. The CAR was calculated using superimposed triplet stimulation during a maximal voluntary contraction (MVC) and calculated by dividing the torque prior to the delivery of the triplet by the maximum torque produced during the superimposition of the triplet (20). Subjects were excluded from further participation if they were unable to achieve a CAR greater than 0.9 at 30° and 90° knee angles.

Furthermore, intermittent isometric torque generation was practiced at either 50%MTC at a 30° (3 subjects) or 90° (3 subjects) knee angle or at 65%MTC at the 30° (2 subjects) knee angle. Pilot studies had indicated that an intensity of 65%MTC at the 30° knee angle resulted in an endurance time comparable to a 50%MTC intensity level at the 90° knee angle. During each following session the intermittent torque

delivery was maintained up to torque failure at one randomly chosen angle and torque level. The second session was not used for analysis to exclude learning effects. To reiterate, each subject performed three fatigue tasks that were included in the analysis: 30°(50%), 90°(50%) and 30°(65%), each on a separate day. Each session lasted 1.5 - 2 hours in total.

Maximal voluntary contraction (MVC) and Maximal torque capacity (MTC)

In each session MVC torque and MTC were determined at three knee angles (30°, 60° and 90°). Subjects were asked to maximally generate isometric torques for about 3 - 4s to determine MVC extension and flexion torque (flexion torque was used for normalizing the antagonist EMG from the biceps femoris). Two to four attempts were made separated by three minutes rest. MVC torque was determined as the peak force from the stable part of the force signal multiplied by the subjects' moment arm. Real - time force was visible on a computer monitor and subjects were vigorously encouraged to exceed their maximal value, which was also displayed. MVC torque was taken as the highest value, which did not exceed preceding attempts by more than 5%, allowing a maximum of 4 attempts. Next, MTC of the quadriceps femoris muscle was determined using electrical stimulation. A superimposed measurement consisting of a triplet applied to a fully relaxed muscle and another triplet superimposed on an MVC was used to determine MTC. The MTC of the muscle was calculated using the following formula:

$$\text{MTC} = \frac{c}{1 - (a/b)} \quad (10)$$

Where (a) is the torque increment due to the superimposed triplet, (b) is the torque obtained when the triplet is applied to the resting muscle, and (c) is the maximal voluntary extension torque just prior to the effect of the superimposed triplet.

If the torque reached just before superimposed stimulation was below 90% of MVC torque more attempts were made with a maximum of 4 attempts. This was done to make the calculation of MTC as accurate as possible. For statistical analysis, in each session, the attempt was used where the highest voluntary torque before the

superimposed triplet was reached. In instances where MVC torque exceeded the calculated value of MTC, MVC torque was taken as MTC.

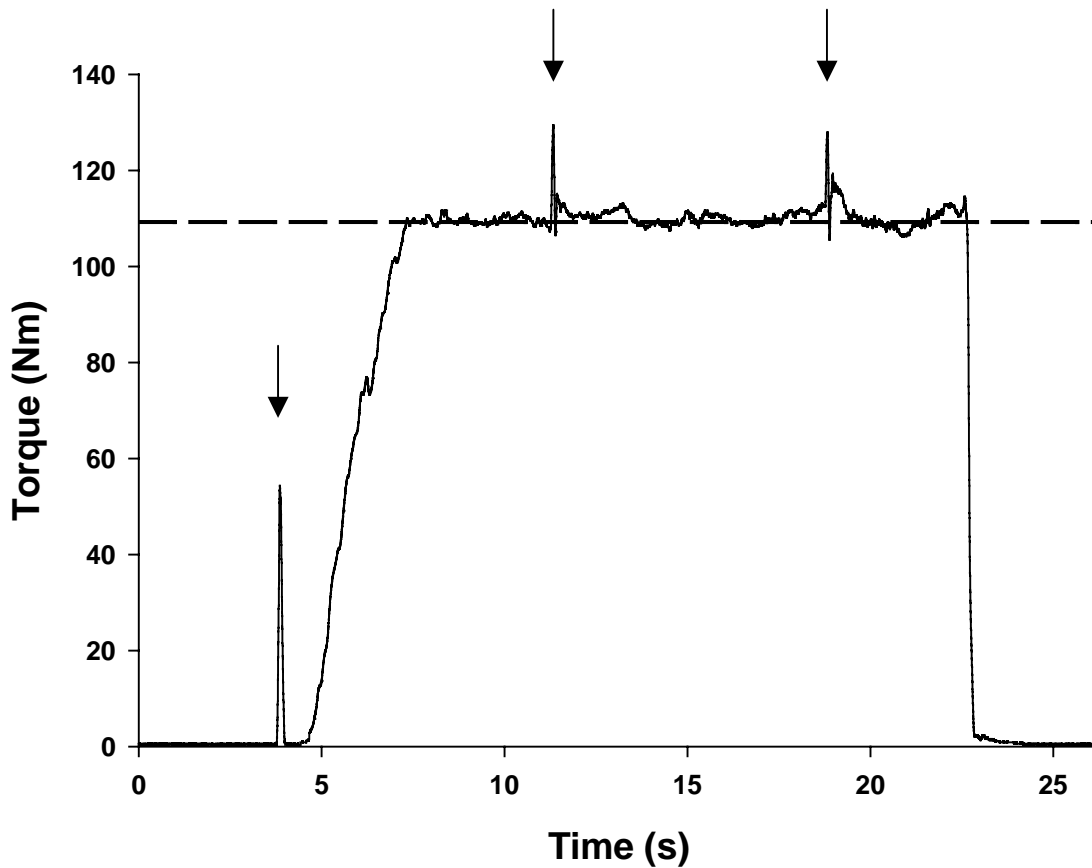


Fig. 1. Typical example of one cycle of the fatigue task. The dashed line denotes the target torque; arrows indicate the timing of the triplets on the relaxed and contracted muscle.

Fatigue task and post - exercise measurements

Subjects were required to perform a fatigue task at either a 30° or 90° knee angle. Prior to this fatigue task blood flow to the right leg was occluded using a sphygmomanometer cuff (400 mmHg) that was placed around the upper thigh as proximal as possible. The fatigue task consisted of the following cycle which was repeated until torque failure: 4.5s of rest were followed by a ~ 3s ramp up phase to either 50% or 65%MTC which was held for 15s (Fig. 1). Exerted and target force were displayed on line on a monitor and subjects were instructed to match exerted with target force. When the target level could no longer be sustained despite vigorous encouragement the test was terminated. The exact end of the fatigue task, torque

failure, was defined afterwards as the point when the exerted torque decreased below 90% of the target level for more than 2s. Endurance time was defined as time spent at target torque. A triplet was applied to the fully relaxed muscle after 3.75s of rest and subsequently every 7.5s for the duration of the fatigue task (Fig. 1). The central activation ratio (CAR) was used to quantify the level of neural activation during the course of the fatigue task. The triplets applied to the relaxed muscle during the fatigue task were used as a measure of muscular fatigue that is independent of voluntary muscle activation. Within 5s after the fatigue task was finished MTC and CAR were determined after which the cuff was deflated.

After 10 minutes MTC and CAR were determined at 30° and 90° knee angles (with 3 min rest in between). Values recorded at this point were normalized to pre-fatigue values to determine the level of recovery.

Surface Electromyography

Electromyographic activity of the vastus lateralis (VL), rectus femoris (RF) and biceps femoris (BF) muscle was recorded using surface EMG electrodes (Ø 17 mm, F - 454AE, Medeq, Gnosis GmbH, Austria). After shaving, roughening and cleansing the skin with 70% ethanol, electrodes were placed on the muscle belly in a bi - polar configuration, parallel to the muscle fiber direction, with an inter - electrode distance of 40 mm. Pairs of electrodes were placed on the RF muscle halfway between the anterior spina iliaca superior and the superior border of the patella. Another pair was placed on the VL muscle two - thirds of the distance between the anterior spina iliaca superior and the lateral side of the patella. Electrode placement for the BF muscle was halfway between the ischial tuberosity and fibula head. Reference electrodes were placed on the left and right patella and also on the right condylus medialis. The locations of all electrodes were marked with a waterproof felt tip pen on the first visit to enable precise electrode application in the remaining sessions. Surface EMG signals were amplified (x 100), digitized (1 kHz), except superimposed measurements (10 kHz) and stored with the force signal on computer disc. All EMG signals were band - pass filtered (10 - 400 Hz). Rectified surface EMG amplitude (rsEMG) was calculated for the VL, RF and BF (VLrsEMG, RFrsEMG and BFrsEMG) for 500 ms segments, during the fatigue task for 5s segments. Segments including

superimposed electrical stimulation were excluded from analysis. Pre - fatigue MVC extension and flexion maximal rsEMG values were set at 100%.

Peak - to - peak M - wave amplitude in response to twitch stimulation at the end of the fatigue task was normalized with respect to pre - fatigue values to check for neuromuscular transmission failure.

Statistics

All results are presented as mean \pm SD. Data were analyzed by means of repeated - measures analysis of variance where appropriate. If significant main effects were observed, Bonferroni tests were performed for post hoc analysis. The level of significance of all statistical analyses was set at $P < 0.05$.

Results

During the familiarization session two subjects had a CAR smaller than 0.9 (0.62 and 0.67) during MVC. They were therefore excluded from further participation and the results of 8 subjects are presented.

Pre - fatigue

MTC and triplet torque recorded at the 60° knee angle were significantly greater than their respective values at the 30° and 90° knee angles, ($P < 0.05$; Table 1). In addition, MTC and triplet torque at the 30° knee angle were not significantly different from their respective values at the 90° knee angle.

Fatigue

As expected, endurance time was significantly longer for 30°(50%) compared to 90°(50%), (Fig. 2). Since endurance times for 90°(50%) and 30°(65%) were not significantly different from each other we succeeded in matching endurance times between the 30° and 90° knee angles. In addition, the endurance time for 30°(50%) was significantly longer than 30°(65%).

The CAR was significantly higher at the end compared to the start of the fatigue task, (Fig. 3). Moreover, as was expected, at the start of the fatigue task the CAR at 30°(65%) was significantly higher than at 90°(50%) and 30°(50%). At the end of the fatigue task no differences in CAR existed among protocol types indicating that central activation was similar at torque failure among protocols.

Table 1. Maximal torque capacity and triplet torque in the pre - fatigue state

| | Knee angle | | |
|-----------------------|---------------|--------------|----------------|
| | 30° | 60° | 90° |
| MTC, N · m | 210.4 ± 35.2* | 303.0 ± 42.8 | 205.94 ± 18.5* |
| Triplet torque, N · m | 76.3 ± 8.9† | 91.5 ± 13.1 | 79.9 ± 11.2† |

Values are means ± SD; n = 8. MTC, Maximal torque capacity. * Significantly different from MTC at 60° (P < 0.05). † Significantly different from triplet torque at 60° (P < 0.05).

As was the case with the CAR no differences in normalized VLrsEMG values existed at torque failure. Although normalized VLrsEMG values did not significantly increase from the start to the end of the fatigue task a trend in that direction was observed, (P = 0.055; Fig. 4A). The lack of significance was probably caused by the 30°(65%) protocol showing no difference across time (Bonferroni post - hoc test). As expected, at the start of the 30°(65%) protocol, normalized VLrsEMG values were significantly higher than those of the 30°(50%) and 90°(50%) protocol.

There was a significant increase in normalized RFrsEMG values from the start to the end of the fatigue task, (Fig. 4B). Normalized RFrsEMG values at the start of the 30°(50%) protocol were significantly lower compared to the 90°(50%) and 30°(65%) protocol. At the end of the fatigue task normalized RFrsEMG was lower for the 30°(50%) protocol compared to the 90°(50%) protocol.

Normalized BFrsEMG values were low for all protocols and did not change significantly from the start to the end of the fatigue task, at the 30° and 90° knee angles their respective values were 7.9 ± 7.8% and 7.5 ± 4.5%. This indicates that co - activation was not an important factor during the course of the fatigue task.

No differences in triplet torque at torque failure, normalized to maximal triplet torque, were found between 30°(50%) and 90°(50%), 47.4 ± 14.6% and 34.5 ± 17.7%, respectively. The triplet torque at 30°(65%), 56.4 ± 17.4%, was significantly higher than 90°(50%) only. These findings indicate that, at torque failure, fatigue of the knee extensor muscles was rather similar among the protocols.

Post - exercise with occluded blood - flow

As expected from the different exercise intensities during the task, post - exercise MVC levels were significantly higher for the 30°(65%) protocol, $69.8 \pm 11.7\%$, compared to the 30°(50%) and 90°(50%) protocol, $54.7 \pm 9.5\%$ and $47.0 \pm 8.1\%$, respectively.

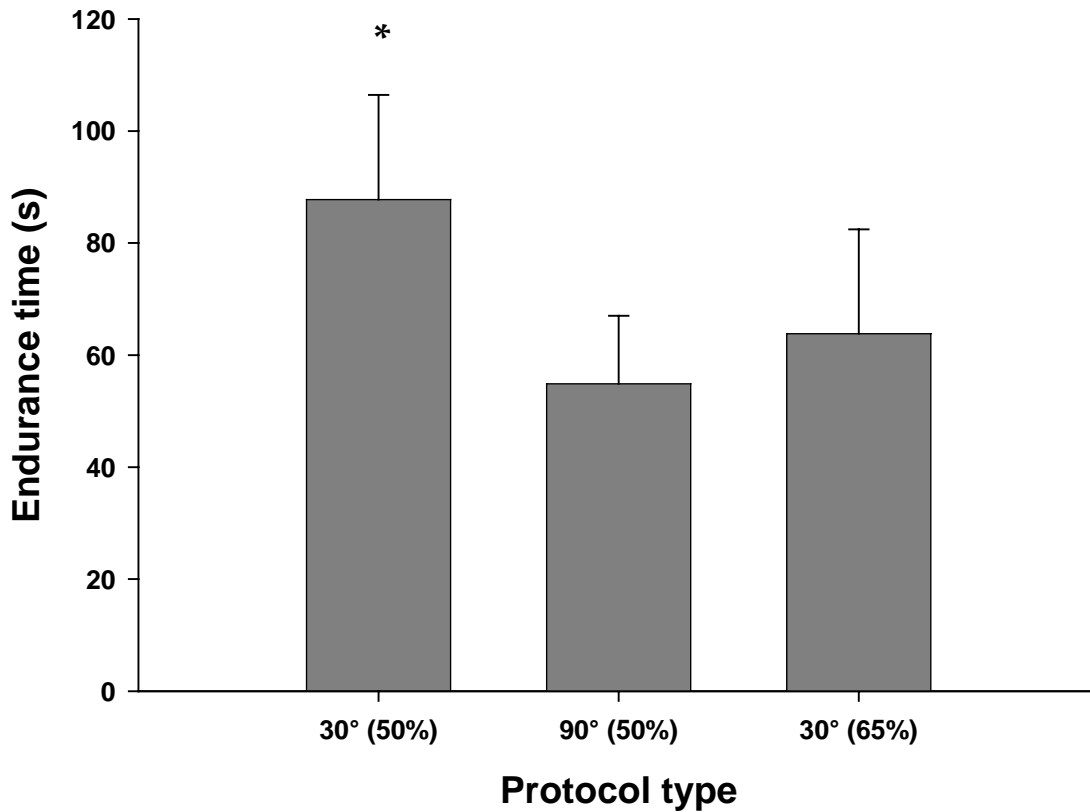


Fig. 2. Endurance times for three different protocol types used: Intermittent isometric exercise was performed at 50% of the maximal torque capacity at 30° [(30°(50%)] and 90° [(90°(50%)] knee angles, and at 65% at the 30° [(30° (65%)] knee angle. Values are mean + SD; n = 8. *Significantly greater than 90°(50%) and 30°(65%) ($P < 0.05$).

During MVC, the post - exercise CAR [0.97 ± 0.05 , 0.99 ± 0.03 , and 0.97 ± 0.02 for 30°(50%), 90°(50%), and 30°(65%), respectively] was similar ($P > 0.05$) compared to the pre - fatigue CAR [0.94 ± 0.05 , 0.98 ± 0.02 , and 0.94 ± 0.07 for 30°(50%), 90°(50%), and 30°(65%), respectively].

VLrsEMG values during MVC post - exercise were not significantly different from pre - exercise (Fig. 5). RFrsEMG values however, were significantly lower

compared to pre - fatigue values. There were no significant differences among protocols. Note that post - exercise rsEMG values were significantly higher than rsEMG values at torque failure for both VL and RF muscles (Fig. 4 and Fig 5). Thus, despite the ongoing occlusion, maximal rsEMG increased within several seconds following exercise, but this did not result in an increase of maximal torque.

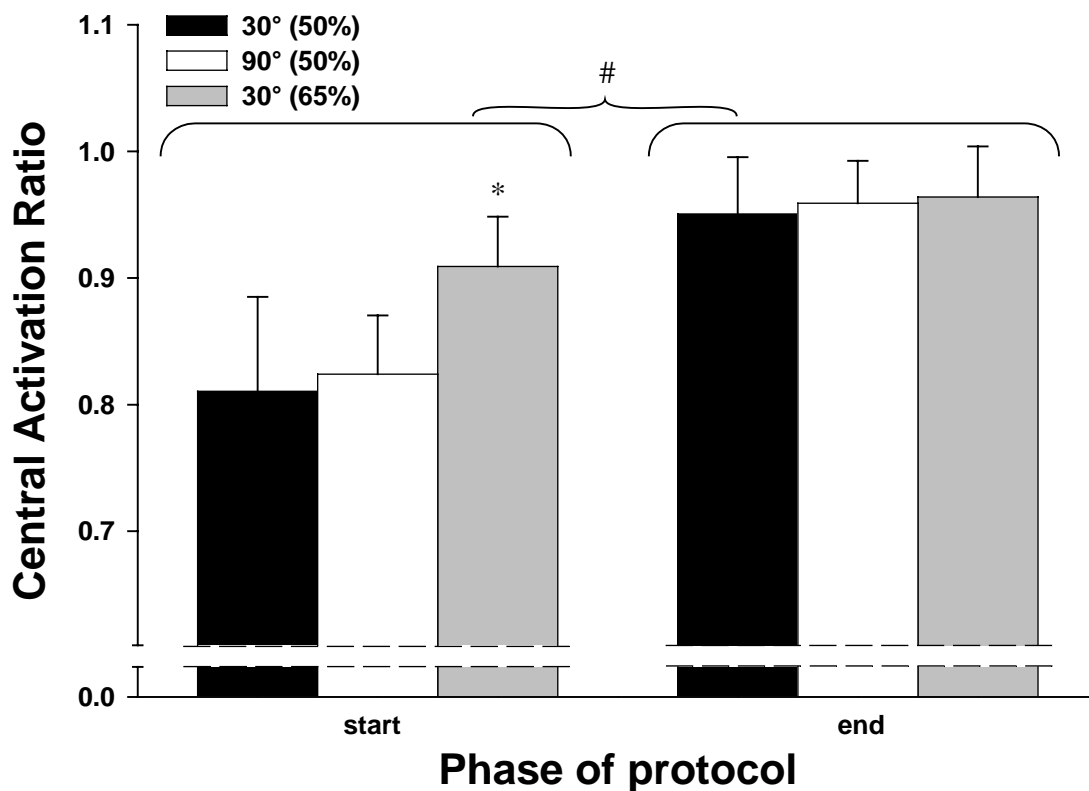


Fig. 3. Central activation ratio (CAR) at the start and end of the fatigue task. Black bars, 30°(50%); white bars, 90°(50%), gray bars 30°(65%). Values are mean + SD; n = 8. *Significantly higher than 30°(50%) and 90°(50%) at the start of the fatigue task ($P < 0.05$). #Significantly higher at torque failure compared with the start of the fatigue task.

Post - exercise normalized M - wave amplitude was not significantly different from pre - exercise values: 92.3 ± 19.3 , 95.5 ± 24.6 , and $90.4 \pm 14.2\%$ for 30°(50%), 90°(50%), and 30°(65%), respectively. This demonstrates that at torque failure, with complete occlusion of blood flow to the knee extensors, neuromuscular transmission remained intact.

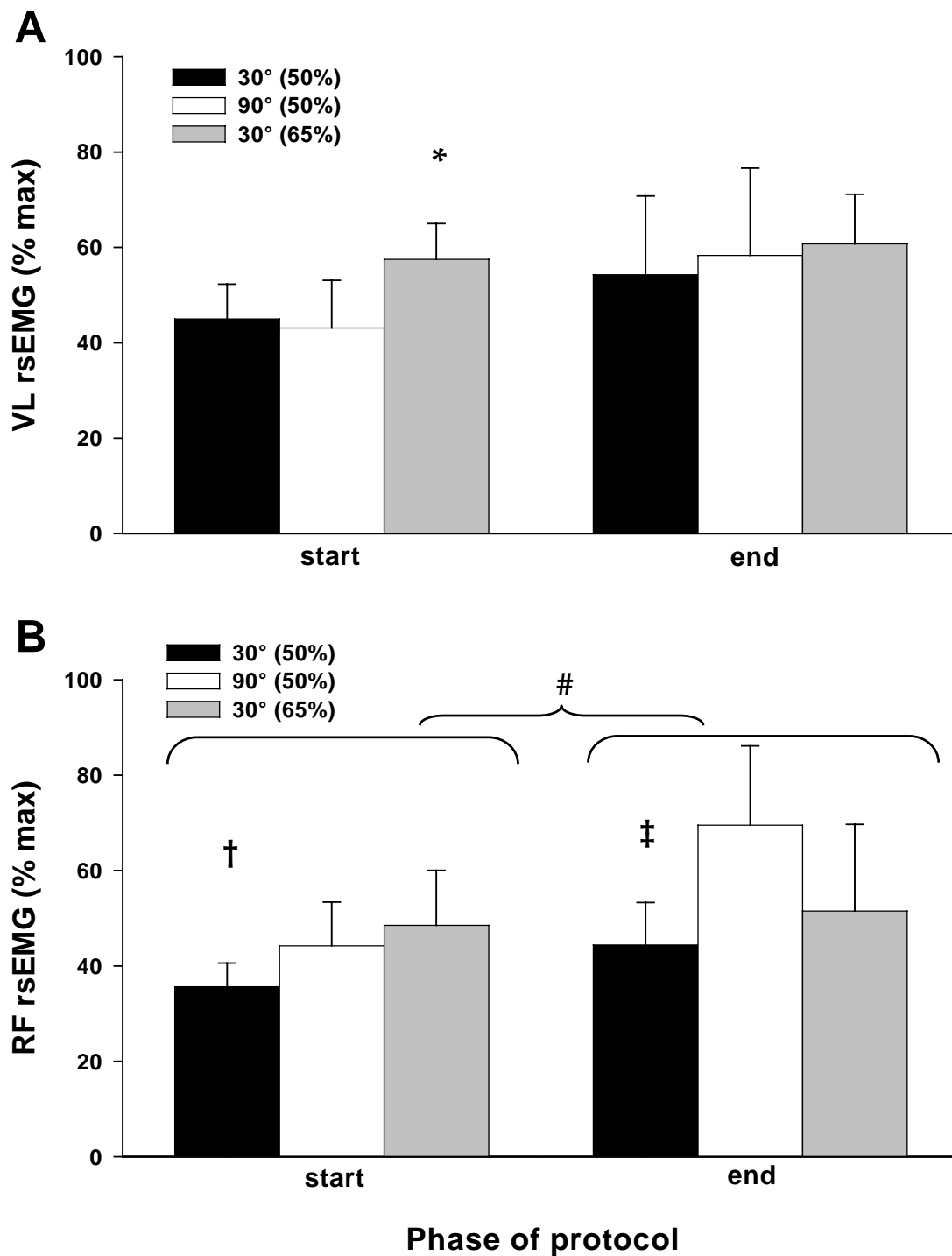


Fig. 4. Vastus lateralis (VL) rectified surface electromyogram (rsEMG) (A) and rectus femoris (RF) rsEMG (B) at the start (first 5s) and end (last 5s) of the fatigue task. Values are normalized to peak rsEMG obtained during pre - fatigue maximal voluntary contraction (MVC). Black bars, 30°(50%); white bars, 90°(50%); gray bars 30°(65%). Values are means + SD; n = 8. *Significantly higher ($P < 0.05$) than 30°(50%) and 90°(50%) at the start of the fatigue task ($P < 0.05$). #Significantly higher at end than at start. †Significantly lower than 90°(50%) and 30°(65%) at the start of the fatigue task. ‡Significantly lower than 90°(50%) at the end of the fatigue task.

Recovery

Ten minutes after deflation of the cuff, triplet torque was not significantly different from pre - exercise values. They had respectively recovered to $94.1 \pm 4.5\%$; $101.8 \pm 7.4\%$, and $99.6 \pm 5.9\%$ for the $30^\circ(50\%)$, $90^\circ(50\%)$, and $30^\circ(65\%)$ task. MTC was marginally, but significantly, lower after 10 minutes of recovery compared to the pre - exercise values: $96.8 \pm 4.7\%$; $95.5 \pm 2.7\%$, and $97.9 \pm 2.9\%$ for $30^\circ(50\%)$, $90^\circ(50\%)$, and $30^\circ(65\%)$, respectively. There were no significant differences among protocols.

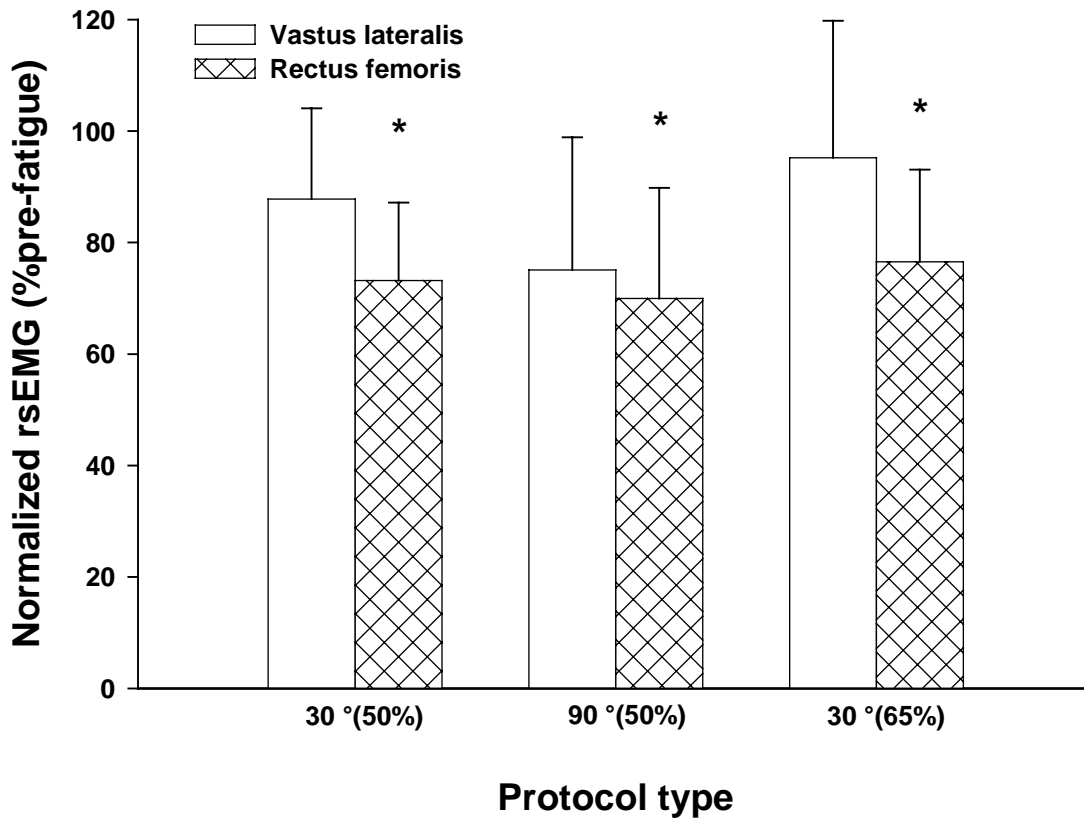


Fig. 5. Post - exercise (blood flow still occluded) vastus lateralis rsEMG (white bars) and rectus femoris rsEMG (hatched bars) during MVC. Values are mean + SD and are normalized to peak rsEMG obtained during pre - fatigue MVCs. *Significantly lower than pre - fatigue value (P < 0.05).

Discussion

In the present study, isometric exercise was sustained for longer at a 30° compared to a 90° knee angle. This occurred despite the exercise being performed at equal relative

intensity levels, similar high levels of muscle activation at torque failure, and full occlusion of the blood supply to the knee extensors. Thus, the main finding of the present study was that we were able to exclude the effects of muscle perfusion, and neural activation on knee angle dependent endurance time.

Neural Activation

The prolonged endurance times for the 30° compared to the 90° knee angle found in the present study correspond well to other studies (14, 22). This difference in endurance time between knee angles does not appear to originate from any differences in intensity at the start of the fatigue task. For both knee angles, at the start of the fatigue task, the equal relative intensity and similar torque levels were reflected by similar CAR and VLrsEMG levels (Fig. 3, 4A). At the 90° knee angle, EMG activity of the VL and RF muscle at 50% MTC corresponds well to other literature (2, 25, 27). (Note that these studies did not use external occlusion. However, since RMS values remained unaffected by the use of a cuff at different knee angles and contraction intensities in pilot studies, we felt it reasonable to compare our results to these studies.) The RFrsEMG levels were lower at the 30° compared to the 90° knee angle at the start of the fatigue task. This could indicate that exercise for the RF was less demanding at the 30° compared to the 90° knee angle. However, since the RF represents only ~ 10% of the total knee extensor volume (34), its influence on knee extensor external torque, and endurance time, will probably be rather small.

A potential limitation of our study was that we did not measure the EMG activity of the vastus medialis muscle, nor is it possible to record surface EMG of the vastus intermedius muscle. Consequently, we cannot exclude preferential activation among the different heads of the quadriceps at different joint angles. However, since the degrees of freedom of the knee joint impose a restriction to extension and flexion, the task was straightforward in nature (isometric knee extension). Thus, it is doubtful that at relatively high force levels the mono-articular head of the vastus lateralis would differ from the vastus medialis. In addition, Weir et al. (35) found no difference in surface EMG of the vastus lateralis and medialis muscle across different joint angles (15°, 45° and 75°). Moreover, at torque failure, CAR values were very high (mean > 0.95) indicating that the force measured of the entire quadriceps was

near maximal thereby excluding a large variation in activation of the separate muscle heads.

It has been suggested that muscle endurance could be limited by the neural activation of muscle (15). In the present study, the high and similar CAR values (mean > 0.95) among knee angles at torque failure indicate that at both knee angles the central nervous system seemed capable of maximally activating the motor neurons supplying the muscle. Evidently, differences in endurance between knee angles are not attributable to differences in central activation. In addition, at torque failure, triplets applied to the relaxed muscle were similar, indicating comparable levels of fatigue at the level of the contractile elements, independent of voluntary drive, at both knee angles.

A priori it was considered possible that a difference in endurance time between long lasting isometric contractions at the 30° and 90° knee angle could contribute to potential differences in muscle activation between the knee angles. In retrospect this seems no longer relevant since at torque failure CAR for the 30°(50%) and 90°(50%) protocol were similar. Nevertheless, it is remarkable that at the 30° knee angle relative torque production had to be increased to 65% MTC (70% is probably an even more accurate percentage) in order for isometric endurance time to become similar to that obtained at 50% of MTC at the 90° knee angle.

A potential error may have occurred in determining the MTC as we assumed a linear relationship to exist between voluntary and superimposed torque. However, since subjects were only allowed to participate if their CAR values exceeded 0.9, any error made in determining the muscles' maximal potential will be minimal. Moreover, it is highly doubtful that the minor error in determining MTC would be knee angle dependent and could therefore account for the large difference in isometric endurance time between the 30°(50%) and 90°(50%) protocol.

VLrsEMG levels were not different between knee angles at torque failure (Fig 4A). The fact that normalized VLrsEMG values failed to reach pre - fatigue maximal values (58.5%, mean of all protocols) could not be ascribed to neuromuscular transmission failure, as the post - exercise M - wave peak to peak value remained high (mean > 92% of pre - fatigue value). The failure of EMG to reach 100% of the pre

- fatigue maximal values, during sub - maximal isometric contractions, has been reported previously (9, 12, 27) and has, in part, been attributed to the cancellation of the interference EMG from overlapping positive and negative phases of action potentials (7).

At torque failure RF surface EMG activity was significantly higher at the 90° compared to the 30° knee angle. This could indicate that neural activation of the fatigued RF muscle at the 90° knee angle was higher, which may reflect a neurophysiological mechanism partly compensating for a mechanical disadvantage (e.g. moment arm, relative position on the muscle length - tension relation) of the RF compared to the VL muscle. However, this remains highly speculative. We applied superimposed electrical nerve stimulation to obtain activation levels of the entire muscle group, but it is not possible to check for potential differences in activation among the different heads of the quadriceps muscle with this technique.

Post - exercise rsEMG values of the VL and RF muscle, recorded during MVC less than 5s after torque failure but with the blood flow to the muscle still occluded, were significantly higher compared to the values at torque failure for all protocols (Fig. 4, 5). In our study, this 'recovery' of EMG occurred without any recovery of torque. Torque recovery was not expected since blood flow was occluded and voluntary activation at torque failure was close to maximal. How can this clear decoupling of EMG and torque be explained? During exercise considerable ion fluxes occur across the sarcolemmal membrane, which may result in high concentrations of potassium in the interfiber spaces (30). Fitch and McComas (11) have suggested that as a result of ionic imbalance across the T - tubular membrane, a breakdown in the excitation - contraction coupling could occur causing failure of inwardly propagated action potentials. Sacco et al. (28) have proposed that a brief respite from contraction, which occurred in our study, could restore a previously defective process of activation. However, in the latter study, and in contrast to the present study, this coincided with force recovery (28). Moreover, and also in contrast to the study of Sacco et al. (28), who electrically stimulated the muscle, neuromuscular transmission failure did not occur in the present study since post - exercise M - wave peak - to - peak values remained high (mean > 92% of pre - fatigue value). Several decades ago

it was suggested that the output of the central nervous system is optimized to the slowing of the muscle contractile speed during fatigue. This so - called 'muscle - wisdom' implies that motor unit firing rate can decrease during a sustained MVC, without contributing to the decline in force output. The present observation of an increase in maximal surface EMG a few seconds following torque failure, during blood flow occlusion, without a concomitant increase of torque therefore may indicate that at torque failure activation was optimized to the slowed contractile properties of the muscle. Indeed, superimposed electrical stimulation indicated that muscle activation was close to maximal at that time. Therefore it is possible that the higher electrical activity (EMG) of the muscle fibers a few seconds following torque failure did not lead to an increase of isometric torque, thereby accounting for the dissociation of the EMG activity and contractile capability of the muscle.

Energy metabolism

The greater endurance at the 30° compared to the 90° knee angle could arise as the result of differences in 1) availability of oxygen for aerobic metabolism or in 2) energy requirement. The extent of muscle perfusion (i.e. oxygenation), which has been shown to cause differences in endurance (23), is an important parameter to take into account. Muscle perfusion can change with intramuscular pressure (29), which in turn may depend on internal muscle force or muscle morphology (16), both of which are knee angle dependent. In the present study, by occluding the blood supply to the knee extensors during the contractions, possible differences in muscle perfusion at different knee angles were eliminated and consequently cannot have influenced endurance time.

It has also been proposed that muscle endurance is related to the number of force producing cross - bridges and its corresponding energy consumption (11). Hence, muscle endurance would depend on actin - myosin filament overlap and fatigue would be greatest at optimum length. Support for this view has been found in frog muscle where a reduced energy cost of contraction has been shown at short compared to optimum length (1, 4, 17). In isolated mammalian muscle however, energy consumption did not change from short to optimum length (24, 32). In contrast, de Haan et al. (8) showed lower energy rich phosphate consumption at high

and low muscle lengths compared to optimum. Baker et al. (5) and Sacco et al. (28), using nuclear magnetic resonance spectroscopy, reported similar rates of ATP use at short and optimum length of the tibialis anterior muscle in humans during contraction. This suggests that the lower fatigability at shorter muscle lengths, such as found in the present study, cannot be explained by differences in energy consumption. However, in the present study voluntary activation at torque failure was close to maximal and there was no indication of neuromuscular transmission failure. Moreover, post - exercise triplet torque was similar among knee angles, which suggests similar levels of fatigue of the contractile elements at both knee angles. Yet, endurance was higher at the 30° compared to the 90° knee angle despite full occlusion of the blood supply to the knee extensors. It therefore seems that isometric exercise is less strenuous at shorter compared to longer muscle lengths. This is supported further by the increase of torque intensity from 50% to 65% MTC that was required to match the endurance time at the 30° to the 90° knee angle in the present study. Moreover, during a long - lasting maximal voluntary contraction, torque declined less steeply at the 30° than the 90° knee angle (unpublished observations). Together these findings do suggest that isometric exercise is energetically less demanding at the 30° compared to the 90° knee angle.

In conclusion, the present findings show that muscle oxygenation and neural activation can be excluded as causes of knee angle dependent differences in muscle endurance. It remains to be shown whether (sub -) maximal isometric exercise is energetically less demanding at extended knee angles.

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**Knee extensor muscle oxygen consumption in relation
to muscle activation**

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Abstract

Recently, fatigability and muscle oxygen consumption (mVO_2) during sustained isometric contractions were found to be less at shorter (30° knee angle; 0° = full extension) compared to longer knee extensor muscle lengths (90°) and, at low torques, less in the rectus femoris (RF) muscle than in the vastus lateralis and medialis. In the present study we hypothesized that these findings could be accounted for by a knee angle - and a muscle - dependent activation respectively. On two experimental days, rectified surface EMG (rsEMG) was obtained as a measure of muscle activation in nine healthy young males. In addition, on day 1 maximal torque capacity (MTC) was carefully determined using superimposed nerve stimulation on brief high intensity contractions ($> 70\%MVC$) at 30 , 60 and 90° knee angles. On day 2, subjects performed longer lasting isometric contractions [$10 - 70\%MTC$] while mVO_2 was measured using near - infrared spectroscopy (NIRS). Maximal mVO_2 was reached significantly ($P < 0.05$) later ($11.0s \pm 6.5s$) at 30° compared to 60 and 90° knee angles indicating a slower increase in mVO_2 . Furthermore, mVO_2 at 30° was significantly less ($57.9 \pm 8.3\%$; average \pm SD, across intensities and muscles) compared to 60 and 90° knee angles. Whereas rsEMG was on average only $18.0 \pm 11.8\%$ ($P = 0.062$) less at the start of the contraction at 30° . At $10\%MTC$ at all knee angles, maximal mVO_2 of the RF occurred significantly later ($28.8 \pm 36.0s$) and showed a significantly smaller increase in rsEMG compared to both vasti. In conclusion, it is unlikely that the tendency for less intense muscle activation could fully account for the $\sim 60\%$ lower oxygen consumption at 30° , but the later increase in RF mVO_2 seemed to be caused by a less strong activation of the RF.

Introduction

During daily activities different muscles are used over a whole range of muscle lengths. When activating skeletal muscle, work is performed by the muscle and concomitantly it consumes oxygen. Thus, it is very relevant to investigate both muscle activation and muscle oxygen consumption during *in vivo* muscle activity at different muscle lengths. Knee extensor endurance is greater at extended (short muscle length) compared to flexed (long muscle length) knee angles during sustained isometric contractions to exhaustion, even at the same relative contraction intensity (9, 14, 18, 22). One reason for this difference could be a difference in maximal voluntary activation between knee angles (6, 30). When relative contraction intensities between knee angles are compared, normalizing to the most accurate maximal value at each knee angle is important. If the maximal voluntary contraction (MVC) is used as a gold standard, and differences in maximal voluntary activation between knee angles occur, differences in the relative contraction intensities between those knee angles will mistakenly be created. Therefore, contraction intensities are better expressed as a percentage of the maximal torque capacity (MTC) rather than MVC.

It has further been shown that the difference in endurance (time to torque failure) between knee angles does not originate from differences in central drive at the point of torque failure or from differences in muscle perfusion (14). However, potentiation, the enhancement of contractile response as a consequence of prior activation, has been suggested to contribute to the increased endurance at extended compared to flexed knee angles (22) as potentiation is greater at short compared to long muscle length in rats (23, 24). Furthermore, potentiation may lead to a constant force output despite a decline in motor unit firing rate (13). Therefore, a muscle length - dependent potentiation could allow for a slower increase in muscle activation at extended compared to flexed knee angles during constant force production. This less intense muscle activity may postpone the recruitment of large fatigable motor units, which may account for the lower muscle oxygen consumption ($m\dot{V}O_2$) found previously at extended compared to flexed knee angles (5). It would

therefore be very interesting to examine muscle activation and oxygen consumption over a range of relative contraction intensities at extended compared to flexed knee angles. In addition, insight into potential mechanisms limiting endurance at different muscle lengths suggested previously (9, 14, 18, 22) may be gained by the simultaneous investigation of muscle activation and oxygen consumption.

Particularly at low contraction intensities there are indications for differences in the muscle activation of the rectus femoris (RF), vastus lateralis (VL) and vastus medialis (VM) muscle. Using EMG, the activity of synergistic motor neuron pools has been shown to alternate during low - force sustained contractions by which constant force production is maintained (15, 31). In addition, the change in root mean square EMG of the VL and VM was twofold greater compared to the RF during sustained low force contractions (7). These variations may arise from the unique role of bi - articular muscles (such as the RF muscle) in controlling the distribution of net moments about the joints (12). In addition, considering the lower $m\dot{V}O_2$ of the RF compared to VL and VM at low contraction intensities (5), an accompanying less intense muscle activation (lower EMG activity) of the RF compared to the VL and VM would be expected.

The first aim of this study was to investigate whether less muscle activation, measured with rectified surface EMG (rsEMG), could account for the lower muscle oxygen utilization at extended compared to flexed knee angles. We hypothesized that at the same relative torque, relative rsEMG would be consistently lower at extended compared to flexed knee angles and similarly, a lower muscle oxygen consumption was expected at the extended knee angle. Secondly, we investigated if differences in muscle activation between the knee extensor group muscles were related to similar differences in muscle oxygen utilization, particularly at low contraction intensities. We hypothesized that at low contraction intensities a relatively lower oxygen consumption of the RF would be accompanied by relatively less rsEMG of the RF compared to the VL and VM.

Methods

Subjects

Thirteen healthy male subjects (mean \pm SD age of 25.4 ± 3.1 years) volunteered to be subjects for this investigation. Before participation, each subject was thoroughly informed about the procedures and provided written informed consent. Only subjects capable of maximal voluntary activation levels $> 90\%$ at each knee angle (see below) were included in the present study. The study was performed according to the Declaration of Helsinki and approved by the local ethics committee. Subjects did not to perform any fatiguing exercise 48 hours prior to measurements.

Experimental procedures

The subjects visited our laboratory on three occasions with at least 2 days in between. The first visit was a habituation session; subjects were familiarized with the set - up, electrical stimulation, and stable constant isometric knee extension with a pressure cuff and visual feedback. The first experimental day (day 1) was used to accurately determine maximal torque capacity (MTC, see below) and record surface EMG of the RF, VL, and VM at high ($> 70\%$ MVC) contraction intensities. During the second experimental day (day 2), muscle oxygen uptake ($m\dot{V}O_2$) and surface EMG of the same muscles were recorded at extended (30° , 0° = full knee extension) and flexed (60 and 90°) knee angles at several submaximal intensities (10, 30, 50, 70%MTC) as well as during two sustained maximal attempts.

Torque measurements

Isometric knee extension torque of the right leg was measured using a custom - built dynamometer. For details see Kooistra et al. (14), but briefly, subjects were seated with their hips at 70° (0° = full extension) and shoulders, hips, and lower thigh were strapped to the dynamometer. Knee joint angles were determined with a handheld goniometer (model G300, Whitehall Manufacturing, California, USA) and the dynamometer arm was positioned so that the indicated knee angles were angles with subjects delivering $\sim 50\%$ of maximal torque. Real - time force applied to the force transducer (KAP, E/200 Hz, Bienfait B.V. Haarlem, The Netherlands, range: 2000 N) was displayed on line on a computer monitor and digitally stored (1 kHz) on computer disc. The force signals were automatically corrected for gravity at each

angle: the average force applied by the weight of the limb to the transducer during the first 50 ms after the start of a recording, with the subject seated in a relaxed manner, was set to zero force by the computer program. The distance from the knee axis to the center of the force transducer was determined for each subject to enable torque calculation.

Electrical stimulation

A cathode (self - adhesive stimulation electrode, 5 × 5 cm, Schwa - Medico, The Netherlands) was placed over the femoral nerve. The anode (13 × 8 cm) was placed over the gluteal fold. The quadriceps femoris muscle was stimulated transcutaneously with rectangular pulses of 100 μ s using a computer - controlled constant current stimulator (Digitimer DS7H, Digitimer Ltd., Welwyn Garden City, UK). Stimulation current was increased until torque measured in response to a triplet (three 100 μ s pulses applied at 300 Hz) leveled off at each knee angle tested. The current (in mA) was then increased by a further 50 mA to ensure supramaximal stimulation (range: 250 - 600 mA). It was assumed that at this point all muscle fibers of the knee extensors were activated. Usually torque generated by the triplet is about ~ 35% of MTC (14).

Maximal voluntary contraction (MVC) and maximal torque capacity (MTC)

Since muscle oxygen consumption ($m\text{VO}_2$, see below) and rsEMG (see below) during constant torque production at fixed percentages of MTC were obtained on day 2, we considered it very important to have highly accurate values for MTC. MTC was preferred to MVC as a 100% value because it eliminates the potential effect of knee angle - dependent differences in maximal voluntary activation. For example, when maximal voluntary activation is less than 100% during an MVC, MVC underestimates the MTC of the muscle. When differences in maximal voluntary activation occur between knee angles (6), using MVC as a 100% value may falsely produce differences in $m\text{VO}_2$ and EMG when torque is set as a percentage of MVC rather than MTC, between knee angles.

During day 1, MVC torque and MTC were determined at three knee angles (30°, 60° and 90°). Subjects were asked to maximally generate isometric torques for about 3 - 4s to determine MVC extension torque. Real - time torque was visible on a

computer monitor and subjects were vigorously encouraged to exceed their maximal value, which was also displayed. MVC torque was determined as the highest stable 1000 ms part of the force signal multiplied by the subjects' moment arm. MVC torque was taken as the highest value, which did not exceed preceding attempts by > 5%, allowing a maximum of four attempts. To quantify MTC as accurately as possible, 5 - 7 voluntary contractions (70 - 100%MVC) were performed with a superimposed triplet. The contractions were performed in random order across knee angles separated by three minutes rest. The increment in torque as a result of the superimposed triplet was plotted as a function of the stable torque plateau reached just before the superimposed stimulation. Following linear regression, the intersection with the x - axis yielded MTC (Fig. 1.). At MTC, all muscle fibers were considered maximally active. The level of voluntary activation was defined afterwards as $MVC/MTC * 100$. Surface EMG of the RF, VL, and VM muscle were recorded during each contraction.

Surface electromyography

Electromyographic activity of the RF, VL, and VM muscle was recorded using surface EMG electrodes (Blue Sensor, Ambu, Ølstykke, Denmark, lead - off area: 1.0 cm²). After shaving, roughening and cleansing the skin with 70% ethanol, electrodes were placed on the muscle belly in a bi - polar configuration, in line with the muscle fiber direction, with an inter - electrode distance of 25 mm. Reference electrodes were placed on bone structures, on each patella and on the lateral epicondyle of the femur of the right leg. The location of each electrode was accurately marked with a waterproof felt tip pen for precise electrode re - application in subsequent sessions. Surface EMG signals were amplified (x 100), digitized (1 kHz), and stored with the force signal on computer disc. All EMG signals were band - pass filtered (10 - 400 Hz). Rectified surface EMG amplitude (rsEMG) was calculated for the RF, VL and VM for 1000 ms segments just before the superimposed stimulation on day 1; on day 2 rsEMG was calculated at torque onset as well as 500 ms before and after $m\dot{V}O_2$ was measured (see below). RsEMG values were normalized to those obtained during the highest MVC on day 1.

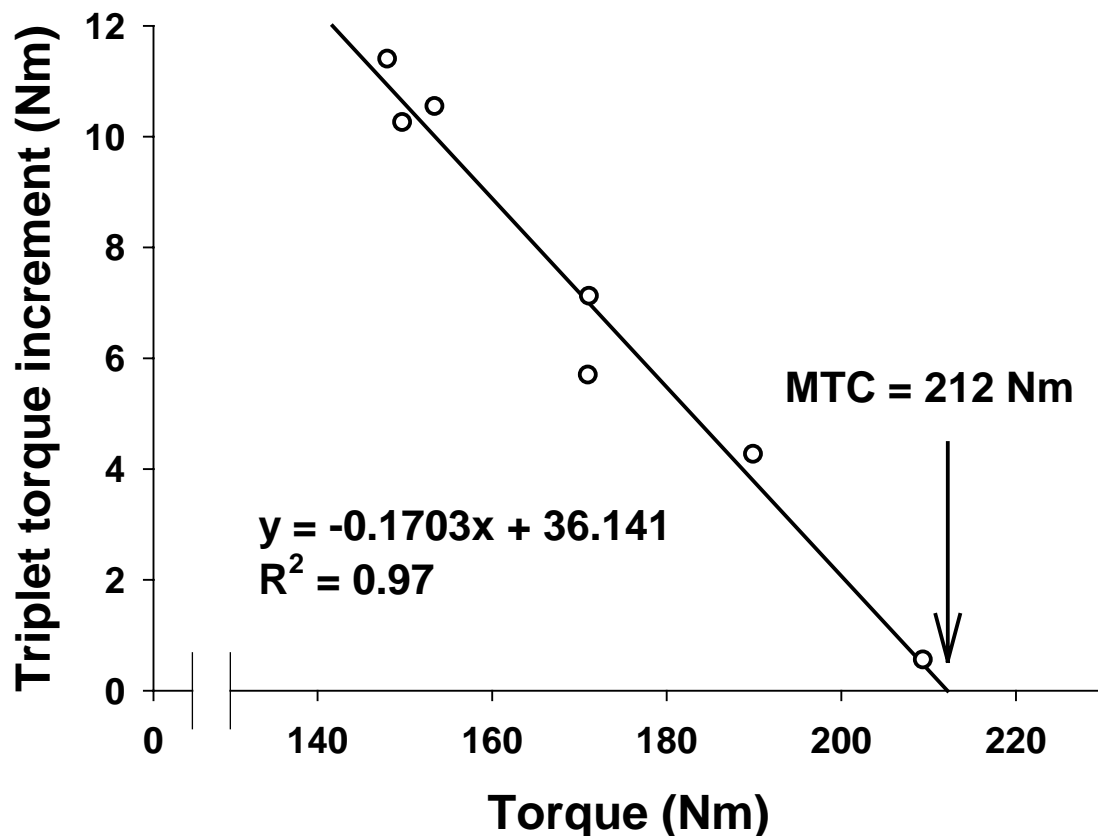


Fig. 1. An example for one subject at the 30° knee angle. The torque increment as a result of the triplet on a voluntary contraction shown as a function of the delivered torque just before the triplet (x - axis). Using linear regression, the intersection at the x - axis is calculated (black arrow), and it was defined as the maximal torque capacity (MTC).

Near - infrared Spectroscopy (NIRS)

On day 2 oxygen consumption of the RF, VL and VM muscle was determined during isometric knee extension contractions by use of a continuous - wave near - infrared spectrophotometer (Oxymon, Artinis Medical Systems, Zetten, The Netherlands), which generated light at 780 and 850 nm (33) and was regularly calibrated by Artinis Medical Systems (Zetten, The Netherlands). The three optode sets were each fixed in a mould with an inter - optode distance of 45 mm (penetration depth of the tissue was therefore 22.5 mm). The moulds were secured to the upper leg with elastic Velcro straps such that the optodes did not move during contraction. The optodes were positioned over the center of the muscle bellies. Note that the surface EMG electrodes were placed in between the NIRS optodes to ensure that the recording volume of the electrodes coincided with that of the NIRS optodes.

With NIRS, the tissue oxygenation level can be measured non - invasively, in fact, the optical change in density of the tissue is measured, which, by modification of the Lambert - Beer law (17), can be transformed into the change in concentrations of oxyhaemoglobin ($[O_2Hb]$) and -myoglobin ($[O_2Mb]$) and deoxyhaemoglobin and -myoglobin ($[HHb]$ and $[HMb]$). Due to the overlap in the spectrum, haemoglobin and myoglobin cannot be measured separately; O_2Hb and HHb will respectively denote the oxygenated and deoxygenated form of both proteins in the present study. The rates of concentration changes of O_2Hb and HHb , or the slopes (Fig. 2) of the $[O_2Hb]$ - and $[HHb]$ - time curves, represent the oxygen consumption per unit time in the muscle (mVO_2 , see also (8)) Blood volume and total Hb (the sum of $[O_2Hb]$ and $[HHb]$) of the m. quadriceps were kept constant by inflation of a pressure cuff (Hokanson SC 10D), which was placed around the most proximal part of the thigh, a few seconds prior to each contraction and deflated a few seconds afterward (for details see (5)). The maximal slopes of the $[O_2Hb]$ - and $[HHb]$ - time signals (which were not statistically different in any of the muscles at any of the knee angles) were averaged, and this absolute value, mVO_{2max} , was calculated at all contraction intensities. Furthermore, mVO_2 was expressed relative to maximal deoxygenation ($\% \cdot s^{-1}$) which is standard procedure (5). By expressing mVO_2 in this manner, potential differences in subcutaneous fat between muscles, which greatly affect NIRS measurement sensitivity (19, 32), are accounted for. Maximal deoxygenation is defined as the absolute difference in $[O_2Hb]$ (and $[HHb]$) when virtually all O_2Hb is converted into HHb . In this study, maximal deoxygenation was determined at the 60° knee angle in rest as it was demonstrated previously to be independent of torque level (0 - 100%MVC) and knee angle (5). NIRS - data were sampled at 10 Hz and stored on computer disc.

Due to the greater endurance at the 30° knee angle, at each intensity after torque onset, the maximal slope of the HHb and HbO_2 signals (mVO_{2max}) was expected to be reached at a later point in time (t_{max}) at 30° compared to 60 and 90° knee angles. For an appropriate comparison of mVO_2 and rsEMG between knee angles, the instance at which they are compared should be the same (Fig. 2.). Hence, for each intensity, when mVO_{2max} was reached at any muscle, at any of the knee

angles, mVO_2 and rsEMG were determined for all muscles at all three knee angles (30, 60 and 90°) for this point in time.

EMG measurements and NIRS

On day 2 isometric knee extensions were performed at the three knee joint angles at 10, 30, 50, and 70%MTC during which rsEMG and mVO_2 were obtained. The $[O_2Hb]$ - and $[HHb]$ - time signals of each muscle were displayed online and each contraction was continued beyond the point at which the maximal slope (mVO_{2max}) of each muscle was reached.

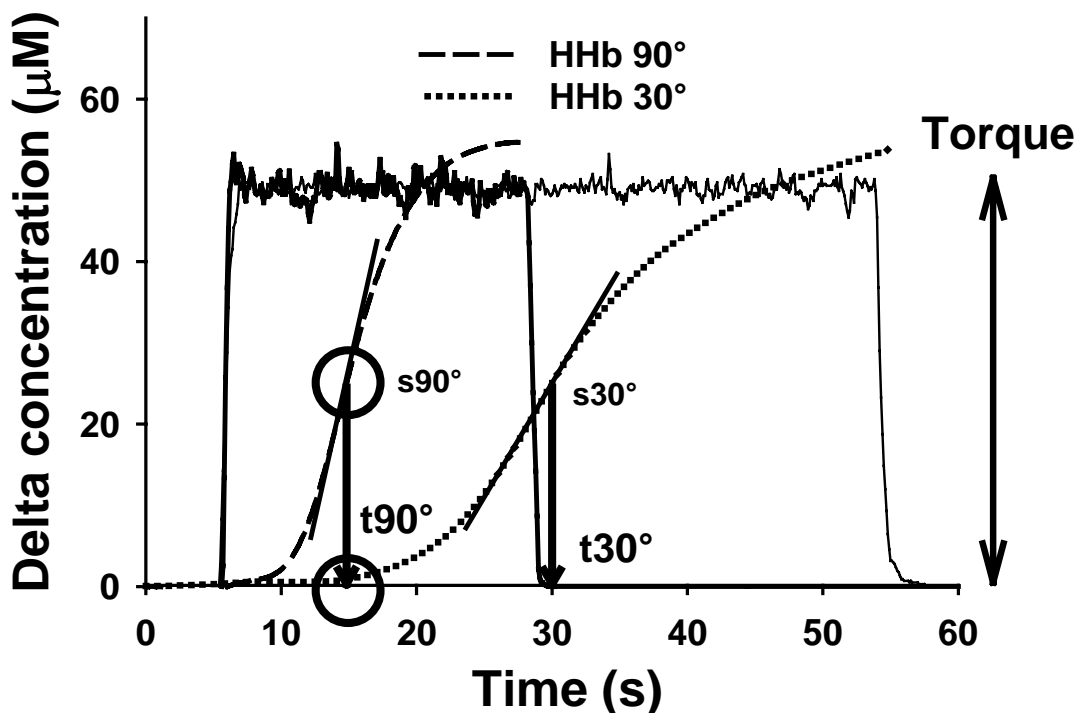


Fig. 2. Near - infrared spectroscopy (NIRS) signals of the vastus medialis (VM) muscle during constant torque production at the 90° (— bold trace) and 30° (— trace) knee angle. The 60° knee angle is not shown for reasons of clarity. Changes in concentration of deoxygenated haemoglobin (HHb, HbO_2 [mirror image of HHb] is not displayed for reasons of clarity) are shown during constant torque production at 30% maximal torque capacity for the 90° (— trace) and 30° (··· trace) knee angle. Maximal oxygen consumption (mVO_{2max}) at each angle occurred at the time (t_{30° and t_{90°) when the slopes (s_{30° and s_{90°) were maximal and is denoted by the two arrows. In this example, muscle oxygen consumption (mVO_2) and EMG were measured at $t = 15s$ (circles) for both 90 and 30° knee angles.

Although we realized maximal voluntary activation might be lower during a sustained MVC (28) compared to the brief MVCs performed on day 1, we wanted

two sustained maximal attempts, where the highest stable torque plateau was used for further analysis. The order of the contraction intensities was randomized, but per subject, the same order of intensities was used at each knee joint angle. All subjects started with a sham measurement at 50%MTC, which was used as warm up. Pilot experiments had shown that the mVO_2 of the first measurement was consistently lower than when the same measurement was repeated. Yet, all measurements thereafter regardless of angle or intensity were very reproducible (that is the difference in mVO_2 of two consecutive measurements at the same intensity was <5%). The subjects received 6 minutes rest between the trials.

Statistics

All results are presented as mean \pm SD. Separate repeated measures analyses of variances (ANOVA, SPSS version 12.0) were used to compare the level of voluntary activation and MTC between the three knee angles tested.

With respect to t_{max} , mVO_2 , and rsEMG an “overall” ANOVA for repeated measures was performed that included the data obtained at the 10 – 70%MTC intensity levels. For significant effects in this overall analysis, subsequent ANOVAs for repeated measures were performed to test for any differences between knee angles as well as differences between muscles.

For the data obtained at 10%MTC, an “overall” ANOVA for repeated measures was performed with respect to t_{max} , mVO_2 , and rsEMG. Bonferroni tests were used for all post hoc analysis. The level of significance of all statistical analyses was set at $P < 0.05$.

Results

During the familiarization session four subjects did not reach > 90% voluntary activation and were excluded from further participation. The results and statistics of nine subjects are presented.

Voluntary activation, MTC and torque

Linear extrapolation of the extra torque as a result of the electrical stimulation on high intensity contractions (> 70%MVC) provided high R^2 values at each knee angle (30°: 0.96 ± 0.03 ; 60°: 0.96 ± 0.03 , and 90°: 0.97 ± 0.03), which did not differ ($P > 0.05$)

between knee angles. The determination of MTC was very accurate since voluntary activation levels were very high, with no differences between knee angles. MTC was significantly ($P < 0.05$) greater at the 60° knee angle compared to the 30 and 90° knee angle, whereas there were no differences between the 30 and 90° knee angle (Table 1).

As expected, during the sustained maximal contraction, maximal voluntary activation at 60° ($83.1 \pm 11.7\%$) and 90° ($74.4 \pm 7.1\%$) knee angles was significantly lower than the voluntary activation during the brief MVCs executed on day 1 (60°: 95.6 ± 2.8 and 90°: $97.1 \pm 2.9\%$). However and to our surprise, maximal voluntary activation at the 30° knee angle ($96.2 \pm 3.8\%$) was very high and not significantly different from day 1 ($95.6 \pm 3.5\%$). Due to the significant difference in maximal voluntary activation between knee angles, further comparisons of mVO_2 and muscle activation between the knee angles will be limited to the 10 - 70%MTC range. At each knee angle, during the sustained contractions at 10, 30, 50 and 70%MTC, average torque was not significantly different from the intended values.

Table 1. Maximal torque capacity (MTC) and voluntary activation

| | Knee angle | | |
|--------------------------|------------------|--------------------|------------------|
| | 30° | 60° | 90° |
| Voluntary activation (%) | 95.6 ± 3.5 | 95.6 ± 2.8 | 97.1 ± 2.9 |
| MTC, N · m | 196.0 ± 40.7 | $273.6 \pm 63.4^*$ | 187.6 ± 29.2 |

All values are means \pm SD. *denotes significantly different from 30 and 90° knee angles, $P < 0.05$, $n = 9$.

Muscle oxygen consumption between knee angles

As expected, mVO_{2max} increased with intensity and, at each intensity, was significantly lower at the 30° knee angle compared to the 60 and 90° knee angle (Fig. 3). At all intensities, t_{max} (the time before mVO_{2max} was reached) was significantly greater at 30° compared to 60 and 90° knee angles (Table 2).

To permit an appropriate comparison between mVO_2 and rsEMG between knee angles, in the present study mVO_2 was also measured at the same point in time for all muscles and angles per intensity. At the 30° knee angle, in line with our 1st

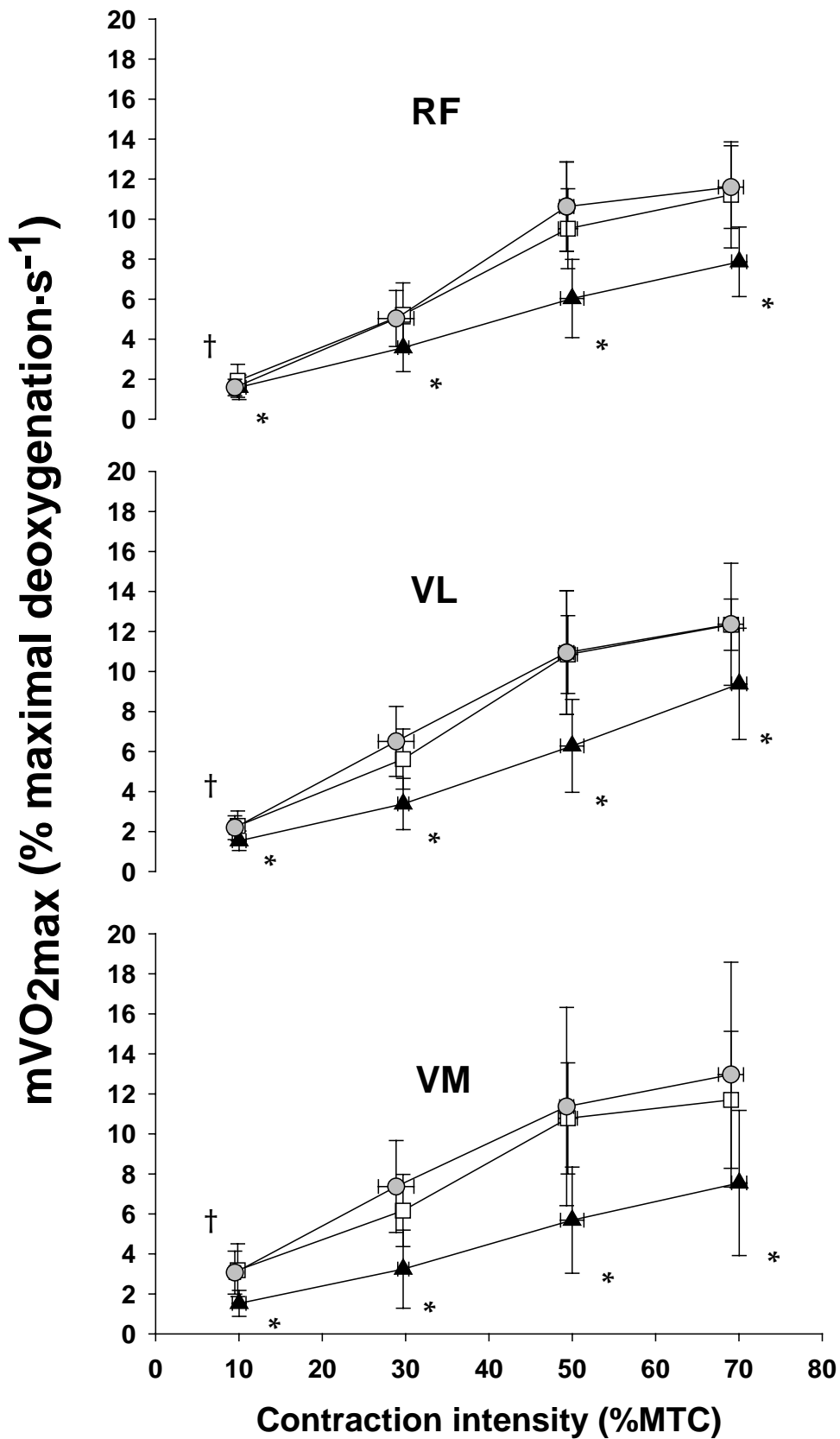


Fig. 3. Maximal muscle oxygen consumption (mVO_{2max}) for the rectus femoris (RF, top panel), vastus lateralis (VL, mid panel) and vastus medialis (VM, bottom panel) muscle at 30° (black triangles), 60° (white squares) and 90° (gray circles) knee angles at different contraction intensities (10 - 70%MTC). * mVO_{2max} significantly lower at the 30° compared to the 60 and 90° knee angle ($P < 0.05$). † Significant main effect of muscle at 10%MTC.

hypothesis, $m\dot{V}O_2$ was significantly lower compared to the 60 and 90° knee angle (Fig 4.). When the $m\dot{V}O_2$ of 60 and 90° was averaged and set to 100% for each intensity level; the $m\dot{V}O_2$ at 30°, across all intensity levels, for RF, VL, and VM was only 47.4 ± 10.2 , 45.3 ± 8.5 , and $33.7 \pm 8.6\%$ respectively.

Table 2. Time to maximal muscle oxygen consumption (t_{max}) of the RF, VL, and VM at different contraction intensities and knee angles

| Muscle | Knee angle | Contraction intensity (%MTC) | | | |
|------------------|------------|------------------------------|---------------|--------------|---------------|
| | | 10 | 30 | 50 | 70 |
| | | t_{max} (s) | | | |
| Rectus Femoris | 30° | 81.9 ± 48.7* † | 22.7 ± 10.5 * | 12.0 ± 4.5 * | 7.5 ± 1.9 * ‡ |
| | 60° | 37.3 ± 18.6 † | 11.3 ± 4.7* | 7.0 ± 3.0* | 4.8 ± 1.1 ‡ |
| | 90° | 39.6 ± 19.0 † | 13.1 ± 6.9* | 5.8 ± 0.9* | 4.3 ± 0.7 ‡ |
| Vastus Lateralis | 30° | 27.3 ± 14.3 * | 17.6 ± 7.9 * | 12.6 ± 2.9 * | 8.8 ± 2.0 * |
| | 60° | 21.3 ± 14.1 | 11.2 ± 1.2 | 7.3 ± 1.0 | 5.8 ± 0.8 |
| | 90° | 18.8 ± 2.9 | 11.0 ± 1.6 | 7.0 ± 1.2 | 5.2 ± 0.7 |
| Vastus Medialis | 30° | 40.0 ± 24.4 * | 24.5 ± 15.8 * | 15.2 ± 5.6 * | 10.3 ± 3.4 * |
| | 60° | 19.0 ± 7.1 | 11.9 ± 1.9 | 7.9 ± 1.5 | 6.2 ± 0.8 |
| | 90° | 18.1 ± 2.8 | 11.1 ± 1.7 | 6.9 ± 1.0 | 5.2 ± 1.0 |

* Significantly lower than 60 and 90° at all intensities and for all muscles ($P < 0.05$); †Significantly greater than VL and VM at 10%MTC; ‡Significantly smaller than VL and VM at 70%MTC, $n = 9$.

Muscle activation between knee angles

In comparing the level of muscle activation between knee angles (with regard to our 1st hypothesis), we felt that the rsEMG at the start of the contraction would represent any initial potential difference in demand between the respective knee angles tested. Surprisingly, and in contrast to muscle oxygen consumption, rsEMG at the start of the contraction was not different between knee angles (Fig 5.). However, there was a trend for a main effect (when all intensities (10 - 70%MTC) were included) for knee angle ($P = 0.062$). Expressed as a percentage of the rsEMG of 60 and 90° (averaged across all intensity levels), rsEMG levels at 30° were: 86.5 ± 15.4 , 80.1 ± 13.1 , and $79.4 \pm 12.9\%$ for the RF, VL, and VM muscle respectively. The rsEMG values obtained at

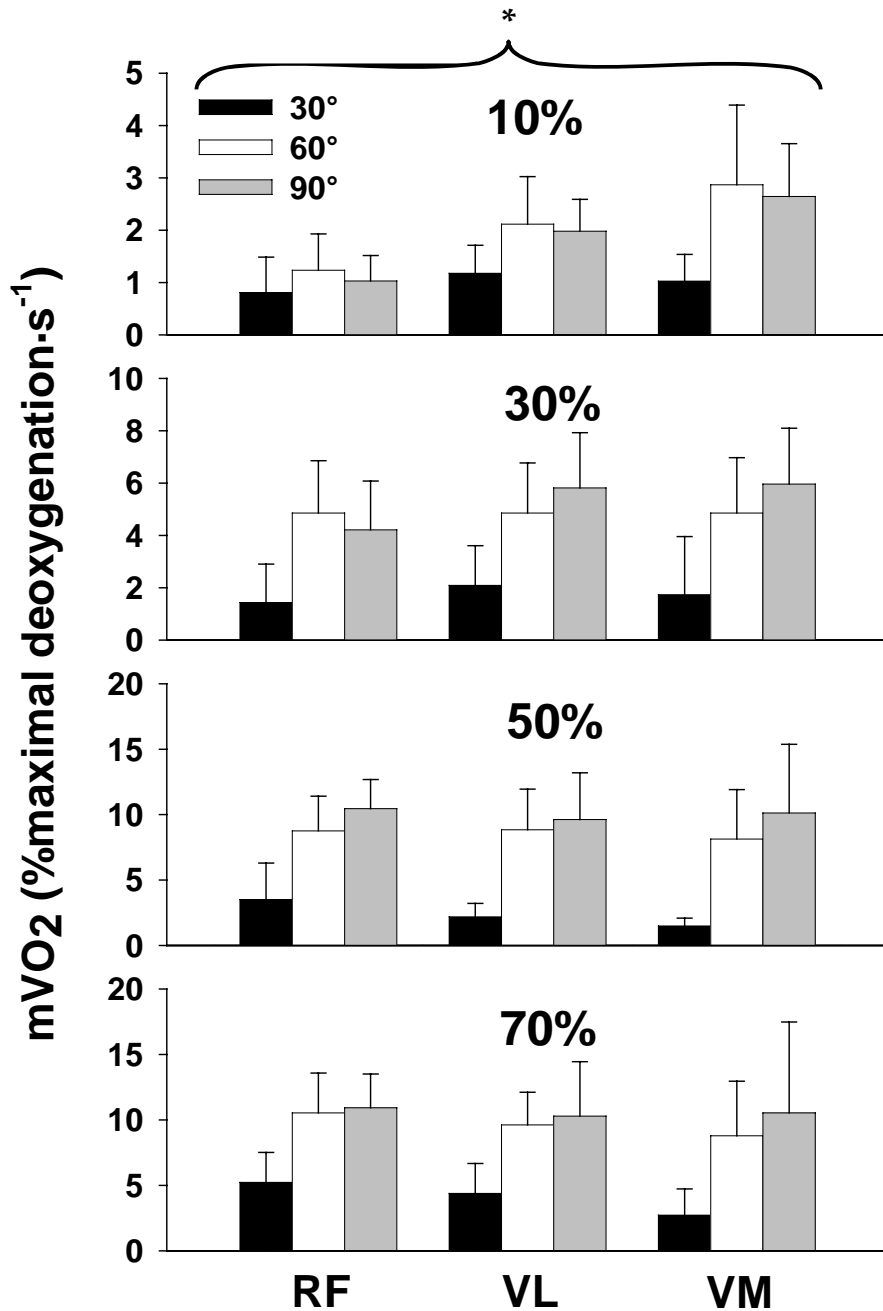


Fig. 4. Muscle oxygen consumption for the rectus femoris (RF, on the left), vastus lateralis (VL, mid) and vastus medialis (VM, right) muscle at different contraction intensities (10 - 70%MTC). At all intensities, mVO₂ was significantly smaller at 30° (black bars) compared to 60 (white bars) and 90° (grey bars) for all muscles ($P < 0.05$). * At 10%MTC there was a significant main effect of muscle and mVO₂ of the RF tended ($P = 0.069$) to be smaller than VM. Note the differences in scale of the y - axis.

the same time point as mVO₂ was reached were comparable ($P > 0.05$) to those measured at the start of the contraction and not different ($P > 0.05$) between knee angles. At the time point mVO₂ was measured, rsEMG levels at 30° were: 84.8 ± 14.7 ,

89.5 ± 13.1, and 85.9 ± 13.2% for the RF, VL and VM muscle respectively, expressed as a percentage of 60 and 90° knee angles.

Muscle oxygen consumption and muscle activation at 10%MTC

We will now focus on probable differences in mVO₂ and rsEMG at 10%MTC (our 2nd hypothesis). With respect to mVO_{2max} there was a significant main effect of muscle with further post - hoc testing showing no differences between muscles. Furthermore, the time before mVO_{2max} was reached (t_{max}) for the RF was significantly greater than that for the VL and VM muscle. The extent to which this occurred was substantial; t_{max} of the RF muscle was 250.2 ± 59.4% of the VL and VM (averaged) muscle across the 60 and 90° knee angles. For each subject, mVO₂ was also measured at the same point in time after torque onset (14.5 ± 2.0s) for all muscle and angles. Similar to mVO_{2max}, a significant main effect for muscle was found. In addition, mVO₂ of the RF tended to be smaller than VM (P = 0.069), but not VL (P = 0.148).

At 10%MTC, rsEMG values were not different between muscles at the start of the contraction. However, from the start of the contraction to the time when mVO₂ was measured, VL rsEMG values increased significantly at 30 and 60° knee angles, and VM rsEMG values at 30, 60 and 90° knee angle, whereas RF rsEMG values showed no change (Fig 6.).

Discussion

In the present study, isometric knee extension contractions at different intensities were compared between knee angles with respect to muscle activation (rsEMG) and muscle oxygen utilization (mVO₂). The first finding of the present study was that a tendency for lower muscle activation at 30° may contribute to, but cannot completely account for the substantially lower mVO₂ at 30° compared to 60 and 90° knee angles. Second, at low intensities of contraction, the slower increase in RF mVO₂, coupled to the slower relative increase of RF rsEMG, suggests a less intense activation of the RF compared to the VL and VM muscles

Muscle oxygen consumption between knee angles

The lower mVO_{2max} at 30° compared to 60 and 90° knee angles in the present study was expected and reaffirms results of an earlier study of ours (5). We measured

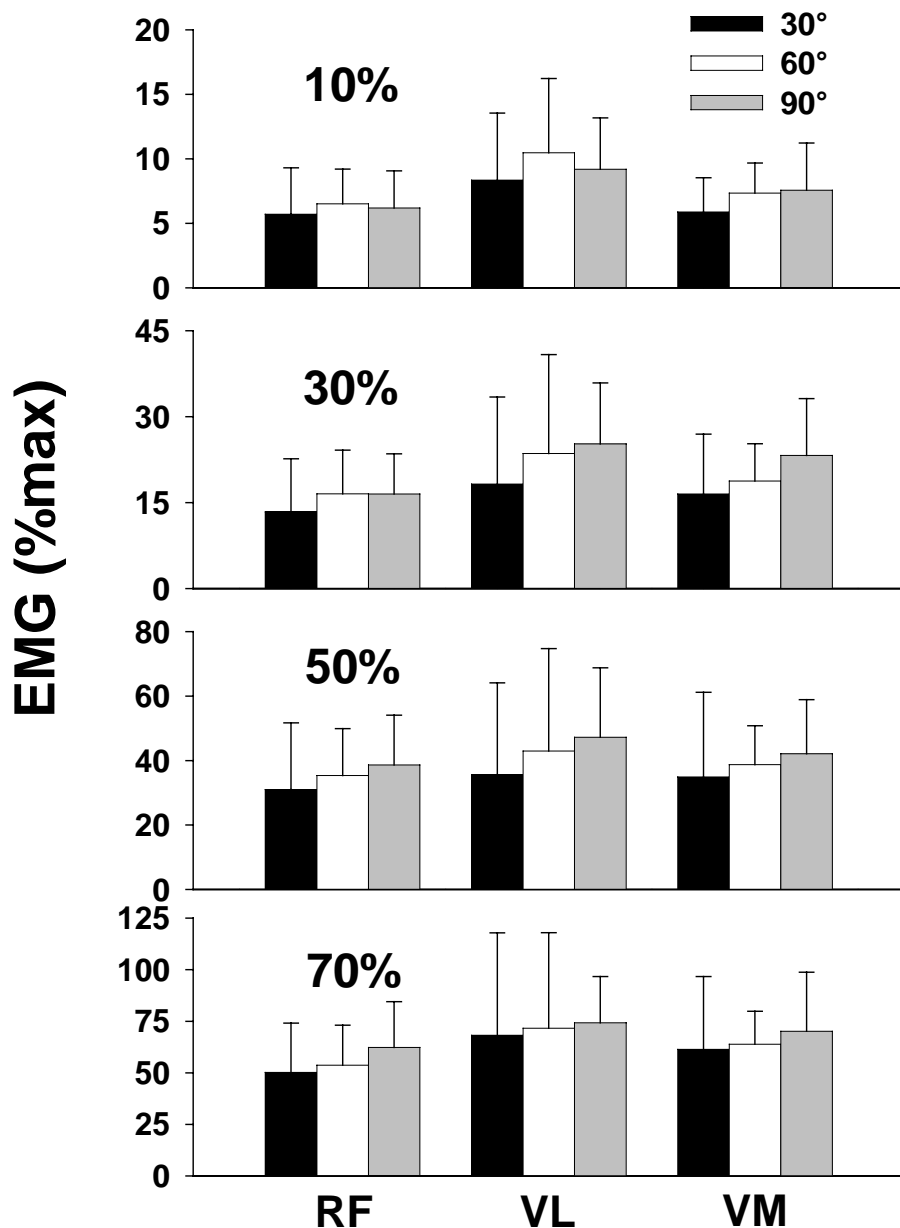


Fig. 5. Rectified surface EMG at the start of contraction. Rectus femoris (RF, on the left), vastus lateralis (VL, mid) and vastus medialis (VM, right) muscle at different contraction intensities (10 – 70%MTC) are shown for 30° (black bars), 60° (white bars), and 90° (grey bars) knee angles. Note the differences in scale of the y - axis.

mVO₂ during complete arterial occlusion and used this as a measure for energy consumption, which is a reasonable assumption as has been argued previously (5)

In addition, after torque onset, at each intensity significantly more time was required to reach mVO_{2max} at 30° compared to the other two joint angles (Table 2), which is a further indication that isometric contractions are less strenuous at 30° . Moreover, by measuring mVO_{2max} for each muscle at each knee angle, the present study shows that the time at which mVO_{2max} occurred after torque onset varied greatly between muscles and knee angles. Because we were interested in comparing task demand at relative torque levels between knee angles, for each subject we compared mVO_2 (and rsEMG) at the same point in time at each intensity level. This revealed that isometric knee extensions are energetically even less demanding at 30° than at 60° and 90° knee angles compared to our previous study, where only mVO_{2max} was studied (5). In fact: averaged from 10 - 70%MTC, mVO_2 at 30° was only $\sim 40\%$ of the mVO_2 that was needed at the 60° and 90° knee angles.

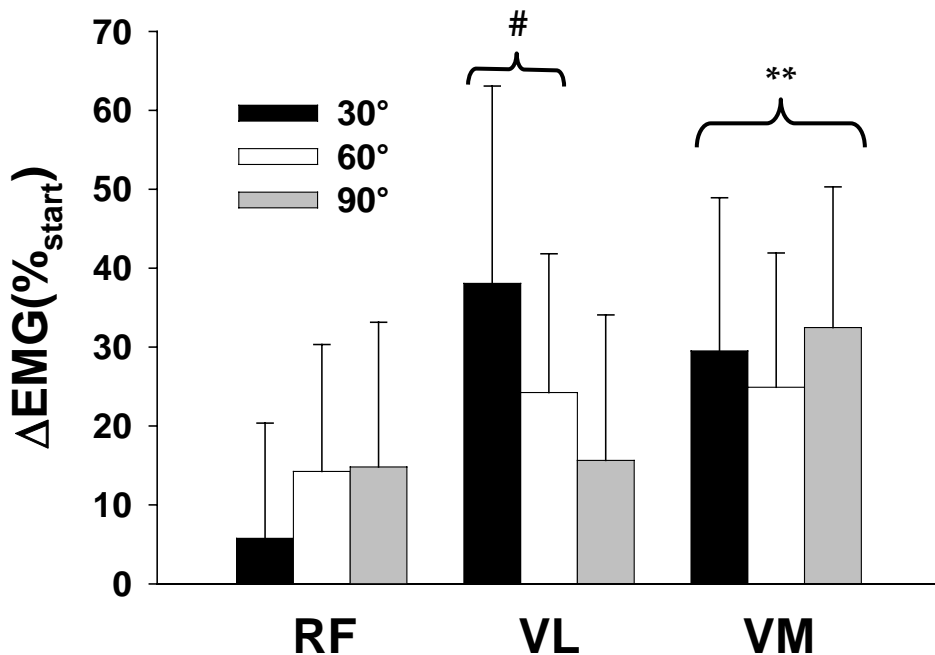


Fig. 6. Increase in rsEMG at 10%MTC of the rectus femoris (RF, on the left), vastus lateralis (VL, mid) and vastus medialis (VM, right) muscle from the start of contraction to the time mVO_2 was measured. # VL increased significantly at 30 (black bars) and 60° (white bars) knee angle ($P < 0.05$). ** VM increased significantly at 30, 60, and 90° (grey bars) knee angle.

It is improbable that the lower mVO_2 at 30° compared to 60° and 90° is the result of an underestimation of the MTC at 30° . As a result of our strict selection

criteria of an activation level of > 90% at each knee angle, voluntary activation levels were very high (Table 1.). The linear extrapolation of the extra torque as a result of electrical stimulation on high intensity contractions (> 70%MVC) resulted in high R² values. Together with the high levels of voluntary activation, this ensured an accurate estimation of the 100% value for MTC at each knee angle and consequently enabled a proper comparison of relative torques between knee angles.

During the sustained maximal attempts on day 2 maximal voluntary activation was lower at the 60° (83.1 ± 11.7%) and 90° (74.4 ± 7.1%) knee angle. This was expected since during sustained maximal contractions, initial voluntary activation is rarely 100%, as seen in the m. biceps brachii for example (28). Surprisingly however, maximal voluntary activation was very high (96.2 ± 3.8%) at the 30° knee angle during the sustained maximal contraction, another indication that maximal contractions at this knee angle seem to be less demanding compared to 60 and 90°.

Muscle activation between knee angles

A potentiation - related relatively lower increase in muscle activation at 30° compared to the other knee angles could contribute to the lower energy consumption at the 30° knee angle. In rat muscle, twitch potentiation is inversely proportional to muscle length, the magnitude of the enhancement being greater at shorter muscle lengths (23, 24). In addition, after a fatiguing contraction in the human quadriceps muscle, peak twitch and doublet force were significantly potentiated at short (35° knee angle) but not at long (75° knee angle) muscle length (22). This higher level of twitch potentiation at extended compared to more flexed knee angles has been suggested to underlie the greater endurance time at extended compared to more flexed knee angles (22). Potentiation of force production, while force output remains constant, is expected to lead to a lower increase in muscle activation; motor unit firing rates may even decrease during constant force production (13). A lower increase in the required muscle activation during constant torque production at 30° compared to the 60 and 90° knee angle, could in turn lead to a decrease of the activation dependent energy consumption. The latter being mainly related to calcium release and re - accumulation by the sarcoplasmic reticulum (10). However, in the

present study, there was only a tendency for rsEMG levels to be lower at 30°. The magnitude of this potential knee angle effect in each muscle (13 - 21%) is small compared to the knee angle effect on $m\dot{V}O_2$ that was found (see above) to be 53 - 64% lower at the 30° knee angle. In addition, such an approximately 15% lower muscle activation at 30° may account for only a small fraction of the ~ 60% lower $m\dot{V}O_2$ at 30° compared to 60 and 90° knee angles. The energy cost of muscle activation during maximal isometric contraction at optimum length is in the order of 30% of the total energy consumption (10). This fraction may be somewhat higher at very low muscle lengths (4), thus, a reduction of muscle activation of about 15%, as suggested by the strong tendency for lower surface EMG found in the present study at the 30° knee angle would, at most, account for a 5 - 10% lower $m\dot{V}O_2$ at 30° compared to 60 and 90° knee angles. This leaves at least 80% of the ~ 60% lower energy consumption at 30° unaccounted for.

How can we account for the remaining 80% of the ~ 60% lower energy consumption at 30° compared to 60 and 90°? A length - dependency of energy consumption might provide an explanation. Despite reports of an unchanged energy consumption at short compared to optimum muscle length in isolated mammalian muscle (20, 29), at very low muscle lengths energy consumption did decrease compared to that found at optimum muscle length (4, 27). Using ^{31}P - MRS, Sacco et al. (26) and Baker et al. (2), reported similar rates of ATP use at short and optimum length of the tibialis anterior muscle in humans during contraction. This might indicate that the relative length changes *in vivo* may be rather small. The knee extensors most likely operate on the ascending limb of the length - tension relationship at 30°, whereas 60 and 90° knee angles will be closer to optimal muscle length (11, 30).

From the above, it seems unlikely that at equal relative intensities the lower $m\dot{V}O_2$ at 30° (short muscle length) would be the result of length - dependent lower energy consumption compared to 90° (long muscle length). The present study shows that the lower $m\dot{V}O_2$ at 30° compared to 60 and 90° cannot be accounted for by differences in muscle activation and consequently the exact explanation remains to be established.

Muscle oxygen consumption and muscle activation at 10%MTC

At the 10%MTC level, we hypothesized that a relatively lower $m\dot{V}O_2$ of the RF would be accompanied by a lower level of muscle activation of the RF compared to both vasti. There was a significant main effect for $m\dot{V}O_2$ between the three muscles at the 10%MTC contraction intensity and post - hoc analysis showed a tendency ($P = 0.069$) for RF $m\dot{V}O_2$ to be lower than VM $m\dot{V}O_2$. These results seem to be less conclusive than those of de Ruiter et al. (5) where RF $m\dot{V}O_2$ was clearly lower than both vasti. This might be explained by the fact that in our study in one out of nine subjects a faster increase in, as well as a higher $m\dot{V}O_2$ at 10%MTC was found in the RF than in the vasti at each knee angle. This accounts for the large standard deviation seen in t_{max} at 10%MTC (Table 2) and the less distinct difference in energy consumption between the RF and the VL and VM muscles, compared to our previous work (5). In the present study the slower increase in RF $m\dot{V}O_2$ (t_{max} of the RF was substantially greater than that of the VL and VM muscles) was not accompanied by less EMG activity of the RF compared to the VL and VM muscle at the start of the contraction at any of the knee angles tested (Fig. 5, top panel.). However, the increase in RF EMG from torque onset to the time $m\dot{V}O_2$ was measured ($14.5 \pm 2.0s$) was less compared to the two vasti, the VL and VM muscle showed increases in EMG activity of 26 and 29%, whereas the RF muscle only showed an increase of 12% (see Fig. 6.).

During low intensity isometric contractions, there are indications of less RF EMG activity compared to both vasti in some studies (1, 3), whereas in others this is not the case (21), or even the opposite (more EMG activity of the RF compared to VL and VM muscles) is seen (25). It is not clear why these differences exist between studies; though speculation, different hip angles ranging from 90° to 120° ($180^\circ =$ full extension), with some authors (21) unfortunately failing to specify hip angle, may be a factor. Because the RF spans both knee and hip joint, both joint angles will influence its muscle length and thereby potentially its activation relative to the other knee extensors (see (25)). Our findings are in agreement with the slow development of the integrated EMG of the RF muscle compared to the VL and VM muscle reported by Kouzaki et al. (16) at the 90° knee angle. Apparently, in the present study, similar levels of muscle activation of the RF, VL, and VM at the start of the

contraction are not necessarily maintained further on in the contraction and it seems as if the relative contribution of the RF to total torque production decreases. Another explanation may be that the RF fatigues less quickly than the VL and VM and therefore requires a comparatively smaller increase in EMG. This dissimilar increase in EMG might lead to the considerably greater t_{max} of the RF, compared to VL and VM muscles at 10%MTC found in the present study. Whatever the mechanism for this finding, at low contraction intensities subtle differences in mVO_2 between muscles were accompanied by differences in EMG, whereas the far more pronounced difference in mVO_2 between knee angles was not accompanied by a comparable difference in EMG.

In conclusion, the present study shows that during isometric contractions (i) The tendency for lower muscle activation at 30° compared to the 60 and 90° may contribute to, but certainly cannot account for the lower muscle oxygen consumption at the 30° knee angle. (ii) At low contraction intensities, the slower increase in mVO_2 of the RF seems to be due to a less intense activation of the RF compared to the VL and VM muscles.

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Conventionally assessed voluntary activation does not represent relative voluntary torque production

R.D. Kooistra, C.J. de Ruitter, and A. de Haan

Abstract

The ability to voluntarily activate a muscle is commonly assessed by some variant of the twitch interpolation technique (ITT) which assumes that the stimulated force increment decreases linearly as voluntary force increases. In the present study, subjects ($n=7$) with exceptional ability for maximal voluntary activation (VA) of the knee extensors were used to study the relationship between superimposed and voluntary torque. This includes very high contraction intensities (90-100% VA), which are difficult to consistently obtain in regular healthy subjects (VA of $\sim 90\%$). Subjects were tested at 30, 60 and 90° knee angles on two experimental days. At each angle, isometric knee extensions were performed with supramaximal superimposed nerve stimulation (triplet: three pulses at 300Hz). Surface EMG signals were obtained from rectus femoris, vastus lateralis and medialis muscles. Maximal VA was similar and very high across knee angles: $97 \pm 2.3\%$ (mean \pm SD). At high contraction intensities the increase in voluntary torque was far greater than would be expected based on the decrement of superimposed torque. When voluntary torque increased from $79.6 \pm 6.1\%$ to 100% MVC, superimposed torque decreased from 8.5 ± 2.6 to $2.8 \pm 2.3\%$ of resting triplet. Therefore, an increase in VA of 5.7% (from 91.5 ± 2.6 to $97 \pm 2.3\%$) coincided with a much larger increase in voluntary torque ($20.4 \pm 6.1\%$ MVC) and EMG ($33.9 \pm 6.6\%$ max). Moreover, a conventionally assessed VA of $91.5 \pm 2.6\%$ represented a voluntary torque of only $79.6 \pm 6.1\%$ MVC. In conclusion, when maximal VA is calculated to be $\sim 90\%$ (as in regular healthy subjects) this probably represents a considerable overestimation of the subjects' ability to maximally drive their quadriceps muscles.

Introduction

When a supramaximal electrical stimulus is applied to the nerve of a muscle during a voluntary isometric contraction, the contractile response of any motor units not yet (fully) recruited will lead to a force increment. Merton (1) first reported a linear decrease of this superimposed force increment as voluntary contraction intensity increased in the adductor pollicis muscle. Hence, as voluntary activation (VA) increases, the force increment as a result of electrical stimulation decreases. For the most accurate assessment of maximal VA, Behm et al. (2) recommended using the maximal voluntary contraction (MVC) with a superimposed electrical stimulus, which is now common practice (3, 4, 5, 6, 7, 8, 9, 10, 11). Maximal VA is generally quantified by expressing the 'twitch' force increment on an MVC as a percentage of resting twitch force. Subtraction from 100% results in a value for maximal VA.

Generally, high levels of VA (~90%) for the knee extensors are achieved by regular healthy subjects (e.g. 4, 6, 7, 9, 10, 11, 12). An important implicit meaning of a VA level of 90% is that 90% of the maximal torque capacity (MTC) is reached, and that at most, a 10% further increase in torque is possible. In our experience however, a greater increase in torque can be observed than the obtained VA level suggested when subjects succeed in improving their MVC in a later attempt during the same session. This suggests that VA can be overestimated using the conventional method of superimposed stimulation. This would have important consequences for conclusions drawn regarding calculated VA. The inability of patient groups to access their muscles' potential could be strongly underestimated. This has implications for the estimations made of the effects of paralytic poliomyelitis on strength and endurance for instance (13). Furthermore, many studies have been performed using target force levels expressed as %MVC (13, 14, 15, 16, 17, 18). For example, the %MVC at which full occlusion of blood flow of the knee extensors occurs during isometric contraction (18). Clearly, knowledge of the potential of a far greater torque increase than for example, the 10% implied by a VA of 90%, is very important.

In our experience, in subjects capable of consistently high levels of maximal VA (~97%), the superimposed torque increment is already very small at high

contraction intensities (~70%MVC). Further increases in voluntary torque show only minor reductions of the superimposed torque increment. This is in accordance with the modeling study of Herbert and Gandevia (19), for the adductor pollicis muscle they show that large increases of motoneuronal excitation near MVC will be accompanied by only minor reductions in the size of the superimposed force. At high contraction intensities therefore, the %VA does not necessarily represent the same percentage of the muscles' maximal torque capacity. The first aim of the present study was therefore to assess the relationship between calculated VA and voluntary torque of the knee extensors at high contraction intensities. To achieve the best possible assessment of the relationship between conventionally assessed VA and voluntary torque (as %MVC), in the present study only those individuals capable of consistently high levels of maximal VA were included.

An additional concern of using the ITT is the dependence of the calculated VA on the size of the resting twitch. Following a single electrical stimulus, tendon slack must first be taken up before resting peak twitch torque is reached. Therefore, during an isometric contraction, where most of the tendon slack has already been taken up, the twitch torque increment will be relatively large compared to the twitch torque at rest (20). By scaling the superimposed twitch to resting twitch torque, calculated VA will thus be lower than it otherwise would have been. The effect of tendon slack on the resting twitch has been reported to be of minor influence on the relationship between the superimposed response and voluntary torque, at flexed knee angles (6, 12, 21). However, tendon slack is greater at short compared to long muscle length (22). Therefore, particularly at short muscle lengths scaling the superimposed torque response to the response obtained at rest may lead to a relatively lower calculated VA at short (extended knee angles) compared to long muscle lengths (flexed knee angles).

In subjects with a high ability for maximal VA a neural drive may be reached that is usually unattainable for regular healthy subjects. Moreover, during voluntary fast isometric torque development, rectified surface EMG levels of the knee extensors may be substantially higher than at the torque plateau of an MVC (7). These findings indicate a potential to increase neural drive at the torque plateau of a MVC (see

also(19)). However, based on the shape of the stimulation frequency - force (23, 24) and pCa - force curve (25), at high force levels large increases in neural drive and calcium are required for a comparatively small increase in force. As EMG is often measured to quantify the neural drive at different contraction intensities, this has important consequences for the EMG - torque relationship. EMG is generally normalized to the EMG obtained during MVC, a disproportionate increase in EMG at very high force levels will consequently lead to a more curvilinear EMG - torque relationship. However, this can only be demonstrated in subjects who are able to consistently reach high levels of neural drive. The shape of a subjects' EMG - torque relationship would therefore be determined by the subjects' ability for VA. This would explain why both linear (26) and non-linear (27) EMG - torque relationships have been reported. Therefore, this study further aimed to investigate the EMG - torque relationship of the knee extensors in subjects with high ability for VA.

Methods

Subjects

Seven healthy male subjects (23-32 yr, 69-83 kg, and 1.73-1.93 m) volunteered to be subjects for this investigation. Before participation, each subject was thoroughly informed about the procedures and provided written informed consent prior to testing. Only subjects capable of >95%VA across knee angles (see below) were included in the present study. The study was performed according to the Declaration of Helsinki and approved by the local ethics committee. Subjects did not perform any fatiguing exercise 48 hours prior to measurements.

Experimental procedures

The subjects visited our laboratory on two occasions with at least one day in between. All subjects were familiar with the set-up, electrical stimulation, and isometric knee extension from previous studies (7, 28). During the first experimental day, several contractions with superimposed stimulation (see below) were performed at 30° (extended), 60 and 90° (flexed) knee angles. At the same time, surface EMG of the rectus femoris (RF), vastus lateralis (VL), and vastus medialis (VM) was recorded. To gain information concerning test-retest variability the

experimental procedure performed on experimental day 1 was repeated during experimental day 2.

Torque measurements

Isometric knee extension torque of the right leg was measured using a custom-built dynamometer (28). Subjects were seated with their hips at 70° (0° = full extension). Shoulders, hips, and lower thigh were strapped to the dynamometer. By very tight strapping of the hips and lower thigh, a contribution of the hip extensors to knee extension was absolutely impossible. Moreover, during contractions subjects had to lean forward about 10 cm with their shoulders (while grasping the strap around their shoulders) pushing their lower back into the back rest. This further guaranteed that no hip extension occurred. The distal part of the shank was strapped to a force transducer (KAP, E/200Hz, Bienfait B.V. Haarlem, The Netherlands) that was attached to the lever arm of the dynamometer. A shin guard ensured subjects could exert maximal forces without discomfort at the shin. The compliance of the dynamometer at the position of the force transducer was 1.4×10^{-4} deg/Nm. The backrest, force transducer height, and its medio-lateral position were adjusted for precise alignment of the knee axis with the axis of rotation of the dynamometer arm. A crank enabled changing of the knee angle of the subject. Knee joint angles were determined with a handheld goniometer (model G300, Whitehall Manufacturing) using the greater trochanter and lateral epicondyle of the femur, and the lateral malleolus of the fibula as references. The dynamometer arm was positioned so that the indicated knee angles were angles in an active state, with subjects delivering ~50% of MVC. During pilot experiments using an electrogoniometer attached to the lateral side of the knee, the changes (active - passive) in knee angle were found to be 3 - 7°, independent of knee angle, with minimal (<1°) changes above ~50% of MVC. Real-time force applied to the force transducer was displayed on line on a computer monitor and digitally stored (1 kHz) on computer disc. The force signals were automatically corrected for gravity at each angle: the average force applied by the weight of the limb to the transducer during the first 50ms after the start of a recording, with the subject seated in a relaxed manner, was set to zero force by the

computer program. Extension torque was calculated by multiplication of force with the individual lever arm and data will be presented as torque.

Electrical stimulation

A cathode (self-adhesive stimulation electrode, 5 × 5 cm, Schwa-Medico, The Netherlands) was placed over the femoral nerve. The anode (13 × 8 cm) was placed over the gluteal fold. The quadriceps femoris muscle was stimulated transcutaneously with rectangular pulses of 100 μs using a computer-controlled constant current stimulator (Digitimer DS7H, Digitimer Ltd., Welwyn Garden City, UK). Stimulation current was increased until torque measured in response to a triplet (three 100 μs pulses applied at 300 Hz) leveled off at each knee angle tested. The current (in mA) was then increased by a further 50 mA to ensure supramaximal stimulation (range: 250-600 mA). At this point, we considered all muscle fibers of the knee extensors to be activated. Pilot studies had shown that triplet torque did not increase after an MVC at any knee angle, indicating that any potential post-activation potentiation did not result in a triplet torque increase (see also Discussion). Usually torque generated by the triplet is about ~35% of the MTC (28). Furthermore, in pilot studies the stimulation current was increased far beyond supramaximal levels (up to 200%) without a subsequent decrease in triplet torque. Hence, the hamstring muscles were assumed not to be activated during supramaximal stimulation of the knee extensors (see also Discussion).

Voluntary activation

During experimental day 1, the level of maximal VA was determined in random order at 30°, 60° and 90° knee angles. At each knee angle subjects were asked to maximally generate isometric torques for about 3 - 4s to determine MVC extension torque. Real-time torque was visible on a computer monitor and subjects were vigorously encouraged to exceed their maximal value, which was also displayed. MVC torque was defined as highest torque within a stable 1000 ms plateau of the torque signal. MVC torque was taken as the highest value, which did not exceed preceding attempts by >5%, allowing a maximum of four attempts to prevent fatigue. In the incidental case a trial had no stable plateau, the trial was repeated. Subjects then performed a series of superimposed measurements consisting of a

triplet applied to a fully relaxed muscle and another triplet superimposed on the stable part of the torque signal (Fig. 1).

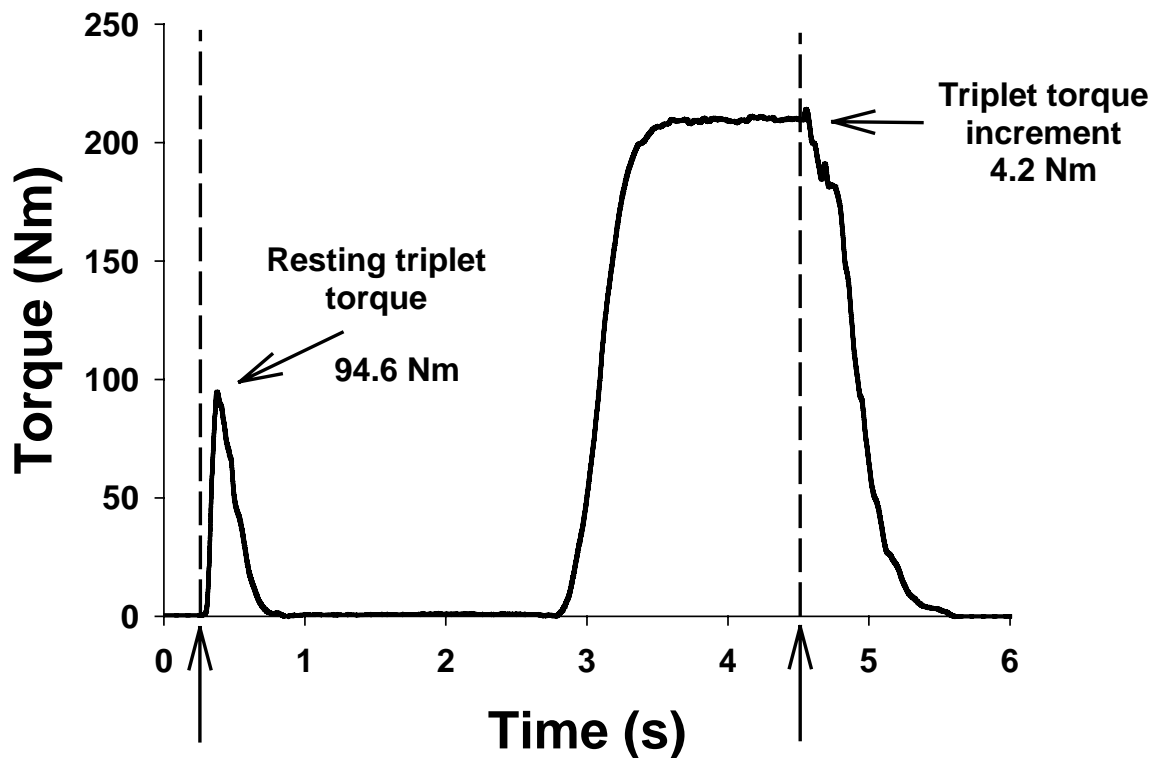


Fig. 1. Typical example of a superimposed contraction. The resting triplet torque as a result of the electrical stimulation as well as the triplet torque increment on a 100%MVC contraction at the 90° knee angle are shown. The timing of the triplet stimulation is shown by the vertical arrows and vertical dashed lines. Voluntary activation (VA) is generally calculated using the following equation: $VA (\%) = 100 - [(triplet \text{ torque increment} / \text{resting triplet torque}) * 100]$. In this particular example voluntary activation was calculated to be: $100 - [(4.2 / 94.6) * 100] = 95.6\%$.

If the torque level was unstable (>1% torque fluctuation) prior (100ms) to stimulation, the contraction was repeated. As all our subjects were very experienced at maintaining stable torque levels at all contraction intensities, it was rarely necessary to repeat a measurement. As an indication, for all subjects less than 1 in 10 contractions (across all knee angles and intensities) were repeated. Several different contraction intensities, with an emphasis toward higher intensities were chosen (25, 50, 70, 80, 90 and 100%MVC). At each knee angle the order of the contraction intensities with superimposed triplet was randomized, and the order of the knee angles was randomized per subject. A three - minute rest period separated contractions. For each superimposed contraction the accepted formula for the

interpolated twitch torque technique (3, 5, 8, 9, 10, 28, 29, 30, 31, 32) was used to determine the level of voluntary activation (VA):

$$VA (\%) = 100 - [(triplet \text{ torque increment} / \text{resting triplet}) * 100]$$

With this method, the triplet torque increment is expressed as a percentage of the resting triplet torque, and subtraction from 100% results in a value for VA (Fig. 1). Maximal VA was obtained from the 100%MVC superimposed contraction. To illustrate the relationship between superimposed and voluntary torque from 90–100%VA only subjects capable of an average maximal VA of >95% across knee angles were included in the present study. MVC torques as close to the muscles' MTC as possible were thus obtained. Due to our strict inclusion criteria (average VA of >95%), at each knee angle we were able to select a contraction at a lower than maximal torque level where the superimposed triplet was ~10% of resting triplet. At this torque level VA is conventionally calculated to be ~90%, and we expressed this torque level as a percentage of MVC. We could thus compare the theoretical 10% torque increase implied by a conventionally calculated VA level of 90% with the torque increase actually achieved by our subjects during MVC. This provides us with a good indication of the potential torque increase that may be possible in regular healthy subjects with a maximal VA level of ~90%. The ~90% is a VA level that is frequently reported in the literature for regular healthy subjects (4, 7, 9, 10, 11, 12, 29).

Furthermore, for each subject and for each superimposed contraction, the increment in torque as a result of the superimposed triplet was plotted as a function of the torque reached just prior to the superimposed stimulation (e.g. Fig. 2 and 3A). Curvilinear relationships between voluntary and superimposed torque are often reported in the literature for group data (e.g. 6, 10, 11, 33). In the present study however, neither a linear nor a curvilinear fit closely followed the data points for any of the individual subjects at any knee angle. However, linear regression was performed on the highest four contraction intensities (70, 80, 90 and 100%) and R² values were calculated at each knee angle, for each subject. This was done to show that although superimposed torque increments were small at high contraction

intensities, the torque increment continued to decrease with contraction intensity (e.g. Fig. 3A and B).

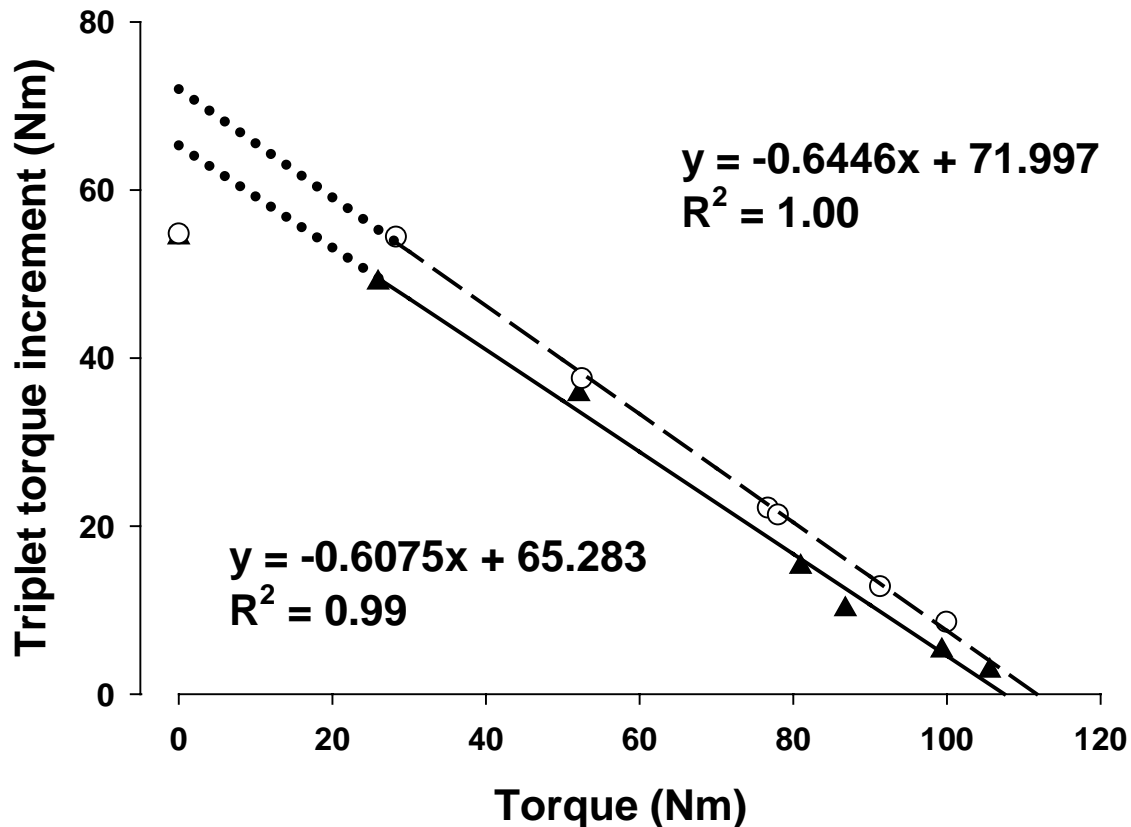


Fig. 2. Torque increment as result of the triplet vs. voluntary torque delivered at the 30° knee angle by a normal healthy subject in a pilot study. The empty circles denote the first day of testing, the black triangles the second day. The dashed and solid line represent linear regression fits for the data points on experimental day 1 and 2 respectively excluding the resting triplet torque at 0Nm. The resting triplet torque is clearly underestimated as it is very similar to the triplet torque increment at ~30Nm. The dotted lines represent backward extrapolation of the regression fits for both experimental days and illustrate the underestimation of the resting triplet torque as a result of tendon slack. For each experimental day the respective equations and R^2 values are shown. Using the interpolated twitch torque technique, voluntary activation (VA) is calculated to be quite high for experimental day 1 (84.4%) and 2 (94.7%).

Surface Electromyography

Electromyographic activity of the RF, VL, and VM muscle was recorded using surface EMG electrodes (Blue Sensor, Ambu, Ølstykke, Denmark, lead-off area: 1.0 cm²). After shaving, roughening and cleansing the skin with 70% ethanol electrodes were placed on the muscle belly in a bi-polar configuration, in line with the muscle fiber direction, with a center to center inter-electrode distance of 25mm. Reference

electrodes were placed on bone structures, on each patella and on the lateral epicondyle of the femur of the right leg. Surface EMG signals were amplified ($\times 100$), digitized (1 kHz), and stored with the force signal on computer disc. All EMG signals were band-pass filtered (10-400 Hz). Rectified surface EMG amplitude (rsEMG) was calculated for the RF, VL and VM for 1000ms segments just before the superimposed stimulation. RsEMG values obtained during the highest torque level on each experimental day were set to 100%.

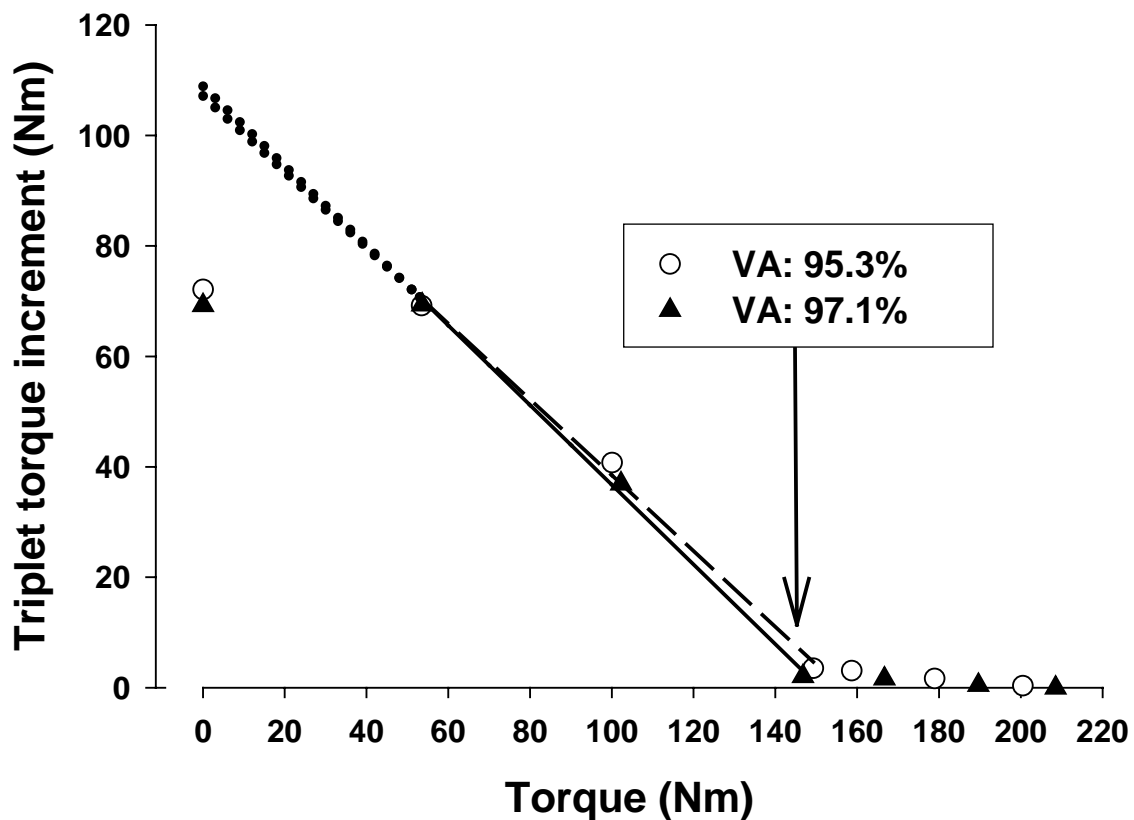


Fig. 3A. The torque increment as a result of the triplet on a voluntary contraction (y-axis) is shown as a function of the voluntarily delivered torque just before the triplet (x-axis). The data points shown are those of subject no. 1, a subject with high ability for maximal voluntary activation, at the 30° knee angle. The open circles denote results from experimental day 1 and the filled triangles from experimental day 2. It is immediately apparent that using a linear or a curvilinear fit over all data points is incorrect for this subject. The dashed and solid lines illustrate linear regression fits that have been performed for a voluntary torque level of ~50 - 150Nm for experimental day 1 and 2 respectively. The dotted lines represent backward extrapolation of the regression fits for both experimental days and illustrate the potential effect of tendon slack on the resting triplet torque, especially when compared to the triplet torque increment at ~60Nm. For clarity, and to illustrate the continued decrease of the triplet torque increment with increasing voluntary torque, the data points of torque levels above 140Nm have been replotted in figure 3B

Statistics

All results are presented as mean \pm SD. Knee angle effects were tested for significance with repeated measures ANOVA. If significant main effects were observed, Bonferroni tests were performed for post hoc analysis. Test-retest reliability was tested for using the intraclass correlation coefficient. The level of significance of all statistical analyses was set at $P < 0.05$.

Results

Torque levels during the superimposed contractions at the 25, 50, 70, 80, 90 and 100%MVC level were not different from the intended values or different between experimental days. Linear extrapolation of the torque increment as a result of the triplet on the high intensity contractions (70, 80, 90 and 100%MVC) provided high R^2 values at each knee angle (30°: 0.89 ± 0.09 ; 60°: 0.90 ± 0.10 , and 90°: 0.87 ± 0.10 averaged over days), which were not different between knee angles or the two experimental days (e.g. Fig. 3B). The continuing consistent decrement of the triplet torque increment with increase in contraction intensity was denoted by the overall negative slope (-0.13 ± 0.05 across days and angles), which was not different between days and angles. The relationship between the triplet torque increment and voluntary torque is shown in figure 4 for the 30, 60 and 90° knee angle.

Table 1. Torque

| | Knee angle | | | ICC |
|-----------------------|-------------------|--------------------|------------------|----------------|
| | 30° | 60° | 90° | |
| MVC, N · m | 186.1 ± 36.6 | $278.4 \pm 44.5^*$ | 189.6 ± 19.7 | $0.99\ddagger$ |
| Triplet torque, N · m | $65.9 \pm 10.0\#$ | 91.6 ± 15.8 | 80.1 ± 10.8 | $0.96\ddagger$ |

All values are means \pm SD, averages across days shown, $n = 7$. Maximal voluntary isometric knee extension (MVC) and resting triplet torque for the 30, 60 and 90° knee angle. *Significantly different from 30 and 90° knee angle ($P < 0.05$); # significantly different from 60° and 90° knee angle ($P < 0.05$). The intraclass correlation coefficient (ICC) was calculated for the measurements made on experimental day 1 and 2 and is shown in the last column. ‡Significant ICC.

There was no difference in resting triplet torque or MVC between experimental days and average values across days are presented. At the 60° knee angle MVC was significantly ($P < 0.05$) greater compared to both 30 and 90° knee

angles (Table 1). Resting triplet torque however, was significantly ($P < 0.05$) lower at 30° compared to 60 and 90° (Table 1). Reproducibility for both MVC and resting triplet torque was very high which was illustrated by high ICC values (Table 1).

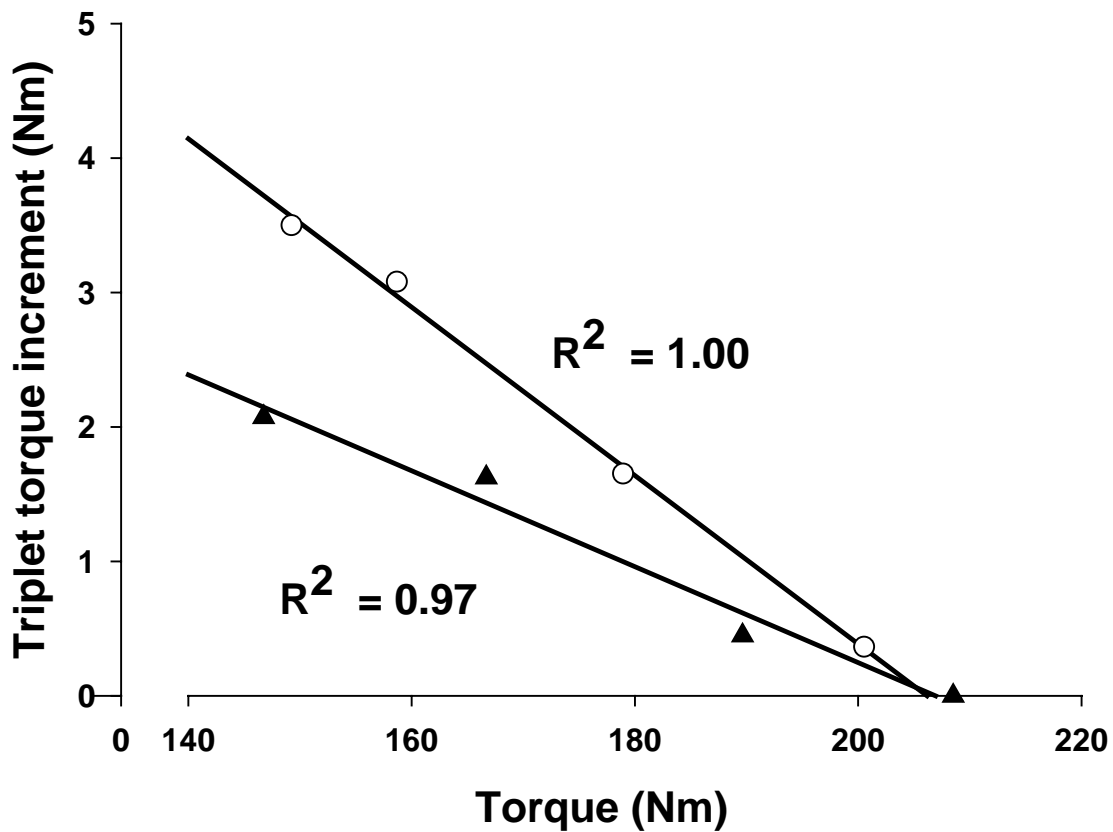


Fig. 3B. Data points from figure 3A have been replotted for torque levels above 140Nm. The torque increment as a result of the triplet is shown as a function of the voluntarily delivered torque just before the triplet (x-axis) for four high intensity contractions (70, 80, 90 and 100%MVC). The open circles represent data points obtained on experimental day 1 and the filled triangles experimental day 2. A linear regression line shows the continued decrease in triplet torque increment with increase in contraction intensity for each set of four data points with corresponding R^2 values.

Voluntary activation

Overall, there was no difference in maximal VA, determined by applying the ITT to the highest MVC, between experimental days and average values across days are presented. The significantly lower triplet torque at 30° compared to the 90° knee angle did not result in a lower maximal VA at 30° compared to 90°. At each knee angle maximal VA was very high, and similar between knee angles (Fig. 5).

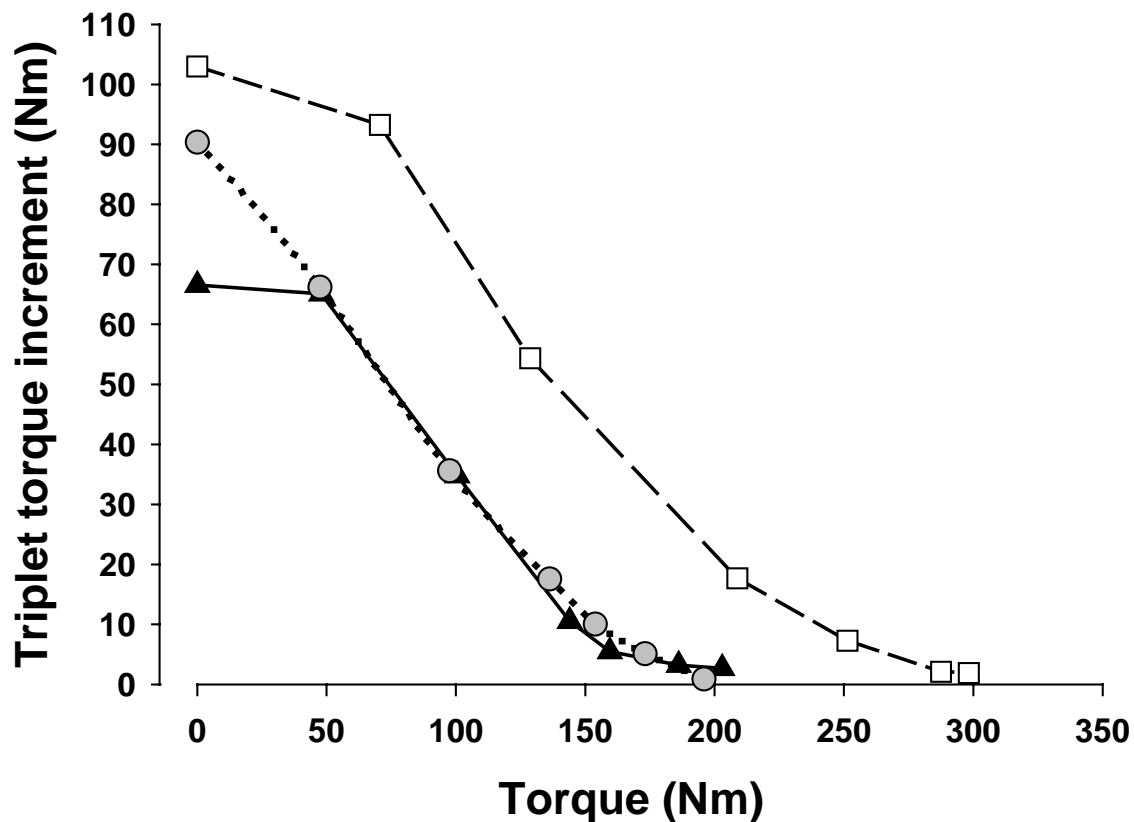


Fig. 4. The torque increment as result of the triplet is shown as a function of voluntary torque for subject no. 5 on experimental day 1. Note that at the higher contraction intensities (>70%) the shape of the curve is similar at the 30° (black triangles, solid line), 60° (white squares, dashed line) and 90° (gray circles, dotted line) knee angle at the higher (>70%MVC) contraction intensities.

Furthermore, at each knee angle, at torques where the triplet increment was $\sim 10\%$ ($8.5 \pm 2.6\%$), VA was calculated to be $\sim 90\%$ ($91.5 \pm 2.6\%$, across angles). This VA level turned out to be significantly higher compared with the relative voluntary torque level at which the triplet was superimposed. The torques at which VA was calculated to be $91.5 \pm 2.6\%$ represented only $79.6 \pm 6.1\%$ of MVC (across knee angles), a finding that was similar at each knee angle ($P > 0.05$). Note that at this torque level, the triplet torque increments were already small ($3.2 \pm 1.1\%$ MVC) compared to the torque generated when the triplet was applied on the resting muscle ($\sim 37\%$ MVC). The key point we want to address with our study is illustrated in figure 6. For both subject no. 1 and 7 a VA level of $\sim 90\%$ was calculated at the 60° knee angle at a torque level of

~250Nm. This suggests a potential further increase of maximal torque by 10 %. However, subject no. 1 was able to significantly increase his torque production by more than 30%.

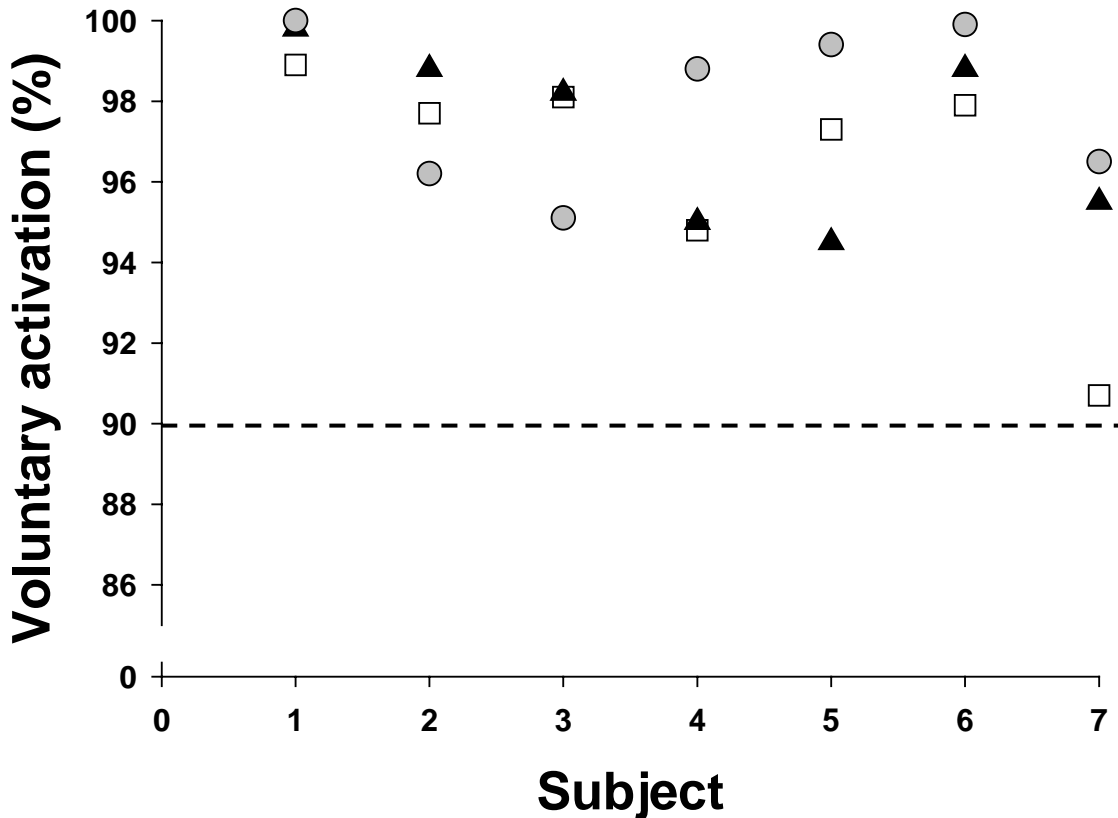


Fig. 5. Average (2 days) maximal voluntary activation (VA) per subject, for the 30° (black triangles), 60° (white squares) and 90° (gray circles) knee angles. Note that only 2 out of a total of 21 data points are below 95%VA. The horizontal dotted line indicates the 90%VA level that is generally obtained for the knee extensors by regular healthy subjects.

EMG

The rsEMG values obtained during MVC were similar between days at each knee angle ($P = 0.96$). For all submaximal contraction intensities, normalized rsEMG values for each muscle (RF, VL and VM) at each contraction intensity was similar between days, hence averaged values across days are presented. Furthermore, no difference between knee angles or muscles was found, and with each increase in contraction intensity there was a significant increase in normalized rsEMG (Fig. 7A, VL shown). An increase of normalized rsEMG of ~21% (across angles and muscles)

was seen for a 25% increase in torque from 25 to 50%MVC (Fig. 7A). When contraction intensities approached MVC, there was a much larger increase in normalized rsEMG (~34%) relative to the torque increase of only ~18% at all knee angles (Fig. 7A). In regular healthy subjects, the highest MVC would have occurred at torque levels where VA would have been calculated to be ~90%, and consequently the EMG - torque relationship would most likely have been considerably less curvilinear. This is illustrated by the two EMG - torque relationships shown in figure 7B for the VL at the 90° knee angle. The first EMG - torque relationship includes all contraction intensities measured, whereas for the second EMG - torque relationship, the 90 and 100%MVC contraction intensities have been removed. In the latter case all rsEMG values are normalized to a torque level that corresponds to the maximal VA level of ~90% (generally obtained by regular healthy subjects), the result being a near linear EMG - torque relationship (Fig. 7B).

As a consequence of the disproportionate increase in EMG as MVC is approached, normalized rsEMG values obtained at intensities below MVC are normalized to a relatively large value. Hence, these normalized rsEMG values are relatively smaller compared to the relative torques at which they have been obtained. For example, at 25 and 50%MVC, normalized rsEMG for the RF, VL, and VM muscle was significantly less than would be expected based on the %MVC at which they were obtained at all knee angles (Fig. 7A, VL shown). By normalizing the rsEMG levels to the rsEMG values obtained at a torque level that corresponds to the VA level of 90% as found in regular healthy subjects, normalized rsEMG values are closer to the line of identity, and a more linear EMG - torque relationship is seen (Fig. 7B).

Discussion

In the present study, the relationship between calculated VA and voluntary torque was assessed in subjects with high ability for maximal VA at different knee angles. At the same time, the EMG - torque relationship in these selected subjects was examined. The first and main finding of the present study was that when VA is calculated to be ~90% (as in regular healthy subjects) this probably represents a

considerable overestimation of the subjects' ability to maximally drive their quadriceps muscles. An additional finding was that, although resting triplet torque was lower at 30° vs. 90°, this was of minor influence on the calculated maximal VA in our subjects. Furthermore, a relatively large increase in normalized rsEMG was observed as MVC was approached, making the shape of the normalized EMG - torque relationship of the knee extensors curvilinear.

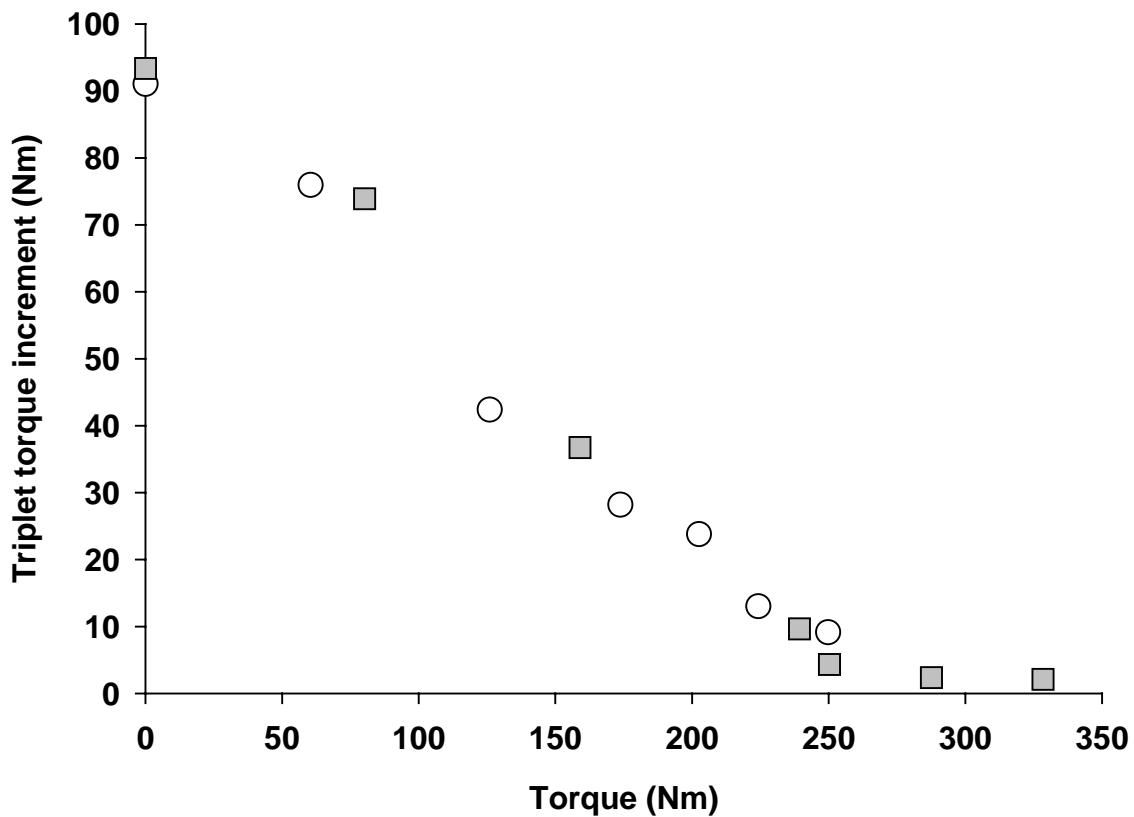


Fig. 6. The torque increment as result of the triplet is shown as a function of voluntary torque for subject no. 1 (gray squares) and 7 (open circles) at the 60° knee angle on day 1. At the 60° knee angle the maximal VA level of subject no. 7 calculated at 250 Nm was 90.7%, which resembled that of a regular subject, and is exceptionally low in our study (see lowest point in Fig. 5). A VA of 90.0% is also calculated at 240 Nm for subject no. 1. In both subjects this implies a further potential increase in torque of 10%, yet a >30% torque increase (to 330Nm) could be demonstrated in subject 1. This is most likely due to the exceptional neural drive of subject 1 (~98%VA) during his best attempts at this knee angle.

Maximal voluntary activation and knee angle

Subjects in the present study exhibited a consistent high level of maximal VA of the knee extensors (Fig. 5), which is higher than the maximal VA levels of 90% that are

generally reported for the knee extensors in regular healthy subjects when applying the ITT (4, 7, 9, 10, 11, 12, 29). Although, full activation of the knee extensors has been reported in the past (21, 34) in those studies a single superimposed twitch was used. Due to the declining signal-to-noise ratio with increase in contraction intensity, the detection of a single superimposed twitch is difficult, and 100% VA may mistakenly have been assumed (33). To improve the signal-to-noise ratio, multiple stimuli have been suggested (35, 36). In the present study triplet stimulation provided considerable resting triplet torque levels at each knee angle (Table 1), which ensured a good signal-to-noise ratio. Furthermore, pilot studies had shown no increase in triplet torque before and after an MVC at any knee angle. By using supramaximal and high frequency (300 Hz) triplet stimulation, we avoided the potential influence of post-activation potentiation on our data.

A limitation of the ITT may be the scaling of the twitch increment to the resting twitch. When muscle is stimulated by a twitch at rest all tendon slack has to be taken up, conversely, for a twitch superimposed on an ongoing isometric contraction no further slack has to be taken up (11). Since calculated VA is determined by the scaling of the triplet torque increment to the resting triplet, calculated VA will be reduced by the influence of tendon slack. As the effect of tendon slack is greater at short vs. long muscle length (22), VA will be influenced to a greater degree at short vs. long muscle length. Besides tendon slack, length-dependent Ca^{2+} sensitivity may also influence the size of the resting triplet by shifting the resting triplet torque - length relationship to shorter muscle length compared to the MVC torque - length relationship. The length-dependent effect of tendon slack and Ca^{2+} sensitivity were reduced by the use of a triplet (37), but not abolished as triplet torque was significantly smaller at the 30° vs. 90° knee angle (Table 1), whereas MVC levels were similar. This implies that despite using triplet stimulation, compared to the MVC, resting triplet torque remains relatively lower at the 30 vs. 90° knee angle. The latter is supported by figure 2 and 3A, where the triplet response is similar at 0 and 25%MVC at the 30° knee angle. It is evident that calculated VA will be influenced by tendon slack, as the resting triplet is influenced

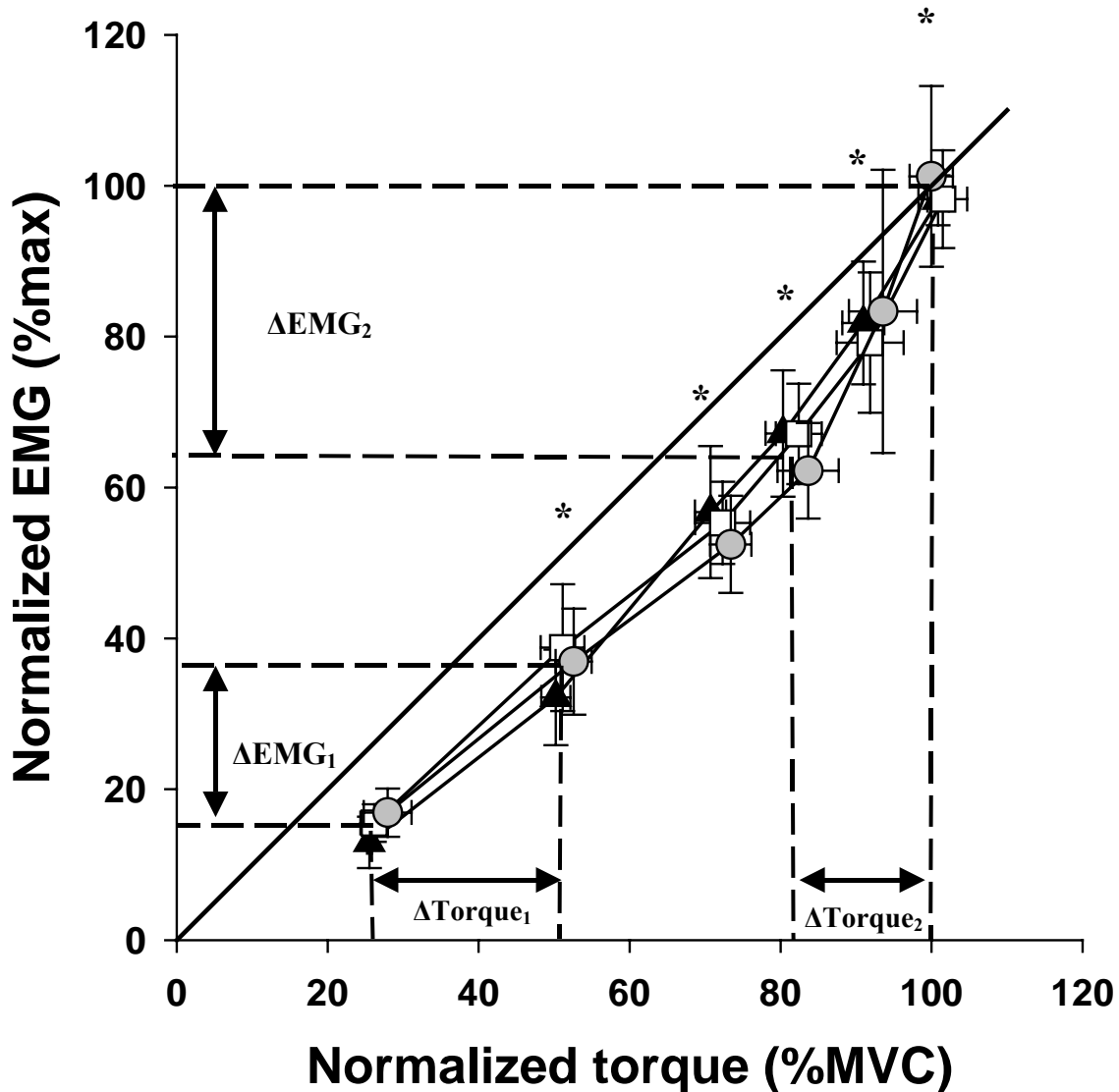


Fig. 7A. Normalized rsEMG levels averaged over the two experimental days vs. normalized torque for the vastus lateralis (VL) muscle at 30° (black triangles), 60° (white squares) and 90° (gray circles) knee angles. * Significantly different ($P < 0.05$) from preceding intensity level. At the lower contraction intensities ΔEMG_1 denotes the increase in EMG (21%, across muscles) that is accompanied by a comparatively larger increase torque (~25%), which is denoted by ΔTorque_1 . Conversely, ΔEMG_2 denotes the much larger increase in EMG (~34%, across muscles) that is accompanied by a comparatively smaller increase in normalized torque (~18%, denoted by ΔTorque_2) as the contraction intensity approaches MVC. Note that on average for ΔTorque_2 , calculated voluntary activation (VA) increased from $91.5 \pm 2.6\%$ to $97.2 \pm 2.3\%$. Thus a 34% increase in normalized rsEMG was accompanied by an 18% increase in torque, for which only a ~5.7% increase in VA (denoted by ΔVA) was calculated. As a consequence of the relatively large increase in EMG as MVC is approached, the rsEMG of contraction intensities below MVC are normalized to a relatively large value and are located well beneath the line of identity.

by tendon slack, especially at short muscle length, whereas the triplet torque increment is not (11).

Despite a lower resting triplet torque at the 30° compared to the 90° knee angle, maximal VA was not different between the 30 and 90° knee angle in our subjects. From the literature it is unclear whether maximal VA is knee angle-dependent. Kubo et al. (38) report a lower level of maximal VA at extended compared to flexed knee angles, whereas Suter and Herzog (11) find maximal VA to be highest at the most extended knee angle (15°). Newman et al. (9) and Babault et al. (4) however, find no effect of knee angle on maximal VA, which is in line with our current findings. Note that due to our selection criteria (average maximal VA of 95%, and at least 90% at each knee angle), maximal VA was very high (~97%) and close to 100%. Potential knee angle-dependent differences in maximal VA could consequently only occur within a very small range. Furthermore, the small size of the superimposed triplet torque makes the calculation of maximal VA insensitive to large differences in the size of the resting triplet torque. By hypothetically increasing the resting triplet torque at 30° by 20%, making it similar to the 90° knee angle, an increase in maximal VA of less than 0.5% is calculated at the 30° knee angle. In subject populations possessing lower maximal VA levels (e.g. patient groups), a relatively greater increment will be obtained on an MVC. This greater increment is more susceptible to the muscle-length dependent influence of, for example, tendon slack on the size of the resting triplet. The influence of tendon slack would have been greater still had a twitch instead of a triplet been used (37), leading to an even greater difference in resting twitch size at 30 vs. 90°. Combined with the use of a resting twitch therefore, a muscle length-dependent effect of tendon slack is expected to significantly influence the calculation of VA in this subject population.

Calculated voluntary activation in relation to relative voluntary torque

For the most accurate assessment of maximal VA, Behm et al. (2) recommended using the MVC with a superimposed electrical stimulus. As mentioned previously, in regular healthy subjects a maximal VA level of ~90% is often reported for the knee extensors using this method (4, 7, 9, 10, 11, 12, 29) implying that the MTC has almost been reached. In the present study, with our selected subject group, we were able to

demonstrate that 90%VA is already calculated at torque levels that represent only ~79% of MVC (across knee angles).

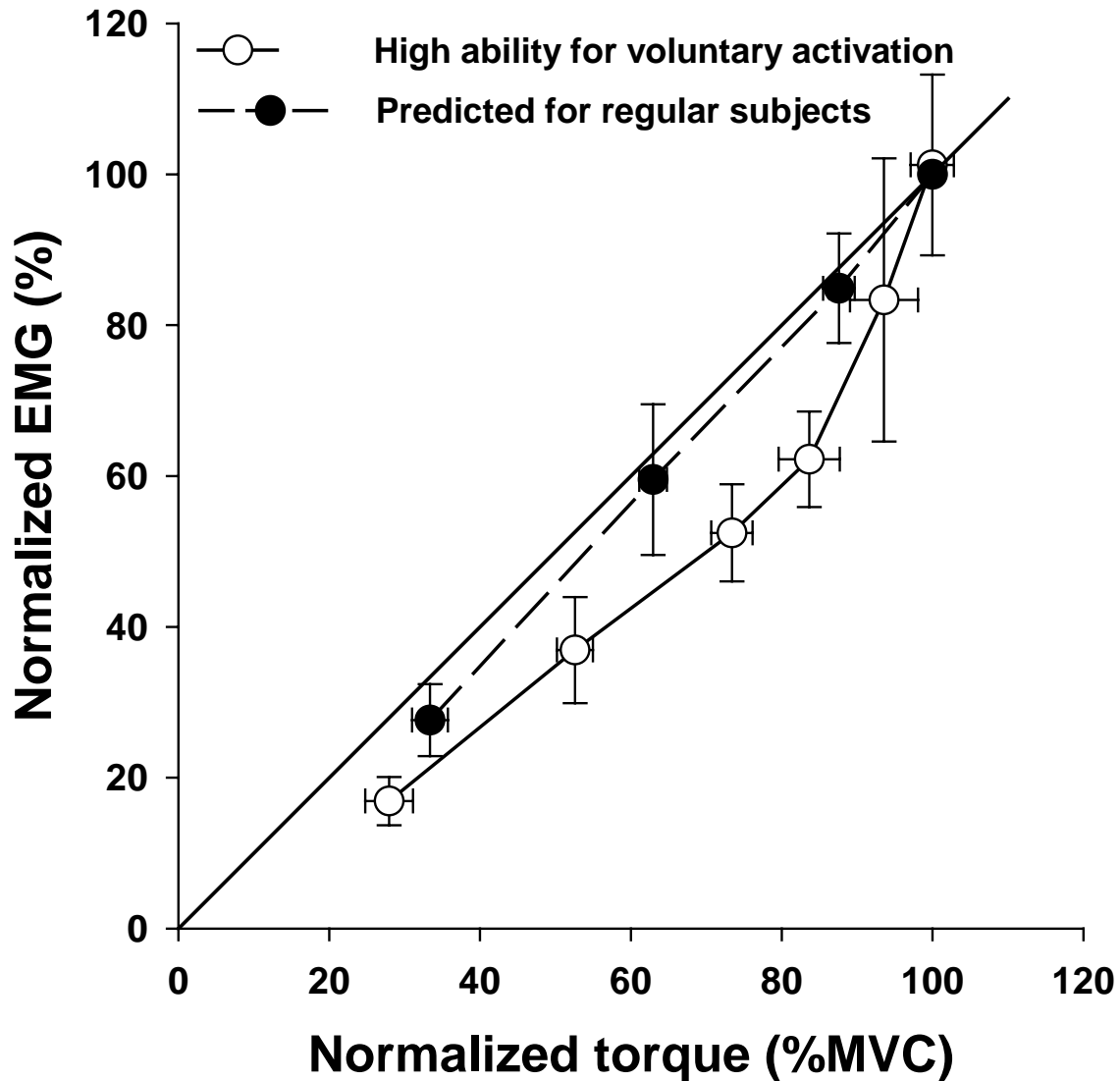


Fig. 7B. Normalized rsEMG levels averaged over days vs. normalized torque for the vastus lateralis (VL) muscle at the 90° knee angle. The white circles represent rsEMG values that have been normalized to the MVC of subjects with a very high ability for voluntary activation (VA). With the black circles, the EMG - torque relationship for regular healthy subjects has been predicted. The black circles denote rsEMG values that have been renormalized to the rsEMG value reached at 90%VA. This is similar to the usual maximal VA in regular individuals. Note that the EMG - torque relationship predicted for regular healthy subjects is linear and closer to the line of identity compared to subjects with very high ability for voluntary activation.

In some subjects, this phenomenon is very pronounced. For the subject shown in figure 3A and B, already at a torque of ~148Nm, VA using the ITT was calculated to

be 95.3 and 97.1% for experimental day 1 and 2 respectively. These are very high VA levels that have been calculated at 148Nm, especially when compared with the MVC of >200Nm that was obtained on both experimental days. Note that the higher part of the curve (in this example over 140Nm), as shown in figure 3B can usually not be obtained in regular healthy subjects. In our experience, we can only demonstrate the existence of this part of the curve in subjects with a very high ability for maximal VA (>99% in this extreme example, subject no. 1 in Fig. 5). This finding strongly suggests that the 90%VA reported for regular healthy subjects is a large underestimation of the MTC of the muscle. A far greater torque increase therefore seems possible than the 10% implied by a calculated VA of 90% (Fig. 6). The main new finding therefore, is that the present study for the first time quantifies the difference between calculated VA and relative voluntary torque.

Experimental and methodological factors

When relating the triplet torque increment to the triplet torque obtained at rest, it is important to maintain supramaximal stimulation during both conditions. In a previous study examining superimposed stimulation of the knee extensors (2), the authors reported significantly lower M-wave amplitudes during a superimposed stimulation on an MVC compared to during rest. They presumed that the contraction of the knee extensors resulted in a displacement of the stimulating electrode from its optimal position over the femoral nerve. In pilot studies of the present investigation, M-wave amplitude at rest and superimposed on an MVC was similar. Moreover, a substantial (50%) increase in stimulation current did not lead to an increase in the size of the triplet increment on high intensity contractions (70 and 80%MVC). This strongly suggests that even during high intensity contractions there was supramaximal stimulation of the femoral nerve during superimposed triplet stimulation. By using multiple stimuli during superimposed stimulation, spinal reflexes may have more time to diminish the superimposed response (19, 39). Modeling suggests that the use of twin stimuli at 100Hz has a minimal effect on the estimates of VA (19). By applying a stimulation frequency of 300Hz in the present study, triplet stimulation occurred within 10ms, which is even less when compared to the use of twin stimuli at 100Hz. The influence of spinal reflexes on the

superimposed stimulation is therefore considered negligible. Furthermore, although the current passes through the sciatic nerve that innervates the hamstring muscles, its stimulation was regarded negligible. This is illustrated by the very small EMG recording of the m. biceps femoris compared to the VL muscle during stimulation of the knee extensors (Fig. 8, data from a previous study of ours (28)). We accordingly assumed a marginal activation, if at all, of the hamstring muscles during superimposed stimulation and therefore no influence on our calculation of VA. In a previous study by our group (7) we measured the contribution of co-activation during isometric knee extensions during brief MVCs and found it to be very small (5-10%). As this finding is in accordance with previous findings (9, 40), we did not measure coactivation in the present study and considered it minimal.

The EMG - torque relationship

As expected, when MVC was approached we observed a relatively large increase in normalized rsEMG (Fig. 7A). This large increase makes the shape of the EMG - torque relationship of the knee extensors curvilinear. This is in contrast to the findings of Woods and Bigland-Ritchie (26) who report a linear EMG - torque relationship, yet in accordance with Alkner et al. (27) and Pincivero and Coelho (41) who show a non-linear relationship for the knee extensors.

Based on a modeling study of motor-unit pools Fuglevand et al. (42) indicate that the difference between muscles that exhibit the linear as opposed to the nonlinear form of the EMG - torque relationship may be related to differences in firing rate behavior rather than due to differences in recruitment organization. It is reasonable to expect that only subjects with a high ability for maximal VA (high neural drive) will achieve very high motor unit firing rates. For this large increase in firing rate however, little gain in torque may be predicted (19). The large increase in excitation, as represented by the disproportionate increase in normalized rsEMG when MVC was approached, confirms the model study by Herbert and Gandevia (19). However, in contrast to their model where essentially 100%MVC was achieved at 60% of maximal voluntary excitation, in the present study we experimentally determined that the disproportionate increase in excitation lead to a more substantial increase in torque (~18%). However, many factors may affect the EMG - torque relationship that have

not been taken into account. Those include, signal cancellation from overlapping positive and negative phases of action potentials (43); the sigmoid relationship between motor unit force and firing rate (19), and the nonlinear distribution of recruitment thresholds (42). Interestingly, in a review de Luca (44) states that the amplitude of the EMG signal should be normalized to values less than 80%MVC. Above this level, the EMG signal is said to be exceptionally unstable and hence, is unable to provide a suitable reference point. As shown in the present study, very large increases in EMG coincide with very small increases in calculated VA (Fig. 7A). This is consistent with unstable EMG measurements for similarly high torque levels.

EMG, superimposed torque and voluntary activation

When combining the non-linearity of the EMG-torque relationship and non-linearity of the superimposed triplet-torque relationship. The EMG-torque relationship indicates that a large amount of extra EMG produces relatively little extra torque at high intensities. The triplet given to evoke the superimposed triplet could be considered as extra EMG, in that three extra action potentials are added. As voluntary EMG is reduced in its effectiveness at producing torque in strong voluntary contractions, the extra activation from the triplet might be reduced similarly. This is in accordance with a model study of the adductor pollicis motoneuron pool (19). The authors predict large increases in motoneuronal excitation for minor increases in voluntary force and VA (as shown by small decreases in the superimposed twitch, their Fig 9C). As mentioned above, this is consistent with the EMG - torque data in the present study (Fig. 7A). The small decreases in modeled interpolated twitch amplitude are also consistent with the current findings, where the triplet torque increment was only $3.2 \pm 1.1\%$ MVC at a torque level that corresponded to $79.6 \pm 6.1\%$ MVC and showed only a minor further reduction in size (to $1.0 \pm 0.9\%$ MVC).

Note that the minor reduction in triplet increment with increasing voluntary torque is, and can only be, observed in those subjects consistently able to achieve high levels of VA, as would be expected from the model (19). This would indicate that these subjects are capable of excitation levels that (almost) evoke their MTC. Having noted this, it is important to point out that a 100% excitation may not be

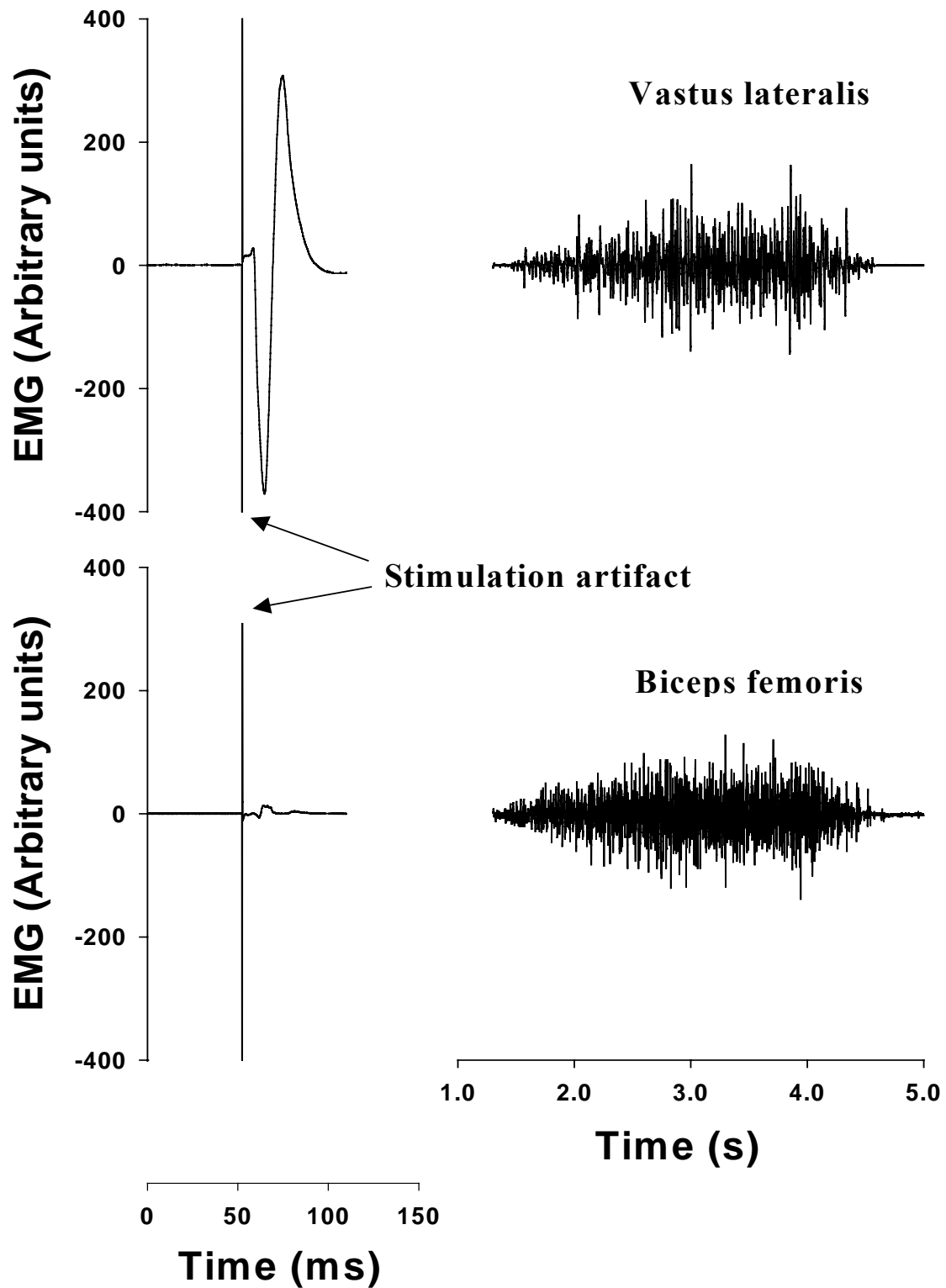


Fig. 8. Representative EMG recordings are shown for the vastus lateralis (VL, upper panel) and biceps femoris (BF, lower panel) muscle. On the left the M-wave for the VL as a result of supramaximal twitch stimulation applied to the n. femoralis. The simultaneous EMG recording for the BF muscle is shown in the lower left panel. On the right, the EMG recording during maximal voluntary extension (top, right) and flexion (lower, right) torque is shown to illustrate that the lower M-wave of the BF is not due a lower sensitivity of the BF recordings compared to the VL recordings.

necessary to evoke the MTC as “only” 60% excitation was modeled to elicit 100% MTC (19). This is in agreement for example with the much greater excitation (EMG) levels attained during voluntary fast isometric knee extensions compared to during the plateau of an MVC (7).

How can we be certain that our selected subjects did indeed approach their true MTC? The only way to truly determine the MTC of a muscle is by the use of tetanic nerve stimulation. In two subjects with exceptional ability for maximal VA (>99%), supramaximal tetanic nerve stimulation (2s, at 150Hz) of the knee extensors was performed and torque levels showed no further increase above MVC (data not presented). Applying supramaximal tetanic nerve stimulation is not recommended however, as it is highly unpleasant and could be harmful to certain vulnerable subject groups (e.g. subjects with an ACL deficiency).

Currently, the ITT is the only feasible available technique used to determine VA of the knee extensors and, as mentioned previously, it is used extensively (2, 3, 4, 5, 6, 7, 9, 10, 11, 32). The findings of the present study indicate that for the knee extensors, despite a commonly reported maximal VA of ~90% for regular healthy subjects, a far greater relative torque increase seems possible than the 10% implied by 90% VA. This has important implications for conclusions that are drawn regarding calculated VA. Especially because many studies have used target force levels expressed as %MVC to investigate: effects of caffeine neuromuscular function (17), the %MVC at which full occlusion of the blood supply occurs (18), and fatigue of the quadriceps in patients with multiple sclerosis for instance (45). Clearly, knowledge of the potential of a far greater torque increase than for example, 10% implied by a VA of 90%, is very important.

In conclusion, when maximal VA is calculated to be ~90% (as in regular healthy subjects) this probably represents a considerable overestimation of the subjects' ability to maximally drive their quadriceps muscles. Moreover, the effect that the length dependent size of the resting triplet has on the calculation of VA with the conventional method is only minimal when VA is greater than 95%. Furthermore, a curvilinear shape of the EMG - force relationship may be caused by a

disproportionately large increase in normalized rsEMG when MVC is approached in subjects with very high capacity to drive their muscles maximally.

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**Knee angle - dependent oxygen consumption of
human quadriceps muscles during maximal voluntary
and electrically evoked contractions**

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Abstract

Fatigability and muscle oxygen consumption ($m\dot{V}O_2$) during sustained voluntary isometric knee extensions are less at extended (30° knee angle; 0° = full extension) vs. flexed knee angles (90°). This lower energy consumption may partially result from lower neural activation at extended knee angles. We hypothesized a smaller difference in $m\dot{V}O_2$ between extended and flexed knee angles during electrical stimulation, where $m\dot{V}O_2$ is maximal, compared to during maximal voluntary contractions (MVC). In eight healthy young males, MVC extension torque was obtained at 30 , 60 and 90° knee angles. Maximal $m\dot{V}O_2$ ($m\dot{V}O_{2max}$) of the rectus femoris, vastus lateralis and medialis muscle was measured using near - infrared spectroscopy during tetanic (10s) and maximal voluntary (15s) contractions (MVC_{15}). For electrically induced contractions, $m\dot{V}O_{2max}$ was reached at similar ($P > 0.05$) times after torque onset ($4.6 \pm 0.7s$) for all knee angles. In contrast, for MVC_{15} contractions $m\dot{V}O_{2max}$ was reached $4.7 \pm 1.1s$ after torque onset at 90° , significantly ($P < 0.05$) earlier compared to 30 and 60° knee angles. At similar times after torque onset, normalized to the value at 90° , $m\dot{V}O_2$ at 30° was $79.0 \pm 9.4\%$ (across muscles) for electrically induced contractions, and only $49.3 \pm 7.8\%$ (across muscles) for MVC_{15} contractions. In conclusion, at similar times after torque onset $m\dot{V}O_2$ is $\sim 50\%$ less for voluntary, and $\sim 20\%$ less for electrically induced contractions at extended vs. flexed knee angles. The $\sim 20\%$ lower $m\dot{V}O_2$ must originate from differences that reside within the muscle. Therefore, 60% ($[50 - 20] / 50 * 100\%$) of the total $m\dot{V}O_2$ difference during voluntary contractions is the result of a lower neural activation at extended knee angles.

Introduction

Time to torque failure during voluntary submaximal isometric knee extensions has been found to be greater at extended (short muscle length) compared with flexed (long muscle length) knee angles (18, 25, 32, 36). Apparently, isometric contractions at extended knee angles are less demanding when compared at the same relative torque level at flexed knee angles.

During voluntary sustained isometric contractions, muscle oxygen consumption ($m\dot{V}O_2$) of the knee extensors, a measure of energy consumption, was found to be lower at extended compared to flexed knee angles (11). This probably contributed to the greater endurance found at extended knee angles in the past (25, 32). The lower $m\dot{V}O_2$ at extended knee angles was accompanied by a somewhat smaller increase in surface EMG during the contraction. A smaller increase in neural activation is thereby implied, possibly due to a greater potentiation at shorter muscle lengths (24). Potentiation, the enhancement of contractile response by prior activation, is greater at short compared to long muscle lengths in rats (38, 40). Since potentiation may lead to constant force output despite a decline in motor unit firing rate (23), a muscle length - dependent potentiation could allow for a slower increase in muscle activation at extended compared with flexed knee angles during constant force production. This less intense muscle activation could delay the recruitment of larger motor units which may account for the lower $m\dot{V}O_2$ and lower fatigability found at extended knee angles (11, 24). However, at submaximal contraction intensities (10 - 70% of the maximal torque capacity [MTC]) a lower muscle activation at extended knee angles could maximally account for only ~ 10% of the 60% lower $m\dot{V}O_2$ at extended knee angles (24). Because it is likely that the position of surface electrodes relative to the muscle changes with changing knee angle, it is hazardous to interpret different changes in surface EMG in terms of knee angle dependent differences in neural activation. Therefore, the present study set out to give a definite quantification of the contribution of knee angle dependent differences in neural activation to the observed differences in $m\dot{V}O_2$.

A difference in maximal voluntary activation between knee angles (11) could also contribute to the knee angle - dependent difference in mVO_2 . Equal relative contraction intensities, derived by normalizing to the MVC at each knee angle, are generally used to investigate and compare endurance at different knee angles (18, 32, 36). When an MVC is performed with a lower maximal voluntary activation at the extended compared with the flexed knee angles, everything else being equal, the relative contraction intensity at the extended knee angle will be less demanding. This could contribute to the greater endurance (18, 25, 32, 36), as well as the lower mVO_2 (11), that has been reported for extended knee angles. A lower maximal voluntary activation has been reported at extended knee angles previously (11), but this finding is not conclusive as maximal voluntary activation has also been suggested to be lowest at optimal knee angle (45).

Although highly unpleasant and only possible using very motivated subjects, measuring knee extensor mVO_2 during electrical activation guaranteed a constant and maximal activation of the muscle fibers. Thus the part of the muscle length - dependent differences in mVO_2 and fatigability explained by factors that reside within the muscle can be determined. By use of tetanic stimulation, the muscle fibers of the knee extensors will be activated maximally. Using near - infrared spectroscopy (NIRS), the mVO_2 of the superficial parts of the knee extensors can be measured (8, 28) during isometric contractions. As there is a consensus that electrically activated human skeletal muscle invokes a greater energy demand compared with voluntary contractions (30, 39, 48, 49), and since the superficial parts of the knee extensors are maximally stimulated by tetanic surface stimulation, the mVO_2 that is elicited should be maximal at extended (30°), flexed (90°) and optimum (60°) knee angle.

By using this approach in the present study, insight into the mechanisms limiting endurance at different knee angles may be gained. This allows us to tease out to which degree mVO_2 is lower at extended knee angles as a result of maximal voluntary activation and other processes (e.g. potentiation) that may affect neural activation, and those which originate within the muscle. The main goal of the present study therefore, is to investigate to what extent the lower mVO_2 at extended knee angles is dependent on differences in neural activation. From a previous study in our

group (11), a lower $m\dot{V}O_2$ is expected at extended knee angles during prolonged MVCs. We hypothesized that when removing the effect of muscle length - dependent differences in neural activation during electrically evoked tetanic contractions, the difference in $m\dot{V}O_2$ between extended and flexed knee angles would be less compared to during prolonged MVCs.

Methods

Subjects

Eight healthy male subjects (23 - 32 yr, 69 - 83 kg, and 1.76 - 1.93 m) volunteered to be subjects for this investigation. Before participation, each subject was thoroughly informed about the procedures and provided written informed consent prior to testing. The study was performed according to the Declaration of Helsinki and approved by the local ethics committee. Subjects did not perform any fatiguing exercise 48 hours prior to measurements.

Experimental procedures

The subjects visited our laboratory for one experimental session of ~ 2 hours. All subjects were familiar with the set - up, electrical stimulation, and isometric knee extension from previous studies (12, 25). Moreover, these subjects had a previously proven very high ability for voluntary activation ~ 97% at all knee angles (11, 12). Each subject performed a prolonged isometric MVC of 15s duration (MVC_{15}) and a 10s tetanic contraction (see below) at extended (30°), optimum (60°) and flexed (90°) knee angles (0° = full extension), in random order. During these contractions, muscle oxygen consumption ($m\dot{V}O_2$, see below) of the rectus femoris (RF), vastus lateralis (VL) and medialis (VM) was measured.

Torque measurements

Isometric knee extension torque of the right leg was measured using a custom - built dynamometer (25). Subjects were seated with their hips at 70° (0° = full extension). Shoulders, hips, and lower thigh were tightly strapped to the dynamometer. The distal part of the shank was strapped to a force transducer (KAP, E/200 Hz, Bienfait B.V. Haarlem, The Netherlands) that was attached to the lever arm of the dynamometer. A shin guard ensured subjects could exert maximal forces without

discomfort at the shin. The backrest, force transducer height, and its medio - lateral position were adjusted for precise alignment of the knee axis with the axis of rotation of the dynamometer arm. A crank enabled changing of the knee angle of the subject. Knee joint angles were determined with a handheld goniometer (model G300, Whitehall Manufacturing) using the greater trochanter and lateral epicondyle of the femur, and the lateral malleolus of the fibula as references. The dynamometer arm was positioned so that the indicated knee angles were angles in an active state, with subjects delivering ~ 50% of maximal torque. During pilot experiments using an electrogoniometer attached to the lateral side of the knee, the changes (active - passive) in knee angle were found to be 3 - 7°, and independent of knee angle, with minimal (< 1°) changes above 50% of maximal torque. Real - time force applied to the force transducer was displayed on line on a computer monitor and digitally stored (1 kHz) on computer disc. The force signals were automatically corrected for gravity at each angle: the average force applied by the weight of the limb to the transducer during the first 50 ms after the start of a recording, with the subject seated in a relaxed manner, was set to zero force by the computer program. Extension torque was calculated by multiplication of force with the individual lever arm and data will be presented as torque.

At each knee angle, subjects were asked to maximally generate isometric torques for about 3 - 4s to determine MVC extension torque. Real - time torque was visible on a computer monitor and subjects were vigorously encouraged to exceed their maximal value, which was also displayed. MVC torque was defined as the highest stable 1000 ms part of the torque signal. MVC torque was taken as the highest value and did not exceed the 2nd best attempt by > 5%. To prevent fatigue a maximum of four attempts were allowed.

At each knee angle, an MVC₁₅ was performed during which mVO₂ (see below) of the RF, VL and VM was measured. As with the brief MVCs, the highest 1000 ms of the MVC₁₅ contraction was defined as the highest value. To ensure arterial occlusion a pressure cuff (400 mmHg, Hokanson SC 10D), placed around the most proximal part of the thigh, was rapidly (< 2s) inflated immediately preceding the MVC₁₅

contraction. A few seconds after the end of the contraction the cuff was deflated (for details see (25)). A rest period of 8 min was maintained between MVC₁₅ contractions.

Electrical stimulation

Electrical stimulation, with rectangular pulses of 100 μ s, was applied using a computer - controlled constant current stimulator (Digitimer DS7H, Digitimer Ltd., Welwyn Garden City, UK). Surface electrodes (self - adhesive, 13 \times 5 cm, Schwa - Medico, The Netherlands) were placed on the anterior surface of the right upper leg, one was placed \sim 1 cm proximal to the superior margin of the patella, and another was placed on the upper part of the thigh. To ensure that at each knee angle a similar muscle mass was stimulated, the stimulation current was increased until maximal torque measured in response to a 150 Hz tetanic contraction (1s duration) was \sim 65%MVC at each knee angle (range of currents used: at 30°: 70-120; at 60°: 105-160, and at 90°: 110-195 mA). At this torque level \sim 65% of the muscle fibers were assumed to be maximally activated at each knee angle. This intensity was chosen as pilot studies had shown that maximal mVO₂ (see below) did not increase when tetanic torque levels were increased over 50%MVC at any knee angle. All (superficial) muscle fibers under the NIRS optodes (see below) were considered to be maximally activated throughout a 10s tetanic contractions at each knee angle. During continuous high frequency stimulation, excessive force loss may occur due to failure of electrical propagation, particularly during stimulation at high (e.g. 150 Hz) frequencies (5). To prevent high frequency fatigue during the 10s tetanic contraction a stimulation frequency lower than 150 Hz was used. However, this lower stimulation frequency still elicited 90-95% of the 150Hz values (13). To accommodate for the rightward shift of the stimulation frequency - force relationship as muscle length decreases (10), stimulation frequencies of 70 Hz at the 30°, 60 Hz at the 60° and 50 Hz at the 90° knee angle were selected. This ensured close to maximal forces (90 - 95% of the 150 Hz values (13) at each knee angle while at the same time preventing the occurrence of high frequency fatigue during the relatively long (10s) contractions.

Arterial occlusion was achieved by the rapid inflation of the pressure cuff immediately preceding the 10s tetanic contraction. The cuff was deflated a few

seconds after the end of the contraction. A rest period of 8 min was maintained between the tetanic contractions of 10s duration. At the end of each 10s tetanic contraction the half - relaxation time was determined. Half - relaxation time was defined as the time taken for the torque to decrease to 50% of active torque after the last stimulation pulse.

In pilot studies we controlled for both MVC and fatigue. An 8 min rest period between contractions was shown to be sufficient to prevent fatigue. This was tested by additional measurements 8 min after the completion of the protocol. At each knee angle, maximal torques as well as torque decline and $m\dot{V}O_2$ (see below) of the voluntary and electrically evoked contractions were similar during these additional measurements compared measurements made earlier during the protocol.

Near - infrared Spectroscopy (NIRS)

Muscle oxygen consumption of the RF, VL and VM muscle was determined during isometric knee extension contractions by use of a continuous - wave near - infrared spectrophotometer (Oxymon, Artinis Medical Systems, Zetten, The Netherlands), which generated light at 780 and 850 nm (47) and was regularly calibrated by Artinis Medical Systems (Zetten, The Netherlands). The three optode sets were each fixed in a mould with an inter - optode distance of 45 mm (penetration depth of the tissue was therefore ~ 22.5 mm). The moulds were secured to the upper leg with elastic Velcro straps such that the optodes did not move during contraction. The optodes were positioned over the center of the muscle bellies.

With NIRS, the tissue oxygenation level can be measured non - invasively, in fact, the optical change in density of the tissue is measured, which, by modification of the Lambert - Beer law (27), can be transformed into the change in concentrations of oxyhaemoglobin ($[O_2Hb]$) and -myoglobin ($[O_2Mb]$) and deoxyhaemoglobin and -myoglobin ($[HHb]$ and $[HMb]$). Due to the overlap in the spectrum, haemoglobin and myoglobin cannot be measured separately; O_2Hb and HHb will respectively denote the oxygenated and deoxygenated form of both proteins in the present study. Blood volume and total Hb (the sum of $[O_2Hb]$ and $[HHb]$) of the m. quadriceps were kept constant by inflation of a pressure cuff. The rates of concentration changes

of O₂Hb and HHb, or the slopes (Fig. 1) of the [O₂Hb] - and [HHb] - time curves, represent the oxygen consumption per unit time in the muscle (mVO₂, see also (15)).

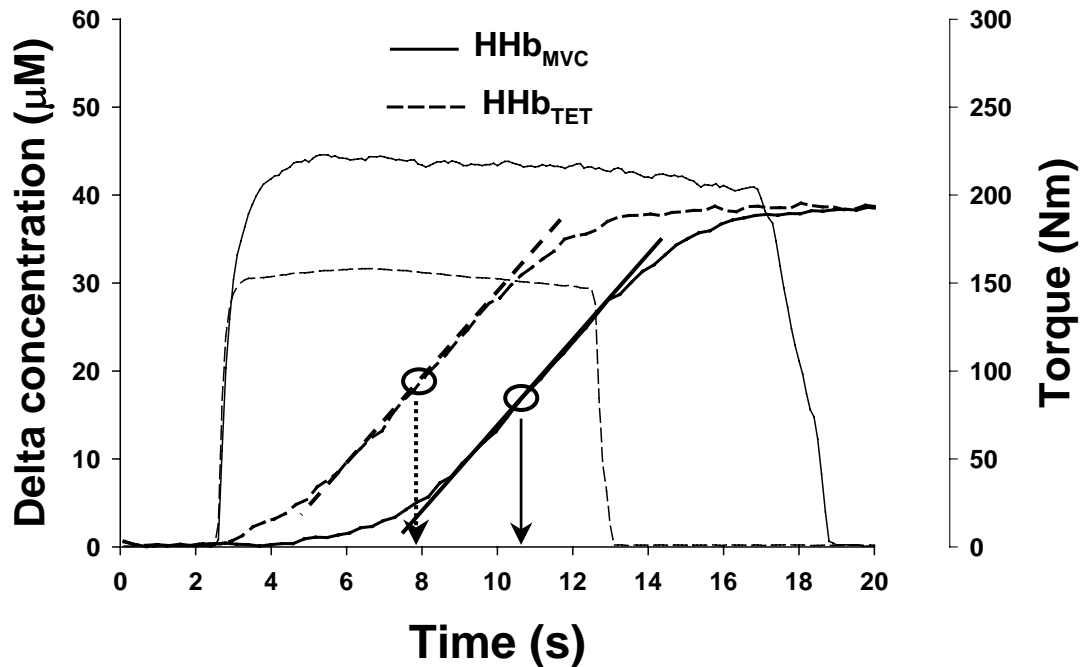


Fig. 1. Torque and near - infrared spectroscopy signals of the vastus medialis (VM) muscle during a long lasting MVC of 15s duration (MVC₁₅ – thick trace) and a tetanic contraction (- - thin trace) at the 30° knee angle. Changes in concentration of deoxygenated haemoglobin (HHb, HbO₂ [mirror image of HHb] is not displayed for reasons of clarity) are shown for the MVC₁₅ (– trace) and the tetanic contraction (- - trace). Maximal oxygen consumption (mVO_{2max}) occurred when the slope for the MVC₁₅ and tetanic contraction was maximal (denoted by the two circles). In this example, mVO_{2max} was similar for voluntary and tetanic contractions (9.5 and 9.7% maximal deoxygenation · s⁻¹, respectively). However mVO_{2max} of the tetanic contraction was reached earlier after torque onset compared with mVO_{2max} of MVC₁₅ and was measured at t = 7.9 and 10.8s (dotted and solid arrow, respectively)

In previous studies (11) a linear portion of the slopes of the [O₂Hb] - and [HHb] - time curve has been found. As contraction intensity increases, this linear portion became shorter, and when approaching maximal levels (especially at flexed knee angles), the duration of this linear portion is only a few seconds. Therefore, the peak slopes of the filtered [O₂Hb] - and [HHb] - time signals (which were not statistically different in any muscle at any of the knee angles) were averaged, and this absolute value, mVO_{2max}, was calculated for every contraction. This usually led to a slightly (a few percent) higher value compared to when the average slope over the several seconds linear portion was used. The time to mVO_{2max} after torque onset was defined

as t_{max} , this allowed us to quantify the differences in time that existed between knee angles in instances for which the [HHb] - and [O₂Hb] - time curves were linear. For an appropriate comparison of mVO₂ between knee angles, the instance at which they are compared should be the same. As t_{max} was smallest and similar across knee angles for the tetanic contractions (see results), mVO₂ was also measured at this point in time (4.5s) for the MVC₁₅ contractions. Furthermore, mVO₂ and mVO_{2max} were expressed relative to maximal deoxygenation (% · s⁻¹) which is standard procedure (11). By expressing mVO₂ in this manner, potential differences in subcutaneous fat between muscles, which greatly affect NIRS measurement sensitivity (34, 46), are accounted for. Maximal deoxygenation is defined as the absolute difference in [O₂Hb] (and [HHb]) when virtually all O₂Hb is converted into HHb. In this study, maximal deoxygenation was determined at the 60° knee angle in rest as it was demonstrated previously to be independent of torque level (0 - 100%MVC) and knee angle (11). NIRS - data were sampled at 10 Hz and stored on computer disc. At the end of the experimental session, skinfold measurements were taken with the subjects seated in the dynamometer with a 60° knee angle. Skinfold thickness was measured using Harpenden skinfold caliper (John Bull; British Indicators, West Sussex, UK), and the average of three measurements taken at the position of the optodes on each muscle was used.

Although we realized maximal voluntary activation might be lower during a 15s MVC compared with a brief 3 - 5s MVC (38), by using subjects with a previously confirmed (11, 12) very high ability for voluntary activation (~ 97%) in combination with strong verbal encouragement, maximal torques and therefore maximal mVO₂ values obtained were as close to the absolute maximum as can be expected during an MVC₁₅.

Statistics

All results are presented as mean ± SD. Tests for significance were performed using a two-factor (knee angle, contraction type) repeated measures ANOVA. If significant main effects were observed, Bonferroni tests were performed for post hoc analysis. The level of significance of all statistical analyses was set at $P < 0.05$.

Results

Torque

MVC, MVC₁₅ and tetanic torque levels were significantly ($P < 0.05$) greater at the 60° compared with the 30 and 90° knee angle (Table 1). As expected, maximal torque achieved during the MVC₁₅ was significantly lower than during the brief MVC at each knee angle (Table 1). When expressed as a percentage of the brief MVC, maximal torque during the MVC₁₅ at the 30° knee angle ($96.2 \pm 8.4\%$) was significantly greater compared with 60° ($87.0 \pm 6.1\%$), and tended ($P = 0.078$) to be greater compared with the 90° knee angle ($83.9 \pm 11.8\%$), indicating the less demanding nature of isometrically contracting at extended compared with flexed knee angles.

As we intended, at each knee angle torque during 150 Hz tetanic stimulation was $\sim 65\%$ MVC ($65.7 \pm 3.9\%$ MVC, across knee angles). As expected torque reached (1s after the start of stimulation) during the 10s tetanic contractions at each knee angle was slightly, yet significantly lower than the 150 Hz torque (Table 1), but relative torque levels were similar between knee angles (30°: 63.0 ± 12.0 ; 60°: 60.9 ± 6.9 , and 90°: $61.6 \pm 3.8\%$ MVC).

Table 1. Torque

| | Knee angle (deg) | | |
|----------------------------|------------------|------------------|----------------|
| | 30° | 60° | 90° |
| MVC, N · m | 193.6 ± 39.8 | 311.0 ± 50.5 * | 206.3 ± 24.7 |
| MVC ₁₅ , N · m | 184.8 ± 32.9 # | 271.7 ± 52.9 * # | 172.3 ± 27.6 # |
| 150 Hz, N · m | 130.4 ± 24.3 | 202.0 ± 26.6 * | 132.1 ± 15.5 |
| Tetanic contraction, N · m | 120.6 ± 25.4 ‡ | 186.9 ± 25.5 * ‡ | 126.6 ± 12.4 ‡ |

All values are means ± SD, n = 8. Maximal voluntary isometric knee extension (MVC), Maximal force during MVC of 15s duration (MVC₁₅), 150 Hz, and tetanic contractions of 10s duration for the 30, 60 and 90° knee angle, respectively. *Significantly greater than 30 and 90° knee angle ($P < 0.05$). #Significantly lower than MVC torque. ‡ Significantly lower than 150 Hz torque.

Half - relaxation times for the 10s tetanic contractions were not different between the 30° (96.4 ± 10.1), 60° (101 ± 10.5) and 90° knee angle (100.3 ± 10.4 ms), respectively. Torque decrease from contraction onset to the end of the 10s tetanic contraction was not significantly different ($P > 0.05$) between knee angles either (30°:

5.0 ± 6.9%; 60°: 5.6 ± 4.7; and 90°: 9.2 ± 4.2%). For the MVC₁₅ contractions however, for torque decrease over the first 10s a main effect for angle was found that seemed to be the result of a greater decrease in torque at 90° (16.0 ± 5.9%) compared with the 30° (9.2 ± 5.8%) and 60° (9.5 ± 5.3%) knee angle. Across knee angles for the first 10s, the overall torque decreases for the MVC₁₅ (11.6 ± 6.3%) was significantly greater compared to that of the tetanic contractions (6.6 ± 5.5%). An example of the time course of the torque traces for both voluntary and tetanic contractions is shown in figure 2.

Maximal deoxygenation and skinfold

As expected, skinfold thickness was significantly higher for the RF muscle (10.3 ± 3.4 mm) compared with the VL (8.0 ± 2.8) and VM (7.4 ± 2.0) muscle and consequently measured maximal deoxygenation of the RF (41.3 ± 16.7 μM) was significantly lower compared with both VL (54.6 ± 16.1), and VM (59.6 ± 9.6) muscles. Moreover, a significant negative linear relationship between skinfold thickness and maximal deoxygenation (r² = 0.77, averaged across muscles) was found.

Table 2. Maximal muscle oxygen consumption during prolonged maximal voluntary contractions.

| | Maximal muscle oxygen consumption (%maximal deoxygenation · s ⁻¹) | | |
|------------------|---|------------------------------|------------------------------|
| | Muscle | | |
| | Rectus femoris | Vastus lateralis | Vastus medialis |
| Knee angle (deg) | | | |
| 30° | 8.2 ± 2.3 * # [4.4 - 11.1] | 11.6 ± 3.2 * [7.0 - 16.2] | 11.9 ± 2.1 * [9.5 - 15.4] |
| 60° | 9.0 ± 2.9 # [3.0 - 12.2] | 12.4 ± 3.7 [6.9 - 16.6] | 12.4 ± 3.4 [7.1 - 16.2] |
| 90° | 10.6 ± 1.6 † [8.0 - 13.4] | 15.4 ± 2.4 [9.9 - 17.6] | 13.4 ± 2.7 [8.3 - 16.7] |

All values are means ± SD, range between brackets, n = 8. Maximal muscle oxygen consumption for the rectus femoris (RF), vastus lateralis (VL) and vastus medialis (VM) muscle during prolonged (15s) maximal voluntary contractions (MVC₁₅). * Significantly lower than the 90° knee angle. # Significantly lower than the VL and VM muscle. † Significantly lower than VL. There was also a trend (P = 0.062) for mVO_{2max} of the RF to be lower than the VM at the 90° knee angle.

Maximal muscle oxygen consumption and time to maximal muscle oxygen consumption

As expected, during both MVC₁₅ and the tetanic contractions mVO_{2max} was significantly lower at the 30° compared with the 90° knee angle (Table 2; Fig. 3). Furthermore, for both contraction types, mVO_{2max} at the 60° knee angle was not significantly different ($P > 0.05$) from mVO_{2max} at either 30 or 90° knee angles (Table 2; Fig. 3). In addition, across knee angles, mVO_{2max} of the RF was significantly lower compared with the VL and VM muscle for both the MVC₁₅ and tetanic contractions.

mVO_{2max} was not different between MVC₁₅ and tetanic contractions at any of the knee angles. However, an overall repeated measures ANOVA revealed that t_{max} was significantly smaller for the tetanic contraction compared with MVC₁₅. The significant interaction effect of contraction type \times knee angle, and subsequent Bonferroni post - hoc testing indicated that at 30° t_{max} was significantly smaller for the tetanic compared to the MVC₁₅ contractions, and strongly tended ($P = 0.056$) to be smaller at the 60° knee angle. At the 90° knee angle however, t_{max} was similar between the electrically induced and voluntary contractions (Fig. 4).

As could subsequently be expected, t_{max} was significantly smaller for the MVC₁₅ contractions at the 90° knee angle compared to the 30° and 60° knee angle. In addition, during the tetanic contractions t_{max} was similar between all knee angles and muscles (Fig. 4).

Muscle oxygen consumption between knee angles

By measuring mVO_2 for the MVC₁₅ contractions at t_{max} for the tetanic contractions we accounted for the knee angle - dependent difference in t_{max} for the MVC₁₅ contractions (see methods). At this point in time, mVO_2 for the MVC₁₅ contraction at 30° was significantly lower than 90°, with a tendency ($P = 0.08$) for mVO_2 at 30° to be lower than the 60° knee angle as well (Fig. 5).

In the present study, we wanted an easy and quantifiable manner in which to compare the difference in mVO_2 between extended (30°) and flexed (90°) knee angles for the two contraction types. For both the MVC₁₅ and the electrically evoked contractions, the mVO_2 at 90° was set to 100%. At the 30° knee angle, mVO_2 for the

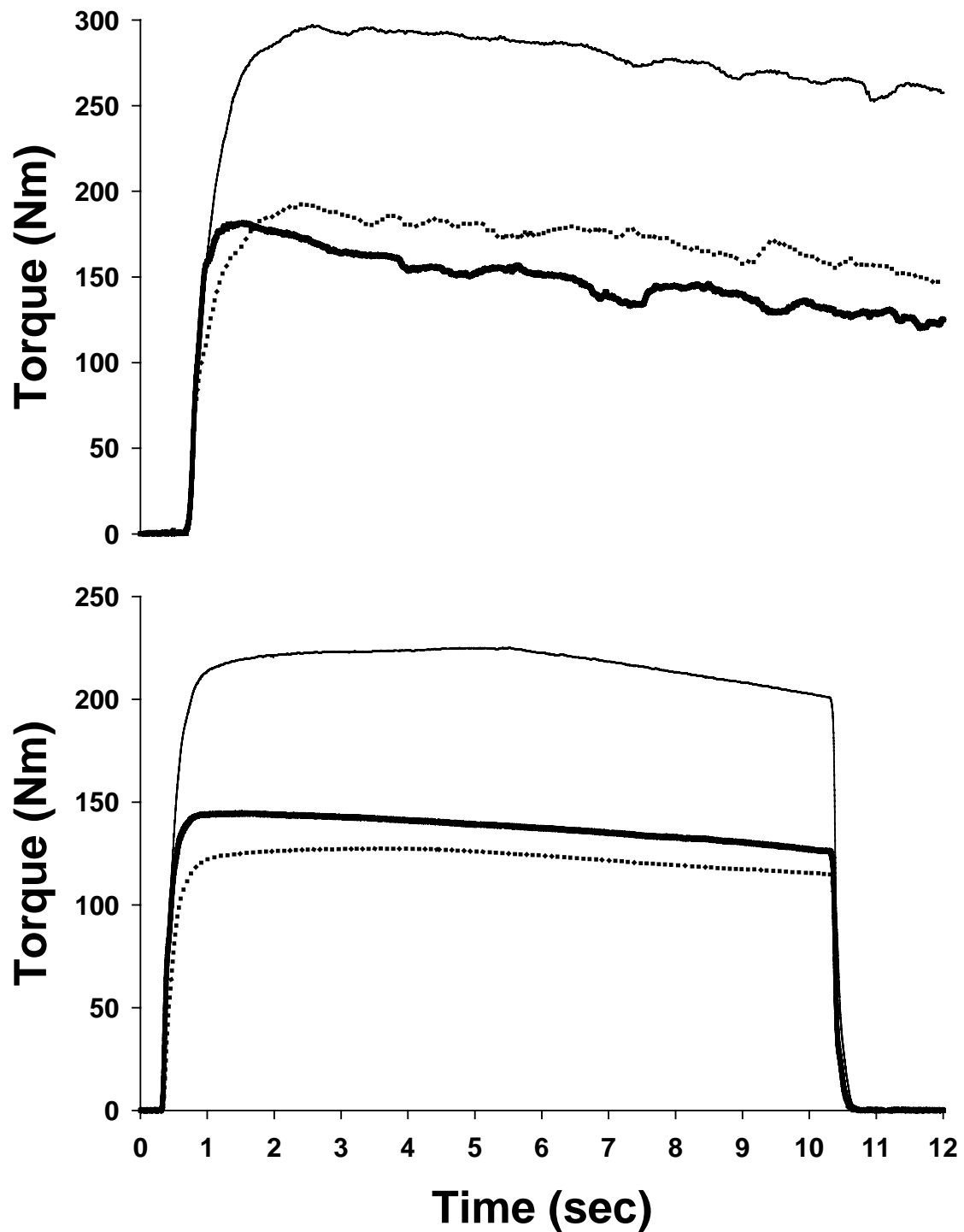


Fig. 2. To give an example of the time course of typical torque traces the maximal voluntary knee extension torque during the 15s duration contractions (MVC_{15}) for the 30° (...), 60° (—, thin trace) and 90° (—, thick trace) knee angle are shown in the upper panel for one subject. The lower panel shows typical torque traces for the 10s tetanic contractions at 30° (...), 60° (—, thin trace) and 90° (—, thick trace) knee angles for another subject.

MVC₁₅ contractions for the RF, VL and VM muscle respectively was only 48.8 ± 8.7 , 45.7 ± 8.2 , and $53.6 \pm 7.9\%$ of the mVO₂ obtained at the 90° knee angle. For the electrically induced contractions, where t_{max} was similar across knee angles, at 30° mVO_{2max} for the RF, VL and VM was 89.4 ± 10.9 , 78.5 ± 8.9 , and $70.4 \pm 8.1\%$ of mVO_{2max} at 90° respectively.

Discussion

In the present study mVO₂ was assessed during MVC₁₅ and tetanic contractions of the knee extensors at 30, 60 and 90° knee angles. The first finding of the present study was that mVO₂ of the knee extensors is ~ 20% less during tetanic contractions at 30° (extended) compared to 90° (flexed) knee angles. This difference must originate from differences that reside in the muscle. Secondly, during voluntary contractions mVO₂ of the knee extensors is ~ 50% less at 30° compared to 90° knee angles. Therefore ~ 60% ($[50 - 20] / 50 * 100\%$) of the total knee angle - dependent difference in mVO₂ during voluntary contractions must originate from knee angle - dependent differences in voluntary activation.

Torque

Throughout the tetanic contractions at all knee angles, we succeeded in maintaining > 50% of MVC, with similar values being reached between knee angles at the start ($61.9 \pm 8.7\%$) and end of the contraction ($57.7 \pm 8.1\%$ MVC, across knee angles). Pilot studies had shown no further increase in mVO_{2max} above 50% MVC for the tetanic contractions at any knee angle. Furthermore, torque decrease and half - relaxation times at the end of the tetanic contractions were similar and small between knee angles. Taken together, this indicated that the tetanic contractions induced a maximal metabolic strain on the knee extensors at each knee angle with only minimal fatigue.

As expected (43) and reported previously (24), relative torque levels reached during the MVC₁₅ contractions were slightly lower compared with that obtained during brief (3 - 4s) MVCs. The relative torque level at the 30° knee angle tended to be higher than the 90° knee angle. Furthermore, for the relative torque decrease of the MVC₁₅ contraction, the main effect of angle that was found seems to be a consequence of the relatively larger relative torque decrease found at the 90° (~ 16%) compared to the

30° knee angle (~ 9%). These above findings suggest that contracting at 90° is more demanding compared to the 30° knee angle. This is also what the subjects report and suggests that the effort and possible neural drive needed to reach MVC during 15s contractions is less at 30° compared with 90° knee angles, as seen in a previous study of ours (24). Although speculative, the relative ease with which an MVC is reached and maintained at the 30° compared to 90° knee angle could be the consequence of: (i) greater inability to access the full maximal torque capacity (MTC) at 30° vs. 90° and/or (ii) other factors such as a potentiation mediated greater contractile response at 30° vs. 90° requiring less voluntary drive at the 30° knee angle.

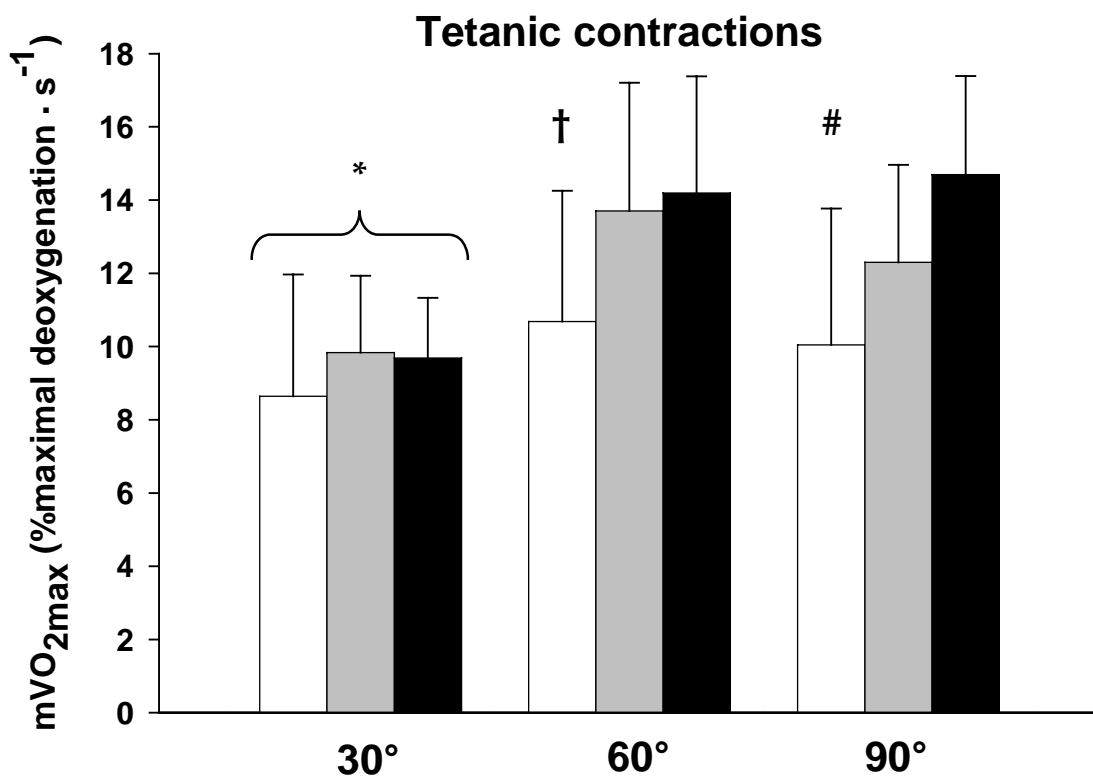


Fig. 3. Maximal muscle oxygen consumption (mVO_{2max}) for the 30° (on the left), 60° (mid) and 90° (right) knee angle for the rectus femoris (RF, white bars), vastus lateralis (VL, grey bars) and vastus medialis (VM, black bars) for 10s tetanic contractions. * Significantly lower than the 90° knee angle. # Significantly lower than VL and VM. † Significantly lower than VL.

Although we exclusively used subjects with a previously proven high ability for maximal voluntary activation (11, 12), substantial underestimations of the MTC are

possible (14). Due to the limitations of the superimposed twitch technique, it cannot be excluded that this underestimation of MTC is relatively greater at the 30° knee angle (11). In that case it may be comparatively easier to reach and maintain an MVC for 15s at extended vs. flexed knee angles. With respect to potentiation, the effect of muscle length - dependent potentiation on neural activation during MVCs is not known. However, a greater twitch contractile response has been reported at short vs. long muscle length after an MVC (50). Combined with other reports of a potentiation - mediated greater contractile response at short muscle length (36, 38, 40), it is not unlikely that potentiation occurs during the course of an MVC₁₅ contraction. A greater effect of potentiation at the 30° vs. the 90° knee angle during MVC₁₅ contractions could allow for less voluntary drive whilst eliciting a similar contractile response during prolonged contractions at 30°. In addition to the possible underestimation of the MTC, a higher potentiation at short muscle lengths would also contribute to making it relatively easier to achieve and sustain the MVC₁₅ at 30° knee angle.

In previous studies by our group we measured the contribution of co-activation during brief MVCs (12) and prolonged isometric contractions of the knee extensors (25) and found it to be very small (5-10%). As these findings are in accordance with previous findings (31, 37), we did not measure co-activation in the present study and considered its effect upon the relative large differences in mVO₂ between knee angles to be minimal.

Muscle oxygen consumption between knee angles

The less demanding nature of contractions at 30° compared to the 90° knee angle is reflected in the lower mVO_{2max} at 30° compared to the 90° knee angle reached during MVC₁₅ contractions (Table 2), a finding that was expected and reaffirms results of an earlier study of ours (11). The current findings are also in line with the consistent findings (18, 25, 35, 36) of a greater endurance at extended knee angles.

For the MVC₁₅ contractions, the time to mVO_{2max} was significantly smaller for the 90° compared to both 30 and 60° knee angles (Fig. 4). After accounting for this difference in t_{max} between knee angles, in line with our hypothesis, mVO₂ at 30° was significantly lower than at 90°, and tended ($P = 0.08$) to be lower compared to the 60°

knee angle (Fig. 5). Expressed as a percentage, at 30° $m\dot{V}O_2$ was only ~ 50% of the $m\dot{V}O_2$ at the 90° knee angle, comparable to the knee angle dependent differences in $m\dot{V}O_2$ during submaximal (10 - 70%max) contractions in our earlier study (24).

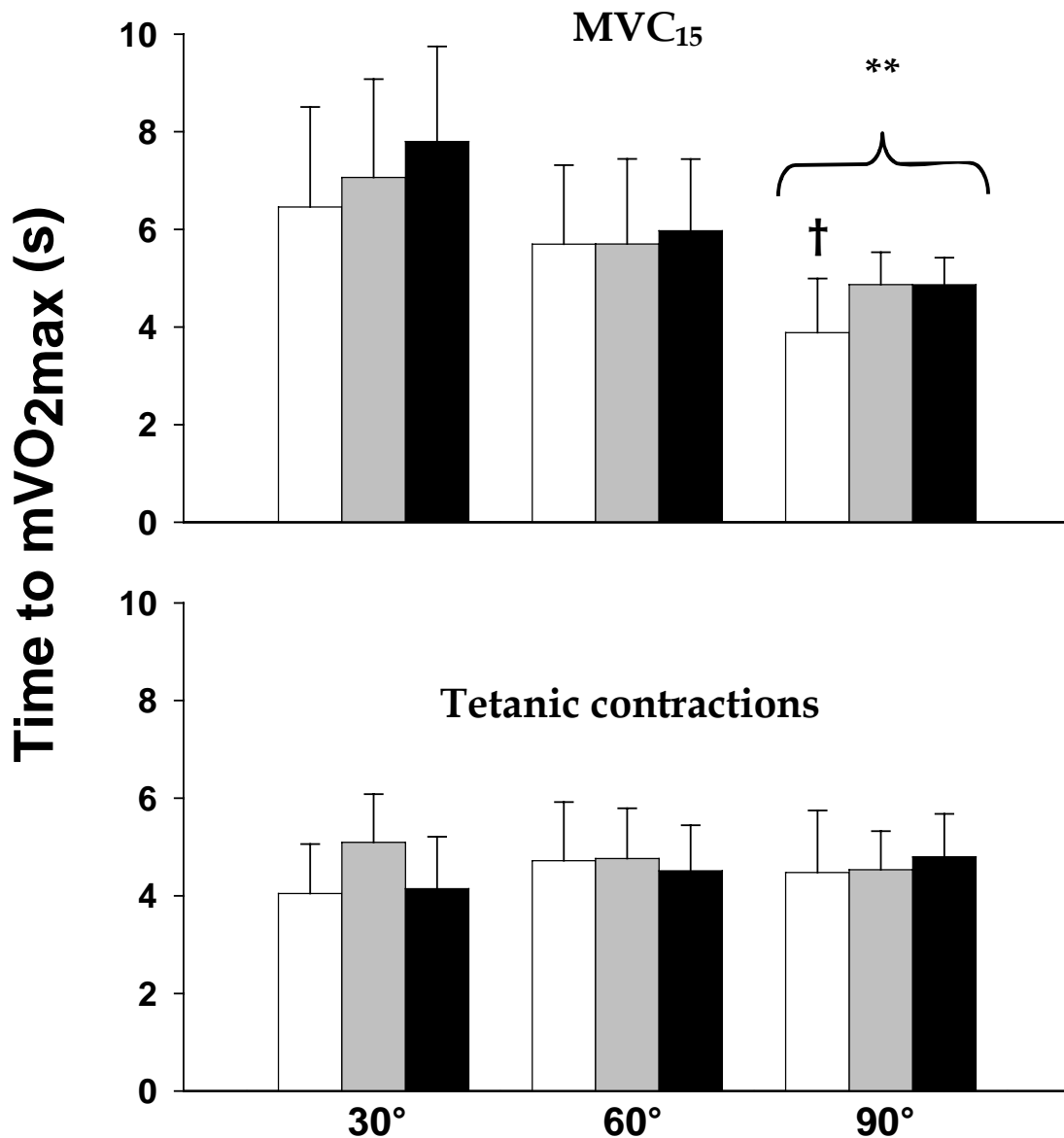


Fig. 4. Time to maximal muscle oxygen consumption from contraction onset (t_{max}) for the 30° (on the left), 60° (mid) and 90° (right) knee angle for the rectus femoris (RF, white bars), vastus lateralis (VL, grey bars) and vastus medialis (VM, black bars) for the 15s MVC (MVC₁₅, top panel) and the 10s tetanic contractions (lower panel). ** Significantly smaller than 30 and 60° knee angle. In addition, for the 15s there was a trend ($P = 0.065$) for 60° to be smaller than 30°. † Significantly smaller than the vastus lateralis muscle. Furthermore, for the MVC₁₅ at the 90° knee angle there was a trend ($P = 0.067$) for t_{max} of the RF to be smaller than VM. Across muscles, t_{max} was significantly smaller for the tetanic contractions compared with the MVC₁₅ at 30°, and a strong trend ($P = 0.056$) was found for t_{max} to be smaller at the 60° knee angle as well.

In the present study we used $m\dot{V}O_2$ as a measure of energy consumption as it was measured under conditions of complete arterial occlusion. This was considered to be a reasonable assumption because of the tight coupling of electron transfer to oxidative phosphorylation, which in turn is recognized to be regulated by some index of the energetic state, such as the free ATP / ADP ratio (6). Consequently, the lower $m\dot{V}O_2$ during isometric knee extension contractions at 30° strongly suggests that energy consumption and the accompanying metabolic changes (decrease in pH and increase of inorganic phosphate) were lower compared to 90°. Although it is impossible to exclude a knee angle-dependent difference in the contribution of non-oxidative energy sources, we have no indications that anaerobic energy consumption is higher at short than longer muscle lengths and could therefore compensate for the lower $m\dot{V}O_2$ at shorter muscle length. It would seem more likely that energy consumption from anaerobic and aerobic sources are closely coupled (17, 33, 44).

As mentioned above, at 30° $m\dot{V}O_2$ was only ~ 50% of the $m\dot{V}O_2$ at the 90° knee angle. This difference may partially result from knee angle - dependent differences in neural activation. During tetanic stimulation of the knee extensors any knee angle - dependent effects of neural activation are abolished. For the electrically induced contractions, $m\dot{V}O_{2max}$ at 30° was ~ 20% lower (across muscles) compared to the 90° knee angle. Because this ~ 20% lower $m\dot{V}O_2$ cannot be related to differences in neural activation between knee angles, it must arise from knee angle - dependent differences that reside in the muscle. Furthermore, 60% ($[50 - 20] / 50 * 100\%$) of the difference in $m\dot{V}O_2$ found during maximal voluntary contractions is therefore related to a lower neural activation at 30°.

When placing the knee extensors under a maximal metabolic strain by applying tetanic stimulation at each knee angle, $m\dot{V}O_2$ is ~ 20% less at 30° vs. 90°. As $m\dot{V}O_2$ is a measure of energy consumption (11), the lower $m\dot{V}O_2$ at 30° implies a muscle length - dependency of energy consumption. If energy consumption were indeed related to the number of force producing cross - bridges, it would depend on actin - myosin filament overlap and hence would be greatest at optimum muscle length. Previous studies on isolated preparations have shown however, that only at *very* low muscle lengths did energy consumption indeed decrease compared with

that found at optimum muscle length (9, 42). Unfortunately, a precise comparison of the above results with the present *in vivo* findings is not straight forward due to the lack of consensus regarding the internal moment arm of the knee joint (4, 22, 26, 51).

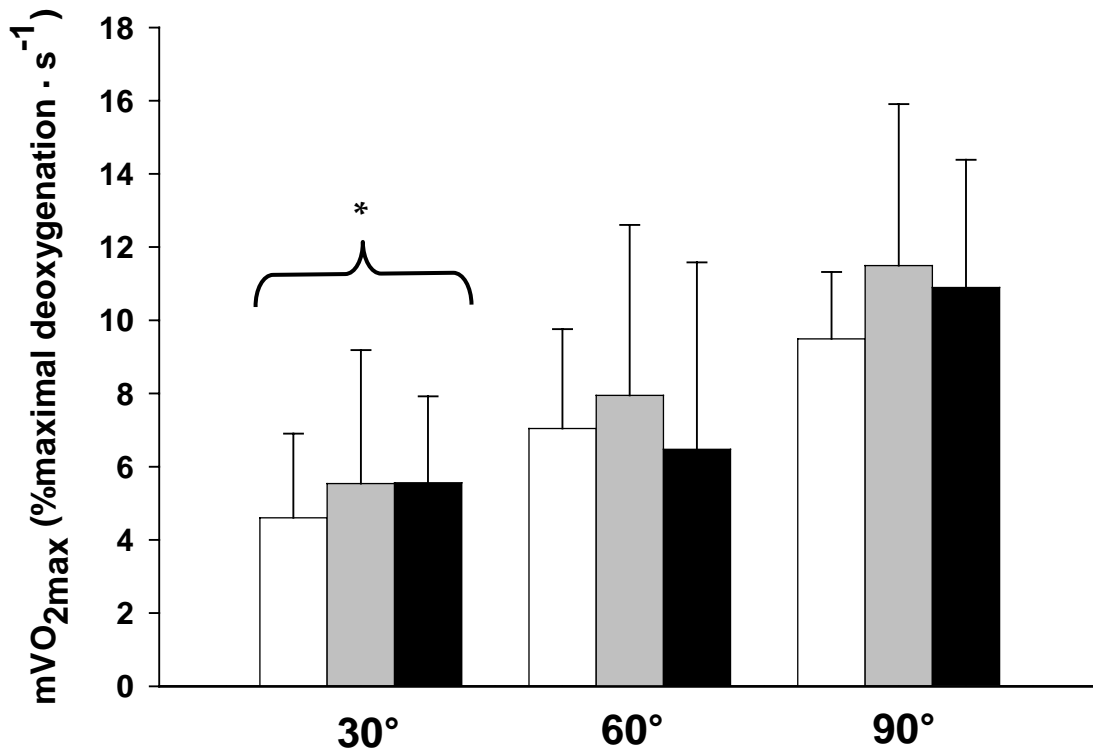


Fig. 5. Muscle oxygen consumption (mVO_2) for the rectus femoris (RF, white bars), vastus lateralis (VL, gray bars) and vastus medialis (VM, black bars), measured ~ 4.5 s after torque onset for the long lasting maximal voluntary contractions (MVC_{15}) at the 30° (left), 60° (middle) and 90° (right) knee angle. *Significantly smaller than the 90° knee angle. There was also a tendency ($P = 0.08$) for mVO_2 at 30° to be smaller compared to the 60° knee angle.

Nevertheless, using those moment arms (4) that result in the largest difference in force production between 30° and 60° knee angles, at the 30° knee angle at least $\sim 65\%$ of force relative to 60° is reached. Hence, it is unlikely that the knee extensors are operating at a length that corresponds to the *very* short muscle length mentioned in for example the study of de Haan et al. (9). It is likely however, that the knee extensors operate on the ascending limb of the length - tension relationship at 30° , whereas 60 and 90° knee angles will be closer to optimal muscle length (20, 45). In addition, using ^{31}P - MRS, Sacco et al. (41) and Baker et al. (3) reported a similar rate of ATP use at short and optimum length of the human tibialis anterior muscle during

contraction. An unchanged energy consumption at short compared with optimum muscle length has also been reported in isolated mammalian (35, 44) and frog muscle (1, 2, 21). As it is unlikely that the knee extensors are operating at the *very* short muscle length mentioned in de Haan et al. (9), the underlying cause(s) for the lower $m\dot{V}O_2$ at the 30° knee angle remains to be elucidated, as a lower number of cycling cross bridges at short muscle length seems improbable (44).

The energy cost of activation, which is ~ 30% at optimal muscle length (19) must also be taken into account when investigating length - dependent differences in energy consumption. Although Ca^{2+} release is lower at lower muscle lengths (7), in the present study a higher stimulation frequency (70 Hz) was used at short compared to long muscle lengths (50 and 60 Hz). This would more than compensate for any lower Ca^{2+} released per stimulation pulse at lower muscle lengths. Hence, a lower energy consumption at short compared to long muscle lengths seems unlikely to be a consequence of a lower energy cost of activation at short muscle length. Therefore, although the exact reason for the ~ 20% lower $m\dot{V}O_2$ found during tetanic contractions at extended knee angles remains to be elucidated, the present results clearly indicate that it resides within the muscles.

The ~ 50% lower $m\dot{V}O_2$ at 30° compared to 90° knee angles during voluntary contractions and the ~ 20% lower $m\dot{V}O_2$ found during tetanic contractions at extended knee angles contribute to explaining the consistent finding (18, 25, 32, 36) of a greater endurance at extended knee angles. The greater endurance at short vs. long muscle length is not restricted to the knee extensors. It appears to be a general phenomenon as it has also been reported for the m. tibialis anterior (15, 41), elbow flexors (29), and the m. triceps surae ((42), unpublished observations).

In conclusion, $m\dot{V}O_2$ is ~ 50% less during MVC_{15} contractions, and ~ 20% less during tetanic contractions of the knee extensors at extended vs. flexed knee angles. The ~ 20% lower $m\dot{V}O_2$ must be a consequence of knee angle - dependent differences originating within the muscle that remain to be elucidated. Therefore ~ 60% of the ~ 50% lower $m\dot{V}O_2$ during voluntary contractions is related to a lower neural activation at the extended knee angle.

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Summarizing discussion

In this thesis we set out to investigate the extent to which the central nervous system is able to adapt activation of the knee extensors to length - and fatigue induced changes of the muscles' contractile properties. Neural activation of the knee extensors was investigated using superimposed electrical stimulation and surface EMG; in addition the energy consumption of the superficial knee extensors was measured using near - infrared spectroscopy. Using this combination of methods insight was obtained in the underlying mechanisms that relate motor control during sub - maximal isometric contractions at different muscle lengths, which have been discussed in the corresponding chapters. Nevertheless, some other issues are worthwhile to address and are discussed below after a brief summary of the main findings of this thesis.

Summary

Knee extensor endurance during sustained isometric contractions had previously been shown to be greater at short vs. longer muscle lengths (8, 14). In **chapter 2** we investigated the role of central activation, using superimposed stimulation, and blood flow in muscle - length dependent endurance (11). Time to torque failure for intermittent isometric knee extensions was $\sim 60\%$ greater at the 30° (short muscle length) vs. the 90° (long muscle length) knee angle (0° = full extension). The use of a cuff, occluding blood flow to the knee extensors throughout the fatiguing contractions, eliminated any potential effects of knee angle - dependent differences in muscle perfusion. At torque failure central activation ratios obtained using superimposed stimulation were very high and similar at both knee angles. The greater endurance at 30° compared to 90° was therefore concluded not to originate from knee angle - dependent differences in central activation at torque failure. It was postulated that (some of) the differences in endurance between knee angles could be related to differences in metabolic cost at different knee angles.

This was confirmed as at extended knee angles fatigability (8, 14) and muscle oxygen consumption ($m\dot{V}O_2$, (4, 10)) during sustained submaximal isometric contractions were found to be less compared to flexed knee angles. Furthermore, at low torques $m\dot{V}O_2$ was reported to be less in the rectus femoris muscle than in the

vastus lateralis and medialis muscle (4). In **chapter 3** we hypothesized that these findings could be accounted for by a respective knee angle - and a muscle - dependent activation. By the simultaneous measurement of rectified surface EMG (rsEMG), as a measure of muscle activation, and mVO_2 during isometric contractions of the m. quadriceps, the contribution of knee angle - and a muscle - dependent activation could be determined. The results show that a tendency for less muscle activation (15 - 20%) contributes, but certainly cannot account for the substantially lower mVO_2 (~ 60%) at 30° vs. 60 and 90° knee angles. Moreover, at low contraction intensities a (s)lower (increase in) m. rectus femoris mVO_2 suggested a less intense activation of this muscle compared to the vasti.

Besides the reports of a muscle length - dependence of fatigue (6, 11, 13, 18), fatigue is also dependent on the relative force level during muscle contraction (7). Equal relative contraction intensities, derived by normalizing to the maximal voluntary contraction (MVC) at each knee angle, are often used to investigate and compare muscle fatigue at different knee angles (8, 14). If the MVC is used as a gold standard, any differences in maximal voluntary activation during the MVC between knee angles will erroneously create differences in the relative contraction intensities between knee angles. This could make it relatively easier to perform isometric contractions at one knee angle compared to another. Generally, the maximal voluntary activation during an MVC, measured using superimposed stimulation, is used as an indication of a subjects' ability to reach the maximal force capacity of the muscle. However, due to uncertainties in the determination of this maximal force capacity (5, 15), the relationship between relative voluntary force and voluntary activation was investigated in **chapter 4**. This type of relationship can only be properly evaluated using subjects with exceptional ability for maximal voluntary activation of the knee extensors. Already at a relative torque level of ~ 79%MVC a voluntary activation level of ~ 92% was calculated in these subjects. A subsequent ~ 34% increase in EMG was measured for a further ~ 18% torque increase for which a mere ~ 6% increase in voluntary activation was calculated. It was therefore concluded that using the conventional interpolated twitch torque method, voluntary activation does not represent relative voluntary torque. In addition, small increases

in relative torque lead to disproportionate (relative to torque) increases in EMG as MVC is approached.

Despite an as accurate as possible determination of the maximal torque capacity of the muscle in **chapter 3** using superimposed stimulation, the findings in **chapter 4** illustrated that very small differences in voluntary activation could still coincide with substantial differences in relative torque. Since $m\dot{V}O_2$ was measured at equal relative intensities at different knee angles in **chapter 3**, a knee angle - dependent effect of neural activation could not be excluded. To definitively exclude this potential knee angle - dependent effect of neural activation on the measurement of $m\dot{V}O_2$, in **chapter 5** $m\dot{V}O_2$ was measured during electrically evoked and maximal voluntary quadriceps contractions. By using this approach insight into the mechanisms limiting endurance at different knee angles was gained. Any differences in $m\dot{V}O_2$ between knee angles during the electrically evoked contractions could be attributed to differences that originated at the level of the muscle. Additional differences in $m\dot{V}O_2$ between knee angles found during the long lasting (15s) MVCs are then a consequence of processes (e.g. maximal voluntary activation) that may affect neural activation. It was hypothesized that during electrical stimulation where $m\dot{V}O_2$ is maximal, the difference in $m\dot{V}O_2$ between extended (30°) and flexed (90°) knee angles would be less compared to MVC contractions. During voluntary contractions $m\dot{V}O_2$ was found to be ~ 50% less at extended vs. flexed knee angles. The ~ 20% lower $m\dot{V}O_2$ found during electrically induced contractions must originate from differences that reside within the muscle. Therefore, 60% ($[50 - 20] / 50 * 100\%$) of the total $m\dot{V}O_2$ difference during voluntary contractions is the result of a lower neural activation at extended knee angles.

What causes the muscle length - dependent fatigue?

Fatigability had been shown to be less at short compared to longer muscle lengths for the m. tibialis anterior (6, 18), the elbow flexors (13), and knee extensors (8, 11, 14) and therefore appears to be a general phenomenon.

In **chapter 2** differences in blood flow and central activation at the point of torque failure were eliminated as possible causes for the lower fatigability at short

muscle length. Potentiation was suggested to be responsible for some of the lower fatigability at short muscle length by Place et al. (17). In their study a greater twitch potentiation at short compared to long muscle length was reported after a voluntary sustained contraction of the knee extensors at 20%MVC. They suggested that a slower development of central fatigue during the sustained contraction at short compared to longer knee extensor muscle length could partly explain the lower fatigability at shorter muscle length.

Futhermore in a recent study by MacNaughton and MacIntosh (12) part of the muscle length - dependence of fatigue was attributed to a relatively greater underestimation of active force at long vs. short muscle lengths. As the underestimation was of minor importance at lengths below optimum muscle length, these findings were of little relevance to the experiments described in the present thesis and other studies investigating fatigue of the knee extensors (8, 14). The knee angles investigated in these experiments most likely correspond to a knee extensor muscle length at or below optimum (9, 21).

Another explanation for the lower fatigability at short vs. longer muscle length could be a reduced energy requirement at short muscle length as at short muscle lengths there would be a lower number of energy consuming cross - bridges (6). However, in studies on isolated mammalian muscle (16, 20) and frog muscle (1, 2) energy consumption was reported to be similar at short and optimum muscle length. Only at *very* short muscle length has energy consumption been reported to be lower compared to optimum muscle length (3, 19). Although the knee extensors studied in this thesis are probably operating on the ascending limb of the length - tension relationship at 30° (9, 21), it seems unlikely that this corresponds to the *very* short muscle length mentioned by de Haan et al. (3) and Sandberg and Carlson (19). Nevertheless mVO_2 , established as a measure for energy consumption (4), was shown to be ~ 60% less at 30° (short muscle length) vs. 60 and 90° (long muscle length) angles in **chapter 3**. Furthermore, in the same chapter it was estimated that at most 10% of this difference in mVO_2 could be accounted for by a difference in muscle activation, measured using surface EMG.

It was shown in **chapter 4** that minor differences in voluntary activation could coincide with substantial differences in relative torque. As explained above, this may have contributed to the ~ 60% difference in $m\dot{V}O_2$ reported between knee angles in **chapter 3**. In **chapter 5**, using only highly motivated subjects capable of high levels of voluntary activation at each knee angle, a smaller yet still substantial ~ 50% difference in $m\dot{V}O_2$ was found between knee angles during long lasting MVCs. In the same chapter neural activation was eliminated as a confounding factor by using electrically evoked contractions of the knee extensors. A ~ 20% lower $m\dot{V}O_2$ remained at the 30° compared to the 90° knee angle during electrical stimulation. The exact mechanism that causes this ~ 20% difference is unclear and remains to be elucidated, but must reside at the level of the muscle as the use of electrical stimulation excluded any knee angle - dependent differences in neural activation. However, 60% ($[50 - 20] / 50 * 100\%$) of the total $m\dot{V}O_2$ difference during voluntary contractions must be the result of a lower neural activation at extended knee angles. The consistent previous findings of a similar energy consumption at short and optimum muscle lengths (1, 2, 16, 20) seem difficult to reconcile with the ~ 20% difference in $m\dot{V}O_2$ reported in this thesis and needs further investigation. The ~ 20% difference in $m\dot{V}O_2$ found in the present thesis however, could account for the consistent finding (8, 11, 13, 14, 17) of a lower fatigability at short compared to long muscle lengths.

De invloed van kniehoek op de prestatie van de m. quadriceps femoris

In dit proefschrift is onderzocht in hoeverre het centraal zenuwstelsel de aansturing kan aanpassen aan lengte - en vermoeidheids geïnduceerde veranderingen van de contractiele eigenschappen van de kniestrekkers. De neurale aansturing van de kniestrekkers werd onderzocht door gebruik te maken van elektrische stimulatie en oppervlakte EMG; tevens werd het energieverbruik van de oppervlakkige kniestrekkers gemeten met behulp van "near - infrared" spectroscopie. Door deze methoden te combineren kon inzicht verkregen worden in de relaties tussen de onderliggende mechanismen die de motoriek reguleren op verschillende spierlengten tijdens submaximale isometrische contracties, welke zijn behandeld in de bijbehorende hoofdstukken. Er zijn echter nog andere onderwerpen die niet aan bod zijn gekomen in de desbetreffende hoofdstukken, deze zijn wel de moeite van het bespreken waard en komen na een korte samenvatting van de voornaamste bevindingen van dit proefschrift ter sprake.

Samenvatting

Het uithoudingsvermogen van de kniestrekkers tijdens langdurig volgehouden isometrische contracties is groter op lange spierlengte in vergelijking tot korte spierlengte (8, 14). In **hoofdstuk 2** werd de rol van centrale activatie, door gebruik te maken van elektrische stimulatie boven op vrijwillige contracties, en doorbloeding op spierlengte afhankelijke vermoeidheid onderzocht. De volhoudtijd voor intermitterende contracties was $\sim 60\%$ langer op een kniehoek van 30° (korte spierlengte) vergeleken met die van 90° (lange spierlengte, 0° = volledig gestrekt). Het gebruik van een manchet, waarmee de bloedtoevoer naar de kniestrekkers werd gestopt tijdens contracties, verhinderde mogelijke effecten van kniehoek afhankelijke verschillen in doorbloeding. Op het moment van opgave waren "central activation ratios", gemeten m.b.v. elektrische stimulatie, zeer hoog en vergelijkbaar tussen kniehoeken. Het grotere uithoudingsvermogen op 30° vergeleken met 90° , kon dus niet veroorzaakt zijn door kniehoek afhankelijke verschillen in centrale activatie op het moment van opgave. Er werd gesuggereerd dat (een gedeelte van) de verschillen

in uithoudingsvermogen gerelateerd konden worden aan verschillen in energieverbruik op verschillende kniehoeken.

Onderzoek naar de kniestrekkers waarbij submaximale isometrische contracties werden volgehouden bevestigden deze suggestie. Daarin werd in een gestrekte kniehoek stand (30°) een lagere vermoeibaarheid (8, 14) en verminderd zuurstofverbruik van de kniestrekkers (mVO_2 , (4, 10)) gevonden vergeleken met kleine kniehoeken. Tevens is aangegeven dat op lage momenten mVO_2 minder was in de rectus femoris spier vergeleken met de vastus lateralis en medialis spier (4). In **hoofdstuk 3** hadden wij als hypothese dat bovenstaande bevindingen verklaard zouden kunnen worden door een respectievelijke kniehoek en spierafhankelijke activatie. Door tegelijkertijd oppervlakte EMG, als maat voor spieractiviteit tijdens de contractie, en mVO_2 tijdens isometrische contracties van de m. quadriceps te meten, kon het aandeel van kniehoek en spierafhankelijke activatie bepaald worden. Uit de resultaten kwam naar voren dat op een kniehoek van 30° een tendens voor een lagere spieractiviteit (15 - 20%) slechts een klein deel van het volledige aanzienlijk (~ 60%) lagere mVO_2 kon verklaren vergeleken met de 60 en 90° kniehoeken. Bovendien suggereerde een lagere, en langzamer toenemende mVO_2 van de m. rectus femoris op lagere momenten, een minder intense activatie van deze spier ten opzichte van de vasti.

Hoofdstuk 4: behalve de bevindingen van een spierlengteafhankelijke vermoeidheid in de literatuur (6, 11, 13, 18), is vermoeidheid ook afhankelijk van het relatieve krachtniveau tijdens de contractie (7). Een gelijk relatief contractie niveau, verkregen door te normaliseren naar de maximaal vrijwillige contractie (MVC) op elke kniehoek, wordt vaak gebruikt om de vermoeidheid op verschillende kniehoeken met elkaar te vergelijken (8, 14). Als de MVC gebruikt wordt als gouden standaard, zal een verschil in maximaal vrijwillige activatie tijdens een MVC voor een verschil zorgen in de relatieve intensiteit tussen kniehoeken. Dit zou het relatief makkelijker kunnen maken om op één kniehoek een isometrische kniestrekking uit te voeren in vergelijking tot op een andere. Over het algemeen wordt de maximaal vrijwillige activatie, gemeten m.b.v. elektrische stimulatie, gebruikt als indicatie voor de vaardigheid van een proefpersoon om zijn/haar maximaal krachtgenererend

vermogen te bereiken. Echter, wegens onzekerheden in de bepaling van dit maximaal krachtgenererend vermogen (5, 15) is de relatie tussen relatieve vrijwillige kracht en vrijwillige activatie onderzocht in **hoofdstuk 4**. Deze relatie kan alleen goed geëvalueerd worden door gebruik te maken van proefpersonen met een uitzonderlijk vermogen voor maximaal vrijwillige activatie van de kniestrekkers. Al op een relatief moment van $\sim 79\%$ MVC werd een vrijwillig activatie niveau van 92% berekend in deze proefpersonen. Een daaropvolgende $\sim 34\%$ toename in EMG werd gemeten voor een toename van $\sim 18\%$ in moment waarvoor slechts een toename van $\sim 4\%$ vrijwillige activatie werd berekend. Hieruit werd geconcludeerd dat bij gebruik van de conventionele geïnterpoleerde "twitch" methode, de vrijwillige activatie niet overeenkomt met relatief vrijwillige moment. Tevens, dat als de MVC bijna bereikt wordt een relatief kleine toename in moment gepaard gaat met een disproportionele (relatief t.o.v. moment) toename in EMG.

Ondanks een zo nauwkeurig mogelijke bepaling van het maximaal genererend vermogen van de spier in **hoofdstuk 3** terwijl gebruik werd gemaakt van elektrische stimulatie, bleek uit de resultaten van **hoofdstuk 4** dat kleine verschillen in vrijwillige activatie gepaard konden gaan met aanzienlijke verschillen in relatief moment. Aangezien $m\dot{V}O_2$ gemeten werd op gelijke relatieve intensiteiten op verschillende kniehoeken in **hoofdstuk 3**, kon een kniehoek - afhankelijk effect van neurale aansturing dus nog steeds niet helemaal worden uitgesloten worden. Om dit mogelijk kniehoek afhankelijke effect van neurale aansturing op de bepaling van $m\dot{V}O_2$ definitief uit te sluiten, werd in **hoofdstuk 5** $m\dot{V}O_2$ gemeten tijdens elektrisch gestimuleerde (en maximaal vrijwillige contracties) van de kniestrekkers. Hierdoor werd inzicht verkregen in de mechanismen die het uithoudingsvermogen beperken op verschillende kniehoeken. Verschillen in $m\dot{V}O_2$ tussen kniehoeken tijdens de elektrische contracties moeten toegeschreven worden aan spierlengte - afhankelijke verschillen in intrinsieke spiereigenschappen. Overige verschillen in $m\dot{V}O_2$ tussen de kniehoeken die gevonden werden tijdens de langdurige (15s) MVCs moeten dan het gevolg van processen (bijv. maximaal vrijwillige activatie) die van invloed zijn op de neurale aansturing. Wij hadden als hypothese dat tijdens elektrische stimulatie waar $m\dot{V}O_2$ maximaal is, het verschil in $m\dot{V}O_2$ tussen niet - gestrekte en gestrekte

kniehoeken minder zou zijn in vergelijking tot tijdens vrijwillige maximale contracties. Tijdens de langdurige vrijwillige contracties werd er een ~ 50% lagere mVO_2 gevonden op gestrekte (30°) vergeleken met niet - gestrekte (90°) kniehoeken. De ~ 20% lagere mVO_2 gevonden tijdens elektrisch gestimuleerde contracties moet zijn oorsprong vinden in spierlengte - gerelateerde verschillen van de spier. Dit betekent dat 60% ($[50 - 20] / 50 * 100\%$) van het totale verschil in mVO_2 tijdens vrijwillige contracties het gevolg is van een lagere neurale aansturing in gestrekte kniehoek stand.

Wat veroorzaakt de spierlengte afhankelijke vermoeidheid?

De vermoeibaarheid van de spier is minder op korte spierlengte in vergelijking tot lange spierlengte in de m. tibialis anterior (6, 18), de elleboogflexoren (13) en de kniestrekkers (8, 11, 14) en het lijkt dan ook een algemeen verschijnsel te zijn.

In **hoofdstuk 2** zijn voor de kniestrekkers verschillen in doorbloeding en centrale aansturing op het moment dat de proefpersoon de contractie niet meer vol kan houden uitgesloten als mogelijke oorzaken voor de lagere vermoeibaarheid op korte spierlengte. Potentiëatie zou een deel van de lagere vermoeibaarheid op korte spierlengte t.o.v. lange spierlengte kunnen verklaren volgens Place et al. (17). In dat onderzoek werd een grotere potentiëatie van de "twitch" op korte spierlengte in vergelijking tot lange spierlengte gevonden na een vrijwillig isometrisch volgehouden contractie op 20% MVC. Zij wijten een deel van de lagere vermoeibaarheid op korte spierlengte aan een langzamere ontwikkeling van centrale vermoeidheid tijdens de volgehouden contractie op korte spierlengte in vergelijking tot lange spierlengte.

Verder werd in een recent onderzoek van MacNaughton en MacIntosh (12) een deel van de spierlengte afhankelijkheid van vermoeidheid toegeschreven aan een relatief grotere onderschatting van de actieve kracht op langere in vergelijking tot kortere spierlengte. Aangezien deze onderschatting van geringe invloed was op spierlengten korter dan optimum lengte, lijken deze bevindingen weinig relevant voor de experimenten zoals beschreven in dit proefschrift, alsmede in dat van andere onderzoeken waarin de vermoeidheid van de kniestrekkers is onderzocht (8, 14). De

knieshoeken die in deze en onze experimenten zijn onderzocht komen waarschijnlijk overeen met een lengte van de kniestrekkers die zich op, dan wel onder optimumlengte bevinden (9, 21).

Ook een verminderd energieverbruik kan een verklaring zijn voor de lagere vermoeibaarheid op korte spierlengte vergeleken met lange spierlengte. Op korte spierlengte zouden er minder energie consumerende “cross - bridges” aangehecht zijn in vergelijking tot lange spierlengte (6). Echter, in onderzoek naar geïsoleerde spieren van zoogdieren (16, 20) en kikkerspieren (1, 2) was het energieverbruik op korte spierlengte en optimum spierlengte vergelijkbaar. Alleen op *hele* korte spierlengte is er een lager energieverbruik op korte in vergelijking tot lange spierlengte gevonden (3, 19). Ook al zijn de kniestrekkers in dit proefschrift op 30° actief op het stijgend been van de kracht - lengte relatie (9, 21), toch is het onwaarschijnlijk dat dit overeenkomt met de *hele* korte spierlengte zoals genoemd door de Haan (3) en Sandberg en Carlsen (19). Desalniettemin is in **hoofdstuk 3** en 5 op de 30° knieshoek (korte spierlengte) een ~ 60% lagere mVO₂ (een maat voor het energieverbruik (4)) gevonden vergeleken met de 60 en 90° knieshoek (langere spierlengte). Verder werd in hetzelfde hoofdstuk ingeschat dat maximaal ~ 10% van dit verschil in mVO₂ verklaard kon worden door een verschil in spieractivatie, gemeten door middel van EMG.

In **hoofdstuk 4** is gerapporteerd dat kleine verschillen in vrijwillige activatie tijdens het bepalen van het MVC gepaard konden gaan met aanzienlijke verschillen in relatief moment. Zoals hiervoor is uitgelegd, kan dit bijgedragen hebben aan het verschil van ~ 60% in mVO₂ dat is gevonden tussen knieshoeken in **hoofdstuk 3**. In **hoofdstuk 5** werd, na het meten van goed gemotiveerde proefpersonen met een uitzonderlijke vaardigheid voor vrijwillige activatie, een kleiner maar nog altijd substantieel verschil van ~ 50% in mVO₂ gevonden tussen knieshoeken tijdens langdurige MVCs. In hetzelfde hoofdstuk werd de invloed van een mogelijke knieshoekafhankelijke neurale aansturing uitgesloten door gebruik te maken van elektrisch opgewekte contracties van de kniestrekkers. Er werd een ~ 20% lagere mVO₂ gevonden bij 30° vergeleken met 90° tijdens elektrisch gestimuleerde contracties. Het exacte mechanisme dat dit verschil van ~ 20% veroorzaakt is

onbekend en moet nog gevonden worden, maar het is wel duidelijk dat deze zich op het niveau van de spier bevindt aangezien het gebruik van elektrische stimulatie kniehoek afhankelijke verschillen in neurale aansturing heeft uitgesloten. Dit betekent wel dat 60% ($[50 - 20] / 50 * 100\%$) van het totale verschil in mVO_2 tijdens vrijwillige contracties het gevolg is een lagere neurale aansturing in gestrekte kniehoek stand. De consistente bevindingen van een vergelijkbaar energieverbruik op korte en optimum lengte (1, 2, 16, 20) lijkt moeilijk te rijmen met het verschil van ~ 20% in mVO_2 gevonden in dit proefschrift, en verlangt derhalve meer onderzoek. Het in dit proefschrift gevonden verschil van ~ 20% in mVO_2 kan echter wel bijdragen aan een verklaring voor de consistente bevindingen van een lagere vermoeibaarheid op korte in vergelijking tot langere spierlengte (8, 11, 13, 14, 17).

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The thank you word

Het zit er dik in dat dit één van de eerste 4-5 bladzijden zijn die je aan het lezen bent van dit proefschrift, wat jammer nou! Dus hup, terug naar de inleiding en ga de overige ~145 bladzijden maar lezen! Dan kan je namelijk pas echt snappen waarvoor de onderstaande mensen in de onderstaande tekst staan aangegeven ...

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