Chapter 1

General introduction
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By voluntarily activating our skeletal muscles, muscle force is produced and transmitted through the tendon to the bone resulting in joint movement. The extent to which skeletal muscles are voluntary activated depends on the force that has to be produced. A number of studies have investigated muscle activation during isometric exercise, but little is known regarding muscle activation during dynamic exercise. Therefore, in this thesis the activation of the quadriceps muscle is investigated during dynamic exercise and a comparison is made with the activation during isometric exercise. This introductory chapter firstly describes the functional unit of movement and the processes of regulating force. Thereafter the techniques used in this thesis to measure muscle activation are described and a brief literature overview is given of muscle activation during exercise. Finally, the specific research questions addressed in this thesis are given.

Motor units and force regulation

The motor unit is the functional unit of movement. It consists of a motoneuron and all the muscle fibres which are innervated by this motoneuron (Figure 1.1). The number of motor units and fibres in a muscle varies from muscle to muscle. The quadriceps muscle may consist of several hundred motor units, each motor unit innervating several thousand fibres. The muscle fibres themselves consist of thick (myosin) and thin (actin) myofilaments, organized as repeated subunits along the length of the fibre. These subunits are called sarcomeres and are the active force generating units of a muscle fibre. When the motoneuron sends a single pulse (action potential) down the axonal branches, all muscle fibres of that motoneuron are activated and will contract at virtually the same moment.

The activation pattern (defined as the number of pulses and the interpulse distance) by which a motoneuron drives its muscle fibres mainly determines the properties of the muscle fibres in the motor unit. It is therefore generally assumed that all muscle fibres of a given motor unit have the same contractile and metabolic properties and that the fibres are of the same type, i.e. contain the same myosin heavy chain proteins (Saltin and Gollnick, 1983). On the basis of physiological properties human muscle fibres can be divided into two major groups, type I and type II fibres. Based on the type of myosin heavy chain, type II fibres can be subdivided into two subgroups, IIA and IIX (Ennion et al, 1995; Sant'Ana Pereira et al, 1995b). Type I fibres are in general slow-oxidative, type IIA fast-oxidative-glycolytic and type IIX fast-
glycolytic fibres, but fibres can also contain more types of myosin heavy chains (hybrid fibres, e.g. type IIAX fibres), leading to a continuum in fibre properties.

![Figure 1.1. Concept of the motor unit. A motor unit consists of the motoneuron and all the muscle fibres which are innervated by this motoneuron. From Jones et al (2004).](image)

There are two main mechanisms by which the force of a muscle can be regulated. The first mechanism is orderly recruitment which is based on the size of the motoneurons. According to the size principle of Henneman et al (1965) the smaller motoneurons, innervating the slow, fatigue resistant type I fibres, are already recruited at low levels of force. As force increases more motoneurons are recruited and the larger motoneurons, innervating the fast, fast fatigable type IIA and IIX fibres, are recruited. Since the small motoneurons innervate only a small number of muscle fibres (small motor units) and the larger motoneurons innervate a larger number of muscle fibres (larger motor units), the orderly recruitment of motoneurons could also be referred to as the orderly recruitment of motor units. The second mechanism of force regulation is by changing the discharge rate of each already activated motor unit, which is referred to as rate coding (e.g. (Person and Kudina, 1972)). During voluntary movements, both recruitment and rate coding are used simultaneously to regulate force. The extent to which these two force regulating mechanisms are used varies between muscles. Recruitment is the main mechanism to regulate force in the larger
proximal muscles, whereas the smaller distal muscles rely heavily on rate coding (Kukulka and Clamann, 1981).

In addition to these two mechanisms to regulate force, the force produced by a muscle is influenced by the length of the muscle, the contraction mode (isometric, shortening or lengthening) and the contraction history. Firstly, according to the force–length relationship force declines when a muscle is at shorter or at longer length compared to its optimum length for force production, at which overlap between actin and myosin filaments is optimal (Gordon et al., 1966; Rassier et al., 1999). Secondly, according to the force–velocity relationship skeletal muscles are intrinsically weaker during shortening and conversely stronger during lengthening compared to isometric contractions (Edman et al., 1978; Katz, 1939). Moreover, skeletal muscle force is known to be depressed during and following shortening and enhanced during and following lengthening, phenomena referred to as respectively shortening induced force depression and lengthening induced force enhancement (Abbott and Aubert, 1952; Edman et al., 1982; Granzier and Pollack, 1989; Marechal and Plaghki, 1979). Thus, the relation between force production and muscle activation is influenced by the contraction mode and muscle length. Therefore, the purpose of the present study was to investigate whether muscle activation is different between isometric and dynamic (shortening and lengthening) contractions, and during isometric contractions at different muscle lengths.

In the present thesis muscle activation was determined for the quadriceps, which consists of four muscle heads: vastus lateralis (VL), vastus medialis (VM), rectus femoris (RF) and the vastus intermedius (VI). All quadriceps muscle heads converge into the patellar tendon and the whole is referred to as the muscle-tendon complex. Length changes of the quadriceps muscle-tendon complex occur when the knee angle is varied. It should, however, be noted that induced changes in the length of the muscle-tendon complex are not equivalent to length changes of the muscle fibres, since the quadriceps muscle fibres are arranged at an angle to the line of action of the muscle (i.e. pennation angle), and because of the compliance of the patellar tendon, part of the length change is taken up by the tendon. Similarly, since the direction of force generation of the muscle fibres is not the same as the direction of pull of the muscle-tendon complex, the force produced by the contraction of the quadriceps muscle fibres is not equivalent to the force output of the muscle-tendon complex. Moreover, the compliant tendon will stretch during contraction of the muscle fibres, thereby determining the length of the muscle fibres and their force output based on the force–length relationship. The compliant tendon also means that the muscle fibres do
not ‘see’ the same velocity of the movement as the whole muscle tendon complex when the muscle is shortening or lengthening. The effect of this is to preserve force when the whole complex is shortening and reduce force during lengthening compared to the forces that would be generated by the fibres alone moving at the same speeds.

The geometric arrangement of muscle fibres at different knee angles has been studied in vivo for the VL muscle (Fukunaga et al., 1997; Ichinose et al., 1997). When the knee angle was passively extended from 10° above to 10° below the optimal knee angle for force production, fascicle (i.e. a group of muscle fibres) length was decreased by ~5 % (from 115 to 110 mm) and pennation angle increased by ~10 % (from 15.5 to 17°). By extending the knee angle passively from 30° above to 30° below the optimal knee angle, fascicle length decreased by ~20 % (from 120 to 100 mm) and pennation angle increased by ~25 % (from 14 to 19°). Fascicle length decreased more (over the same range) when voluntary contractions were performed, for example fascicle length decreased from 115 to 90 mm (~25 %) at 10 % MVC. Moreover, larger pennation angles were observed during contractions at 10 % MVC, up to 21° at a knee angle 30° below optimum. A limitation of the present thesis was that, due to methodological considerations, most of the data were obtained by inducing only small variations in knee angle (20°), and thus muscle fibre length, during the dynamic contractions. During daily life movements such as walking and cycling, variations in knee angle were respectively ~60° and ~35°. Therefore, differences in muscle activation during isometric and dynamic exercise found in the present thesis may be an underestimation of the differences in daily life movement.

**Measuring muscle activation**

There are different ways to identify whether the muscle fibres within a muscle are, or have been, active during movement. The first method involves the measurement of metabolites that change with activity, using human muscle biopsy samples obtained at rest and following movement (Figure 1.2). Muscle contractile activity depends on the available energy in the form of ATP. Since skeletal muscle contains only a small amount of ATP, which would be used up within a few seconds during maximal activation, ATP has to be replenished rapidly. The main energy source during the first few seconds of exercise is phosphocreatine (PCr), which is therefore a temporary energy buffer. ATP is rapidly resynthesised from PCr, which is broken down to creatine (Cr) by donating a phosphate group (P) to ADP:

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\text{PCr} + \text{ADP} + \text{H}^+ \leftrightarrow \text{Cr} + \text{ATP}
\]
Another way to replenish ATP is the oxidation of glycogen and/or fat. During high-intensity exercise most of the energy comes from glucose, which is stored as intramuscular glycogen. During the sequence of reactions in which glycogen is oxidized into H₂O and CO₂, ATP is produced which can be used for exercise. During longer term low-intensity exercise the oxidation of fat, which is available to the muscle fibre as fatty acids, into H₂O and CO₂ also provides energy in the form of ATP. The degree of depletion of glycogen following exercise in different fibre types is frequently used as an indication of fibre activation (see (Saltin and Gollnick, 1983)). The glycogen content of different muscle fibres can be measured using the Periodic Acid Schiff (PAS) staining on cross sections of muscle biopsies. A decrease in the PAS staining intensity in a fibre during exercise compared to rest (pre exercise) is used as an indication that the fibre was activated. Rates of energy utilization are different in different fibre types. Moreover, other substrates such as glucose and fatty acids are also available in the muscle fibre, thus different muscle fibre types will use glycogen at different rates and in different proportion relative to the other substrates (Essén, 1978; Kernell et al, 1995). Therefore, the amount of glycogen depletion does not provide an indication of the extent by which muscle fibres have been activated. Moreover, the glycogen depletion method has the limitation that, due to the relatively high concentration of glycogen in human muscle fibres, changes are only detectable after some minutes of exercise. Therefore, recently a new method was developed to measure PCr and Cr in single fibre fragments, which were dissected from muscle biopsy samples (Karatzafiri et al, 1999; Sant'ana Pereira et al, 1995a). Similarly to the glycogen depletion method, a decrease in the PCr/Cr ratio during exercise compared to rest is taken as a marker for fibre activation. The advantage of this method is that a decrease in PCr is directly linked to the ATP resynthesis during exercise (Infante et al, 1965) and therefore the PCr/Cr ratio method is a useful indicator of fibre activation in very brief exercise (Beltman et al, 2004b).

Figure 1.2. Part of a freeze-dried muscle biopsy sample, with on top a dissected single fibre fragment of approximately 2 mm.
The second method to investigate muscle fibre activation is to record the electromyographic (EMG) activity of single motor units (Figure 1.3), using a needle or fine wire electrodes inserted into the muscle (e.g. de Luca et al, 1982; de Ruiter et al, 2004; Desmedt and Godaux, 1977; Milner-Brown et al, 1973; Person and Kudina, 1972). When an action potential (electrical signal) from the motoneuron reaches the membrane of a muscle fibre that is innervated by this motoneuron, the action potential is propagated along the length of the muscle fibre and calcium (Ca$^{2+}$) is subsequently released into the cytoplasm of the fibre. The released Ca$^{2+}$ leads to a series of events resulting in the attachment of the myosin to the actin filament forming the so called cross bridge. The sarcomeres generate force by the sliding of the actin filament towards the centre of the sarcomere, which is referred to as the sliding filament theory (Huxley, 2000). According to this sliding filament theory the force generated is proportional to the extent of overlap between actin and myosin. The action potentials propagating along the length of the muscle fibre introduce an electric current in the surrounding tissue and can therefore be measured using intramuscular needle or wire electrodes inserted into the muscle. The dominant spikes (motor unit action potentials) obtained with this method come from the muscle fibre closest to the tip of the electrode and reflect the discharge pattern of the motor unit to which that particular fibre belongs (see Figure 1.3).

![Figure 1.3. Recording of single motor unit activation. A, needle electrode with tip close to one fibre (shaded). B, electrical activation from a number of fibres with the shaded fibre giving the large regular spikes. From Jones et al (2004).](image)

The advantage of using fine wire electrodes, inserted into the muscle through a hypodermic needle which is subsequently withdrawn, instead of needle electrodes is that the position of the wires is relatively stable within the muscle belly during contractions and the method is painless. Besides the intramuscular EMG measurements, the electrical potentials can also be detected by the use of surface
electrodes placed on the skin near the activated muscle fibre. Since many muscle fibres from numerous motor units are located within a given recording area of the surface electrode, the surface EMG signal is a summation of all the motor unit potentials detected.

All methods described above to detect muscle activation have their advantages and limitations. The force produced by a single muscle is the result of the number of activated motor units and their discharge rates. The advantage of the intramuscular EMG method is that it is possible to discriminate between the relative contribution of the two force generating mechanisms. In theory this is also possible with the use of the PCr/Cr ratio method and the glycogen depletion method, since a faster and/or larger decline in PCr/Cr ratio and glycogen content could indicate rate coding. It is however difficult to make the distinction between recruitment and rate coding using the measurement of metabolites since single muscle fibre cannot be followed over time and there is considerable variation in the starting levels. With intramuscular EMG techniques, the same single motor unit can be studied repeatedly, which is therefore an advantage of this method. Furthermore, the number of biopsies which can be taken from one muscle (subject) is limited and the techniques to determine glycogen content and PCr/Cr ratios of single fibres are laborious. The advantage of measuring metabolites is that muscle fibre activation could be determined during contractions at high force levels, which is more difficult with the use of single motor unit EMG due to simultaneous discharging of many motor units in the area of the electrode (McComas, 1996). Therefore, motor unit discharge rates are often measured during low force contractions, implying that mainly the activation pattern of the type I fibres is determined and that it is difficult to assess the activation pattern of type II fibres. Moreover, using the glycogen depletion method or the PCr/Cr ratio method muscle fibre activation could be determined during dynamic contractions, which is also difficult with the use of intramuscular EMG, since the shortening of the muscle fibres can move the electrodes (McComas, 1996). Therefore, thus far motor unit discharge rates have mainly been measured during isometric contractions.

**Muscle activation during exercise**

A number of studies have, using the glycogen depletion method and more recently the PCr/Cr ratio method, demonstrated the orderly activation of the different fibre types with increasing force level, with the type I fibres activated at low force levels and, with higher forces, type IIA and subsequently IIAX fibres are activated (Beltman et al, 2004a; Gollnick et al, 1973; Gollnick et al, 1974; Vøllestad et al, 1984). It is however
not entirely clear to which extent recruitment and rate coding of the different fibre types, type IIAX and IIX in particular, are used at different exercise intensities.

Beltman et al (2004a) showed with the use of the PCr/Cr ratio method a predominant activation of type I fibres during repeated isometric contractions of the quadriceps muscle at low force (39% of the maximal voluntary contraction, MVC), whereas a substantial activation of type II fibres was only detected at the high exercise intensities (72% MVC and higher). In contrast, Vøllestad et al (1984) used the glycogen depletion method and found that both type I and II fibres were activated during a longer term dynamic cycling exercise at constant pedalling rate and at 75% of the maximal oxygen uptake ($V_{O2max}$). Since cycling at 75% $V_{O2max}$ requiring only ~40% of the maximal dynamic force exerted on the pedals (Sargeant and Jones, 1995), there seems to be an inconsistency between the studies of Beltman et al (2004a) and Vøllestad et al (1984) in the submaximal exercise intensity at which type II fibres are activated (expressed as percentage of the maximal muscle force). An explanation for the additional recruitment of type II fibres found in the study of Vøllestad et al (Vøllestad et al, 1984) but not in the study of Beltman et al (2004a) could be related to the methodologies used to determine muscle fibre activation (glycogen depletion method vs. PCr/Cr ratio method) or to type of exercise performed (dynamic vs. isometric) in the different studies. We therefore decided to compare the isometric exercise performed in the study of Beltman et al (2004a) with the dynamic exercise in the study of Vøllestad et al (1984). Thus, the first aim of this thesis was to assess type I and II fibre activation from the very beginning and throughout longer term cycling exercise (Chapter 2). Furthermore, by using both the new potentially more accurate PCr/Cr ratio method and the frequently used glycogen depletion method to indicate fibre activation, we aimed to compare both methods.

During the cycling exercise only shortening contractions are performed by the quadriceps muscle, whereas in daily life lengthening contractions are also performed. Therefore, we were interested to determine whether there are differences in muscle activation, defined as the number of motor units recruited and their discharge rates, between shortening, lengthening and isometric contractions. For this purpose we used intramuscular EMG to determine recruitment and discharge rates of single motor units. Although it is known from human experiments that maximal stimulated force is lower during shortening and higher during lengthening (de Ruiter et al, 1998; de Ruiter et al, 2000), the maximal voluntary lengthening force does not always exceed the isometric force, which has been attributed to the incomplete voluntary activation due to a neural inhibition mechanism (Babault et al, 2001; Beltman et al, 2004c; Pinniger et al, 2000;
Westing et al., 1990). Nevertheless, according to the force – velocity relationship skeletal muscles are intrinsically weaker during shortening and stronger during lengthening (Edman et al., 1978; Katz, 1939). In addition to these force – velocity related differences in intrinsic muscle strength, force is depressed and enhanced during and following respectively shortening and lengthening due to shortening induced force depression and lengthening induced force enhancement (de Ruiter and de Haan, 2003; de Ruiter et al., 1998; de Ruiter et al., 2000; Lee et al., 1999; Oskouei and Herzog, 2005; Oskouei and Herzog, 2006a).

A number of studies have investigated muscle activation during voluntary submaximal isometric, shortening and lengthening contractions with the use of single motor unit EMG. Although some studies reported a selective recruitment of fast motor units during lengthening contractions (Howell et al., 1995; Linnamo et al., 2003; Nardone et al., 1989), most studies found no reversal of the recruitment order during lengthening and shortening contractions (Kossev and Christova, 1998; Moritani et al., 1987; Pasquet et al., 2006; Søgaard et al., 1996; Tax et al., 1989). Moreover, motor unit discharge rates were found to be lower during submaximal lengthening compared to shortening and isometric contractions at the same absolute torque (Del Valle and Thomas, 2005; Howell et al., 1995; Kossev and Christova, 1998; Linnamo et al., 2003; Søgaard et al., 1996). In contrast, higher (Pasquet et al., 2006; Søgaard et al., 1998; Tax et al., 1989) and similar (Del Valle and Thomas, 2005; Søgaard et al., 1996) discharge rates were found during shortening compared to isometric contractions. In only a few studies the same single motor unit was followed during the different contractions (Howell et al., 1995; Pasquet et al., 2006; Stotz and Bawa, 2001) but in these studies force – velocity related differences in intrinsic muscle strength were not taken into account, resulting in differences in relative contraction intensity (i.e. force level as percentage of maximum) at the different contraction modes (isometric, shortening and lengthening). Therefore, the second aim of this thesis was to establish to which extent differences in quadriceps muscle activation (i.e. the number of motor units recruited and their discharge rates) between isometric, shortening and lengthening contractions could be fully explained by differences in intrinsic muscle strength based upon the force – velocity relationship (Chapter 3).

The effects of shortening induced force depression and lengthening induced force enhancement can be most clearly investigated during an isometric contraction following respectively shortening and lengthening. In this way, the depression of force following shortening (de Ruiter and de Haan, 2003; Lee and Herzog, 2003; Rousanoglou et al., 2007) and the enhancement of force following lengthening (Lee and
Herzog, 2002; Oskouei and Herzog, 2006a; Oskouei and Herzog, 2006b) have been demonstrated during voluntary contractions. Furthermore, muscle activation measured with the use of surface EMG during voluntary submaximal contractions was found to be higher following shortening (Rousanoglou et al., 2007) and lower following lengthening (Oskouei and Herzog, 2006a; Oskouei and Herzog, 2006b). There is however no information available regarding whether motor unit discharge rates are adapted to the lower and higher force capacities following respectively shortening and lengthening. Therefore, the third aim of the present study was to establish at the motor unit level whether quadriceps muscle activation was different during isometric contractions following shortening and lengthening compared to isometric reference contractions at the same joint angle (i.e. muscle length) and the same absolute torque (i.e. force times moment arm) (Chapter 4). Any change in activation immediately following shortening or lengthening may already have been present during the shortening or lengthening phase of the contraction and could therefore, in addition to the force – velocity related differences, contribute to potential differences in muscle activation between isometric, shortening and lengthening contractions.

Finally, differences in muscle activation can be present during submaximal contractions at different muscle lengths, since force is lower at muscle lengths below and above the optimum length for maximal force production (Gordon et al., 1966; Rassier et al., 1999). During contractions at the same relative (% MVC at each joint angle) torque Bigland-Ritchie et al (1992) and Del Valle & Thomas (2004) found no changes in discharge rate at short compared to long muscle lengths. However, since different populations were studied at short and long muscle lengths, it could not be excluded that in these studies additional motor units were recruited at low muscle lengths without the necessity of increasing the discharge rate of already activated motor units. Alternatively, an increase in discharge rate could have been masked by lower discharge rates of newly recruited motor units. There are only two studies in which the discharge behaviour of the same motor units was investigated at different muscle lengths. In the study of Christova et al (1998) higher motor unit discharge rates were found for the majority of the motor units studied at short compared to longer muscle lengths, during contractions at the same relative torque. In contrast, Pasquet et al (2005) found no changes in discharge rate between the different muscle lengths, however, discharge rates were obtained just above recruitment threshold and thus at different absolute and relative torques at the different muscle lengths. Additionally, in that study lower recruitment thresholds were found at the shorter compared to the longer muscle lengths, indicating that additional motor units were recruited. However,
both the ‘short’ and the ‘long’ muscle lengths in the study of Pasquet et al (2005) were probably on the ascending limb of the force – length relationship and therefore there is no information available whether motor unit recruitment thresholds are lower and discharge rates are higher at the muscle lengths above the optimum length. Moreover, the derecruitment threshold of the same motor units at different muscle lengths has never been studied before. The fourth aim of this thesis was therefore to investigate the effect of joint angle on the recruitment and derecruitment thresholds and discharge rates of the same single motor units during contractions at the same absolute torque (Chapter 5).
Outline of this thesis

Specific research questions addressed in the present thesis:

1. **What is the activation pattern of type I and II fibres from the very beginning throughout longer term exercise determined by measurement of the glycogen content and the PCr/Cr ratio and which method is more accurate in determining fibre activation?** In chapter 2 the experiment is described in which the glycogen content and the PCr/Cr ratio were determined, using muscle biopsies, in type I and type II fibres of the quadriceps muscle at rest and after 1, 10 and 45 min of cycling at 75% \( V_{O2\text{max}} \). Forces exerted on the pedals were measured, using an isokinetic ergometer, to determine the percentage of the maximal muscle force exerted on the pedals at the specific pedalling rate (90 rpm) during the imposed exercise intensity.

2. **Can the differences in muscle activity between isometric, shortening and lengthening contractions be fully explained by differences in intrinsic muscle strength based upon the force – velocity relationship?** Chapter 3 describes the experiment in which quadriceps muscle activation was investigated with the use of surface EMG and single motor unit EMG with the contractions in the different modes performed at the same percentage of the maximal torque capacity and comparable joint angle. Single motor unit EMG was recorded from the vastus lateralis muscle. Differences in maximal torque capacity between isometric, shortening and lengthening contractions were determined using superimposed electrical stimulation. The data in Chapter 3 as well as in Chapters 4 and 5 are obtained from the same experiments and subjects.

3. **Are there at the motor unit level differences in quadriceps muscle activation between isometric contractions following shortening and lengthening and isometric reference contractions?** In chapter 4 the experiment is described in which quadriceps muscle activation, quantified on the basis of surface EMG and single motor unit EMG, was assessed during isometric contractions following shortening and lengthening and during isometric reference contractions at the same joint angle and the same absolute torque. Single motor unit EMG was recorded from the vastus lateralis muscle.

4. **Are there differences in recruitment and derecruitment thresholds and discharge rates of the same single motor unit at different joint angles (i.e. muscle lengths) during submaximal isometric contractions at the same absolute torque?** In chapter 5 the experiment is described in which both single motor unit EMG and surface EMG are measured during submaximal isometric
contractions at different joint angles. Isometric contractions were performed at the same absolute torque and at joint angles at which the maximal torque capacities were less than at optimum angle. Single motor unit EMG was recorded from the vastus lateralis muscle.

Finally, in chapter 6 the main results are summarized and discussed.
References


