THE INTER-INDIVIDUAL VARIABILITY IN HUMAN MUSCLE STRENGTH AND IN THE RESPONSE TO RESISTANCE TRAINING

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PUBLICATIONS

The following parts of the thesis have been published or submitted for publication:

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Chapter 4: **Erskine RM**, Jones DA, Williams AG, Stewart CE & Degens H. What causes *in vivo* muscle specific tension to increase following strength training? *Exp Physiol* Submitted.

Other publications associated with the thesis:

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Seynnes OR, **Erskine RM**, Maganaris CN, Longo S, Simoneau EM, Grosset JF & Narici MV (2009). Training-induced changes in structural and mechanical properties of the patellar tendon are related to muscle hypertrophy but not to strength gains. *J Appl Physiol* 107(2): 523-30.

Abstracts and conference proceedings:

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Erskine RM, Jones DA & Degens H (2008). Quadriceps specific tension in young men is highly variable. *Proceedings of the 13th Annual Congress of the ECSS* 13:104P (Estoril, Portugal).

ABSTRACT

Inter-individual differences in strength are not fully explained by muscle size, suggesting that muscle specific tension [force per unit physiological cross-sectional area (PCSA)] varies between untrained individuals. Furthermore, many reports demonstrate greater gains in muscle strength than size following resistance training, thus indicating an increase in specific tension. Moreover, there is considerable variation in the response to training that may have a genetic origin. The aims of the work described in this thesis were *i)* to examine the degree of variability in muscle specific tension; *ii)* to investigate whether specific tension changed following resistance training; *iii)* to quantify the variability in the response of human muscle to resistance training; *iv)* to identify gene variants that may be associated with the variable training responses. In a group of untrained young men and before and after 9 weeks of resistance training, the quadriceps femoris (QF) muscle specific tension was calculated from the maximum isometric voluntary contraction (MVC) torque, taking into account the contribution of voluntary activation, antagonist muscle co-activation, moment arm length, QF volume, muscle fascicle length and pennation angle. Correcting for these factors made little difference to the between subjects variance of MVC torque, thus demonstrating that muscle specific tension varies considerably between individuals. Resistance training increased QF muscle force much more than PCSA, indicating that most of the increase in force was a result of increased specific tension. This increase was not associated with a change in myosin heavy chain expression and was not accompanied by an increase in single fibre specific tension, or an increase in power per unit muscle volume measured during isokinetic cycling. The results are consistent with an increase in lateral force transmission in the muscle. Substantial variation in the training responses was observed and the final part of this thesis is concerned with linking this variation with specific gene variants.

ABBREVIATIONS AND EXPRESSIONS

1 RM	Single repetition maximum
ACSA	Anatomical cross-sectional area
*f*CSA	Muscle fibre cross-sectional area
d_{PT}	Patellar tendon moment arm
F_t	Patellar tendon force
L_f	Muscle fascicle length
mATPase	Myosin adenosine triphosphatase
MRI	Magnetic resonance imaging
MVC	Maximum voluntary contraction
MyHC	Myosin heavy chain
PCSA	Physiological cross-sectional area
QF	Quadriceps femoris
RF	Rectus femoris
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
TFCP	Tibio-femoral contact point
VI	Vastus intermedius
VL	Vastus lateralis
VM	Vastus medialis
V_m	Muscle volume
W_{max}	Maximum power output
W_{peak}	Peak power output
θ_p	Muscle fascicle pennation angle

Contents

Chapter 1

General introduction

Of all the tissues in the human body, skeletal muscle is probably one of the most conveniently studied since its outputs, force and movement, are relatively easy to measure. Consequently, there is an extensive body of work documenting the differences in strength between healthy individuals of the same age (Maughan *et al.*, 1983; Kanehisa *et al.*, 1994; Hubal *et al.*, 2005; Williams *et al.*, 2005), between people of different ages (Hakkinen *et al.*, 1998; Akima *et al.*, 2001; Morse *et al.*, 2008), between men and women (Kanehisa *et al.*, 1994; Akima *et al.*, 2001; Hubal *et al.*, 2005), between athletes of different types (Hakkinen *et al.*, 1984; Tesch & Karlsson, 1985; Blazevich *et al.*, 2003) and of the effects of both resistance (Rutherford & Jones, 1986; Jones & Rutherford, 1987; Narici *et al.*, 1989; Narici *et al.*, 1996b; Reeves *et al.*, 2004a) and endurance (Campos *et al.*, 2002; McCarthy *et al.*, 2002; Putman *et al.*, 2004) training. At the subcellular level, the regular arrangement of the contractile proteins, actin and myosin, has made it possible to understand in great detail how the interaction of these two molecules results in the characteristic outputs of force and movement. There are, however, lacunae in our knowledge when it comes to bridging the gap between what is known at the molecular and subcellular level and what is observed at the level of the whole human muscle. The work described in this thesis is concerned with three such areas; 1) the relationship between muscle size and the force and power that it can generate; 2) the effects of strength training on these relationships; 3) the variation between individuals in their response to resistance training.

The considerable variation in muscle strength between healthy, untrained individuals of similar age (Maughan *et al.*, 1983; Chapman *et al.*, 1984; Kanehisa *et al.*, 1994; Bamman *et al.*, 2000; Akima *et al.*, 2001; Fukunaga *et al.*, 2001) appears to be only partly explained by differences in muscle size, which implies that the maximum force per unit cross-

sectional area (CSA) of muscle varies between people. There are numerous neural and peripheral factors that might explain this inter-individual variability in force per unit CSA, such as differences in the level of voluntary activation, antagonist muscle co-activation, moment arm length, muscle fibre length and pennation angle, and fibre-type composition. Despite considerable interest in these matters over the last 50 years, the contribution of each of these factors to the variation in strength in the untrained state is still not known. Furthermore, if the force of a maximally activated muscle were proportional to its CSA, as is conventionally thought (see below), then resistance training would be expected to increase muscle strength and size proportionately. Yet, the literature abounds in studies that report larger gains in strength than muscle CSA following progressive resistance training (Ikai & Fukunaga, 1970; Moritani & deVries, 1979; Young *et al.*, 1983; Jones & Rutherford, 1987; Davies *et al.*, 1988; Narici *et al.*, 1989; Narici *et al.*, 1996b; Ferri *et al.*, 2003). As with the variation in force per unit CSA mentioned above, there could be several explanations for this phenomenon but the precise role that each factor plays in increasing muscle strength has yet to be established. Furthermore, there appears to be considerable variation in the way human muscle responds to resistance training, with some people experiencing much greater gains in strength and muscle size than others (Hubal *et al.*, 2005) but the causes for this variance are as yet unknown. This variability in training response has received little attention in the scientific literature possibly because these observations have been considered artefacts of measurement error or due to variations of effort invested by the participants in the training programme. More recently, however, there has been great interest in this variable training response in relation to differences in genotype (Thomis *et al.*, 2004; Clarkson *et al.*, 2005a; Pescatello *et al.*, 2006; Charbonneau *et al.*, 2008). One major deficiency of these studies, however, is that relatively simple measures of training outcome were often employed, such as the maximum load that can be

lifted during one repetition (1 RM) or maximum voluntary contraction (MVC) and anatomical cross-sectional area (ACSA), rather than the true maximum muscle force and physiological CSA (PCSA). Information obtained from a smaller, more controlled study might help determine the main factors that contribute to the variable increase in strength following resistance exercise. Such factors include changes in muscle volume, muscle architecture, muscle fibre-type composition, as well as neural adaptations. The extent to which these factors respond to training is likely to be determined by different genes, and elucidation of the most important factor(s) may help future investigations discover gene variants that are associated with the response to resistance training.

An overview of skeletal muscle structure and function

The main function of skeletal muscle is to enable movement by generating force and acting on the bone to which the muscle is attached. Skeletal muscle comprises numerous bundles of long, thin cells, or muscle fibres, each containing a multitude of myofibrils. Each myofibril is composed of myofilaments (comprising the contractile proteins, actin and myosin) and a variety of structural proteins, all arranged in a regular configuration throughout the length of the myofibril, so as to form a series of contractile components, or sarcomeres (Fig. 1.1). The length of the myofibril and thus the muscle fibre is determined by the number of sarcomeres arranged in series, while the fibre cross-sectional area (*f*CSA) is primarily determined by the number of sarcomeres arranged in parallel (Fig. 1.2).

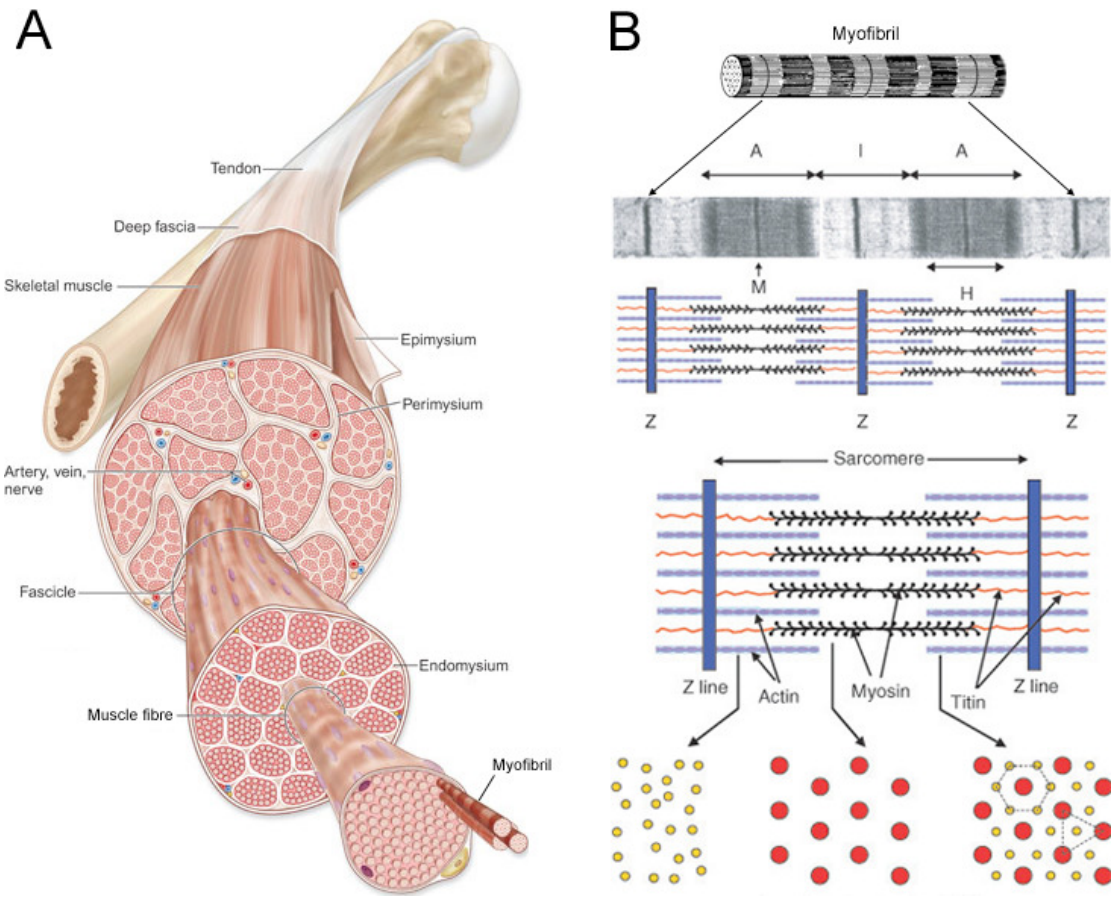

Fig. 1.1 Adapted from Jones *et al*. (2004). An overview of skeletal muscle structure, from the whole muscle to the individual myofibril **(A)** and from the myofibril to the contractile proteins that constitute the sarcomere **(B)**, illustrating the striated appearance of skeletal muscle via an electron micrograph of two sarcomeres and the longitudinal and cross-sectional arrangement of the thick (myosin) and thin (actin) filaments

According to the sliding filament theory (Huxley, 1957), the interaction between the thick (myosin) and thin (actin) filaments causes the sarcomere and therefore the muscle fibre to contract and produce force. The maximal amount of force that can be generated is dependant upon the number of sarcomeres arranged in parallel (Fig. 1.2), while the number of serial sarcomeres, and therefore the fibre length, is one of the determinants of the maximal speed at which the fibre can contract. Thus, as power output is the product of force x velocity, both muscles in the example given in Fig. 1.2 should give the same power output, as both have the same volume (muscle length x CSA). Therefore, muscle CSA is

expected to be the predominant factor that determines the variability in maximum voluntary force produced between people, while muscle volume is likely to be the main determinant in explaining inter-individual differences in power output. However, the determinants of *in vivo* muscle structure and function are a lot more complex, as the relationship between muscle CSA and force output only applies to a maximally activated muscle. Furthermore, muscle fibres do not all have the same contractile properties and the addition of non-contractile material is likely to affect the ability to generate force and power at the whole muscle level.

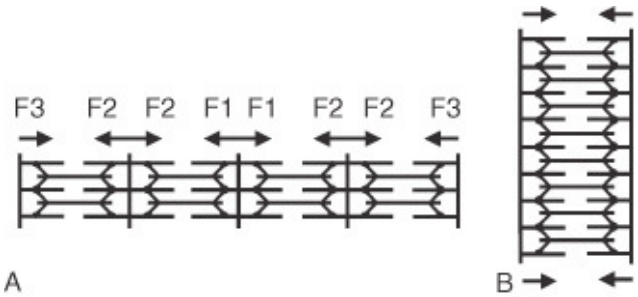

Fig. 1.2 (Jones, 1992). Force generated by sarcomeres in (**A**) series and in (**B**) parallel. In **A**, four sarcomeres are arranged in series. When activated, the forces F1 and F2 are opposed, leaving only F3 to exert force at the ends of the muscle. In **B**, the same number of actin and myosin filaments are arranged in parallel to give four times the isometric force of **A**

Voluntary muscle activation

To contract and generate force, each muscle fibre is innervated by one of the many axonal branches of a motoneuron. All the individual muscle fibres that are innervated by the same motoneuron form a motor unit, which will differ in size and type, according to the total number of fibres innervated and the contractile characteristics of those fibres (Fig. 1.3). Once an action potential has passed down the axonal branches to the neuro-muscular junction, the release of acetylcholine from the synapse causes depolarisation of the sarcolemma (the muscle fibre plasma membrane), via interaction of acetylcholine with its

receptors on the post-synaptic membrane. The action potential is propagated along the surface membrane and into the transverse-tubuli, which leads to the release of calcium ions (Ca^{2+}) from the sarcoplasmic reticulum. The Ca^{2+} binds to troponin C on the actin filament, causing a conformational change of the tropomyosin that exposes the myosin binding sites. This whole process is called excitation-contraction coupling, as the excitation by neural stimulation is coupled to the resulting muscle contraction. The myosin heads attach to the actin filament and, with the hydrolysis of adenosine tri-phosphate, the cross bridge power stroke occurs, drawing the Z-lines of the sarcomere closer together, thus causing the muscle to contract.

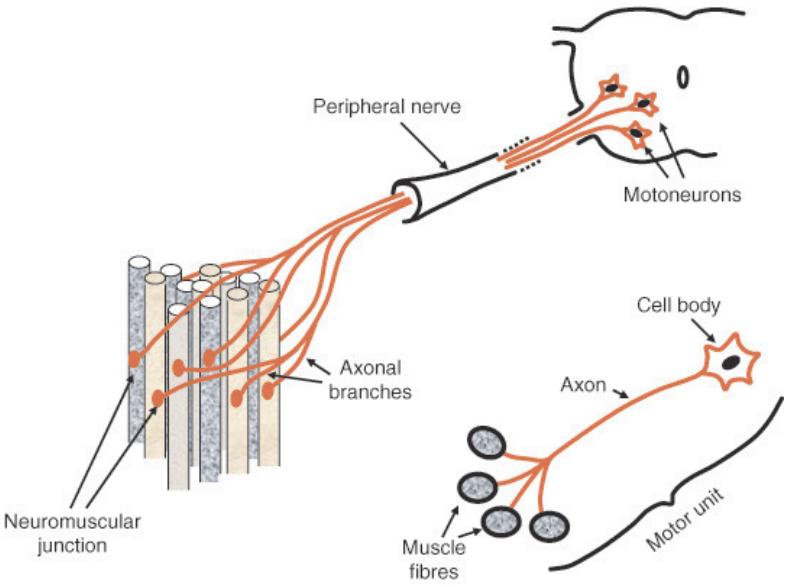

Fig. 1.3 (Jones *et al.*, 2004). Concept of the motor unit. A motor unit consists of the motoneuron and all the scattered muscle fibres that it innervates

The level of muscle activation depends on the number of motor units recruited *(orderly recruitment of motor units)* and on the firing frequency of the motoneurons that activate the muscle fibres in their units *(rate coding)*. The smaller motoneurons innervating slow muscle fibres are predominantly recruited at low force levels but as the force intensity increases, the larger motoneurons innervating the faster muscle fibres are recruited, which

is known as *Henneman's size principle* (Henneman *et al.*, 1974). Maximal activation of a muscle requires that all motor units are recruited and that they are each stimulated at their optimal firing frequency. There is evidence to suggest that untrained individuals are incapable of fully activating their muscle voluntarily (Moritani & deVries, 1979; Hakkinen *et al.*, 1998), while other investigators have reported 100% activation in their participants prior to training (Komi & Buskirk, 1972; Thorstensson *et al.*, 1976; Jones & Rutherford, 1987; Davies *et al.*, 1988). The reason for this discrepancy is unclear but differences in methodologies used to assess voluntary activation might offer an explanation. For example, recording electromyography (EMG) from a single point overlying the muscle of interest is associated with many technical problems, such as the positioning of the surface electrodes relative to the muscle and the impedance of the skin and underlying tissues, all of which are critical in determining the size of the EMG signal. The interpolated twitch technique (ITT, Fig. 1.4) and variations on this method have been used extensively to assess activation (Rutherford *et al.*, 1986b; Jones & Rutherford, 1987; Allen *et al.*, 1995; Allen *et al.*, 1998; Harridge *et al.*, 1999; Folland *et al.*, 2000; Scaglioni *et al.*, 2002; Reeves *et al.*, 2004a, b; Williams *et al.*, 2005; Bampouras *et al.*, 2006; Folland & Williams, 2007). Comparing the force obtained by supramaximal electrical stimulation of a muscle at rest with the additional force obtained by superimposing this level of stimulation during an MVC, allows the level of voluntary muscle activation to be reliably estimated.

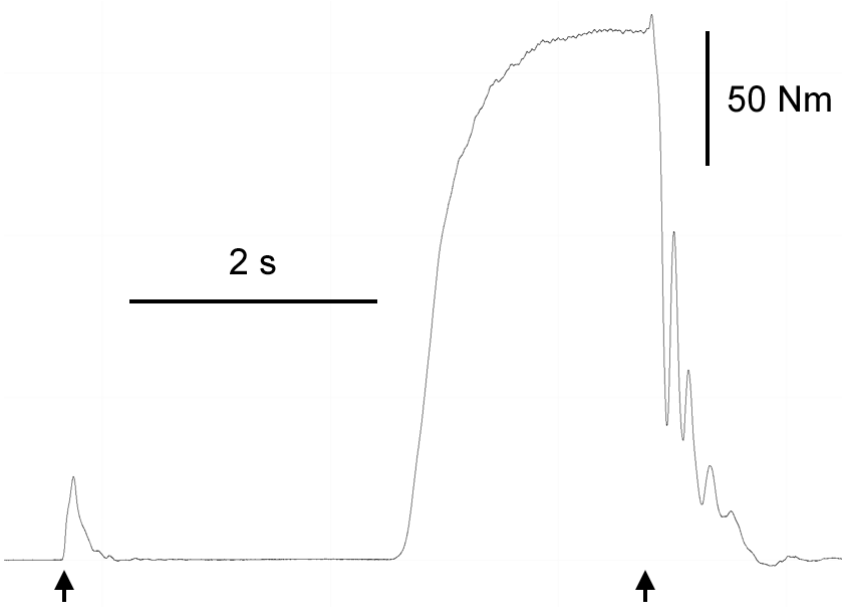

Fig. 1.4 Superimposed electrical stimulation used to determine the level of voluntary muscle activation. The quadriceps femoris muscle was stimulated with a single twitch (represented by an arrow) before and during MVC. This torque trace was taken from one of the participants of the studies described in the current thesis

The consequence of not accounting for voluntary activation capacity could be an inaccurate assessment of maximum muscle force, and inter-individual differences in activation capacity will likely contribute to the inter-individual variability in muscle strength. Furthermore, activation capacity has been shown to increase following resistance training (Scaglioni *et al.*, 2002; Reeves *et al.*, 2004a) and might explain a proportion of the strength gain following training. A variable increase in the level of voluntary activation (Scaglioni *et al.*, 2002) will undoubtedly have implications for a variable response to strength training.

Antagonist muscle co-activation

When a joint moment is created, the generated torque is the result of the action of several muscles, namely the agonists, all pulling in the same direction over that joint and a certain amount of force produced by antagonist muscles, pulling in the opposite direction. It is

thought that antagonist muscle co-activation around the knee occurs in order to stabilise the joint and reduce shearing movements (Hortobagyi & Devita, 2006). The level of antagonist co-activation varies between healthy, untrained people of similar age (Carolan & Cafarelli, 1992; Macaluso *et al.*, 2002; De Vito *et al.*, 2003; Reeves *et al.*, 2004a). Therefore, differing levels of antagonist muscle co-activation between untrained individuals are likely to explain a certain amount of the inter-individual variability in agonist muscle strength. Similarly, decreased antagonist muscle co-activation following strength training, as shown by some investigators (Carolan & Cafarelli, 1992; Hakkinen *et al.*, 1998; Hakkinen *et al.*, 2000), would have implications for the calculated training-induced change in muscle force.

Muscle-tendon moment arm

To obtain a realistic estimate of maximally activated muscle force *in vivo*, not only is it essential that the levels of voluntary muscle activation and antagonist muscle co-activation are accounted for but also the muscle-tendon moment arm, which is determined by the anatomical constraints of the skeleton (Maganaris *et al.*, 2001). During knee joint extension, it is the patellar tendon moment arm (d_{PT}) that acts as the leverage of the effective force transmitted to the tibia during contractions of the quadriceps femoris muscle (Fig. 1.5). Using sophisticated methods, such as magnetic resonance imaging (MRI) and X-ray video, the d_{PT} has been accurately quantified *in vivo* in humans (Baltzopoulos, 1995; Tsaopoulos *et al.*, 2006). However, the d_{PT} has been shown to differ greatly from person to person (Tsaopoulos *et al.*, 2007b), which would have implications for inter-individual variability in joint moment. Moreover, d_{PT} is known to change according to the knee joint angle (Baltzopoulos, 1995) and as MVC torque changes as a function of joint angle (Sale *et al.*, 1982; Reeves *et al.*, 2004b), accurate measurements of

maximum muscle force are dependent upon estimations of d_{PT} at the joint angle at which peak MVC occurs (the optimum angle). Furthermore, the optimum angle has been shown to change as a consequence of resistance training (Reeves *et al.*, 2004b), which would have implications for the calculated change in maximum muscle force.

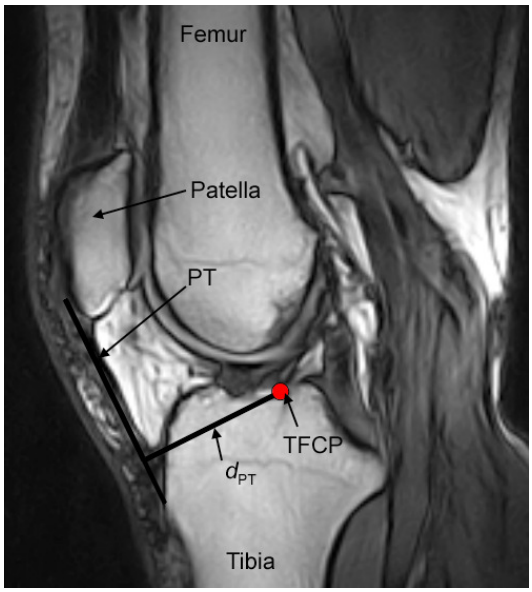

Fig. 1.5 A sagittal MRI scan of the knee joint, from which the patellar tendon (PT) moment arm (d_{PT}) can be measured. d_{PT} is defined as the perpendicular distance from the tibiofemoral "contact point" (TFCP) to the PT. This scan was taken from one of the participants in the studies described in the current thesis

Length-tension relationship and physiological cross-sectional area

Although maximally activated skeletal muscle force is governed by the number of parallel-aligned sarcomeres, force is determined by the degree of overlap between the actin and myosin filaments within the sarcomere (Gordon *et al.*, 1966), as shown in Fig. 1.6. If the sarcomere length is too short or too long, i.e. the interaction of the actin and myosin filaments either exceeds or does not reach optimum overlap for producing maximum tension (~2.5 μm in human muscle fibres), force output decreases (Fig. 1.6). Therefore, to measure the true maximum isometric force of a muscle, it is essential that the muscle is at

its optimum length for producing maximum force. Thus, it follows that when measuring the muscle force over a joint, the joint should be set at the angle at which the muscle fibres of the muscle or group of muscles are at optimum length for producing maximum force (Maganaris *et al.*, 2001; Reeves *et al.*, 2004a, b).

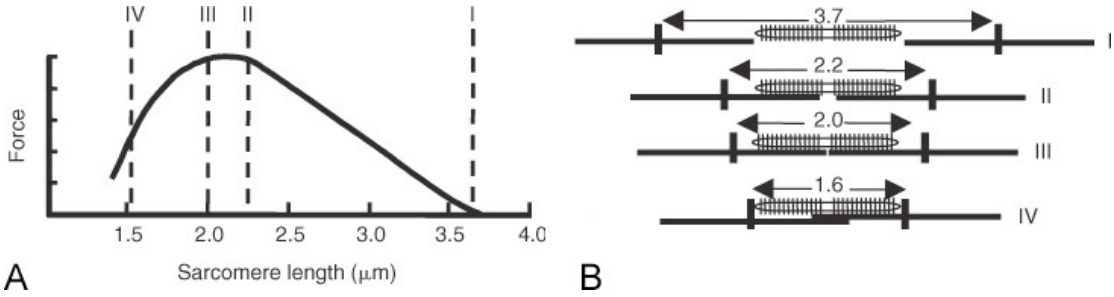

Fig. 1.6 Adapted from Jones *et al.* (2004). **A**, Isometric force generated at different sarcomere lengths (I-IV). **B**, arrangement of filaments at the different lengths. At lengths less than 2.0 μm thin filaments begin to overlap and at still shorter lengths the thick filaments come into contact with the Z lines. Values are for frog muscle (Gordon *et al.*, 1966); mammalian thin filaments are slightly longer, so the corresponding sarcomere lengths are: I, 4.0 μm; II, 2.5 μm; III, 2.4 μm; IV, 1.6 μm

Furthermore, the possibility exists that fibre length is increased as a consequence of a training-induced addition of serial sarcomeres (Reeves *et al.*, 2004b, a). Such a change in length would shift the fibre length-tension relationship to the right (Fig. 1.7), which would affect the joint angle at which peak MVC is measured (Reeves *et al.*, 2004b). An additional factor that must be recognized when measuring muscle force *in vivo* is tendon compliance and how that affects the muscle length-tension relationship. In short, a more compliant tendon would allow greater shortening of the muscle fibres at a set joint angle, thus altering the joint angle-MVC relationship to a less extended joint angle. Resistance training, however, is known to increase tendon stiffness (Reeves *et al.*, 2003b; Kongsgaard *et al.*, 2007; Seynnes *et al.*, 2009), thus reducing the optimum joint angle following training (Reeves *et al.*, 2004b). This further highlights the importance of measuring muscle

strength at the joint angle at which peak MVC is obtained.

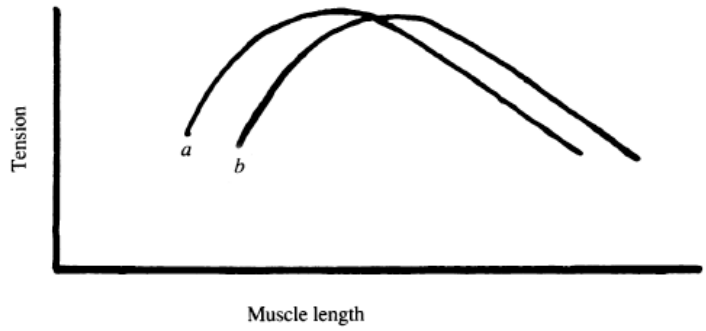

Fig. 1.7 Adapted from Jones *et al.* (1989). The possible effect of resistance training on muscle fibre length and the consequences for the length-tension relationship. Training may lead to an increase in the numbers of serial sarcomeres in each muscle fibre and a subsequent shift in the length-tension relationship to the right. a, before training; b, after training.

Muscle architecture

Due to the technical difficulties of measuring the cross-sectional area of the total number of muscle fibres [represented by the PCSA (Powell *et al.*, 1984)] of complex human muscles *in vivo*, the anatomical CSA (ACSA, the area of muscle perpendicular to the longitudinal axis of the limb) has often been used as an index of muscle size (Maughan *et al.*, 1983; Chapman *et al.*, 1984; Kanehisa *et al.*, 1994; Akima *et al.*, 2001; Hubal *et al.*, 2005). However, ACSA only explains ~50% of the variability in MVC between untrained individuals (Maughan *et al.*, 1983; Chapman *et al.*, 1984; Bamman *et al.*, 2000), which could be due to both neural and architectural factors. The majority of skeletal muscles have a complex architecture, that is to say the muscle fibre lengths and angle at which the fibres insert into the aponeurosis, known as the pennation angle, differ between muscles (Alexander & Vernon, 1975) and for the same muscle between individuals (Kawakami *et al.*, 2006). Using ultrasonography it is now possible to assess the architecture of human muscles *in vivo* (Henriksson-Larsen *et al.*, 1992; Rutherford & Jones, 1992; Kawakami *et*

al., 1993). Each bundle of muscle fibres, known as a muscle fascicle, is encased by a sheath of connective tissue called the perimysium (Fig. 1.1). Thus, the fascicular paths can be identified from sonographs as the interspaces between the echoes arising from the surrounding perimysial tissue and the fascicle pennation angle is defined as the angle between the fascicular path and the aponeurosis of the muscle (Fig. 1.8).

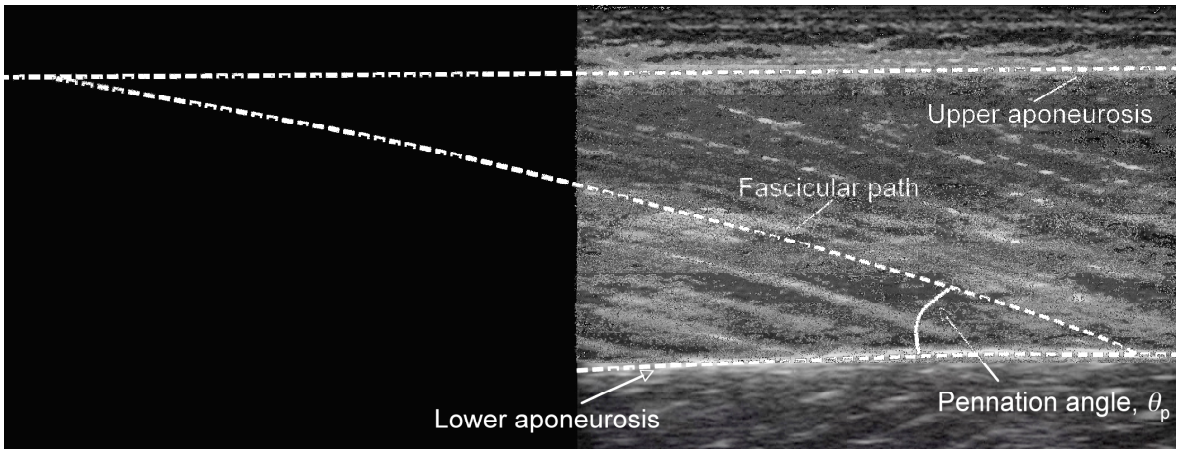

Fig. 1.8 A sagittal ultrasound image of the vastus lateralis (VL) muscle, highlighting the fascicular path, the fascicle pennation angle (θ_p) and the upper and lower aponeuroses. This scan was taken from one of the participants in the studies of the current thesis

The pennate arrangement of muscle fibres allows a greater amount of contractile material to attach to a limited area of tendon (Alexander & Vernon, 1975; Gollnick *et al.*, 1981; Aagaard *et al.*, 2001), thus increasing the force generating capacity of the muscle. However, a trade-off exists between the increase in force generating capacity and the concomitant reduction in force resolved at the tendon as a function of the cosine of the pennation angle, where net tendon force will continue to increase up to a pennation angle of 45° (Alexander & Vernon, 1975). The larger the fascicle pennation angle, the less representative the ACSA is of the PCSA (Alexander & Vernon, 1975), a point illustrated in Fig. 1.9. Using a combination of MRI and ultrasonography, PCSA can be estimated *in*

vivo from the ratio of muscle volume to fascicle length (Maganaris *et al.*, 2001; Reeves *et al.*, 2004a). Further, to obtain an accurate assessment of the muscle strength-size relationship, it is vital that the PCSA is estimated when the muscle fibres are at their optimum length for generating maximum force (Chow *et al.*, 1999; Maganaris *et al.*, 2001; Reeves *et al.*, 2004a).

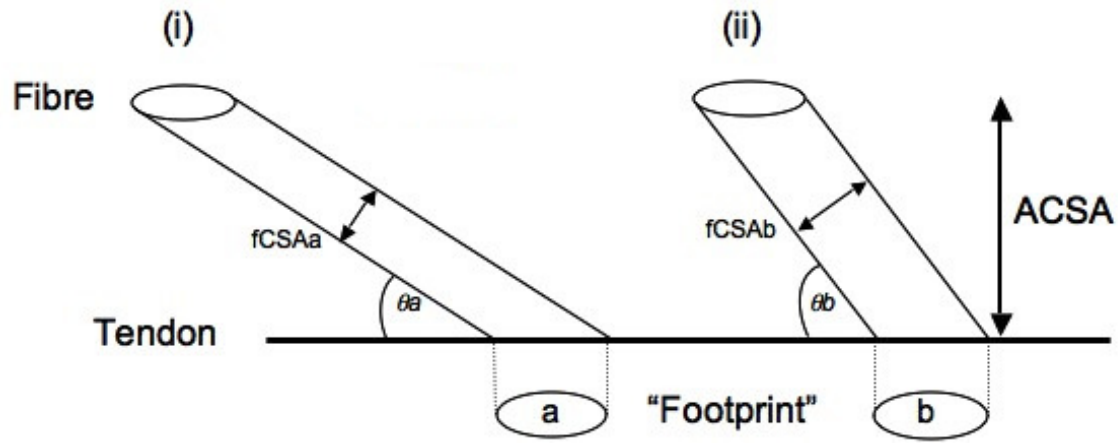

Fig. 1.9 Effect of pennation angle on the size of muscle fibre that can attach to the tendon. θa and θb are angles of pennation; fCSA and ACSA are the fibre and anatomical cross-sectional areas, respectively; "Footprint" is the area of tendon occupied by each muscle fibre. Increasing the pennation angle from θa to θb allows more contractile material to attach to the tendon (causing an increase from fCSAa to fCSAb) without increasing the footprint, thus and enabling the fibre to produce more force. However, as the fibre length has decreased from (i) to (ii), so the ACSA remains the same, thus demonstrating how differences in muscle architecture between people can affect muscle force output and how ACSA can underestimate PCSA

A number of studies have demonstrated that strength training induces an increase in muscle fascicle pennation angle (Kawakami *et al.*, 1995; Aagaard *et al.*, 2001; Reeves *et al.*, 2004a), which has been attributed to muscle fibre hypertrophy (Staron *et al.*, 1990; Staron *et al.*, 1994; Aagaard *et al.*, 2001; Campos *et al.*, 2002). However, not only does muscle fascicle pennation angle vary between untrained individuals (Kawakami *et al.*, 2006) but also in the extent to which it changes with training (Reeves *et al.*, 2004b). This

emphasizes the importance of measuring muscle architecture, in order to obtain an accurate account of the inter-individual variability in strength and the true changes in muscle force.

Muscle fibre type

As described above, skeletal muscle is composed of different types of motor units, depending on the contractile properties of the innervated muscle fibres. Human muscle fibres can be broadly classified into three types (I, IIa and IIx) using their histochemical staining patterns when staining for myosin adenosine triphosphatase (mATPase) after pre-incubations at different pH (Brooke & Kaiser, 1970). Although this staining procedure shows the distribution of particular fibres within a cross-section, it appears problematic in distinguishing hybrid fibres, such as type IIa/IIx. An alternative method is to determine the myosin heavy chain (MyHC) isoform composition of a muscle (Fig. 1.10). The advantage is that the proportions of the different MyHC isoforms within a muscle can be readily obtained, although information on how these isoforms or fibres are distributed within the muscle is not accessible.

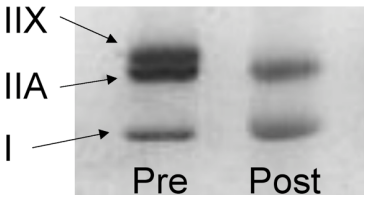

Fig. 1.10 Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) protein separations showing MyHC I, MyHC IIA, and MyHC IIX bands from the vastus lateralis muscle of one participant before (Pre) and after (Post) nine weeks of resistance training. This example was taken from the study described in Chapter 4 of the current thesis

For fibres of the same length, it is the mATPase in the globular (S-1 unit) region of the myosin molecule of the different MyHC isoforms that is the basis for the differences in

speed of shortening of fibres. In fact, there is a close correlation between the maximal shortening velocity of a muscle and its mATPase activity (Barany, 1967), Fig. 1.11.

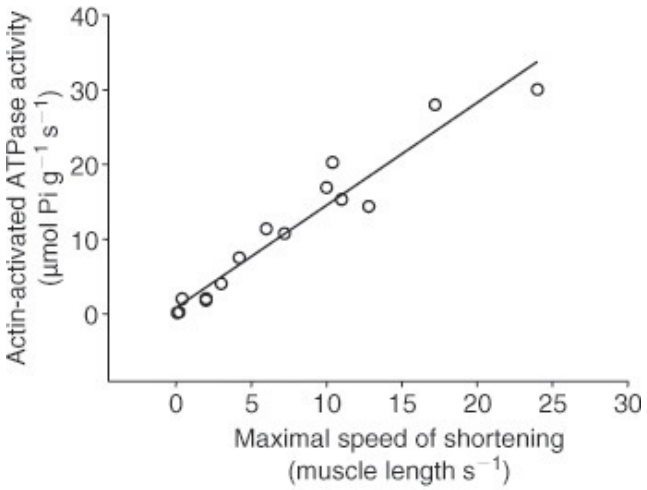

Fig. 1.11 (Jones *et al.*, 2004). Relationship between maximal shortening velocity and actin-activated myosin ATP-ase activity from various animal species [redrawn from the data of Barany (1967)]

The different fibre types each have their own myosin isoform (Staron & Pette, 1986; Staron, 1991; Adams *et al.*, 1993b; Fry *et al.*, 1994) and the greater mATPase activity of muscle fibres containing purely MyHC IIX allows them to contract faster than fibres comprising only MyHC IIA, which in turn have faster contractile properties than pure MyHC type I fibres (Bottinelli *et al.*, 1996; Li & Larsson, 1996; Bottinelli & Reggiani, 2000; D'Antona *et al.*, 2006; Degens & Larsson, 2007). Consequently, type II fibres are able to produce more force at any given shortening velocity than type I fibres. Therefore, there is an upward shift in force-velocity relationship from type I to type II fibres, which leads to type II fibres generating a higher power output than slower type I fibres, as illustrated in Fig. 1.12. In addition to speed, there are studies that suggest that type IIX and type IIA fibres are intrinsically stronger than type I fibres (Bottinelli *et al.*, 1996; D'Antona *et al.*, 2006; Pansarasa *et al.*, 2009), although this is not a universal conclusion (Larsson & Moss, 1993; Ottenheijm *et al.*, 2005; Gilliver *et al.*, 2009). Moreover, resistance training

may increase the specific tension of isolated single fibres in human muscle following resistance training (Parente *et al.*, 2008; Pansarasa *et al.*, 2009). If so, this suggests that overload training causes an increase in the packing of contractile material within the muscle fibre (Penman, 1970). However, other investigations have reported no change in the specific tension of single human muscle fibres following resistance training (Trappe *et al.*, 2000; Trappe *et al.*, 2001a; Widrick *et al.*, 2002), thus demonstrating the need for further clarification.

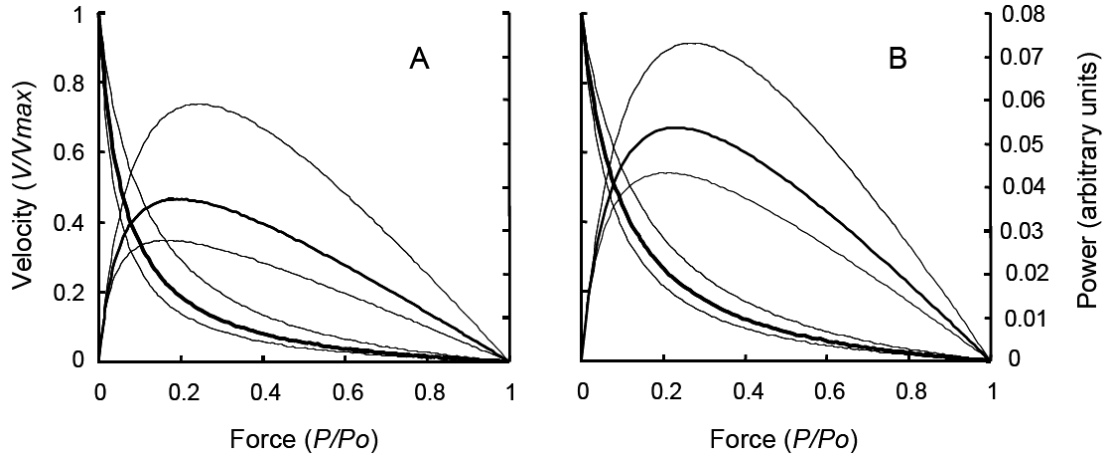

Fig. 1.12 (Gilliver *et al.*, 2009). Variation in the curvature of the force-velocity relationship and the consequences for power of human single muscle fibres. Values for force and velocity have been normalised for maximum isometric force (P_o) and unloaded shortening velocity (V_{max}). **A**, type I fibres; **B,** type IIA fibres. The darker lines are the mean values for the particular fibre type, the lighter lines indicate the range of values.

The evidence of a resistance training-induced alteration in the human fibre-type profile is equivocal; some cross-sectional studies suggest that chronic strength training leads to a transition towards a faster fibre-type composition (D'Antona *et al.*, 2006; Kesidis *et al.*, 2008), while longitudinal resistance training studies have shown either no change (Pansarasa *et al.*, 2009) or a shift from type IIX to IIA MyHC expression and a transition

from IIx to IIa muscle fibre-type composition (Adams *et al.*, 1993b; Roman *et al.*, 1993; Fry *et al.*, 1994; Staron *et al.*, 1994; Campos *et al.*, 2002). Therefore, further investigations are required to clarify the relationship between muscle fibre-type composition and the variance in strength and power observed in untrained human skeletal muscle, as well as the adaptation response of muscle to resistance training.

Genetic predisposition

Evidence from studies on human monozygotic and dizygotic twins suggests that heritability plays an important role in determining the phenotype in untrained muscle (Arden & Spector, 1997; Loos *et al.*, 1997; Thomis *et al.*, 1998b) and in the response to strength training (Thibault *et al.*, 1986; Thomis *et al.*, 1998a). Thus, it appears that a large proportion of the variability in muscle phenotypes in the untrained state, and in the response to strength training, might be attributed to genetic factors. The variants of two genes have received particular attention in recent years for their possible association with human muscle strength and size: the angiotensin I-converting enzyme (ACE) gene (Folland *et al.*, 2000; Thomis *et al.*, 2004; Williams *et al.*, 2005; Pescatello *et al.*, 2006; Charbonneau *et al.*, 2008) and the alpha-actinin-3 (ACTN3) gene (Clarkson *et al.*, 2005a; Clarkson *et al.*, 2005b; Delmonico *et al.*, 2007). The evidence for genotype-muscle phenotype and genotype-training response interactions is, however, inconclusive and more detailed work is required to appropriately determine the association between genotype and muscle force, PCSA and specific tension, and how the genotype affects the response to training.

Aims and objectives of the project

Various factors have been discussed in this chapter that may individually or collectively provide an explanation for the inter-individual variability in untrained muscle strength, the disproportionate training-induced change in muscle strength and size and the variable response of human muscle to resistance training. The specific aims and objectives of the overall project were:

1. To clarify the relationships between strength, muscle activation, muscle size and architecture in untrained individuals and to determine to what extent these factors explain the inter-individual variability in muscle strength. This is addressed in the work described in Chapter 2.
2. To examine the effect of resistance training on the relationships outlined in Aim 1 and to assess to what extent changes in neural activation and muscle architecture explain the training-induced change in muscle strength and size. This topic is explored in the work described in Chapter 3.
3. The third aim was to investigate possible explanations for an increase in specific tension as a result of training. Specifically, a change in fibre-type composition, augmented specific tension of single muscle fibres and evidence of an increase in lateral force transmission as potential reasons were examined in Chapter 4.
4. To quantify the variation in human muscle responses to resistance training, regarding changes in muscle force, PCSA and specific tension. This is addressed in Chapter 5.
5. To investigate possible associations between candidate gene polymorphisms and muscle size, strength and power, and how the different polymorphisms affect the response to resistance training. This topic is explored in the work described in Chapters 6 and 7.

Chapter 2

The inter-individual variability in muscle strength

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In vivo specific tension of the human quadriceps femoris muscle.

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Abstract

It is not known to what extent the inter-individual variation in human muscle strength is explicable by differences in specific tension. To investigate this, a comprehensive approach was used to determine *in vivo* specific tension of the quadriceps femoris (QF) muscle (Method 1). Since this is a protracted technique, a simpler procedure was also developed to accurately estimate QF specific tension (Method 2). Method 1 comprised calculating patellar tendon force (F_t) in 27 young, untrained males, by correcting maximum voluntary contraction (MVC) for antagonist co-activation, voluntary activation and moment arm length. For each component muscle, the physiological cross-sectional area (PCSA) was calculated as volume divided by fascicle length during MVC. Dividing F_t by the sum of the four PCSAs (each multiplied by the cosine of its pennation angle during MVC) provided QF specific tension. Method 2 was a simplification of Method 1, where QF specific tension was estimated from a single anatomical CSA and vastus lateralis muscle geometry. Using Method 1, the variability in MVC (18%) and specific tension (16%) was similar. Specific tension from Method 1 (30 ± 5 N·cm^{-2}) was similar to and correlated with that of Method 2 (29 ± 5 N·cm^{-2}; $R^2 = 0.67$; $P < 0.05$). In conclusion, most of the inter-individual variability in MVC torque remains largely unexplained. Furthermore, a simple method of estimating QF specific tension provided similar values to the comprehensive approach, thereby enabling accurate estimations of QF specific tension where time and resources are limited.

Introduction

Muscle strength changes during growth, development and ageing and also as a result of training, disuse or disease (Degens *et al.*, 1995; D'Antona *et al.*, 2003; Elder *et al.*, 2003; Payne *et al.*, 2004; Reeves *et al.*, 2004a; Stubbings *et al.*, 2008). In addition, there is considerable variation in muscle strength between healthy, untrained individuals of similar age (Maughan *et al.*, 1983; Chapman *et al.*, 1984; Kanehisa *et al.*, 1994; Williams *et al.*, 2005), part of which is due to differences in muscle size. However, some of the variation remains unexplained, implying differences in maximum force per unit cross sectional area of muscle between people.

The primary determinant of the maximum force a muscle can generate is the number of sarcomeres in parallel, which is represented by the muscle's physiological cross sectional area (PCSA). However, other extrinsic factors such as the level of voluntary muscle activation, antagonist muscle co-contraction, moment arm length, the angle at which the muscle fibres insert into the tendon and the infiltration of non-contractile material within the muscle may influence this relationship and thus the observed variance in strength.

For animal muscle measured both *in situ* and *in vitro*, the specific tension (muscle force per PCSA) is in the region of 20-30 $N \cdot cm^{-2}$ (Close, 1969; Barany & Close, 1971; Lieber *et al.*, 1986; de Haan *et al.*, 1992; Degens *et al.*, 1995) and for human single muscle fibres measured *in vitro*, specific tension values lie between 10 and 40 $N \cdot cm^{-2}$ (Degens *et al.*, 1999; Widrick *et al.*, 2002; Cristea *et al.*, 2008; Luden *et al.*, 2008). However, for human muscle studied *in vivo*, the specific tension has been reported to range from 10 to 100 $N \cdot cm^{-2}$ (Narici *et al.*, 1988; Maganaris *et al.*, 2001) and it is never clear whether such discrepancies represent intrinsic differences in muscle fibre contractility (such as variation

in muscle fibre type composition and/or myofibrillar density), are a function of variation in muscle structure or are the result of difficulties in accurately determining PCSA and/or muscle force. Human muscles can be characterised as either parallel- or pennate-fibred, i.e. the muscle fibres are predominantly arranged either in parallel or at an angle to the line of pull of the tendon. In parallel-fibred muscles, the anatomical CSA (ACSA) may provide an adequate estimation of the PCSA (Davies *et al.*, 1988; Kawakami *et al.*, 1994) but in pennate muscles, such as the quadriceps femoris muscle (QF), ACSA has been shown to underestimate PCSA (Alexander & Vernon, 1975; Wickiewicz *et al.*, 1983). Using a combination of magnetic resonance imaging (MRI) and ultrasonography, it is possible to measure muscle volume and fascicle length in order to estimate the PCSA of human pennate muscles *in vivo* (Maganaris *et al.*, 2001; Reeves *et al.*, 2004a). To determine human muscle specific tension reliably *in vivo*, it is necessary to obtain maximal isometric muscle force at optimum fibre length, accounting for antagonist co-activation and voluntary activation of the agonist and to obtain accurate measurements of PCSA (Chow *et al.*, 1999; Maganaris *et al.*, 2001).

Despite recent technological advances, no study has comprehensively accounted for all of the aforementioned extrinsic factors when assessing the specific tension of the human QF *in vivo* and thereby provided a more accurate representation of the inter-individual variance in the force-generating capacity of this muscle. The first aim of the present investigation was, therefore, to determine the specific tension of QF *in vivo* in a relatively large group of untrained individuals, taking into account the muscle architecture of each of the component muscles of the QF during MVC and other factors that affect force production. By doing so, it was hypothesised that the variation in QF specific tension would be much reduced compared to the variability in MVC torque. However, measuring

the geometry of all four component heads during MVC would require a great deal of time and effort from each participant. Alternatively, as the vastus lateralis muscle (VL) is the predominant of the four muscles (Alexander & Vernon, 1975; Wickiewicz *et al.*, 1983; Scott *et al.*, 1993), assessing VL geometry alone may provide an accurate estimate of the QF group as a whole, thereby saving time and resources. Although the VL has previously been considered representative of the total QF group (Trappe *et al.*, 2001b; Reeves *et al.*, 2004a; Gorgey *et al.*, 2006), accurate *in vivo* measurements of fascicle lengths and pennation angles of the four QF component muscles during MVC have yet to be reported and it remains to be seen whether the assumption that the VL is representative of the whole QF muscle is valid in this context. In addition, despite MRI currently being the most accurate and reliable method to measure muscle volume *in vivo*, it is a costly and time-consuming process. However, a simpler alternative exists, whereby QF volume may be estimated from a single QF ACSA (Morse *et al.*, 2007a). Therefore, the second aim of the study was to determine a minimal set of measurements that could be used to provide acceptable estimates of QF specific tension much more easily.

Materials and methods

Participants

Twenty-seven untrained males, aged 21.3 ± 3.4 years, height 177.0 ± 5.8 cm, body mass 76.2 ± 10.1 kg (mean ± SD), participated in the study after written informed consent was obtained. Participants were excluded if they had a history of lower-limb fracture, their age was outside the range of 18-39 years, they had taken part in strength training within the 12 months prior to the study, had used dietary supplements or performance enhancing aids or if they were considered to be in ill health (assessed from the responses to a general health

questionnaire). The study was approved by Manchester Metropolitan University Local Ethics Committee.

General experimental design

Two different methods were used to calculate specific tension of the human QF and all measurements were performed on the right limb. Method 1 was the most detailed assessment of specific tension, where QF PCSA was calculated by accounting for the optimum fascicle length (L_f) and volume of each of the four constituent muscles. Patellar tendon force (F_t) was determined from MVC, accounting for antagonist muscle co-activation, the level of voluntary muscle activation, patellar tendon moment arm (d_{PT}) and optimum muscle fascicle pennation angle (θ_p). Method 2 was identical to Method 1, except that the VL muscle geometry at MVC was assumed to be representative of the mean QF architecture. Additionally, QF volume was estimated from a single ACSA and the femur length, as described by Morse *et al*. (2007a). Participants were asked to avoid performing any strenuous physical activity for up to 72 hours prior to partaking in two separate testing sessions within a 14-day period. The initial session was used to familiarise the participants with the test procedures and equipment and the reliability of the main measurements was determined from the test-retest results obtained from the two testing sessions.

Method 1

Maximal voluntary isometric contractions

Participants sat on a rigid chair of a dynamometer (Cybex Norm, Cybex International, New York, USA) with a hip angle of 85 degrees (180 degrees corresponding to supine position) and were firmly strapped at the hip, distal thigh and chest with inextensible straps to minimise movement. The seat length was adjusted for each participant to align the rotational axis of the dynamometer with the lateral femoral epicondyle. The lever arm of the dynamometer was firmly attached to the lower leg with inextensible straps and gravitational corrections were performed to account for the effect of leg and dynamometer lever arm weight on moment measurements. Participants were asked to perform a minimum of two MVCs (if the second attempt was >10% higher than the first, a third attempt was made to ensure a true MVC was achieved) at three separate knee joint angles (70, 80, and 90 degrees of knee flexion; 0 degrees equalling full extension). The order of joint angle was randomly selected and the angle at which the highest MVC occurred was deemed the optimum angle. Visual and verbal feedback was provided during and after each MVC, which lasted two-three seconds and there was a rest-interval of 60 seconds between each contraction. The range of joint angles investigated was based on results from a pilot study that indicated the optimum knee joint angle for this population was 80.5 ± 2.1 degrees of knee flexion.

Antagonist co-activation

To determine the extent of antagonist (hamstring) muscle co-activation during a maximal voluntary isometric knee extension, the electromyographic (EMG) activity of the biceps femoris (long head) was recorded, as this muscle has previously been shown to be representative of the knee flexor muscle group (Kellis & Baltzopoulos, 1999). This

procedure has been reported in detail elsewhere (Reeves *et al.*, 2004a). Briefly, once the biceps femoris muscle was identified and the skin surface impedance reduced to <5 kΩ, two bipolar silver chloride surface electrodes (Neuroline, Medicotest, Rugmarken, Denmark) were placed 20 mm apart along the sagittal axis over the muscle belly and one reference electrode was positioned over the lateral tibial condyle. The exact location of the electrodes was marked on the participant's skin and recorded on an acetate sheet with a permanent marker to ensure precise electrode repositioning in the trial that followed the familiarisation session. The root mean square of the raw EMG signal was calculated over one second around the peak torque during each maximum voluntary isometric knee extension at all three joint angles. Maximal agonist EMG activity of the biceps femoris was recorded during isometric knee flexion at the same joint angles (Kellis & Baltzopoulos, 1997) and the torque produced by the hamstrings during knee extension was estimated assuming a linear relationship between torque and EMG activity (Reeves *et al.*, 2004a). The estimated antagonist torque obtained at the optimum knee extension joint angle was used to calculate the maximum overall knee extension torque.

Voluntary muscle activation

The procedure for determining maximal voluntary muscle activation using the interpolated twitch technique (ITT) has been described previously (Rutherford *et al.*, 1986b; Williams *et al.*, 2005). The participants were seated in the strength-testing chair as explained above. The level of voluntary activation was assessed with the knee joint fixed at 90 degrees of knee flexion. Electrical stimulation was administered via two 7.5 cm x 12.5 cm self-adhesive electrodes (Versastim, Conmed, New York, USA), placed distally (anode) and proximally (cathode) over the QF. The QF was stimulated in a relaxed state with 100 mA pulses of 200 μs which were increased in 50 or 100 mA increments (Digitimer stimulator,

model DS7, Welwyn Garden City, UK) until no further increase in twitch torque was observed. This current was used two minutes later to elicit a single twitch whilst the participant was in the resting state, followed five seconds later by a second twitch during MVC. True maximal torque (TMT) was calculated as:

$TMT = MVC_{CORR} / (1-t/T)$

where *t* is the amplitude of the superimposed twitch, T the value of the twitch before the MVC and MVC_{CORR} the MVC corrected for co-activation. The percentage activation was given by:

$100 \cdot (1-t/T)$

Torque signals, electrical stimuli, and EMG activity were displayed on a computer monitor (Macintosh, G4, Apple Computer, Cupertino, USA), interfaced with an acquisition system (AcqKnowledge, Biopac Systems, Santa Barbara, USA) used for analogue-to-digital conversion, at a sampling frequency of 2 KHz. The activation level estimated using the above approach depends not only on the voluntary effort applied during contraction but also on the stiffness of the series elastic component, which is a function of joint angle (Bampouras *et al.*, 2006). However, no difference in QF voluntary activation or the rate of resting twitch torque development at knee flexion angles between 70-90 degrees was found in a subsample of seven participants from the same population (Table 2.1). Therefore, the level of activation at 90 degrees knee flexion was applied to the MVC_{CORR} torque value at the optimum joint angle to determine TMT.

Table 2.1 Voluntary muscle activation capacity and rate of torque development (RTD) measured at 70, 80 and 90 degrees knee joint flexion; n = 7; mean ± SD

Knee flexion angle (deg)	Voluntary activation (%)	RTD ($Nm \cdot s^{-1}$)
70	93.1 ± 2.2	861.3 ± 302.2
80	94.2 ± 3.6	852.9 ± 257.9
90	93.3 ± 2.5	859.0 ± 224.4

Patellar tendon moment arm

To calculate patellar tendon forces, the torque developed at the knee must be divided by the patellar tendon moment arm length (d_{PT}). The procedure used to measure d_{PT} has been described elsewhere (Tsaopoulos *et al.*, 2006). Briefly, d_{PT} was quantified using a 0.2-T magnetic resonance imaging (MRI) scanner (G-Scan, Esaote Biomedica, Genoa, Italy). Sagittal and coronal-plane knee scans were acquired using a Turbo 3D T1-weighted sequence with the following scanning parameters: time of repetition 40 ms; time to echo 16 ms; matrix 256 x 256; field of view 180 mm x 180 mm; slice thickness 3.4 mm; interslice gap 0 mm. The knee was scanned at rest with the participant in the supine position and, due to the size of the coil, the joint had to be fully extended. The scans were imported to a dicom viewer (Osirix 2.7.5, Osirix Foundation, Geneva, Switzerland) and images in the coronal plane were used to identify the appropriate sagittal scans, which were used to locate the tibiofemoral contact point (TFCP, the midpoint of the shortest distance between the two femoral condyles and the tibia plateau). d_{PT} was defined as the length of the perpendicular line between the axis of the patellar tendon and the TFCP (Tsaopoulos *et al.*, 2006). Previously reported values of d_{PT} length at full extension (0 degrees knee flexion) and angles of 70, 80 and 90 degrees knee flexion (Baltzopoulos, 1995) were used to provide the necessary ratios with which to estimate d_{PT} length at optimum knee joint angle, i.e.

1. $d_{PT\ 70\ deg} / d_{PT\ 0\ deg} = 1.08$
2. $d_{PT\ 80\ deg} / d_{PT\ 0\ deg} = 1.03$
3. $d_{PT\ 90\ deg} / d_{PT\ 0\ deg} = 0.99$

The appropriate ratio was applied to the d_{PT} measured at full extension to provide the estimated d_{PT} at optimum joint angle, which was subsequently multiplied by 1.14 to account for a 14% increase in d_{PT} from rest to MVC (Tsaopoulos *et al.*, 2007a).

Muscle volume

The upper leg was scanned using the same MRI protocol used to scan the knee, except that slice thickness was set at 2.8 mm. Contiguous axial ACSA scans were taken from the tibiofemoral joint to the iliac crest perpendicular to the femur, with the participant in the supine position. The scans were subsequently transferred to a computer and the ACSA of each of the four QF heads was manually outlined (Osirix 2.7.5, Osirix Foundation, Geneva, Switzerland) in ~15 scans at 3.08 cm intervals along the femur length (from the lateral tibiofemoral point of contact) and plotted against femur length (Fig. 2.1). Subsequently, a spline curve was fitted to the ACSA data points associated with each of the four component muscles (vastus lateralis, VL; vastus intermedius, VI; rectus femoris, RF; vastus medialis, VM) and muscle volume was calculated as the area under the curve, a technique that displays a strong test-retest reliability (Seynnes *et al.*, 2008). MRI was performed prior to strength testing to ensure that volume measurements were not influenced by any exercise-induced fluid shifts.

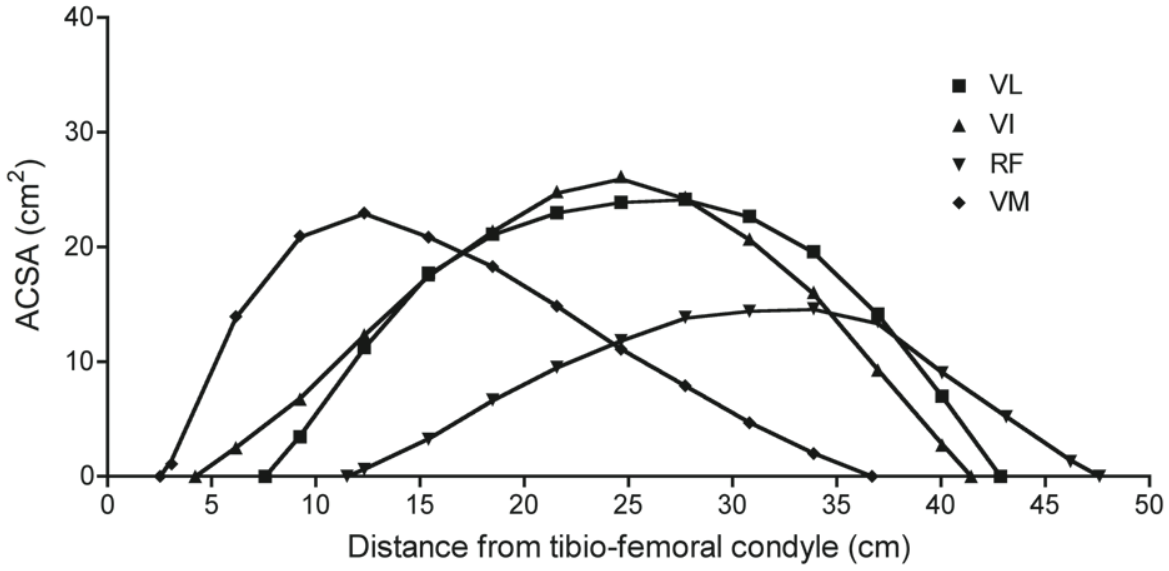

Fig. 2.1 A typical plot illustrating how the volumes of the vastus lateralis (VL), vastus intermedius (VI), rectus femoris (RF) and vastus medialis (VM) muscles were calculated using Method 1. Anatomical cross-sectional areas (ACSAs) of the four heads were measured every 3.08 cm along the femur length from the tibio-femoral condyle (*X*-axis origin) and plotted against femur length. A spline curve was then fitted to the points associated with each muscle and the muscle volume was calculated as the area under the curve

Muscle architecture

Muscle architecture was examined in the four constituent QF muscles via ultrasonography (MyLab25, Esaote, Genova, Italy) during MVC at optimum joint angle, with the participant seated in the strength-testing chair, as detailed above. While the participant was sitting at rest, the origin and insertion points and the lateral and medial boundaries of each muscle were identified using ultrasonography and the distances between the points were measured. Thus, four sites, each corresponding to 50% of the appropriate muscle length along the mid-sagittal plane of the muscle, were marked on the skin surface. A 10-15 MHz linear array probe was coated with a water-soluble transmission gel and positioned perpendicular to the skin surface on one of the measuring sites. The participant was then asked to perform an isometric knee extension to MVC at the pre-determined optimum joint angle. Ultrasound scans were acquired at the appropriate depth and focus to optimise

image quality and sampling frequency, which was maintained at or above 28 Hz and each series of scans was recorded in Audio Video Interleave format. An external voltage trigger was used to synchronise the series of ultrasound scans with the acquisition system, so that the single ultrasound image corresponding to the maximal isometric knee extension torque could be identified and exported for subsequent analysis. This procedure was repeated for each of the remaining three component muscles. Measurements of muscle fascicle length (L_f) and pennation angle (θ_p) were performed on selected images using digitizing software (NIH ImageJ, version 1.39b, National Institutes of Health, Bethesda, USA). θ_p was measured as the angle between the muscle fascicular paths and their insertion into the deep aponeurosis. L_f was measured by tracing the fascicle echo from origin to insertion, taking into account any curvature along the fascicle length. If one end of the fascicle extended beyond the acquired ultrasound image, the fascicle length was estimated by extrapolating the superficial and lower aponeuroses as well as the fascicle (Fig. 2.2). For every ultrasound image obtained, θ_p and L_f were measured on at least three fascicular paths and the average of these measurements gave the θ_p and L_f for each muscle. In the case of the bi-pennate RF muscle, only the lateral θ_p and L_f was analysed, assuming that the muscle architecture for the medial side was identical.

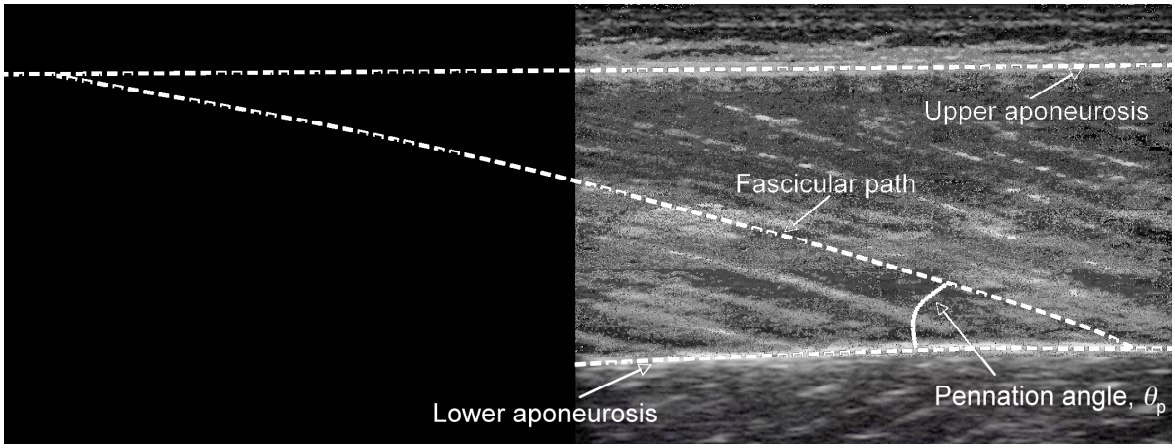

Fig. 2.2 A representative ultrasound image of the vastus lateralis muscle in the sagittal plane, highlighting the fascicular path, the fascicle pennation angle (θ_p) and the upper and lower aponeuroses

To compare the architecture of each component muscle (VL, VI, RF and VM) with that of the QF muscle group as a whole, the L_f and θ_p of each constituent muscle was multiplied by the ratio of its respective volume to total QF volume. The sum of these values represented the weighted mean QF L_f and θ_p at optimum joint angle.

Physiological cross-sectional area (PCSA)

The PCSA of each of the four constituent muscles of the QF was calculated by dividing the component muscle volume by its respective optimum L_f. The total RF volume was divided by the lateral L_f. The complete QF PCSA was given by the sum of the PCSAs of the four constituent muscles.

Specific tension

The force transmitted from the muscle fibre to the tendon is reduced as a result of the fibre pennation angle. Consequently, a *reduced* PCSA (*r*PCSA) of the complete QF muscle was determined as the sum of the PCSAs of the four constituent muscles, each multiplied by the cosine of the appropriate θ_p, thus:

$$r\text{PCSA}_{QF} = \sum_{i=1}^{n} \text{PCSA}_i \bullet \cos\ \theta_{pi}$$

where rPCSA$_{QF}$ is the total *reduced* physiological cross-sectional area of the quadriceps femoris muscle, PCSA$_i$ is the muscle PCSA of each of the four heads of the quadriceps femoris muscle and cos θ_{pi} is the cosine of the optimum fascicle pennation angle in each one of the four heads of the quadriceps femoris muscle. QF specific tension was then obtained as follows:

QF specific tension = F_t / rPCSA$_{QF}$

where F_t is patellar tendon force (TMT divided by the patellar tendon moment arm length).

Method 2

Method 2 differed from Method 1 in that the QF volume was estimated from a single QF ACSA (Fig. 2.3). With the participant relaxed in the supine position, femur length was measured as the distance from the tibiofemoral point of contact to the proximal insertion of the VL muscle, which was identified via ultrasonography (MyLab25, Esaote, Genova, Italy). Oil-filled beads were attached to the skin at the measurement location (corresponding to 40% of femur length from the distal end), in order that the correct MRI scan could be easily identified (Fig. 2.3). The participant was scanned in the supine position and five serial axial slices were acquired around the position of the beads using a spin echo T1-weighted protocol with the following scanning parameters: time of repetition 200 ms; time to echo 1.9 s; matrix 256 x 256; field of view 180 mm x 180 mm; slice thickness 5 mm; interslice gap 5 mm. QF volume was then estimated by using the ACSAs of the constituent QF muscles from a single slice taken at 40% of the femur length, together with a series of regression equations, as described in detail by Morse *et al.* (2007a). This technique has been shown to consistently underestimate QF volume by 551 cm^3 (Morse *et al.*, 2007a). Therefore, once QF volume was assessed using this procedure,

551 cm^3 was added to the QF volume of each participant to provide a more accurate estimation.

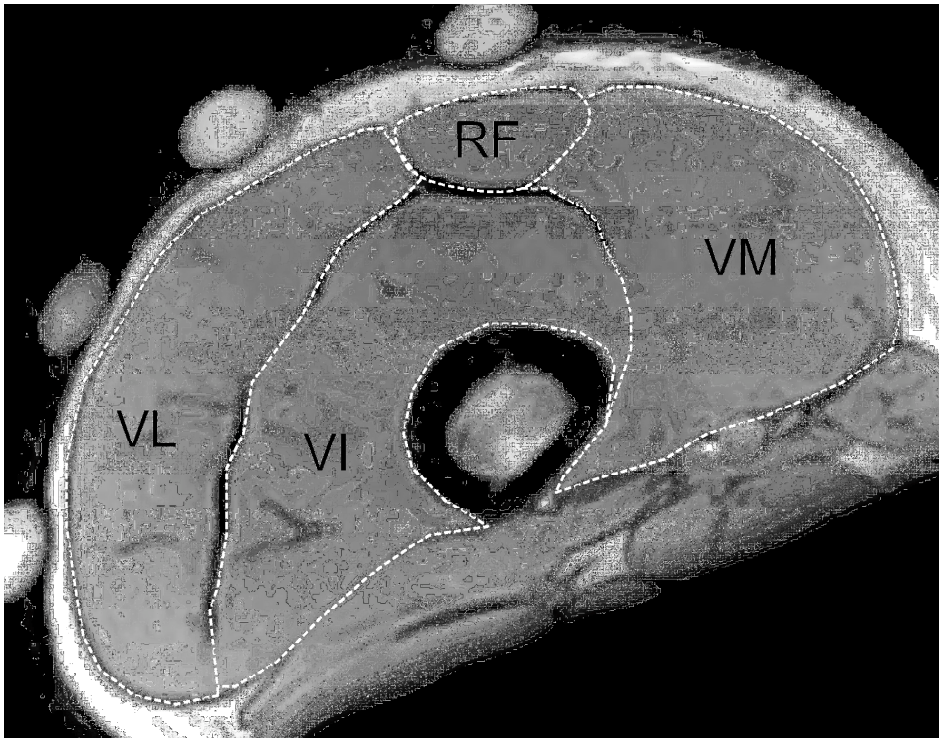

Fig. 2.3 A typical transverse magnetic resonance imaging scan of the quadriceps femoris muscle at 40% femur length. The boundaries of the four component muscles: vastus lateralis (VL); vastus intermedius (VI); rectus femoris (RF); vastus medialis (VM) have been highlighted

QF PCSA was calculated by dividing the estimated QF volume by the fascicle length (L_f) of the VL only (Fig. 2.2); VL L_f measured during MVC at optimum joint angle was assumed to be representative of the mean L_f for the entire QF. QF *r*PCSA was then calculated as:

QF PCSA $\cdot \cos \theta_{pVL}$

where VL θ_p was assumed to be representative of the mean QF θ_p.

QF specific tension was subsequently calculated as follows:

QF specific tension = F_t / $r\text{PCSA}_{QF}$

Statistics

The test-retest reliability of the main assessments used in Method 1 and Method 2 was determined by calculating the respective coefficients of variation (CV) and intraclass correlation coefficients (ICC), which are presented in Table 2.2. In order to compare the inter-individual variability in MVC knee joint torque with that of QF specific tension, the single sample CV ($100 \cdot [\text{standard deviation} \cdot \text{mean}^{-1}]$) was calculated for these variables. Linear regression analyses were performed to determine the relations between Method 1 and Method 2 regarding QF PCSA and specific tension. Bland-Altman plots were made to demonstrate the level of agreement between Method 1 and Method 2 concerning the calculation of QF PCSA and specific tension. The standard error of the estimate (SEE) of the regression equation was assessed to determine the accuracy of the estimate of PCSA and specific tension using Method 2 compared to Method 1 and was calculated using the following equation, where SD is the standard deviation of the values for Method 2:

$$SEE = SD \cdot \sqrt{(1-R^2)}$$

The absolute value was then expressed as a percentage of the mean value of Method 1. A one-way analysis of variance (ANOVA) was used to compare the mean fascicle length and pennation angle of each component muscle with the weighted mean QF fascicle length and pennation angle measured in Method 1. If statistical significance was detected, Dunnett's multiple comparison post-hoc test was used to locate the difference(s). Statistical significance was accepted when $P < 0.05$.

Table 2.2 Reliability of the main measurements and calculations used in Methods 1 and Method 2

Variable	Method 1		Method 2	
	CV (%)	ICC	CV (%)	ICC
MVC torque	3.92	0.95	M1	M1
Voluntary Activation	2.61	0.73	M1	M1
Antagonist co-activation	14.39	0.89	M1	M1
Moment arm length	1.57	0.95	M1	M1
Tendon force	3.99	0.92	M1	M1
Femur length	N/A	N/A	1.69	0.86
QF ACSA	N/A	N/A	1.12	0.99
QF Volume	0.81	0.99	1.73	0.99
θ_p (VL; VI; RF; VM)	5.1; 5.7; 5.8; 6.5	0.86; 0.97; 0.90; 0.94	M1 (VL)	M1 (VL)
L_f (VL; VI; RF; VM)	3.5; 4.0; 4.7; 5.4	0.92; 0.94; 0.87; 0.79	M1 (VL)	M1 (VL)
QF PCSA	5.76	0.93	5.00	0.90
QF specific tension	8.79	0.74	9.11	0.69

CV, coefficient of variation; ICC intraclass correlation coefficient; MVC, maximal voluntary contraction; QF, quadriceps femoris muscle; VL, vastus lateralis muscle; VI, vastus intermedius muscle; RF, rectus femoris muscle; VM, vastus medialis muscle; θ_p, fascicle pennation angle; L_f, fascicle length; PCSA, physiological cross-sectional area; M1, as for Method 1

Results

The mean values and associated degrees of variance for MVC torque, antagonist muscle co-activation, level of voluntary muscle activation, patellar tendon moment arm length and tendon force that were used in both Method 1 and Method 2 to calculate tendon force are presented in Table 2.3. The single sample coefficients of variation for MVC torque, specific tension (Method 1) and specific tension (Method 2) were 17.8%, 16.2% and 16.5%, respectively.

Table 2.3 Variables used in the calculation of tendon force; n = 27; mean ± SD

MVC (N·m)	264 ± 47
Antagonist co-activation (%)	16.5 ± 8.6
Voluntary activation (%)	92.8 ± 3.7
d_{PT} (cm)	4.8 ± 0.3
Patellar tendon force (N)	6335 ± 957

MVC, Maximal voluntary contraction; d_{PT}, patellar tendon moment arm

Table 2.4 provides information on the measured volume, ACSA and PCSA of each of the four constituent QF muscles and the value for the total QF. In addition, L_f and θ_p at the optimum joint angle are reported for each of the component muscles, together with the weighted mean QF value (accounting for the ratio of constituent muscle volume to total QF volume). The VL optimum L_f and θ_p did not differ from the weighted mean QF L_f and θ_p ($P > 0.05$; Table 2.4). Table 2.5 presents QF volume, PCSA and specific tension values obtained from the two methods.

Table 2.4 Morphological characteristics of the vastus lateralis (VL), vastus intermedius (VI), rectus femoris (RF), vastus medialis (VM) and total quadriceps femoris muscle (QF); n = 27; Mean ± SD. * significantly different ($P < 0.05$) to weighted mean QF value (applicable to L_f and θ_p only)

Variable	VL	VI	RF	VM	QF
V_m (cm^3)	674 ± 123	580 ± 103	339 ± 61	461 ± 69	2054 ± 261
ACSA (cm^2)	18.3 ± 3.5	20.1 ± 3.2	4.0 ± 1.4	22.3 ± 3.2	64.7 ± 7.9
L_f (cm)	9.1 ± 1.2	10.5 ± 1.7*	69.0 ± 1.4*	10.5 ± 1.3*	9.4 ± 7.0
θ_p (°)	15.7 ± 2.4	12.7 ± 3.8*	27.9 ± 7.6*	22.9 ± 6.6*	18.5 ± 2.6
PCSA (cm^2)	75.1 ± 14.5	56.5 ± 13.7	49.8 ± 10.9	44.4 ± 6.4	224.9 ± 27.1

V_m, Muscle volume; ACSA, anatomical cross-sectional area at 40% femur length; L_f, fascicle length; θ_p, fascicle pennation angle; PCSA, physiological cross-sectional area calculated according to Method 1

Table 2.5 Quadriceps femoris muscle (QF) volume, physiological cross-sectional area (PCSA) and specific tension calculated according to Method 1 and Method 2. Values are mean ± SD; n = 27

Method	QF Volume (cm^3)	QF PCSA (cm^2)	QF Specific tension ($N \cdot cm^{-2}$)
1	2054 ± 261	226 ± 28	30.3 ± 4.9
2	2117 ± 238	236 ± 34	29.1 ± 4.8

QF volume estimated using Method 2 was significantly correlated with that measured using Method 1 ($R^2 = 0.90$; $P < 0.0001$; Fig. 2.4). Likewise, PCSA estimated using Method 2 correlated with that calculated using Method 1 ($R^2 = 0.59$; $P < 0.0001$; SEE = 9.9%; Fig. 2.5a). Bland–Altman analysis concerning QF PCSA demonstrated that the respective bias was -10.6 cm^2 and the 95% limits of agreement were between 33.1 and 54.4 cm^2 (Fig. 2.6a).

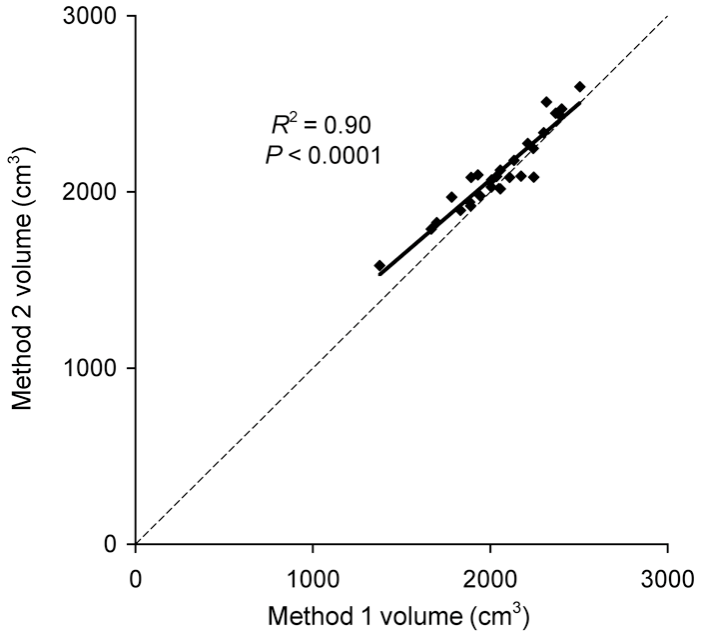

Fig. 2.4 The relationship between measured (Method 1) and estimated (Method 2) quadriceps femoris muscle volume; $R^2 = 0.90$; $P < 0.0001$; SEE = 3.7%; n = 27

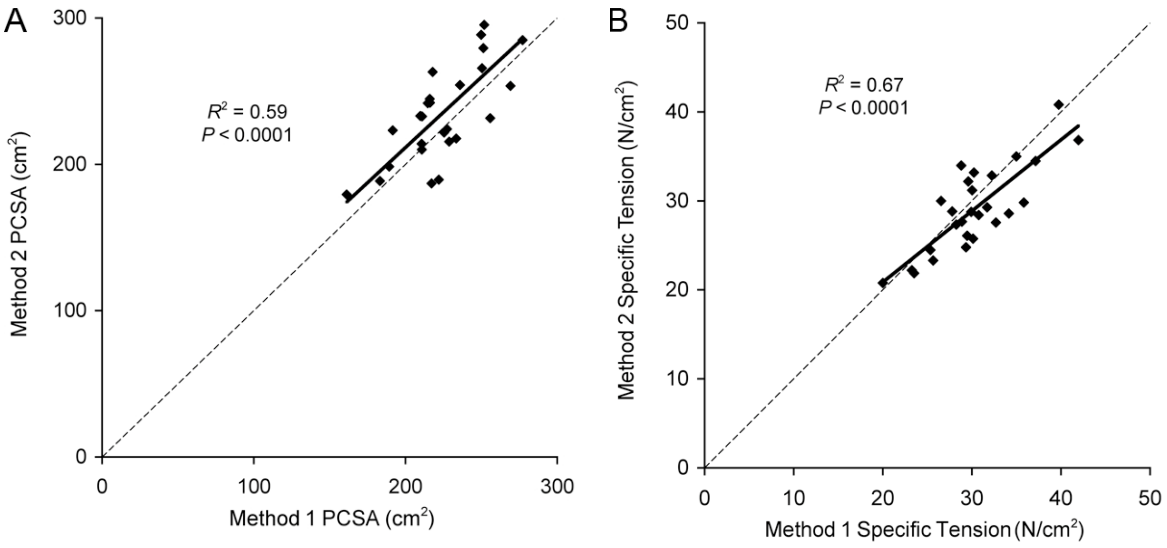

Fig. 2.5 The relationship between calculated (Method 1) and estimated (Method 2) quadriceps femoris muscle PCSA (A) and specific tension (B); SEE = 9.9% (A) and 9.1% (B); n = 27

As shown in Fig. 2.5b, the specific tension values derived from Method 1 correlated significantly with the estimates obtained from Method 2 (R^2 = 0.67 P < 0.0001; SEE = 9.1%). Bland–Altman analysis demonstrated that the respective bias was 1.2 N·cm^{-2} and the 95% limits of agreement were between -4.6 and 6.9 N·cm^{-2} (Fig. 2.6b). Furthermore, Method 1 and Method 2 produced similar values regarding the inter-individual variability in QF volume, PCSA and specific tension (Table 2.5).

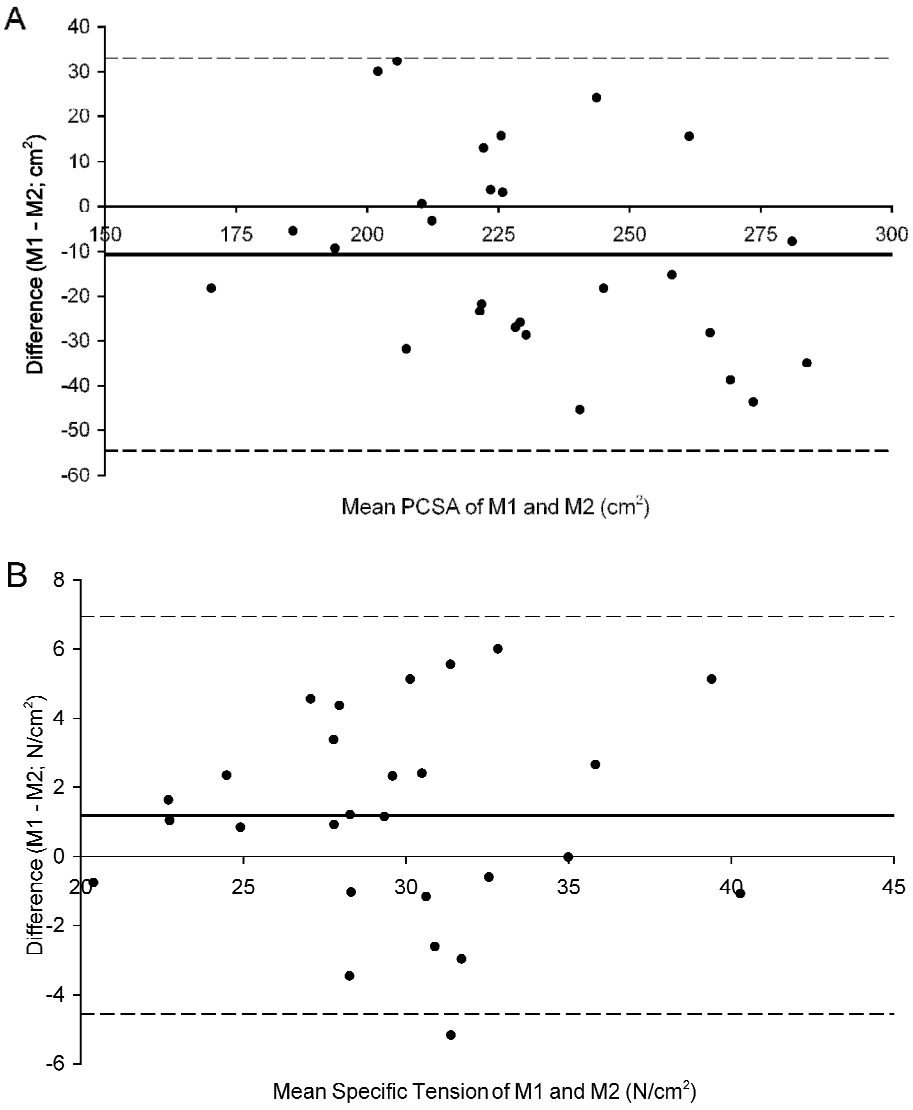

Fig. 2.6 Bland-Altman plots showing the bias (—) and 95% limits of agreement (---) between Method 1 and Method 2 regarding the calculation of quadriceps femoris muscle PCSA (A) and specific tension (B); n = 27

Discussion

The hypothesis of the present study was that much of the expected variability in MVC knee joint torque might be explained by variation in extrinsic factors that have not been routinely measured, such as differences in the architecture of the four component muscles in the quadriceps femoris muscle group (QF), voluntary muscle activation, antagonist muscle co-activation and patellar tendon moment arm length. Because the list of possible factors affecting the measured force is extensive, the second aim of the current

investigation was to determine a minimal set of measurements that may be used to provide reliable estimates of QF specific tension *in vivo*. In short, the results of Method 1 of the present investigation have shown that, in a relatively large and homogeneous group of participants, variation in these extrinsic factors explains little (~2%) of the variance in maximum isometric knee joint torque. As calculated using Method 1, the variability in QF specific tension is 16%, which is similar to that reported in animal muscle (Degens *et al.*, 1995). Further, the results of the present study suggest that reliable comparisons can be made on the basis of a comparatively small number of measurements of muscle strength and size.

In order to calculate PCSA and specific tension accurately, muscle architecture and tendon force should be assessed during maximal activation at the optimum joint angle, i.e. when the muscle fibres are at optimum length for producing maximum force (Chow *et al.*, 1999; Maganaris *et al.*, 2001). The present study is the first to address this issue in the QF using purely *in vivo* measurements of fascicle length for each of the four heads at maximum activation (Method 1). The results demonstrate that there are differences in fascicle length and pennation angle between the four component muscles at MVC, although VL architecture did not differ from the mean QF architecture (Table 2.4). This suggests that, although there may be differences in architecture between QF component muscles, VL geometry measured during MVC at the optimum joint angle is typical of the QF muscle group as a whole.

The complex geometry of the four QF constituent muscles makes the *in vivo* calculation of the respective PCSAs and subsequent QF specific tension in Method 1 of the present study a sedulous process that is probably not suitable for large cohorts or where frequent

repeated measurements are required. Examining the variability in strength in a given population requires a considerable sample size (Maughan *et al.*, 1983; Kanehisa *et al.*, 1994; Bamman *et al.*, 2000; Akima *et al.*, 2001; Hubal *et al.*, 2005) and thus the time available to perform extensive measurements on each individual is limited. Obtaining a single QF ACSA assessment, however, is relatively straightforward and previous studies have generally compared this measure of muscle size with MVC to evaluate the muscle size-strength relationship *in vivo* (Ikai & Fukunaga, 1970; Maughan *et al.*, 1983; Chapman *et al.*, 1984; Maughan *et al.*, 1984; Kanehisa *et al.*, 1994; Akima *et al.*, 2001). However, ACSA underestimates the PCSA of pennate muscles and net joint torque does not give a true representation of the maximum force generated by the muscle. Therefore, ACSA and MVC may give a false indication of the muscle size-strength relationship and the nature of any training-induced changes (Kawakami *et al.*, 1995).

To circumvent some of the problems associated with making multiple measurements, a simpler method was employed in which QF muscle volume was calculated from a single ACSA and the geometry of the VL muscle was considered representative of the mean QF muscle architecture (Method 2). This method produced similar PCSA and specific tension values (236 cm^2 and 29 $N \cdot cm^{-2}$, respectively) to the more comprehensive approach used in Method 1 (226 cm^2 and 30 $N \cdot cm^{-2}$, respectively). In addition, the PCSA and specific tension values calculated using Method 2 were highly correlated with those obtained using Method 1 and the SEE for PCSA and specific tension estimated using Method 2 was relatively small at just 10 and 9%, respectively. The Bland-Altman plot in Fig. 2.6a indicates that Method 2 overestimated QF PCSA calculated using Method 1 by just 11 cm^2 (4%) and the limits of agreement are relatively small, suggesting that Method 2 provided similar estimates of QF PCSA to Method 1. The slight overestimation was due primarily to

an initial 3% overestimation of QF volume. Consequently, Method 2 underestimated the QF specific tension calculated with Method 1 by only 1 $N \cdot cm^{-2}$ (4%) with a small variation around this bias (Fig. 2.6b). It appears, therefore, that Methods 1 and 2 are well matched for calculating QF PCSA and specific tension.

The fact that Method 1 and Method 2 yielded very similar specific tension values and neither method appreciably reduced the inter-individual variability observed in MVC torque suggests that most of the variability in specific tension is related to factors other than differences in moment arm length, muscle architecture, voluntary activation and/or antagonist co-activation. The implication is, therefore, that differences reside in the intrinsic force generating capacity of the muscle fibres themselves. Inter-individual differences in QF muscle fibre type composition may explain some of the variance in QF specific tension, as type II muscle fibres have been shown to be intrinsically stronger than type I fibres (Bottinelli *et al.*, 1996). Another possible explanation for some of the variability in QF specific tension is a variable infiltration of non-contractile tissue, such as intramuscular fat and connective tissue (Kent-Braun *et al.*, 2000; Holmback *et al.*, 2002; Macaluso *et al.*, 2002), which has been reported to constitute 6% of the total QF volume measured *in vivo* in young women (Macaluso *et al.*, 2002). By not differentiating between intramuscular contractile and non-contractile material in the present study, the volume of muscle contractile material may have been slightly overestimated, which would have led to an overestimation of PCSA and an underestimation of specific tension. Furthermore, although the mean composition of intramuscular non-contractile material is relatively small in young individuals (Macaluso *et al.*, 2002), the variation around the mean is comparatively high and different amounts of intramuscular non-contractile material between people may well affect the overall variability in muscle specific tension. In

addition, differences in connective tissue content between people may lead to inter-individual differences in the degree of lateral transmission of force from muscle fibres to the tendon, which would also affect the variability in specific tension (Jones *et al.*, 1989).

The QF specific tension reported using Method 1 of the present study (30 $N \cdot cm^{-2}$) is slightly higher than that reported elsewhere for the same (Narici *et al.*, 1992; Reeves *et al.*, 2004a; Gorgey *et al.*, 2006) and other muscles, such as the soleus and tibialis anterior (Maganaris *et al.*, 2001). As well as differences in muscle fibre-type composition between different muscle groups (Johnson *et al.*, 1973), variations in electrical muscle stimulation protocols (more specifically the pulse duration or frequency of stimuli) used to generate a joint moment may partly explain the variability in specific tension values reported between some studies (Maganaris *et al.*, 2001; Gorgey *et al.*, 2006). In addition, voluntary muscle activation was determined using the ITT in the present study, while a combination of T2-MRI and neuromuscular stimulation has been used for the same purpose in several other investigations (Adams *et al.*, 1993a; Kendall *et al.*, 2006). The latter method generally demonstrates a lower voluntary muscle activation capacity (~75%) than the ITT (~90%) and hence would lead to differences between studies when calculating muscle specific tension *in vivo*.

Conclusions

Specific tension of the human QF muscle has been calculated *in vivo* from the most comprehensive set of measurements to date (Method 1) and the results indicate that most of the inter-individual variance in MVC torque arises from factors other then differences in the structure and geometry of the muscle. Possibly linked to this observation is the fact that a simpler approach (Method 2) provided an accurate estimate of QF PCSA and QF specific

tension and may be useful where time and resources are limited. It should be noted that the conclusions from the present study are based on a relatively homogeneous population and the methods and conclusions may therefore need to be validated for other populations.

Chapter 3

Resistance training increases *in vivo* quadriceps femoris muscle specific tension in young men

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Abstract

The present study investigated whether *in vivo* human quadriceps femoris (QF) muscle specific tension changed following strength training by comprehensively determining QF maximal force and physiological cross-sectional area (PCSA). Seventeen untrained men (20 ± 2 yrs) performed high-intensity leg-extension training 3 times a week for 9 weeks. Maximum tendon force (F_t) was calculated from maximum voluntary contraction (MVC) torque, corrected for agonist and antagonist muscle activation, and moment arm length (d_{PT}) before and after training. QF PCSA was calculated as the sum of the four component muscle volumes, each divided by its fascicle length (L_f). Dividing F_t by the sum of the component muscle PCSAs, each multiplied by the cosine of the respective fascicle pennation angle (θ_p), provided QF specific tension. MVC torque and QF activation increased by 31% ($P < 0.01$) and 3% ($P < 0.05$), respectively, but there was no change in antagonist co-activation or d_{PT}. Subsequently, F_t increased by 27% ($P < 0.01$). QF volume increased by 6% but L_f did not change in any of the component muscles, leading to a 6% increase in QF PCSA ($P < 0.05$). θ_p increased by 5% ($P < 0.01$) but only in the vastus lateralis muscle. Consequently, QF specific tension increased by 20% ($P < 0.01$). An increase in human muscle specific tension appears to be a real consequence of resistance training rather than being an artefact of measuring errors but the underlying cause of this phenomenon remains to be determined.

Introduction

The maximum force generated by skeletal muscle is primarily determined by the number of sarcomeres arranged in parallel, which is represented by the physiological cross sectional area (PCSA) of the muscle (Close, 1972; Powell *et al.*, 1984). Consequently, an increase in PCSA should be accompanied by a proportional increase in force generating capacity as is observed in experiments on animal muscle subjected to chronic overload (Degens *et al.*, 1995; Hornberger & Farrar, 2004). In human muscle, however, resistance training is commonly reported to induce a proportionally larger increase in strength than muscle size (Ikai & Fukunaga, 1970; Moritani & deVries, 1979; Young *et al.*, 1983; Jones & Rutherford, 1987; Davies *et al.*, 1988; Narici *et al.*, 1989; Narici *et al.*, 1996b; Ferri *et al.*, 2003). One possible explanation for this discrepancy between animal and human may be the difficulty of assessing maximum human muscle force and PCSA *in situ*, compared to the fewer experimental constraints encountered in animal models.

As well as assessing neural drive to the muscle and antagonist muscle co-contraction, a thorough assessment of the peripheral factors affecting the force generating capacity of a muscle is critical in accurately estimating *in vivo* muscle specific tension (maximum muscle force per PCSA). These factors include muscle volume, optimum muscle fibre length, muscle fibre pennation angle (the angle of fibre insertion into the aponeurosis) and moment arm length (Maganaris *et al.*, 2001; Reeves *et al.*, 2004a; Erskine *et al.*, 2009). Anatomical CSA (ACSA), the area of muscle perpendicular to the longitudinal axis of the limb, underestimates the PCSA of pennate-fibred muscles (Alexander & Vernon, 1975). Nevertheless, this measure has frequently been used to assess the effects of strength training on quadriceps femoris (QF) muscle size (Young *et al.*, 1983; Jones & Rutherford, 1987; Narici *et al.*, 1989; Narici *et al.*, 1996b; Ferri *et al.*, 2003) since there are technical

difficulties in assessing *in vivo* PCSA of the total QF muscle group, which comprises four pennate muscles of different sizes and optimum architecture (Chow *et al.*, 1999; Erskine *et al.*, 2009).

Although a training-induced increase in fibre pennation angle (Kawakami *et al.*, 1995; Aagaard *et al.*, 2001; Reeves *et al.*, 2004a) is thought to allow more contractile material to be accommodated along a given tendon length, thereby increasing the PCSA and allowing greater force production (Gollnick *et al.*, 1981; Aagaard *et al.*, 2001), it simultaneously reduces the force exerted by the fibre that is resolved along the tendon (Alexander & Vernon, 1975; Narici *et al.*, 1996a; Degens *et al.*, 2009). This demonstrates the importance of considering changes in muscle architecture when determining the training-induced changes in muscle specific tension *in vivo*.

In addition to changes in muscle architecture and volume (Aagaard *et al.*, 2001; Reeves *et al.*, 2004a; Kubo *et al.*, 2006), neural adaptations to resistance training, such as an increased voluntary muscle activation (Scaglioni *et al.*, 2002; Reeves *et al.*, 2004a) and reduced co-activation of antagonist muscles (Carolan & Cafarelli, 1992; Hakkinen *et al.*, 1998; Hakkinen *et al.*, 2000) can lead to a change in maximum voluntary contraction (MVC) torque following training. A failure to fully account for possible changes in muscle architecture, activation or co-activation with training may lead to erroneous conclusions of the effects of strength training on *in vivo* muscle specific tension. Consequently, the aim of the present study was to elucidate whether *in vivo* QF specific tension was affected by high-intensity resistance training using a comprehensive assessment of individual component muscle volume and architecture, degree of voluntary muscle activation, antagonist muscle co-activation and moment arm length. It was hypothesised that QF

specific tension would remain the same after strength training. Thus, previous reports of a disproportionate increase in muscle strength and size with strength training would be explicable by changes in factors that have not been routinely measured in the past.

Materials and methods

Participants

Seventeen untrained males, aged 20.1 ± 2.3 years, height 176.9 ± 4.7 cm, body mass 76.1 ± 10.1 kg (mean ± SD), participated in the study after written informed consent was obtained. Participants were excluded if they had a history of lower-limb fracture, their age was outside the range of 18-39 years, they had taken part in resistance training within the 12 months prior to the study, had used dietary supplements or performance enhancing aids or if they were considered to be in ill health (assessed from the responses to a general health questionnaire). The study was approved by Manchester Metropolitan University Local Ethics Committee. All volunteers were familiarised with the test procedures and equipment within a 14-day period prior to starting the study and participants were asked to maintain their habitual dietary intake and level of physical activity over the course of the investigation.

Experimental design

All *in vivo* measurements used to calculate maximum patellar tendon force (including MVC at optimum knee joint angle, voluntary QF muscle activation, antagonist muscle co-activation and patellar tendon moment arm), QF muscle physiological cross-sectional area [including muscle volume and fascicle length (L_f) measurements of each component QF muscle (VL, vastus lateralis; VI, vastus intermedialis; RF, rectus femoris; VM, vastus medialis)] and QF muscle specific tension [accounting for the fascicle pennation angle (θ_p) of each constituent QF muscle] of the right limb before and after resistance training have

been described in detail as per Method 1 in Chapter 2.

Progressive resistance training

The training programme comprised unilateral knee extension exercises performed three times a week for a period of nine weeks on the right leg. Thus, each participant performed a total of 27 sessions and each session lasted 10-15 min. At the start of each week, the single repetition maximum (1 RM) was determined on a standard leg-extension machine (Technogym SpA, Gambettola, Italy) and the training intensity was set relative to the 1 RM. Therefore, the training load was increased progressively throughout the training period, according to the re-assessed 1 RM. Each training session consisted of a warm-up set of 10 repetitions at 40% of the 1 RM, followed by four sets of 10 repetitions at 80% of the 1 RM, with a 2 min rest period between each set. Each repetition required approximately one second to lift and one second to lower the load through a range of 110 to 20º of knee flexion (0º = full extension). All training sessions were supervised and appropriate verbal encouragement was given throughout each session.

Statistics

The test-retest reliability (coefficient of variation, CV and intraclass correlation coefficient, ICC) of all the measurements used to calculate *in vivo* QF specific tension has been reported in Chapter 2 (Erskine et al., 2009). Paired samples Student's *t*-tests were used to investigate pre- to post-training differences in MVC torque, voluntary muscle activation, antagonist muscle co-activation, optimum knee joint angle, patellar tendon moment arm length, maximum patellar tendon force, total QF volume and total QF PCSA. A two-factor within subjects ANOVA was used to investigate the effect of time (2 levels; pre- and post-training) on QF component muscle (4 levels; VL, VI, RF and VM) for each of the

following variables: muscle volume, L_f, θ_p and PCSA. If a training effect was detected, paired samples Student's *t*-tests were used to locate post-training changes in each muscle. Differences and effects were considered significant when $P < 0.05$. All data are presented as mean ± standard deviation (SD) unless otherwise stated.

Results

Maximum patellar tendon force

After training, MVC knee joint torque increased by 30.8 ± 8.6 % ($P < 0.01$; Table 3.1) and QF voluntary activation capacity increased by 2.6 ± 4.6 % ($P < 0.05$; Table 3.1). However, there was no change in the level of antagonist muscle co-activation, optimum knee joint angle or d_{PT} (Table 3.1). Correction of MVC torque for all of these factors gave the maximum patellar tendon force, which increased by 27.4 ± 10.5% ($P < 0.01$; Table 3.1).

Table 3.1 Variables used to calculate maximum patellar tendon force before and after training

Variable	Before training	After training	Change (%)
MVC (N·m)	269 ± 50	350 ± 57	30.8 ± 8.6**
Optimum knee angle (°)	81.2 ± 3.4	81.8 ± 4.2	0.8 ± 3.5
Co-activation (%)	13.5 ± 3.2	13.2 ± 2.9	-1.7 ± 9.9
Activation (%)	93.4 ± 3.0	96.0 ± 3.5	2.6 ± 4.6*
d_{PT} length (cm)	4.8 ± 0.3	4.8 ± 0.3	0.2 ± 2.0
F_t (N)	6296 ± 988	8023 ± 826	27.4 ± 10.5**

MVC Maximum voluntary contraction; d_{PT} patellar tendon moment arm; F_t maximum patellar tendon force; n=17; *$P < 0.05$ and ** $P < 0.01$ significant training-induced change

Muscle architecture

L_f did not change in any of the four component QF muscles following the nine-week intensive training programme (Table 3.2). VL muscle θ_p increased by 5.1 ± 8.8% following the training period ($P < 0.01$; Table 3.2) but the small increases in θ_p of the remaining three heads did not reach statistical significance (Table 3.2).

Table 3.2 Muscle architecture assessed before and after training

	Before training	After training	Change (%)
VL L_f	8.8 ± 1.1	8.8 ± 1.2	0.3 ± 9.0
VI L_f	10.3 ± 1.9	10.4 ± 1.6	1.7 ± 14.1
RF L_f	7.2 ± 1.7	7.4 ± 1.9	1.6 ± 9.4
VM L_f	10.3 ± 1.2	10.3 ± 1.3	0.6 ± 7.6
VL θ_p	16.2 ± 2.4	16.9 ± 2.4	5.1 ± 8.8**
VI θ_p	13.2 ± 3.2	13.8 ± 3.1	6.8 ± 17.9
RF θ_p	26.5 ± 4.6	27.0 ± 4.2	2.5 ± 9.9
VM θ_p	24.5 ± 6.8	24.9 ± 7.4	1.8 ± 8.3

VL, vastus lateralis; *VI*, vastus intermedius; *RF*, rectus femoris; *VM*, vastus medialis; L_f fascicle length (cm); θ_p fascicle pennation angle (°); $n = 17$; $^{**}P < 0.01$ significant training-induced change

Muscle volume

Volumes of the individual component QF muscles (VL, VI, RF and VM) before and after training are shown in Table 3.3. Training had a similar significant effect by increasing the volumes of all four muscles ($P < 0.05$), resulting in a 5.5 ± 2.6% increase in total QF muscle volume ($P < 0.01$).

Physiological cross-sectional area and specific tension

The PCSAs for the RF and VM muscles increased significantly by 8.1 ± 11.7% and 7.4 ± 9.9% ($P < 0.05$), respectively and, although not reaching statistical significance, the VL and VI muscle PCSAs showed a tendency to increase by 4.4 ± 10.4% ($P = 0.09$) and 4.7 ± 8.0% ($P = 0.06$), respectively (Table 3.3). As there was no interaction between training and component muscle PCSA, all four muscles appear to have responded similarly to training, leading to a 5.5 ± 5.8% increase in whole QF muscle PCSA ($P < 0.05$). Systematically assessing maximally activated QF muscle force and PCSA before and after training led to

a 20.1 ± 13.1% increase in QF specific tension ($P < 0.01$), from 29.5 ± 4.1 N·cm^{-2} before to 35.5 ± 5.1 N·cm^{-2} after training.

Table 3.3 Muscle volume and physiological cross-sectional area (PCSA) assessed before and after training

	Before training	After training	Change (%)
VL volume (cm^3)	677 ± 94	704 ± 106	3.9 ± 3.2**
VI volume (cm^3)	586 ± 117	609 ± 118	4.0 ± 2.5**
RF volume (cm^3)	345 ± 56	375 ± 57	8.9 ± 5.4**
VM volume (cm^3)	466 ± 55	500 ± 57	7.4 ± 4.1**
QF volume (cm^3)	2074 ± 221	2188 ± 245	5.5 ± 2.6**
VL PCSA (cm^2)	78.1 ± 13.0	81.7 ± 16.4	4.4 ± 10.4
VI PCSA (cm^2)	58.5 ± 15.3	60.0 ± 14.7	4.7 ± 8.0
RF PCSA (cm^2)	49.6 ± 11.5	53.3 ± 12.2	8.1 ± 11.7*
VM PCSA (cm^2)	45.6 ± 5.1	49.0 ± 6.9	7.4 ± 9.9*
QF PCSA (cm^2)	231 ± 24	245 ± 34	5.5 ± 5.8*

VL, vastus lateralis; *VI*, vastus intermedius; *RF*, rectus femoris; *VM*, vastus medialis; $n = 17$; *$P < 0.05$ and **$P < 0.01$ significant training-induced change

Discussion

The present study sought to explain the disproportionate change in human quadriceps femoris (QF) muscle strength and size that is often observed following resistance training. It was hypothesised that a training-induced gain in knee joint MVC torque would be explained by changes in factors that have not been routinely measured in the past, such as changes in volume and architecture of the four component muscles of the quadriceps femoris (QF) group, together with changes in voluntary QF muscle activation antagonist muscle co-activation and changes in the patellar tendon moment arm length, thus indicating no change in QF muscle specific tension. Contrary to this hypothesis, however, *in vivo* QF muscle specific tension was found to increase by 20% following nine weeks of

high-intensity resistance training.

The 31% increase in MVC observed in the present study is comparable to that reported by others following a similar period of resistance training of the QF (Hakkinen *et al.*, 1998; Seynnes *et al.*, 2007). MVC torque, however, is not a measure of the force produced by a maximally activated muscle. By assessing the voluntary activation capacity of the agonist muscle, the co-activation of the antagonist muscles and the length of the moment arm, maximal muscle force can be estimated *in vivo* (Maganaris *et al.*, 2001; Reeves *et al.*, 2004a; Erskine *et al.*, 2009). It has been suggested that greater neural adaptations than muscle hypertrophy might be the main reason for the disproportionate changes in muscle strength and size following strength training (Moritani & deVries, 1979; Staron *et al.*, 1991; Staron *et al.*, 1994). However, voluntary QF muscle activation in the present study increased by only 3%, which was similar to previous reports of increased muscle activation after resistance training (Scaglioni *et al.*, 2002; Reeves *et al.*, 2004a). Furthermore and in line with previous studies (Hortobagyi *et al.*, 1996; Reeves *et al.*, 2004a; Reeves *et al.*, 2005), there was no change in the level of antagonist muscle co-activation following strength training. Therefore, it would appear that neural adaptations played a minimal role in explaining the 31% increase in MVC torque. In addition, although patellar tendon moment arm length (d_{PT}) changes as a function of knee joint angle (Baltzopoulos, 1995), no change in optimum joint angle was observed in the current investigation, thus d_{PT} was not altered following training. Consequently, correcting MVC for voluntary muscle activation, antagonist muscle co-activation and d_{PT} gave a 27% increase in the maximum patellar tendon force, demonstrating that training-induced changes in voluntary activation, co-activation and moment arm contributed little to the 31% increase in MVC torque.

As the force generating capacity of a muscle is thought to be primarily determined by the number of sarcomeres in parallel, which is represented *in vivo* by the muscle PCSA (Close, 1972), muscle hypertrophy is the factor most likely to account for this increase in maximally activated force. By dividing the volume of each component muscle by its fascicle length, PCSA of the whole QF muscle was able to be determined before and after training in the present study, thus providing the critical measure of *in vivo* muscle hypertrophy. In line with previous reports (Aagaard *et al.*, 2001; Reeves *et al.*, 2004a; Kubo *et al.*, 2006), whole QF volume increased by 6% and, as fascicle length did not change with training in any of the four component QF muscles, QF PCSA increased by 6%, accordingly. However, this relatively small degree of muscle hypertrophy explained only 22% of the increase in maximum muscle force resolved at the tendon, suggesting that other training-induced adaptations played a larger role.

Although an increase in fascicle pennation angle (θ_p) allows more contractile material to attach to the tendon (Gollnick *et al.*, 1981; Aagaard *et al.*, 2001), less of the muscle fibre force is simultaneously resolved along the tendon (Alexander & Vernon, 1975; Narici, 1999; Degens *et al.*, 2009). Thus, taking into account the change in θ_p in each of the four component QF muscles is likely to improve the estimate of the change in force produced by the actual QF muscle fibres. The small training-induced increases in θ_p of the constituent QF muscles only reached statistical significance in the VL muscle, which reflected a 6% increase in QF PCSA. However, such small increases in θ_p would have minimal limiting effects on the force resolved at the tendon, thereby not greatly affecting the change in QF specific tension observed in the present study.

Contrary to the original hypothesis of the present study it was not possible to reconcile the

large increase in knee extension torque with the smaller increase in QF muscle volume by allowing for changes in fascicle length and pennation angle of the component muscles, together with training-induced changes in activation and co-activation or alterations in optimal knee angle and moment arm. Consequently, it is concluded that the training resulted in a real increase in specific tension of the QF muscle, which accounted for most of the increase in MVC observed in the present study. A study on an elderly human population found specific tension of the VL muscle to increase by 19% following 14 weeks of resistance training (Reeves *et al.*, 2004a). In this particular study, the force generated by the VL muscle was estimated by assigning the patellar tendon force according to the relative VL PCSA obtained from another study (Narici *et al.*, 1992), assuming that the relative QF component muscle PCSAs would not be affected by age or training status. The current investigation demonstrated that all four constituent QF muscles did respond similarly to resistance training, although the relative component muscle PCSAs differed from those reported previously (Narici *et al.*, 1992).

The source of the increase in *in vivo* QF specific tension cannot be deduced from the present results but various possibilities exist. An increase in the packing density of myofilaments would cause an increase in specific tension of single human muscle fibres, which has recently been reported following resistance training (Parente *et al.*, 2008; Pansarasa *et al.*, 2009), although others have observed no such a change (Trappe *et al.*, 2000; Widrick *et al.*, 2002). Furthermore, it is possible that a change in muscle fibre-type composition and/or preferential muscle fibre hypertrophy of type II fibres (Staron *et al.*, 1994; Campos *et al.*, 2002) might affect the change in whole muscle specific tension, as single fibre specific tension has been shown to be fibre-type dependant in human muscle (Bottinelli *et al.*, 1996; D'Antona *et al.*, 2006; Pansarasa *et al.*, 2009). Alternatively, one

possibility that has yet to be tested is that there may be a change in the type of attachment between the contractile muscle fibres and the surrounding connective tissue through which force is transmitted to tendons and bones. It has been suggested that if there were an increase in lateral force transmission from sarcomeres along the length of the fibre, rather than only from the end sarcomeres, there would be an increase in the isometric force generated by the muscle with little or no increase in the cross sectional area, thus increasing *in vivo* specific tension (Jones *et al.*, 1989).

Conclusion

The results of the present study demonstrate that, by systematically accounting for factors such as changes in volume and architecture of the four component muscles of the QF muscle group, voluntary QF muscle activation, co-activation of the antagonist muscles and patellar tendon moment arm length, *in vivo* QF muscle specific tension increased following resistance training. The cause of this increase in specific tension remains unknown but future studies should investigate the potential influences of a change in muscle fibre-type composition or an increase in lateral force transmission to obtain a more complete overview of how human muscle responds to resistance exercise.

Chapter 4

What causes *in vivo* muscle specific tension to increase following strength training?

Abstract

It is not known why *in vivo* muscle specific tension increases following resistance training in humans but changes in muscle fibre-type composition, single fibre specific tension or lateral force transmission might provide explanations. The latter would increase specific tension but decrease contraction velocity, thus not affecting maximum power per unit muscle volume (W_{max}/V_m). *In vivo* muscle specific tension, W_{max} and V_m were determined in m. quadriceps femoris of 42 young men, while myosin heavy chain (MyHC) isoform composition and single fibre specific tension of m. vastus lateralis was established in a sub-group (n = 17) before and after high-intensity leg-extension resistance training ($3 \cdot wk^{-1}$ for 9 wks). Following training, *in vivo* muscle specific tension increased by 17% but W_{max}/V_m remained unaltered. There was no relationship between the training-induced decrease in MyHC IIX and the change in specific tension *in vivo*. Isolated single fibre specific tension did not change for either type I or type II fibres following training. To conclude, it does not appear as though a change in fibre-type composition can explain an increase in muscle specific tension observed *in vivo*. Furthermore, no support was found for a training-induced increase in myofibrillar protein density. However, no change in W_{max}/V_m suggests that a change in lateral force transmission might explain this phenomenon.

Introduction

A common observation with resistance training studies in humans is that muscle strength increases more than size (Ikai & Fukunaga, 1970; Moritani & deVries, 1979; Young *et al.*, 1983; Jones & Rutherford, 1987; Davies *et al.*, 1988; Narici *et al.*, 1989; Narici *et al.*, 1996b; Ferri *et al.*, 2003), suggesting that the intrinsic force-generating capacity of the muscle increases as a result of progressive overload. By overcoming previous technical difficulties in assessing *in vivo* physiological cross-sectional area (PCSA) and maximum force of human pennate muscles, it has been shown that *in vivo* muscle specific tension (maximum force per unit PCSA) does increase in both young (Chapter 3) and older (Reeves *et al.*, 2004a) individuals. However, the underlying cause for this increase is not known and it remains to be seen whether changes in the determinants of muscle fibre contractile properties, namely myosin heavy chain (MyHC) isoform composition, and/or the way in which force is transmitted from the muscle fibre to the tendon can account for a training-induced change in muscle specific tension.

In the untrained human quadriceps femoris (QF) muscle there is evidence to suggest that the intrinsic strength of type II fibres is twice that of type I fibres (Young, 1984; Grindrod *et al.*, 1987) and several investigations of single human muscle fibres have indicated that the specific tension of type II single fibres may well be higher than that of the slower type I fibres (Bottinelli *et al.*, 1996; D'Antona *et al.*, 2006; Pansarasa *et al.*, 2009). A shift in muscle fibre-type composition towards an apparently stronger type II fibre-type might therefore explain why *in vivo* specific tension increases following resistance training. The chronically overloaded muscles of bodybuilders express a higher MyHC IIX isoform composition and a higher specific tension of type II single fibres compared to untrained controls (D'Antona *et al.*, 2006). However, following significant increases in strength,

some longitudinal resistance training studies have reported a transition in fibre-type composition from the faster IIx to IIa fibre-type (Adams *et al.*, 1993b; Staron *et al.*, 1994; Andersen & Aagaard, 2000; Campos *et al.*, 2002), while other investigators have observed no such change (Pansarasa *et al.*, 2009). Therefore, it is unclear whether or not an increase in specific tension of human muscle can be explained by a change in muscle fibre-type composition following strength training. Furthermore, a training-induced increase in whole muscle specific tension may also be realised if the specific tension of individual fibres is increased, although it is equivocal whether this does (Parente *et al.*, 2008; Pansarasa *et al.*, 2009) or does not (Trappe *et al.*, 2000; Trappe *et al.*, 2001a; Widrick *et al.*, 2002) occur in isolated human muscle fibres.

Muscle force is not solely transmitted along the axis of the muscle fibre but also laterally to the tendon via the matrix of connective tissue that encases muscle fibres (Street, 1983; Patel & Lieber, 1997) and intra-sarcolemmal protein complexes known as costameres (Danowski *et al.*, 1992). An increase in collagen synthesis (Miller *et al.*, 2005), as well as an increase in costameric proteins (Woolstenhulme *et al.*, 2006; Kosek & Bamman, 2008), which occurs in human muscle as a result of resistance training might result in an increased number of attachments between the extra-cellular matrix and intermediate sarcomeres that would serve to effectively increase the number of parallel sarcomeres per PCSA while reducing the functional length of the muscle fibres (Jones *et al.*, 1989). The consequence of this is that specific tension of the muscle fibres would increase, while the maximum shortening velocity would decrease. Since maximum muscle power (W_{max}) is a product of force and contraction velocity, W_{max} normalised to muscle volume (V_m) would be expected to remain unchanged in such circumstances (Jones *et al.*, 1989).

Furthermore, Chapter 2 demonstrated that QF muscle specific tension in young healthy men can be reliably estimated *in vivo* using an assessment of QF V_m from a single anatomical CSA of the QF muscle, together with the fascicle length (L_f) and pennation angle (θ_p) of the VL muscle (Method 2) (Erskine *et al.*, 2009). It remains to be seen, however, whether this method remains valid when compared to the more comprehensive method (Method 1) after strength training. The first aim of the present study was therefore to determine whether a minimal set of measurements could provide acceptable estimates of QF specific tension following resistance training. If so, the second aim was to determine whether an expected increase in human muscle specific tension following resistance training could be explained by changes in muscle MyHC isoform composition, single fibre specific tension and/or lateral force transmission, the latter being reflected by an unaltered W_{max}/V_m. It was hypothesized that enhanced *in vivo* muscle specific tension following resistance training would be due to increased lateral transmission of force and that the increase in specific tension would not be accompanied by an increase in W_{max}/V_m or expression of fast MyHC.

Materials and methods

Participants

Forty-two untrained males, aged 20 ± 3 years, height 178 ± 6 cm, body mass 74 ± 9 kg (mean ± SD), participated in the present study after written informed consent was obtained. Participants were excluded if they had a history of lower-limb fracture, their age was outside the range of 18-39 years, had taken part in strength training within the 12 months prior to the study, had used dietary supplements or performance enhancing aids or if they were considered to be in ill health (assessed from the responses to a general health questionnaire). The study was approved by the local ethics committee at Manchester Metropolitan

University. Volunteers were familiarized with all the test procedures and equipment within a 14-day period prior to the baseline measurements and, as far as possible, testing and training sessions occurred at the same time of day for each participant. Furthermore, participants were asked to maintain their habitual dietary intake and level of physical activity over the course of the study.

Experimental design

In vivo specific tension of the right QF muscle was calculated in 42 individuals before and after 9 weeks of leg-extension resistance training using a method (Method 2) that has been validated against a more comprehensive method (Method 1) in the untrained state (Chapter 2). To investigate whether Method 2 provided accurate estimates of QF specific tension post-training, the physiological cross-sectional area (PCSA) and specific tension values obtained using Method 2 were compared with those obtained from Method 1 in a sub-sample (n = 17) of the current cohort before and after training (Fig. 4.1). Peak power (W_{peak}) was determined during short, all-out sprints on an isokinetic cycle ergometer (Sargeant *et al.*, 1981) in the total cohort (n = 42) prior to and following the nine weeks of training. Muscle biopsies were taken from the vastus lateralis (VL) muscle in a sub-sample (n = 17) of participants before and after training to determine changes in MyHC isoform composition and the specific tension of isolated single muscle fibres.

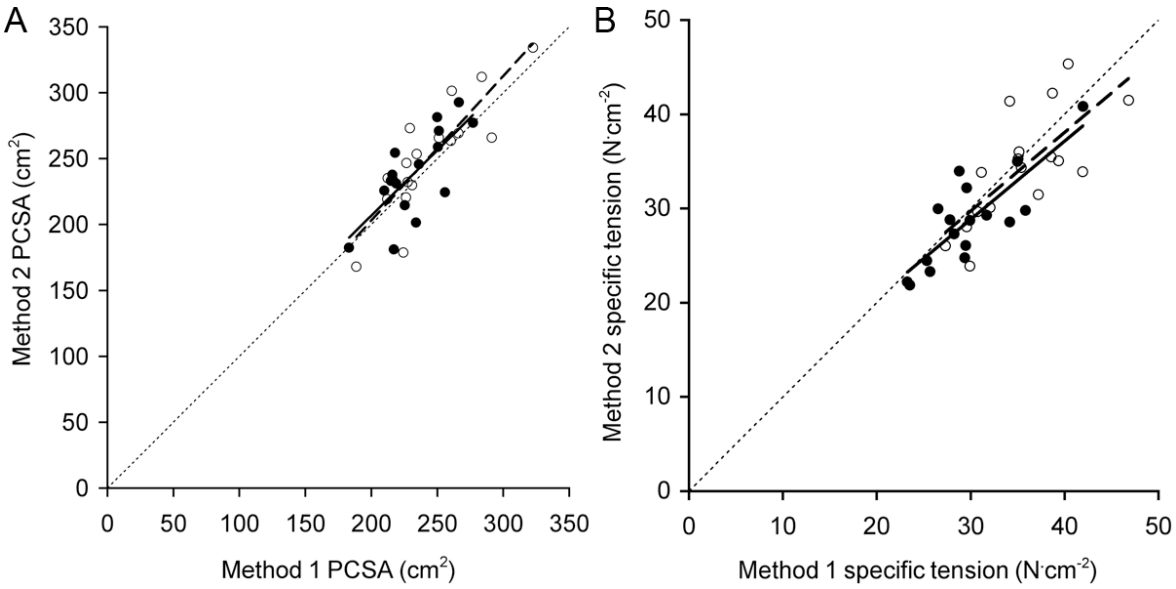

Fig. 4.1 The relationship between the comprehensive Method 1 and the simplified Method 2 regarding **(A)** QF muscle physiological cross-sectional area (PCSA) before (●; R^2 = 0.53; P < 0.01) and after (○; R^2 = 0.72; P < 0.01) training and **(B)** QF muscle specific tension before (●; R^2 = 0.66; P < 0.01) and after (○; R^2 = 0.53; P < 0.01) training; n = 17

Progressive resistance training

The strength training protocol for this study has been described in detail in Chapter 3.

Maximum force

The method used to calculate maximum patellar tendon force (F_t) has been described in detail as per Method 2 in Chapter 2.

Physiological cross-sectional area

The method used to calculate QF muscle physiological cross-sectional area (PCSA) has been described in detail as per Method 2 in Chapter 2.

In vivo specific tension

The method used to calculate QF muscle specific tension has been described in detail as per Method 2 in Chapter 2.

Power output

Peak power output (W_{peak}) of the lower right limb was determined using a modified isokinetic cycle ergometer (Lode Standard, The Netherlands), driven by a 2.24 kW motor through a variable speed gearbox and with strain gauges within the pedals (Sargeant *et al.*, 1981; Rutherford *et al.*, 1986a; Beelen *et al.*, 1994). Handlebar and saddle settings were individually adjusted and recorded for subsequent tests and toe-clips were used to secure the participant's feet to the pedals. The test started with a 5 min warm-up at a pedal frequency of 90 revolutions per minute (RPM) and with the pedals connected to an external load of 60 W. The external cranks were then connected to the electric motor and the participant performed unloaded isokinetic sprints at five predetermined, randomly assigned pedal frequencies (50, 70, 90, 110 and 130 RPM), where the participant attempted (unsuccessfully) to accelerate the system by exerting maximal voluntary force during each revolution. Each sprint lasted for 6 s and was separated by a 5 min resting period. During the low-to-medium speed sprints (50-90 RPM), the participant was secured to the saddle of the ergometer with a non-extensible waist-strap to prevent movement from the seated position as a consequence of leg forces exceeding body mass. Pedal forces in the horizontal (x) and vertical (y) planes (relative to the pedal surface) were measured using strain gauges inserted in the pedal and the pedal angle (relative to the crank) and crank angle (0° = vertical position) was recorded using incremental encoders. The x and y pedal forces, together with the pedal and crank angles and the pedal frequency were continuously recorded at a rate of 140 samples per second. Although participants performed a two-

legged sprint, W_{peak} was calculated in the right (trained) limb only. The method used to calculate W_{peak} has been adapted from a previous study that used a similar protocol (Beelen *et al.*, 1994). The highest x and y forces, together with the corresponding pedal and crank angles, were used to calculate peak tangential force (F_{tan}) during each sprint, thus:

F_{tan} (N) = ($F_y \cdot \cos \theta_{ped}$) - ($F_x \cdot \sin \theta_{ped}$)

where F_y is the vertical force and F_x is the horizontal force exerted on the pedal; cos θ_{ped} is the cosine of the pedal angle; sin θ_{ped} is the sine of the pedal angle (Fig. 4.2).

Tangential velocity (V_{tan}) was calculated as follows:

V_{tan} ($m \cdot s^{-1}$) = $2\pi r \cdot$ (RPM / 60)

where r is the length of the crank (0.177 m).

Peak power output (W_{peak}) was subsequently calculated at each pedal frequency according to the following equation:

W_{peak} (W) = $F_{tan} \cdot V_{tan}$

The coefficient of variation for repeated measurements using this technique was 3.8%.

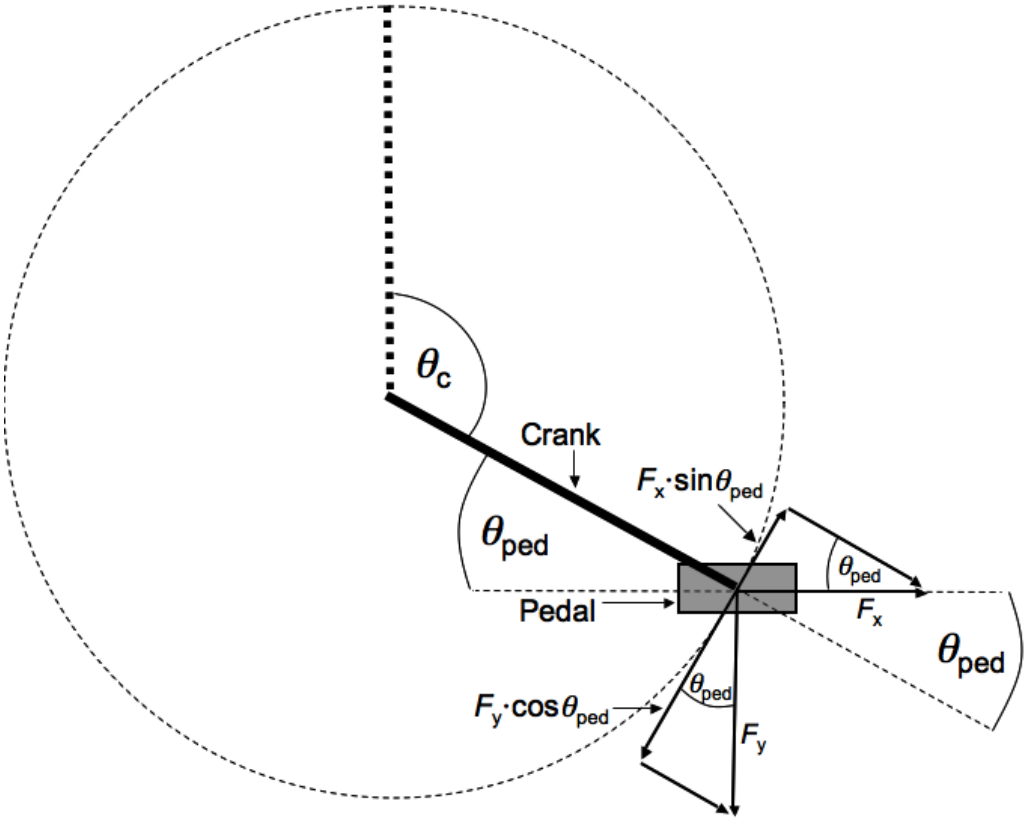

Fig. 4.2 The calculation of tangential force (F_{tan}) during each crank revolution [adapted from Beelen *et al.* (1994)]. The crank angle (θ_c) and pedal angle (θ_{ped}) were continuously recorded throughout the test, as were the forces applied to the pedal in the vertical (F_y) and horizontal (F_x) planes, which were multiplied by the cosine and sine of θ_{ped}, respectively, to provide F_{tan}. Thus, $F_{tan} = (F_y \cdot \cos\theta_{ped}) - (F_x \cdot \sin\theta_{ped})$

Muscle biopsies

A muscle sample was obtained from the VL muscle of 17 of the original 42 participants before and after training. Following application of local anaesthetic, the biopsy was harvested with a conchotome (Dietrichson *et al.*, 1980) mid-way between the knee and greater trochanter and immediately frozen in liquid nitrogen, then stored at -80°C until subsequent analyses.

Myosin heavy chain isoform composition

A 10 μm cryostat section from each pre- and post-training muscle sample was obtained and processed in sodium dodecyl sulphate (SDS) sample buffer (Laemmli, 1970). Subsequently, 10 μl of denatured sample was analysed using SDS-polyacrylamide gel electrophoresis (SDS-PAGE). The stacking and running gels both contained 30% glycerol, as well as 4% and 6% polyacrylamide, respectively. The gels were run at 120 V for 27 hours at 15°C. MyHC isoforms were identified using a silver stain plus kit (BioRad, Hercules, California, USA), according to manufacturers instructions, and the relative quantities of protein were determined by densitometry (The Discovery Series, Quantity One 1-D Analysis Software, BioRad Laboratories Inc., Hercules, USA). The coefficient of variation of repeated measurements using this technique was 2.9%.

Specific tension of isolated single muscle fibres

The experimental procedure and composition of the different solutions have been described in detail elsewhere (Degens *et al.*, 1999; Gilliver *et al.*, 2009). Briefly, on the day of the experiments muscle bundles were incubated for 20 min in a relaxing solution containing 1% TritonX-100 to ensure membrane permeabilisation. The bundle was then transferred to relax solution and single muscle fibres dissected and attached to a force transducer (Aurora 403A, Aurora Scientific Inc., Canada) and isotonic lever (Aurora 312C) in the fibre test set-up (Aurora 400). The sarcomere length was set at 2.60 μm and fibre CSA (*f*CSA) was determined while the fibre was suspended in air, assuming a circular shape and correction was made for swelling. Before maximal activation in a solution with pCa 45, the fibre segment was transferred to a solution with less EGTA than the relax solution for at least 30 s. The maximum isometric force (P_o) was recorded and fibres were excluded if the force declined before reaching a plateau or if the sarcomere

length had changed by > 0.1 μm. A total of 108 fibres fulfilled these criteria (59 before and 49 fibres after training). Specific tension was calculated as P_o divided by *f*CSA. At the end of the experiments, the fibre segments were dissolved in sample buffer (Laemmli, 1970) and stored at -80^{0}C until they were characterised for MyHC isoform composition by SDS-PAGE (see above).

Statistics

The test-retest reliability (coefficient of variation, CV and intraclass correlation coefficient, ICC) of all the measurements used to calculate *in vivo* QF specific tension has been reported in Chapter 2 (Erskine *et al.*, 2009). Paired samples Student's *t*-tests were used to investigate pre- and post-training differences in all the variables included in Table 4.1. Independent *t*-tests were used to determine differences between the entire cohort (n = 42) and the sub-sample (n = 17) in all variables before and after training. Linear regression analyses established the correlation coefficient before and after training between Method 1 and Method 2 regarding QF PCSA and specific tension, as well as between QF specific tension and MyHC IIX isoform composition, between V_m and W_{max} and between W_{max}/V_m and MyHC isoform composition. A repeated measures ANOVA was used to investigate pre and post-training differences in MyHC I, IIA and IIX isoform composition, as well as pre and post-training differences in W_{peak} and W_{peak}/V_m at all five pedal frequencies. If a training interaction was found, paired Student's t-tests were used to identify the pre- to post-training difference(s) and Bonferroni multiple correction was applied where appropriate. A two-way ANOVA was used to determine differences in specific tension between fibres of different types and changes in response to training. Due to the small number of hybrid (I/IIA and IIa/IIX) and IIX MyHC fibres, these fibres were not included in the analysis and comparisons were limited to MyHC I and IIA fibres. Statistical

significance was accepted when $P < 0.05$. All data are presented as mean ± standard deviation (SD) unless otherwise stated.

Results

Muscle specific tension

There was no difference between the total cohort ($n = 42$) and the sub-sample ($n = 17$) regarding any of the variables either before or after training (Table 4.1). The sub-sample is therefore considered representative of the entire cohort. As shown in Table 4.1, MVC torque increased by 27 ± 10% following training ($P < 0.01$). QF voluntary activation capacity increased by 3.5 ± 5.1% ($P < 0.01$), whereas the level of antagonist muscle co-activation did not change significantly after training and, likewise, there was no change in the patellar tendon moment arm (d_{PT}). Consequently, maximally activated muscle force increased by 23 ± 11% ($P < 0.01$). After training, QF volume (V_m) increased by 5.5 ± 3.5% ($P < 0.01$) but fascicle length (L_f) of the VL muscle did not change. As a result, QF PCSA also increased by 6.4 ± 4.5% ($P < 0.01$). VL muscle pennation angle (θ_p) increased by 13.0 ± 9.8% ($P < 0.01$) and having accounted for all these factors, a 17 ± 11% increase in QF specific tension was observed following training ($P < 0.01$).

Table 4.1 *In vivo* measured and calculated variables before and after training; mean ± SD

	Total cohort (n = 42)		Sub-sample (n = 17)	
Variable	Before training	After training	Before training	After training
MVC (N·m)	248 ± 50	315 ± 64**	244 ± 55	315 ± 66**
Co-activation (%)	13.9 ± 5.2	13.6 ± 5.2	13.8 ± 4.1	13.5 ± 4.0
Activation (%)	92.9 ± 4.7	96.0 ± 3.9**	91.3 ± 5.1	94.9 ± 4.0**
d_{PT} length (cm)	4.7 ± 0.3	4.7 ± 0.3	4.7 ± 0.2	4.7 ± 0.2
F_t (N)	5809 ± 1067	7152 ± 1386**	5896 ± 1151	7316 ± 1357**
QF volume (cm^3)	2084 ± 243	2200 ± 277**	2116 ± 257	2237 ± 309**
VL L_f (cm)	8.9 ± 1.4	8.9 ± 1.4	9.1 ± 1.3	9.0 ± 1.5
VL θ_p (º)	16.1 ± 2.3	18.0 ± 2.5**	16.0 ± 2.2	18.0 ± 2.4**
QF PCSA (cm^2)	238 ± 41	253 ± 43**	235 ± 35	252 ± 41*
QF specific tension ($N \cdot cm^{-2}$)	25.9 ± 5.3	30.3 ± 6.7**	26.5 ± 5.4	30.8 ± 5.3**

MVC maximal voluntary contraction torque; d_{PT} patellar tendon moment arm; F_t maximum patellar tendon force; *QF* quadriceps femoris muscle; *VL* vastus lateralis muscle; L_f fascicle length; θ_p fascicle pennation angle; *PCSA* physiological cross-sectional area; *P < 0.05 and **P < 0.01 significantly different from baseline

Myosin heavy chain isoform composition

The proportion of type IIX MyHC decreased from 29 ± 15% to 19 ± 16% (P < 0.05) after training. Although this must necessarily be accompanied by an increase in at least one of the other MyHC isoforms, the increases in the proportions of MyHC I and MyHC IIA did not reach statistical significance (Fig. 4.3). Furthermore, MyHC IIX isoform composition did not correlate with QF specific tension either before or after training (P > 0.05).

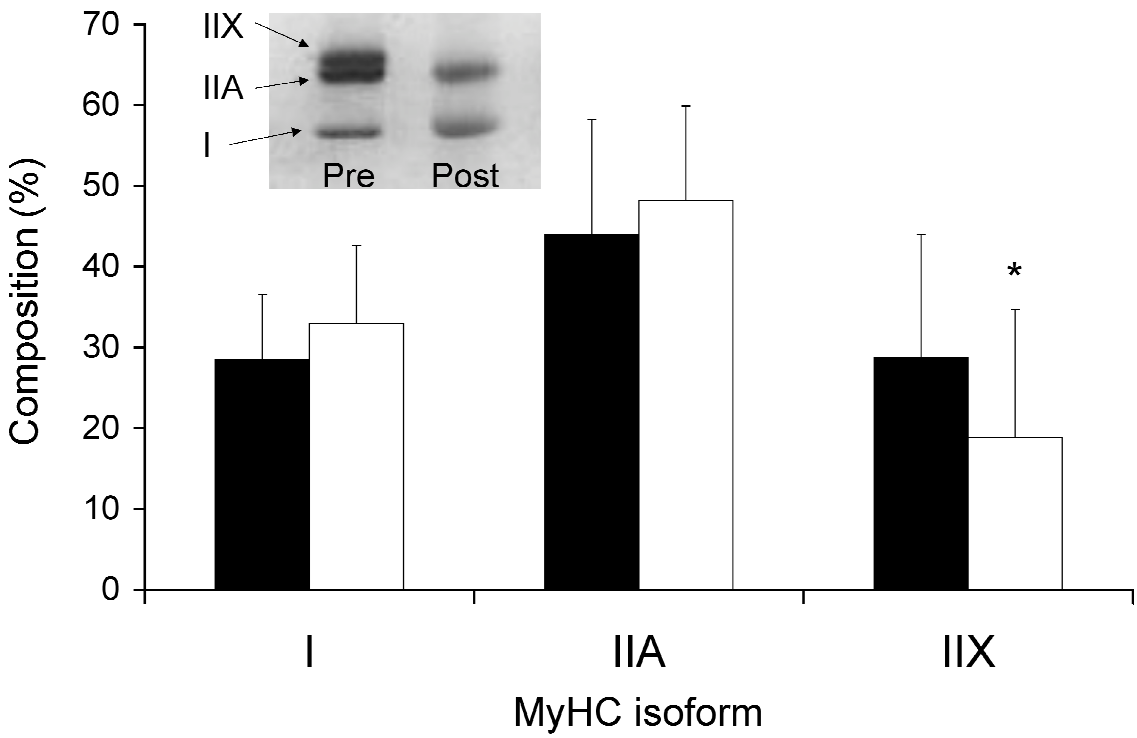

Fig. 4.3 Myosin heavy chain (MyHC) isoform composition of the vastus lateralis muscle ■ before and □ after training; n = 17; values are mean ± SD; *significantly different from baseline ($P < 0.05$); inset illustrates SDS-PAGE gel separations, showing MyHC I, MyHC IIA and MyHC IIX bands from one participant before (Pre) and after (Post) 9 weeks of resistance training

Specific tension in single muscle fibres

Before training, 4 pure type IIX fibres were isolated from the pre-training biopsies but no such fibres were found after training, although 2 IIA/IIX hybrid fibres were isolated after training but none of this hybrid type was isolated before training. Furthermore, 3 I/IIA hybrid fibres were isolated before training, while 3 were isolated post-training. A two-way ANOVA revealed that there was no effect of training on the specific tension of single fibres ($P > 0.05$), nor did one fibre type change its specific tension more than the other ($P > 0.05$). In addition, there was no difference in specific tension between type I and type IIA fibres ($P > 0.05$).

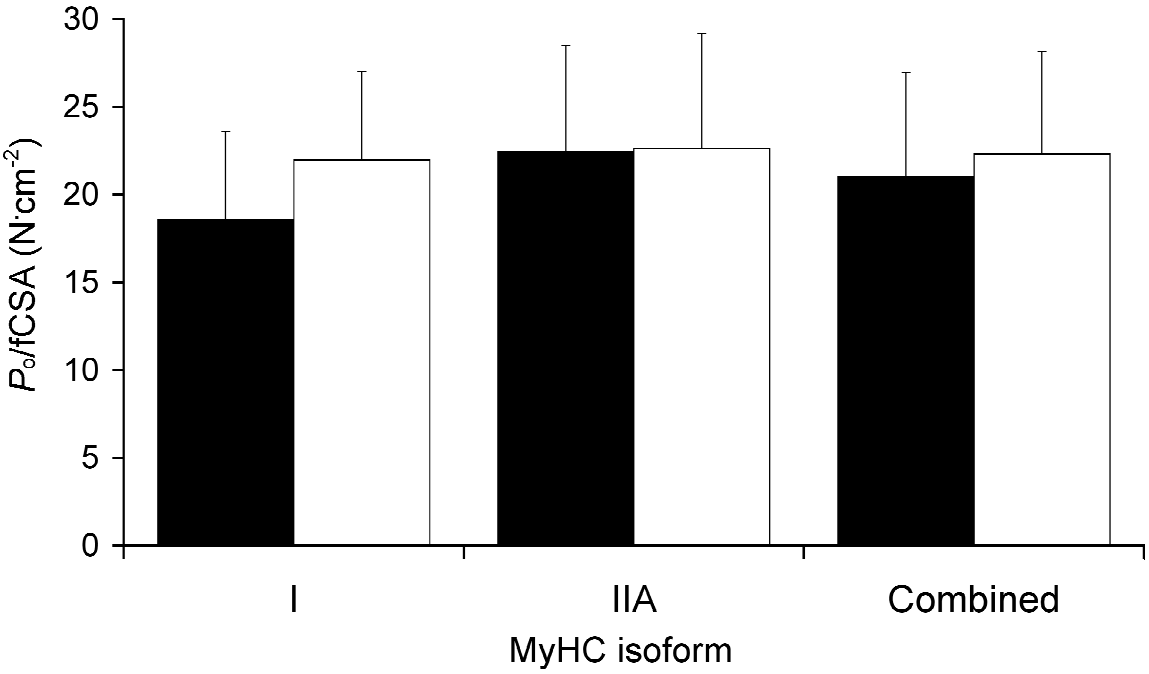

Fig. 4.4 Specific tension (P_o/*f*CSA) of skinned single vastus lateralis muscle fibres of myosin heavy chain (MyHC) isoform type I (n = 19 before training; n = 21 after training), IIA (n = 33 before training; n = 24 after training), and all fibres combined (n = 59 before training; n = 49 after training); ■ before training; □ after training; values are mean ± SD

Power output

From the five pedal frequencies tested (50, 70, 90, 110 and 130 RPM), peak power output (W_{peak}) was the highest (W_{max}) at 110 RPM, both before and after training (Fig. 4.5A) and a 5% increase ($P < 0.05$) in W_{peak} was observed at this pedal frequency only (Fig. 4.5A). W_{peak} normalised to muscle volume (W_{peak}/V_m) did not change with training at any pedal frequency ($P > 0.05$; Fig. 4.5B). Furthermore, neither W_{max} nor W_{max}/V_m correlated with MyHC IIX isoform composition either before or after training ($P > 0.05$) and there were no relationships between the training-induced changes of these factors ($P > 0.05$).

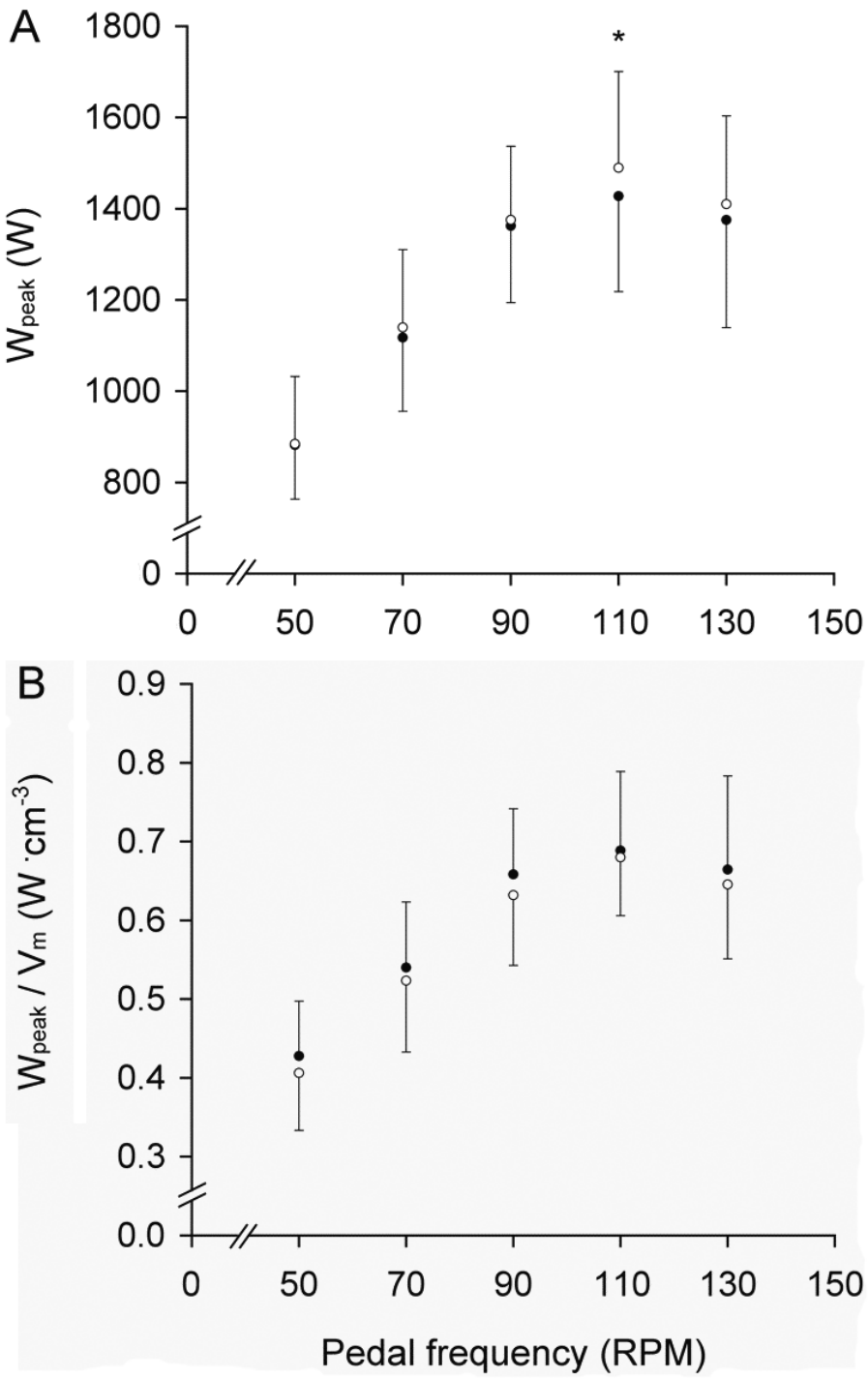

Fig. 4.5 (**A**) Peak power output (W_{peak}) and (**B**) W_{peak} normalised to QF muscle volume (W_{peak}/V_m) measured on an isokinetic cycle ergometer at 50, 70, 90, 110 and 130 revolutions per minute (RPM) before (●) and after (○) resistance training; n = 42; values are mean ± SD; *significant change from baseline ($P < 0.05$)

Discussion

The current investigation confirms that *in vivo* human quadriceps femoris (QF) muscle specific tension increases following resistance training and it was hypothesized that this occurs as a consequence of increased lateral transmission of force from sarcomeres along the length of the fibre to the extracellular matrix and, ultimately, to the tendon. One prediction of this idea is that whilst specific tension would increase, the consequent shortening of the functional length of the fibre would decrease the maximal velocity of shortening and that, consequently, maximal power output per unit of muscle volume would be unchanged as a result of training. The results of the present study substantially support this idea. Although an increase in specific tension could possibly be related to an increased expression of fast MyHC, the decreased proportion of the fastest IIX MyHC found in the present study would, if anything, result in a decrease in specific tension.

In vivo muscle specific tension

The method used to calculate *in vivo* QF specific tension before and after strength training in the present study was based on an estimate of QF muscle volume from a single anatomical CSA of the QF muscle, together with the fascicle length (L_f) and pennation angle (θ_p) of the VL muscle (Method 2). This method has provided similar QF muscle specific tension values to those obtained using a more comprehensive approach (Method 1), where the volume and optimum architecture of each component muscle of the QF muscle group was considered in a large cohort of untrained young men (Erskine *et al.*, 2009). By validating these two methods on a subgroup of 17 participants, the present study has demonstrated that Method 2 provides valid estimations of QF specific tension after resistance training as well as in the untrained state, which is largely because strength training affects the volume and architecture of all four component QF muscles to a similar

extent (Chapter 3). The advantage of using Method 2, however, is that much less time is required than when using the more exhaustive Method 1 and is therefore better suited for large-scale investigations. Similar to previous findings for young (Chapter 3) and elderly (Reeves *et al.*, 2004a) individuals, the present study demonstrated that *in vivo* specific tension of the QF muscle increased by 17% following short-term resistance training. This increase occurred after accounting for changes in the extrinsic factors that affect the estimation of QF muscle PCSA and maximally activated muscle force.

Changes in myosin heavy chain isoform composition and its impact on specific tension

Based on a comparison of bodybuilders and control subjects, it has been suggested that strength training may cause a shift in fibre-type composition towards the reputedly stronger type II fibre-type (D'Antona *et al.*, 2006). The present study, however, observed a decrease in MyHC IIX isoform composition, which is in accord with the findings of previous short-term resistance training investigations (Adams *et al.*, 1993b; Fry *et al.*, 1994; Staron *et al.*, 1994; Andersen & Aagaard, 2000; Campos *et al.*, 2002) and would thus be expected to result, if anything, in a reduction rather than an increase in specific tension. However, several studies, including the present study, have shown no differences in specific tension between fibre types of single human muscle fibres (Larsson & Moss, 1993; Ottenheijm *et al.*, 2005; Gilliver *et al.*, 2009). Furthermore, any training-induced reduction in MyHC IIX might be outweighed by preferential hypertrophy of the type II fibres compared to type I fibres (Staron *et al.*, 1994; Campos *et al.*, 2002). It is therefore not surprising that no relationship was found between MyHC isoform composition and specific tension either before or after training, nor between the changes in MyHC composition and changes in specific tension in the current investigation. It seems unlikely, therefore, that changes in fibre type composition underlie the increase in specific tension

seen with training in the present study.

Specific tension of isolated single fibres and the effect of resistance training

A training-induced increase in muscle specific tension observed *in vivo* could conceivably occur due to augmented packing of contractile material (Penman, 1970). Such an increase would be reflected in an augmented specific tension at the single fibre level, which has been observed by some investigators (Parente *et al.*, 2008; Pansarasa *et al.*, 2009) but not others (Trappe *et al.*, 2000; Trappe *et al.*, 2001a; Widrick *et al.*, 2002) following resistance training in human muscle. In accordance with the latter investigations, the present study observed no increase in specific tension of isolated single fibres from the vastus lateralis muscle. Although the reasons for this discrepancy are unclear, it may be that the disparity is related to the duration of the respective training programmes. In those investigations where a significant increase in single fibre specific tension was reported, participants were trained for a period of 12 months (Parente *et al.*, 2008; Pansarasa *et al.*, 2009). However, where no increase was observed, individuals took part in a training programme that lasted for a duration of 12 weeks (Trappe *et al.*, 2000; Trappe *et al.*, 2001a; Widrick *et al.*, 2002), while the participants of the present study performed high-intensity resistance training for nine weeks in total. Whatever the explanation for the contrasting results, it appears unlikely that the training-induced increase in muscle specific tension observed *in vivo* in young men in the present study was due to an increase in myofibrillar protein density, as this would have been manifested in an augmented specific tension at the single fibre level.

Power output and its significance regarding specific tension

The fact that QF specific tension increased by 17% while W_{max}/V_m did not change with training implies a 17% slowing of the QF muscle, which could have occurred as a

consequence of a decrease in L_f and/or shortening velocity of the muscle. VL L_f, however, did not change with training. The decreased proportion of MyHC IIX isoform seen after training is expected to have some consequences for the maximum velocity of shortening and thus for W_{max} of the QF muscle. Muscle fibres composed exclusively of the MyHC IIX isoform have faster contractile properties than either MyHC I or IIA isoforms (Larsson & Moss, 1993; Bottinelli *et al.*, 1996; Bottinelli & Reggiani, 2000; D'Antona *et al.*, 2006). It is possible, therefore, that the apparent slowing of the muscle and the moderate increase in W_{max} compared to the increase in specific tension may be due to this change in MyHC expression. However, pure type IIX fibres are relatively uncommon in the VL muscle of untrained young men (Widrick *et al.*, 2002; D'Antona *et al.*, 2006; Yu *et al.*, 2007) and it is likely that most of the MyHC IIX in the present study would have been present in hybrid type IIA/IIX fibres, which are more prevalent than type IIX fibres (Klitgaard *et al.*, 1990; Larsson & Moss, 1993; Widrick *et al.*, 2002) and the difference in contractile velocity between type IIA/IIX and the type IIA fibres is less than it is between type IIX and IIA fibres (Bottinelli *et al.*, 1994; Widrick *et al.*, 2002; D'Antona *et al.*, 2006; Yu *et al.*, 2007). In addition, there was no relationship between either W_{max} or W_{max}/V_m and MyHC isoform composition before or after training, or between the training-induced changes in these variables. Consequently it seems unlikely that the change in MyHC IIX isoform composition was responsible for a lack of change in W_{max}/V_m.

A slowing of the muscle is consistent with the development of additional lateral transmission of force from sarcomeres along the length of the fibres. Such a change would explain the increase in specific tension without an increase in W_{max}/V_m (Jones *et al.*, 1989). An increase in attachments between intermediate sarcomeres and the extracellular matrix might arise from an increase in collagen synthesis (Miller *et al.*, 2005) and/or proteins that

constitute structural complexes known as costameres (Woolstenhulme *et al.*, 2006; Kosek & Bamman, 2008), which have been shown to play a key role in the lateral transmission of muscle fibre force (Danowski *et al.*, 1992). An increase in lateral force transmission would have increased the "effective PCSA" of the muscle, thus increasing specific tension by allowing the muscle to produce more force without the need for additional contractile material. However, the functional length of the muscle fibres would have been reduced, thus decreasing the maximum shortening velocity and preventing W_{max} from increasing to the same extent as specific tension (Jones *et al.*, 1989). It is acknowledged, however, that power output measured during the leg-extension phase on an isokinetic cycle ergometer is not necessarily generated only by the QF muscle group, as the hip extensors and calf muscles may also contribute, but it has previously been shown that power measured in this way is related to thigh volume (Sargeant *et al.*, 1981) and it seems unlikely that the contribution of synergist muscles would decrease as a result of training to the extent that they exactly counteracted any increase in power of the QF muscle.

Conclusions

Following nine weeks of high-intensity resistance training, a 17% increase in *in vivo* muscle specific tension was observed, which was not explicable by the concomitant fast-to-slow shift in MyHC isoform composition. However, a training-induced increase in lateral force transmission could account for the change in specific tension, as power output normalised to muscle volume was unaltered after training despite a marked increase in isometric strength.

Chapter 5

The variable change in muscle specific tension following resistance training

Abstract

Considerable variation exists in the response of human muscle to resistance training and there are numerous methods of adaptation. Therefore, the primary aim of this study was to quantify the range of responses concerning the training-induced change in muscle force (*F*), physiological cross-sectional area (PCSA) and specific tension (*F*/PCSA). Fifty-three untrained young men performed progressive leg-extension training 3 times a week for 9 weeks. Maximum quadriceps femoris (QF) muscle *F* was determined from maximum voluntary contraction (MVC) torque, the level of voluntary muscle activation, antagonist muscle co-activation and patellar tendon moment arm length (d_{PT}). QF muscle specific tension was established from *F* and measurements of muscle volume and architecture. MVC increased by 26 ± 11% ($P < 0.0001$; range: -1 – 52%), while *F* increased by 22 ± 11% ($P < 0.0001$; range: -1 – 44%). PCSA increased by 6 ± 4% ($P < 0.001$; range: -3 – 18%) and specific tension increased by 17 ± 11% ($P < 0.0001$; range: -5 – 39%). In conclusion, training-induced changes in muscle force and PCSA varied substantially, giving rise to a highly variable change in muscle specific tension. In searching for mechanisms underlying these responses, it is important that the complexity of muscle adaptation to strength training is clearly appreciated.

Introduction

It is well established that resistance training induces gains in both strength and muscle size in humans. However, even in relatively small sample sizes, it is evident that inter-individual variation exists in these changes (Jones & Rutherford, 1987; Narici *et al.*, 1996b; Seynnes *et al.*, 2009). Most previous studies have focused on the mean muscle response to overload and the variability was assumed to be experimental error associated with the measurements made or variation in the commitment of the subjects. More recently, however, there has been an increasing awareness that the variable training response is a "real" phenomenon and understanding the reasons for it may give insights into the mechanisms controlling muscle growth and changes in strength. In addition there could be practical benefits in knowing who might or might not respond since training is an important aspect of sport and rehabilitation. Consequently there is considerable interest in determining whether variants of particular genes are associated with the way in which human muscle adapts to strength training (Folland *et al.*, 2000; Clarkson *et al.*, 2005a; Pescatello *et al.*, 2006; Delmonico *et al.*, 2007; Charbonneau *et al.*, 2008; Bray *et al.*, 2009). However, for a genetic study to provide useful information it is necessary to first characterize the variability in response, since there are several ways in which a muscle may increase in strength. Broadly, a muscle may increase in quantity [defined as the physiological cross sectional area (PCSA)] or quality (characterized as specific tension; force per unit PCSA) and there may also be questions concerning muscle activation and the co-contraction of agonist and antagonist muscles, as well as muscle architecture.

A large-scale investigation has reported substantial variability in the response to 12 weeks of unilateral resistance training of the elbow flexor muscle group in 243 young men (Hubal *et al.*, 2005). Relative training-induced changes in strength varied between individuals

from 0% to 150% and from -24% to 149%, according to the maximum load lifted in one repetition (1 RM) and the maximum isometric voluntary contraction (MVC), respectively. Relative changes in muscle anatomical CSA, on the other hand, ranged from -3% to 56%. Thus, not only does the response to strength training vary considerably between individuals but the amount of variation appears to differ, according to the variable measured. Although a proportion of the gain in 1 RM will undoubtedly be due to an increase in muscle force, an unknown amount is likely to result from increased co-ordination of additional muscles during training (Rutherford & Jones, 1986), and the acquisition of this skill might differ between people. Furthermore, MVC is not necessarily a true measure of the maximum isometric force produced by a fully activated muscle. To assess this *in vivo*, the ability of an individual to fully activate their muscle, as well as the level of antagonist muscle co-activation and the length of the moment arm are critical measurements (Maganaris *et al.*, 2001; Reeves *et al.*, 2004a; Erskine *et al.*, 2009). Moreover, as most human skeletal muscles are pennate in that the muscle fibres are aligned at an angle to the line of pull of the tendon (Alexander & Vernon, 1975), the muscle PCSA is the most appropriate measure to provide an accurate assessment of training-induced muscle hypertrophy, as this parameter represents the total CSA of all the constituting fibres of a muscle at right-angles to their long axes, and is the main determinant of maximum muscle force (Close, 1972; Powell *et al.*, 1984). Therefore, an accurate quantification of the full range of responses of human muscle to resistance training requires the force and PCSA of a maximally activated muscle or group of muscles to be determined, thus enabling the extent of variable change in muscle specific tension to be established.

The aim of the present study was to quantify the range of human muscle responses to resistance training with regard to changes in QF muscle strength and size. In doing so, it

was expected that the inter-individual variability in strength changes following resistance training would be reduced by accounting for training-induced alterations in voluntary activation, antagonist muscle co-activation, muscle volume and architecture.

Materials and methods

Participants

Potential participants were excluded if they had history of lower-limb fracture, their age was outside the range of 18-39 years, had taken part in strength training within the 12 months prior to the study, had used dietary supplements or performance enhancing aids or if they were considered to be in ill health (assessed from the responses to a general health questionnaire). The study was approved by the Manchester Metropolitan University local ethics committee. Fifty-three untrained males, aged 20 ± 3 years, height 178 ± 5 cm, body mass 77 ± 11 kg (mean ± SD), were eligible to participate in the study after written informed consent was obtained. Volunteers were familiarized with all test procedures and equipment within a 14-day period prior to the baseline measurements and, as far as possible, testing and training sessions occurred at the same time of day for each participant.

Habitual physical activity level

The habitual physical activity level (PAL) of each participant was assessed by questionnaire (Baecke *et al.*, 1982) prior to commencing the nine-week training period. The overall PAL was scored using a scale from 1 to 5 points, where 1 was the least active, 3 was intermediate and 5 was extremely active. Participants were asked to maintain their PAL and habitual dietary intake over the course of the study.

Experimental design

Maximum quadriceps femoris (QF) muscle force, physiological cross-sectional area (PCSA) and specific tension muscle were determined in previously untrained individuals, as described in detail using Method 2 in Chapter 2 (Erskine *et al.*, 2009), before and after nine weeks of leg-extension resistance training (described in Chapter 3).

Statistics

The test-retest reliability (coefficient of variation and intraclass correlation coefficient) of all the measurements used to calculate QF specific tension has been reported previously (Erskine *et al.*, 2009). Post-training differences in MVC, co-activation, voluntary activation, maximum force, muscle volume, muscle fascicle length, muscle fascicle pennation angle, muscle PCSA and specific tension were analyzed using paired Student's *t*-tests. Pearson correlations were used to determine relations between the changes in maximum force and specific tension and between the changes in PCSA and specific tension. Statistical significance was accepted when $P < 0.05$. All data are presented as mean ± standard deviation (SD) unless otherwise stated. The variable change in MVC, maximum force, PCSA and specific tension was reported for each parameter as the single sample CV [$100 \cdot (SD \cdot mean^{-1})$]. Individuals were defined as high or low responders to resistance training if their relative post-training changes were, respectively, ≥ 1 SD or ≤ 1 SD.

Results

Habitual physical activity level

The physical activity level (PAL) of the 53 participants prior to starting the strength-training period was 2.7 ± 0.3 points, described as slightly less than "intermediate" in terms

of PAL scores (Baecke *et al.*, 1982). PAL scores did not correlate with any of the muscle changes recorded after training.

Single repetition maximum

Following nine weeks of high-intensity unilateral leg-extension exercise, the 1 RM increased by 68 ± 30% ($P < 0.001$; Table 5.1), with individual changes ranging from 18 to 113%.

Table 5.1 Measured and calculated variables before and after training; $n = 53$; mean ± SD

Variable	Before training	Post training
1 RM (kg)	54.3 ± 10.9	88.2 ± 12.0*
MVC (N·m)	246 ± 51	310 ± 62*
Co-activation (%)	13.9 ± 4.9	13.6 ± 4.9
Activation (%)	92.8 ± 5.2	96.2 ± 3.8*
d_{PT} length (cm)	4.8 ± 0.3	4.8 ± 0.3
F (N)	5722 ± 1036	6968 ± 1343*
V_m (cm^3)	2080 ± 254	2201 ± 277*
L_f (cm)	8.9 ± 1.3	8.9 ± 1.4
θ_p (°)	16.1 ± 2.3	18.2 ± 2.9*
PCSA (cm^2)	237 ± 41	251 ± 43*
Specific tension ($N \cdot cm^{-2}$)	25.6 ± 5.1	29.8 ± 6.4*

MVC maximal voluntary contraction torque; d_{PT} patellar tendon moment arm; F maximum muscle force; V_m quadriceps femoris muscle volume; L_f fascicle length; θ_p fascicle pennation angle; *PCSA* quadriceps femoris muscle physiological cross-sectional area; *$P < 0.001$ significantly different from baseline

Maximum isometric QF force

Maximum voluntary contraction (MVC) torque increased by 26 ± 11% ($P < 0.001$; Table 5.1) and the full range of individual responses is shown in Fig. 5.1; the CV for the change

in MVC was 42%. Eight participants were designated high responders (≥ 1 SD) and 8 were classed as low responders (≤ 1 SD). QF voluntary activation increased by 3.8 ± 5.2% (P < 0.001; range: -5 – 18%; Table 5.1), while antagonist muscle co-activation did not change significantly with training (range: -13 – 15%; Table 5.1). Similarly, d_{PT} did not change with training (range: -4.8 – 4.4%; Table 5.1). Consequently, maximum isometric QF force increased by 22 ± 11% (P < 0.001; Table 5.1). The complete account of individual responses is shown in Fig. 5.2 (CV = 51%), from which it can be seen that 11 of the participants were high responders (of which only 5 were also high responders in relation to changes in MVC) and 10 were low responders (of which only 6 were also low responders concerning changes in MVC).

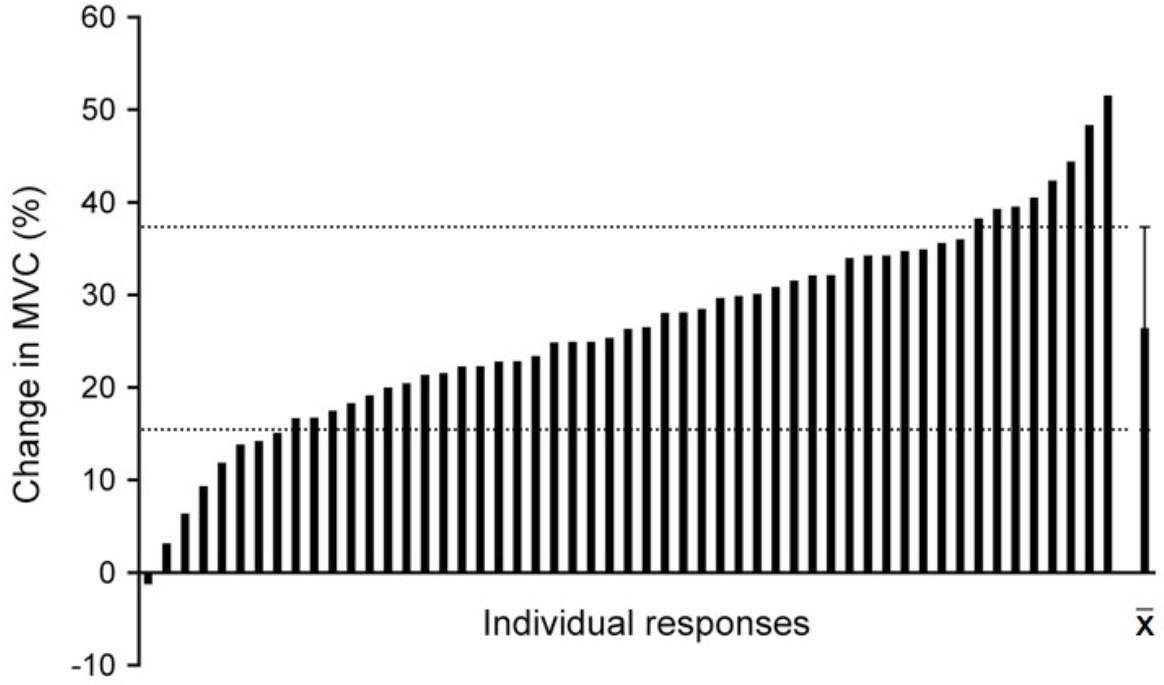

Fig. 5.1 The range of individual changes in MVC relative to baseline values; dotted lines represent ± 1 SD from the mean ($\bar{x}$), from which high responders (≥1 SD) and low responders (≤1 SD) were determined; n = 53

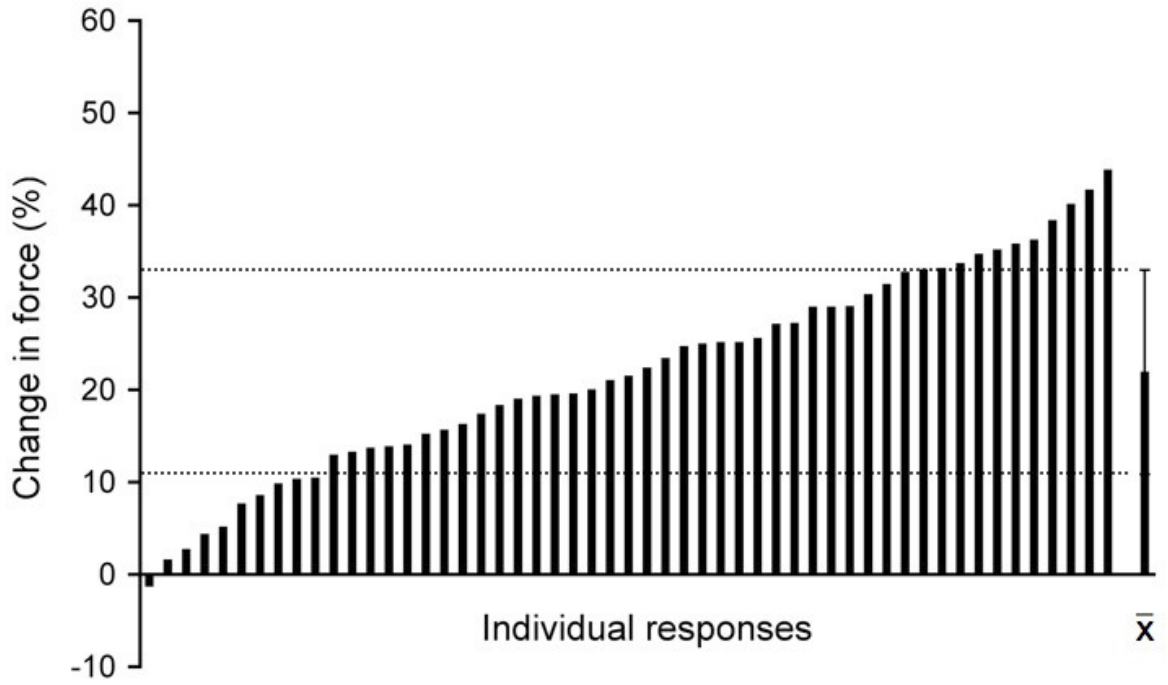

Fig. 5.2 The range of individual changes in maximum muscle force relative to baseline values; dotted lines represent ± 1 SD from the mean ($\bar{x}$), from which high responders (≥1 SD) and low responders (≤1 SD) were determined; n = 53

Physiological cross-sectional area and specific tension

Following training, QF muscle volume increased by 5.6 ± 3.4% (P < 0.001; range: 0 – 16%; Table 5.1). Since VL muscle L_f did not change (range: -9 – 13%; Table 5.1), QF PCSA increased by 5.7 ± 4.5% (P < 0.001; Table 5.1) and the full range of responses is shown in Fig. 5.3 (CV = 75%), from which 8 individuals were defined as high responders and 8 were low responders. VL muscle θ_p increased by 13 ± 10% (P < 0.001; range: -5 – 39%; Table 5.1) and when all these factors were taken into account, QF muscle specific tension increased by 16.5 ± 11.2% after training (P < 0.001; Table 5.1). The CV for the change in specific tension was 68% and all the individual changes are shown in Fig. 5.4, including the high (n = 9) and low (n = 12) responders. None of the PCSA low responders were classified as low responders according to change in force or specific tension, although interestingly, one was classed as a high responder regarding change in muscle

force. However, 75% of the low specific tension responders were also classified as low responders according to changes in muscle force. Regarding specific tension high responders, 89% were also high muscle force responders, of which only one was classified as a high responder regarding a change in PCSA. The discrepancy between high and low PCSA and specific tension response groups is supported by the lack of correlation between the training-induced changes in PCSA and maximum force (Fig. 5.5A), while the similarity between high and low force and specific tension response groups is supported by the strong relationship between the changes in specific tension and muscle force (Fig. 5.5B).

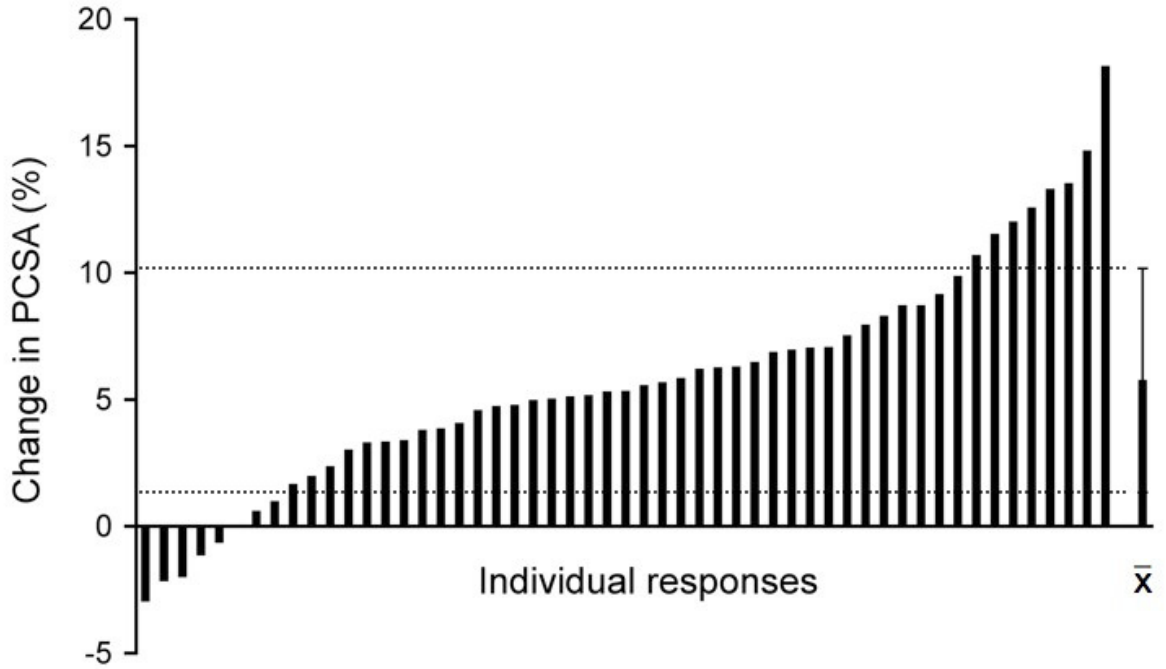

Fig. 5.3 The range of individual changes in muscle PCSA relative to baseline values; dotted lines represent ± 1 SD from the mean ($\bar{x}$), from which high responders (≥1 SD) and low responders (≤1 SD) were determined; $n = 53$

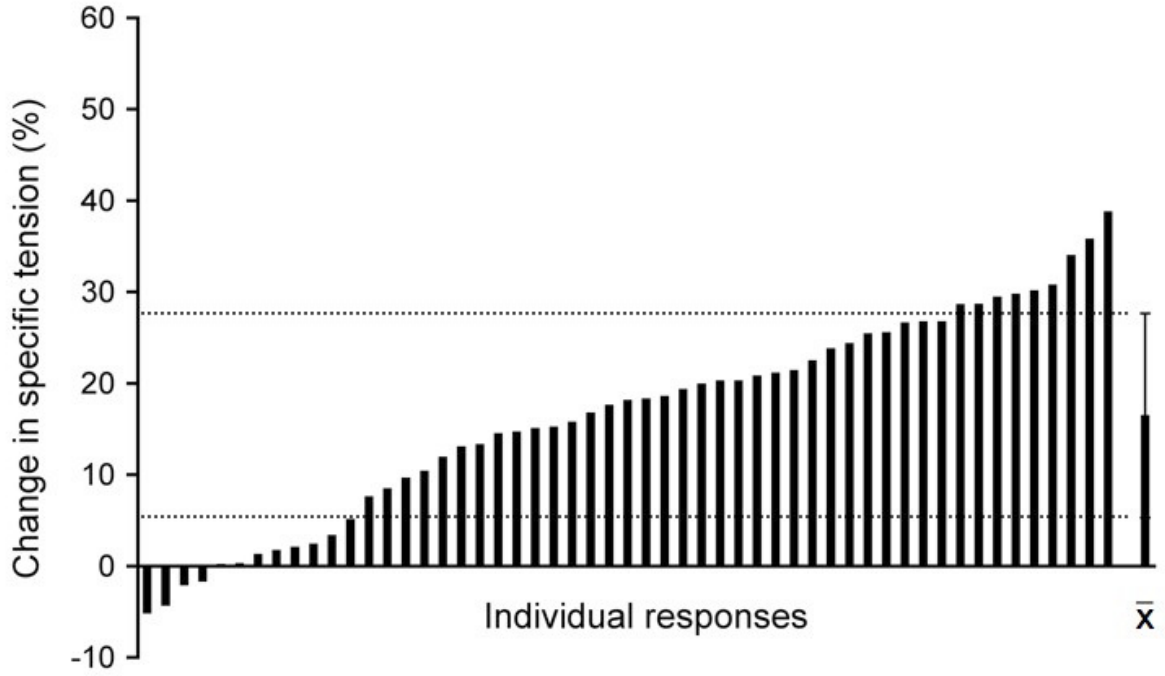

Fig. 5.4 The range of individual changes in muscle specific tension relative to baseline values; dotted lines represent ± 1 SD from the mean ($\bar{x}$), from which high responders (≥1 SD) and low responders (≤1 SD) were determined; n = 53

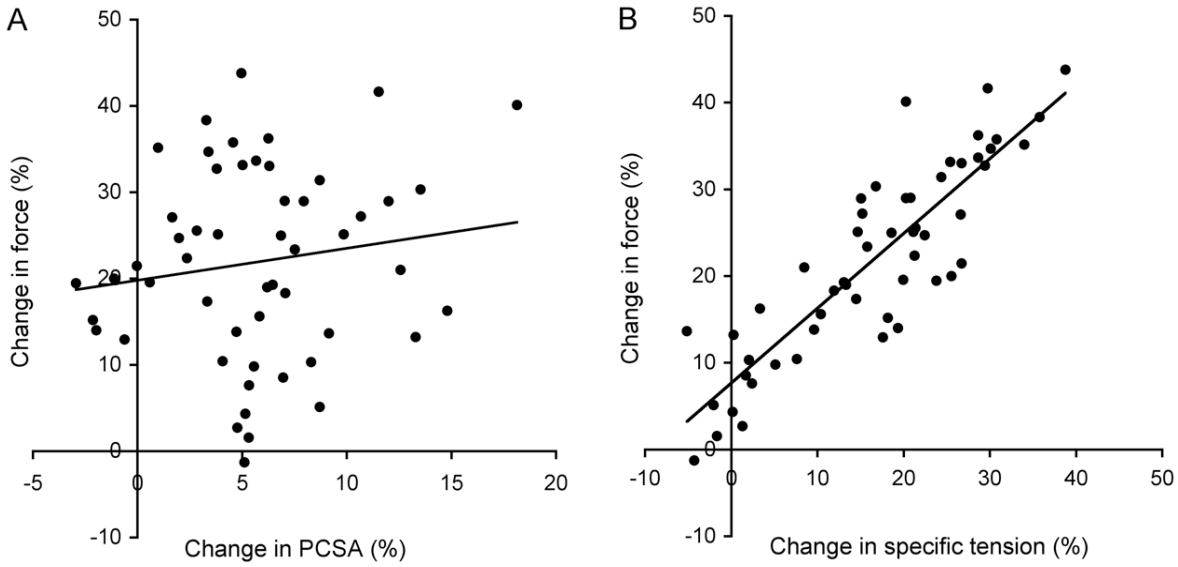

Fig. 5.5 The change in muscle force as a function of the change in physiological cross-sectional area (PCSA); R^2 = 0.02; $P > 0.05$ (**A**); and the change in muscle force as a function of the change in muscle specific tension; $R^2 = 0.76$; $P < 0.0001$ (**B**); $n = 53$

Discussion

The present study is the first to quantify the complete range of responses of human muscle to resistance training, regarding changes in maximum force, PCSA and specific tension. The main conclusion is that the major variation in strength as a result of training is due to variations in the change in specific tension of the muscle.

Since it has been suggested that prior training status affects the response to strength training (Kraemer *et al.*, 2002), it might be assumed that the variable response to strength training observed in the present study was influenced by different levels of habitual physical activity (PAL) or training status prior to the commencement of training. However, as well as all participants being screened prior to taking part in the study (see *Materials and methods*) and their PAL reaching less than an intermediate score, training-induced changes in knee joint torque and muscle specific tension did not correlate with either baseline levels or PAL. Therefore, it is maintained that the range of responses to resistance

training observed in the present study in QF muscle specific tension is a real reflection of how the QF muscle responds to the particular type and intensity of resistance training performed in the current investigation.

The present study observed a large variable change (CV = 45%) in the unilateral leg extension single repetition maximum (1 RM), which was similar to the previously reported variability (CV = 55%) in the unilateral preacher curl 1 RM in young men (Hubal *et al.*, 2005). Numerous resistance training studies in humans have used the 1 RM as a method of determining the effect of resistance training on muscle strength (Riechman *et al.*, 2004; Hubal *et al.*, 2005; Kostek *et al.*, 2005; Hand *et al.*, 2007; Charbonneau *et al.*, 2008). However, although the 1 RM is a relatively simple task to perform, it involves not just the intrinsic strength of the muscle group but also the actions of synergistic and stabilizing muscles (Rutherford & Jones, 1986), thus questioning its suitability for detailed physiological assessment. In contrast, maximum voluntary isometric contraction (MVC) provides a more accurate assessment of muscle strength and the 26% increase in MVC knee extension torque observed in the present study is comparable to that found in previous investigations into the effects of training on the knee extensors (Rutherford *et al.*, 1986a; Jones & Rutherford, 1987; Narici *et al.*, 1989) and other muscle groups (Cureton *et al.*, 1988; Kawakami *et al.*, 1995; Hubal *et al.*, 2005). Great care was taken in the present study to ensure that the subjects were similar in their body size, age and habitual physical activity before training, that participant compliance was good and the training supervised by the same investigators throughout. Nevertheless, the range of training responses for MVC torque seen (CV = 42%) was large but somewhat less than that reported previously for the upper limb (CV = 107%) (Hubal *et al.*, 2005). However, changes in MVC do not necessarily reflect changes in the contractile properties of the muscle itself, and for this it

is necessary to determine the true muscle force, taking into account changes in voluntary muscle activation, antagonist muscle co-activation and moment arm length (Maganaris *et al.*, 2001; Reeves *et al.*, 2004a; Erskine *et al.*, 2009). Yet, rather than reducing the variation, taking these factors into account increased the variability of the training response (CV of 51% for muscle force versus 42% for MVC torque).

As muscle force is primarily determined by the number of sarcomeres arranged in parallel, which is represented by the PCSA (Close, 1972; Powell *et al.*, 1984), it is important to know how resistance training affects PCSA in order to ascertain a complete overview of the variable response of human muscle to overload. Previous studies have used whole muscle volume (Kanehisa *et al.*, 2002; Charbonneau *et al.*, 2008) or ACSA (Tracy *et al.*, 1999; Ferri *et al.*, 2003; Hubal *et al.*, 2005) to determine the effect of resistance training on muscle size. However, unlike PCSA, neither of these two indices of muscle size represents the CSA of the total number of fibres at right angles to their axes in pennate muscles. Although the mean increase in PCSA in the present study was relatively small (6%), it was evident that this change varied greatly between people (CV = 75%). Of the eight low hypertrophic responders in the present study, six exhibited no change or even a decrease in PCSA. In 89% of the participants, however, a varying degree of hypertrophy did occur and eight were classified as high responders, of which only two were also classified as high responders regarding a change in maximum force. Moreover, one of the low hypertrophic responders was in the high response group for change in maximum force.

Change in muscle force did not correlate with the variable change in PCSA and it is evident therefore that while there is a variable hypertrophic response, this does not explain the variability in the force response to training. The lack of correlation between changes in

muscle PCSA and force is most probably due to inter-individual differences in the way muscle adapts to resistance training. For example, some people may have increased their strength through an increase in contractile material and therefore a proportional increase in muscle PCSA, while other may have increased the amount of force transmitted laterally, leading to larger increases in force than PCSA. However, it is acknowledged that certain assumptions were made in the calculation of in vivo muscle PCSA, which might have affected the lack of relationship between the changes in muscle force and PCSA after training. Muscle PCSA was calculated by dividing muscle volume by fascicle length Muscle PCSA was calculated by dividing muscle volume by fascicle length (Haxton, 1944; Alexander & Vernon, 1975; Maganaris *et al.*, 2001; Reeves *et al.*, 2004a), with the latter measured during MVC in the mid-sagittal plane of the central portion of each muscle. Therefore, it was assumed that the muscle was cylindrical in shape and that its fascicles were a constant length. Although this assumption might be contested, previous *in vivo* (Narici *et al.*, 1996a; Maganaris *et al.*, 1998; Maganaris & Baltzopoulos, 1999) and cadaveric (Friederich & Brand, 1990) findings in human muscles support the assumption that fascicle length is relatively constant along the length of a muscle. Furthermore, this calculation of muscle PCSA does not account for a possible change in intramuscular fat content, myofibrillar packing or in muscle fibre-type composition. The first two possibilities could feasibly lead to an under or overestimation of muscle PCSA, which would lead to erroneous calculations of muscle specific tension. However, it has been shown that the level of non-contractile material in the muscle of young, healthy male individuals is relatively small (Holmback *et al*., 2002), while in Chapter 4 and in previous studies (Trappe *et al*., 2000; Trappe *et al*., 2001a; Widrick *et al*., 2002) it has been demonstrated that the specific tension of isolated single muscle fibres does not change following 9-12 weeks of resistance training, thus suggesting that there is no change in

myofibrillar packing following short-term strength training. Some investigations have provided evidence that single fibre specific tension is fibre-type dependent (Bottinelli *et al*., 1996; D'Antona *et al*., 2006; Pansarasa *et al*., 2009), thus leading to the possibility that a training-induced change in fibre-type composition might cause a change in the muscle force-size relationship. However, previous studies (Larsson & Moss, 1993; Ottenheijm *et al*., 2005; Gilliver *et al*., 2009) and the results from the study in Chapter 4 have suggested that there is no difference in isolated single fibre specific tension between fibre-types. Furthermore, Chapter 4 demonstrated no relationship between the training-induced changes in muscle specific tension and changes in myosin heavy chain isoform composition. Therefore, it is unlikely that a change in fibre-type composition would have influenced the lack of correlation between training-induced changes in muscle force and PCSA.

Not surprisingly, in these circumstances the increase in force was found to correlate well with the increase in specific tension of the muscle but the cause of this varied increase in specific tension is not clear. A change in fibre-type expression appears to be an unlikely explanation since it has been shown in Chapter 4 of the current thesis, as well as in previous studies that strength training tends to decrease the expression of the fast IIX myosin isoform (Adams *et al.*, 1993b; Roman *et al.*, 1993; Fry *et al.*, 1994; Staron *et al.*, 1994; Campos *et al.*, 2002) and there appears to be no difference between the specific tension of type IIA and IIX fibres (Bottinelli *et al.*, 1996; D'Antona *et al.*, 2006; Pansarasa *et al.*, 2009). Another possibility is that skeletal muscle in these individuals adapts by increasing its ability to transmit force laterally to the tendon. This increase might occur through an increase in attachments between intermediate sarcomeres and the extracellular matrix (Jones *et al.*, 1989), either as a consequence of an increase in collagen synthesis

(Miller *et al.*, 2005) or proteins that constitute structural complexes known as costameres (Woolstenhulme *et al.*, 2006; Kosek & Bamman, 2008). The consequence of this is that the "effective" PCSA of the muscle would increase, thus allowing muscle force to increase without additional contractile material being required (Jones *et al.*, 1989) although the effective length of the fibres would decrease and the power remain relatively constant (Chapter 4).

Conclusions

The results from the present study clearly demonstrate that the response of human muscle to high-intensity resistance training varies considerably between previously untrained young men. Large differences in the hypertrophic response of the muscle were not related to the variable response of muscle force, giving rise to variable increases in the specific tension of the muscle. In searching for mechanisms underlying these variable changes, whether of a genetic or other nature, future investigations should focus not only on factors involved in muscle growth but also those that affect the quality, or force generating capacity, of skeletal muscle.

Chapter 6

The impact of ACE and ACTN3 genotype on the variable muscle response to strength training

Abstract

It is equivocal whether angiotensin converting enzyme (ACE) and α-actinin-3 (ACTN3) genotypes are associated with human muscle strength, power and size, and the variable response to resistance training. To determine whether a detailed analysis of muscle phenotypes could resolve this issue, quadriceps femoris (QF) maximum muscle force, volume, physiological cross-sectional area (PCSA), specific tension (force per PCSA) and power output (W_{max}) were determined before and after 9 weeks of leg-extension training in a large group of untrained young men genotyped for the ACE I/D and the ACTN3 R/X polymorphisms. Greater increases in the single repetition maximum (1 RM) and W_{max} were observed in ACE I/I homozygotes compared to D-allele carriers. The ACTN3 R-allele was associated with greater 1 RM, muscle volume and W_{max} but not with the response to training. To conclude, the ACE I/D polymorphism does explain a certain amount of the variability in the muscle response to strength training. The ACTN3 R/X polymorphism, on the other hand, explains part of the variability in muscle volume and power output but not the variable change in muscle power, strength or hypertrophy following resistance training.

Introduction

Genetic factors appear to influence human muscle phenotype (Arden & Spector, 1997; Loos *et al.*, 1997; Thomis *et al.*, 1998b) and the response to strength training (Thibault *et al.*, 1986; Thomis *et al.*, 1998a). The identification of genes that are associated with muscle strength and the responses to resistance training would enhance our understanding of how human skeletal muscle adapts to loading and why some people are more prone to muscle wasting diseases or are more likely to attain elite athletic performance than others. The angiotensin I-converting enzyme (ACE) and the α-actinin-3 (ACTN3) genes have been considered prime candidates for such investigations.

The primary role of ACE in the renin-angiotensin system is to convert angiotensin I (Ang I) to Ang II (Rigat *et al.*, 1990). As well as causing vasoconstriction, Ang II also affects renal sodium reabsorption and aldosterone production. Moreover, the resulting Ang II also appears to regulate smooth (Geisterfer *et al.*, 1988; Berk *et al.*, 1989) and cardiac (Sadoshima *et al.*, 1993; Ishigai *et al.*, 1997) muscle growth. ACE is also expressed in skeletal muscle (Reneland & Lithell, 1994), where Ang II has been shown to modulate skeletal muscle hypertrophy in response to mechanical load (Gordon *et al.*, 2001). Detection of either the presence (insertion allele, I) or absence (deletion allele, D) of a 287 amino acid base pair fragment within intron 16 on chromosome 17 has enabled a functional polymorphism of the ACE gene to be identified in humans (Rigat *et al.*, 1990). Accordingly, an individual can be classified as having one of three ACE genotypes; I/I, D/D, or I/D, where carriers of the D allele express higher ACE activity than I/I carriers (Rigat *et al.*, 1990; Tiret *et al.*, 1992; Danser *et al.*, 1995; Williams *et al.*, 2005; McCauley *et al.*, 2009). Reports of a larger proportion of D-allele carriers among elite power athletes compared to endurance athletes and sedentary subjects has led to the hypothesis that

carriers of the D-allele in the normal population would have larger muscle size and hence strength (Myerson *et al.*, 1999; Nazarov *et al.*, 2001; Woods *et al.*, 2001).

While significant associations have been found between ACE genotype and muscle size (Charbonneau *et al.*, 2008) or strength (Williams *et al.*, 2005) in untrained individuals, other investigators have observed no such association (Folland *et al.*, 2000; Thomis *et al.*, 2004; Giaccaglia *et al.*, 2008; McCauley *et al.*, 2009). Similarly, some studies have not reported an association between the ACE genotype and strength gains (Thomis *et al.*, 2004; Williams *et al.*, 2005; Pescatello *et al.*, 2006), while those that did observe an association reported that carriers of the D allele had the greatest gains in strength (Folland *et al.*, 2000; Giaccaglia *et al.*, 2008). Although this could be caused by a greater hypertrophic response, there are no reports of ACE genotype specific muscle hypertrophy following resistance training (Thomis *et al.*, 2004; Pescatello *et al.*, 2006; Charbonneau *et al.*, 2008).

A common single nucleotide polymorphism (SNP) of the human ACTN3 gene results in either an arginine (R) or a stop codon (X) being located at amino acid 577 in exon 16 (North & Beggs, 1996). Consequently, three ACTN3 genotypes exist; R/R, R/X, or X/X and, although the R577X genotype frequency varies between ethnic groups, the frequency for the X/X genotype in the human population is approximately 18% (Mills *et al.*, 2001). Individuals homozygous for the X-allele (X/X genotype) are unable to produce ACTN3, a cytoskeletal protein found only in type II muscle fibres that binds and attaches actin filaments to the Z-line (Beggs *et al.*, 1992; North *et al.*, 1999; Mills *et al.*, 2001). A deficiency in this protein might impair the performance of the type II fibres (MacArthur & North, 2007) that are able to contract faster and produce more power than type I muscle

fibres (Bottinelli *et al.*, 1996; Widrick *et al.*, 2002; Gilliver *et al.*, 2009). Therefore, the ACTN3 R577X SNP is thought to be functionally significant in determining skeletal muscle strength and power, a hypothesis supported by an observed under-representation of X/X homozygotes among elite power athletes (Yang *et al.*, 2003). Investigations into an association between ACTN3 genotype and untrained human muscle strength have produced conflicting results, with some investigators observing a positive relation (Clarkson *et al.*, 2005a; Vincent *et al.*, 2007), while others found no association (McCauley *et al.*, 2009). Moreover, a positive association has been reported between the ACTN3 X/X genotype and changes in strength (Clarkson *et al.*, 2005a), while another study found that the R/R genotype was associated with a greater change in power (Delmonico *et al.*, 2007) following resistance training. However, no study to date has investigated the effects of ACTN3 genotype on maximally activated muscle force or muscle specific tension [maximum force per unit physiological cross-sectional area (PCSA)], or indeed the effects of resistance training on the above phenotypes. Such a detailed analysis of muscle phenotypes might provide novel information about the effects of ACTN3 genotype on inter-individual variability in muscle function and the variable response to overload training.

The aims of the present study were to investigate the association of ACE and ACTN3 genotypes with maximum QF muscle force, power, volume, PCSA and specific tension before and after resistance training in previously untrained men. It was hypothesized that the ACE D-allele would be associated with larger muscle volume, PCSA and maximum force before, and greater hypertrophy and strength gains following, resistance training. It was further hypothesized that the ACTN3 R-allele would be positively associated with higher strength and power values, as well as greater training responses in those variables.

Materials and methods

Participants

Potential participants were excluded if they had a history of lower-limb fracture, their age was outside the range of 18-39 years, had taken part in strength training within the 12 months prior to the study, had used dietary supplements or performance enhancing aids or if they were considered to be in ill health (assessed from the responses to a general health questionnaire). The study was approved by the Manchester Metropolitan University local ethics committee. Fifty-one untrained males, aged 20 ± 3 years, height 178 ± 5 cm, body mass 77 ± 11 kg (mean ± SD) participated in the present study after written informed consent was obtained. The characteristics of the participants according to ACE and ACTN3 genotypes are presented in Table 6.1 and Table 6.2, respectively. Volunteers were familiarized with all test procedures and equipment within a 14-day period prior to the baseline measurements and, as far as possible, testing and training sessions occurred at the same time of day for each participant.

Habitual physical activity level

The habitual physical activity level (PAL) of each participant was assessed by questionnaire (Baecke *et al.*, 1982), as described in Chapter 5.

Experimental design

Maximum QF muscle force, volume, physiological cross-sectional area (PCSA) and specific tension were determined using Method 2 of Chapter 2, before and after nine weeks of high-intensity leg-extension resistance training (described in Chapter 3), in 51 previously untrained men. Maximum power output (W_{max}) of the right limb was

determined before and after training in a sub-sample (n = 40) at 110 RPM during maximal sprints on a modified isokinetic cycle ergometer, as described in detail in Chapter 4. In addition, all participants were genotyped for the ACE I/D and the ACTN3 R577X polymorphisms.

Blood sampling

A 10-mL blood sample was drawn from a superficial forearm vein and collected into 10-mL EDTA tubes (BD Vacutainer Systems, Plymouth, UK). The blood was then aliquotted into 2-mL tubes (Eppendorf AG, Hamburg, Germany) and stored at -80°C until subsequent analysis.

DNA extraction and determination of ACE and ACTN3 genotype

DNA extraction was performed automatically using a QIAcube® (Qiagen, Crawley, UK) and QIAamp® DNA blood kit (Qiagen, Crawley, UK), following the QIAamp® spin protocol for DNA purification from whole blood. Real-time quantitative polymerase chain reaction (Q-PCR) DNA amplification was performed to determine genotype of the ACE and ACTN3 genes.

ACE I/D and ACTN3 genotyping: In each well of a 96-well plate, the 10-µL reaction volume included 5 µL Genotyping Master Mix (Applied Biosystems, Foster City, USA), 4.3 µL nuclease-free H_2O (Qiagen, Crawley, UK) and 0.5 µL genotyping assay mix. For the ACE I/D polymorphism, a three-primer system was used to eliminate mistyping (Shanmugam *et al.*, 1993) that can occur with a two-primer system (Rigat *et al.*, 1990). In this case, the 0.5 µL genotyping assay mix included the following primers: 5'-CCCATCCTTTCTCCCATTTCTC-3'; 5'-AGCTGGAATAAAATTGGCGAAAC-3'; 5'-

CCTCCCAAAGTGCTGGGATTA-3', and probes, VIC-5'-AGGCGTGATACAGTCA-3'-MGB (I allele specific) and FAM-5'-TGCTGCCTATACAGTCA-3'-MGB (D allele specific) (Applied Biosystems, Foster City, USA). Regarding the ACTN3 R/X SNP, 0.5 µL of TaqMan® SNP genotyping assay mix (rs1815739) was used, which included the appropriate primers and TaqMan® probes, VIC® and FAM® (Applied Biosystems, Foster City, USA). Subsequently, 0.2 µl DNA sample was pipetted into each well, except for two wells that served as negative controls, into which 0.2 µL nuclease-free H_2O was pipetted. The well-plate was covered with an optical seal (Microseal 'B' adhesive seal, BioRad Laboratories, Hercules, USA) and centrifuged at 6000 x g (8000 RPM) for 1 min, then placed into a Q-PCR machine (Chromo4 Real-Time PCR Detection System, BioRad Laboratories, Hercules, USA). DNA amplification was then performed using the following PCR protocols. ACE I/D polymorphism: 50 cycles of incubation at 92°C for 15 s (denaturation) and annealing at 57°C for 1 min. ACTN3 SNP: denaturation at 95°C for 10 min, followed by 40 cycles of incubation at 92°C for 15 s and annealing at 60°C for 1 min. ACE and ACTN3 genotypes were ultimately determined using Opticon Monitor 3.1 software (BioRad Laboratories, Hercules, USA). All samples were analysed in duplicate and in all cases there was agreement between genotype for samples from the same subject.

Statistics

The test-retest reliability (coefficient of variation and intraclass correlation coefficient) of all the measurements used to calculate QF specific tension has been reported in Chapter 2 (Erskine *et al.*, 2009). ACE and ACTN3 genotype frequencies were tested for compliance with the Hardy-Weinberg principle using a χ^2 test. To determine whether associations existed between ACE or ACTN3 genotype and 1RM, MVC, QF muscle force, power, QF PCSA and volume measurements before or after training, repeated measures analysis of

covariance (ANCOVA) was used. The within-subjects factor was time (2 levels: pre- and post-training) and the between subjects factor was ACE or ACTN3 genotype (3 levels: I/I; I/D; D/D or R/R; R/X; X/X). All ANCOVAs included either baseline QF muscle PCSA or BMI as covariates. All significant associations from the main ANCOVA model were subjected to pairwise statistical analyses among each of the three genotype groups (either I/I; I/D; D/D or R/R; R/X; X/X) with Bonferroni correction. In the case of QF muscle specific tension, repeated measures ANOVA with Scheffé post-hoc test was used. If a genotype x training interaction was found, a one-way ANOVA and Scheffé post-hoc test was used to detect genotype differences in the pre-training status and the training response. In addition, based on the hypothesis that ACE D-allele carriers would express higher baseline values and training-induced changes in muscle phenotypes than ACE I/I homozygotes, the results for ACE D/D and I/D genotypes were pooled and compared with those of I/I homozygotes. Likewise, the results for ACTN3 R/R and R/X genotypes were pooled and compared with those of ACTN3 X/X homozygotes. In this instance, the same statistical analyses were used as described above but the between-subjects factor had only 2 levels (either ACE D-allele carrier and I/I genotype or ACTN3 R-allele carrier and X/X genotype). Statistical significance was accepted when $P < 0.05$. All data are presented as mean ± standard deviation (SD) unless otherwise stated.

Results

ACE and ACTN3 genotypes

The ACE I/D genotype frequencies were 11.8%, 51.0%, and 37.3% for ACE I/I, I/D, and D/D, respectively (Table 6.1), and were in Hardy-Weinberg equilibrium (p = 0.373; q = 0.627; $\chi^2 = 0.418$, $P = 0.811$). The ACTN3 genotype frequencies were 39.2%, 47.1%, and 13.7% for R/R, R/X, and X/X, respectively (Table 6.2) and were also in Hardy-Weinberg

equilibrium for the investigated population (p = 0.628; q = 0.372; χ^2 = 0.002, *P* = 0.999). Furthermore, the ACE and ACTN3 genotype frequencies for the sub-sample of individuals who completed the power output tests (*n* = 40) did not differ from the main group.

Table 6.1 Physical characteristics and physical activity level (PAL) of participants according to angiotensin I-converting enzyme (ACE) genotype

	ACE genotype			
Variable	I/I	I/D	D/D	I/D + D/D
	(*n* = 6)	(*n* = 26)	(*n* = 19)	(*n* = 45)
Age (years)	20.5 ± 2.1	19.8 ± 2.0	21.0 ± 4.4	20.3 ± 3.3
Body mass (kg)	73.3 ± 12.1	76.2 ± 12.5	74.9 ± 7.2	75.7 ± 10.5
Height (cm)	177.2 ± 7.2	178.6 ± 5.9	177.8 ± 5.1	178.3 ± 5.5
BMI ($kg \cdot m^{-2}$)	23.3 ± 2.8	23.8 ± 2.9	23.7 ± 2.0	23.7 ± 2.6
PAL	2.8 ± 0.5	2.7 ± 0.3	2.8 ± 0.3	2.7 ± 0.3

I insertion allele; *D* deletion allele; *BMI* body mass index

Table 6.2 Physical characteristics and physical activity level (PAL) of participants according to α-actinin-3 (ACTN3) genotype

	ACTN3 genotype			
Variable	R/R	R/X	X/X	R/R + R/X
	(*n* = 20)	(*n* = 24)	(*n* = 7)	(*n* = 44)
Age (years)	20.5 ± 2.4	20.7 ± 3.9	18.7 ± 1.1	20.6 ± 3.3
Body mass (kg)	76.2 ± 6.5	76.5 ± 12.6	69.3 ± 12.2	76.3 ± 10.2
Height (cm)	179.1 ± 4.8	178.1 ± 5.8	175.4 ± 7.4	178.6 ± 5.3
BMI ($kg \cdot m^{-2}$)	23.8 ± 2.0	24.0 ± 2.9	22.5 ± 3.0	23.9 ± 2.4
PAL	2.9 ± 0.4	2.7 ± 0.4	2.6 ± 0.2	2.8 ± 0.4

R/R wild-type homozygote; *R/X* heterozygote; *X/X* mutant homozygote; *BMI* body mass index

Habitual physical activity level

Physical activity levels (PAL) were not associated with either the ACE I/D or ACTN3 R577X polymorphisms (Table 6.1; 6.2) and the mean PAL scores for each ACE and ACTN3 genotype are described as slightly less than "intermediate" (Baecke *et al.*, 1982). Furthermore, PAL scores did not have a significant effect on any of the muscle changes as a result of training.

Strength parameters before and after training

Single repetition maximum (1 RM) There was not only a significant effect of training ($P < 0.05$), but also a significant training x ACE genotype interaction ($P < 0.05$) on the 1 RM values that had been corrected for baseline muscle PCSA. Subsequent ANCOVAs revealed that the baseline 1 RM values were similar for all ACE genotypes but that the increase in 1 RM was larger in I/I carriers than individuals of both I/D and D/D genotypes (Table 6.3). There was a significant effect of training ($P < 0.001$) and ACTN3 genotype ($P < 0.05$) regarding the 1 RM (Table 6.4). Ensuing ANCOVAs demonstrated that, while 1 RM values were not significantly different between ACTN3 genotype before training, both ACTN3 R/R and R/X genotypes had higher 1 RM values than X/X homozygotes following training (Table 6.4). As there was no training x ACTN3 genotype interaction ($P > 0.05$), individuals of all three genotypes responded similarly to training (Table 6.4). When ACTN3 R/R and R/X genotypes were pooled, it was found that the 1 RM (corrected for baseline muscle PCSA) was higher in carriers of the R-allele than in X/X homozygotes ($P < 0.05$; Table 6.4).

Maximum isometric voluntary contraction MVC torque (corrected for baseline muscle PCSA) was independent of ACE genotype, both at baseline and following training (Table

6.3). The absence of training x genotype interaction for either ACE or ACTN3 genotype ($P > 0.05$) indicates that for each polymorphism, all three genotypes responded similarly to training (Table 6.3; 6.4). However, when ACTN3 R/R and R/X genotypes were pooled and compared with X/X genotype, a significant genotype effect was observed ($P < 0.05$), which was evident as a lower MVC in X/X than in the R carriers (Table 6.4).

Maximum isometric force Once MVC torque had been adjusted to account for voluntary muscle activation level, antagonist muscle co-activation and moment arm length, and had been corrected for baseline QF PCSA, there was no difference between the genotypes of either the ACE or ACTN3 polymorphisms regarding baseline maximum QF muscle force and the training-induced increase in force (Table 6.3; 6.4).

Muscle size before and after resistance training

Muscle volume Having accounted for BMI, neither baseline muscle volume nor the training-induced changes in volume differed between ACE genotypes (Table 6.3). There was a significant effect of ACTN3 genotype ($P < 0.01$) on QF muscle volume (Table 6.4). Subsequent ANCOVAs revealed that QF muscle volume was higher in individuals of R/R genotype than X/X homozygotes before and after training (Table 6.4). As there was no training x genotype interaction ($P > 0.05$), individuals of all three genotypes responded similarly to training (Table 6.4). Furthermore, when R/R and R/X genotypes were pooled, carriers of the R-allele were found to have greater QF muscle volume than X/X homozygotes ($P < 0.05$; Table 6.4).

Physiological cross-sectional area Dividing QF muscle volume by muscle fascicle length provides a measure of the physiological cross-sectional area (PCSA). No differences were

seen in the QF muscle PCSA between the ACE or ACTN3 genotypes, either before or after training, or in the training-induced increases in PCSA (Table 6.3; 6.4).

Muscle specific tension before and after training

Taking into account muscle fascicle pennation angle, maximum muscle force and PCSA, the specific tension of the QF muscle was found to be independent of ACE and ACTN3 genotypes before and after training. Furthermore, QF muscle specific tension increased similarly between ACE and ACTN3 genotypes (Table 6.3; 6.4).

Table 6.3 Pre and post-training values, as well as training-induced changes in muscle strength and size phenotypes in participants according to angiotensin I-converting enzyme (ACE) genotype

	ACE genotype			
Variable	I/I	I/D	D/D	I/D + D/D
	(*n* = 6)	(*n* = 26)	(*n* = 19)	(*n* = 45)
Pre 1 RM (kg)	47.0 ± 7.6	55.4 ± 11.3	54.7 ± 11.2	55.1 ± 11.1
Post 1 RM (kg)	94.0 ± 4.2	89.2 ± 12.3	85.9 ± 12.8	87.9 ± 12.5
Δ 1 RM (%)	103 ± 26*	65 ± 27	61 ± 31	63.2 ± 28.0
Pre MVC (N·m)	227 ± 27	250 ± 56	251 ± 51	251 ± 54
Post MVC (N·m)	272 ± 39	319 ± 64	312 ± 63	316 ± 63
Δ MVC (%)	20.1 ± 10.8	28.5 ± 11.6	24.6 ± 8.7	26.9 ± 10.5
Pre *F* (N)	5359 ± 983	5883 ± 1114	5669 ± 997	5793 ± 1060
Post *F* (N)	6260 ± 1311	7245 ± 1355	6862 ± 1351	7083 ± 1351
Δ *F* (%)	16.8 ± 11.8	23.5 ± 10.8	21.1 ± 11.2	22.5 ± 10.9
Pre V_m (cm^3)	2034 ± 279	2114 ± 279	2068 ± 221	2095 ± 254
Post V_m (cm^3)	2145 ± 248	2245 ± 315	2174 ± 230	2215 ± 281
Δ V_m (%)	5.8 ± 3.5	6.1 ± 3.6	5.2 ± 3.1	5.7 ± 3.4
Pre PCSA (cm^2)	229 ± 33	239 ± 42	242 ± 42	241 ± 41
Post PCSA (cm^2)	239 ± 40	255 ± 44	254 ± 43	255 ± 43
Δ PCSA (%)	3.9 ± 3.6	6.9 ± 5.0	5.0 ± 3.7	6.1 ± 4.6
Pre ST ($N \cdot cm^{-2}$)	24.6 ± 4.8	26.1 ± 5.4	24.8 ± 5.1	25.6 ± 5.3
Post ST ($N \cdot cm^{-2}$)	27.9 ± 6.5	30.5 ± 6.2	29.0 ± 6.8	29.9 ± 6.5
Δ ST (%)	13.5 ± 12.7	17.3 ± 10.1	16.0 ± 12.6	16.8 ± 11.2

I insertion allele; *D* deletion allele; *1 RM* single repetition maximum (accounting for baseline muscle PCSA); *MVC* maximal voluntary contraction torque (accounting for baseline muscle PCSA); *F* maximum muscle force (accounting for baseline muscle PCSA); V_m quadriceps femoris muscle volume (accounting for BMI); *PCSA* quadriceps femoris muscle physiological cross-sectional area (accounting for BMI); *ST* quadriceps femoris muscle specific tension; all post-training changes were significantly different from baseline values ($P < 0.05$); *$P < 0.05$ significantly different from other genotypes

Table 6.4 Pre and post-training values, as well as training-induced changes in muscle strength and size phenotypes in participants according to α-actinin-3 (ACTN3) genotype

	ACTN3 genotype			
Variable	R/R	R/X	X/X	R/R + R/X
	(*n* = 20)	(*n* = 24)	(*n* = 7)	(*n* = 44)
Pre 1 RM (kg)	55.3 ± 9.6	56.1 ± 12.5	45.7 ± 4.5	55.8 ± 11.2†
Post 1 RM (kg)	92.5 ± 11.2†	88.9 ± 11.4†	77.1 ± 9.9	90.5 ± 11.3†
Δ 1 RM (%)	70.7 ± 28.2	63.6 ± 31.9	71.0 ± 32.7	66.8 ± 30.1
Pre MVC (N·m)	263 ± 54	246 ± 48	211 ± 40	253 ± 51†
Post MVC (N·m)	321 ± 62	313 ± 62	273 ± 57	317 ± 61
Δ MVC (%)	22.8 ± 10.8	28.0 ± 10.7	28.7 ± 9.8	25.6 ± 10.9
Pre *F* (N)	5960 ± 1026	5674 ± 976	5351 ± 1370	5804 ± 998
Post *F* (N)	6998 ± 1357	7053 ± 1290	6726 ± 1763	7028 ± 1306
Δ *F* (%)	17.3 ± 10.9	24.5 ± 10.5	25.7 ± 10.6	21.2 ± 11.1
Pre V_m (cm^3)	2197 ± 244†	2053 ± 246	1895 ± 185	2118 ± 253†
Post V_m (cm^3)	2317 ± 268†	2183 ± 266	1974 ± 176	2244 ± 272†
Δ V_m (%)	5.5 ± 3.9	6.4 ± 3.1	4.3 ± 2.6	5.9 ± 3.5
Pre PCSA (cm^2)	253 ± 47	229 ± 37	238 ± 40	240 ± 43
Post PCSA (cm^2)	267 ± 44	242 ± 45	250 ± 16	254 ± 46
Δ PCSA (%)	6.1 ± 4.0	5.7 ± 5.3	5.6 ± 3.2	5.9 ± 4.7
Pre ST ($N \cdot cm^{-2}$)	25.3 ± 6.0	26.1 ± 3.8	23.7 ± 6.8	25.7 ± 4.9
Post ST ($N \cdot cm^{-2}$)	28.3 ± 7.2	31.1 ± 5.3	28.5 ± 7.7	29.8 ± 6.3
Δ ST (%)	11.7 ± 12.1	19.0 ± 9.6	21.0 ± 10.5	15.7 ± 11.3

R/R wild-type homozygote; *R/X* heterozygote; *X/X* mutant homozygote; *Pre* before training; *Post* after training; Δ relative change after training; *1 RM* single repetition maximum (accounting for baseline muscle PCSA); *MVC* maximal voluntary contraction torque (accounting for baseline muscle PCSA); *F* maximum muscle force (accounting for baseline muscle PCSA); V_m quadriceps femoris muscle volume (accounting for BMI); *PCSA* quadriceps femoris muscle physiological cross-sectional area (accounting for BMI); *ST* quadriceps femoris muscle specific tension; all post-training values were significantly different from baseline ($P < 0.05$); † $P < 0.05$ significantly different from X/X genotype

Power output and genotype

There were no significant differences between the three ACE genotypes either before or after training regarding maximum power output (W_{max}) corrected for BMI. However, ACE I/I homozygotes demonstrated a greater increase in W_{max} compared to their D-allele counterparts ($P < 0.05$; Table 6.5). Regarding ACTN3 genotype, W_{max} was higher in individuals of R/R genotype than X/X homozygotes before training, while after training both R/R and R/X genotypes expressed greater W_{max} values than X/X homozygotes ($P < 0.05$; Table 6.6). As there was no training x genotype interaction, individuals of all three genotypes responded similarly to training, although there was a small, non-significant increase in W_{max} in the groups expressing the R-allele and a slight, non-significant decrease in the group expressing the X/X genotype (Table 6.6). When R/R and R/X genotypes were combined, carriers of the R-allele were found to generate more W_{max} than X/X homozygotes ($P < 0.05$). Furthermore, the 16% higher W_{max} observed in R-allele carriers (R/R + R/X) compared to X/X homozygotes before training was similar to the 20% larger baseline MVC values in R-allele carriers compared to individuals of the X/X genotype (Table 6.4; 6.6).

Table 6.5 Pre and post-training values, as well as training-induced changes in power output in participants according to angiotensin-I converting enzyme (ACE) genotype

	ACE genotype			
Variable	I/I	I/D	D/D	D/D + I/D
	(*n* = 4)	(*n* = 22)	(*n* = 14)	(*n* = 36)
Pre W_{max} (W)	1322 ± 227	1437 ± 210	1432 ± 185	1435 ± 198
Post W_{max} (W)	1531 ± 197	1471 ± 243	1472 ± 174	1472 ± 216
Δ W_{max} (%)	16.4 ± 5.9*	2.9 ± 12.3	3.5 ± 10.9	3.1 ± 11.6

I/I homozygote; *I/D* heterozygote; *D/D* homozygote; *Pre* before training; *Post* after training; Δ relative change after training; W_{max} maximum power output; * $P < 0.05$ significantly different from D-allele carrier; values are mean ± SD

Table 6.6 Pre and post-training values, as well as training-induced changes in power output in participants according to α-actinin-3 (ACTN3) genotype

	ACTN3 genotype			
Variable	R/R	R/X	X/X	R/R + R/X
	(*n* = 16)	(*n* = 19)	(*n* = 5)	(*n* = 35)
Pre W_{max} (W)	1478 ± 195*	1424 ± 198	1251 ± 158	1449 ± 19*
Post W_{max} (W)	1526 ± 151*	1513 ± 226	1190 ± 86	1519 ± 193*
Δ W_{max} (%)	4.1 ± 10.7	6.8 ± 11.8	-3.5 ± 14.3	5.6 ± 11.2

R/R wild-type homozygote; *R/X* heterozygote; *X/X* mutant homozygote; *Pre* before training; *Post* after training; Δ relative change after training; W_{max} maximum power output; * $P < 0.05$ significantly different from X/X genotype; values are mean ± SD

Discussion

It is controversial whether the ACE I/D and ACTN3 R/X polymorphisms are associated with human muscle strength, size and power, and the variable response to resistance training. This controversy might be related to the fact that inter-individual differences in strength and the variance in training-induced strength gains are not only influenced by muscle size but also the muscle specific tension (Chapter 5), a factor that is often overlooked. Therefore, the present study not only assessed muscle force and power but also muscle volume, PCSA and specific tension. It was hypothesised that the ACE D-allele would be related to higher baseline values of muscle size, strength and power, and greater training-induced gains in these phenotypes. Further, it was hypothesized that the ACTN3 R-allele would be associated with greater values of baseline strength and power and greater training-induced gains compared to the X/X genotype. Contrary to what was hypothesised, ACE I/I genotype was associated with larger increases in dynamic strength and power compared to carriers of the ACE D-allele but as expected, the ACTN3 R-allele was associated with greater muscle volume and power output. However, there were no differences between any of the three ACE or ACTN3 genotypes regarding muscle specific tension, nor were there any differences in the training-induced changes in muscle force, volume or specific tension.

A good indication of the maximum strength produced by a muscle is obtained from a maximum voluntary isometric contraction (MVC). In line with the reports of other investigations (Thomis *et al.*, 2004; Pescatello *et al.*, 2006; McCauley *et al.*, 2009) there was no association between ACE genotype and MVC knee joint torque before training in the present study, although one previous study did observe higher MVC values in untrained D-allele carriers than in individuals of I/I genotype (Williams *et al.*, 2005).

Furthermore, some investigators have reported no ACE genotype specific training-induced gains in MVC (Thomis *et al.*, 2004; Williams *et al.*, 2005), while others have reported an I-allele dependent increase in isometric MVC following progressive resistance training (Pescatello *et al.*, 2006). These apparent discrepancies might be related to the fact that MVC and changes thereof do not necessarily reflect changes in the force generating capacity of the muscle itself, which can be calculated by accounting for voluntary muscle activation, antagonist muscle co-activation and moment arm length (Maganaris *et al.*, 2001; Reeves *et al.*, 2004a; Erskine *et al.*, 2009). By excluding the possible bias of inter-individual differences in voluntary activation, co-activation and moment arm length, the results from the present study suggest that ACE genotype does not play a major role in determining maximally activated muscle force or the extent to which resistance training affects muscle force production.

D-allele carriers express greater ACE activity (Rigat *et al.*, 1990; Tiret *et al.*, 1992; Danser *et al.*, 1995; Williams *et al.*, 2005; McCauley *et al.*, 2009) and ACE positively affects skeletal muscle hypertrophy by converting Ang I to Ang II (Gordon *et al.*, 2001). Therefore, it is conceivable that D-allele carriers might have larger untrained muscles and be able to increase muscle size more than non-D allele carriers. Indeed, a previous study found D/D genotype carriers to have larger QF muscle volumes than their I/I counterparts (Charbonneau *et al.*, 2008). Yet, the present study observed no association between ACE genotype and QF muscle PCSA or volume. Similar negative findings have been reported for ACE genotype and the anatomical CSA (ACSA) of the elbow flexor muscle group (Thomis *et al.*, 2004; Pescatello *et al.*, 2006).

In contrast to expectation and other studies showing no association between ACE genotype

and 1 RM gains (Thomis *et al.*, 2004; Pescatello *et al.*, 2006; Charbonneau *et al.*, 2008), the present study demonstrated that I/I homozygotes exhibited the largest gains in 1 RM. It should be noted that the 1 RM is a measurement of dynamic strength and may thus be a better index of muscle power than force. Indeed, the larger increase in 1 RM among ACE I/I genotype corresponded with the observation that I/I homozygotes increased their maximum power output more than ACE D-allele carriers. As power is the product of force x velocity and muscle volume is determined by the number of sarcomeres arranged both in series and parallel, it might be expected that I/I homozygotes increased their muscle volume more than D-allele carriers. However, neither the present investigation nor a previous study (Charbonneau *et al.*, 2008) observed an ACE genotype specific hypertrophy response regarding QF muscle volume.

This evidence indicates that the significance of the ACE I/D polymorphism in determining human skeletal muscle size and the extent of hypertrophy is minimal. Thus, the associations between ACE I/I genotype and a greater increase in 1 RM as well as maximum power output cannot be explained by muscle hypertrophy. Although the reasons for these unexpected findings cannot be inferred from the present results, training-induced changes in muscle fibre-type composition (Adams *et al.*, 1993b; Roman *et al.*, 1993; Fry *et al.*, 1994; Staron *et al.*, 1994; Campos *et al.*, 2002) might offer a solution, as the force- and power-velocity relationships of isolated human muscle fibres are fibre-type dependent (Bottinelli *et al.*, 1996; Gilliver *et al.*, 2009).

The maximum force of a muscle is primarily determined by the muscle PCSA and is, in pennate muscles, modulated to some extent by the fascicle pennation angle, as the force resolved at the tendon is reduced as a function of pennation angle (Alexander & Vernon,

1975; Degens *et al.*, 2009). By accounting for these factors, it has been shown that *in vivo* specific tension of the QF muscle varies considerably between untrained individuals (Chapter 2), while Chapter 5 illustrated that substantial variation exists in the change in QF specific tension following resistance training. In the present study, no association was observed between ACE genotype and untrained muscle specific tension, or the training-induced change in specific tension. Although the reasons for a lack of correspondence between ACE genotype and muscle PCSA, maximum force and specific tension are unclear, the theoretical physiological significance of genotype-dependent ACE activity in skeletal muscle is to modulate muscle size, rather than the intrinsic force of the muscle.

A possible genetic association with muscle strength, power and size, and the variable response to resistance training, lies with the ACTN3 R/X SNP. The present study demonstrated that carriers of the ACTN3 R-allele performed higher MVC values than their X/X counterparts before training, suggesting that R-allele carriers are stronger than X/X homozygotes. However, this genotype dependence was lost after training and no association was observed with a training-induced change in MVC. A previous study made a similar observation concerning an ACTN3 R/X genotype association with isometric MVC of the elbow flexors in young women (Clarkson *et al.*, 2005a). Therefore, the present study corrected MVC torque for the level of voluntary muscle activation, antagonist muscle co-activation and moment arm length to ascertain whether maximum muscle force was more strongly associated with ACTN3 genotype. However, there was no genotype association with maximum force, either before training, or with a training-induced change in force. The possibility remained that inter-individual differences or changes in muscle size and/or fibre pennation angle were obscuring any relation between ACTN3 genotype and muscle force. Therefore, maximum QF muscle force was corrected for muscle PCSA

and the angle of fibre pennation, in order to calculate the specific tension of the QF muscle. However, no ACTN3 genotype association was observed. This would suggest that the ACTN3 protein has little relevance in determining isometric muscle force.

However, the possibility exists that ACTN3 allows greater force transmission at the Z line during high-velocity muscle contraction (Yang *et al.*, 2003; MacArthur & North, 2004), thus enabling more force and ultimately more power to be produced during shortening contractions. Therefore, muscle function assessments that incorporate dynamic force or power generation would be more appropriate to detect an effect of ACTN3 genotype on muscle force or power. The present study observed greater 1 RM values in ACTN3 R-allele carriers than in X/X homozygotes, but there was no genotype association with the training-induced increase in 1 RM. In contrast, a previous study found no association between ACTN3 genotype and 1 RM before or after strength training but contrary to expectation and to the results of the present study, a greater 1 RM training response was observed in female X/X homozygotes than in carriers homozygous for the R-allele (Clarkson *et al.*, 2005a). However, the accuracy of the 1 RM assessment in determining the dynamic strength of a muscle group will undoubtedly be influenced by the level of muscle activation and the additional muscles involved in the exercise, such as the synergistic and stabilizing muscles (Rutherford & Jones, 1986). Thus, a certain proportion of the change in 1 RM might be due to the development of skill involved in lifting weights rather than a change in muscle strength *per se*. Therefore, a more accurate assessment of power output would be required to support the 1 RM results of the present study.

In line with the hypothesis of the current study, carriers of the ACTN3 R-allele were found to generate more power than X/X homozygotes both before and after strength training.

However, contrary to what was hypothesized, no association was observed between ACTN3 genotype and a training-induced change in power output. Conversely, a previous study on an older population (aged 50-85 years) found that while peak leg-extension power was higher in untrained X/X homozygotes than in carriers of the R-allele in females but not in males, both female and male carriers of the R-allele increased their peak power more than X/X homozygotes following leg-extension resistance training (Delmonico *et al.*, 2007). The reasons for the disparities between the results of the present study and those of other reports are not immediately clear, although differences in experimental methodologies and populations studied might have played a role.

Muscle fibre CSA of ACTN3 deficient mice is significantly smaller compared to their wild-type counterparts that are able to produce the ACTN3 protein (Chan *et al.*, 2008). If this were the case in human muscle, ACTN3 R-allele carriers would be expected to have larger muscles than X/X homozygotes and indeed QF muscle volume corrected for BMI was significantly greater in carriers of the R-allele both before and after training in the present study. Surprisingly, however, this difference was not observed in muscle PCSA, either before or after training. Furthermore, a previous study reported no association between elbow flexor muscle ACSA and ACTN3 genotype in either men or women (Clarkson *et al.*, 2005a). Thus, it is possible that the larger muscle volume in R-allele carriers was due to these individuals having longer muscle fascicles than their X/X counterparts. Indeed, once corrected for BMI, there was a tendency ($P = 0.09$) for the fascicle length of the vastus lateralis muscle in R-allele carriers to be longer than that of X/X homozygotes before training (data not shown), while the association was stronger following training ($P < 0.05$). Therefore, the ~16% difference in power output between carriers of the R-allele and X/X homozygotes could be due to the ~12% larger muscle

volume in R-allele carriers compared to non R-allele carriers. This is further supported by the ~20% larger mean baseline MVC value in R-allele carriers compared to individuals of the X/X genotype.

Conclusions

The results from the present study demonstrate that ACE genotype is not associated with QF muscle volume, PCSA, maximum force or *in vivo* muscle specific tension prior to strength training. ACE I/I homozygotes, however, expressed larger increases in dynamic strength and power output after training. This indicates that the ACE I/D polymorphism does explain a certain amount of the variability in the muscle response to strength training, which is one of the major concerns of this thesis. Concerning the ACTN3 polymorphism, larger muscle volume, greater strength and power were found in carriers of the ACTN3 R-allele compared to X/X homozygotes, although muscle specific tension was unrelated to ACTN3 genotype. No ACTN3 genotype differences were observed in the responses to resistance training and so it is unlikely that the variable response to resistance training is related to ACTN3 genotype. However, polymorphisms of genes encoding proteins involved in muscle cell structure may help explain the inter-individual differences in the intrinsic strength of skeletal muscle and the variable change in muscle specific tension.

Chapter 7

General discussion

Muscle strength and the effects of training have been of interest to man since mythological times when it is said that Milo of Croton first introduced the concept of progressive resistance training (Chiras, 2005). This idea of progressive muscle overload training was more recently developed by DeLorme and colleagues (Delorme, 1945; Delorme *et al.*, 1948; Delorme & Watkins, 1948; Delorme *et al.*, 1950) and since then there has been a voluminous literature on the topic. However, one common and intriguing observation is that there are considerable differences between individuals in their muscular strength and also in their response to resistance training. In maximally activated parallel-fibred muscles, that is where the fibres are aligned parallel to the line of pull of the tendon and run from one end of the muscle to the other, the strength and power of the muscle are directly related to the cross sectional area and volume of the muscle, respectively. However, there are a host of other factors that modulate the contractile properties of the muscle *in vivo* and when it comes to training adaptations it is important that these are all considered when trying to explain the overall change in muscle function. This has been the main purpose of the work described in this thesis.

The general aims of the current thesis were to investigate the differences in strength between untrained individuals and to perform a detailed examination of the response of human muscle to resistance training concerning gains in muscle strength and size and the extent to which these responses vary from person to person. Chapter 1 provides a review of the factors that could affect the strength of a muscle and how they might change with training. Chapter 2 focuses on investigations designed to explain the inter-individual variation in maximum voluntary isometric contraction (MVC) knee joint torque in a large homogenous cohort of young men by systematically accounting for a variety of neuromuscular parameters. The main conclusion was that the differences in MVC torque

could not be explained by variation in muscle volume, architecture (fascicle length and pennation angle), activation or moment arm length. The work described in Chapter 3 examined the effect of overload training of the quadriceps femoris (QF) muscle with the aim of determining whether the gains in strength can be explained by an increase in size of the muscle or whether there is an increase in specific tension after taking into account the possible changes in muscle architecture, activation or moment arm. The results clearly indicate that the gains in strength with training were mainly as a result of an increase in specific tension [muscle force per unit physiological cross-sectional area (PCSA)] with increases in muscle size being a minor contributing factor. Chapter 4 explored whether this increase in specific tension could be related to changes occurring within the muscle itself, such as an alteration of muscle fibre-type composition, an increase in single fibre specific tension or a change in the way force is transmitted from muscle fibre to the tendon. Measurements of power output on an isokinetic cycle ergometer suggest that the effect of strength training is to increase isometric force more than power, which is consistent with an increase in force transmission from sarcomeres along the length of the fibre rather than those just at the ends, sometimes known as “lateral transmission” of force. Chapter 5 is concerned with the extent of variability in the response of human muscle to strength training, with regard to individual changes in muscle force, PCSA and specific tension. It is clear that most of the variation in the gains in strength occurs as a result of variation in specific tension and not size of the muscle. Chapters 6 and 7 explored the possibility of an association between specific gene variants and the inter-individual variability in muscle strength, size and power, and the variable changes of those phenotypes in response to resistance training. The results from these chapters suggest that neither the angiotensin I-converting enzyme (ACE) I/D gene polymorphism, nor the α-actinin-3 (ACTN3) R577X

single nucleotide polymorphism (SNP) is responsible for the variable response to strength training.

The inter-individual variability in untrained muscle strength

Around half of the difference in MVC knee joint torque between untrained individuals has been typically associated with the anatomical CSA (ACSA) of the QF muscle (Maughan *et al.*, 1983; Chapman *et al.*, 1984; Maughan *et al.*, 1984; Kanehisa *et al.*, 1994; Akima *et al.*, 2001). However, ACSA is known to underestimate the PCSA of pennate muscles (Alexander & Vernon, 1975) and it is the PCSA that represents the CSA of the total number of muscle fibres perpendicular to their long axes, which in turn is thought to be the primary determinant of the force produced by a maximally activated muscle (Powell *et al.*, 1984). By measuring muscle architecture in each of the component QF muscles, together with the patellar tendon force during maximal muscle activation at the optimum knee joint angle, the work described in Chapter 2 demonstrated how it is possible to determine maximum force, PCSA and specific tension of the QF muscle when the muscle fibres in the whole muscle group were at their optimum length for producing peak force. However, the variance in QF specific tension was only slightly less than that of MVC knee joint torque. Thus, the results of this work show that the variability in knee joint moment in an homogenous group of untrained individuals was not appreciably reduced by accounting for inter-individual differences in voluntary muscle activation (assessed with the interpolated twitch technique, ITT), antagonist muscle activation (quantified by means of electromyography, EMG), patellar tendon moment arm length (measured using magnetic resonance imaging, MRI), muscle volume (calculated using MRI) and muscle fascicle length and pennation angle (determined via ultrasonography). The implication, therefore, is that differences reside in the inherent force generating capacity of the muscle fibres

themselves. As type II muscle fibres have been shown to be intrinsically stronger than type I fibres (Bottinelli *et al.*, 1996; D'Antona *et al.*, 2006; Pansarasa *et al.*, 2009), a possibility is that some of the variance in QF specific tension was due to inter-individual differences in QF muscle fibre-type composition. However, the results reported in Chapter 4 suggest that *in vivo* muscle specific tension is not associated with myosin heavy chain (MyHC) isoform composition in the untrained state, thus fibre-type composition appears to be an unlikely cause of the differences in specific tension between people. It remains to be seen, however, whether differences in connective tissue content and/or costameric density between people leads to inter-individual differences in the degree of lateral transmission of force from muscle fibres to the tendon, which would affect the variability in specific tension (Jones *et al.*, 1989).

The effect of resistance training on in vivo muscle specific tension

Larger increases in muscle strength than size have commonly been reported following short-term resistance training (Ikai & Fukunaga, 1970; Moritani & deVries, 1979; Young *et al.*, 1983; Jones & Rutherford, 1987; Davies *et al.*, 1988; Narici *et al.*, 1989; Narici *et al.*, 1996b; Ferri *et al.*, 2003). In an attempt to explain this phenomenon, the study described in Chapter 3 comprehensively investigated the effect of resistance training on human muscle *in vivo* by systematically accounting for factors not routinely measured in the past, such as voluntary muscle activation, antagonist muscle co-activation, moment arm length, muscle volume and architecture before and after a period of high-intensity leg-extension training. In doing so, the expectation was that *in vivo* QF muscle specific tension would not change following training. Greater neural adaptations than muscle hypertrophy have been proposed as reasons for this discrepancy (Moritani & deVries, 1979; Staron *et al.*, 1991; Staron *et al.*, 1994) but, as shown in Chapter 3, voluntary muscle activation

increased by a mere 3% and antagonist muscle co-activation did not change with training. An alternative solution to the problem might have resided in the method used to determine the extent of muscle hypertrophy, which has previously been defined as the change in muscle volume (Kanehisa *et al.*, 2002; Charbonneau *et al.*, 2008) or ACSA (Davies *et al.*, 1988; Tracy *et al.*, 1999; Ferri *et al.*, 2003) but neither of those indices of muscle size represent the CSA of the total number of muscle fibres perpendicular to their long axes. This is represented by the muscle PCSA, which largely determines the maximum force produced by a muscle (Powell *et al.*, 1984). However, by assessing *in vivo* volume of the four component QF muscles, together with their respective fascicle lengths (Chapter 3), the change in QF muscle PCSA did not correspond to the increase in maximally activated muscle force. Thus, in Chapter 3 it is shown that *in vivo* QF muscle specific tension does increase following short-term resistance training, implying that overload training increases the inherent force generating capacity of the muscle.

Although the underlying cause of this increase in intrinsic force remained elusive, one possibility was that a training-induced change in fibre-type composition might affect the change in muscle specific tension, as isolated single fibre specific tension has been shown to be fibre-type dependant in human muscle (Bottinelli *et al.*, 1996; D'Antona *et al.*, 2006; Pansarasa *et al.*, 2009). Furthermore, a training-induced increase in the packing of contractile material within the muscle fibres, which would be reflected in augmented specific tension of isolated single fibres (Parente *et al.*, 2008; Pansarasa *et al.*, 2009), could feasibly increase the muscle specific tension calculated *in vivo*. Alternatively, an increase in the number of attachments, whether in the form of collagen (Miller *et al.*, 2005) or costameric proteins, e.g. desmin and dystrophin (Woolstenhulme *et al.*, 2006; Kosek & Bamman, 2008), between myofibrils and the surrounding connective tissue, could increase

the amount of force generated by the contractile component of muscle, thus increasing muscle specific tension (Jones *et al.*, 1989). In the latter case, however, additional inter-myofibrillar attachments would reduce the effective velocity of muscle contraction, thus leading to no change in power output (Jones *et al.*, 1989). Therefore, the work described in Chapter 4 investigated whether a training-induced increase in muscle specific tension observed *in vivo* could be explained by changes in muscle fibre-type composition, determined by establishing the relative constitution of the different variants of the myosin heavy chain (MyHC) molecule using gel electrophoresis, increased myofibrillar protein density, revealed through the assessment of isolated single fibre specific tension, and/or *lateral force transmission*, estimated by assessing *in vivo* maximum power output before and after training. However, in accordance with previous studies (Staron *et al.*, 1990; Fry *et al.*, 1994; Campos *et al.*, 2002) a decrease in the fast type IIX MyHC isoform composition was observed following training, which if anything would have reduced muscle specific tension. Furthermore, this change in MyHC IIX isoform composition was not associated with the increase in muscle specific tension. Additionally and in accordance with some previous studies (Trappe *et al.*, 2000; Trappe *et al.*, 2001a; Widrick *et al.*, 2002) but not with others (Parente *et al.*, 2008; Pansarasa *et al.*, 2009), no change in single fibre specific tension was observed following resistance training in Chapter 4. Thus, it appears unlikely that increased packing of contractile material was the cause of the training-induced increase in muscle specific tension observed *in vivo* in Chapters 3 and 4. However, the significantly larger increase in isometric force than absolute power output (Chapter 4) is consistent with the hypothesis that a training-induced development of additional lateral transmission of force from sarcomeres along the length of the fibres would increase specific tension at the expense of contraction velocity, thus leaving power output per unit muscle volume unaltered. Although it has not yet been shown in human muscle, it has been

demonstrated that force is not only transmitted to the tendon along the axis of the muscle fibre but also laterally to adjacent fibres via the matrix of connective tissue that encases muscle fibres (Street, 1983). Further evidence that lateral force transmission occurs comes from the identification of intra-sarcolemmal protein complexes, known as "costameres", that are circumferentially aligned along the length of the muscle fibre (Pardo *et al.*, 1983). It is thought that the main function of costameres was to mechanically link peripheral myofibrils to the sarcolemma, thus maintaining the integrity of the muscle cell during phases of contraction and relaxation (Pardo *et al.*, 1983). Moreover, it has been shown that these protein structures also actually transmit muscle fibre force laterally (Danowski *et al.*, 1992), thus directing a certain amount of fibre force through the extra-cellular matrix to adjacent muscle fibres and ultimately to the tendon (Morris & Fulton, 1994; Patel & Lieber, 1997; Huijing, 1999; Rybakova *et al.*, 2000).

The variable response to resistance training

It is evident that considerable variability exists in the response of human muscle to resistance training, concerning both the change in muscle strength and size (Hubal *et al.*, 2005). However, with numerous studies investigating possible associations between these variable training responses and genetic predisposition, it is vital to characterise the variability appropriately, since there are several ways in which a muscle may increase in strength and size. Although commonly used to characterise muscle strength (Riechman *et al.*, 2004; Hubal *et al.*, 2005; Kostek *et al.*, 2005; Hand *et al.*, 2007; Charbonneau *et al.*, 2008), neither an MVC nor the maximum load lifted in a single repetition (1 RM) necessarily represent the true maximum force produced by the muscle, as neither parameter accounts for voluntary muscle activation, antagonist muscle co-activation or moment arm length, all of which may change variably between people as a result of

training. Furthermore, the variable hypertrophic response to resistance training is often reported as the relative change in ACSA (Clarkson *et al.*, 2005a; Hubal *et al.*, 2005; Pescatello *et al.*, 2006). However, this index of muscle size underestimates PCSA in pennate muscles (Alexander & Vernon, 1975) and may therefore provide misleading information regarding a change in muscle size. In addition, a muscle may increase in quantity, defined as the PCSA, or in its intrinsic force generating capacity, characterized as specific tension, which is affected by the angle of fascicle pennation. By accounting for training-induced changes in the ratio of optimum muscle fascicle length to muscle volume, as described in Chapter 5, it was possible to obtain a more accurate account of the variability in muscle hypertrophy by estimating changes in muscle PCSA. Taking maximum muscle force, PCSA and fascicle pennation angle into account, it was shown that the change in muscle specific tension following training was highly variable, demonstrating that the adaptation of human muscle to resistance training varies considerably regarding changes in both muscle force and hypertrophy. The cause of a variable change in specific tension is not known but it could be related to a variable change in fibre-type expression, although this appears unlikely since in Chapter 4 it was demonstrated that there was no association between the change in MyHC IIX and *in vivo* muscle specific tension. Another possibility is that, in some individuals, skeletal muscle adapts to overload through muscle hypertrophy, while others increase the number of lateral attachments within the muscle that would serve to increase to the force generating capacity but decrease the effective shortening velocity (Jones *et al.*, 1989), thus resulting in no change in power output per unit muscle volume, as demonstrated in Chapter 4.

Methodological considerations of the current work

It is acknowledged that the lack of a control group to assess the reliability of the post-training results in Chapters 3, 4 and 5 is a limitation of those experimental chapters. Although it might have been advisable to use the contra-lateral leg as a control, there is considerable evidence of a cross-over effect with training of one limb, causing strength increases in the contra-lateral untrained limb (Komi *et al.*, 1978; Moritani & deVries, 1979; Zhou, 2000; Patten *et al.*, 2001). As an alternative, a systematic assessment of the test-retest reliability of all the *in vivo* measurements used to determine *in vivo* QF muscle specific tension using the familiarisation and baseline results is reported in Chapter 2 as the coefficient of variation and intraclass correlation coefficient (ICC). The ICCs ranged from 0.73 to 0.99, thus demonstrating the high reproducibility of these measurements. Furthermore, other investigators have reported good reproducibility for these *in vivo* measurements and have included control groups as further evidence that the changes observed following a period of resistance training are indeed a consequence of training and not measurement error (Reeves *et al.*, 2003a; Reeves *et al.*, 2003b, 2004b, a; Morse *et al.*, 2007b). Furthermore, the test-retest correlation coefficient for the assessment of *in vivo* power output and myosin heavy chain isoform composition were reported in Chapter 4 as 3.8% and 2.9%, respectively, thus demonstrating the high reliability of these measurements.

To accurately calculate *in vivo* QF muscle specific tension, it was necessary to quantify the level of voluntary QF muscle activation during MVC, which was achieved using the interpolated twitch technique (ITT), a method that has been used extensively for this purpose (Rutherford *et al.*, 1986b; Jones & Rutherford, 1987; Harridge *et al.*, 1999; Folland *et al.*, 2000; Scaglioni *et al.*, 2002; Reeves *et al.*, 2004a, b; Williams *et al.*, 2005).

The interpolated twitch technique (ITT) assesses the level of voluntary muscle activation essentially by assessing to what extent the motoneuron drive to the muscle is activated. Voluntary activation is therefore defined as the force produced during a maximal voluntary contraction and is given as the percentage of maximum possible force. The ITT, however, does not take into account the descending drive to the motoneurons and does not distinguish between motor unit recruitment and firing rates that would affect the potential maximal force output of a muscle. Reports of a non-linear inverse relationship between superimposed and voluntary force have questioned the validity of the ITT in determining voluntary activation (Bulow *et al.*, 1993; Dowling *et al.*, 1994; Lyons *et al.*, 1996). However, by carefully controlling the factors thought to contribute to non-linearity in this relationship the validity and reliability of this technique is improved. Firstly, the stimulating electrodes were carefully placed over the QF muscle, so as to minimise the stimulation of synergist and antagonist muscles during the ITT protocol. Secondly, the QF muscle was stimulated at a joint angle of 90° knee flexion, thus reducing the effect of slack in the series elastic component on the calculated activation level when assessed at smaller joint angles (Becker & Awiszus, 2001; Kubo *et al.*, 2004; Bampouras *et al.*, 2006). Furthermore, due care was taken to minimise compliance in the isokinetic dynamometer system by adding a counter-weight to the lever arm. Thirdly, it has been suggested that a single superimposed twitch is unable to detect sub-optimal motor-unit firing rates (Maffiuletti *et al.*, 2002). However, it has been shown that the effect of stimuli number is negligible as there is no difference in the calculated level of activation when one, two, four, five or eight stimuli are administered (Behm *et al.*, 1996; Bampouras *et al.*, 2006). Although the aforementioned precautions taken during the current project do not remove all limitations of the ITT, it is one of the best available techniques to obtain an insight into voluntary activation, and hence inclusion of the ITT is better than not using it at all.

While it is acknowledged that the estimation of hamstring co-activation during an isometric knee extension MVC using surface EMG measurements is subject to a variety of technical issues, care was taken to maximise test-retest reliability of this assessment. Re-location of EMG electrodes in subsequent tests was done as accurately as possible by marking the original electrode location in relation to anatomical landmarks on a sheet of acetate, and skin surface impedance was reduced to below 5 kΩ. However, it is not known how training would have affected levels of subcutaneous and intramuscular fat, which would have also influenced the EMG signal.

It has been demonstrated using cadaver knees that the tension developed in the QF tendon is ~1.5 times higher than in the patellar tendon over the range of joint angles used in the present study, i.e. that the patello-femoral joint does not function as a frictionless pulley (Buff *et al.*, 1988). If this were the case *in vivo*, untrained QF muscle specific tension values using Method 1 described in Chapter 2 would have been ~45 $N \cdot cm^{-2}$, considerably higher than previous values determined with animal muscle (Close, 1972; Degens *et al.*, 1995). Although this discrepancy in forces at the QF and patellar tendons should be acknowledged, applying ratios gained from cadaveric data may well give a false indication of the tendon force relationship *in vivo*. Moreover, this issue does not affect the comparison of Methods 1 and 2 in Chapter 2 and, as there was no change in joint angle with training (Chapter 3), its influence on post-training changes in tendon force would be negligible (assuming that any frictional resistance around the knee is unchanged by training).

One of the novel approaches of this thesis is that the geometry (fascicle length and pennation angle) of each of the four component QF muscles was assessed using two-dimensional (2D) ultrasonography during MVC at optimum knee joint angle. As rotation of the ultrasound probe relative to the plane of contraction is known to affect the measured fascicle length (Klimstra *et al.*, 2007), care was taken to eliminate probe movement during each measurement trial. Following each assessment, the ultrasound scan was checked for probe movement and if this was observed, the trial was repeated. Although it is acknowledged that 2D images would not account for possible fascicle rotation, the ultrasound images were of good quality and fascicles were easily identifiable and able to be tracked along their lengths within the field of view. Muscle PCSA was calculated by dividing muscle volume by fascicle length (Haxton, 1944; Alexander & Vernon, 1975; Maganaris *et al.*, 2001; Reeves *et al.*, 2004a), with the latter measured in the mid-sagittal plane of the central portion of each muscle. Therefore, it was assumed the muscle was cylindrical in shape and that its fascicles were a constant length, which is supported by previous *in vivo* (Narici *et al.*, 1996a; Maganaris *et al.*, 1998; Maganaris & Baltzopoulos, 1999) and cadaveric (Friederich & Brand, 1990) findings in human muscles. In the resting state, however, it has been shown that *in vivo* QF muscle architecture varied along the muscle length (Blazevich *et al.*, 2006). Although measuring muscle geometry at multiple sites in each constituent muscle is feasible in resting conditions, obtaining such extensive measurements during an MVC is problematic; it would require at least 12 MVCs (assuming 3 sites per muscle), which is likely to induce errors due to the development of muscular fatigue (in addition to the MVCs performed at each of the remaining two joint angles).

When using MRI to measure QF muscle volume in young women, it has been reported that a small proportion (~6%) constitutes non-contractile material (Macaluso *et al.*, 2002). Therefore, it is recognised that by not accounting for this component in the current thesis, it is possible that the volume of contractile material was slightly overestimated, thus leading to an overestimation of PCSA and an underestimation of specific tension. However, as the non-contractile component of the ankle dorsiflexor muscle group is 25% higher in young women than in men of the same age (Holmback *et al.*, 2002), it is likely that the level of intramuscular fat in the male participants of the present study was negligible and the influence on the calculation of muscle PCSA, if any, would have been minimal.

Implications for future research

The work described in this thesis explored the possible reasons for a large variation in inter-individual strength (Maughan *et al.*, 1983; Chapman *et al.*, 1984; Maughan *et al.*, 1984; Kanehisa *et al.*, 1994; Akima *et al.*, 2001) and found that differences in voluntary activation, antagonist co-activation, moment arm length, muscle volume and muscle architecture could not fully explain this variance. Therefore, future studies should focus on investigating possible factors causing inter-individual differences in the intrinsic force of the muscle. Although the present thesis has demonstrated that inter-individual differences in muscle fibre-type composition are unlikely to explain this variability, quantification of intramuscular lateral attachments in the form of connective tissue or costameres might offer an explanation for this variance in muscle specific tension, based on differences in the extent of lateral force transmission.

The observed increase in muscle specific tension following resistance training described in Chapter 3 suggests that previous reports of disproportionate increases in muscle strength and size following resistance training (Ikai & Fukunaga, 1970; Moritani & deVries, 1979; Young *et al.*, 1983; Jones & Rutherford, 1987; Davies *et al.*, 1988; Narici *et al.*, 1989; Narici *et al.*, 1996b; Ferri *et al.*, 2003) are not explained by changes in voluntary activation, antagonist co-activation, moment arm length, muscle volume, or architecture. As described in Chapter 4, no association was found between the increases in muscle specific tension and change in MyHC isoform composition, neither was there a change in power output per unit muscle volume. Consequently, a change in lateral force transmission remains a strong possibility for explaining these observations. Training-induced changes in muscle collagen or costameric protein content could provide a possible mechanism for this increase in muscle specific tension. Furthermore, it is feasible that resistance training causes an increase in myofilament packing, thus increasing the specific tension of the individual muscle fibres. This possibility could be examined by determining the contractile properties of isolated human muscle fibres before and after a period of resistance training. However, Chapter 4 of the current thesis and recent reports have provided equivocal results (Trappe *et al.*, 2000; Widrick *et al.*, 2002; Parente *et al.*, 2008; Pansarasa *et al.*, 2009), thus demonstrating the need for clarification on this issue. Comparing the force generated by an isolated bundle of fibres, where lateral transmission would be expected to play a role, with the force from single fibres where lateral transmission is not a factor, might throw some light on this question.

The results described in Chapter 5 illustrate the extent of variation in the response to resistance training (at least in the short term), regarding a change in maximum force, muscle PCSA and specific tension. As a result, a greater understanding of the way in

which human muscle adapts to strength training was achieved. Future investigations into possible mechanisms underlying these variable responses should consider the variable changes in force, PCSA and specific tension ascertained in Chapter 5 of this thesis. The discovery of genetic polymorphisms that identify high and low responders to strength training would considerably improve our understanding of the factors that regulate muscle size and strength. However, in the search for associations between gene variants and the muscle response to resistance training, it is important to distinguish between gains in strength and increases in muscle size, since it would appear that these are two separate responses to training. No significant associations were observed concerning the ACE I/D polymorphism or the ACTN3 R577X SNP and the variability in muscle specific tension or the variable response to resistance training, although the ACTN3 genotype does appear to be related to muscle volume and power output. It is suggested that future studies should investigate variants of genes encoding cytoskeletal proteins that are involved in the lateral transmission of muscle force. Such studies might provide further evidence for lateral force transmission underlying the differences in specific tension and the variable response to resistance training.

Conclusions

Specific tension of the human QF muscle has been calculated *in vivo* from the most comprehensive set of measurements to date and the results indicate that most of the inter-individual variance in MVC torque arises from factors other then differences in the activation, size and geometry of the muscle. By systematically accounting for these factors, *in vivo* QF muscle specific tension was shown to increase following resistance training. This increase was not found to be associated with a change in fibre-type composition or an increase in isolated single fibre specific tension but, as power output per muscle volume

remained unaltered after training, a change in lateral force transmission is a conceivable mechanism for a training-induced increase in muscle specific tension. Furthermore, it has been clearly demonstrated that the response of human muscle to high-intensity resistance training varies considerably between previously untrained young men. Large differences in the hypertrophic response of the muscle were not related to the variable response of muscle force, giving rise to variable increases in muscle specific tension. Certain candidate gene variants have provided evidence of genetic predisposition influencing muscle strength, size and power but not the human muscle response to resistance training. However, it is acknowledged that the genetic determinants of muscle phenotype and the response to overload are likely to be polygenic and more studies are required to elucidate new candidate gene variants. Moreover, in searching for mechanisms, whether of a genetic or other nature, underlying these differences or changes in muscle function, it is important that the complexity of the muscle response is clearly appreciated.

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