Effects of Ageing and Physical Activity on Regulation of Muscle Contraction

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Abstract

The aims of this study were to investigate the mechanisms underlying (1) the ageing-related motor handicap at the whole muscle, cellular, contractile protein and myonuclear levels; and (2) ageing-related differences in muscle adaptability.

In vivo muscles function was studied in the knee extensors. Decreases were observed in isokinetic and isometric torque outputs in old age in the sedentary men and women and elite master sprinters. A 20-week long specific sprint and resistance training successfully improved the maximal isometric force and rate of force development in a subgroup of master sprinters.

In vitro measurements were performed in muscle biopsies from the vastus lateralis muscle. Immunocytochemical and contractile measurements in single membrane permeabilized muscle fibres demonstrated ageing- and gender-related changes at the myofibrillar level. In sedentary subjects, data showed a preferential decrease in the size of muscle fibres expressing type IIa MyHC in men, lower force generating capacity in muscle fibres expressing the type I MyHC isoform in both men and women and lower maximum velocity of unloaded shortening (V_{o}) in fibres expressing types I and IIa MyHC isoforms in both men and women. The master sprinters also experienced the typical ageing-related reduction in the size of fast-twitch fibres, a shift toward a slower MyHC isoform profile and a lower V_{o} of type I MyHC fibres, which played a role in the decline in explosive force production capacity. The fast-twitch fibre area increased after the resistance training period. A model combining single muscle fibre confocal microscopy with a novel algorithm for 3D imaging of myonuclei in single muscle fibre segments was introduced to study the spatial organisation of myonuclei and the size of individual myonuclear domains (MNDs). Significant changes in the MND size variability and myonuclear organization were observed in old age, irrespective gender and fibre type. Those changes may influence the local quantity of specific proteins per muscle fibre volume by decreased and/or local cooperativity of myonuclei in a gender and muscle fibre specific manner.

In conclusion, the ageing-related impairments in in vivo muscle function were related to significant changes in morphology, contractile protein expression and regulation at the muscle fibre level. It is suggested that the altered myonuclear organisation observed in old age impacts on muscle fibre protein synthesis and degradation with consequences for the ageing-related changes in skeletal muscle structure and function. However, the improved muscle function in response to a 20-week intense physical training regime in highly motivated physically active old subjects demonstrates that all ageing-related in muscle function are not immutable.

Keywords: ageing, sarcopenia, sprint training, progressive resistance training, in vivo function, isokinetic dynamometer, in vitro function, single permeabilised fibre, myonuclear domain, confocal microscopy

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Dedication

To my Family
This thesis is based on the following papers which are referred to by their Roman numerals:


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<tbody>
<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
</tr>
<tr>
<td>COX</td>
<td>Cytochrome c oxidase (complex IV)</td>
</tr>
<tr>
<td>CSA</td>
<td>Cross sectional area</td>
</tr>
<tr>
<td>DHPR</td>
<td>Dihydropyridine receptor</td>
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<tr>
<td>EGTA</td>
<td>Ethylene glycol tetraacetic acid</td>
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<tr>
<td>EMG</td>
<td>Electromyography</td>
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<td>ETC</td>
<td>Electron transport chain</td>
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<td>iEMG</td>
<td>Integrated electromyography</td>
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<tr>
<td>KE</td>
<td>Knee extensor</td>
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<tr>
<td>MND</td>
<td>Myonuclear domain</td>
</tr>
<tr>
<td>mtDNA</td>
<td>Mitochondrial DNA</td>
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<tr>
<td>MU</td>
<td>Motor unit</td>
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<tr>
<td>MyHC</td>
<td>Myosin heavy chain</td>
</tr>
<tr>
<td>MyLC</td>
<td>Myosin light chain</td>
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<tr>
<td>NN</td>
<td>Nearest neighbour</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
</tr>
<tr>
<td>RyR</td>
<td>Ryanodine receptor</td>
</tr>
<tr>
<td>SDH</td>
<td>Succinate dehydrogenase (complex II)</td>
</tr>
<tr>
<td>SERCA</td>
<td>Sarco/endoplasmic reticulum Ca(^{2+})-ATPase</td>
</tr>
<tr>
<td>SPRT</td>
<td>Sprint and resistance training</td>
</tr>
<tr>
<td>SR</td>
<td>Sarcoplasmic reticulum</td>
</tr>
<tr>
<td>ST</td>
<td>Specific tension</td>
</tr>
<tr>
<td>Tm</td>
<td>Tropomyosin</td>
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<tr>
<td>Tn</td>
<td>Troponin</td>
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<tr>
<td>(V_0)</td>
<td>Maximum velocity of unloaded shortening</td>
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<td>PTM</td>
<td>Posttranslational modifications</td>
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Introduction to skeletal muscle

The skeletal muscle cell

The muscle cell, composed of myofibrils, is the force producing unit in skeletal muscle. Myosin and actin are the major constituents in the myofibril. Each myosin molecule is surrounded by six actin molecules. The myofibrils occupy approximately 2/3 of the cell volume (Hoppeler and Fluck, 2002) and it is estimated that an adult muscle cell of 50 μm in diameter contains up to 2000 myofibrils.

The sarcomere is the functional unit of the muscle cell and is composed of thick filaments of myosin associated at the M-line, and the thin filaments of actin that are anchored to the Z-disc. The essential role of the sarcomere is to generate force by the ATP-driven movement of myosin heads along the actin filaments (Alberts et al., 1994).

Myosin is the most abundant protein in the skeletal muscle cell. The human genome presents a significant degree of polymorphism with over 40 different myosin genes (Hodge and Cope, 2000), all having the capacity to move along actin molecules and generate force while hydrolyzing ATP (Caiozzo, 2002). Skeletal muscle myosin is constituted of two myosin heavy chains (MyHC) subunits of approximately 220kDa and four myosin light chains (MyLC) of approximately 20kDa each. Ten myosin isoforms have presently been identified in the mammalian muscle: ß-slow (I), α-cardiac, slow-tonic, embryonic, foetal, IIa, IIx (IId), IIb and two super-fast isoforms in jaw closing and extraocular muscles. Of those, ß-slow (I), IIa and IIx are expressed in adult human skeletal muscle fibres (Schiaffino and Reggiani, 1996).

The ATPase activity of different myosin isoforms has a different sensitivity to alkali or acid preincubations, a property that is utilised in the enzyme-histochemical fibre typing. The velocity of the muscle fibre contraction is dependant on the myosin isotype. Based on the myosin isotype, the human skeletal muscle fibres are classified in type I, IIa, IIx and hybrid fibre types I-IIa and IIax. The unloaded shortening velocity $V_o$ (see Methods) in muscle fibre segments is dependent on the MyHC composition and increases in the order I<IIa<IIa<IIax<IIx (Larsson and Moss, 1993). A modulatory effect of essential MyLC isoform expression on $V_o$ has been demonstrated in avian (Reiser et al., 1988) and rabbit muscles (Sweeney et al., 1986), while the
effect has not been confirmed in human skeletal muscle fibres (Larsson & Moss, 1993).

Fibre type transitions (Pette and Staron, 1997, 2000) occur in an ordered manner and are thought to represent the capacity of the skeletal muscle fibre to adapt to altered internal and external demands.

Mitochondria and oxidative stress

Most of the ATP needed for muscle contraction is generated in the mitochondria. Mitochondria contain oxidative enzymes and can be found dispersed in-between the myofibrils (intermyofibrillar mitochondria, IM) or beneath the sarcolemma (subsarcolemmal mitochondria, SS) (Adhihetty et al., 2006). The double membrane of mitochondria contains the electron transport chain (ETC) that utilizes oxygen as the terminal electron acceptor and generates energy in the form of adenosine triphosphate (ATP) (Alberts et al., 1994). The cytochrome oxydase (COX) enzyme (complex IV) comprises 13 subunits. Three of those are encoded by mtDNA (Alberts et al., 1994). Thus, fibres that are COX-negative represent fibres with mitochondrial genome abnormalities. The succinate dehydrogenase (SDH) enzyme complex (complex II) is a nuclear-encoded complex of the electron transport chain; therefore, negative SDH staining intensity is a reflection of nuclear genome mutations.

Mitochondrial function decreases with age as a result of oxidative damage and results in mitochondrial uncoupling, i.e., leakage of electrons from the ETC (electron transport chain) (Harper et al., 2004). Accumulation of mtDNA mutations promote apoptosis in normal ageing skeletal muscle cells via the caspase -3 pathway (Kujoth et al., 2005). The mitochondrial genome of humans accumulates with time due to deletions and mutations in an exponential manner (Packer and Cardenas, 1999), especially in metabolically very active and postmitotic fixed tissues such as the skeletal muscle.

The loss of COX activity in some muscle fibres with age coincides with non-functional mtDNA and mitochondrial energy loss. COX-negative fibres are rare in young persons, but occur more often in skeletal muscle in persons over 50 years of age, which may account for the overall loss of muscle mass and decrease in muscle function. Increased amount of mitochondrial abnormalities with age is often associated with single fibre atrophy, branching and increased oxidative damage (Wanagat et al., 2001).

Nuclei and myonuclear domain

The skeletal muscle cell contains hundreds of nuclei that share a common cytoplasm (syncitium). The nuclei are generally situated at the exterior of the
muscle cell, beneath the sarcolemma and become incapable of further mitosis as the fibre matures. Every nucleus determines the transcriptional activity within the nearest neighbourhood in the cytoplasm, the myonucleus domain (MND) (Pavlath et al., 1989). Myonuclei in the adult skeletal muscle have been reported to be stationary (Bruusgaard et al., 2003), in order to minimize the transport distances in the sarcoplasm. The protein synthesis occurs in a pulsatile manner (Newlands et al., 1998). The myonuclear domain size is smaller at the tips of the fibre (Rosser et al., 2002) and presents fibre type specificity, with smaller domains for slow contracting muscle fibres (Allen et al., 1999).

**Muscle contraction**

Muscle contraction (twitch, power stroke, ATPase activity)

The sarcoplasmic reticulum (SR) is organised as a network of channels around the myofibrils and releases Ca\(^{2+}\) into the cytosol through the ryanodine receptors (RyR). Sarcolemmal invaginations constitute a second network of fine tubule, the T-tubule, which encompasses the myofibrils and contains extracellular fluid. The role of the T-tubule is to conduct the action potential that initiates the release of Ca\(^{2+}\) from the SR. The action potential from the neuron innervating the muscle cell activates the dihydropyridine receptors (DHPR) in the T-tubule, which in turn activate the ryanodine receptors to release Ca\(^{2+}\) from the SR. The increased Ca\(^{2+}\) concentration in the close vicinity of the contractile proteins causes Ca\(^{2+}\) to bind to the regulatory protein troponin (Tn), which starts the initiation of muscle contraction. The sarco/endoplasmic reticulum Ca\(^{2+}\) pumps (SERCA) are ATP driven pumps that transport Ca\(^{2+}\) back into the lumen of the SR during muscle relaxation (Bagshaw, 1993).

A muscle contraction (also known as a muscle twitch or simply, twitch) occurs when a muscle cell (muscle fibre) shortens, due to a sudden increase in cytosolic Ca\(^{2+}\) concentration. Slow twitch human muscle fibres are fatigue resistant and contain mainly slow type I myosin, while the fast twitch fibres contain IIa or IIx MyHC, or a combination of both. Slow twitch fibres are called oxidative, due to the large number of mitochondria. Lowest number of mitochondria is found in the IIx fibres (glycolitic), with the IIa fibres in-between type I and IIa fibres. The Ca\(^{2+}\) transient is also different in different fibre types. Two isoforms of SERCA pumps exist. SERCA 1 is found in fast fibres and SERCA 2 in slow fibres. The Ca\(^{2+}\) pumps density is higher in fast fibres than in slow fibres (Bortolotto and Reggiani, 2002).
The sliding filament theory states that thick filaments of myosin will slide longitudinally past the thin actin filament, which results in muscle shortening. The I-band (where the actin filaments are present) shortens, while the A-band remains at the same length during the contraction.

The myosin head has the capability to hydrolyse ATP in the presence of actin. Different myosin isoforms hydrolyses the ATP at different reaction rates and are related to the velocity at which a muscle fibre shortens. The cross-bridge model considers muscle contraction as the result of a cyclic interaction during which the cross-bridges pull the actin filament across the myosin, in a reaction step called the power-stroke. The myosin filament remains stationary during the contraction, with each cross-bridge acting as a force generator. The number of the cross-bridges, as well as the force generated by the individual cross-bridges, determines the amount of force generated by the whole muscle fibre (Alberts et al., 1994).

The motor unit and registration of motor unit activity

The motor unit (MU) is the CNS’ smallest functional unit of force development control. A motor unit (Fig. 1) includes a single α-motor neuron and all the muscle fibres that it innervates.

Alpha-motoneurons are in direct contact with skeletal muscle fibres at the motor end-plate level, and are directly responsible for the initiation of the muscle contraction. All fibres within a MU are of the same type and will contract at the same time. The size of a motor unit varies with the muscle
function. A large muscle such as the quadriceps femoris has a high force output but requires relatively low fine-tuning of movements and is organised into larger motor units. The motor units are recruited depending on the force they must produce, with the smallest motor units (including type I fibres) being recruited before the larger fast-twitch motor units (including type II fibres) (Henneman et al., 1965). Depending on the time required for a contractile cycle, the MUs may be slow, intermediary fatigue resistant and fast fatiguing, reflecting the characteristics of the fibre types innervated by the same α-motoneuron (Bortolotto and Reggiani, 2002).

Neurological modifications with age contribute to a decline in voluntary force production and are associated with changes in muscle mass. A decrease in the number of excitable MUs associated with an increase in the area of the remaining MU’s, as well as slower axonal conduction velocity, were observed beyond the seventh decade of life (Vandervoort, 2002). The loss of muscle mass is primarily due to a loss of α-motoneurons and incomplete reinnervation of individual muscle fibres by adjacent α-motoneurons (Larsson and Ansved, 1995), leading to angular fibres and fibre type grouping.

Electrodes placed on the skin over the muscle permit the registration of the electrical variation in MUs. Limitations of the method are recognised, since smaller variations are not detected. An underestimation of the signal is also known to occur, due to the fact that the positive and negative potentials of the MUs cancel each other out (Duchateau et al., 2006).

Force generation is controlled by selective activation of units and differential firing range and frequency. Smaller MUs are composed of slow twitch fibres and are recruited first. The firing frequency range is also lower in slow units. Within that range, the force generated by a motor unit increases with increasing firing frequency. If an action potential reaches a muscle fibre before it has completely relaxed from a previous impulse, then force summation will occur. By this method, firing frequency affects muscular force generated by each motor unit. At slower pace of exercise, slow-twitch fibres are selectively utilized because they have the lowest threshold for recruitment. An increase in activity level will require the recruitment of the larger fast-twitch units. In general, as the intensity of exercise increases in any muscle, the contribution of the fast fibres will increase (Bagshaw, 1993).

Neuromuscular plasticity with ageing and activity level

The term sarcopenia- (from the Greek meaning "poverty of flesh") is used for the involuntary loss of skeletal muscle mass and strength with increasing age.
The aetiology of sarcopenia is difficult to study; the causes of the impairment in muscle function are multiple and occur at the whole muscle, cellular, contractile protein and transcriptional level. However, appropriate life-long physical exercise programs are demonstrated to influence positively the skeletal muscle sarcopenic manifestations (Fig. 2). The cellular and molecular mechanisms, through which those interventions act, are currently being investigated.

Figure 2. Ageing and physical activity influence the sarcopenic processes at the molecular and nuclear levels.

Changes at the whole-muscle level

The muscular performance in humans reaches its peak during the third decade of life. Longitudinal studies indicate a constant decrease in strength of approximately 1-3 % per year (Frontera et al., 2000a; Frontera et al., 2008). The decrease in force production is linear until the sixth decade, followed by an accelerated decrease after the seventh decade of life in both sedentary (Larsson et al., 1979) and long- and short-distance runners (Korhonen et al., 2003; Michaelis et al., 2008). A decrease in isokinetic and isometric muscle force is observed to occur with age (Borges, 1989; Gur et al., 2003; Jubrias et al., 1997; Lanza et al., 2003; Larsson et al., 1979; Rantanen et al., 1994; Stalberg et al., 1989). The muscle mass is a determinant of strength (Frontera et al., 1991; Frontera et al., 2000b) and it was shown that the decrease in muscle mass is both gender (Akima et al., 2001) and muscle specific (Frontera et al., 1991; Grimby et al., 1982; Lanza et al., 2003). Further, higher age is associated with a reduced capacity to produce force rapidly (Hakkinen et al., 1995; Harridge et al., 1996; Korhonen et al., 2003). This aspect is of primary importance, since the ability to develop force rapidly is decisive for avoiding falls and fall-related injuries (Aagaard et al., 2002),
being the dominant cause of morbidity and mortality in the increasing proportion of elderly citizens.

**Effects of training**

Physical activity is the preferred stimulus of the skeletal muscle, and resistance training has a particularly beneficial influence on the quality/mass of the neuromuscular system (Trappe, 2001). Resistance training increases the muscular strength in previously untrained old men and women (Aniansson et al., 1984; Charette et al., 1991; Frontera et al., 1988), with a similar increase in men and women after the initial period (Lexell et al., 1995). Also in frail nonagenarians, resistance training is beneficial, with improvements in muscle mass, strength and gait speed (Fiatarone et al., 1990; Harridge et al., 1999). Further, resistance training is associated with ameliorated neuromuscular function, as shown by improvements in explosive force production and rate of force development (Hakkinen and Hakkinen, 1995; Hakkinen et al., 1998b; Hakkinen et al., 2001). The ageing process is immutable and the hypertrophic potential of old muscle decreases after the eight decade of life (Slivka et al., 2008). It can be speculated that the intensity and duration of the stimulus, as well as differences in age, gender and habitual activity level of the subjects may be factors that decide the size of the hypertrophic effects of resistance training (Aagaard et al., 2001b; Frontera et al., 1988; Hakkinen et al., 2002; Shoepe et al., 2003; Trappe et al., 2000; Widrick et al., 2002). Nothing is known about the effects of resistance training in already trained elite sprinters at older age.

**Neural adaptations**

In parallel with the hypertrophic mechanisms, neural adaptations contribute to the improved muscle performance in response to resistance training. The neural mechanisms, are indicated to be more important at the beginning of the training period (Frontera et al., 2003; Hakkinen et al., 1992; Moritani and deVries, 1979), with an increasing importance of the hypertrophic mechanisms, depending on the initial training status of the subjects, during the later phases of adaptation (Hakkinen and Hakkinen, 1995). Other factors, such as an increase in individual fibre-specific tension, a decrease in antagonist muscles coactivation, an improved co-ordination and an increased neural drive may also contribute to the increments in strength in old untrained men (Ferri et al., 2003).
Changes at the single fibre level

**Fibre type transitions**

The quantitative and qualitative changes with age observed at the whole-muscle level reflect the changes at the single muscle fibre level. A decrease in number of both type I and II muscle fibres (Lexell et al., 1988) along with a preferential age-related fibre atrophy of type II fibres (Larsson, 1982; Larsson et al., 1978; Tomonaga, 1977) is described, leading to a progressive decrease in the type II-to-type I fibre area ratio (Larsson et al., 1979; Lexell et al., 1988). Ageing is associated with adaptations such as atrophy and slow-to-fast fibre transition (D'Antona et al., 2003; Degens and Alway, 2006). A transition from slow-to-fast (MyHC I > MyHC IIa > MyHC IIax> MyHC IIx) was observed in slow-twitch fibres, where a percentage of the slow myosin is degraded and replaced by faster MyHC isoforms, mainly IIx (Baldwin and Haddad, 2001). The number of hybrid fibres that co-express at least two different MyHC types increases with age due to altered transcriptional events (Andersen, 2003; Andersen et al., 1999). Fibre type transitions (Pette and Staron, 1997, 2000) occur in an ordered manner and are thought to represent the capacity of the skeletal muscle fibre to adapt to changed internal and external requirements.

**Quantitative changes**

While the CSA of type I was not affected by ageing or physical level, it is shown that CSA of type IIa fibres is smaller in the old sedentary individuals. Muscle hypertrophy in humans as a result of RT is suggested to occur by addition of myofibrils, resulting in enlarged muscle fibre size (Allen et al., 1999). Sprint training in male athletes is thought to induce a bi-directional transformation in MyHC isoform, with a decrease in type I and IIx in the favour of type IIa MyHC isoform (Andersen et al., 1994b). Resistance training protocols demonstrate an increase in CSA and P₀ in young (D'Antona et al., 2006; Shoepe et al., 2003; Widrick et al., 2002) and old (Trappe et al., 2000) individuals. The hypertrophic effects of the resistance training vary between different studies. It can be speculated that the intensity and duration of the stimulus, as well as differences in age, gender and habitual activity level of the subjects may be factors that decide the size of the hypertrophic effects of resistance training (Aagaard et al., 2001a; Frontera et al., 1988; Shoepe et al., 2003; Trappe et al., 2000; Widrick et al., 2002). Nothing is known about the hypertrophic adaptations at the single fibre level in old age after life-long physical performance.

**Qualitative changes**

The maximum force generated by the muscle fibres (P₀) is not dependent on the MyHC isoforms, but on the number of cross-bridges per area unit, the
force developed by every cross-bridge and the proportion of cross-bridges producing force (Schiaffino and Reggiani, 1996). Total myofibrillar volume is directly proportional to muscle mass (scaling factor 0.98). Skeletal muscle sarcomeres are built very similarly in all mammalian species, so the total number of cross-bridges that the myosin heads can form with actin is directly proportional to muscle mass. Deviations of the ATP demand for muscle contraction from direct proportionality to body mass must therefore depend mainly on size-dependent differences in cross-bridge cycling rates (Hoppeler 2002). An ageing-related decline in the capacity of single muscle fibres to generate force independent of changes in fibre size is observed in type I and IIA fibres (Larsson et al., 1997b), but the activity level has a modulatory influence on the specific tension (D'Antona et al., 2003; D'Antona et al., 2007). Maximally activated skinned fibres of older rats have a reduced fraction of myosin heads in the strong-binding structural state (Lowe et al., 2001a; Lowe et al., 2002) resulting in a reduced force generating capacity per cross-bridge. A decrease in myosin concentration and a reduction in the fraction of strongly bound myosin heads may affect P0/CSA in skinned single fibre segments. The strong correlation between relative myosin content in human single fibres and P0/CSA suggests that a major determinant of the lower specific tension in older men is the reduced number of cross-bridges (D'Antona et al., 2003).

Maximum unloaded shortening velocity (V0) is one of the most important design parameters of the muscle fibres, and the velocity of the muscle fibre contraction is depending closely on the MyHC isoform expressed within a fibre and the actin-activated myosin ATP-ase activity (Bottinelli et al., 1996; Larsson and Moss, 1993; Schiaffino and Reggiani, 1996). The V0 variability within fibres expressing the same MyHC isotype is relatively small, with no overlap between different cell types (Larsson et al., 1999). A modulatory effect on shortening velocity of the alkali MyLC has been demonstrated in avian (Reiser et al., 1988) and rabbit muscles (Sweeney et al., 1986). Studies in human muscle show no significant relationship between V0 and MyLC isoform composition, and it was suggested the existence of other factors that may co-vary to modulate the V0 (Larsson et al., 1997a; Larsson and Moss, 1993). An age-related decrease in V0 (D'Antona et al., 2003; D'Antona et al., 2007; Larsson et al., 1997a) and also gender-related differences with age (Krivickas et al., 2001b) are generally recognised, even though not always confirmed (Frontera et al., 2008; Trappe et al., 2003).

Modifications at the protein level
A reduction in protein synthesis with age (Balagopal et al., 1997; Yarasheski et al., 1993) is paralleled by enhanced proteolysis, especially under conditions of muscle unloading such as microgravity or prolonged bed rest (Booth and Criswell, 1997; Booth et al., 1994). It was shown that RT itself, rather
than protein supplementation, has an anabolic effect and plays a role in maintaining muscle mass and strength in older men (Candow et al., 2006; Castaneda et al., 2001).

Ageing has a negative impact on the velocity of type I myosin molecule \(V_f\) in rodents and humans (Hook et al., 2001), while resistance training increases the velocity of type IIa myosin molecule in both young and elderly previously untrained (Canepari et al., 2005).

Myosin can be directly or indirectly modified by reaction with oxidized carbohydrates and lipids (Ramamurthy et al., 2001; Ramamurthy et al., 1999; Thompson et al., 2006; Thorpe and Baynes, 2003).

Oxidative stress is a condition in which the production of ROS is exceeding the antioxidant enzyme production. The subsequent protein damage is speculated to affect the single fibre characteristics. The PTM-adducts generated tend to accumulate in ageing cells and act as amplifiers of oxidative damage in ageing and diabetes (Jakus, 2000; Li et al., 2007), which gradually contributes to inflammatory processes with accumulation of more ROS and AGE products (Ramasamy et al., 2005). The effect of the PTMs damage on cellular proteins is further enhanced by diminished capacity of cellular repair and regeneration in aged cells. The proteasome function, which is the main mechanism of selective degradation of oxidized proteins, was reported to decrease with age in both slow-twitch (Husom et al., 2004) and fast-twitch rat-skeletal muscle (Ferrington et al., 2005). Site-specific oxidative changes in the myosin molecule and changes in myosin:actin ratio (Prochniewicz and Thomas, 2001; Prochniewicz et al., 2005) are at least in part, responsible for changes in the contractile properties of the skeletal muscle fibre without a change in MyHC isotype.

In old age, numerous proteins undergo a number of post-translational modifications which may affect enzymatic activity, stability and digestibility (Mooradian and Wong, 1991). The slowing of contractile speed in old age has therefore been suggested to be related to a posttranslational modification of the motor protein myosin (Hook et al., 2001; Lowe et al., 2001a; Mooradian and Wong, 1991; Ramamurthy et al., 2001). The slow turnover rate of myosin and the additional decrease in turnover rate in old age makes myosin a potential target for posttranslational modifications (Balagopal et al., 1997). Skeletal muscle proteins are exposed to reactive oxidative species and biological ageing is associated with modifications of different muscle proteins, such as the accumulation of nitrotyrosine in the sarcoplasmic reticulum (Viner et al., 1996) and non-enzymatic glycosylation of myosin (Syrový and Hodný, 1992). Non-enzymatic glycosylation of myosin by a reducing sugar has been shown to have a negative effect on myosin function (Ramamurthy et al., 2001; Ramamurthy et al., 2003). Further, specific mutations in the regulatory \(\beta\)-tropomyosin regulatory protein has been shown to result in lower number of force generating cross-bridges (Ochala et al., 2007), resulting in muscle weakness in the absence of muscle wasting. Also, preliminary
results obtained during the present project indicate a preferential accumulation of nitrated β-tropomyosin with ageing in men (unpublished results).

Ageing-related loss-of-function of critical regulatory proteins are consistent with observed increases in intracellular Ca\(^{2+}\) levels within senescent cells, where the levels of free calcium have been found to increase two-fold in senescent animals. The altered Ca-transient in the sarcoplasmic reticulum was shown to occur differentially in slow and fast fibres in rat, contributing to altered contractile properties with ageing (Larsson and Salviati, 1989).

Myonuclear spatial organisation

Ageing is commonly associated with a smaller satellite cell population (Kadi et al., 2004a) and reduced satellite cell activation, indicating an impaired adaptability of myonuclear reorganization in old age (Kadi et al., 2004b). A relationship between myonuclear number and fibre size has been reported in trained young (Kadi et al., 1999) and old individuals (Hikida et al., 2000; Manta et al., 1987), but not in untrained elderly subjects (Hikida et al., 1998), reflecting an ageing- and/or atrophy-related deterioration of this relationship (Ohira et al., 1999). In humans, there are very few reports in the literature on the effects of ageing and gender on myonuclei number and organisation in different fibre types. Contradictory results demonstrating a constant (Vassilopoulos et al., 1977) or a decreased (Manta et al., 1987) amount of cytoplasm per nucleus in old age are reported.

The information on myonuclear organisation changes with age and gender in different fibre types in humans is extremely scarce. Changes in the cytoplasmic volume related to each myonucleus are related to physical condition, skeletal muscle fibre type and even location of the nucleus on the same fibre type.
Aims of the present investigation

1. To study the MyHC isoform expression and distribution in different age-, gender- and training groups (I, II, III)
2. To explore the mechanisms underlying the ageing-related slowing of $V_0$ and reduced force generating capacity at the single fibre level in different age-, gender- and training groups (I, II, III)
3. To examine the interaction of life-long sprint training and specific designed sprint and resistance training on the isometric and dynamic force production (II, III)
4. To quantify the gender- and fibre-type related variation during ageing in the spatial arrangement of the myonuclei (IV)

Materials and methods

Subjects (I, II, III, IV)

The effects of ageing and gender (I) were studied in 13 young (20-43 yr) and 22 old (65-85yr) sedentary, healthy men and women, with no history of dementia, metabolic disease, locomotor or neuromuscular disease. Smokers or those who had been treated with hormones during the last year were excluded from the study. The study was approved by the ethical committee at the Karolinska Institute and the Pennsylvania State University Institutional Review Board.

Sixteen young (18-33 yrs) and 75 master-aged (40-84 yrs) male sprinters, all members of different track and fields Finnish associations volunteered for the age study in sprinters (II). All the athletes had a long-term sprint training background and success in national and international championships in 100- to 400 m events. The young adult sprinters (personal records, 100 m: 10.97±0.07; 200 m: 21.92±0.19; 400 m: 49.54±0.84 s) were selected on the criterion that their age-adjusted sprint performance resembled that of the master athletes. The runners were matched for the relative performance level. The 60 m sprint times were 109±0.4%, 110±1.1%, 107±1.2%, 109±0.9%, and 109±1.6% of the indoor age-based world record times for 18-33-, 40-49-, 50-59-, 60-69-, and 70-79-yr-old runners, respectively.
The resistance training study (III) was part of a larger investigation on the performances of 72 40- to 84-year-old male sprint athletes participating in the baseline measurements and subsequently randomized into an experimental (EX, $N=40$) and a control (CTRL, $N=32$) group. A subset of twelve 52- to 78-year-old elite sprinters with no previous background in heavy strength training was chosen for the third study. At the end of the training period, the athletes were randomly assigned to an experimental group that participated at a 20-week combined sprint and progressive training (SPRT) program ($N=7$) or control ($N=4$) group, that continued with accustomed sprint-based training. Three participants at the first study did not meet the medical requirements and were not included in any further training study. All subjects were healthy as determined by reference to their detailed medical histories. Men over 55 yrs underwent further medical examination for cardiovascular diseases. The analyses were based on resting electrocardiograms and blood pressure measurements. They all gave a written consent and were fully informed about procedures, potential risks, and benefits associated with participation. The studies were approved by the Ethics Committee of the University of Jyväskylä and conformed to the Declaration of Helsinki.

The changes at the nuclear level with age in different gender and fibre types (IV) were studied in six young males (21-35 yrs), six old males (72-82 yrs), four very old males (89-96 yrs), six young women (24-32 yrs), six old women (65-83 yrs) and five very old women (89-96 yrs). All the subjects included were healthy sedentary that had no history of metabolic, locomotor or neuromuscular disease. The study was approved by the Ethic Committee of the Karolinska Institutet, Stockholm.

Sprint training program (II)

The participants were members of the Finnish track and field organizations and had a continuous long-term sprint training background and success in international or national championships in 100-400 m sprinting events. The young adult sprinters (II) were selected so that their age-adjusted sprint performance resembled that of the master athletes (see Methods, II).

Training characteristics and competition performances of the subjects were studied by means of a questionnaire and personal interview (Korhonen et al., 2003) (II, Tab. 2). A non-linear decrease in the training amount and intensity occurred with age from the younger groups to the 40-49 yrs group. The decrease was accompanied by a reduction in sprint training intensity, with a larger relative portion of training consisting of aerobic “warm-up” running.

Periodised training program (III)

The combined strength and sprint training program (SPRT) was designed by researchers and coaches in collaboration and utilized knowledge obtained
from earlier studies in young adult athletes (Delecluse, 1997; Joch, 1992; Kraemer and Häkkinen, 2002). The periodised training program was designed to reduce the potential for overtraining and to optimize the adaptation (III, Fig 1). The training program consisted of two 11- and 9-week periods that were further divided into three 3-4 week phases with variations in training intensity, volume and type.

The aim of the strength training was to increase maximal and explosive strength and promote muscle hypertrophy. The first four weeks of training involved low intensity and high volume strength endurance/hypertrophy exercises (3-4 sets x 8-12 repetitions at 50-70% of 1-RM) to prepare the muscles for more intensive training in the following phases. In the second and third phases, maximal strength (2-3 x 4-6 reps at 70-85%), and explosive-type weight lifting (2-3 x 4-6 reps at 35-60%) and plyometric exercises (2-3 x 3-10 reps) were undertaken and alternated within a week to allow recovery from different types of exercise stress. Plyometric training acts on both the musculotendinous and neurological levels to increase the power output, without necessarily increase the strength output. During the latter half of the training program, the 3-phase protocol was repeated with a slight progressive increase in training intensity aimed at inducing a further overload stimulus and to peak maximal and explosive strength at the end of the training period. Maximal strength and plyometric exercises had already been included in the first phase (weeks 12-14) in the second training period. Strength training was performed two times per week on non-consecutive days and each session lasted 50-90 minutes.

The strength training focused on the leg extensor and hamstring muscle groups and the main exercises that were performed at the beginning of the training sessions included leg press and/or half squat on machines, clean pull (from knee height) and/or stiff leg deadlift (Romanian lift) using free weights. The supplementary dynamic exercises (using whole range of motion) were hip extension, hip flexion, knee flexion, knee extension and ankle plantar flexion on machines. In addition, each training session included 2-4 exercises for the other main muscle groups of the body (trunk extension, trunk flexion, bench press, push press, and sprinting arm movements with and without hand weights). Plyometric exercises, utilized as a part of explosive strength training, progressed over the training period from low intensity vertical jumps to horizontal bounding exercises. These exercises were performed at the beginning of the speed training sessions.

The aim of the sprint training was to increase acceleration and maximum speed abilities in running. In general, it followed the athletes’ usual training regimen but the overall volume was decreased when strength exercises were incorporated into the program. The schedule for sprint training was similar in the first and second half of the training period. Sprint training was started with a combination of low intensity, high volume speed-endurance intervals (3-5 x 200-250 m at 75-85% of max speed) and acceleration practices from
the standing start position (4 x 30 m at 80%) to develop the requisite muscular and metabolic base for subsequent training. In the second and third phases, maximum speed exercises were added and intensified gradually up to almost competitive pace (2-3 x 30-80 m at 90-98%) while the total running distance covered was decreased. In addition, exercises for explosive starting and high acceleration from starting blocks (2-4 x 30 m at 90-98%) were included. Each sprint training session included drills to improve coordination and running technique. Speed training was performed two times weekly on non-consecutive days and the session duration was from 50 to 90 minutes.

Subjects completed training logs describing all their training parameters (number of repetitions, sets, loads, distances, times of exercises) to monitor progress and to provide motivation for maximal effort during the study. The logs were collected every 5th week during the field testing sessions. The overall training adherence rate in EX, calculated as the percentage of training sessions successfully completed, was 86±4% for strength training and 83±6% for sprint training across the 20-week study period. In EX, the average training hours and frequency over the training period were 2.1±0.2 h and 1.6±0.1 times per week for strength training and 2.1±0.3 h and 1.6±0.1 times per week for sprint training. Other exercises (ball games, aerobic running, skiing) were performed 0.5±0.2 h and 0.6±0.2 times per week. The controls maintained their previous run-based training schedules.

**In vivo measurements**

**Anthropometry and muscle architecture (II, III)**

Body height was measured with a height gauge and body mass with a balance beam scale. Total body fat percentage was assessed using bioelectrical impedance (Spectrum II; RJL Systems, Detroit; MI, U.S.A). Thigh length was measured with a ruler as the distance from the lateral condyle of the femur to the greater trochanter. Thigh circumference was measured at 50% thigh length using a tape. Muscle thickness and fascicle length were determined at the midregion of the vastus lateralis muscle (biopsy site) by a B-mode ultrasound instrument (SSD-1400, Aloka, Japan) as described elsewhere (Kubo et al., 2003). Briefly, during the ultrasound scanning procedure a 5 cm linear-array probe (7.5 MHz) was positioned perpendicular to the surface of the muscle and in the ultrasound images the subcutaneous adipose tissue layer, superior and inferior aponeurosis and a number of muscle fibre fascicles between aponeuroses were identified. Muscle thickness was determined as the distance from the adipose tissue-muscle interface to the intermuscular interface. The muscle fibre pennation angle was measured as the angle between the fibre fascicle and the deep aponeuroses. From the muscle thickness and fibre pennation angle, the fibre fascicle length across the deep
and superficial aponeuroses was estimated as: Fibre fascicle length = Muscle thickness x sin (fibre pennation angle)\(^1\).

**Muscle strength (I, II, III)**

*Isokinetic strength (I)*

Maximal voluntary dynamic knee extensor strength in healthy untrained young and old men and women was measured using an isokinetic micro-computer controlled dynamometer (KinCom H500, Chattanooga Corp. Chattanooga, Tenn., USA), immediately following the muscle biopsy. During the test the subject was sitting on a couch with 90 degrees flexion of hip and knee. The subjects sat on the couch of the isokinetic dynamometer with the right thigh fixed with straps, the lower leg fixed to the dynamometer’s lever arm, and the knee joint axis aligned with the rotational axis of the lever arm. The subjects were instructed to avoid rotation or movement in the upper body during the testing session. Further, a trigger torque of 50 Nm had to be overcome by the subject before the rotational movement of the lever arm was initiated, the initial acceleration rate was controlled prior to attaining the preset speed of movement, and torque was only measured in the part of the torque recording where the pre-set speed was constant (Gransberg and Knutsson, 1983).

Torque at maximum voluntary effort does not vary appreciably between repeated isokinetic movements since the torque is set by the upper limit of the voluntary strength. Therefore, the following procedure was used for each selected speed to ensure that torque recordings were accepted only when maximal voluntary effort seemed likely. At each preselected speed of movement, the torque-angle curve of a maximal voluntary contraction was superimposed on preceding records. When three records matched closely, they were accepted as maximum voluntary activations, and the average torque at each angular position was calculated. The thigh of the tested leg was strapped in a horizontal position and pelvis fixed and supported against tilt. The arms were kept crossed over the chest. The knee joint was aligned to the rotational axis of the dynamometer and the lower leg was attached to the lever arm just above the malleolus. Knee extensions were performed in concentric actions in a range of motion between 90 and 0 degrees at angular velocities of 30 and 180°/sec, during perpetual verbal encouragement. Between each contraction there was a pause of approximately 20 seconds. Recordings were accepted when three consecutive measurements at each movement velocity showed high reproducibility. The average torque was calculated between 75° and 50° of knee angle to avoid the acceleration and deceleration phases, and corrected for gravitation. Torque over the same knee angle range, i.e., muscle length range, was compared at the different speeds of movement (30 and 180°/sec). A 20 second rest followed each maximal contraction. The subjects had no prior experience with the isoki-
netic dynamometer and were familiarized with testing procedures performing three consecutive submaximal warm-up trials for each speed.

Unilateral isometric strength (III)
Unilateral isometric torque of the knee extensors and flexors of the resistance trained master sprinters was measured on the dominant leg by means of a David 200 dynamometer, as described by (Hakkinen et al., 1998b). The subject was in a sitting position with 90º knee and 110º hip angles and on verbal command exerted maximal force for a period of ~4.0 s. Three to four trials were recorded until there was no further improvement in peak torque. Concentric force of leg extensors was measured with one repetition maximum method (1 RM) using a half squat exercise in the Smith machine (Hakkinen et al., 2002). The test involved the subject bending the knees with a loaded bar on the shoulder to down 90º (controlled with auditory signal), maintaining the position for ~1 second, and then extending up on a command. The highest load lifted was determined as the subject’s 1 RM. Two subjects in the experimental group and one control subject declined to perform the squat test due to worry of injury.

Bilateral isometric strength (II)
Maximal bilateral isometric strength and force-time parameters of the knee extensor muscles were measured using an electromechanical dynamometer (Hakkinen et al., 1998b). In the test, the subjects were in a seated position with 107º knee and 110º hip angles (180º = full extension). On a verbal command, the subjects performed a maximal isometric leg extension as fast as possible over 2.5-4 s. Each testing session consisted of two practice contractions followed by three to four maximum-effort trials with 1-1.5 min rest periods.

The force signal was recorded on a computer (486 DX-100) and subsequently digitized and analyzed by a Codas computer system (Data Instruments). Maximal isometric force (F_{max}) was defined as the highest force value recorded during the contraction. The entire force-time curve was analyzed according to the guidelines of Viitasalo et al. (Viitasalo and Komi, 1978; Viitasalo et al., 1980). In the force-time curves, the times taken to increase force from contraction onset to the levels of 100, 250, 500, 750, 1000, 1500 and 2000 N (absolute scale) and the times needed to increase force from the start of contraction to 10-90% of F_{max} in 10% increments (relative scale) were calculated. The maximal rate of force development (RFD) was determined as the greatest increase in force in a given 50 ms time period. Normalised maximal rate of force development (normalised RFD), a measure of the slope of the force-time curve when normalised with respect to maximal force, was obtained by dividing the absolute RFD by the F_{max} for the subject (expressed as % of F_{max} per second) (Thelen et al., 1996). The
test-retest $r$ values of the two best efforts were within the range 0.96-0.99 for $F_{\text{max}}$ and 0.85-0.98 for RFD and the coefficient of variations were between 2.7-4.2% for $F_{\text{max}}$ and 4.9-9.0% for RFD in the different age groups.

**Dynamic explosive strength (II, III)**

Dynamic explosive strength was evaluated by means of a vertical counter-movement jump (Asmussen and Bonde-Petersen, 1974). The test was performed on a contact mat (Newtest, Oulu, Finland) connected to a digital timer ($\pm 0.001$ s) which recorded the flight time of the vertical jump. The height of rise of the body’s centre of gravity was calculated from the flight time (Bosco et al., 1983). During the jump the hands were kept on the hips to minimize differences in technique. After the practice jumps the subjects performed three to four maximal trials, separated by 1-1.5 min rest, and the highest jump with an acceptable technique was used for the analyses. The flight time of the two highest jumps showed $r$ and CV values within the range of 0.94-0.99 and 0.6-2.2% in the different age groups. Further, the explosive force in resistance trained sprinters (III) was assessed by jumping tests as follows. The squat jump required the subject perform vertical jump on the force platform from a static squat position of ~90° knee angle (Asmussen and Bonde-Petersen, 1974). Three to five trials were recorded and the highest vertical displacement value evaluated from the flight time was used in the analyses. For the triple jump test, carried out on a long jump place, the subjects began by standing on a plate (height 5 cm) with toes over the edge (Mero et al., 1981). Using arm swings at the start the subjects performed three jumps forward as far as possible with alternative left- and right-leg contacts and landed on two legs on the sand of the final jump. The reactive jump test was performed two to three times and involved a series of vertical jumps for ~5 s on a contact mat keeping the legs as extended as possible and to emphasize the use of ankle plantar flexors (Bret et al., 2002). The subjects were instructed to jump as high as possible while minimizing the contact times. The contact and flight times of each jump were measured to determine mechanical power per kg body weight (Bosco et al., 1983), and the mean of two best consecutive jumps were taken in the analysis.

**Sprint performance (II, III)**

A number of 86 subjects participated in the sprint and strength performance tests. Sprint performance was determined by standing-start 30 m (II) and 60 m (II, III) sprint trials performed on an indoor tartan running track. Times for the sprint tests were measured using double beam photocell gates connected to an electronic timer (starting line was 0.7 m behind the first photocell gates). The testing session was preceded by a ~30-45 min general warm-up such as the subjects were accustomed to (jogging, stretching) and submaximal practice runs to familiarize them with the procedures. The subjects performed two maximum-effort trials at both sprint distances with 5-7 min rest
between runs. During the sprint tests all subjects wore spiked track shoes. For the 60 m sprints the test-retest coefficient of reliability ($r$) varied from 0.93 to 0.98 and the coefficient of variation (CV) from 0.7 to 0.9% in the different age groups.

**Force production of running (III)**

Vertical and horizontal ground reaction forces, contact times and stride rate were measured during the maximal speed phase (30-40 m) using a 10-m long force platform (TR-testi, and Kistler: natural frequency ≥150 Hz, nonlinearity ≤ 1%, cross talk ≤2%). The force platform signals were sampled at 500 Hz and stored on microcomputer via an AT Codas A/D converter card (Dataq Instruments). The average 10-m running velocity over the force platform and the 60-m trial times were obtained by using double-beam photocell gates (starting line 0.7 m behind the first photocell gates). The average stride length was calculated by dividing the 10-m running velocity by the stride rate.

The ground reaction force variables were analysed by custom-built software. The transition point from negative to positive value in the horizontal force-time curve was used to divide the contact time and vertical and horizontal force components into the braking and propulsion phases (Mero and Komi, 1986). Average vertical and horizontal forces were integrated with respect to time phases and then combined to obtain the average resultant force for the braking and propulsive phases separately. The amplitudes of average resultant forces were normalised to body weight (N/N body weight). Rate of force development (RFD) for the braking and propulsive phases was calculated by dividing the average resultant forces by the respective contact times (Karamanidis and Arampatzis, 2005). The first four contacts of the fastest trial were averaged and used for the final analysis.

**EMG activity (III)**

EMG activity during isometric knee extension, dynamic squat 1-RM and squat jump tests was recorded from the agonist muscles of the vastus lateralis (VL) and vastus medialis (VM) and from the antagonist muscles of biceps femoris (BF; long head) of the dominant leg using the previously described procedures (Hakkinen et al., 1998b) with slight modifications. Briefly, to reduce skin impedance (< 5 kΩ), the skin area was shaved, rubbed with sandpaper and cleaned with alcohol based tissue pad. Bipolar surface electrodes (Beckman miniature skin electrodes; 2 cm interelectrode distance) were attached longitudinally to the belly of the muscles on the motor point areas determined using anatomical landmarks. The positions of the electrodes were marked with small ink dots to ensure the consistency in electrode placement in the pre and post measurements. The EMG signals were amplified (gain 200, low-pass cut-off frequency of 360 Hz/3 dB) and col-
lected together with force signals (at 1000 Hz) on a computer with the Codas TM A/D data acquisition system (Dataq Instruments). The nonsmoothed EMG was then full wave rectified, integrated (iEMG, in µV/s), averaged, and time-normalised for the peak force phase of the isometric knee extension (over a period of 1 s around the peak torque) and for the whole concentric phases of the dynamic half squat 1 RM and squat jump exercises. The iEMG was calculated for the VL and VM separately and then averaged for the final analyses. The iEMGs of the VL and VM of dynamic actions were also expressed as percentage of their maximum isometric activity level of knee extension. This normalization allows evaluation of neural adaptation without comparison of absolute EMG values. The EMG activity was also measured during isometric knee flexion for the BF, when it acted as an agonist. The iEMG of the BF from knee flexion exercise was analyzed as described for VL and VM in the isometric knee extension. To evaluate the degree of antagonist BF coactivation during isometric knee extension, squat 1-RM and squat jump exercises, the iEMG of the BF was expressed relative to that measured in maximal isometric knee flexion contraction. The BF iEMG data had to be excluded from two subjects in the experimental group due to distorted EMG signals.

In vitro methods

Muscle biopsy (I, II, III, IV)

The needle biopsy with suction from the dominant leg (I & III) and the percutaneous conchotome (II) methods were used under local anaesthesia to obtain biopsies from the middle portion of vastus lateralis muscle. Biopsies for the third study were taken both before and after a 20-week indoor combined sprint and progressive training period (SPRT).

Each biopsy typically contained muscle fibre segments of 200-800 muscle fibres and weighed 50-120 mg. The biopsies were placed in relax solution (as described in Single fibre measurements) at 4°C and dissected free of fat and connective tissue. From each biopsy 5-12 bundles of ~50-250 fibres were tied with surgical silk to glass capillary tubes at slightly stretched lengths (110%). Bundles were chemically skinned for 24 h in skinning solution containing 50% (v/v) glycerol at 4°C and were subsequently stored at -20°C for up to 3 weeks before use. Treatment with cryoprotectant (2.0 M sucrose) was used for long-term storage at -190°C. For this purpose, the bundles were detached from the capillary tubes and snap frozen with isopentane cooled by liquid nitrogen.

Skinning of single muscle fibres is the procedure that permeabilizes the surface membrane. Chemical skinning with glycerol is used to disrupt all the membrane structures of the mammalian muscle fibre, i.e., the membranes of
the sarcoplasmic reticulum, T-tubules and sarcolemma are rendered permeable while the contractile structures remain intact. The glycerol skinning disrupts the excitation-contraction coupling and allows the study of actin-myosin interaction without the influence of muscle architecture and the spatial orientation of the fibres, connective tissue, neural influences and sarcolemmal regulatory proteins. The specific role of Ca\(^{2+}\), Mg\(^{2+}\), ATP, EGTA, and other compounds in the regulation of muscle contraction is well defined and the buffering capacity of the various solutions can be rigorously controlled (Fabiato, 1988). Thus, controlled experimental conditions are created to obtain reliable data concerning unloaded shortening velocity and peak tension normalised to cross sectional area of skinned single muscle fibres.

**Single-fibre measurements (I, II, III)**

The experimental procedure was described in detail elsewhere (Moss, 1979). Fibres were bathed in a relaxing solution for 30 min prior to the experiment, leaving an average fibre segment length of 2.02±0.51 mm (mean ± SD, range 1.05-3.60 mm) exposed to the solution between connectors to a force transducer (Model 403, Cambridge Technology, Inc., U.S.A.) and a DC torque motor (Model 300H, Cambridge Technology, Inc.). The device was mounted on the stage of an inverted microscope (Zeiss Axiovert-35, Carl Zeiss, Oberkochen, Germany). When the fibre was in the relaxing solution, sarcomere length (SL) was set to 2.79±0.01 \(\mu\)m (range 2.72-2.84 \(\mu\)m) (with slightly differences between the studies) by adjusting the overall segment length. The segments were observed at a magnification of 320×. Prints of the fibre segments were taken with a videoprinter (P71E, Mitsubishi Electric Corp., Japan). Sarcomere length, segment width and the length of the segment between the connectors were measured directly from the microscope via a TV-overlay with the aid of a digitizer connected to a microcomputer (Videoplan, Kontron Bildanalyses GmbH, Munich, Germany). The final magnification with the image analysis system on the TV screen was 1.480×. Fibre SL was measured routinely in the fibres during maximal activation. Fibre depth was measured by recording the vertical displacement of the microscope nosepiece while focusing on the top and bottom surfaces of the fibre. Cross-sectional fibre area (CSA) was calculated from the width and depth, assuming an elliptical circumference. Every width and depth value represented the average of three different measurements.

The accuracy of the measurements done by the same observer was verified by comparing 200 average values for depth calculated at two different sarcomere lengths (\(r^2=0.99\)) (III). Specific tension was calculated as maximum tension (\(P_o\)) normalised to CSA, and was corrected for the 20% swelling that is known to occur during skinning (Moss, 1979). Relaxing and activating solutions contained (in mM): 4 MgATP, 1 free Mg\(^{2+}\), 20 imidazole, 7 EGTA, 14.5 creatine phosphate and sufficient KCl to adjust the ionic strength to
The pH was adjusted to 7.0. The free Ca\(^{2+}\) concentrations were 10\(^{-9}\) M (relaxing solution) and 10\(^{-4.5}\) M (maximum activation solution) and are expressed as pCa (-log[Ca\(^{2+}\)]). Apparent stability constants for Ca\(^{2+}\)-EGTA were corrected for temperature and ionic strength (Fabiato, 1988). The computer program of Fabiato (Fabiato, 1988) was used to calculate the concentrations of each metal, ligand and metal-ligand complex. Immediately preceding each activation, the fibre was immersed for 10-20 s in a solution with a reduced Ca\(^{2+}\)-EGTA buffering capacity (Moisescu and Thieleczek, 1978). This solution was identical to the relaxing solution except that EGTA was reduced to 0.5 mM, which resulted in more rapid attainment of steady tension during subsequent activation and helped to preserve the regularity of cross-striations during activation.

The maximum velocity of unloaded shortening (\(V_0\)) was measured by the slack-test procedure (Edman, 1979) at 15°C. When a steady tension was reached in the activation solution (pCa 4.5) slacks (\(\Delta L\)) of amplitudes between 8.5-14.0% of the fibre length exposed to the activation solution were rapidly (1-2 ms) applied. The time (\(\Delta t\)) required to take up the imposed slack was measured from the onset of the length step to the beginning of tension redevelopment. The fibre was re-extended after each slack while relaxed in order to minimize non-uniformity of sarcomeres. \(V_0\) was calculated as the slope for a straight regression line, using at least four measurements. Maximum active tension (\(P_0\)) was calculated as the difference between the total tension in the activating solution (pCa 4.5) and the resting tension measured in the same segment while in the relaxing solution. Fibres were excluded from analyses if \(r\) for the fitted line was less than 0.97, if \(P_0\) changed more than 10% from first to final activation, or if SL during isometric tension development changed by more than 0.10 \(\mu m\) compared with SL when the fibre was relaxed, according to established acceptance criteria (Moss, 1979).

**Single fibre fluorescent staining (IV)**

Eight to 10 skinned single fibre segments from each individual biopsy were dissected free from the muscle bundle. Each fibre was attached to the connectors of an experimental device, leaving a length of approximately 1200 \(\mu m\) skinned single fibre segment exposed to the media in an incubation chamber. The chamber was created on a borosilicate cover glass 24x50 mm (VWR International) with the help of a hydrophobic marker (Daido Sangyo Co. Ltd. Japan). The chamber so created was filled with 50 \(\mu l\) relaxing solution. Every fibre was attached parallel to the floor of the incubation chamber between two connectors manipulated by micrometrical devices. During every step of fibre preparation, care was taken to avoid any contact between the fibres and the underlying glass slip, in order to preserve the initial arrangement and number of the myonuclei. The fibres were incubated for 10
min in 0.1% Triton X-100 (Sigma Aldrich Chemie Gmbh, Germany) in relax solution. After a brief wash in relax solution, the fibres were incubated for 45 min in rhodamine phalloidin (Invitrogen, Molecular probes, Oregon, USA) (1:200) in relax solution containing protease inhibitor (1:1000). The fibres were again washed 3x5 min in relax solution before incubating for 5 min with DAPI (1:1000 in relax solution), followed by a final wash for 5 min in relax solution before data acquisition. All incubation steps and fibre measurements were performed at room temperature. At the end of the experiment, the fibres were placed in SDS sample buffer for MyHC type analysis.

Confocal microscopy and digital image analysis of myonuclear organisation (IV)
The experimental device containing the fibre to be analyzed was mounted on the stage of a Zeiss confocal microscope (Zeiss 510 Meta, Zeiss Gmbh, Germany). The sarcomere length was set to 2.74 ± 0.11 μm by adjusting the overall fibre length, by using the pattern created by the rhodamine phalloidin staining. The length of each fibre segment captured was 450 μm. Tridimensional stacks were created by collecting consecutive XY images in the Z-direction, from the top to the bottom of the fibre. The optical slice was set to 4.3 μm. For detection of nuclei by DAPI, laser line 405 nm was used with HFT 404/514 and BP filter 420-480 nm. The phalloidin-rhodamine labelled actin was detected by laser line 543 nm, HFT 488/543 and LP 550 nm emission filter (Fig. 3).

Figure 3. Changes in myonuclear organisation with age in single fibre segments expressing different MyHC isoforms in men and women. (A) Type I fibre of a young woman, 32 yrs. old; (B) Type IIa fibre of a young man, 31 yrs. old; (C) Type I fibre of an old woman, 78 yrs. old; (D) Type IIax fibre of an old woman, 67 yrs. old; (E) Type I fibre of an old woman, 90 yrs. old. The bar length denotes 50 μm.
The data was collected at a depth of 8 bit and 3-D images were reconstructed using the Zeiss software. The final proportions for the reconstructed images were set to 0.90x0.45x0.45 in order to standardize the subsequent data analysis. The spatial distribution of the nuclei and the spatial coordinates of each nucleus centre were determined manually by using Imaris (ver. 5.7.2) graphical analyzing software and considering each fibre according to a general elliptical model (GEC) (Fig. 4A). The cytoplasmic volume and size of the myonuclear domains corresponding to each myonucleus (Fig. 4B) were determined by using custom made software, developed at the Centre for Digital Data Analysis, Uppsala.

The GEC fibre model is constructed in a multi-step approach so that any excess anisotropic signal from the fibre can be compensated for. First, the dimensions of the fibre volume are arranged so that the y-axis is oriented along the length of the fibre, i.e., browsing through the fibre cross-sections follows the y-axis. Secondly, the image elements in the volume (voxels) are merged into cubic voxels of dimension 0.9×0.9×0.9 μm³, by averaging together four adjacent voxels, and their intensities, for each xy-plane. A linear model of a general fibre is applied to the image data. The modelling represents a segmentation of the fibre from the background, and allow for parametric representation of the fibre surface. The modelling is done by first creating a weight volume G to weight the original image intensities, before the parameters representing a fibre model are extracted from the weighted fibre volume. The parameters needed to represent the GEC is the centre points at each end of the fibre, the lengths of major and minor axis for an ellipse at each end of the fibre, and the angle between the major axes and the x-axis at each end of the fibre.

In the first step, the fibre volume is traversed slice-wise along the y-axis, and a fuzzy c-means (FCM) (Bezdek, 1981) thresholding is applied to every xz-slice individually. FCM classifies all grey level pixels, in every slice individually, into two clusters representing foreground (fibre), and background. The two clusters are then separated by a threshold in-between the two clusters. The pixels in the current xz-slice, with grey levels above the threshold are considered to contain information about the stained muscle fibre, and the excess anisotropic signal. The main reason why FCM is applied slice-wise along the y-axis is twofold. First, the FCM method always requires an input signal, which is the case along the fibre. Secondly, the signal variation for a subset of adjacent slices low, even though a single slice may contain low signal intensity. Thus, the FCM output should be similar, providing robustness to the model. Unfortunately the foreground cluster will also include pixels that are not part of the fibre, but are part of the signal artefact. For each segmented fibre slice region a centre point, major axis length, minor axis length, and orientation of the major axes (in relation to the x-axis), are calculated. The z-positions of the centre points are likely to be overestimated, with regard to the signal artefacts along the z-axis. The five
arrays (of length y) with the centre points c (coordinates in three dimensions), the major (a) and minor (b) axes (lengths), and the orientation ϑ (angle) are each fitted with a line in the least square sense. The five linear parameter arrays describe a linear model of the fibre, and the artefact. An ellipse centred on the centre point c is defined by,

\[
\frac{(x-c_x)^2}{a^2} + \frac{(z-c_z)^2}{b^2} = 1,
\]

where a rotation of ϑ degrees around the centre of the ellipse is a separate operation. To counter the effect of the artefacts in the z-direction, the initial linear model is used to create a normally distributed weight volume G of the interior, and exterior of the fibre. The volume G is then used to weight the grey levels of the original fibre volume, with intention to lower the effect of the artefacts in the z-direction. To accomplish that, G is shifted down, in the z direction, by δz voxels. A single slice \( G_y \) of the model G, before rotation is defined as,

\[
G_y(x, y) = \exp \left[ -\frac{1}{2} \left( \frac{(x-c_{xy})^2}{a_y^2} + \frac{(z-c_{yz} - \delta z)^2}{b_y^2} \right) \right],
\]

where (x, z) are the pixel coordinates in slice y, c_{xy} and c_{yz} are the x, and z coordinates of the centre points of \( G_y \), a_y is the length of the major axis in slice y, and by is the length of the minor axis in slice y. Every \( G_y \) slice is then rotated according to the angle given by first degree polynomial approximated orientation of the major axes, in relation to the x-axis.

In the second step, the grey levels (normalised to [0, 1]) in the original fibre volume are weighted with G, element by element. Finally, the FCM method is applied to the product. The pixels belonging to the fibre get a membership value closer to 1 (one), and pixels belonging to the background get a membership value closer to 0 (zero). The output from the FCM method is segmented into fibre and background by thresholding the slice at threshold in between the two clusters. From the pixels determined to belong to the fibre, the centre points, major and minor axes, and the orientation of the major axes in relation to the x-axis, are again calculated. The extracted values of the five parameters, for all slices, are again fitted with lines in a least squares sense. The final model, i.e., the GEC, is then generated from these parameter values. The GEC is stored as a binary volume with the same size as the down-sampled original fibre volume, where voxels with the value 1 (one) belongs to fibre, and voxels with the value 0 (zero) belongs to the
background. The GEC is then used together with the predetermined nuclei positions to calculate high level features.

A myonuclear domain is defined as all voxels within the fibre that are closest to an individual nucleus. For every nucleus a myonuclear domain is calculated (Fig. 3) by starting at the centre points of each nucleus, and calculating the distance to the surrounding voxels using a distance transform (DT) with an Euclidean metric (Friedman et al., 1997).

![Figure 4](image)

*Figure 4. Fibre model of fibre and myonuclear domains. (A) A GEC model of a fibre segment. The centroids of the myonuclei (blue spheres) merged with the surface rendering. (B) The myonuclear domains (MND) extracted.*

The value in each voxel in the DT-volume contains the distance to the closest nucleus. The DT-volume is then segmented using a watershed algorithm (Vincent and Soille, 1991) that partitions the DT-volume into labelled regions, i.e., the myonuclear domains.

The volume containing the myonuclear domains (MND) is made up of voxels with integer values (labels) denoting which nuclei a particular voxel has been assigned. The background outside the fibre is assigned the label 0 (zero). The myonuclear domains within the MND that share one, or more, sides with the borders of the image volume are identified and their labels are stored separately. The individual volumes of the myonuclear domains are calculated by counting all voxels for each label, except for the background, and disregarding the labels indicating that the domain is in contact with the image volume. The resulting array elements are multiplied with the volume of a voxel (0.9×0.9×0.9 µm³) before interpretation. The myonuclear domains that touch the border of the image volume are not counted, since they most likely are overestimated due to the lack of knowledge of the positions of nuclei outside the image volume.

The contact area between an individual myonuclear domain (MND) and the surface of the fibre is calculated by using weighted surface elements (Lindblad, 2005) to avoid overestimation of the surface measurement. The algorithm measures the surface areas shared between the fibre and the myonuclear domains (Axelsson and Svensson, 2008). Again, domains that are in contact with the border of the image volume are disregarded, and the measured surfaces are multiplied with the voxel side area 0.9×0.9µm².
The cross-section of the GEC varies along the length of the fibre. An average cross-section for each image volume is calculated by dividing the volume of the GEC with the length of the fibre volume along the y axis. The measured average cross-sectional area is multiplied with the voxel side area 0.9×0.9 μm².

The set of distances from every nucleus in an image volume to its nearest neighbour is calculated using the manually extracted coordinates (in 3D) from the nuclei volume image. The resulting array contains the distance values to nearest neighbours and the measured values are multiplied with the voxel side length 0.9 μm.

**SDS-PAGE (I; II, III, IV)**

The MyHC composition was determined after the mechanical measurements (I, II & III) by 6% SDS_PAGE. The total acrylamide and bis concentrations were 4% (w/v) in the stacking gel and 6% in the running gel, and the gel matrix included 30% glycerol. The ammonium persulphate concentrations were 0.04% and 0.029% in the stacking and separation gels, respectively, and the gel solutions were degassed (<100 millitorr) for 15 min before casting. Polymerization was activated by adding TEMED to the stacking (0.1%) and separation gels (0.07%). Sample loads were kept small to improve the resolution of the MyHC bands and electrophoresis was performed at 120 V for 22-24 h with a Tris-glycine electrode buffer (pH 8.3) at 150 °C (GE 600 vertical slab gel unit, Hoefer Scientific Instruments, USA, for details see (Damiani et al., 1996).)

**Homogenate electrophoresis (II, III)**

The MyHC content of the biopsy samples (II, III) was determined by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) as described elsewhere (Andersen and Aagaard, 2000) with slight modifications. For the analysis, 10-15 cryosections (10 μm) from each biopsy were placed into 700 μL of a lysine buffer containing 10% (vol/vol) glycerol, 5% (vol/vol) 2-mercaptoethanol, 2.3% (mass/vol) SDS in 62.5mM Tris·HCl buffer (pH 6.8) and heated for 10 min at 60 °C. A small amount of the muscle extracts (3-10μL) was loaded into each lane of the SDS-PAGE gel system consisting of stacking gel with 3% acrylamide and separating gel with 6% acrylamide and 30% glycerol. The gels were run on an electrophoresis device (Bio-Rad Protean II xi Cell) at 4 °C at a constant voltage of 70 V for 42 h. Following the run, the gels were fixed for 24 h in 5% acetic acid and 50% methanol, stained using Coomassie blue, and destained in 7.5% acetic acid and 5% methanol overnight or until the background was clean. In the stained gels three distinct protein bands could be separated and identified as MyHC I, IIA, or IIX isoforms according to their migration characteristics.
The relative proportion of each MyHC isoform in a biopsy sample was determined using a densitometric system (Cream 1D, Kem-En-Tec aps, Copenhagen, Denmark). Examples of SDS-polyacrylamide gel and densitometric tracings illustrating separation of the MyHC isoforms in samples from a younger and an older subject are shown in Figure 1B. It has been shown previously that the slowest migration protein band in humans is analogous to the MyHC IIx, not the MyHC IIb isoform, in rats (Pereira Sant'Ana et al., 1997) and therefore the MyHC IIx nomenclature is used in this study.

**Myofibrillar ATPase histochemistry (II, III)**

Serial 10 µm thick transverse sections (II, III) were cut on a cryostat (Leica CM 3000) at -24 °C, mounted on glass slides and stained for myofibrillar ATPase after acid (pH 4.37, 4.60) and alkaline (pH 10.30) preincubations (Brooke and Kaiser, 1970). Six different fibre types (I, IC, IIC, IIA, IIAB, and IIB) were identified according to Staron et al. (Staron et al., 2000). However, because in each age group the type IC and IIC represented <1.0% and <0.5% of the fibre pool, respectively, they were not included in the analyses. Figure 1A and B (II) illustrate cross-sections from a young and an old muscle stained for myosin ATPase activity after alkaline pre-incubation, discriminating between slow and fast fibres.

The fibre area and relative proportion of the various fibre types were analysed from the stained cross-sections using a microscope combined with a computer-assisted image analysis system (Tema, Scanbeam, Hadsund, Denmark) (Sipila et al., 2004). Relative fibre type distribution was calculated from an average of 508±29 fibres in each biopsy sample. The measurements of fibre cross-sectional area comprised an average of 228±15 type I, 162±10 type IIA, 60±5 type IIAB, and 53±6 type IIB fibres.

Frozen samples from the vastus lateralis muscle (I) were cut perpendicular to the longitudinal axis of the muscle fibres into 10 µm cross-sections with a cryotome (2800 Frigocut E, Reichert-Jung GmbH, Heidelberg, Germany) at -200°C. The sections were stained for the demonstration of myofibrillar ATPase activity (EC 3.6.1.3) after alkaline (pH 10.3) and acid (pH 4.6 and 4.3) preincubations and classified into types I, IIA, and IIB (Dubowitz, 1985). Cytochrome c oxidase (COX) and succinate dehydrogenase (SDH) stainings were performed according to Seligman et al. (Dubowitz, 1985) and Dubowitz (Dubowitz, 1985).

**Statistical analysis**

Means and standard errors (SEM) of means and standard deviations (SD) were calculated from individual values by using standard procedures. Student’s unpaired t-test was when comparing two groups. When the normality test failed, the Wilcoxon or Mann-Whitney rank sum test was used to test for the differences in single fibre characteristics, MyHC, muscle performance.
and iEMG before and after training within the study groups. Linear and curvilinear regression analyses were performed to determine the association between age and the dependent variables. Pearson correlation coefficients were used to measure the association between MyHC I isoform content and relative type I fibre area as well as correlations of strength, sprint and jump performance with MyHC II content (II, III). Analysis of variance (ANOVA) was used to compare the age groups in terms of their physical characteristics, training and selected performance characteristics and properties of single muscle fibres (II) and pre- and post- isometric and dynamic force measurements (III), followed by post hoc tests used for the specific comparisons. Two-way analysis of variance (ANOVA) was performed to test for the effect of gender and age and followed by post-hoc procedures. When the normality test failed (III), a statistical procedure was used in which the inter-individual and between-group variances were considered. Differences were considered significant at P<0.05.
Results and comments

Age and physical characteristics (I, II, III, IV)
Height and body weight in healthy sedentary men and women (I) did not differ between young and old, but both the young and old men were heavier and taller (P<0.01) than the women. In the fourth study, the men were taller and heavier than women irrespective of age.

Body height and mass were lower in the older sprinter groups (II), while the percentage body fat did not differ with age. Thigh length did not change with age but the thigh circumference of vastus lateralis muscle decreased with age. The estimated fascicle length of the vastus lateralis muscle did not differ with age. The physical characteristics of the master sprinters participating at SPRT remained unaffected during the 20-week training period.

Comments: Body fat measurements in sedentary were not conducted in sedentary men and women. Available data indicate that the circumference of the vastus lateralis muscle may not change up to 65-years-of-age (Larsson et al., 1979). At the same time, the amount of fat free- and mean muscle mass decreases with age in sedentary men and women (Frontera et al., 1991), indicating an increased fat content.

In vivo measurements results

Muscle strength in healthy untrained men and women (I)
Maximal voluntary knee extensor (KE) strength values were 19-47% higher (P<0.05-0.01) in men than in women irrespective of age and speed of movement (I, Tab. 1). At a slow speed of movement (30°/s), average torque declined (P<0.001) more in men than in women (44 vs. 33%). At a faster speed of movement (180°/s), the decline in average torque was higher (P<0.001) in women compared to men (55 vs. 46%), resulting in an age- and gender-related difference (P<0.01) in the fast:slow torque ratio (I, Tab. 1).
Comments: The isokinetic movements were performed at a controlled acceleration and the movement started only when the force applied on the lever arm of the dynamometer was higher than a pre-set torque. This method reduces the artefacts in measuring the peak torque and gives an accurate index of dynamic muscle strength (Gransberg and Knutsson, 1983). Changes in MyHC distribution show large individual variances and cannot account for the observed decrease in isokinetic force, in accordance with other studies (Jubrias et al., 1997).

Muscle strength in sprint-trained young and old athletes (II)
The isometric and dynamic performance decreased with age in the master sprinter groups compared to young controls (II, Tab. 2).

The maximal bilateral isometric force ($F_{\text{max}}$) and vertical jump performance decreased linearly with age. The rate of decrease was 8.3%/decade in $F_{\text{max}}$ and 11.1%/decade in vertical jump height. The rate of lengthening in the 60 m sprint times with age was curvilinear, showing an accelerated rate around age 65-70.

The force-time curves on the absolute scale indicated an ageing-related lengthening ($P<0.001$) in the times needed to reach specific force levels up to 2000 N (II, Fig. 6A). The absolute maximal rate of force development (RFD decreased ($P<0.001$) by 9.7% per decade from the youngest to the oldest group. The time taken to reach RFD increased ($P<0.001$) from 54±4 ms (18-33 yr) to 104±12 ms (70+ yr) with advancing age.

When the force in the force-time curves was calculated as a percentage of the maximal force produced, there continued to be an ageing-related increase ($P<0.001$-0.05) in the time needed to reach 10-80% of $F_{\text{max}}$, with the significance of association being strongest at force levels between 10-50% of the maximum (II, Fig. 6B). Moreover, adjustment for $F_{\text{max}}$ also decreased the influence of age on the rate of force development (normalised RFD), although a small but significant decline ($P<0.05$) remained. In all age groups normalised RFD occurred early in the force-time curve, i.e., before 25% of maximal force was reached (range 21.1-24.9%).

Comments: The isometric force production is depending on the muscle mass and the number of force-producing cross-bridges and force produced per cross-bridge (Cooke, 1997). However, the decrease in specific force with age explain only a half of the decrease in isokinetic force production with age (Jubrias et al., 1997). It was early suggested that the decrease in muscle force may depend on other factors than the decrease in muscle mass (Vandervoort and McComas 1986). The fact that muscle fascicle length remained constant in the studied sprinters eliminates one of the possible explanations of reduced force production with ageing. Both young and old athletes trained dynamically, respecting the specificity of training and testing.
Muscle strength in resistance-trained old sprinters (III)

The maximum isometric and dynamic strength in master sprinters changed after the 20-week combined strength and sprint training period (SPRT). The maximal isometric torque during unilateral knee extension increased by 21% (P<0.05) and by 40% in knee flexion exercises (P<0.01) in EX (III, Fig. 2, A and B respectively). Maximum dynamic strength, evaluated by bilateral concentric 1-RM squat, increased by 27% (P<0.001, N=5). Significant changes were also observed in explosive and reactive strength, i.e., a 10% (P<0.01), 4% (P<0.01) and 29% (P<0.01) increases were observed in squat jump, triple jump, and the mechanical power of reactive jump test, respectively.

In CTRL, no significant changes were observed in any of these parameters.

Comments: The randomization of subjects for the SPRT study (III) was done after conducting the measurements, resulting in four controls and seven experimental subjects. Only twelve individuals were randomly selected for the present study, due to practical aspects posed by the time limitation. During two intensive days, a large number of measurements had to be done on elderly, and biopsies were prepared for histochemical as well as single fibre measurements. One subject from the group of twelve was excluded from the analysis due to lower compliance to the training protocol. The fact that two master athletes did not participated at the 1-RM measurements (III) could have influenced the power of the statistical test. Given these circumstances, it is worth noting that the SPRT programme caused a significant increase in the isometric and dynamic force of already trained athletes.

Force production during maximal running in resistance-trained old sprinters (III)

The progressive training programme induced changes in the force production during running in master sprinters. An 8% increase (P<0.05) was observed in the propulsive phase of the contact in EX (III, Fig. 3). The ground contact times of the braking and propulsive phases decreased by 9% (P<0.01) and 5% (P<0.05), respectively. As a consequence of these changes there was a significant increase in the rate of resultant force development in both the braking (12%, P<0.05) and propulsive (14%, P<0.05) phases. In addition, maximum 10-m running speed improved 4% (P<0.01) and 60-m sprint time improved 2% (P<0.01). Stride length of the maximum speed phase increased 3% (P<0.05), but no significant change occurred in stride frequency.

In CTRL, no significant changes were observed in either the ground reaction force characteristics (III, Fig. 3B) or in any of the other studied parameters of running. When comparing percentage changes over the training pe-
period, the propulsive phase RFD (P<0.05), maximum 10-m speed (P<0.05), stride length (P<0.05) and overall 60-m sprint time (P<0.01) differed between the two groups.

Comments: Running velocity is the product between stride length and stride rate. The stride length has a major importance in determining the running velocity (Mero and Komi, 1986). The decrease in running speed with age in both males and females runners is due mainly to a decrease in stride rate, while the stride rate decreases first after the age of 80 (Korhonen et al., 2003).

The increase in force production is sport specific. Higher percentage changes in rate of force production in EX indicate that the SPRT program increases the sport performance by increasing strength and force.

EMG activity in resistance-trained master athletes (III)

The absolute and normalised iEMG activity levels during isometric and dynamic knee extension were measured before and after the 20-week period in EX and CTRL (III, Fig. 4). In EX, a 9% increase (P<0.05) was observed in the iEMG of the VL and VM muscles in the squat jump. No significant changes were observed in the maximum iEMGs of the VL and VM in the concentric half squat 1-RM or during isometric knee extension. The maximum iEMGs of the BF during the isometric knee flexion remained unaltered. Nonsignificant increases were observed when the iEMGs of the VL and VM obtained in dynamic squat 1-RM and squat jump actions were normalised to their maximum isometric activity level of knee extension. In addition, no change was observed in the antagonist BF iEMG coactivity (relative to maximum agonist values of the BF) during isometric knee extension or squat jump exercises.

In CTRL, no significant alterations were observed in any of the muscle activity parameters. The percentage change of pre- to post-measurement in the squat jump iEMG differed (P<0.05) between the two groups.

Comments: The iEMG technique permits to register the activity of all neurons in the electrodes contact area. The larger neurons of the fast MUs are closer to the skin surface and may influence the outcome more than the deeper and smaller neurons innervating the slow fibres. At the same time, it can be mentioned that the measurements were done in training-accustomed, highly motivated athletes, which guarantees a maximal voluntary contraction. Therefore, when comparing the EX and CTRL groups, the higher percentage change in the EX represents an indirect indication that the neural system either had gained an improved ability of to recruit more MUs at the same time or had an increased firing frequency, or both.
In vitro measurements results

MyHC isoform content (II, III)

The MyHC isoform composition in male young and old sprinters revealed an ageing-related increase in the relative content of MyHC I (P<0.01) and a decrease in that of MyHC IIx (P<0.05), while no age difference was observed in MyHC IIa expression (II, Fig. 5). MyHC I isoform content was associated with the histochemically determined relative area of type I fibre (r=0.92, P<0.001).

The 20 week SPRT in old sprinters determined no change in the relative content of type I, IIa and IIx MyHC isoforms in either EX or CTRL (III, Tab. 2). The IIx MyHC isoform was not detected in pre- or post-biopsies in three subjects in EX and in one subject in CTRL.

Comments: The histochemically determined fibre area is highly associated to the fibres myosin content. The fact that no IIx MyHC was detected may be due to the absence or very low amounts of IIx MyHC in trained subjects. The IIx MyHC expression occurs primarily in hybrid fibres together with the IIa MyHC. The Coomassie staining technique used for the analysis gives a higher relative error percentage when the staining is weak. It was suggested that IIx is the “default” gene during inactivity (Goldspink, 1998).

Relation between dynamic force production and MyHC isoform expression (II)

The dynamic performance of the young and old sprinters, as shown by vertical jump height and the 60 m running times measurements, correlated with MyHC II content for the overall sample. The absolute maximal RFD was associated with the total relative MyHC II (IIa+IIx) isoform content, when both young and old subjects were considered (r=0.28, P<0.01). The times taken to reach the specific force levels between 100 N and 1500 N were also related to MyHC II content (r=-0.41- -0.31, P<0.001-0.01). Adjusting for age, the times to the force levels up to 1000 N remained correlated with MyHC II (r= -0.35- -0.32, P<0.01), whereas the effect of MyHC II on absolute RFD was no longer statistically significant. Furthermore, the correlation coefficients between F_{max} and MyHC II did not reach the level of statistical significance whether unadjusted or adjusted for age.

The association of normalised force production with MyHC II content was also evaluated, in order to take into account differences in the strength levels of the athletes. For the overall sample, a relationship between normalised RFD and MyHC II content was found (r=0.27, P<0.05). In addition, the times taken to reach the force levels of 10-70% of F_{max} were associated with
MyHC II, the correlation being progressively weaker with increasing force levels (10-40%, $r=-0.41$ - $-0.38$, $P<0.001$; 50%, $r=-0.33$, $P<0.01$; 60-70%, $r=-0.28$ - $-0.23$, $P<0.05$). After controlling for age, normalised RFD ($r=0.23$, $P<0.05$) and the times taken to reach 10-50% of $F_{\text{max}}$ ($r=-0.35$ - $-0.26$, $P<0.01$-0.05) continued to show a significant correlation with MyHC II content.

Comments: The MyHC isoform composition was performed in muscle homogenates. The increased proportion of slow type I My is in line with results observed in normally active (Klitgaard et al., 1990; Short et al., 2005) and endurance trained (Klitgaard et al., 1990) subjects. Higher type I MyHC content is associated with increased relative area of type I fibre (Klitgaard et al., 1990), which is also confirmed in the present study. The slow motor units, constituted by type I fibres, are recruited before the faster motor units (Henneman and Mendell, 1981b). Thus, changes in then amount and properties of type I fibres may influence the dynamic force output.

Histochemically determined fibre cross-sectional area and fibre type distribution in male sprinters (II, III)

There was a decrease ($P<0.001$) of type II fibre area with age in old sprinters compared to young sprinter groups (II, Fig. 2B-D), while the area of type I fibres remained unaffected. Consequently, there was a decline in type IIA/I (6.7%/decade), IIAB/I (7.5%/decade) and IIB/I (11.3%/decade) (II, Fig. 3A). Within the age groups, type I fibres were smaller than type IIA and/or IIAB fibres in the three youngest age groups, but were larger than type IIAB and IIB in the two oldest groups (II, Fig. 3B). Among the fast fibre population, the area of type IIA fibre was larger ($P<0.001$-0.01) than that of type IIAB and type IIB in all age groups, while a difference between type IIAB and type IIB was observed in the 40- to 49-yr ($P<0.01$) and 50- to 59-yr ($P<0.05$) groups (data not shown).

The relative fibre type distribution of the histochemically determined fibre types in young and old sprinters did not differ with age, but the relative area occupied by the type I fibre increased with ageing (II, Fig. 4). Within the fast fibre subtypes no age differences in relative fibre type area were observed, although there was a trend towards a decrease ($P=0.053$) in the area of type IIB fibres with age. Among fast fibres, type IIA fibres occupied the largest area ($P<0.001$) in all age groups, whereas no differences were seen in the relative areas of types IIAB and IIB.

The relative fibre type distribution in older sprinters (III) remained unaltered after the resistance training period in EX, but the mean of histochemically determined CSA of muscle fibres in EX increased 19.8% compared to CTRL ($P<0.05$) (III, Tab. 2). In enzyme-histochemically identified fibre
types, a significant change was only observed in type II and type IIA fibres, demonstrating an increase of 17 and 20%, respectively (P<0.05). Larger cross-sectional areas were also observed in type I (19%), IIB (17%) and type IIB (33%) fibres, but these changes were not statistically significant (P=0.1-0.3).

In CTRL, no changes were observed in either muscle fibre type distribution or cross-sectional areas (P=0.3-0.6). The percentage changes over the experimental period in the cross-sectional area of type II and type IIA fibres were significantly (P<0.05) different between EX and CTRL.

Comments: The type I fibre type distribution has been observed to increase with age in untrained healthy men in cross-sectional studies (Larsson et al., 1978) while others observed no change (for ref. see (Frontera et al., 2000a). The differences may be due to different activity background of the subjects, the selection bias, coexpression of different MyHC isoforms and misclassifying of fibres through histochemical methods or to the bias observed between muscle biopsies (Frontera et al., 2000a).

Single muscle fibre contractile properties in young, old and oldest groups (I, II, III)

Accepted fibres (I, II, III)

A gender- and ageing-related trend was observed in the relative number of fibres discarded from the analyses (I), i.e., a lower number of fibres met the criteria for acceptance in the old men compared with young men and old women (I, Tab. 2). The number of discarded fibres due to sarcomere length non-uniformity and break during the maximum activations was higher in old men, but not in old women. The type II fibres were overrepresented among the discarded fibres in men, but not in women. Further, many of the discarded fibres expressing fast MyHC isoforms in the old men showed a "wrinkled" sarcolemma (I, Fig. 3).

The number of successful single fibre experiments was not different between the young and old sprinters, neither between master sprinters before and after the 20-weeks SPRT period.

Comments: The acceptance criteria introduced almost three decades ago (Moss, 1979) were used in all single fibre analysis, so diminishing the risk for taking into account erroneous results. In general, physical activity was shown to preserve the quality of the single fibres, reducing the occurrence of by example Z-line streaming, loss of thick filament and loss in areas showing mitochondrial abnormalities that may occur after unaccustomed exercise.
A gender and age-related difference in type I fibre quality of men was hypothesized to depend on intrinsic defects in single fibres (Frontera et al., 2000b).

**Muscle fibre size and force (I, II, III, IV)**

The CSA of fast muscle fibres in healthy sedentary individuals was larger (P<0.01) in slow muscle fibres than in fast fibres in the young men, but smaller (P<0.05) in the young women (I). In old age, there was no significant difference in fibre size between fast- and slow-twitch muscle fibres, irrespective gender. Within each gender, type I muscle fibre CSA did not differ between young and old individuals, but muscle cells expressing the type I MyHC isoform were larger in the men (I, Tab. 3). The ageing-related decline in muscle fibre CSA was gender specific, being primarily confined to muscle fibres expressing the Ila MyHC isoform in the men (I, Tab. 3). The P0 of type IIA fibres decreased with age in both men and women, with men having stronger fibres (P<0.001) than women in both young and old age (P<0.01). An increase (P<0.01) in the type I fibre size and a decrease (P<0.05) in the type Ia fibre size with age was observed in both genders in the fourth study.

The sprint-trained master-athletes (II) presented lower CSA than young controls, in both type I (21%, P<0.05%) and Ila (37%, n.s.) fibre types. The decrease was accompanied by lower P0 in both fibre types.

Single fibre atrophy was reversed by a 20-week SPRT period in master sprinters (III), resulting in a 25% increase in the CSA of all fibres (n.s., p=0.075), with a 40% increase (P<0.05) in CSA in type Ila fibres EX. The 22% larger CSA in type I MyHC fibres at the end of the 20-week training period was not significant. In CTRL, average CSA was unchanged.

The increase in CSA was accompanied by an increase in maximum force by 27% in type I and 58% in type IIA fibre types (n.s.). No changes could be observed in type II/I fibre CSA ratio either in EX (0.87±0.05 vs. 0.88±0.06) or CTRL (1.09±0.18 vs. 1.09±0.16) (III, Tab. 2).

The CSA of single fibre segments studied in the fourth project showed an increase (P<0.01) in old men and old women, while a significant decrease (P<0.05) in type Ila muscle fibre CSA was observed in the old men and old women. This resulted in a significant (P<0.001) decrease in the type II/I fibre area ratio in both the old men (from 1.09±0.12 to 0.77±0.08) and old women (from 1.25±0.12 to 0.75±0.11).

Comments: Single fibre CSA measurements were performed at a fixed sarcomere length. This is advantageous compared with analyses of muscle biopsy cross-sections where sarcomere length can be highly variable, influencing muscle fibre CSA in an unpredictable way (Larsson, 1990; Larsson et al., 1978; Larsson and Skogsberg, 1988). The major disadvantage with sin-
Single muscle fibre CSA measurements is the small number of analysed fibres compared with morphometrical measurements of muscle cross-sections. Muscle fibre size was measured in all fibres assuming an elliptical cross-sectional area. The force related to CSA was corrected for the 20% swelling known to occur in skinned muscle fibres.

At the single fibre level, the CSA area of type I and II fibres vary in a non-random manner within the vastus lateralis muscle of young (Lexell and Taylor, 1989) but not old men (Lexell and Taylor, 1991). Actually, the standard deviation (SD) of the histochemically determined CSA of type I and IIA muscle fibres increases with age and is higher for type II than for type I muscle fibres (Lexell and Taylor, 1991). A larger number of fibres, not fulfilling the criteria for acceptance during contractile measurements, were observed in old age (I).

**Single muscle fibre contractile velocity (I, II, III)**

The $V_0$ in untrained men and women (I), young and old sprinters (II) and resistance trained master sprinters (III) was dependent on the MyHC expressed and increased in the order: $\beta$/slow (type I) $\rightarrow$ I/Ila $\rightarrow$ I/IIa $\rightarrow$ IIx MyHCs. In old age (I), $V_0$ decreased (P<0.05) in muscle cells expressing the type I and Ila MyHC isoforms in both men and women. A gender-related difference was observed in muscle cells expressing the type I MyHC isoform, i.e., $V_0$ was higher (P<0.05) in men than in women irrespective age (I, Tab. 4).

An ageing-related difference in contractile speed was observed in muscle cells expressing the type I MyHC isoform in sprinters (II), i.e., $V_0$ was 24% slower (P<0.05) in the muscle fibres from the older subjects. The $V_0$ values of muscle fibres expressing the Ila MyHC isoform were not significantly different between younger and older subjects (II, Tab. 3).

The 20-week combined sprint and resistance training period did not affect the $V_0$ in master sprinters from the EX and CTRL groups, in either type I or type Ila muscle fibre segments (III, Tab. 1B).

Comments: The criteria originally introduced by Moss (Larsson et al., 1997b; Larsson and Moss, 1993; Moss, 1979) for determining which contractile measurements should be included in analyses were adopted in all studies (see Materials and Methods). All muscle fibres that did not meet the acceptance criteria were discarded from analyses. Inclusion of fibres with high end compliance or sarcomere length non-uniformity would otherwise have resulted in erroneously high $V_0$ values. Single muscle fibres $V_0$ remains unchanged by the skinning procedure (Eddinger et al., 1985) and is similar in adjacent segments of the skinned fibre segments (Larsson et al., 1997a).
Single muscle fibre specific tension (I, II, III)

A decrease in ST was observed in type I fibres in both men and women (I, Tab.4). A decrease of similar magnitude could be observed in type IIa fibres on both men and women, but the differences were not significant (P=0.07). A gender-related difference in specific tension was observed in muscle fibres expressing the type I and IIa MyHC isoform, i.e., higher (P<0.05-0.01) specific tensions were observed in the women irrespective age.

There was a tendency towards increased ST in both fibre types with age in sprint trained athletes, but the difference was not significant (II, Tab. 3).

The specific tension in resistance trained master athletes (EX) did not change in single muscle fibres expressing type I and IIa MyHCs. The ST values in the control group were similar to the values observed in EX for both type I and type IIa fibres (III, Tab. III).

Comments: Muscle fibre force $P_0$ at maximum thin filament activation ($pCa$ 4.5) is proportional to the muscle fibre CSA. Therefore $P_0$ is normalised to fibre CSA of individual fibres, i.e. specific tension ST.

Oxidative stress and ageing

Oxidative stress (I)

There was an ageing-related increase in the number of muscle fibres affected by oxidative damage, in both men and women (P<0.05) (I, Fig. 4). The COX$^-$ fibres were observed in all fibre types and there was no significant overrepresentation of COX$^-$ in any specific fibre type identified by mATPase stainings in either men or women.

Comments: The release of $H_2O_2$ in skeletal muscle increases with age (Capel et al., 2005). Faster ageing was predicted in more active cells due to increased ROS production with increased activity, but research shows paradoxically better longevity for more active cells, due to mild mitochondrial uncoupling (Amara et al., 2007). It may be expected that the type II cells would be more affected by oxidative damage. In vivo results in human muscle show that mitochondrial dysfunction may cause the preferential loss of the type II muscle fibres (Conley et al., 2007). Data available also suggest that the different fibre types are specifically affected by oxidative damage, contributing to the preferential atrophy of type II fibres with age and fibre type specific modification of contractile properties with age. The type II
muscle fibres seem to generate a larger amount of H$_2$O$_2$ (Anderson and Neufer, 2006), but this has not always being confirmed (Leary et al., 2003).

Myonuclear spatial arrangement (IV)

The myonuclei were either round or elliptical, independent on age, gender or fibre type (IV, Fig. 1). Aggregated nuclei were observed in elderly irrespective gender and fibre type, but not the young (IV, Fig. 2).

Comments: A number of 25 fibres out of 233 were discarded from the analysis, due to suspected damages of the myonuclei or inconsistent fluorescent signal. A strict fibre type dependent myonuclear shape was reported in mouse muscle fibres, with myonuclei from fast-twitch EDL fibres having elliptical nuclei aligned to the longitudinal axis of the fibre and slow-twitch soleus fibres having more rounded nuclei (Bruusgaard et al., 2003). An altered nuclear morphology and aggregation of nuclei into long chains were observed in both old men and women (Brack et al., 2005) and old mice (Bruusgaard et al., 2006). The reorganization of nuclei in ageing human skeletal muscle supports the idea that the positioning of myonuclei is plastic in adult muscle (Ralston et al., 2006), but the exact mechanisms are unknown. Microtubuli, the intermediate filament desmin, as well as blood vessels have all been shown to be involved in the localization and/or anchoring of nuclei in muscle fibres (Bruusgaard et al., 2006; Ralston et al., 2006). Aggregation of myonuclei has been reported in response to long-term denervation (Viguie et al., 1997) and the ongoing denervation-reinnervation process in ageing skeletal muscle (Larsson and Ansved, 1995) may accordingly play an important role for the aggregation of myonuclei observed in the old men and women in this study.

Myonuclear domain size and nearest neighbour values (IV)

The number of nuclei per mm fibre length increased significantly (P<0.001) in old age in type I fibres, while no significant difference was observed in muscle fibres expressing the IIa MyHC isoform.

In type I muscle fibres, MND size was not affected by ageing. A significant (P<0.05) age and gender interaction was observed in MND size of type IIa fibres in men and women, due to a larger decrease in women (IV, Tab. 2). The fibre size was a better predictor of MND size in men than in women (r$^2$=0.25-0.69; P<0.05-0.001). An increased occurrence of small and very large MNDs was observed in old age, irrespective gender and fibre type (IV, Fig. 6).
The higher variability in the MND size was related to the myonuclear spatial organisation, or nearest neighbour distances (NN). The NN-changes normalised to fibre size (NN/CSA) were lower (P<0.001) in type I fibres in elderly men and women, and similar changes were observed in the type IIa fibres (IV, Fig. 7). The NN/CSA value was higher (P<0.05) in young women than in young men.

Comments: The volume of each fibre was calculated according to a general elliptical model (GEC) and differed by 1-3% from the volume calculated manually by measuring the width, depth and length of the fibre. During the technical procedures, care was taken to avoid fibre contact with the incubation chamber, in order to preserve the myonuclei organisation. The myonuclei closest to the ends of the fibre segment were omitted from the analyses since these MNDs may reach outside the fibre segment and give rise to erroneously small MND sizes. The NN-value represents the mean of the distances between the myonuclei in three dimensions. In our model, the NN-value is a multifactorial unit and it depends on the myonuclear number, myonuclear distribution in the fibre and fibre size.
Discussion

The effects of ageing and gender on muscle performance

The average isokinetic torque at the knee extensor muscle level decreased by 33-55% with age in both men and women irrespective speed of movement, but a larger average torque decrease was observed in women at higher speeds of movement. Men were stronger than women at both slow and faster speeds of movement. Those changes were related to the decrease in type IIa muscle fibre area in men, in accordance with previous observations demonstrating a preferential atrophy of fast-twitch muscle fibres (Larsson, 1982; Larsson et al., 1978; Tomonaga, 1977). In vivo muscle function is significantly related to the MyHC isoform expression in the muscle fibres (Gur et al., 2003) and to quantitative loss of muscle mass (Frontera et al., 2000a; Jubrias et al., 1997). The ageing- and gender-related differences in isokinetic torque were paralleled by higher muscle fibre force ($P_0$) values in muscle fibres expressing type I, type I/IIa, type IIa and type IIax MyHC isoforms from the men. The higher $P_0$ values in the men were secondary to the larger size of the muscle fibres in the men and there were no gender-related differences at the single muscle fibre level when $P_0$ was normalised to fibre CSA.

Muscle loss alone cannot not fully account for the decrease in force production (Jubrias et al., 1997). The force generating capacity at the single muscle level, i.e., the muscle fibre force normalised to fibre size (ST), decreased significantly with age in slow muscle fibres in both genders, and a similar trend was observed in the type IIa muscle fibres. Women had higher ST in both fibre types, irrespective age. The 20-30% decline in specific tension in the most frequently recruited muscles cells expressing the β/slow (type I) MyHC isoform, belonging to motor units of the slow-twitch type, contribute significantly to the ageing-related 30-55% decline in maximum voluntary strength.

The lower torque production at the whole muscle level was also paralleled by lower maximum velocities of unloaded shortening ($V_0$) in fibre types expressing the type I and IIa MyHC isoforms in both men and women. Lower shortening velocities with age (D'Antona et al., 2003; Larsson et al., 1997a) and lower values in women than in men in both fibre types (Krivickas et al., 2001b) are previously documented. Ageing-relating
changes in myosin structure (D'Antona et al., 2003; Hook et al., 2001) are indicated to affect the contractile speed of muscle fibres.

Mechanisms underlying the ageing-related changes in sprint performance

There was a decrease in the maximal isometric (F\text{max}) and dynamic force development in master sprinters, compared to younger subjects. Their 60-m sprint times decreased linearly until the age around the 65 years, and showed an accelerated decline rate after this age. The ageing-related slowing of normalised rate of force production (RFD) and time to reach 10-80\% of F\text{max} in old sprinters were associated with a decrease in the relative content of MyHC II isoforms, as indirectly indicated by the fibre size, muscle thickness and myosin isoform content findings. Several other studies have reported that older subjects exhibit slower isometric force production, whether determined by maximal voluntary (Bemben et al., 1991; Clarkson et al., 1981; Hakkinen et al., 1995; Thelen et al., 1996) or electronically stimulated contractions (Connelly et al., 1999; Harridge et al., 1997).

The decrease in power output and slowing of movement is likely to reflect the differences in intrinsic properties between type I and type II MyHC fibres. Fibres with a fast MyHC have significantly higher shortening velocity (Harridge et al., 1996; Larsson and Moss, 1993) and develop tension faster (Harridge et al., 1996; Metzger and Moss, 1990) than fibres expressing the slow MyHC isoforms. A reduction in the shortening velocity of single MyHC I fibres was also observed. This could influence the rate of force production during the initial phase of muscle contraction when the slower motor units are recruited first (Henneman and Mendell, 1981a). Despite the impaired force production capacity, the maximal and fast force production in old sprinters remained comparable to that of untrained men tested by the same methods (Hakkinen et al., 1998a; Hakkinen et al., 1998b). The oldest sprinters had F\text{max} and RFD values which were approximately 31\% and 47\% higher, respectively, than those of 70-yr-old non-athletes. These values were actually at the same level as the values obtained for untrained subjects at the age of 40 yrs. Moreover, in terms of dynamic explosive strength, the oldest subjects in this study had vertical jump values twice as high as those reported earlier for untrained men aged 71-73 yrs (Bosco and Komi, 1980).
The influence of specific resistance training on force production in running in elite master athletes

In a follow up study, a subgroup of master sprinters improved maximal isometric and dynamic leg strength, explosive jump performance and force production in running, after completing a 20-week sprint and resistance training (SPRT) special designed programme. Both neural and hypertrophic mechanisms were observed to contribute to increase force development in running. The 10% increase in explosive force during squat jump in EX was accompanied by a 9% increase in the integrated EMG (iEMG) of the leg extensors. Also, a large increase was observed in isometric and dynamic strength, but those changes were not paralleled by significant changes in iEMG. As discussed previously, muscle mass has a major impact on the force development (Frontera et al., 2000a). A significantly increased (40%) CSA of type IIa single fibres and considerably higher (22%) area of type I single fibres were observed, proving the efficiency of the training programme and leading to improvements in maximal and explosive strength even in world-class master sprinters. The increase in power production was expected (Peterson et al., 2006; Stone et al., 2003; Wisloff et al., 2004), while the development in explosive jump performances surprises, considering the long training history in sprint and plyometric training of master sprinters. Most probably, the explosive power improvement is due to the specific character of the training programme, explosive types of weight training, and plyometric exercises. Strength training can improve the sprinting speed in young adult athletes (Andersen et al., 1994a; Blazevich and Jenkins, 2002; Delecluse et al., 1995; Harris et al., 2000). Combined heavy and explosive weight training improves strength performance in both young (Harris et al., 2000; Newton et al., 2002) and older (Hakkinen et al., 1998b; Izquierdo et al., 2001; Newton et al., 2002) subjects.

We also investigated how strength gains are transferred to force production of running. The results suggest that the older athletes could produce 8% higher ground propulsive force with shorter contact times in response to the training program, leading to an increase in the rate of force development during both propulsive and braking phases. Our interpretation is that the gains in maximal strength allowed the muscles to withstand greater amount of impact forces leading to faster transition from the braking to the propulsion phase.

Resistance training adaptations in old age: muscular vs. neural mechanisms

We observed in this study that the significant increases in maximal isometric torque in unilateral knee extension and knee flexion in resistance trained master sprinters were not accompanied by changes in isometric knee exten-
sion and flexion iEMG. The changes in the agonist iEMG were not followed by changes in the antagonist iEMG. This seems to indicate that improvement in isometric torque-producing capacity in response to training is due to muscle adaptations independent of neural drive to muscles. However, we cannot rule out the possibility that other neural adaptations have occurred. For example, it is possible that there have been increased muscle activity levels in other agonist muscles and/or improved coactivation of synergists, which could have mediated increased torque-production capacity. In previous studies on untrained subjects, a reduction in the co-activation of the antagonists after strength training has also been detected. The results of this study, however, indicated no change in the BF coactivation in either dynamic or isometric contractions. Finally, the enhanced isometric torque in the absence of a change in iEMG may result from unilateral isometric testing mode. In a previous study, bilateral training of knee extensors has been found to increase maximal iEMG when measured during bilateral test but not when measured unilaterally (Hakkinen et al., 1995).

In healthy young and old men and women, progressive training loads has been shown to result in increased iEMG activity during the whole 6 mo training period (Hakkinen et al., 1998b). Increased maximal force productions associated with increased EMG activity were attributed to an increased neural drive from the higher neural centres (Yue and Cole, 1992) or spinal cord circuitry in humans (Carroll et al., 2002). Early improvements in strength after RT in women (Frontera et al., 2008) and elderly (Kosek et al., 2006) were to a larger extent related to neural changes than in young controls. At the same time there is evidence of incomplete activation on untrained very old during maximal voluntary effort, which may be due to the different initial training skills of the subjects (for ref. see (Deschenes and Kraemer, 2002). Resistance training was indicated to have a beneficial effect by influencing the neural function through increased firing rate and the muscular force production (Leong et al., 1999).

The adaptability of skeletal muscle from old individuals to respond to exercise by improving strength and restoring muscle fibre size has been shown to be similar to those in younger subjects at the whole muscle level (Frontera et al., 1988) (Fiatarone et al., 1990; Kosek et al., 2006). However, the nature of resistance training induced molecular adaptations of skeletal muscle may differ in old compared with young subjects and our understanding of these processes remain incomplete (Jozsi et al., 2000)

Changes in single fibre size and MyHC isoforms content with age and activity level
The quantitative loss in muscle CSA with ageing has a major impact on the decrease in muscle strength seen with advancing age (Frontera et al., 2000a).
The type IIa fibre size in sedentary men and women and sprint-trained athletes decreased significantly with age, while an increase was observed after the resistance training period. Consistent with several previous studies in untrained (Larsson et al., 1979; Lexell et al., 1988; Tomonaga, 1977) as well as in endurance-trained humans (Klitgaard et al., 1990; Proctor et al., 1995; Widrick et al., 1996) we observed that the reduction in fibre area in sedentary and sprint trained subjects is mainly confined to fast fibres, leading to a decrease in the type II-to-type I fibre area ratio with age. Muscle homogenates of young and old sprinters showed an ageing-related increase in the relative proportion of slow MyHC I with a concomitant decrease in MyHC IIx content. It is generally reported that in normal active (Hameed et al., 2003; Klitgaard et al., 1990; Short et al., 2005) and endurance-trained older people (Klitgaard et al., 1990) electrophoretic studies of muscle homogenates show a shift towards greater relative MyHC I content in the vastus lateralis.

The preferential decrease in type II fibre area started as early as at ~30 years of age even in highly trained sprinters, resembling results obtained from untrained people (Lexell et al., 1988; Oertel, 1986). A lower volume and intensity of sprint training, and moreover resistance training, are contributory factors to the preferential decline of fast fibre areas in sprinters, due to lower overload stimulus for high-threshold type II fibres. However, resistance training protocols in both young and old have proved efficient in stimulating muscle fibre hypertrophy, with subsequent strength gains and increases in myofibrillar protein turnover (Frontera et al., 1988). In support of the major influence of resistance training on muscle, we observed that sprinters in their sixties that were able to maintain an aggressive strength-training program, the type II-to-type I area ratio was equal to that found in younger athletes and significantly larger than in runners of the same age who had no such strength training background. In these older sprinters, the cross-sectional areas of the fast fibres were maintained close to the values found in the youngest group.

Maximum velocity of unloaded shortening and specific tension in response to ageing and training

The shortening velocity of type I and IIa muscle cells decreased in elderly men and women. The $V_0$ was higher in men than in women, irrespective age. The ageing-related slowing of contractile velocity was also confirmed in type I fibres in master sprinters, while no change was observed in type IIa fibres. Large, but not significant, changes towards a higher $V_0$ of type I (22.0 %) and IIa (5.2 %) fibres were observed after the 20-week SPRT, restoring the unloaded contractile velocity of single fibre segments close to the values...
observed in the young sprinters. The \( V_0 \) variation was larger in elderly, both sedentary and trained.

Maximum velocity of unloaded shortening is a very important design parameter of skeletal muscle fibre and it is primarily determined by MyHC isoform expressed in the fibre (Bottinelli et al., 1996; Harridge et al., 1996; Larsson et al., 1979; Larsson et al., 1997a). The lower maximum velocity of unloaded shortening (\( V_0 \)) in type I and IIa fibres observed in experimental animal studies (Li and Larsson, 1996) was confirmed in sedentary humans (D’Antona et al., 2003; Krivickas et al., 2001b; Larsson et al., 1997a; Ochala et al., 2006). A gender-related difference was also reported, with lower \( V_0 \) in muscle cells expressing type I and IIa MyHC isoforms in old women than in old men (Krivickas et al., 2001b). It is suggested that the gender-related difference in regulation of muscle contraction contributes to the greater frailty and impairment of muscle function reported in old women than in old men. The ageing-related decline in \( V_0 \) of type I fibres was not reversed in sprint trained athletes, indicating that lower activity level with age is not responsible for the lower \( V_0 \) values. The observed alterations in \( V_0 \) may reflect a change in the myosin function, as indicated by in vitro motility experiments in aged mice, rats and humans (D’Antona et al., 2003; Hook et al., 1999; Hook et al., 2001). The effect was suggested to depend on decreased myosin density or changes that may affect the intrinsic properties of the contractile proteins (Hook et al., 2001).

The exact mechanisms underlying the observed gender-related differences in regulation of muscle contraction are not known. Balagopal and colleagues (Balagopal et al., 1997) observed that the correlation between muscle strength and MyHC synthesis rate is stronger in women and suggested that protein synthesis in sarcopenia could be regulated by different parameters in men and women. Hormonal influences, e.g., oestrogen levels, may play a role in this difference (Phillips et al., 1993), possibly by influencing the \( \text{Ca}^{2+} \) kinetics, myosin ATPase activity, number of active cross-bridges or force generated per cross-bridge (Sarwar et al., 1996; Wattanapermpool and Reiser, 1999). Oxidation of cysteines has been put forward as a mechanism underlying structural-functional changes of myosin in old age (Lowe et al., 2001b; Prochniewicz et al., 2005). However, the increased oxidative damage with ageing in sedentary subjects was not confined to a specific gender or fibre type.

In addition to the slowing in contractile speed in master sprinters, an ageing-related loss in specific tension has been reported in sedentary individuals at the single muscle fibre level (D’Antona et al., 2003; Krivickas et al., 2001a; Larsson et al., 1997a), but no significant difference in specific tension was observed between the strength- and sprint-trained young and old men in this study. The ageing-related loss in specific tension at the single muscle fibre level may be a consequence of a more sedentary lifestyle in old age. The myosin content per muscle fibre volume (D’Antona et al., 2003;
Marx et al., 2002) and the number of strongly bound cross bridges (Lowe et al., 2001a) decrease in old age. However, the ageing-related decline in specific tension at the single muscle fibre level was not observed in old sprinters. The specific tension increases in response to strength training in old sedentary women (Frontera et al., 2003). The absence of a change in specific tension during the 20-week PRT indicates that this intervention did not have a significant impact on the number or force-generating capacity of the cross-bridges in the already trained master athletes, but the force generating capacity in master athletes is maintained at the same level as in untrained young men.

Ageing- and gender specific changes in the myonuclear spatial organisation

The MND size of the type I fibres did not differ in old age in men and women. The MND decrease observed in the type IIa fibres confirm earlier observations in fast-twitch mouse muscle (Brack et al., 2005) and is a reflection of the faster decrease in type IIa fibre size than in nuclear number. However, the constant MND size in the larger type I fibres in the significant larger type I fibres indicate that other mechanisms may be contributing to the ageing-related fibre atrophy and it was actually demonstrated that moderate fibre changes in human skeletal muscle may occur without a change in the myonuclear number (Kadi et al., 2004b). We observed a considerable variability in the size of the MNDs, related to the impaired myonuclei organisation with ageing, which seems to be gender- and fibre type-specific. Increased MND size variability with ageing is the most important finding of our study. It reflects the changes in the myonuclear spatial organisation, quantified by the NN values. Higher MND size variation may be a contributing factor to altered myofibrillar protein synthesis since all nuclei are not simultaneously transcriptionally active for a given protein (Newlands et al., 1998). The altered spatial organisation in old age may influence the local quantity of DNA per muscle fibre volume by decreased activity and local cooperativity, thus directly influencing the amount and quality of the contractile proteins.
Conclusions

This study provides new information about the ageing neuromuscular system at several levels. We present evidence of specific ageing- and gender-related differences in regulation of muscle contraction at the whole-muscle and cellular level in healthy sedentary men and women. We also present new data about how strength gains are transferred to force production of running in elite master sprinters. The sprint-trained master athletes experienced the typical ageing-related reduction in the size of fast fibres, a shift toward a slower MyHC isoform profile and a lower $V_0$ of type I MyHC fibres, which played a role in the decline in explosive force production capacity. The muscle fibre and strength characteristics were preserved at a high level in the oldest runners, underlining the favourable impact of sprint exercise on ageing skeletal muscle. It is reported for the first time about the influence of ageing and systematic physical training on single fibre characteristics in sprint-power trained athletes. Adding strength training stimulus to the training program improved maximal, explosive and sport-specific force production in elite master sprinters. These improvements were primarily related to hypertrophic muscular adaptations. Further, a successful new method was elaborated for quantifying changes at the myonuclear level. Altered myonuclear spatial organisation may lead to specific gender- and fibre type differences in the DNA quantity per fibre volume. Local impairments in myofibrillar protein synthesis are a potential mechanism underlying the decreased force-generating capacity at the single muscle fibre level in humans.

I. In healthy untrained subjects, ageing is related to a decline in voluntary force, paralleled by a decrease in the type II muscle fibre area in old age in the men but not in the women. This indicates that the preferential decrease in the size of fast-twitch muscle fibres is of minor importance for the ageing-related decline in voluntary force. An ageing-related decline in the maximum velocity of unloaded shortening was observed in muscle cells expressing both type I and IIa MyHCs irrespective gender. Quantitative and qualitative changes in regulation of muscle contraction at the single muscle fibre level contribute to the ageing-related decline in \textit{in vivo} muscle function in both men and women.
II. Ageing was associated with decreased dynamic force and a change towards slower MyHC phenotype and gene expression even in life-long trained master sprinters. Specific resistance training had however a beneficial effect on the muscle mass and dynamic force production in master sprinters and preserved the quality of contractile proteins, as shown by the unchanged ST in both type I and IIa fibres. The lifelong dedication to sprint training in male athletes could not prevent the age-specific slowing of type I fibres, atrophy of type IIa fibres, and the decrease in sprint and strength performance.

III. The process of sarcopenia may be delayed, but not completely compensate for by intense physical exercise. The atrophy of type II fibres was reversed through specially designed training. Significant increases in maximal isometric and dynamic leg strength, explosive jump performances and force development in running were associated with increased iEMG activity and hypertrophy of type II and type IIa fibres. The trained master sprinters represent a valuable model for estimating the effects of resistance training in old age. Combined resistance and sprint training programs at high intensities in highly motivated physically active men proved useful in improving the explosive force at old age.

IV. New findings show that the myonuclei number and spatial organization are in a dynamic state during the ageing process. The variability of the MND size increases with ageing irrespective gender and fibre type. This may influence the local quantity of specific proteins per muscle fibre volume by decreased and/or local cooperativity of myonuclei in a gender and muscle fibre specific manner.
Future studies

Given the positive effects of the resistance training, it is even more important to fully elucidate the mechanisms of muscle adaptation at old age and the beneficial of custom adapted training programs in ageing and disease. Quantification of oxidized proteins and post-translational modification at the contractile protein level by means of mass spectrometry is still a developing field. Those changes are a part of the ageing process and play an active role in sarcopenia. Exciting new possibilities are opened by the study of transcriptional activity in syncitial skeletal muscle by the new method used in this study. Future studies will deepen our understanding about the spatio-temporal changes in the skeletal muscle during muscle wasting in ageing and disease at the nuclear level.
The work described in this dissertation was carried out by the author at the Uppsala University and in the supervisor’s laboratory at the Department of Clinical Neurophysiology at University Hospital in Uppsala, in collaboration with researchers at the Imaging Centre, Uppsala University and Swedish University of Agricultural Sciences, and Jyväskylä University in Finland. This thesis would not have been possible without these stimulating and fruitful collaborations.

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