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Properties and function of the tendon-muscle complex in rheumatoid arthritis

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Properties and function of the tendon-muscle complex in rheumatoid arthritis

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Abstract

Rheumatologic conditions featuring systemic inflammation are characterised by profound loss of physical function, which is in part caused by skeletal muscle wasting. This thesis aims to add to the current knowledge on disability in rheumatoid arthritis (RA) and ankylosing spondylitis (AS) by determining the physiological properties of the tendon-muscle complex in these conditions, and by investigating causes of muscle loss in RA at the cellular level.

The results section is divided into five main research chapters (chapters 3 to 7).

Chapter 3 reports that physiological properties of muscle that determine specific force are preserved in a community-based population with stable RA compared to a healthy age- and sex-matched control group. Despite having deficits in physical function, no differences in vastus lateralis (VL) specific force, contractile properties, voluntary activation capacity and contraction velocity were observed in the RA patients. Body composition using DXA shows a trend towards lower appendicular lean mass and increased total body fat in patients relative to controls, and consistent with this, VL physiological cross-sectional area (PCSA) is reduced with RA (results published in Medicine and Science in Sports and Exercise 2010;42:2149-55).

A considerable proportion of patients with RA are cachectic which indicates that processes have taken place which alter their muscle. Therefore, in **Chapter 4** a group of cachectic RA patients was chosen to assess whether parameters of muscle quality are changed in these patients. However, the results show that even in cachectic RA patients,

muscle specific force and activation are not compromised compared with healthy age and sex-matched controls and thus are unlikely to contribute to the observed reduced function. As expected, VL PCSA and force were reduced (albeit non-significantly), and pennation angle also tended to be lower in RA. No differences were observed for muscle fibre fascicle length (results published in The Journal of Rheumatology 2010;37(2):282-84).

Chapter 5. To investigate intracellular processes in the muscle of RA patients, biopsies were taken from the VL of patients with stable RA, healthy controls, and patients with active RA before and 3 months after achieving disease control. No differences were found in the distribution of myosin heavy chains (MHC) and in caspase-3, a marker of muscle apoptosis and atrophy, between stable RA patients and healthy controls. Thus, in concurrence with chapter 3 and 4, intracellular muscle quality was preserved in stable RA.

In patients with active RA before and after disease control with the first-line disease-modifying antirheumatic drug methotrexate or with anti-TNF agents (etanercept or adalimumab), there was also no significant difference in caspase-3. However, pAkt, a key factor promoting muscle hypertrophy, was suppressed in patients with active RA compared to controlled disease in the same patients 3 months later, and could therefore be one of the principal reasons for muscle loss during the active phase of RA. In contrast to this, atrogin-1, a marker of muscle atrophy, was low in active disease as well, and IκBα, a downstream transcription factor of TNF-α, did not change in patients with active disease before and after disease control. Thus, the complex mechanism leading to muscle atrophy in RA warrants further investigation.

Chapter 6 presents the case of a localised knee effusion in the context of active, newly diagnosed RA. It shows that an inflammatory knee effusion markedly impairs physical function, leads to quadriceps wasting and adversely affects patellar tendon (PT) stiffness and Young's modulus (YM, a measure of tendon stiffness normalised for tendon size) locally. In addition to loss of muscle quantity, and in contrast to the observations in stable RA in chapter 3 and 4, this case study indicates impaired muscle quality in newly diagnosed active RA. At reassessment of these parameters one year later, resolution of disease activity and continued physical activity resulted in partial recovery of muscle quality and physical function, but did not resolve the tendon abnormalities. Furthermore, the development of reduced PT stiffness in the contralateral leg at 1 year suggests a systemic effect of the inflammatory process on tendons in RA (results published in Arthritis Care and Research, 10 May 2011, epub ahead of print).

Chapter 7 investigates this systemic effect by assessing the properties of the PT alongside muscle characteristics and physical function in a group of stable RA patients and in a group of patients with stable ankylosing spondylitis (AS). Adverse changes in PT properties are demonstrated in both RA and AS compared to age- and sex-matched healthy controls. PT stiffness and physical function are significantly lower in RA and AS patients, whilst there is no significant difference in force production between patients and controls. In AS, but not RA, PT cross-sectional area is significantly larger leading to reduction in YM. AS, but not RA, therefore leads to PT thickening without increasing PT stiffness, suggesting that PT thickening in AS is a disorganised repair process (results submitted for publication).

This research is the first to measure qualitative characteristics of muscle and tendon in inflammatory arthropathies. The key findings of this thesis are:

- Muscle properties are preserved in stable RA, even in muscle-wasted patients.
- MHC distribution and the apoptosis marker caspase-3 are similar in patients with stable RA and healthy controls. Markers of muscle hypertrophy, but also of muscle atrophy, are upregulated in active RA, with no significant change in caspase-3 and IκBα between active and controlled disease.
- In active RA with an acute knee joint effusion, local VL muscle properties are adversely affected, but improve with control of the disease activity; in the acute phase, reductions in PT stiffness only occur locally on the leg affected by the joint effusion, but in time these effects spread systematically i.e. to the contralateral PT.
- PT stiffness is reduced in both RA and AS patients with stable disease. However, PT size is only increased in AS, highlighting the different pathologies of the two conditions.

Publications and presentations

Original papers:

Verena Matschke, Peter Murphy, Andrew Lemmey, Peter Maddison, Jeanette Thom. Muscle quality, architecture, and activation in cachectic patients with rheumatoid arthritis. *Journal of Rheumatology* (2010): 37(2):282-284

Verena Matschke, Peter Murphy, Andrew Lemmey, Peter Maddison, Jeanette Thom. Skeletal muscle properties in rheumatoid arthritis patients. *Medicine and Science in Sports and Exercise* (2010): 42: 2149-2155

This paper was invited to be highlighted as the article of the month by the American College of Sports Medicine by a topical commentary:
Verena Matschke. New evidence on skeletal muscle properties encourages exercise for rheumatoid arthritis patients. *ACSM Sports Medicine Bulletin* 11 February 2011.

Verena Matschke, Jeanette Thom, Andrew Lemmey, Peter Maddison, Jeremy Jones Inflammatory joint effusion alters the properties of the tendon-muscle complex in rheumatoid arthritis: A case study. *Arthritis Care and Research*, epub ahead of print May 2011

Jennifer Cooney, Rebecca-Jane Law, **Verena Matschke**, Jonathan Moore, Andrew Lemmey, Yasmeen Ahmed, Jeremy Jones, Peter Maddison and Jeanette Thom. Benefits of exercise in rheumatoid arthritis. *Journal of Aging Research* (2011): 681640

Verena Matschke, Jeremy Jones, Andrew Lemmey, Peter Maddison and Jeanette Thom. Patellar tendon properties and lower limb function in rheumatoid arthritis and ankylosing spondylitis. *under review*

Additional paper contribution:

Francesco Sartor, Helma De Morree, **Verena Matschke**, Samuele Marcora, Athanasios Milousis, Jeanette Thom, Hans-Peter Kubis. High-intensity exercise and carbohydrate-reduced energy-restricted diet in obese individuals. *European Journal of Applied Physiology* (2010): 110(5):893-903

Conference poster presentations:

Verena Matschke, Jeanette Thom, Peter Murphy, Andrew Lemmey and Peter Maddison. Muscle quality in rheumatoid arthritis. *European Conference of Sport Science, Estoril, Portugal 2008*

Verena Matschke, Jeanette Thom, Peter Murphy, Andrew Lemmey and Peter Maddison. Muscle quality in rheumatoid arthritis. *British Society for Rheumatology Conference, Liverpool, UK 2008*

Verena Matschke, Andrew Lemmey, Jeremy Jones, Peter Maddison and Jeanette Thom. Patellar tendon properties and lower limb function in rheumatoid arthritis and ankylosing spondylitis. *British Society for Rheumatology Conference, Glasgow, UK 2009*

Verena Matschke, Andrew Lemmey, Jeremy Jones, Peter Maddison and Jeanette Thom. Patellar tendon properties and lower limb function in inflammatory arthropathies. *Human and Exercise Physiology Themed Meeting of The Physiological Society, King's College London, UK 2009*

Verena Matschke, Jeremy Jones, Andrew Lemmey, Peter Maddison and Jeanette Thom. Inflammatory joint effusion alters the properties of the tendon-muscle complex in rheumatoid arthritis: a case study. *British Society for Rheumatology Conference, Birmingham, UK 2010*

Further poster presentations at Musculoskeletal Research Day, Manchester 2007, North Wales Clinical Research Symposium and Open Day 2008, and North West Rheumatology Club Meeting, Manchester 2009.

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Verena Matschke, Jeanette Thom, Peter Murphy, Andrew Lemmey and Peter Maddison. Effect of muscle strength on physical function in rheumatoid arthritis patients. *North West Rheumatology Club Meeting, Liverpool Medical Institution 2007*

Verena Matschke, Andrew Lemmey, Jeremy Jones, Peter Maddison and Jeanette Thom. Patella tendon properties and lower limb function in rheumatoid arthritis and ankylosing spondylitis. *North West Rheumatology Club Meeting, Deganwy 2009*

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Abbreviations

ACSA	anatomical cross-sectional area
AIDS	acquired immune deficiency syndrome
ALM	appendicular lean mass
AS	ankylosing spondylitis
BASDAI	Bath Ankylosing Spondylitis Disease Activity Index
BF	body fat
BMI	body mass index
BSR	British Society fir Rheumatology
CCP	cyclic citrullinated peptide
CSA	cross-sectional area
CT	computer tomography
DAS	disease activity score
DMARD	disease-modifying antirheumatic drug
DXA	dual x-ray densitometry
EGTA	ethylene glycol tetraacetic acid
EMG	electromyography
ESR	erythrocyte sedimentation rate
FOXO	forkhead box class O
GPa	Gigapascal
HAQ	Health Assessment Questionnaire
HLA-B27	human leucocyte antigen B27
ICC	intraclass correlation coefficient
IGF-I	insulin growth factor I
IκBα	inhibitor-kappa B alpha
IL-1	interleukin 1
IL-6	interleukin 6
Lf	fibre fascicle length
LFN	leflunomide
MAFbx	muscle atrophy F-box
MHC	myosin heavy chain
MQ	muscle quality
MRI	magnetic resonance tomography
mTOR	mammalian target of rapamycin
MTX	methotreaxate
MVC	maximal voluntary contraction
n	number
NF-κB	nuclear factor-kappa B
Nm	Newtonmeter
NSAID	non-steroidal anti-inflammatory drug
pAkt	phospho-Akt
pIκBα	phospho-IκBα
PCSA	physiological cross-sectional area
PMSF	phenylmethylsulfonyl fluoride
PT	patellar tendon
QoL	quality of life
RA	rheumatoid arthritis

RADAI-5	modified rheumatoid arthritis disease activity index
RF	rectus femoris
SDS-PAGE	sodium dodecyl sulphate-polyacrylamide-gel electrophoresis
SEM	standard error of the mean
SF-36	36 question Short-Form Health Survey
SSZ	sulfasalazine
TNF-α	tumour necrosis factor alpha
VI	vastus intermedius
VL	vastus lateralis
VM	vastus medius
VOL	volume
YM	Young's modulus
θ	pennation angle

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Chapter 1: General introduction

Impaired physical function and disability are characteristic of patients with rheumatoid arthritis (RA) (Young et al. 1987). The continual development of powerful disease-modifying antirheumatic drugs (DMARDs) over the past 4 decades has substantially improved control of disease activity and reduced progression of joint damage and deformity, so that many patients are able to maintain an active life in the community. However, even in stable disease with minimal joint symptoms most patients suffer limitations of physical function, often leading to work disability (Eberhardt et al. 2007; Allaire et al. 2008).

Escalante and Del Rincon (Escalante and del Rincón 1999) developed a hierarchical regression model of disability in RA which accounted for 59% of the variance in RA disability by: disease activity, joint inflammation, joint damage and pain, psycho-social factors and demographics. However, this model did not assess the influence of skeletal muscle mass and its physiological properties on disability in RA. Recently Giles et al. (Giles et al. 2008a; Giles et al. 2008c) demonstrated a strong association between body composition in RA (i.e. reduced muscle mass, high percent body fat) and disability. The prevalence of muscle wasting in RA, i.e. rheumatoid cachexia, is high compared to the general population, probably due to the catabolic effect of pro-inflammatory cytokines (e.g. tumor necrosis factor-α (TNF-α), and the down-regulation of anabolic factors for muscle (e.g. insulin-like growth factor I (IGF-1) (Roubenoff et al. 1992; Roubenoff et al. 1994; Munro and Capell 1997; Lemmey et al. 2001; Lemmey et al. 2009). Patients lose an average of 15% of lean mass and 30-80% of strength and physical function (Roubenoff et al. 1994; Rall et al. 1996). However, the intracellular processes

downstream from TNF-α and IGF-I that might lead to loss of muscle mass in RA, such as Akt signalling and induction of caspases, have not yet been elucidated. In addition, whilst histochemical analyses in the 1970s have shown a preferential loss of type 2 fibres in RA (Haslock et al. 1970; Nordemar et al. 1976a; Nordemar et al. 1976b; Magyar et al. 1977), this has not been revisited since the advent of major changes in the treatment of RA with a wide range of DMARDs and anti-TNF antibodies. In addition, the biochemical determination of myosin heavy chain composition has not yet been done in the muscle of this population.

Extensive research has been conducted on the factors that determine muscle force production and function of the tendon-muscle complex in healthy populations, and the adverse changes that occur due to ageing and disuse (Morse et al. 2005b; Stevens et al. 2006). A similar, comprehensive assessment of the physiological characteristics of skeletal muscle and tendon in patients with RA is absent, which is surprising in view of the recent developments in RA treatment. Consequently, it is not known whether the catabolic effect of RA results in alterations in skeletal muscle properties, e.g. muscle specific force and architecture, muscle activation capacity, contractile properties, power production and tendon stiffness, which may then contribute to the reduced physical function in patients with RA.

Furthermore muscle loss associated with systemic inflammation is not exclusive to RA but also occurs in other forms of inflammatory arthropathy including spondyloarthropathies such as ankylosing spondylitis (AS). AS shares the features of joint inflammation and loss of physical function with RA, but is characterised by its primary pathology at the enthesis, i.e. the tendon insertion to bone (Marcora et al.

2006a; Benjamin et al. 2007; Benjamin and McGonagle 2009). The effect of this condition on the physiological properties of the tendon-muscle complex is also unknown.

Finally, exercise training which restores muscle mass has been shown to substantially improve physical function and reduce disability in patients with RA without increasing disease activity (de Jong et al. 2003; Marcora et al. 2005; Lemmey et al. 2009). Thus, encouragement to exercise now features more in routine rheumatology care, and an increasing number of patients, especially those with milder, well-controlled disease, attend public gyms.

By investigating the muscle and tendon properties in RA and AS, and intracellular processes of muscle apoptosis, inflammation and atrophy as well as myosin heavy chain (MHC) distribution in RA, this thesis aims to add to the understanding of the factors underlying disability in these patients. Thus, this research should lead to more effective interventions for restoring muscle function thereby aiding the rehabilitation of RA patients and improving their quality of life.

The following chapter details the clinical background of RA and AS as well as the current knowledge on rheumatoid cachexia and its functional consequences. The properties of the muscles and tendons which determine the quality of the tendon-muscle complex will be explained, with examples from the healthy and ageing population as well as from studies on disuse. Chapter 3 onwards describe the investigations of these parameters in patients with RA and AS conducted during this doctoral work.

Chapter 2: Background

The role of muscle in rheumatoid arthritis

Rheumatoid arthritis in clinical practice

RA is the most common form of chronic inflammatory arthritis with a prevalence of up to 1% of the adult population worldwide, and is three times more common in women. It is primarily known for inflammation of the lining of peripheral joints (proliferative synovitis) which causes significant destruction of articular cartilage and subchondral bone. The disease often begins between the 4^{th} and 6^{th} decade of life, but its onset can be at any age (Silman and Pearson 2002; Kvien 2004).

The aetiology of the condition is not fully understood, but is believed to be a complex interaction between genetic and environmental factors such as infectious agents, smoking, and colder climate (Silman and Pearson 2002; Worthington 2005). These may contribute to the known dysregulation and augmentation of the immune system with heavy leucocytic infiltration (T cells, plasma cells, macrophages) of the synovium, and the production of autoantibodies that act against synovial antigens and induce synovial hyperplasia. Mediators of this process are proinflammatory cytokines such as interleukin 1 (IL-1) and interleukin 6 (IL-6), with TNF-α playing a central role, leading to the production of catabolic enzymes and eventual destruction of articular cartilage, bone, tendons and ligaments (Roubenoff et al. 1992; Sweeney and Firestein 2004).

The major clinical features of RA are joint pain, swelling, early morning stiffness, and impaired physical functioning. There is usually a symmetrical involvement of peripheral joints, in particular metacarpophalangeal, proximal interphalangeal, and metatarsophalangeal joints, as well as wrists, shoulders, knees, ankles and elbows. The destructive process results in irreversible joint damage and deformity.

Advances in research in the past decades have facilitated more aggressive management of the condition with DMARDS (e.g. methotrexate (MTX), sulfasalazine (SSZ), leflunomide (LFN)) and biologic agents (e.g. the anti-TNF agents etanercept, adalimumab and infliximab, and the B-cell depleting agent rituximab) (Deighton 2005; Ledingham and Deighton 2005; Chakravarty et al. 2008; Deighton et al. 2010; Bukhari et al. 2011). This has led to marked improvements in the control of disease activity and to slowing of the progression of radiological damage for many patients. Treatment has to be long term because no cure has yet been found. In addition to drug treatment, patients are seen by physiotherapists, occupational therapists and podiatrists for assessment and advice on exercise, joint protection and management of daily activities (Hennell and Luqmani 2008; Luqmani et al. 2009). Occasionally, joint replacement surgery is required to restore joint function and to reduce pain.

In addition to joint destruction, systemic inflammation and serious extra-articular manifestations such as cardiovascular and respiratory disease and osteoporosis greatly contribute to the morbidity and reduced life expectancy (Lee and Weinblatt 2001; Turesson et al. 2003; Naz and Symmons 2007). Many patients are also affected by loss of muscle strength and mass which will be further explained in the next section.

Disability in rheumatoid arthritis

Despite the developments of medical therapy in RA most patients still experience significant functional limitations and disability (Escalante and del Rincón 1999; Kvien 2004). 35% of patients are unable to work within 10 years from diagnosis (Eberhardt et al. 2007; Allaire et al. 2008) and 50-80% are disabled within 20 years (Wolfe and Hawley 1998; Scott 2000). This is a major burden for society and the economy, with poor physical function being the main predictor of the large cost of this disease (Lajas et al. 2003).

Disability in RA is not only a consequence of local joint inflammation and damage, but is a multifactorial process. A model for disability for RA patients was developed by Escalante and Del Rincon (Escalante and del Rincón 1999). Using hierarchical regression analysis, 59% of the variance in disability was accounted for by disease activity, joint inflammation, joint damage and pain, psycho-social factors and demographics. Among the 41% of unknown contributors to RA disability, other components of the musculoskeletal system are likely to play a role, in particular muscle strength and muscle mass which are known to be reduced in RA. The literature describes strength reductions to 30-80% of normal (Ekdahl and Broman 1992; Hakkinen et al. 1995; Madsen et al. 1998), whereas muscle mass is reported to be lower in RA by approximately 14-16% compared to the healthy population when measured with the potassium-40 method (Roubenoff et al. 1994; Roubenoff 2000; Rall et al. 2002) or whole body dual x-ray densitometry (DXA) (unpublished observations of Lemmey et al.). Muscle mass is the main predictor for muscle strength, and associations of both mass and strength with poor physical function have been repeatedly

demonstrated in the general and RA populations (Hakkinen et al. 1995; Stucki et al. 1998; Hakkinen et al. 2001). In adapting Escalante and del Rincon's model to include muscle as an independent variable, Lemmey et al. found that muscle mass accounts for a further 9% of the disability in RA, and is second only to disease activity as a predictor of disability in patients with established disease (manuscript in preparation). Recently, Giles et al. demonstrated that altered body composition in RA is strongly associated with adverse functional outcome (Giles et al. 2008a). In this study there was a significant negative association of muscle mass with reduced physical function, and a significant positive association of fat mass with reduced physical function, and both independently predicted disability in RA as measured by HAQ (Health Assessment Questionnaire; see chapter 3, methods).

Rheumatoid cachexia

This altered body composition found in RA and characterised by low muscle mass and increased body fat has been termed "rheumatoid cachexia" (Roubenoff et al. 1992). It is thought to be a consequence of the systemic nature of the disease and the elevated circulating levels of proinflammatory cytokines (Roubenoff et al. 1992; Roubenoff et al. 1994; Rall et al. 1996; Rall et al. 2002; Walsmith and Roubenoff 2002; Rall and Roubenoff 2004). These proinflammatory cytokines, in particular TNF-α, lead to negative nitrogen balance and elevated protein breakdown (Walsmith and Roubenoff 2002). Depletion of lean body mass takes place early in the course of the disease, probably because cytokines are particularly abundant in the synovial fluid and the systemic circulation until control of disease activity has been achieved by medication (Roubenoff et al. 1992). For example, Marcora et al. showed that significant muscle loss is generally present within six months of onset of symptoms (Marcora et al.

2006b), and Westhoven et al. demonstrated significant loss of muscle mass in early RA patients within the first 13 months from diagnosis (Westhovens et al. 1997). In consequence, 50-65% of RA patients are severely muscle-wasted (Roubenoff et al. 1994; Munro and Capell 1997; Marcora et al. 2005; Lemmey et al. 2009). This is a worrying finding because loss of muscle mass, as in other catabolic diseases, causes not only weakness and disability but also impaired immune and pulmonary function and increased mortality (see Kotler 2000 for review). Moreover, generalised osteoporosis, glucose intolerance and low aerobic capacity in RA patients are related to muscle loss and disability (Svenson et al. 1988; Sambrook et al. 1995).

Cachexia is generally understood to mean severe loss of muscle mass with consequent marked loss of weight. In RA this can be confusing since the loss of muscle mass is accompanied by increased body fat which obscures the loss of muscle and results in little or no weight change. A very high proportion (> 50%) of RA patients are both muscle-wasted and obese (Roubenoff 2000; Marcora et al. 2005; Lemmey et al. 2009), in particular with a shift to abdominal fat (Westhovens et al. 1997; Inaba et al. 2007; Elkan et al. 2009; Giles et al. 2010). This is likely to add to the morbidity of patients, since central fat distribution is known to be correlated with an adverse lipoprotein profile and coronary heart disease (Inaba et al. 2007; Giles et al. 2010). In the healthy population, a combination of obesity and sarcopenia (the gradual loss of muscle mass seen in ageing) increases the risk of having 3 or more physical disabilities 12-fold and 9-fold in healthy elderly women and men respectively (Morley et al. 2001), and is linked to other clinically relevant outcomes such as mortality (Heitmann et al. 2000) and length of stay in hospital (Kyle et al. 2003).

In addition to the consequences of systemic inflammation in RA, the catabolic effect of glucocorticosteroids, which are part of the treatment of many patients with RA, adds to both the depletion of muscle and the increase in body fat (Roubenoff et al. 1990). Furthermore, the majority of RA patients are physically less active than the general population thereby adding another adverse effect on muscle mass for these patients (Roubenoff et al. 2002). Fortunately muscle loss in RA is at least in part reversible, and a number of intervention studies have demonstrated that high intensity exercise is effective in partially restoring muscle mass and strength with concomitant improvement in function (Hakkinen et al. 2005; Marcora et al. 2005; Lemmey et al. 2009).

In the healthy population, in ageing and in disuse, successful recovery of muscle strength and function is dependant on more than the sole induction of muscle hypertrophy. A range of parameters that influence the quality and function of the tendon-muscle complex are known to be altered with ageing and disuse, and their plasticity enables them to adapt and improve with exercise. These parameters have never been investigated in patients with RA. It is therefore possible that adverse changes of the physiological properties of muscle and tendon are also taking place in rheumatoid muscle, however this is unknown. The physiological factors contributing to muscle and tendon quality are detailed in the next section.

Properties and plasticity of the tendon-muscle complex

Skeletal muscle and tendons are constantly adapting to the functional demands imposed by load-bearing activities. Therefore, regular contractile activity is the necessary stimulus to preserve the integrity, function and quality of muscle. Changes in physical

activity and the physiological processes of ageing can alter a range of factors known to contribute to the 'quality' of a muscle; including the intrinsic muscle strength (i.e. the strength as determined by factors within the muscle such as contractile properties), the activation capacity of the muscle (neural factors) and the co-activation of antagonist muscles, pennation angle (muscle architecture), tendon stiffness, and fibre composition (Maganaris et al. 2001; Maganaris et al. 2004; Morse et al. 2004; Morse et al. 2005a; Onambele et al. 2006). A few studies have provided information on muscle activation and fibre type distribution in RA (Nordemar et al. 1976a; Nordemar et al. 1976b; Bearne et al. 2002), but no comprehensive investigation of the physiological muscle and tendon properties of RA patients - which is the aim of this thesis - has been undertaken so far. Therefore, to help explain the principle characteristics of the tendon-muscle complex, examples from the healthy population and effects of ageing and disuse will be used in the following section.

Muscle strength

Muscle strength is a major determinant of the quality of a muscle. Reductions in maximum isometric strength of between 35-47% have been demonstrated in the elderly in the lower limbs, and to a lesser degree in the upper limbs (Young 1985; Vandervoort and McComas 1986; Hortobagyi et al. 1995). As mentioned before, isometric muscle strength is reduced in patients with RA by 30-80%, and associated with reduction in muscle mass by approximately 15%, as well as with impairment of physical function (Nordemar et al. 1976a; Nordemar et al. 1976b; Ekdahl and Broman 1992; Roubenoff et al. 1994; Hakkinen et al. 1995; Madsen et al. 1998; Roubenoff 2000; Bearne et al. 2002; Rall et al. 2002). The variability of the extent of strength reduction in RA in the above studies can be explained by different assessment methods and disease severity.

However, absolute strength is not an accurate measure of muscle quality and differs with body height and habitus, and with the size and architectural characteristics of the muscles (Samson et al. 2000; Ikegawa et al. 2008). Since the major force-generating potential of a muscle is its size, the measurement of strength normalised to muscle size will reflect more accurately the quality of a muscle.

Muscle specific force

The maximal voluntary isometric force that can be produced per size of a given muscle is termed muscle specific force, and determines the intrinsic force-producing capacity of a muscle (Reeves et al. 2004).

The gold standard of calculating muscle specific force for knee extension is via the following formula: vastus lateralis (VL) force/ VL physiological cross-sectional area (PCSA), with the VL muscle being used as representative of the quadriceps muscle group (Narici et al. 1992). A range of components are included in the calculation of muscle specific force. The absolute VL force takes into account maximal knee extension torque, patellar tendon moment arm length, and co-contraction torque of the antagonist biceps femoris muscle estimated from electromyographic (EMG) activity (Reeves et al. 2004). VL PCSA takes into account muscle volume and architecture, i.e. fibre fascicle length and pennation angle. All these parameters (and research on these is lacking in RA) are necessary to accurately assess muscle specific force, and will be explained forthwith.

Muscle architecture and physiological cross-sectional area

To determine muscle volume, magnetic resonance tomography (MRI) or computer tomography (CT) can be used. Alternatively, muscle volume can be estimated by measuring muscle total length and anatomical cross sectional area (ACSA, Figure 1) in several places by ultrasonography (Reeves et al. 2004). With this method, the volume of the muscle portion between each two ACSA scans is calculated with a formula for truncated cones (Baratta et al. 1988). The volume of the entire muscle is determined by adding up the inter-ACSA volumes.

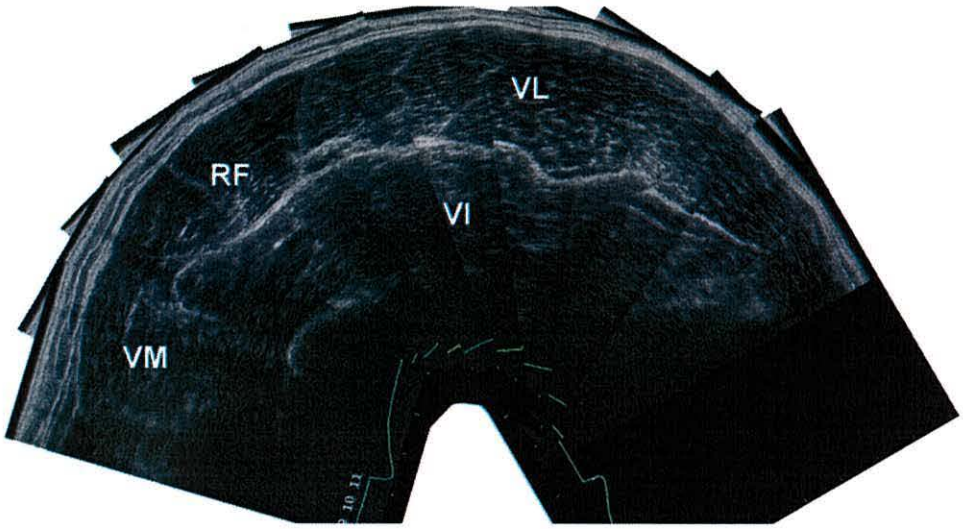

Figure 1. Anatomical cross-sectional area from the midregion of vastus lateralis (VL), vastus intermedialis (VI), vastus medialis (VM) and rectus femoris (RF) muscles, by ultrasonography.

In pennate muscles such as the quadriceps, the most accurate method used to assess muscle size for the calculation of specific force is PCSA. PCSA is the cross-section of the muscle at right angles to the direction of the muscle fibre fascicles, and its

calculation takes into account ACSA, total muscle length, the length of the muscle fibre fascicles and their angle of insertion into the aponeurosis of the muscle (pennation angle θ, Figure 2), which can also be assessed by ultrasound (Reeves et al. 2004).

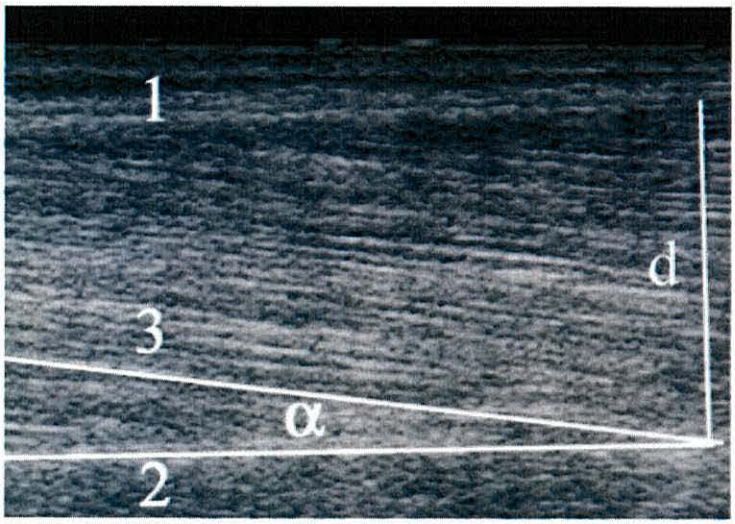

Figure 2. Sagittal-plane sonograph of the vastus lateralis muscle with pennation angle (θ), superficial aponeurosis (1), deep aponeurosis (2), fibre fascicles (3); θ is the angle of insertion of the fibre fascicle into the deep aponeurosis of the VL. Lf is calculated from θ and the muscle thickness d by the equation: Lf = d/sin θ .

These architectural characteristics of muscle influence its force development. For example, within a physiological range, the larger the pennation angle, thereby enabling more fibres to be placed in the muscle in parallel, the higher the force generated (Narici et al. 1992); and the longer the fibre fascicle, the higher the rate of muscle shortening and therefore the rate of force production (Lieber and Friden 2000; Morse et al. 2005b).

The plasticity of muscles allows for significant adaptations of their geometry according to the amount and type of loading (Reeves et al. 2006). Thus, high intensity resistance

training in young and in elderly individuals is associated with increases of fascicle angle of vastus lateralis and triceps brachii, and high-speed training results in increases of fascicle length (Blazevich 2006). The normal ageing process is accompanied by reductions in both fascicle angle and length, and detraining (for example prolonged bed rest) also leads to reduction in these parameters (Kawakami et al. 2000; Narici et al. 2003). So far no study has investigated the architectural characteristics of rheumatoid muscle.

Agonist activation and antagonist coactivation

The degree of activation of a muscle influences its force generation. In young adults, close to 100% activation can often be achieved, whereas in the elderly, activation is compromised due to remodelling of the muscle fibres and their innervation (Morse et al. 2005a). In general, activation capacity levels >90% are considered optimal. Voluntary activation capacity is determined by comparing the force evoked by electrical twitches superimposed on maximal voluntary isometric contractions to post-contraction twitches of the resting muscle (Thom et al. 2005) and can be calculated as follows:
Voluntary activation (%) = (1 – (superimposed doublet torque/post MVC doublet torque)) x 100 (Harridge and White 1993; Allen et al. 1995).

The reduction in activation capacity in the elderly is due to a progressive decline in motor neurone number, and atrophy (reduction in size) of individual muscle fibres (Lexell 1995; Frontera et al. 2000; D'Antona et al. 2003). In immobilisation, muscle fibres also decrease in size due to reduced neural drive, but the number of motor neurons does not decline (Reeves et al. 2006).

Studies dating back 20 years ago investigated the effect of joint effusions on activation of the quadriceps muscle, and showed that fluid in the knee joint leads to reflex muscle inhibition as seen on EMG (Fahrer et al. 1988; Wood et al. 1988). This was confirmed in a recent study (Reeves and Maffulli 2008). In RA, Bearne et al. assessed activation capacity in patients who had knee involvement (Bearne et al. 2002). This study found 8% lower muscle activation capacity ($p<0.001$), together with reduced muscle strength, in RA compared to healthy controls. However, it was not clearly identified in this study whether the reduction in activation capacity was due to an alteration of intrinsic muscle properties, or secondary to pain, fatigue, or joint swelling. An investigation of muscle activation capacity in RA with controlled disease activity and where local knee pain or swelling is not confounding the results has been outstanding until the present thesis.

In addition, antagonist co-contraction has not previously been assessed in RA. Co-contraction of the knee flexor muscles with voluntary quadriceps contraction helps to maintain joint stability (Baratta et al. 1988). Antagonist co-contraction is higher in older compared with young individuals, thereby contributing to reduced specific force of the quadriceps (Hortobagyi et al. 2000; Macaluso et al. 2002; Reeves et al. 2004).

Contractile properties, velocity-specific power, and fibre type distribution

Power is the product of force generation and speed of muscle contraction (Evans 2000). In the elderly, reduced power output is related more to loss of function than strength, for example in complex movements such as in locomotion and balance tasks which need the ability to react quickly to changes of body position. Contractile properties are an indicator of the speed of contraction of the muscle. Their characteristics are determined by fibre type distribution and the intrinsic myosin heavy chain composition of the

muscle tissue. Contractile properties including peak torque, time to peak torque and half relaxation time are assessed by supramaximal resting twitches (Thom et al. 2005). Changes of the contractile properties can occur with ageing and disuse. In ageing, a reduction in the size and decrease in the number of muscle fibres as well as remodelling of the motor units resulting in an increase in fibre type grouping (i.e. fibres completely surrounded by fibres of uniform histochemical type instead of alternating with other fibre types) is seen in muscle biopsies (Lexell 1995). Lexell et al. found that in particular type 2 (fast-twitch) fibres are smaller in old compared to young individuals, with the size of type 1 (slow-twitch) fibres being less affected. In disuse, both fibre types suffer atrophy, with predominant type 1 atrophy at an early stage and type 2 atrophy later (Scelsi et al. 1982). Histochemical studies of muscle in RA were undertaken more than 30 years ago and show a preferential type 2 fibre atrophy (Haslock et al. 1970; Nordemar et al. 1976a; Nordemar et al. 1976b; Magyar et al. 1977). Since then, RA disease management has undergone major developments which might have influenced fibre type distribution. However, no study has reinvestigated the former findings in recent years. Hence, the effect of current treatments for RA on fibre type distribution is unknown. The biochemical myosin heavy chain composition which underlies different fibre types has also not been determined for RA patients up to now.

Intracellular processes of muscle atrophy and apoptosis

In addition to fibre type distribution, changes within each muscle fibre can lead to alterations in the muscle's force production and function. Each muscle fibre is composed of a mixture of myosin heavy chains (MHCs), with slow MHC isoforms predominating in type 1 fibres and fast isoforms in type 2 fibres. Staron et al. described a typical MHC distribution of the vastus lateralis muscle with 36% slow myosin isoform

MHC I, 41% of fast isoform MHC IIa and 23% very fast isoform MHC IIx in young men (with 44% MHC I, 34% MHC IIa and 22% MHC IIx in young women) (Staron et al. 2000). In ageing, MHC distribution is generally shifted towards slow isoforms (Klitgaard et al. 1990; Harridge et al. 1995; Andersen et al. 1999; Pearson et al. 2006), which is in keeping with the reduced speed of muscle contraction on EMG and the reduced power in this population (Thom et al. 2005; Klass et al. 2008). In contrast to this, low physical activity levels and immobilisation lead to reduced expression of MHC I, whilst the faster MHC IIa and IIx isoforms are increased (D'Antona et al. 2003; Gallagher et al. 2005; Trappe 2009). Studies on skeletal muscle in cardiac failure and inflammatory bowel disease have also shown a shift of the MHCs from slow to fast types (Vescovo et al. 1998; Staron et al. 2000; Cuoco et al. 2008). In RA, myosin heavy chain composition has not yet been examined.

On the cellular level, several key signalling pathways regulate protein synthesis and degradation in the muscle (Figure 3, reproduced from (Sharples and Stewart 2011)). Dysregulation of these processes, consisting in particular in inhibition of protein synthesis, is thought to be responsible for muscle atrophy in ageing and disuse. In the accelerated muscle loss seen in catabolic diseases such as cancer, acquired immune deficiency syndrome (AIDS), severe cardiac or renal failure, chronic inflammatory arthritides and chronic obstructive pulmonary disease, these mechanisms are thought to be involved and triggered by inflammatory cytokines and a lack of IGF-1 (Kotler 2000; Lemmey et al. 2001; Macdonald et al. 2004; Schmitt et al. 2007; Vogiatzis et al. 2007; Glass and Roubenoff 2010; Glass 2010).

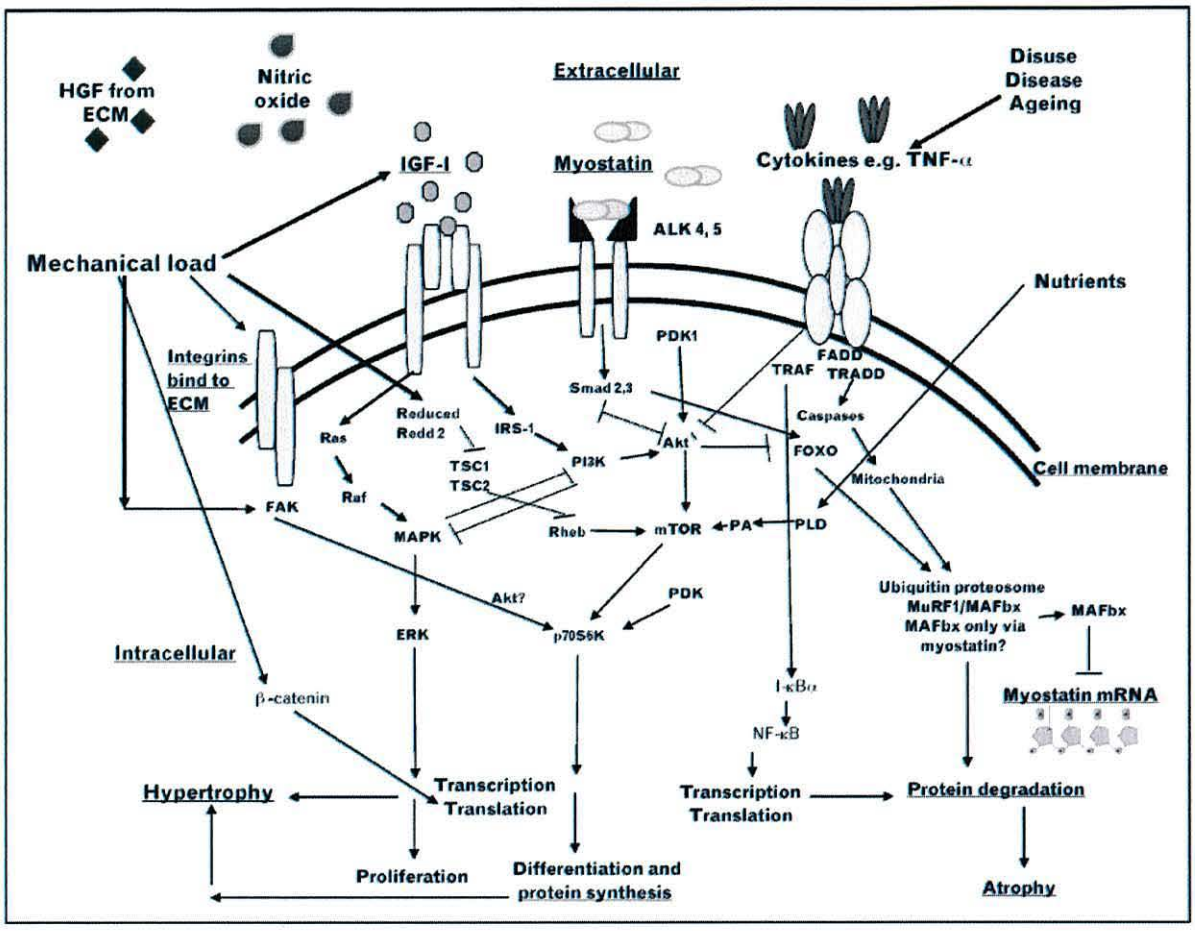

Figure 3. The regulation of protein synthesis and muscle hypertrophy versus protein degradation and muscle atrophy, reproduced from Sharples and Stewart 2011 with approval of the authors. The figure highlights the influence of TNF-α and IGF-1 on skeletal muscle protein turnover and illustrates their downstream effector pathways, including Akt, atrogin-1, caspases and IκBα, which will be investigated in chapter 5 of this thesis.

For example, TNF-α and other cytokines induce apoptosis through activation of caspases, and induce protein breakdown through inhibition of Akt activation, which is involved in the muscle hypertrophy process. Downstream of Akt and of caspase signalling, a reduction in atrogin-1 (also called MAFbx) promotes muscle atrophy. Akt activation is also impaired when IGF-1 is reduced. IGF-1 is considered as the main anabolic hormone for skeletal muscle and is expressed in liver as well as in skeletal muscle (Adams 2002). Finally, inhibitor-kappa B alpha (IκBα) is a transcription factor

downstream of TNF-α signalling which activates nuclear factor-kappa B (NF-κB) and thereby protein degradation and muscle atrophy (Potthoff et al. 2007).

In RA, increased levels of TNF-α are present in the synovial fluid and the circulating blood. Therefore, protein degradation and apoptosis represent possible pathways by which TNF-α induced muscle wasting could occur (Sharma and Anker 2002). In addition, IGF-1 has been shown to be reduced in the peripheral circulation of RA patients (Lemmey et al. 2001). However, further investigations of the intracellular processes in rheumatoid muscle which might be implicated in the catabolic processes are still outstanding. These will be the focus of chapter 5 of this thesis.

Tendon stiffness and Young's modulus

Finally, the force production of a muscle, and physical function in general, is not only influenced by the characteristics of the muscle tissue alone, but also by the properties of the tendon through which it is attached to bone. The function of a tendon is determined by its stiffness, i.e. its elastic properties (Onambele-Pearson and Pearson 2007). When the force of the contracting muscle is transmitted via the tendon, the resulting elongation of the tendon attenuates the impact of the contraction on the connected bone. The force output is thereby reduced by a small amount, but this is stored as elastic energy and released on relaxation of the muscle (Onambele-Pearson and Pearson 2007). Thus, this mechanism plays an essential part in the efficient performance of complex movements. Tendon properties also influence joint stability and the ability to make postural adjustments (Onambele et al. 2006), and consequently play a major role in maintaining balance and preventing falls. Studies using ultrasound have investigated the physiological properties of tendons, especially the load-bearing patellar and achilles tendons, in healthy populations, as well as their adaptation to high intensity exercise, to immobilisation, and changes with ageing (Reeves et al. 2003; Kubo et al. 2004; Onambele et al. 2006). In the elderly, and after immobilisation, tendon stiffness and size are reduced due to altered collagen content and cross-linking and reduced collagen fibril diameter and number (Carroll et al. 2008; Couppe et al. 2009).

The effects of ageing on tendon properties are still somewhat controversial as studies in aging have shown no change, or even increases in tendon CSA with age (Magnusson et al. 2003; Maganaris et al. 2004). This may be due to differences in the assessment methodology and/or in the physical activity of the respective participants. Resistance training leads to increases in tendon stiffness in young (Kubo et al. 2001) and older

adults (Reeves et al. 2003). Intrinsic adaptations of the tendon material properties are mainly responsible for the observed increases (Reeves et al. 2003; Seynnes et al. 2009), with additional increases in PT CSA in some training studies (Kongsgaard et al. 2007; Couppe et al. 2008; Seynnes et al. 2009). The calculation of Young's modulus is also used to investigate the intrinsic quality of a tendon by taking into account its length and cross-sectional area. For example, Reeves et al. showed that resistance training for 3 months improved Young's modulus in elderly by 69% (Reeves et al. 2003).

Tendon involvement in inflammatory arthropathies

In rheumatoid arthritis and other inflammatory arthropathies, the cytokine-induced synovial inflammation extends to the surrounding tendinous structures including synovial tendon sheaths, tendon insertions to bone (entheses) and the tendons themselves, resulting in clinical features of tendinopathy (McGonagle et al. 1998; Benjamin and McGonagle 2009; Emad et al. 2009; McQueen 2009). The corresponding structural alterations of tendons and peritendinous tissues found on magnetic resonance and ultrasound (US) imaging include thickening and hypervascularity of the tendon and enthesis and in tendons with a synovial sheath, thickening and synovial proliferation of the sheath with effusions (Falsetti et al. 2009; McQueen 2009). The physiological and functional consequences of these changes have hitherto not been investigated.

Ankylosing spondylitis and the enthesis concept

The spondylarthropathies are a group of conditions whose predominant feature is enthesitis. Enthesitis is defined as the inflammation of tendons, ligaments, and joint

capsules at the site of their insertion into bone (Ball 1971; Benjamin et al. 2007). This can lead to pain, stiffness and swelling of peripheral joints. In addition, involvement of the spine is common and characterised by inflammation of the sacroiliac joints and spinal ligaments, resulting in typical radiographic changes (narrowing, sclerosis, and ankylosis). Because of these characteristic spinal features, the prototype of the spondyloarthropathies has been termed ankylosing spondylitis (AS). This condition typically starts in young men in their twenties or thirties and is less common in women. It is frequently associated with psoriasis, inflammatory bowel disease and uveitis (Smith et al. 2006). Patients with advanced AS have the classic appearance of marked thoracic kyphosis, loss of body height, and often severe restriction of movement/loss of flexibility throughout the spine. As with RA, patients with AS have impaired physical function due to exacerbated muscle loss (Marcora et al. 2006a). In the treatment of AS, non-steroidal anti-inflammatory drugs (NSAIDS) play a major role, and DMARDS and biologic agents are used for severe disease.

Despite the similarities between RA and AS with regards to their autoimmune aetiology, the symptoms and consequences of articular and periarticular inflammation, including muscle wasting, important differences in the pathology of the conditions have to be taken into account. At the joint level, erosions and joint destruction are more common in RA, whereas bone formation is a hallmark of AS (Appel et al. 2009). In the soft tissues, RA is associated with tendinopathy in over 50% of patients (McQueen et al. 2005), whilst in AS the enthesis is the primary site of the autoimmune process (Benjamin and McGonagle 2009).

In AS, autoantibodies are directed against fibrocartilage antigens of the enthesis (Braun et al. 2000). The enthesis is also the site where stress is concentrated in the tendon-muscle complex, and is therefore prone to microdamage (Benjamin and McGonagle 2009). Additionally, it is assumed that genetic factors in spondylarthropathies such as human leucocyte antigen B27 (HLA-B27) lead to deposition of adjuvant molecules derived from bacteria preferentially at the damaged enthesis, followed by abnormal tissue repair responses (Benjamin and McGonagle 2009). These responses lead to thickening of the tissue and fibrocartilage formation in tendons (enthesophytes) and ligaments (such as syndesmophytes in the axial skeleton) and account for the gradual ankylosing of joints and vertebrae with loss of movement. In conjunction with this, inflammation of the synovium and tenosynovium in spondylarthropathies is thought to be a secondary reaction (Benjamin and McGonagle 2009).

Tendon pathology in rheumatoid arthritis

In contrast to this, the synovium in RA is thought to be the primary antigenic target; hence the tenosynovitis, i.e. inflammation of the synovium of tendon sheaths, which is a common and early feature of the disease, especially in the flexor and extensor tendons of the hand and wrist (Grassi et al. 1998; Kaibara et al. 2008). Involvement of the enthesis is less common and seen only in tendons that are undergoing high mechanical loads and in close proximity to synovial spaces i.e. the patellar and achilles tendons (Falsetti et al. 2009). This suggests that inflammatory changes at the enthesis and tendon in RA are probably secondary to adjacent synovitis, with local diffusion of inflammatory cells and molecules.

Summary of background and aims

Recent studies have shown that the quality of the tendon-muscle complex undergoes adverse changes with age and disuse in healthy populations. In particular, ageing has been shown to result in a decrease in muscle architecture, tendon stiffness, muscle activation capacity and an increase in the co-contraction of antagonist muscles (Reeves et al. 2003; Morse et al. 2005a; Morse et al. 2005b; Onambele et al. 2006). However, although muscle strength and mass have been shown to be lower in RA, e.g. (Madsen et al. 1998; Marcora et al. 2005), it is not known whether changes in the quality of the tendon-muscle complex of RA patients occur, and if so whether these changes contribute to the impaired function of these patients and whether they are disease-specific or also present in other inflammatory arthritides such as AS. In addition, the triggers and pathways for rheumatoid cachexia at the cellular level have yet to be determined.

The aims of this thesis are:

- To investigate whether muscle quality (including muscle specific force, muscle architecture, voluntary activation capacity and antagonist co-contraction, contractile properties and velocity-specific power) as well as intracellular metabolic processes (caspase-3, MHC composition) are impaired in patients with stable RA (chapter 3-5).
- To assess whether muscle quality, muscle intracellular processes and tendon properties (tendon stiffness and Young's modulus) are altered in acute RA (chapters 5 and 6).

- To determine whether tendon properties are altered in stable RA and whether they are different from the tendon properties of other inflammatory arthropathies (i.e. ankylosing spondylitis) (chapter 7).

Chapter 3: Skeletal muscle properties in rheumatoid arthritis patients

Introduction

As detailed in the previous chapter, disability in patients with RA is a multifactorial process involving various unaccounted factors. Loss of lean body mass plays an important role in impaired physical function, and exercise studies in RA have shown promising results in restoring muscle mass, and as a consequence, strength and function (Nordemar et al. 1976b; van den Ende et al. 1996; de Jong et al. 2003; Lemmey et al. 2009). However, no comprehensive assessment of the muscle characteristics has been undertaken to determine whether qualitative changes in muscle also contribute to RA disability.

The present study investigates the properties of skeletal muscle in community-based patients with RA with controlled disease. There is a multitude of factors that constitute muscle quality: skeletal muscle specific force, i.e. maximal isometric force per cross-sectional area of the muscle; maximal concentric force production; contractile properties and activation capacity; and architectural characteristics of the muscle. This study is the first to assess these factors in patients with RA. The results are presented alongside measures of body composition and physical function.

Patients and methods

Participants

Twenty-three patients with a diagnosis of RA according to the American Rheumatism Association 1987 revised criteria (Arnett et al. 1988) were recruited from the Rheumatology outpatient clinics of the North West Wales NHS Trust. These included a subset of patients who were found to be cachectic and were included in a separate analysis (chapter 4) (Matschke et al. 2010a). Inclusion criteria were disease duration of at least three years and stable disease activity (i.e. no flare or change in medication for the last three months). Exclusion criteria were: the presence of any other catabolic disease; high dose steroid therapy (i.e. >10 mg of Prednisolone daily) or a recent steroid injection; and joint replacement or current pain or swelling in the knee joints. The recruited RA patients were age- and sex-matched with 23 healthy volunteers. We initially recruited 25 patients but had to exclude two: one participant did not attend for the assessment, the other was tested but no matching healthy control was found. This led to a final cohort of 23 matched pairs. Written informed consent was given by all participants. The study was approved by the North Wales Health Authority Research Ethics Committee, and was conducted in compliance with the declaration of Helsinki.

Involvement in the study necessitated two appointments for participants: the first for body composition measurements, physical function tests and questionnaires, and the second for assessment of muscle size, strength and activation.

Disease activity

The RA patients completed the modified rheumatoid arthritis disease activity index (RADAI-5) (Leeb et al. 2008), a patient questionnaire assessing global disease activity over the past six months and currently in terms of swollen and tender joints, arthritis pain, general health, and duration of morning stiffness, with scores from 0 = no disease activity to 10 = active disease. Antecubital venous blood was analysed for erythrocyte sedimentation rate (ESR), a measure of systemic inflammation, in the Haematology Department of Gwynedd Hospital.

Body composition

Body composition was assessed by whole body dual energy X-ray absorptiometry (DXA) and bioelectrical impedance spectroscopy (BIS). In brief, upon presentation at the laboratory, body mass was measured on a calibrated balance scale (Seca, Hamburg, Germany) in a standard way with patients only in their underwear, barefoot, fasted (for at least 6 hours) and voided (Eston and Reilly 1996). Whole body DXA was then performed using a pencil-beam scanner (QDR1500, Hologic, Bedford, Massachusetts) to determine total and regional (left and right arm, left and right leg, trunk, head) lean and fat masses. The combined lean mass of arms and legs, called appendicular lean mass (ALM), is a proxy measure of total body skeletal muscle mass (Kim et al. 2002). The BIS measure (Hydra 4200, Xitron Technologies, San Diego, California) was used to ensure euhydration (Fuller et al. 2001; Marcora et al. 2005).

Physical function and quality of life

Physical function was assessed objectively with the 30-second chair sit-to-stand and 8-foot up and go lower body function tests derived from the "Senior Fitness Test" (Rikli

and Jones 2001). In addition, the participants performed a 50-foot walk and a single leg balance test (Mian et al. 2007). Subjective physical function was ascertained by the modified Health Assessment Questionnaire (mHAQ) and the 36 question Short-Form Health Survey (SF-36). The mHAQ scores 0-3, with higher scores for increased disability, and includes questions on an individual's difficulty in performing various activities of daily living (ADL's) (Pincus et al. 1999). The SF-36 provides a subjective evaluation of an individual's health status through questions on physical function, emotional health and quality of life (Keller et al. 1999), with physical component summary scores of 22-59, and mental component summary scores of 11-62 (higher scores mean better function). Habitual physical activity was assessed by a questionnaire developed by Saltin & Grimby (Saltin and Grimby 1968), which has previously been used in RA and ageing populations (Schroll 2003; Marcora et al. 2005). In this questionnaire, scores from 1 "being sedentary" to 4 "being involved in heavy physical activity" are given separately for occupational and recreational activities, providing a total score between 2 and 8.

Muscle strength and electromyography (EMG)

Following familiarisation with test procedures on the first laboratory visit, muscle strength and EMG were assessed during their second visit. For these, the participants sat upright on a Humac Norm isokinetic dynamometer

Figure 4; CSMI Medical Solutions, Stoughton, USA) with their right leg strapped to the dynamometer arm above the ankle with additional straps secured to prevent extraneous movement at the hips and shoulders. The hip angle was fixed at 90°. To assess maximal isometric knee extension and flexion torque (Nm), the knee joint angle was fixed at 70°

from full leg extension as this is the optimum angle for force production by the quadriceps muscles (Narici et al. 1992).

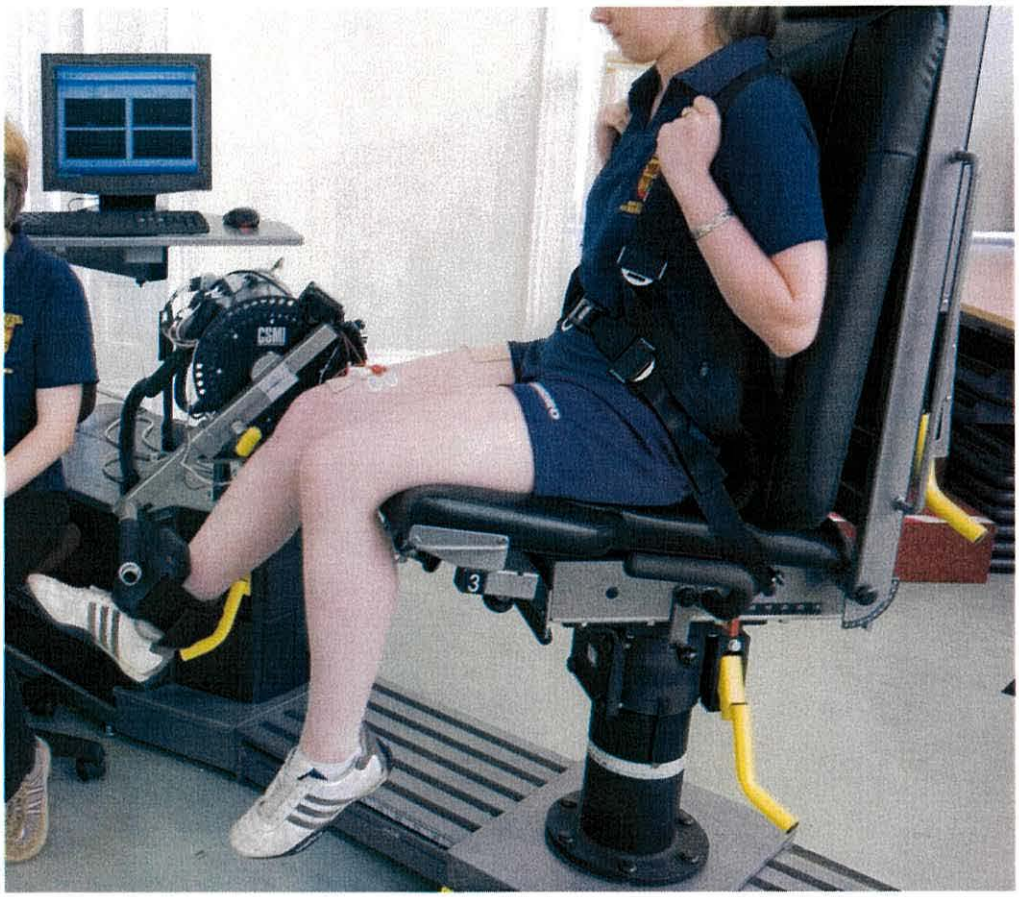

Figure 4. Setup of the isokinetic dynamometer to perform isometric and isokinetic quadriceps contractions.

After a set protocol of warm-up contractions at increasing torque levels (1 contraction at each 25%, 50% and 75% and 100% of maximum torque), participants performed three rapid maximal voluntary isometric contractions (MVCs) of the knee extensors. For these, the participants were instructed to push as fast and as hard as they could against the dynamometer arm. When voluntary torque peaked, participants were asked to maintain their effort for 2-3s. Participants then performed three rapid maximal isometric

knee flexions. Feedback was provided to the participants through a real time display of the muscle torque on a computer screen. There was at least 1 minute rest between each MVC to minimise fatigue, and verbal encouragement was given during each effort.

The contraction with the highest torque was used for analysis. The vastus lateralis (VL) muscle force was then calculated as representative of the quadriceps muscle group using established methods which take into account torque, patellar tendon moment arm length, and antagonist co-contraction torque estimated from electromyographic (EMG) activity (Reeves et al. 2004). Self-adhesive Ag-AgCl electrodes, 10 mm in diameter (Ambu, Denmark), placed over the VL and the long head of the biceps femoris (BF), recorded RMS EMG activity (root mean square of the raw EMG signal) during the MVCs (Figure 5). The anode-cathode configuration was kept consistent with an inter-electrode distance of 2 cm.

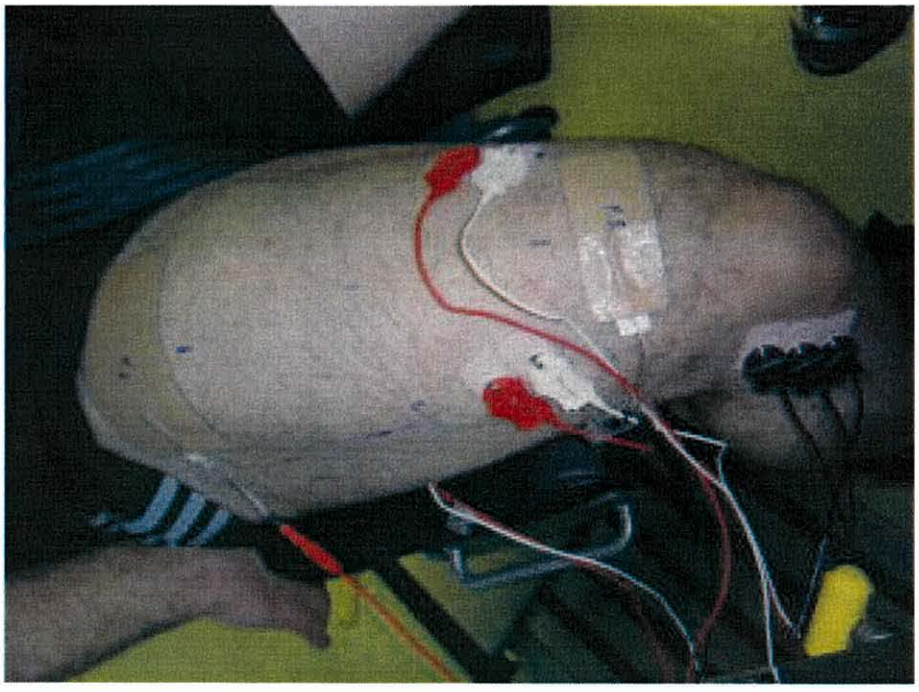

Figure 5. Setup for EMG recording via electrodes (red and white) over VL, VM and BF. Stimulation pads (beige) over the proximal and distal quadriceps.

BF antagonist co-contraction torque was calculated as follows:

BF torque = (BF EMG during knee extension / BF EMG during knee flexion) * knee flexion torque (Pearson and Onambele 2005).

Quadriceps force was calculated as follows:

Quadriceps force = (BF torque + knee extension torque) / estimated patellar tendon moment arm length (Reeves et al. 2004).

The relative contribution of the VL to quadriceps force was assumed from previous data by Narici et al. (Narici et al. 1992).

Voluntary muscle activation capacity and contractile properties

To detect deficits in muscle fibre recruitment, three additional MVCs of the knee extensors were performed by participants with superimposed and post-contraction supramaximal double twitches. Two single twitches with a constant inter-twitch interval of 10ms were used to create a doublet. The twitches were applied percutaneously from a DSV Digitimer Stimulator (Digitimer Ltd., Herts., U.K.) over the proximal (anode) and distal (cathode) regions of the quadriceps muscles using rubber stimulation pads (38 mm × 89 mm and 76 mm × 127 mm; Versastim; Conmed, Utica, NY) (Thom et al. 2005). The strongest voluntary contraction was used for analysis. Voluntary activation capacity of the quadriceps muscle group was calculated as follows:

Voluntary activation (%) = (1 – (superimposed doublet torque/post MVC doublet torque)) x 100 (Harridge and White 1993; Allen et al. 1995).

A series of 4 supramaximal resting single twitches was performed to assess contractile properties including peak torque, time to peak torque and half relaxation time (calculated from the mean of the 2nd, 3rd and 4th twitches) (Thom et al. 2005).

Muscle volume, architecture, physiological cross-sectional area (PCSA) and muscle specific force

In order to estimate muscle volume (VOL), VL muscle anatomical cross sectional area (ACSA; Figure 1, chapter 2) was measured by ultrasonography at 25%, 50% and 75% of the muscle length using a 7.5 MHz linear probe (MyLab50, Esaote, Firenze, Italy). The ultrasound probe was then placed along the VL to gain images of the muscle architecture at rest, which in turn were used to calculate the physiological cross-sectional area (PCSA). Previous studies have shown this to be a reliable method (Reeves et al. 2004), since PCSA takes into account muscle geometry (pennation angle, θ, and fibre fascicle length, Lf; Figure 2, chapter 2) and is at right angles to the direction of the fibre fascicles. In contrast, using ACSA alone can result in overestimation and underestimation of muscle size with training and detraining, respectively, as ACSA changes variably along the length of the muscle with the most pronounced changes occurring in the mid-region of a muscle (Reeves et al. 2004). Using PCSA therefore provides a more accurate measure of muscle size for the assessment of the mechanical stress in a muscle, as it will correctly relate the force developed along the fibres (Narici et al. 1992).

θ was defined as the angle of insertion of the fibre fascicle into the deep aponeurosis of the VL. Lf was calculated from θ and the muscle thickness (d, measured as the distance between the two aponeuroses of the VL) by the equation: $Lf = d/\sin\theta$ (Kumagai et al.

2000). PCSA was calculated as VOL/Lf, and specific force was calculated by normalising VL force to VL PCSA. The estimation of Lf with this method is associated with an error of 2 - 7% (Muraoka et al. 2001; Finni et al. 2003). After obtaining resting ultrasound images of muscle architecture, the ultrasound probe was held in the same position during three maximal voluntary knee extension contractions to assess the extent of changes to θ and Lf during contraction.

Concentric torque and velocity specific power

Participants then performed 4 MVCs of the knee extensors at velocities of 50°/s and 100°/s to assess maximal concentric torque and enable calculation of velocity specific power as follows:

Velocity specific power = maximum torque generated at each velocity * velocity in radians.

Also assessed were the angle of maximum concentric torque and the concentric torque at 70° knee flexion.

Statistics

In the absence of appropriate data for RA and AS patients, to determine the sample size, statistical power was calculated from maximal voluntary isometric muscle force differences between young and middle aged healthy individuals (Onambele et al. 2006). Assuming normal distribution, 2 groups, equal variance, common standard deviation for each group, 2–tailed test, alpha = 0.05, and power = 0.80, this analysis gave a requirement of 10/group. Depending on normality of distribution of the data, either Student's paired t test or Wilcoxon test were used to determine differences between the patient and matched control group. All statistical analyses were performed by SPSS

software version 14.0. Values are presented as means±SEM. Significance was accepted at the level $P<0.05$, with a P of 0.05-0.10 considered a trend.

Intraclass correlation coefficients (ICC's) were employed to assess the reliability of the ultrasound measurements. The analysis of the US pictures for θ, muscle depth and VL ACSA was performed blinded on two separate occasions. ICC's were r = 0.81 - 0.95 on a subsample of 10 participants. Correlations between functional capacity and power produced during concentric MVCs were calculated with simple regression analysis.

Results

Clinical characteristics

The RA patients and healthy controls were well matched for sex, age, habitual physical activity level, and body size (Table 1). All patients had stable disease, with a mean RADAI-5 score of 3.1±0.3 (range 1.0 - 5.4), indicating low disease activity. ESR was 19.1± 2.5 mm/hr (range 3-41), representing normal levels for this age group or low grade inflammation. Twelve patients (52%) were rheumatoid factor positive. The average disease duration for patients was 12.9±1.8 years. Treatment for RA was as follows: 18 patients (78%) were taking methotrexate, 3 of them combined with an anti-TNF agent (2 infliximab, 1 adalimumab); 3 (13%) were taking sulfasalazine; 9 (39%) NSAIDS; 6 (26%) prednisolone (range 1-10mg daily, average dose 7.3mg).

Body composition

A trend towards reduction of ALM by 1kg (5.8%, p=0.10) was observed in the patient group compared to the controls, despite a tendency toward higher BMI (by 4.4%, n.s.)

and body weight (by 2.5%, n.s., Table 1). Body fat (BF) was significantly higher in the patients (Table 1).

	RA patients	Healthy controls	P
Age (years)	60 ± 2, range 22-72	60 ± 3, range 22-76	0.95
Habitual physical activity (2-8)	4.87 ± 0.14	4.35 ± 0.26	0.09
Height (m)	1.65 ± 0.01	1.68 ± 0.02	0.29
Weight (kg)	75.5 ± 3.1	73.6 ± 2.6	0.66
Body mass index (kg/m^2)	27.6 ± 0.9	26.4 ± 0.99	0.45
Appendicular lean mass (kg)	16.3 ± 0.9	17.3 ± 0.9	0.10
Total body fat (%)	41.1 ± 2.2	35.9 ± 2.1	**<0.05**

Table 1. Anthropometric, body composition, and habitual physical activity data of patients with RA (n = 23; 16 women) and age- and sex-matched healthy controls. Results presented as mean ± SEM. Significant p values are presented in bold.

Physical activity and function

As expected, objective physical function was reduced in the RA group relative to the matched healthy controls: sit-to-stand by 10.5% (number of repetitions 12.6±0.7 vs 14.0±0.5, p=0.09), 8-foot up and go by 17.3% (6.1±0.3 vs 5.2±0.2s, p<0.01), 50-foot walk by 25.3% (9.3±0.5 vs 7.5±0.3s, p<0.001), and one-leg standing balance by 27.0% (49.0±5.0 vs 67.1±4.9s, p=0.01); Figure 6), as were the subjective measures of self-assessed physical function: mHAQ (0.64±0.07 vs 0.11±0.06, p<0.001) and SF-36

physical component summary score (38.2±1.9 vs 51.3±1.1, $p<0.001$). Similarly, patients scored lower on psychological quality of life factors with a mean SF-36 mental component summary score of 39.8±1.5 compared to 44.2±0.9 for the controls ($p<0.05$). Both the RA and the control groups reported low habitual physical activity levels, although the healthy controls tended to be even more sedentary than the patients (Table 1).

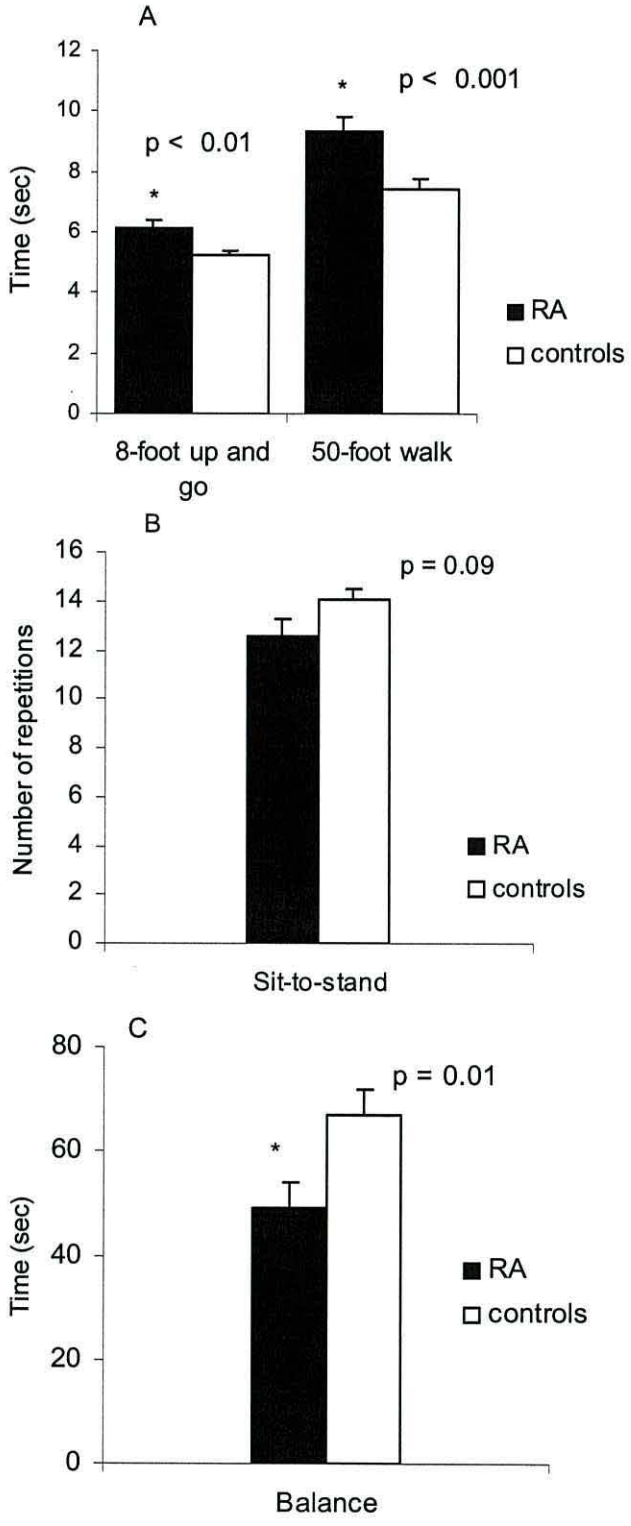

Figure 6. Significantly impaired physical function in RA patients compared to healthy controls. In panel A, the lower the time the better the physical function; in panels B and C, the higher the number or the longer the time, the better the physical function. Results presented as mean ± SEM.

Muscle specific force and architecture

Muscle physiology data are presented in Table 2. Compared to the healthy controls, the patients with RA had a smaller PCSA (by 13.9%, $p<0.05$). Patients had a non-significant reduction of 6.0% in VL force compared to their controls ($p=0.35$). Overall, specific force, calculated by normalising VL force to VL PCSA, was not different between the groups ($p=0.40$, Table 2).

In the RA group, resting θ was 12.5% less ($p<0.05$) than in the controls, whilst Lf tended to be longer (12.1%, $p=0.06$) (Table 2). At maximal contraction, θ increased by 24.6% in the patient group and by 16.2% in the control group, and Lf decreased by 19.1% in the patients and 19.0% in the controls with no differences between the groups ($p=0.78$ and $p=0.16$, respectively).

Muscle contractile properties and voluntary activation capacity

During electrical stimulation of the quadriceps at rest, peak torque, time to peak torque and half-relaxation time were similar in the RA and the control groups ($p=0.68$-0.99). In addition, no difference was found in voluntary muscle activation capacity between the groups ($p=0.86$) (Table 2).

	RA patients	Healthy controls	P
VL force (N)	785.6 ± 49.0	835.9 ± 45.0	0.36
VL volume (cm^3)	446.8 ± 22.5	461.4 ± 16.6	0.47
VL PCSA (cm^2)	34.2 ± 1.9	39.7 ± 2.0	**<0.05**
VL θ at rest (°)	8.85 ± 0.3	10.11 ± 0.3	**<0.05**
VL θ at contraction (°)	11.7 ± 0.5	12.1 ± 0.5	0.78
VL Lf at rest (cm)	13.4 ± 0.5	12.0 ± 0.5	0.06
VL Lf at contraction (cm)	11.3 ± 0.6	10.1 ± 0.6	0.16
Voluntary activation capacity (%)	82.3 ± 2.8	84.0 ± 2.8	0.86
VL specific force (N/cm^2)	24.0 ± 1.7	22.0 ± 1.5	0.40
Twitch peak torque (Nm)	20.1 ± 1.5	19.4 ± 1.1	0.71
Twitch time to peak torque (s)	0.12 ± 0.00	0.12 ± 0.00	0.70
Twitch half-relaxation time (s)	0.10 ± 0.01	0.10 ± 0.01	0.99
Maximum power at speed of 50°/s (W)	96.7 ± 8.2	100.8 ± 5.3	0.61
Maximum power at speed of 100°/s (W)	159.7 ± 14.3	157.7 ± 9.0	0.99
Angle of max. power at 50°/s (°)	57.0 ± 1.8	58.6 ± 1.8	0.50
Angle of max. power at 100°/s (°)	54.5 ± 1.4	56.5 ± 1.9	0.28

Table 2. Muscle physiological data of patients with RA (n = 23; 16 women) and age- and sex-matched healthy controls. Results presented as mean ± SEM. VL = vastus lateralis, PCSA = physiological cross-sectional area, Lf = fibre fascicle length.

Concentric torque and velocity specific power

RA and control groups were not different regarding their maximum concentric torque at 50°/s and 100°/s (p=0.61 and p=0.99, respectively) and velocity specific power (p=0.61 and p=0.99, respectively) (Table 2). Similarly, there was no difference in the angle of maximum torque and power at both speeds (p=0.50 and p=0.28, respectively) (Table 2), and in the concentric torques measured at 70° of knee flexion (87.1±7.1 vs 93.0±7.8 Nm, p=0.45 at 50°/s, and 65.3±4.9 vs 69.5±5.9 Nm, p= 0.43 at 100°/s) (Figure 7).

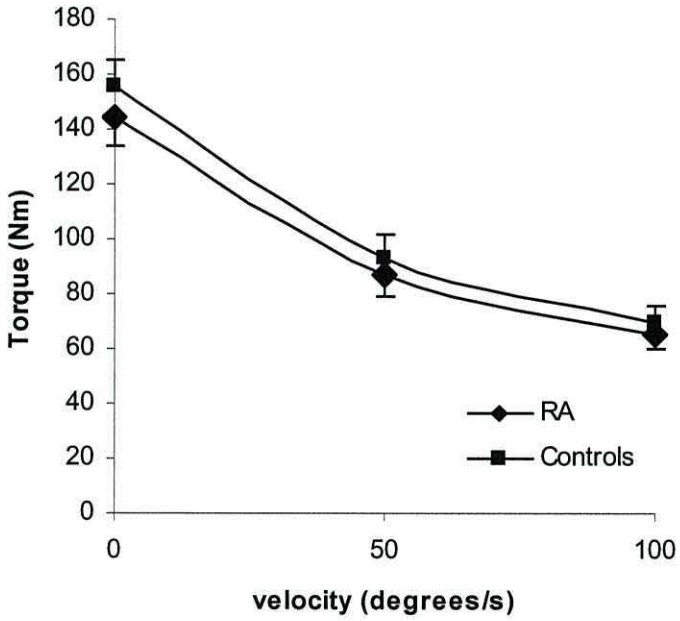

Figure 7. Isometric and concentric quadriceps torque at knee flexion angle of 70°. Results are means ± SEM.

Discussion

The present study demonstrates that in patients with stable RA, physiological properties of skeletal muscle including specific force, contractile properties, activation and concentric force and power are not compromised and thus not likely contributors to

reduced physical function in RA. This is important and encouraging information for rheumatology health professionals and sports scientists involved in designing exercise training for RA patients, and the results are a step forward in piecing together the jigsaw of interactions between muscle function and disability in RA.

In common with most RA patients, our patient cohort had impaired physical function, with the degree of impairment comparable to our previous findings (Marcora et al. 2005; Lemmey et al. 2009). This study also confirms the typical aberrant body composition of RA patients with reduced ALM (by 1kg, albeit in this instance a non-significant decrease) and increased body fat (41.1% in RA vs 35.9% in non-RA) (Marcora et al. 2005; Lemmey et al. 2009). For example, Giles et al. found ALM to be significantly reduced by 1.4 kg in men with RA compared to non-RA controls (n=72 in each group) and a non-significant ALM reduction of 0.3 kg in women with RA compared to non-RA (n=117 in each group) with concomitant significantly higher body fat percentage (42.1% in RA vs 38.7% in non-RA) (Giles et al. 2008b).

In accordance with the results on muscle mass and physical function, force production tended to be reduced in the RA group in the current study. Other factors intrinsic to muscle that influence physical function were then assessed and it was established that muscle specific force was preserved. In muscle physiology research, specific force (force per unit muscle) is the main determinant of the quality of a muscle. The unit of muscle can be given as volume, ACSA, or as PCSA, which takes into account the length of the fibre fascicles and their direction as determined by pennation angle θ. PCSA has been shown to be a better predictor of intrinsic muscle force than ACSA or volume (Narici et al. 1992), since muscle force is dependent on the number of

sarcomeres in parallel (more sarcomeres leads to a larger angle of insertion of fibres into the deep aponeurosis of the muscle and thus greater force), and on the number of sarcomeres in series (more sarcomeres leads to longer fibre fascicles and thus to increased velocity of contraction (Morse et al. 2005b). In this study, PCSA was significantly reduced in RA patients and was accompanied by minor architectural changes with a smaller θ and a non significant trend to a longer Lf in the patient group. Force normalised to PCSA (and ACSA and volume) however did not reveal any change in specific force. The alterations in muscle architecture were small in absolute values and therefore probably insufficient to have functional consequence. This was further confirmed by the finding that the trend to a longer Lf did not lead to a change in contraction velocity measured with resting twitches, concentric torque or velocity-specific power. Another explanation could be that the resting muscle architecture is less predictive of muscle function than the architecture of the contracted muscle, which in our study was not significantly different between patient and control groups i.e. the minor changes that we found in resting θ were not present in the contracted muscle.

In the current study, the significant reduction of PCSA in the patient group compared to the control group corresponded to the lower muscle mass on DXA, whereas ACSA and VOL were not different between the groups. Unlike DXA or MRI, ultrasound does not account for intramuscular fat. In the elderly, intramuscular fat and connective tissue are increased compared to young people (Kent-Braun et al. 2000). Thus, ACSA and volume measures alone might represent an overestimation of muscle content.

Our results are in contrast to findings in ageing and disuse, where a disproportionate loss in muscle strength and in consequence lower specific force are typical features, and

these are associated with reductions in Lf and θ̣ (Morse et al. 2005b). This suggests that the process of muscle loss in ageing and disuse is different from the muscle loss that results from a chronic inflammatory condition like RA. The fact that Lf in our study tended to be longer rather than shorter in the patient group might represent an adaptation, possibly due to alterations in tendon compliance which warrant further investigation (see chapters 6 and 7).

In keeping with the preserved specific force there were no differences in other factors that affect muscle force and function, namely activation capacity, antagonist co-contraction and contractile properties. Muscle activation capacity is typically reduced in old age (Morse et al. 2005a) and following prolonged disuse (Stevens et al. 2006). Studies on knee osteoarthritis and joint replacement and after traumatic knee joint damage (Hurley et al. 1997; Urbach and Awiszus 2002) have also found reductions in activation capacity. The observation that in our study activation capacity was not different between RA patients and matched healthy controls suggests that our patients' efforts were not inhibited by guarding, pain or fatigue, and that they had not lost the capacity to contract their muscles through disuse. In contrast, Bearne et al. (Bearne et al. 2002) found muscle activation to be 8% lower in an RA compared to a control group. This disparity in results might be explained by their selecting patients with confirmed knee involvement, and age differences between their patient and control groups (patients were on average 6 yrs older). In contrast, our patients did not have obvious knee involvement, as those with knee pain or swelling were excluded, had a low mHAQ score, and our groups were age-matched.

Matching for age and sex was important because of the wide age range of the participants, and helped to exclude age- and sex-related differences in force-production and other parameters. Similarly, groups were aligned for habitual physical activity to avoid disparate effects of disuse or exercise training. If anything, the controls were even more sedentary than the patients in this study ($p=0.09$).

Despite the preserved specific force and ability to recruit muscle fibres of our RA patients, their functional capacity was still significantly impaired. To fully understand the role of muscle in RA disability, further research is required, in particular on tendon stiffness, and characteristics at cellular level like myosin heavy chain distribution and intramuscular protein metabolism (see chapters 5-7). To minimize extrinsic influences on muscle such as joint deformity or presence of a joint effusion that might lead to malalignment and thereby altered mechanics of the joints, we only included patients with stable disease and excluded those with knee swelling or pain. However, assessing physiological muscle characteristics in active RA pre-treatment (this will be investigated in chapters 5 and 6) or in persistently active RA in patients who have not responded well to DMARDS (this will be investigated in chapter 5) would also be relevant, albeit difficult in view of the confounding factors of joint pain, swelling, fatigue and disuse that accompany active RA.

In conclusion, this study shows that physiological muscle properties are preserved in patients with stable RA, and thereby suggests that rheumatoid muscle has the potential to respond to physical training in a similar way to healthy muscle. This is in accordance with the success that high intensity exercise training studies have shown in increasing muscle quantity and strength in RA patients and in the near identical training responses

(of strength, muscle mass and fat mass) of RA patients and age-matched healthy controls performing the same training (Hakkinen et al. 2005; Marcora et al. 2005; Lemmey et al. 2009). With the properties of rheumatoid muscle being apt to respond "normally" to exercise training, the results of our study further promote the inclusion of exercise in the routine management of stable RA patients.

Chapter 4: Muscle specific force, architecture and activation in cachectic rheumatoid arthritis patients

Introduction

In chapter 3 we showed that muscle properties are not impaired in a community-based population with stable RA. In this study cohort, muscle mass showed a trend to be lower in RA compared to healthy controls, but this trend did not reach statistical significance. If muscle quality is affected by the inflammatory process in RA, this may only be apparent in patients who are significantly muscle-wasted. In the following study we therefore chose a subgroup of patients from the first study who were muscle-wasted as per the definition for sarcopenia by Baumgartner, i.e. with their appendicular skeletal muscle mass being less than two standard deviations below the mean of a young reference group (Baumgartner et al. 1998).

As detailed in chapter 2, impaired physical function is characteristic of patients with RA and is strongly correlated with muscle mass, the main predictor of muscle strength (Giles et al. 2008a). Muscle wasting is more prevalent and severe in patients with RA (termed "rheumatoid cachexia") than in the general population, perhaps due to increased muscle protein catabolism induced by inflammatory cytokines (Roubenoff et al. 1992).

The aim of this study was to determine whether muscle specific force, voluntary muscle activation capacity and muscle architecture (as per chapter 3) are compromised in cachectic RA patients compared to healthy age- and sex-matched controls.

Patients and methods

Fourteen cachectic RA patients (disease duration ≥ 3 years) were recruited from the Rheumatology clinics of the North West Wales NHS Trust. Patients with pain or swelling in the knee joints, disease flare or change in medication in the previous three months, other catabolic diseases or knee joint replacement were excluded. Significant muscle wasting ("cachexia") was determined following assessment of appendicular lean mass (ALM) by whole body dual energy X-ray absorptiometry (DXA) using the definition of Baumgartner et al. (appendicular skeletal muscle mass less than two standard deviations below the mean of a young reference group) (Baumgartner et al. 1998). Age- and sex-matched healthy controls were recruited from the local community.

Maximal voluntary isometric knee extension and flexion torques (Nm) of the right leg (knee joint angle 70°, hip angle 90°, arms crossed) were determined on an isokinetic dynamometer (CSMI Medical Solutions, Stoughton, USA). Vastus lateralis (VL) force was calculated taking into account maximal voluntary torque, patellar tendon moment arm length (Onambele-Pearson and Pearson 2007) , and antagonist co-contraction was estimated from electromyographic (EMG) activity (Reeves et al. 2004). Superimposed and post-contraction supramaximal percutaneous double twitches from a DSV Digitimer Stimulator (Digitimer Ltd., Herts., U.K.) were applied over the quadriceps to determine voluntary activation capacity (Reeves et al. 2004).

Ultrasonography was used to assess VL volume (VOL; from VL length and VL anatomical cross-sectional area) and muscle architecture i.e. pennation angle (θ) and fibre fascicle length (Lf)

Figure 8), which in turn determined physiological cross-sectional area (PCSA = VOL/Lf) (Reeves et al. 2004). The primary measure, muscle specific force, was calculated as VL force/PCSA (Reeves et al. 2004).

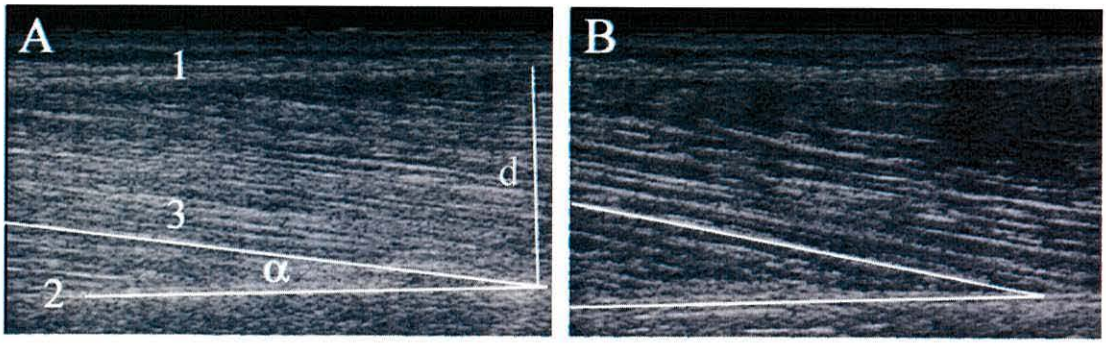

Figure 8. Sagittal-plane sonographs of the vastus lateralis muscle of a patient with rheumatoid arthritis (A) and a healthy control (B). Note the greater pennation angle (θ) in the muscle of the healthy control. 1 = superficial aponeurosis, 2 = deep aponeurosis, 3 = fibre fascicles; θ is the angle of insertion of the fibre fascicle into the deep aponeurosis of the VL.

Further measures were: disease activity using the modified rheumatoid arthritis disease activity index (RADAI-5) (Leeb et al. 2008) and erythrocyte sedimentation rate (ESR); objective physical function as described previously (Rikli and Jones 2001; Mian et al. 2007); the Modified Health Assessment Questionnaire (mHAQ) (Pincus et al. 1999) and Short-Form Health Survey (SF-36). Additionally, a questionnaire used previously in RA and ageing populations (Saltin and Grimby 1968) to assess habitual physical activity was used to exclude physically very active participants (>6 on a scale from 2-8).

Depending on normality of the data, student's paired t test or Wilcoxon test were used to detect differences between patient and control groups ($P<0.05$). All statistical analyses were performed by SPSS software version 14.0. Significance was accepted as $P<0.05$, with a $P = 0.05$-0.10 considered a trend. Values are presented as means±SEM.

Results

All patients were on disease-modifying anti-rheumatic medication and had low disease activity (Table 3). The groups were well matched for age and habitual physical activity (Table 4). Relative to controls, patients had reduced objective and self-assessed physical function (Table 4), less ALM and smaller VL volume, and trends toward lower PCSA, lower force, and a smaller pennation angle (Table 5). However, there were no differences in either muscle specific force or voluntary muscle activation capacity (Table 5).

Disease duration (years)	12.7 ± 2.7
RADAI-5 score (0-10)	2.94 ± 0.33, range 0.8 – 6
ESR (mm/hr)	22.9 ± 3.0, range 6 – 41
Anti-rheumatoid medication (no. of patients):	
Methotrexate	11
Sulfasalazine	1
Etanercept + Methotrexate	2
Prednisolone (dose range 1-7.5mg/day)	4
NSAIDS	5

Table 3. Clinical characteristics of RA patients (n = 14; 11 women). Results presented as mean ± SEM.

	RA patients	Healthy controls	P	% difference: patients to controls
Age (years)	61.6 ± 3.3, range 22-72	62.2 ± 3.5, range 22-76	0.31	1.0
Physical activity (2-8)	4.71 ± 0.19	4.64 ± 0.34	0.86	-1.5
BMI (kg/m^2)	25.8 ± 0.8	27.2 ± 1.5	0.45	5.2
Appendicular muscle mass (kg)	14.1 ± 0.8	16.2 ± 0.7	**0.003**	12.7
Total body fat (%)	42.2	38.8	0.30	-8.8
Sit-to-stand (number)	12.6 ± 1.0	14.4 ± 0.7	0.15	12.4
8-foot-up-and-go (s)	6.4 ± 0.4	5.5 ± 0.2	**0.03**	-17.3
50-foot-walk (s)	9.6 ± 0.7	7.6 ± 0.4	**0.01**	-25.7
Single leg balance (s)	42.4 ± 5.5	58.6 ± 6.3	0.07	27.4
mHAQ (0-3)	0.63 ± 0.08	0.18 ± 0.04	**0.001**	-253
SF-36 physical component summary score (22-59)	39.3 ± 2.1	50.7 ± 1.2	**< 0.001**	22.4
SF-36 mental component summary score (11-62)	40.2 ± 1.54	44.6 ± 0.9	0.10	9.8

Table 4. Demographics, body composition, and physical function data of sarcopenic RA patients (n = 14; 11 women) compared to age- and sex-matched healthy controls. Results presented as mean ± SEM.

	RA patients	Healthy controls	P	% difference: patients to controls
VL force (N)	691.6 ± 58.4	785.5 ± 44.5	0.10	12.0
VL volume (cm^3)	391.2 ± 21.4	445.0 ± 13.3	**0.01**	12.1
VL PCSA (cm^2)	31.3 ± 2.3	37.5 ± 2.0	0.07	16.6
VL pennation angle (°)	8.5 ± 0.4	9.7 ± 0.4	0.07	12.0
Voluntary activation capacity (%)	80.2 ± 3.6	82.1 ± 3.8	0.81	2.4
Muscle specific force (N/cm^2)	23.0 ± 2.1	21.9 ± 1.9	0.70	-5.0

Table 5. Muscle specific force and quality data of sarcopenic RA patients (n = 14; 11 women) compared to age- and sex-matched healthy controls. Results presented as mean ± SEM.

Discussion

In this study we demonstrated that muscle specific force and muscle activation capacity are preserved even in RA patients with significantly impaired physical function and reduced muscle mass. Muscle wasting was a selection criterion in our study. This is a phenomenon seen more frequently in RA patients (Roubenoff et al. 1992) than in the healthy population, and thought to reflect systemic effects of inflammatory cytokines on muscle tissue. In our patients the relative reduction of muscle mass of 13% is in accordance with other studies (Roubenoff et al. 1992).

This finding leads to two important conclusions: Firstly it confirms that muscle loss in RA is a process that differs from that seen in ageing and disuse, wherein muscle specific force and activation capacity are also reduced (Roubenoff et al. 1992). Secondly, it helps to explain why rheumatoid muscle retains the same capacity to adapt to physical training as healthy muscle, thus emphasising the potential of high intensity exercise to restore muscle quantity and physical function in patients with established RA (Hakkinen et al. 2005; Marcora et al. 2005; Lemmey et al. 2009).

In determining muscle specific force, we used definitions of force and size that are standard in muscle physiology research. These take into account architectural features (Lf and θ), influencing the mechanical output of the muscle, and factors affecting force production (co-contraction of antagonist muscles) (Reeves et al. 2004). Since the trend towards lower force levels in our RA patients corresponded with loss of PCSA, the force normalised for PCSA (i.e. muscle quality) was not compromised. Although θ

tended to be smaller in the patient group, this architectural change was not sufficient to influence the force output.

Similarly, muscle activation capacity was not different between our groups, contrasting with Bearne et al. (Bearne et al. 2002) who found 8% lower muscle activation ($p<0.001$) in RA patients with confirmed involvement of the knee joint compared to healthy controls. However, their results may have been compromised by confounding factors such as fatigue, pain and joint effusions on muscle force and activation, whereas we excluded patients with active disease and local knee inflammation. Although our data cannot be extrapolated to patients with persistently active disease, our stable RA patients are a relevant population to study because in rheumatological practice most patients only start exercising once disease control has been achieved with medication.

There are limitations to this study. Firstly, the wide age range of the participants and the inclusion of both genders contributed to the variability of force levels. Secondly, the work intensive nature of muscle specific force assessments necessarily limited the subject numbers.

This is the first study to report on muscle specific force of the cachectic rheumatoid muscle. In conclusion, even in patients with significant muscle loss, muscle specific force and the ability to recruit muscle fibres are not compromised. Therefore, these factors are unlikely to contribute to the disability seen in RA patients. Thus, this confirms the findings of the first study (chapter 3). However, further research is required to determine other factors that might influence physical function in RA, for example MHC composition or intracellular processes that determine protein turnover in

the rheumatoid muscle, and tendon characteristics. These will be addressed in the next chapters in patients with stable and with active RA.

Chapter 5: Biochemical adaptations in skeletal muscle of patients with stable and active RA

Introduction

The molecular mechanisms underlying muscle loss in inflammatory arthropathies (rheumatoid cachexia) are not fully understood. Raised levels of inflammatory cytokines, such as TNF-α, IL-1, and IL-6 have been implicated in the accelerated loss of muscle mass seen in RA (Roubenoff et al. 1994). TNF-α is thought to be the principal mediator, possibly through increased apoptosis of myocytes (Sharma and Anker 2002). Moreover, anabolic pathways are impaired in rheumatoid cachexia. Locally produced (i.e. muscle) insulin-like growth factor-I (IGF-1) is considered to be the main anabolic hormone responsible for maintaining and increasing adult skeletal muscle (Adams 2002). Interference of TNF-α signalling with pathways controlling muscle IGF-1 expression and action are likely mechanisms impairing anabolic processes in muscle (Frost et al. 2003).

However, the precise downstream intramuscular processes induced by circulating inflammatory mediators are still largely unknown in rheumatoid cachexia, which is alarming given the high prevalence of cachexia among RA patients and its detrimental effects on physical function and quality of life. In addition, the potential sensitivity of different myosin heavy chains to cachexia in RA is not known, though some histological studies have suggested a preferential atrophy of type 2 fibres in RA (Nordemar et al. 1976a; Nordemar et al. 1976b).

In synovial cells in RA, the downstream intracellular signalling pathways of TNF-α are mediated via NF-κB and involve activation of IκBα, leading to protein degradation (Okazaki et al. 2005), and activation and cleavage of caspases, which can induce apoptosis (Smith et al. 2010). Disease-modifying anti-rheumatic drugs have been shown to alter cytokine-driven processes in the synovium, although their effects on muscle metabolism have not been examined. Methotrexate (MTX), a folate antagonist, has been routinely used in the treatment of RA since the 1980s and is now the most commonly used first-line disease-modifying drug (Kinder et al. 2005), though its anti-inflammatory properties are still not clearly understood. One of its proposed mechanisms includes inhibition of IκBα phosphorylation and thereby of NF-κB activation (Majumdar and Aggarwal 2001). Anti-TNF agents have been available for RA since the late 1990s. These very effective drugs are currently indicated only for severely afflicted patients (Deighton 2005; Ledingham and Deighton 2005). As with MTX, the effect of anti-TNF therapy on muscle metabolism is not known.

The objectives of our investigation were to determine the underlying mechanisms of muscle wasting in RA. To achieve this, two separate studies were performed. The cross-sectional study 1 compared MHC distribution and the expression of caspase 3 in stable RA patients with healthy controls. The longitudinal study 2 investigated intracellular markers of muscle atrophy (atrogin-1, IκBα), hypertrophy (pAkt) and apoptosis (caspase-3) in muscle biopsies of patients with active RA before and three months after disease control with either MTX or anti-TNF antibody therapies.

Patients and methods

Participants

For the cross-sectional study 1, 17 patients (age 60.7 ± 2.3 years, 11 women) with stable RA and disease duration of at least 3 years were recruited from the outpatient clinics of the Department of Rheumatology, North West Wales NHS Trust, as were 13 healthy controls (age 61.7 ± 2.5 years, 8 women). All had a muscle biopsy performed for cross-sectional comparison of their skeletal muscle myosin heavy chain composition Assessment of patients' disease activity was by DAS28. The study was approved by the local NHS ethics committee and conformed to the declaration of Helsinki.

For the longitudinal study 2, 10 RA patients with active disease (age 57.9 ± 3.6 years, 7 women) gave written informed consent. Of these, seven had established RA with a clinical indication for treatment with an anti-TNF agent. Thus, according to the current British Society for Rheumatology (BSR) guidelines (Ledingham and Deighton 2005), they met the American Rheumatism Association 1987 revised classification criteria for RA (Arnett et al. 1988) and had a 28-joint disease activity score (DAS28; details see below) of >5.1 despite prior treatment with at least two DMARDS. All patients were previously naïve to anti-TNF treatment. The other 3 patients enrolled had a recent diagnosis of RA (1-2 weeks, with joint symptoms <6 months), and had yet to commence first-line therapy with MTX to achieve disease control. Excluded were patients with chronic arthritis diagnosed before the age of 16 years, or affected by other catabolic or potentially confounding concurrent medical conditions. The study was

approved by the local NHS ethics committee. All procedures conformed to the declaration of Helsinki.

Muscle biopsies from the vastus lateralis were performed prior to and 3 months after starting medication (MTX or anti-TNF-α agent, respectively). Disease activity was measured by DAS28. This score is widely used in clinical practice and consists of a composite measure of clinical parameters including the number of tender joints (0-28), the number of swollen joints (0-28), the erythrocyte sedimentation rate (ESR) (mm/hr) and general health perceived by the patient measured on a visual analogue scale from 0 to 100 mm. Scores >5.1 indicate high, >3.2 to ≤5.1 moderate and ≤3.2 low disease activity (Prevoo et al. 1995). In addition, functional parameters were assessed by the modified HAQ and SF-36 questionnaires (Keller et al. 1999; Pincus et al. 1999) and objective lower body physical function tests including the 30-second chair sit-to-stand, 8-foot up and go (Rikli and Jones 2001), 50-foot walk, and single leg balance tests (Mian et al. 2007), and habitual physical activity was estimated by questionnaire (Saltin and Grimby 1968). Following 3 months of treatment, all measures including the muscle biopsy were repeated.

Muscle biopsy procedure. Samples were taken at a constant depth - standardised from the point of entering the muscle tissue - from the mid-portion of the VL (anterolateral aspect of the middle third of the thigh) of the dominant leg under local anaesthesia (2% lignocaine) using a bioptic gun with a 14 gauge needle (TruCore® II Biopsy Instrument, Angiotech Gainesville, FL, USA). The tissue was snap-frozen in liquid nitrogen. The follow-up samples after 3 months were taken 1 cm away from the first biopsy site.

Tissue homogenisation The frozen muscle specimens (approximately 40 mg) were pulverised in 150 µL of frozen Cell-disruption Buffer (PARIS kit, Ambion, Austin, TX, USA) under nitrogen cooling using a micro-dismembrator (1,900 rpm, 15 s) (Sartorius-Stedim Biothec, Goettingen, Germany). Samples were centrifuged (20,000xg, 5 min, 4°C) to extract the soluble proteins.

MHC extraction and electrophoresis. To estimate MHC isoform expression, high resolution sodium dodecyl sulphate-polyacrylamide-gel electrophoresis (SDS-PAGE) (Kubis et al. 1997) was performed. Myosin was extracted from the tissue pellets with myosin extraction buffer (0.6 M KCl, 1 mM ethylene glycol tetraacetic acid (EGTA), 10 mM sodium phosphate dibasic, 1 mM phenylmethylsulfonyl fluoride (PMSF), pH 6.8) at 0°C and ultrasound (3 s pulses followed by 3 s cooling; 10 cycles with 5 min pause on ice between each cycle) (Ultrasonic Processor VCX 130, Sonics & Materials INC, Newtown, CT, USA). After 20 min incubation on ice, the samples were centrifuged (20,000x*g*, 20 min, 4°C). The resulting supernatant was diluted 1:10 with ice cold H_2O and 1 mM PMSF and incubated overnight at 0°C, then centrifuged (20,000x*g*, 20 min, 4°C) to precipitate to precipitate high molecular weight proteins, including actinomyosin complexes, which were resuspended in 50 µL extraction buffer. Protein concentration of the suspension was determined by Lowry assay (Sigma–Aldrich, Saint Louis, MO, USA).

MHCs were separated with SDS–PAGE (separating gel: $T = 9\%$, $C = 1.3\%$ and 34% glycerol, with a maximum voltage of 400 V for 36 h, at 12 mA) and gels were silver stained (Kubis et al. 1997). Protein bands were quantified by densitometry (Gel Doc 2000 and software Quantity One-4.6.3, Bio-Rad, Hercules, CA, USA).

Western blot analysis. Biochemical expression of caspase-3, IκBα and phospho-IκBα, phospho-Akt and Atrogin-1 was determined by using standard SDS-PAGE and Western blotting methods. Supernatant was boiled for 4 min. 50μg of protein were loaded and separated on 10% SDS-PAGE, and then transferred onto a nitrocellulose membrane. The blots were incubated with primary and secondary antibodies as detailed in Table 6.

Protein to be detected	Primary antibody	Secondary antibody
Intact caspase-3 with cleaved caspase-3 component	Caspase-3 antibody #9662, Cell Signaling, 1:1000 (recommended dilution), in 5% milk buffer	Anti-rabbit IgG antibody, Sigma, 1:3000, in 5% milk buffer
pAkt	Phospho-Akt (Thr308) antibody, Cell Signaling, 1:1000 (recommended dilution), in 5% milk buffer	Anti-rabbit IgG antibody, Sigma, 1:3000, in 5% milk buffer
Atrogin-1 (MAFbx)	MAFbx (L-15):sc-27645, Santa Cruz, 1:200 (range 1:100-1:1000) in 5% BSA buffer	Anti-goat IgG antibody, Santa Cruz, 1:2000 (range 1:500-1:10000), in 5% milk buffer
IκBα	IκBα (44D4) Rabbit mAb #4812, Cell Signaling, 1:1000 (recommended dilution), in 5% BSA buffer	Anti-rabbit IgG antibody, Sigma, 1:16000 (recommended dilution), in 5% milk buffer
pIκBα	Phospho-IκBα (Ser32) (14D4) Rabbit mAb #2859, Cell Signaling, 1:1000 (recommended dilution), in 5% BSA buffer	Anti-rabbit IgG antibody, Sigma, 1:3000 and 1:15000, in 5% milk buffer

Table 6. Primary and secondary antibodies used for Western blotting.

The immunocomplexes were visualised by enhanced chemoluminescence detection using Supersignal West Dura Extended Duration Substrate or Supersignal West Femto Maximum Sensitivity Substrate (Thermo Scientific, Pierce Biotechnology, Rockford, IL, USA). The signal bands were scanned and analysed using densitometry (Gel Doc 2000 and software Quantity One-4.6.3, Bio-Rad, Hercules, CA, USA).

Statistical analysis. Comparisons between the muscle of stable RA patients and healthy controls (study 1) were performed by unpaired t-tests, and between patients with active RA before and after starting treatment (study 2) by paired t-tests. $P < 0.05$ was considered statistically significant. Data were analysed using the Statistical Package for Social Sciences version 14.

Results

Stable RA patients versus healthy controls (study 1). The results for the cross-sectional comparison of patients with stable RA and healthy controls are presented in Table 7. The groups were similar for age and BMI. Although the patients had higher habitual physical activity scores compared to the healthy controls, the scores of both patient and control group were within the low to moderate physical activity levels, and none of the participants were exercising regularly or intensely. Of the 17 RA patients, 12 were on monotherapy with MTX, 2 were on monotherapy with SSZ, and 3 were on an NSAID only. Seven patients took an NSAID in addition to their DMARD. Disease activity was under control as the average DAS28 score for patients was low at 3.1. No significant differences in MHC distribution or in the level of intact caspase-3 were found between the stable RA patient group and the healthy control group.

Stable RA – study 1

	RA patients (n=17, 11 women)	Healthy controls (n=13, 8 women)	P value
Age (years)	60.7 ± 2.3, range 43-82	61.7 ± 2.5, range 49-73	0.77
Physical activity (2-8)	4.7 ± 0.1	3.9 ± 0.2	**< 0.05**
BMI (kg/m^2)	27.5 ± 1.3	27.6 ± 0.8	0.97
DAS28 score	3.1 ± 0.2	NA	
MHC type I (%)	41.0 ± 4.1	41.0 ± 6.9	1.0
MHC type IIa (%)	39.4 ± 0.3	46.8 ± 6.9	0.27
MHC type IIx (%)	20.0 ± 0.4	12.2 ± 6.5	0.37
Intact caspase-3 (pixels/mm^2)	17845 ± 2701	23145 ± 2966	0.19

Table 7. Anthropometric and habitual physical activity data, clinical characteristics, MHC distribution and intact caspase-3 levels of stable RA patients and healthy controls. NA = not assessed. Results presented as mean ± SEM.

Active RA before and after disease control (study 2). The patients with active RA and their rheumatoid medications are detailed in Table 8.

Active RA – study 2

patient	Baseline rheumatoid medication	Added medication
Anti-TNF 1	MTX, NSAID	Adalimumab
Anti-TNF 2	Prednisolone 10mg, NSAID	Adalimumab
Anti-TNF 3	Prednisolone 10mg	Etanercept
Anti-TNF 4	Tacrolimus	Etanercept
Anti-TNF 5	SSZ, LFN, prednisolone 10mg, NSAID	Adalimumab
Anti-TNF 6	MTX, HCQ, NSAID	Adalimumab
Anti-TNF 7	None	Adalimumab
New RA 1	None	MTX
New RA 2	None	MTX
New RA 3	None	MTX

Table 8. Baseline and added medication of patients with active RA. MTX = methotrexate, SSZ = sulfasalazine, LFN = leflunomide, HCQ = hydroxychloroquine, NSAID = non steroidal anti-inflammatory drug.

The characteristics of the 10 patients of the longitudinal study with active RA before and 3 months after beginning new medication are pooled in Table 9. Response to treatment was confirmed by a significant decrease in DAS28 scores from high to moderate disease activity ($p<0.01$). In the physical function tests, only sit-to-stand improved significantly after gaining disease control, although performance in the

50-foot walk tended to improve. Self-rated physical function and quality of life did not change significantly during the assessed period.

Active RA – study 2

	Patients with active RA before disease control (n=10, 7 women)	Patients with RA after disease control	P value
Age (years)	57.9 ± 3.6		
Physical activity (2-8)	3.2 ± 0.3	3.3 ± 0.3	0.34
BMI (kg/m^2)	27.5 ± 1.5	27.8 ± 1.6	0.28
DAS28 score	6.2 ± 0.4	3.4 ± 0.3	**< 0.01**
Sit-to-stand (number)	9.1 ± 1.6	10.5 ± 1.3	**0.02**
8-foot-up-and-go (s)	11.1 ± 3.6	8.9 ± 2.0	0.23
50-foot-walk (s)	13.3 ± 2.8	12.5 ± 2.5	0.10
Single leg balance (s)	37.7 ± 9.8	36.9 ± 9.2	0.83
mHAQ (0-3)	0.96 ± 0.18	0.87 ± 0.18	0.61
SF-36 physical component summary score (22-59)	34.0 ± 3.1	36.0 ± 3.8	0.27
SF-36 mental component summary score (11-62)	36 ± 0.8	36.0 ± 3.7	0.82

Table 9. Anthropometric and habitual physical activity data, clinical and functional characteristics of patients with active RA pre and post control of disease activity. Results presented as mean ± SEM.

Western blots. The results of the Western blots are presented in Table 10. This table includes pooled results (n=10) because of the low recruitment number of patients with new RA (n=3).

	Active RA – study 2 (n=10, 7 women)			Participants qualifying for anti-TNF treatment, (n=7, 4 women)			Participants with a new diagnosis of RA (n=3 women)		
	before disease control	after disease control	P value	before disease control	after disease control	P value	before disease control	after disease control	P value
Intact caspase-3 (pixels/mm^2)	179.2 ± 23.7	182.9 ± 33.2	0.93	200.0 ± 29.7	214.6 ± 41.2	0.80	130.8 ± 23.3	108.9 ± 27.3	0.30
Cleaved caspase-3 component (%)	40.3 ± 3.0	37.3 ± 2.1	0.20	37.7 ± 2.8	33.9 ± 1.3	0.37	48.8 ± 5.4	45.5 ± 2.5	0.43
pAkt (pixels/mm^2)	4184 ± 1068	6582 ± 857	0.07	3279 ± 519	6757 ± 1077	**0.03**	6295 ± 3469	6175 ± 1671	0.96
Atrogin-1 (pixels/mm^2)	7947 ± 940	10298 ± 936	0.06	7260 ± 899	10419 ± 1250	0.07	9550 ± 2400	10015 ± 1468	0.77
IκBα (pixels/mm^2)	3144 ± 303	2897 ± 425	0.56	3315 ± 349	3214 ± 545	0.87	2745 ± 639	2158 ± 485	0.12

Table 10. Intracellular markers of muscle apoptosis, hypertrophy and atrophy in active RA before and 3 months after disease control. Results presented as mean ± SEM.

There was no significant difference in intact caspase-3 at baseline compared to the post test, indicating no change in apoptosis with disease control. Caspase-3 cleavage products could be visualised at 17-19kD in addition to the intact caspase at 30kD. These breakdown products of intact caspase indicate the degree of caspase activation. There was no significant difference in intact caspase or the cleaved component between the pre test and post test.

A significantly lower level of pAkt was demonstrated in patients starting anti-TNF at baseline compared to post test ($p = 0.03$, Table 10, Figure 9 and Figure 10). This indicates suppression of a key stimulus for muscle hypertrophy in chronic active RA. This was however not seen in the group of patients with new RA.

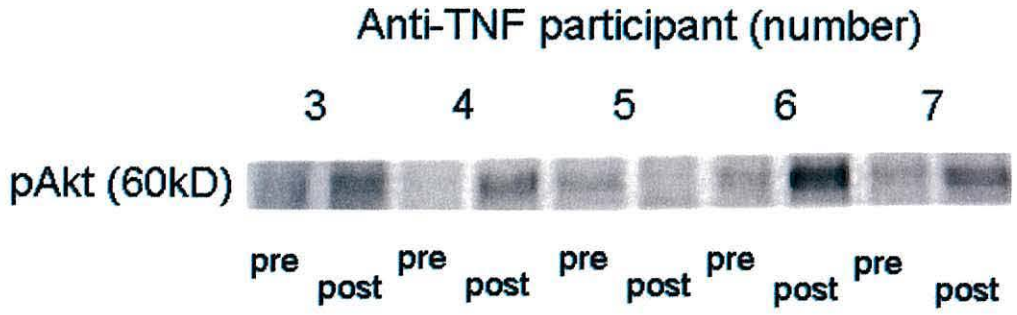

Figure 9. Western blot visualising pAkt. Representative findings of 5 participants with active RA before and 3 months after starting anti-TNF treatment are shown.

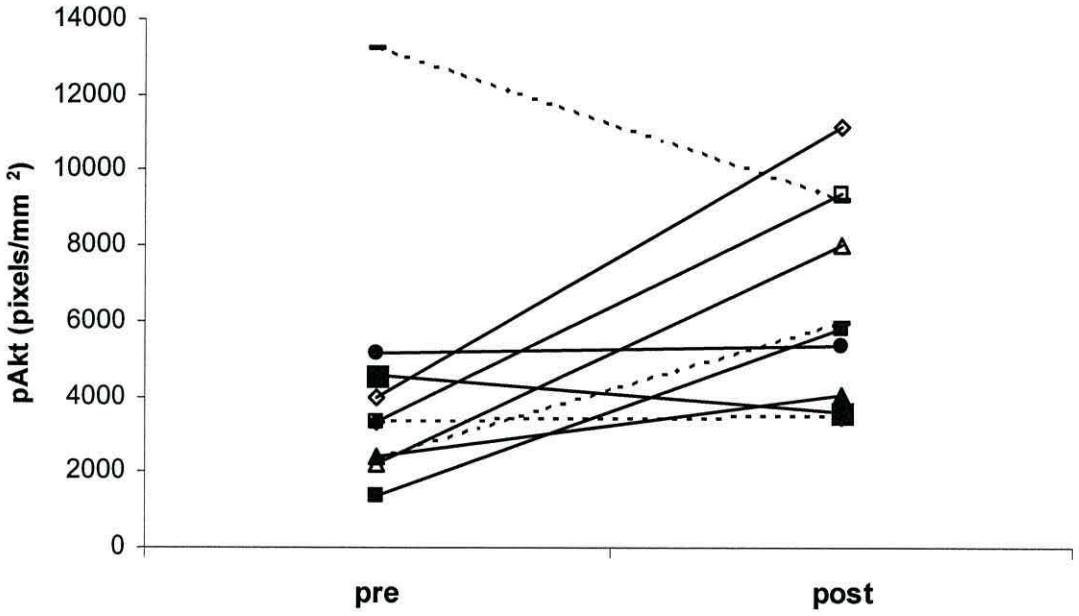

Figure 10. pAkt of all 10 participants (chronic active RA solid lines, new RA dashed lines) pre and 3 months post starting medication for disease control.

Atrogin-1, which promotes protein degradation and thereby muscle atrophy, was low at baseline in the group of patients starting anti-TNF compared to the post test values ($p = 0.07$, Table 10, Figure 11). This effect is contrary to the result of pAkt but could indicate a general suppression of protein synthesis, and thus also of markers of protein degradation, in chronic active RA. Again, this phenomenon was not seen in the group of new RA patients.

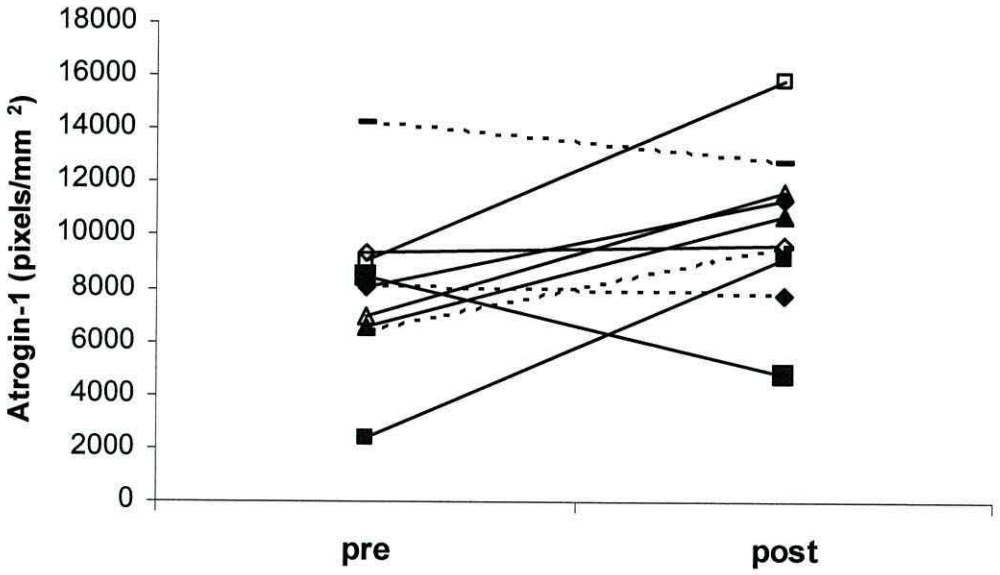

Figure 11. Atrogin-1 of all 10 participants (chronic active RA solid lines, new RA dashed lines) pre and 3 months post starting medication for disease control.

Total IκBα appeared unchanged in our analysis (Table 10). However to assess the status of active IκBα the phosphorylated part of the marker needs to be assessed. Initial attempts were made to visualise pIκBα, however, due to cross-reactivity of unspecific proteins with the secondary antibody the protein band for pIκBα could not be identified clearly.

Discussion

These studies are the first to examine myosin heavy chain composition and markers of protein metabolism and apoptosis in RA. Several notable findings emerged. Firstly, MHC distribution is similar in patients with stable RA compared to healthy controls. Secondly, caspase-3 levels are not different in stable RA patients compared to healthy

controls, nor in patients with active RA pre and post disease control. Thirdly, pAkt is suppressed in chronic active RA, indicating inhibition of protein synthesis in these patients when disease is not controlled. Atrogin-1, a marker of muscle atrophy, was also suppressed in these patients. In newly diagnosed but treatment-naïve RA, there was no suppression of either Akt or atrogin-1prior to disease control. There was no difference in IκBα in all active RA patients between the two time points.

The fact that MHC distribution in stable RA patients is similar to healthy controls of similar age and physical activity levels agree with the results on muscle quality in chapters 3 and 4 of this thesis. A shift towards fast or slow isoforms would be expected to have an influence on the speed of contraction of the whole muscle and thereby on the results of force, velocity-specific power and contractile properties. In other words, if these parameters of muscle quality are not different, then it would be anticipated that MHC distribution would not differ either. (Note: most of the stable RA patients and healthy controls from this biopsy study (13 patients and 10 controls) had also taken part in the study in chapter 3). The results of the present study are in contrast to earlier reports of a preferential atrophy of type 2 muscle fibres in RA (Haslock et al. 1970; Nordemar et al. 1976a; Nordemar et al. 1976b; Magyar et al. 1977). Since type 2 fibres contain mainly fast MHC isoforms, this would suggest that a shift towards MHC type IIa and IIx would take place in RA. This apparent controversy could be due to the identification method of fibre types with histochemistry used by Nordemar and the other authors, which is unable to precisely classify hybrid fibres, i.e. fibres consisting of more than one type of MHC. Also, these publications, among the first to investigate exercise in RA, were from the 1970s when the treatment for RA included long periods of immobilisation, and exercise was generally discouraged for fear of exacerbating

disease activity and joint damage; a view that has since changed in rheumatology practice (de Jong et al. 2003). Therefore, effects of chronic disuse are likely to have influenced the fibre type shift in older studies. In addition, it is possible that long periods of high disease activity and prolonged high dose corticosteroid treatment (common in the 1970s) influenced fibre type shifts in these studies. However, MHC distributions have yet to be examined in active RA.

In a subanalysis to the present study, the results of the subgroup of patients with cachexia were compared to the healthy controls. All 17 stable RA patients from the present study had participated in another study (chapter 3) in the previous year and therefore their appendicular lean mass was known. Of these, 11 patients were cachectic as per the definition by Baumgartner et al. (1988). Similar to the results of the whole group, the comparison of this subgroup to the healthy controls did not show any significant differences for MHC distribution or caspase-3 levels (results not shown).

In the study assessing active RA before and 3 months after achieving disease control (study 2), a range of markers representative of pathways promoting muscle atrophy, hypertrophy and apoptosis (see Figure 3, chapter 2) were assessed. Increases for both pAkt and atrogin-1 were observed with disease control in the subgroup of anti-TNF treated patients. Akt is a key factor promoting skeletal muscle hypertrophy in the IGF-1/phosphatidylinositol-3 kinase/Akt pathway (Glass 2010). Phosphorylation of Akt to its active form pAkt is stimulated by IGF-1, and inhibited by TNF-α (Bodine et al. 2001b; Rommel et al. 2001; Sharples and Stewart 2011). In a rat model of cardiac cachexia in which the ubiquitin-proteasome protein degradation pathway was activated,

anti-TNF treatment counteracted this pathway and thereby reduced skeletal muscle wasting (Steffen et al. 2008).

The downstream markers of Akt include the E3 ubiquitin ligase atrogin-1, which is upregulated under atrophy conditions and induces ubiquitinylation and subsequent degradation of proteins by the ubiquitin-proteasome pathway (Bodine et al. 2001a). In a report on quadriceps muscle biopsies in patients with stable chronic obstructive pulmonary disease, an increase in activation of this pathway was found, including upregulation of pAkt and FOXO-1 protein levels and of atrogin-1 and muscle ring finger (MuRF, another atrophy-inducing ubiquitin ligase) mRNA level compared to healthy controls (Doucet et al. 2007). In cancer cachexia, this pathway is similarly activated (Schmitt et al. 2007). In the present study, contrary to the expected effect, atrogin-1 was suppressed in active disease compared to disease control after 3 months. It was interesting to note that both the suppression of pAkt and atrogin-1 were only seen in the severely affected patients with chronic active disease and difficult to control disease activity who had failed to respond to previous DMARD treatment. It is possible that in these patients a general suppression of protein synthesis, and thus even the synthesis of the proteins that induce atrophy, is taking place. The multitude of longterm medications that these patients were taking, as well as the specific nature of their condition of severe, chronic and difficult to control disease activity might be influencing this observation.

Another possibility is that other pathways, for example activation of the forkhead box class O (FOXO) family of transcription factors through myostatin, a transforming growth factor-β family member, are responsible for the increase in atrogin-1 in the post

test (Lee et al. 2004; Glass 2010; Sharples and Stewart 2011). Furthermore, another downstream pathway of pAkt (i.e. through mammalian target of rapamycin; mTOR) might be the predominant mechanism of muscle loss in active RA. This would suggest a predominant inhibition of protein synthesis, rather than increased protein breakdown, in active RA. However, the mTOR pathway was not explored in this study. It has to be mentioned that in the group of 3 newly diagnosed RA patients one individual had much higher levels of pAkt at baseline and after starting MTX than the other two, and this might be skewing the results of this group. However, with an n=3 only it is not possible to say that the individual with the higher levels is an outlier and further studies with higher numbers of participants with new RA are needed.

In addition, the present study did not show any differences in IκBα and caspase-3 in active RA. These markers are known to be upregulated in inflamed rheumatoid synovium and induced by TNF-α and other inflammatory cytokines (Okazaki et al. 2005; Smith et al. 2010).

Limitations and strengths. Methodological limitations were antibody cross reactions in the Western blot for pIκBα. In addition, as mentioned above, the interactions between markers of atrophy and hypertrophy are complex and the relevance of these pathways to rheumatoid cachexia needs to be further explored. More detailed analysis was beyond the time constraints of this PhD thesis, but the samples of this study will be further investigated at Manchester Metropolitan University, School of Healthcare Sciences, under the direction of Prof. Claire Stewart. Optimization of the antibodies for pIκBα will also be performed in Manchester. An analysis of the mRNA levels of the respective transcription factors could also yield further information.

The recruitment of the patients was limited by the strict guidelines and funding limitations for anti-TNF medication whereby only a small number of patients qualify for this special treatment each year. Whereas recruitment of patients with longstanding RA has been very successful for the various research studies of this department (even for investigations involving challenging physical tests, exercise training interventions, and muscle biopsies), as reflected in the number of participants in the stable RA group of the present study, the recruitment of patients with newly diagnosed RA proved difficult. These patients might be less prepared to take part in a research study as they have yet to come to terms with their diagnosis and build up a relationship with their rheumatology team.

A strong point of this study is that the patients with active RA were at the worst end of the spectrum of disease activity (average DAS28 score 6.2), and they consequently qualified for treatment with clinically highly effective anti-TNF agents. Therefore any pathological effects of the disease process and any effects of the disease treatment on muscle would be expected to be seen in these patients.

In conclusion, the results of the present study represent the first steps to elucidate the complex intracellular network that leads to rheumatoid cachexia. The normal MHC distribution in stable RA confirms the similar physiological quality of rheumatoid and healthy muscle. Although anti-TNF treatment alone does not result in an increase in muscle mass in previous studies (Marcora et al. 2006b; Metsios et al. 2007; Engvall et al. 2010), the present study indicates that anti-TNF treatment can reverse the inhibition of Akt phosphorylation in chronic active RA. High intensity exercise has been

consistently successful in inducing muscle hypertrophy in RA, and the combination of exercise with anti-TNF medication might enhance this effect. Although more investigations are required, this study suggests that effective control of disease activity could help to counteract mechanisms that suppress protein synthesis in the muscle in RA.

Chapter 6: Adverse changes in tendon-muscle physiology and physical function caused by an isolated active rheumatoid knee effusion: a case study

Introduction

As previously explained, many patients with rheumatoid arthritis (RA) have reduced muscle mass, probably due to the catabolic effects of systemic inflammation and disuse (Roubenoff et al. 1994). This loss of muscle leads to reduced strength and physical function (Roubenoff et al. 1994; Lemmey et al. 2009). Whereas the results from chapter 3 and 4 (Matschke et al. 2010a; Matschke et al. 2010b) indicate that muscle quality is not compromised in patients with stable RA, even when cachexia is present, the properties of the tendon-muscle complex in patients with active disease remain to be investigated. So far it is known that the presence of effusion in the knee joint results in reflex inhibition of the quadriceps as demonstrated by reduced EMG activity and diminished strength (Fahrer et al. 1988; Wood et al. 1988).

The wider characteristics of the tendon-muscle complex have not been investigated in active RA or in joint effusions of other aetiologies. As explained in chapter 2, electrophysiological methods are used in exercise science to investigate tendon-muscle properties in healthy populations. These include assessments of muscle specific force (force/cross-sectional area); architecture (fibre fascicle length and pennation angle); voluntary activation capacity; contractile properties; and tendon stiffness. Application of these techniques in ageing and disuse has demonstrated adverse changes in tendon and

muscle properties which result in impaired function but respond well to exercise training (Reeves et al. 2003; Stevens et al. 2006). To our knowledge these electrophysiological methods have not been applied in active RA, probably due to difficulties arising from confounding factors such as pain, fatigue, and the acute effects of inflammation in other joints.

Case description

A fit 53 year old female marine biologist presented to the rheumatology clinic with a 6 month history of a swollen stiff left knee (Figure 12). There were no other musculoskeletal symptoms, and she was otherwise well and taking no medication. Examination showed a large left knee effusion. The right knee and all other joints were normal. Further investigation revealed an elevated ESR (39 mm/hour), anti-CCP antibody positivity, no abnormality on x-ray of the left knee, and a RADAI-5 score of 4.4 (0-10). A diagnosis of CCP-positive RA was made.

This case provided the opportunity to investigate the local and systemic effects of an active inflammatory knee effusion on physical function and the tendon-muscle complex, as in spite of the large effusion the patient had no pain or fatigue and had kept up regular exercise in the gym and as part of her vigorous outdoor occupation. Thus disuse and pain had minimal or no influence on tendon-muscle testing, which requires full cooperation and maximal effort from the participant.

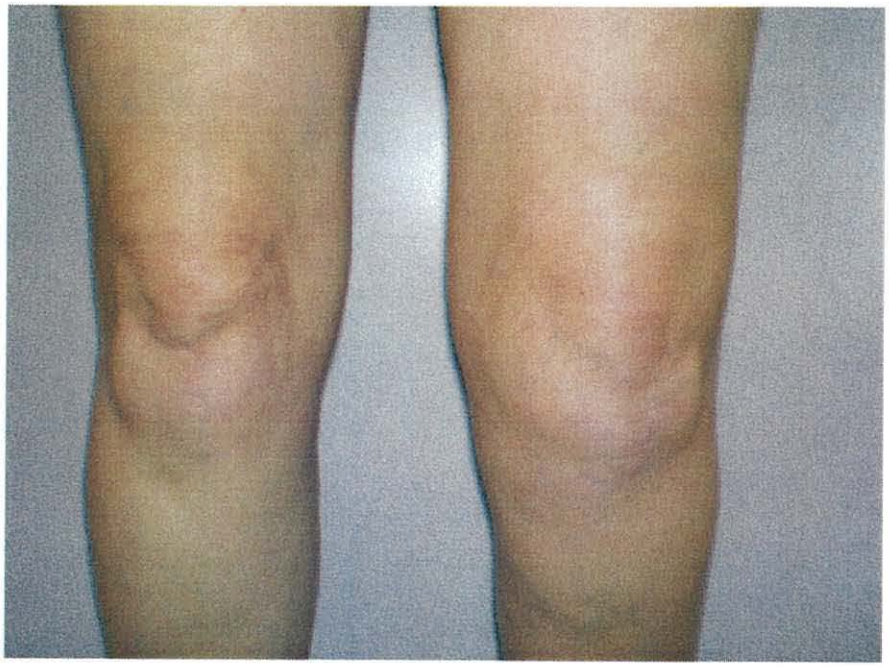

Figure 12. The patient's knees at diagnosis. Note the swelling i.e. effusion in the left knee joint.

After the initial assessment of the tendon-muscle properties, the left knee was aspirated yielding 30 ml of synovial fluid and a long-acting corticosteroid (Depomedrone 80mg) was injected intraarticularly. The effusion settled without recurrence and the participant was able to maintain her usual physical activity. One year later she remained well with no symptoms. Examination of both knees showed no effusions, the other joints were normal, ESR was 2 mm/hour, and RADAI-5 was 1. All baseline measures were repeated at 12 months.

Methods

All investigations were carried out at the Bangor University exercise physiology laboratory. The participant gave informed written consent. The study was conducted in compliance with the Helsinki declaration.

Maximal isometric and concentric quadriceps strength was measured on each leg separately with the knee joints at 90° on an isokinetic dynamometer (CSMI Medical Solutions, Stoughton, USA), with concurrent electromyography of vastus lateralis (VL) and biceps femoris (BF). Calculation of quadriceps force took into account moment arm and BF coactivation torque (Onambele-Pearson and Pearson 2007). Percutaneous electrical stimulation of the quadriceps at rest determined contractile properties (electrically evoked peak force, time to peak force and half-relaxation time) and was superimposed on maximal voluntary quadriceps contraction (MVC) to determine voluntary activation capacity (Thom et al. 2005). Quadriceps anatomical cross-sectional area (ACSA) was measured using ultrasonography (US) to determine specific force (force/ACSA). Concentric force and velocity-specific power were determined from the best of 4 consecutive concentric quadriceps contractions at 50°/s (Matschke et al. 2010b).

Patellar tendon (PT) stiffness was determined using ultrasound as described by Onambele et al. (Onambele-Pearson and Pearson 2007). The distance between the apex of the patella and the superior aspect of the tibial tuberosity was taken as resting PT length (Figure 13). PT excursion from its proximal and distal bone attachments was assessed during three ramped MVCs building up to maximum force with increasing effort over 4-5 seconds, with the ultrasound probe held in the sagittal plane. Images were analysed using digitizing software (Image J, NIH, Bethesda, MD, USA). Tendon force-elongation relationship was assessed at intervals of 12.5% of the maximal force, and fitted with second-order polynomial functions forced through zero. Tendon stiffness

was calculated from the slope of the tangent (1st derivative of the polynomial function) at the level of maximum force.

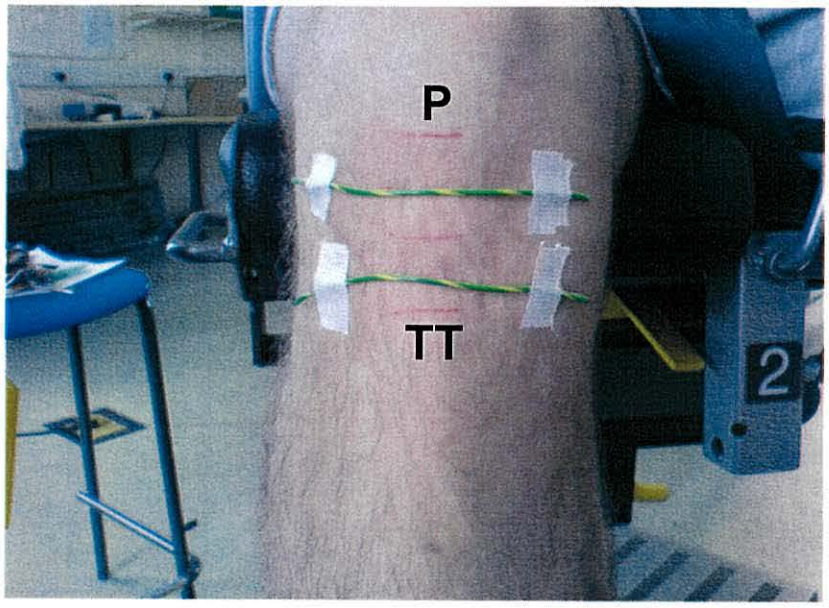

Figure 13. Skin markers at 25% and 75% of PT length are used to assess the tendon elongation from the distal end of the patella (P) and from the proximal end of the tibial tuberosity (TT).

Ultrasound images taken in the axial plane at 25%, 50% and 75% of the PT length were used to determine PT CSA (Figure 14). Three measurements were taken at each level and averaged. Young's modulus (YM), was calculated as follows: YM = tendon stiffness x (resting tendon length/tendon CSA) (Reeves et al. 2003).

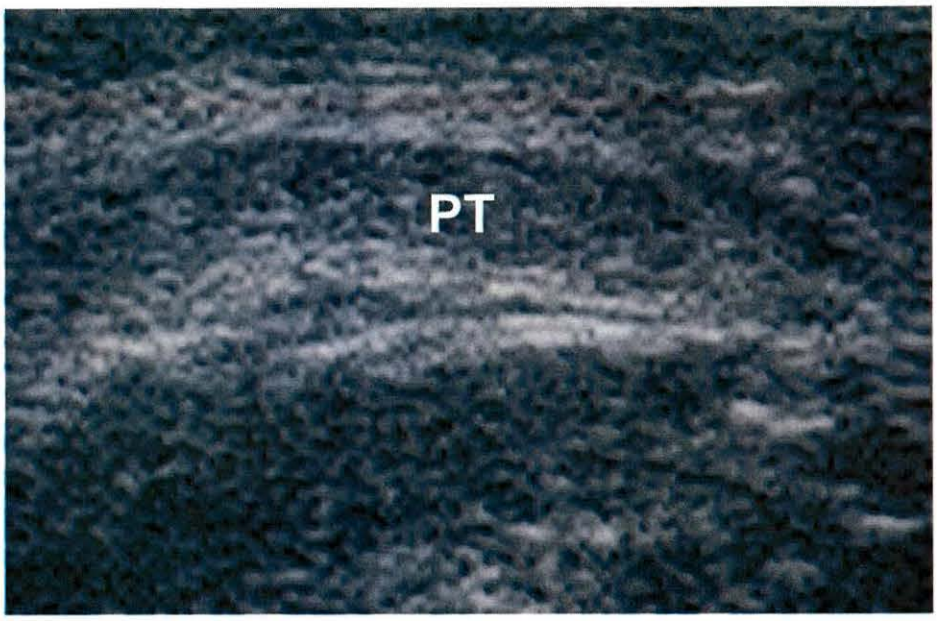

Figure 14. Transverse view of patella tendon (PT) to determine PT cross-sectional area

Lower body physical function was assessed by 30-second sit-to-stand, 8-foot up and go, 50-foot walk, and one-leg standing balance tests (Rikli and Jones 2001).

At baseline the results of the affected leg were compared with those of the unaffected leg. At follow up the results were compared with the previous data for the same leg. Ultrasound images were blinded for analysis.

Baseline results

Table 11 shows results. The effusion caused considerable physical impairment. The sit-to-stand result of 10 corresponds to the 15^{th} percentile of a healthy 60 year old female reference population (Rikli and Jones 2001).

	At baseline		1 year follow up	
Sit-to-stand (n per 30 s)	10		13	
8-foot up-and-go (s)	5.22		4.81	
50-foot walk (s)	7.22		6.06	
Balance (s)	101		120	
	Unaffected leg	Affected leg	Unaffected leg	Affected leg
Quadriceps isometric force (N)	5068	2863	4888	4530
Quadriceps ACSA (cm^2)	53.9	37.3	51.2	41.0
Muscle specific force (N/cm^2)	94.0	76.7	95.6	110.5
Concentric torque at 50 °/s (Nm)	129.4	59.3	136.6	103.6
Velocity-specific power	112.9	51.8	119.3	90.4
Voluntary activation capacity (%)	90.6	93.0	92.9	98.7
Patellar tendon stiffness (N/mm)	7242	4088	4944	3647
Patellar tendon CSA (mm^2)	1.03	1.01	1.05	1.06
Young's modulus (GPa)	3.99	2.27	2.00	1.61
Resting twitch peak torque (Nm)	17.9	-	21.0	17.3
Time to peak torque (s)	0.085	0.083	0.104	0.084
Half-relaxation time (s)	0.042	0.047	-	-

Table 11. Quality of the tendon-muscle complex in the affected and unaffected legs, physical function and disease activity at baseline and at 1 year follow up in the 53 year old female RA participant. - indicates missing values for contractile properties.

At baseline the quadriceps ACSA of the affected leg was 31% less than that of the unaffected leg. Voluntary activation capacity of both quadriceps was over 90%, demonstrating maximal muscle contraction was not inhibited. Despite this, muscle quality was considerably impaired on the affected side, with isometric and concentric forces being lower by 44% and 55%, respectively. This force reduction could not be explained by the reduced quadriceps ACSA alone because the specific force (force/ACSA) was reduced by only 18% (Figure 15). Contractile properties were equal on both limbs, making it unlikely that the reduction in specific force was due to fibre type differences. PT stiffness (Figure 16) and YM were reduced in the affected leg by 48% and 43% in comparison to the unaffected leg. There was no difference in PT CSA.

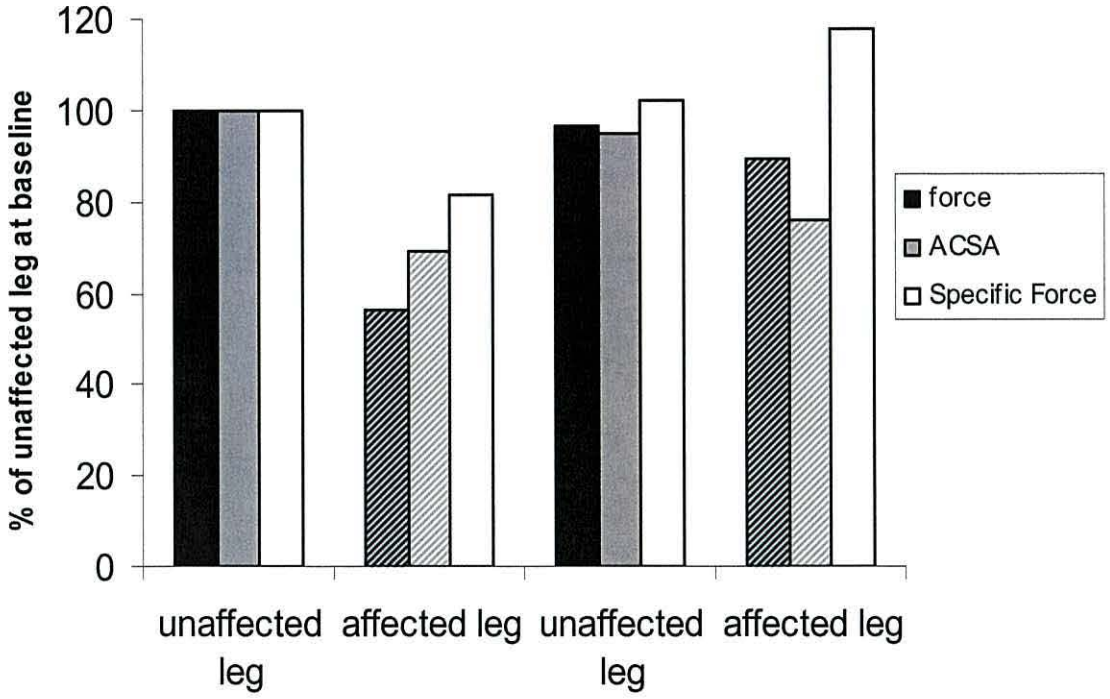

Figure 15. Quadriceps isometric force, ACSA and muscle specific force in the affected (hashed) and unaffected legs (solid) at baseline and at 1 year follow up in the 53 year old female RA participant.

Reassessment at 1 year

Objectively assessed physical function had improved substantially (sit-to-stand by 30%, 8-foot up and go by 8%, 50-foot walk by 16%, one-leg standing balance by 18%).

In the affected leg isometric force had improved by 36.8%, concentric torque by 42.7%, and ACSA by 9%, while PT stiffness (Figure 16) and YM had worsened by a further 10% and 29%, respectively.

In the unaffected leg, PT stiffness (Figure 16) had deteriorated by 27% and YM by 50%, compared with baseline. There were minor reductions in isometric force (-3.6%) and quadriceps ACSA (-5.1%) (Figure 15).

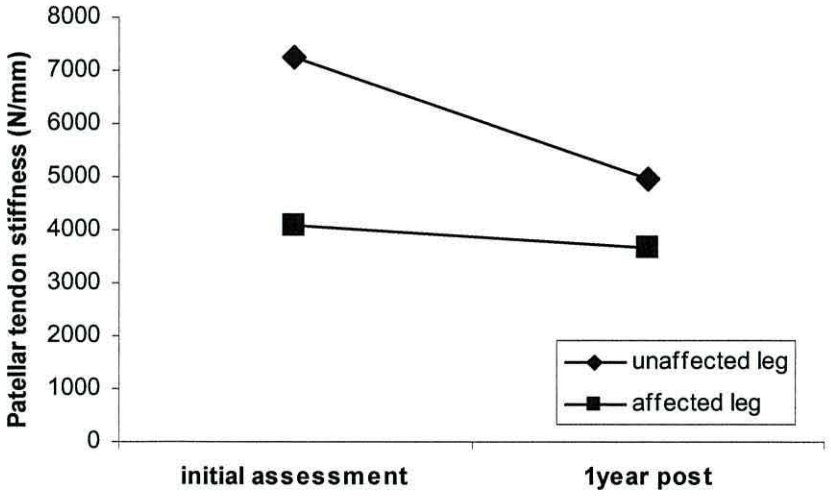

Figure 16. Patellar tendon stiffness in the affected and unaffected legs at baseline and at 1 year follow up in the 53 year old female RA participant.

Discussion

This case demonstrates the dramatic consequences of an active inflammatory knee effusion on physical function and on the physiology of the local tendon-muscle complex. In spite of the patient's very active lifestyle, the presence of knee effusion greatly diminished her physical function as shown by the poor sit-to-stand test performance. Conversely, her function improved with resolution of the effusion a year later. Specific electrophysiological examination showed an adverse effect on PT stiffness. Acutely this was only seen in the affected leg, but at 1 year was evident in both legs. The acute changes could be a local effect of the joint effusion, whilst the more long term bilateral changes suggest a systemic effect of the inflammatory process. Our results also showed that the presence of an effusion resulted in quadriceps wasting, a dramatic loss of force production - not due to pain, and impaired muscle quality as shown by reduced muscle specific force.

Tendons are extensible structures that reversibly deform when a mechanical load is applied. The content and organisation of the extracellular matrix (collagen, elastin, water) enable the tendons to store elastic strain energy and return it on recoil; a unique mechanical property which is essential for locomotion and complex functional tasks (Onambele-Pearson and Pearson 2007). The extent of elongation of a tendon to loading, i.e. the tendon stiffness, influences the performance of the attached muscle, and thereby determines the magnitude and speed with which force is transmitted from muscle to bone. Thus, stiffer tendons result in increased and faster force production, whereas the opposite effect is seen with more compliant tendons, since increased elongation of the tendon requires further shortening of the muscle fibres and can cause electromechanical

delay (Pearson and Onambele 2006). Outside a characteristic elastic range, tendons become less efficient for muscle output and motor control, and more vulnerable to injury (Magnusson et al. 2003; Magnusson et al. 2008). Tendon stiffness is reduced in ageing and following immobilisation due to a concomitant decrease in collagen and increase in the more extensible elastin content (Reeves et al. 2003). In contrast, increases in tendon stiffness are found following exercise training (Reeves et al. 2003).

Our case is, we believe, the first to show reduction of tendon stiffness in active inflammatory joint disease. In patients with stable established RA we have also demonstrated reduced tendon stiffness when compared with controls of similar age, sex, and physical fitness (chapter 7, manuscript submitted). RA is known to cause synovial inflammation, hypertrophy of tendon sheaths, and on occasions synovial tissue infiltration of the tendon itself (Jain et al. 2001). However, the PT does not have a tendon sheath. Asymptomatic PT enthesitis with associated PT thickening has been described in RA (Genc et al. 2005). However, in our study comparing stable RA patients with healthy controls we could not demonstrate PT thickening (chapter 7, manuscript submitted). Similarly, in this case study, no PT thickening was observed. The reduced PT stiffness in the affected leg at baseline could be a result of mechanical effects of the knee effusion or a detraining effect with the reduced muscle force leading to reduced tendon loading. Local diffusion of inflammatory cells and cytokines from knee joint synovitis may have contributed to the adverse effects in the tendon. Interestingly, one year later, despite recovery of the muscle properties and continual physical activity there was no recovery of tendon stiffness. Furthermore, the tendon stiffness in the unaffected leg was also reduced. This strongly suggests a systemic effect

of RA on tendon. Tendons have slower metabolism than muscle and this might be a factor in delaying recovery (Williams 1986).

Our findings of reduced muscle force and size in the affected leg with a dramatic reduction in physical function are in accord with other studies on the effects of pathological and simulated knee effusions on quadriceps and EMG activity. In most reports the presence of an effusion resulted in reduced strength and EMG activity when compared to the same joint with no effusion. The EMG changes indicate that quadriceps muscle reflex inhibition cause the reduction in strength. In chronic effusions, strength is also affected by muscle wasting (Reeves and Maffulli 2008). In our participant, force was diminished more than muscle size, resulting in reduced muscle specific force. This phenomenon is also seen with immobilisation and ageing, where changes in muscle architecture, activation, and fibre type play an important part (Thom et al. 2005; Stevens et al. 2006). Exercise training initially increases strength via increased neural drive, followed by muscle hypertrophy (Reeves et al. 2003). Thus, muscle specific force rises more quickly during the early stages of exercise training due to enhanced fibre recruitment. In our case, after treatment of the effusion and continued physical activity, the quadriceps improved more in strength than in size. We have previously shown that muscle specific force is unimpaired in patients with stable established RA (chapter 3 and 4) (Matschke et al. 2010a; Matschke et al. 2010b), but it might have been reduced in the active stage of the disease and subsequently recovered.

Presumably the difference in muscle size and force between the affected and unaffected leg is a result of excess synovial fluid causing mechanical distension of the knee joint.

Rheumatoid cachexia might also play a role, but cannot be commented upon in this case in the absence of information about body composition before disease onset.

The findings of this case study highlight several important points: firstly, knee joint effusion results in dramatic physical impairment; secondly, early local and subsequent systemic effects of RA reduce PT stiffness; thirdly, in addition to loss of muscle quantity, there is impaired muscle quality in acute RA, a characteristic not evident in stable RA (chapter 3 and 4) (Matschke et al. 2010a; Matschke et al. 2010b); fourthly, resolution of disease activity and continued physical activity led to partial recovery of muscle and physical function, but did not resolve the tendon abnormalities; and finally, this case highlights the adverse effect of an inflammatory joint effusion on the tendon-muscle complex and the consequent need for early intervention including joint aspiration, steroid injection and continuing physical activity to minimise disability in these patients. Further areas of research suggested by this study include clarification of the role tendon abnormalities play in the impaired physical function of RA (this is investigated in chapter 7), the effect of tendon-focused exercise training, and the effect of medication on rheumatoid tendons.

Chapter 7: Patellar tendon properties and lower limb function in rheumatoid arthritis and ankylosing spondylitis

Introduction

The case study of the previous chapter indicated that PT stiffness and YM are adversely affected locally in an active rheumatoid knee joint effusion and systemically 1 year later despite control of disease activity. The present chapter investigates in a larger group of participants whether the physiological properties of the human patellar tendon are chronically compromised in the context of stable inflammatory arthropathies *in vivo*.

In addition to RA patients, a group of patients with ankylosing spondylitis (AS) was recruited, a condition known to primarily affect the enthesis, i.e. the insertion of tendon to bone. This was done in order to determine whether RA and AS have different effects on tendon function. The research was conducted as two separate studies comparing stable RA patients with matched healthy controls, and stable AS patients with matched healthy controls. This was done because of the differences in gender prevalence between RA (more women) and AS (more and younger men). To our knowledge, this is the first time *in vivo* assessment of patellar tendon properties with ultrasound has been done in participants with arthropathies. Additionally, assessment of muscle size, muscle specific force (muscle force normalised to muscle size) and neural activation of the muscle with electromyography was performed.

Patients and methods

Participant characteristics and disease activity

Eighteen patients with RA according to the American Rheumatism Association 1987 revised criteria (Arnett et al. 1988) and 12 patients with AS according to the European Spondylarthropathy Study Group criteria (Dougados et al. 1991) were recruited from the Rheumatology outpatient clinics of the North West Wales NHS Trust, as were, respectively, 18 and 12 age- and sex-matched healthy volunteers. Inclusion criteria for all patients were: disease duration of at least three years; and stable disease activity (i.e. no flare or change in medication for the last three months). Exclusion criteria were: the presence of any other catabolic disease; high dose steroid therapy (i.e. >10 mg prednisolone daily) or a recent steroid injection; joint replacement or current pain or swelling in the right knee. The study was approved by the North Wales Health Authority Research Ethics Committee and conducted in compliance with the Helsinki declaration.

Disease activity was assessed in RA patients by the modified rheumatoid arthritis disease activity index (RADAI-5) (Leeb et al. 2008), and in AS patients by the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) (Garrett et al. 1994). RADAI-5 measures global RA disease activity of the past six months, and current disease activity in terms of swollen and tender joints, arthritis pain, general health and duration of morning stiffness. BASDAI measures AS disease activity of the past 1 week, in terms of fatigue, spinal pain, peripheral joint pain and swelling, areas of localised tenderness (e.g. at the site of tendons and ligaments), and duration and severity of morning stiffness. Both RADAI-5 and BASDAI result in a score from 0 = no disease

activity to 10, respectively. Peripheral blood erythrocyte sedimentation rate (ESR) was determined in the Haematology Department of Gwynedd Hospital.

Habitual physical activity and physical function

A questionnaire on habitual physical activity (Saltin and Grimby 1968) was administered to all participants. This questionnaire has been used previously in RA and ageing populations (Marcora et al. 2005) and gives separate scores (1=sedentary to 4= vigorous physical activity) for work and leisure time which are then summed to give a final score of 2-8. Objective physical function of the lower body was assessed by the 30-second chair sit-to-stand, the 8-foot-up-and-go (Rikli and Jones 2001), the 50-foot-walk, and single leg balance tests (Mian et al. 2007). The Modified Health Assessment Questionnaire (mHAQ) (Pincus et al. 1999) and the 36 questions Short-Form Health Survey (SF-36) (Keller et al. 1999) provided information on subjective physical function and health-related quality of life (QoL), respectively.

Quadriceps muscle force and patella tendon stiffness

As participants sat upright on a Humac Norm isokinetic dynamometer (CSMI Medical Solutions, Stoughton, USA), their right leg was strapped to the dynamometer arm above the ankle, and additional straps were secured to prevent extraneous movement at the hips and shoulders. The knee joint angle was fixed at 90° from full leg extension and the hip angle at 90° (Narici et al. 1988). Patella tendon stiffness was then determined using the method of Onambele et al. (Onambele-Pearson and Pearson 2007). After a set protocol of warm-up contractions, participants performed three ramped maximal voluntary isometric knee extension contractions (MVC), building up to maximum force with increasing effort over 4-5 seconds. During these contractions, participants crossed

their arms over their chest to avoid the addition of arm muscle force to the quadriceps force measurements. Verbal encouragement was given. The US 7.5 MHz linear probe (MyLab50, Esaote, Firenze, Italy) was positioned sagittally over the patella tendon (PT) and three video clips were recorded for PT excursion from the proximal and the distal attachment of the tendon to the bone, respectively (Figure 17). An external marker fixed on the skin to detect accidental movement of the probe against the skin; when this occurred, recordings were repeated. The recordings were aligned by synchronization of force and US data. Feedback was provided to the participants through a real time display of muscle torque on a computer screen. There was at least 1 minute rest between each MVC to minimise fatigue. US images were blinded and analysed using digitizing software (Image J, National Institute of Health, Bethesda, MD, USA).

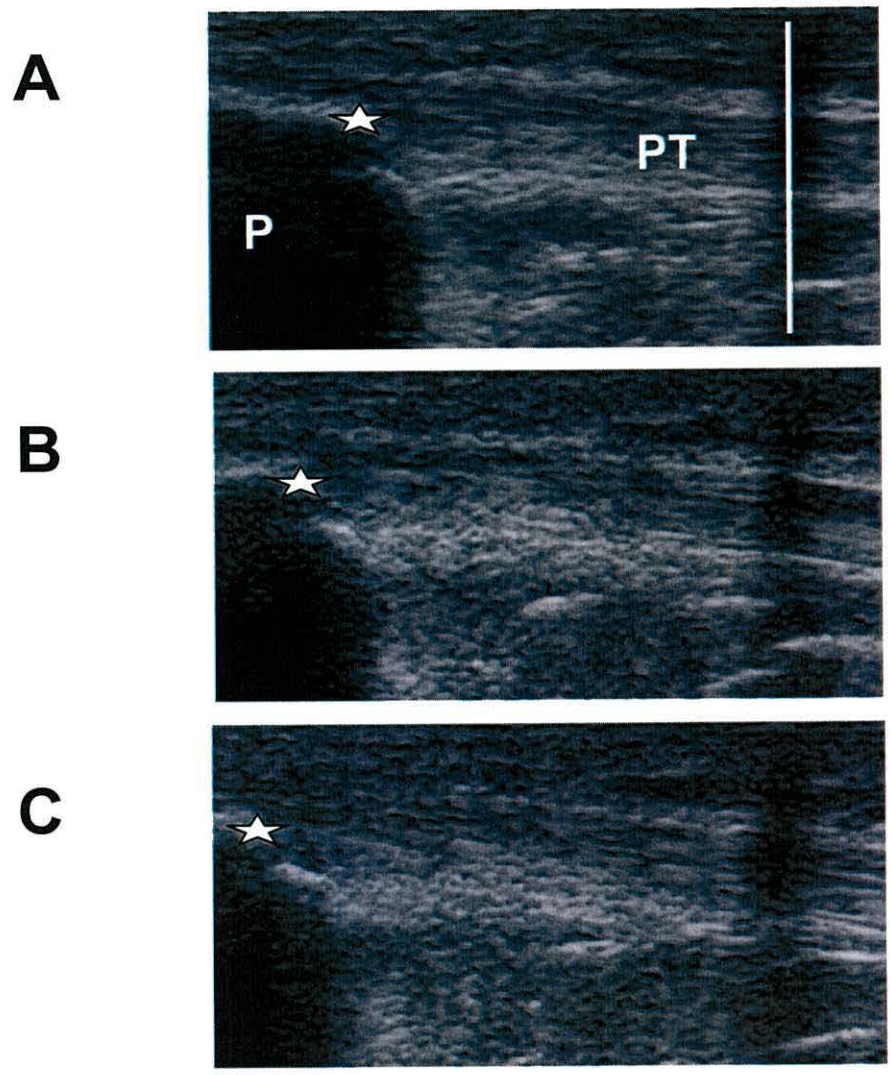

Figure 17. Illustration of patellar (P) tendon (PT) elongation from a skin marker (vertical line) to the tendon insertion at the patella (star) using US at rest (A) and during contractions (B and C) to assess proximal displacement. Distal displacement (from the skin marker to the tendon insertion at the tibial tuberosity) was assessed in a similar way.

The contraction with the highest torque was used for analysis. Calculation of quadriceps muscle force accounted for torque, patellar tendon moment arm length (Onambele-Pearson and Pearson 2007) and antagonist co-contraction (which was estimated from electromyographic (EMG) activity (Reeves et al. 2004; Onambele-Pearson and Pearson

2007). EMG activity (root mean square of the raw EMG signal) was recorded through self-adhesive Ag-AgCl electrodes (Ambu, Denmark) over the vastus lateralis (VL) and the long head of the biceps femoris (BF) during the extension MVCs and during three subsequent maximal isometric knee flexions. The latter data were used to correct extension torque for the effect of knee flexor muscle co-contraction.

The following equations were used to calculate quadriceps force:
BF torque = (BF EMG during knee extension / BF EMG during knee flexion) * knee flexion torque (Pearson and Onambele 2005).
Quadriceps force = (BF torque + knee extension torque) / estimated patellar tendon moment arm length (Reeves et al. 2004).

The tendon force-elongation relationship was assessed at intervals of 12.5% for maximal force development and fitted with second-order polynomial functions forced through zero (Onambele-Pearson and Pearson 2007), as shown in Figure 18. Tendon stiffness for each participant was calculated at the level of maximum force of each individual's maximum force from the slope of the tangent (1st derivative of the polynomial function) at this force level.

Patella tendon length, patella tendon cross-sectional area and Young's modulus
Patella tendon length was defined as the distance between the apex of the patella and the superior aspect of the tibial tuberosity visualised on sagittal-plane ultrasound images with the knee joint at 90°. Ultrasound images taken in the axial plane at 25%, 50% and 75% of the patella tendon length were used to determine PT CSA. At each level, three measurements were averaged. The mean of all CSA measurements at all levels was then

averaged for the calculation of Young's modulus (YM = tendon stiffness * (tendon length/tendon CSA) (Reeves et al. 2003).

Quadriceps muscle cross-sectional area and muscle specific force

To estimate muscle size, quadriceps anatomical cross sectional area (ACSA) was measured by ultrasonography at 50% of the muscle length (Reeves et al. 2004). ACSA was measured separately for each of the four muscles of the quadriceps (VL, vastus medialis VM, vastus intermedius VI, rectus femoris RF) and then added together. In this way, non-contractile tissue between the muscles was not included in estimations of muscle tissue. Muscle specific force was calculated by normalising force to quadriceps ACSA.

Statistics

All statistical analyses were performed using SPSS software version 14.0 (Chicago, IL). Depending on normality of distribution of the data, differences between the patient and matched control groups were determined by either student's paired t test or Wilcoxon test. Unless otherwise stated, values are presented as means ± SEM. Significance was accepted at the level $P<0.05$.

Results

Participant characteristics and disease activity

The anthropometric characteristics of the participants are summarised in Table 12. All patients had stable disease with low disease activity scores (DAS). ESR levels corresponded to normal levels for this age group or low grade inflammation.

In the RA group, 8 patients were rheumatoid factor positive, disease duration was 14±2.3 years, and DAS by RADAI-5 was 3.3±0.4. With regard to medication, 15 RA patients (83%) were taking methotrexate (MTX), 2 of them in combination with an anti-tumour necrosis factor (TNF) agent (1 infliximab, 1 adalimumab), and 1 in combination with sulfasalazine (SSZ). One patient was on SSZ monotherapy. Eight patients (44%) were also taking a non-steroidal anti-inflammatory drug (NSAID) and 5 (28%) prednisolone (range 1-10 mg/day; average dose 7mg).

In the AS group, disease duration was 20.7±3.9 years and DAS by BASDAI score 3.0±0.6. Three patients were on disease modifying antirheumatic drugs (DMARDs) (1 MTX, 2 SSZ), two of them in combination with an NSAID; one patient was on the anti-TNF agent etanercept; five patients were on an NSAID only and 3 patients required no medication for their arthritis. Five patients had conditions known to be frequently associated with AS: ulcerative colitis (n=1), Crohn's disease (n=1), and psoriasis (n=3).

	RA study			AS study		
	RA patients (n=18)	**Healthy controls (n=18)**	**P value**	**AS patients (n=12)**	**Healthy controls (n=12)**	**P value**
Age (years)	59 ± 2.8	58 ± 3.2	0.35	53.7 ± 3.3	54.8 ± 3.3	0.68
Height (m)	1.65 ± 0.01	1.69 ± 0.03	0.21	1.66 ± 0.03	1.74 ± 0.02	<0.001
Weight (kg)	75.1 ± 3.3	73.8 ± 3.2	0.98	79.0 ± 4.2	78.2 ± 2.9	0.89
Body mass index (kg/m^2)	27.4 ± 1.0	26.0 ± 1.2	0.61	28.7 ± 1.07	25.7 ± 0.9	0.08

Table 12. Participant anthropometric characteristics. Presented are the data of RA patients (n = 18; 13 women) and their age- and sex-matched healthy controls (n = 18) as well as the data of AS patients (n = 12; 4 women) and their age- and sex-matched controls (n = 12). Results presented as mean ± SEM.

Habitual physical activity and physical function

There were no significant differences in the self-reported habitual physical activity levels between either of the patient groups and their matched controls (Table 13). Despite this, the laboratory tests based objective physical function was significantly reduced in RA patients (8-foot up and go by 17.2%, 50-foot walk by 25.7%, and one-leg standing balance by 27.4%) and in AS patients (sit-to-stand reduced by 25.4%, 8-foot up and go by 15.8%, 50-foot walk by 19.5%) compared to their controls (Table 13). Similarly, both patient groups reported poorer subjective, self-assessed physical function, measured by mHAQ and the SF-36 physical component summary score, relative to their respective healthy control groups. The AS group also had a reduced score on psychological QoL factors from the SF-36 mental component summary score compared to their matched controls (Table 13).

	RA study			AS study		
	RA patients (n=18)	**Healthy Controls (n=18)**	**P value**	**AS patients (n=12)**	**Healthy Controls (n=12)**	**P value**
Habitual physical activity (range 2 – 8)	4.9 ± 0.7	4.4 ± 1.4	0.17	4.6 ± 0.4	4.3 ± 0.3	0.46
30-sec sit-to-stand (n)	12.8 ± 0.8	13.7 ± 0.4	0.3	11.8 ± 0.7	15.8 ± 1.1	**0.002**
8-foot-up-and-go (sec)	6.0 ± 0.3	5.2 ± 0.2	**0.03**	5.3 ± 0.3	4.6 ± 0.2	**0.03**
50-foot-walk (sec)	9.2 ± 0.5	7.4 ± 0.3	**0.004**	7.9 ± 0.4	6.6 ± 0.4	**0.03**
One-leg balance (cumulative) (sec)	50.2 ± 5.6	66.9 ± 6.3	**0.04**	74.9 ± 4.5	78.7 ± 6.2	0.62
mHAQ (range 0–3)	0.60 ± 0.06	0.18 ± 0.03	**<0.001**	0.56 ± 0.12	0.17 ± 0.0	**<0.01**
SF-36 physical component (range 22-59)	38.1 ± 3.3	51.3 ± 1.9	**<0.001**	41.6 ± 3.6	50.7 ± 1.6	**0.04**
SF-36 mental component (range 11-62)	39.6 ± 2.8	43.2 ± 1.5	0.11	34.7 ± 3.1	44.3 ± 1.3	**0.03**

Table 13. Habitual physical activity and subjective and objective physical function of RA and AS patients and their respective healthy controls. Results presented as mean ± SEM.

Patella tendon properties

Figure 18 shows increased elongation of the PT in both patient groups at defined force levels relative to their respective control groups, as demonstrated by a right-shift of the force-elongation curves of the patient groups. This indicates a reduction in tendon stiffness (i.e. the gradient to the curve).

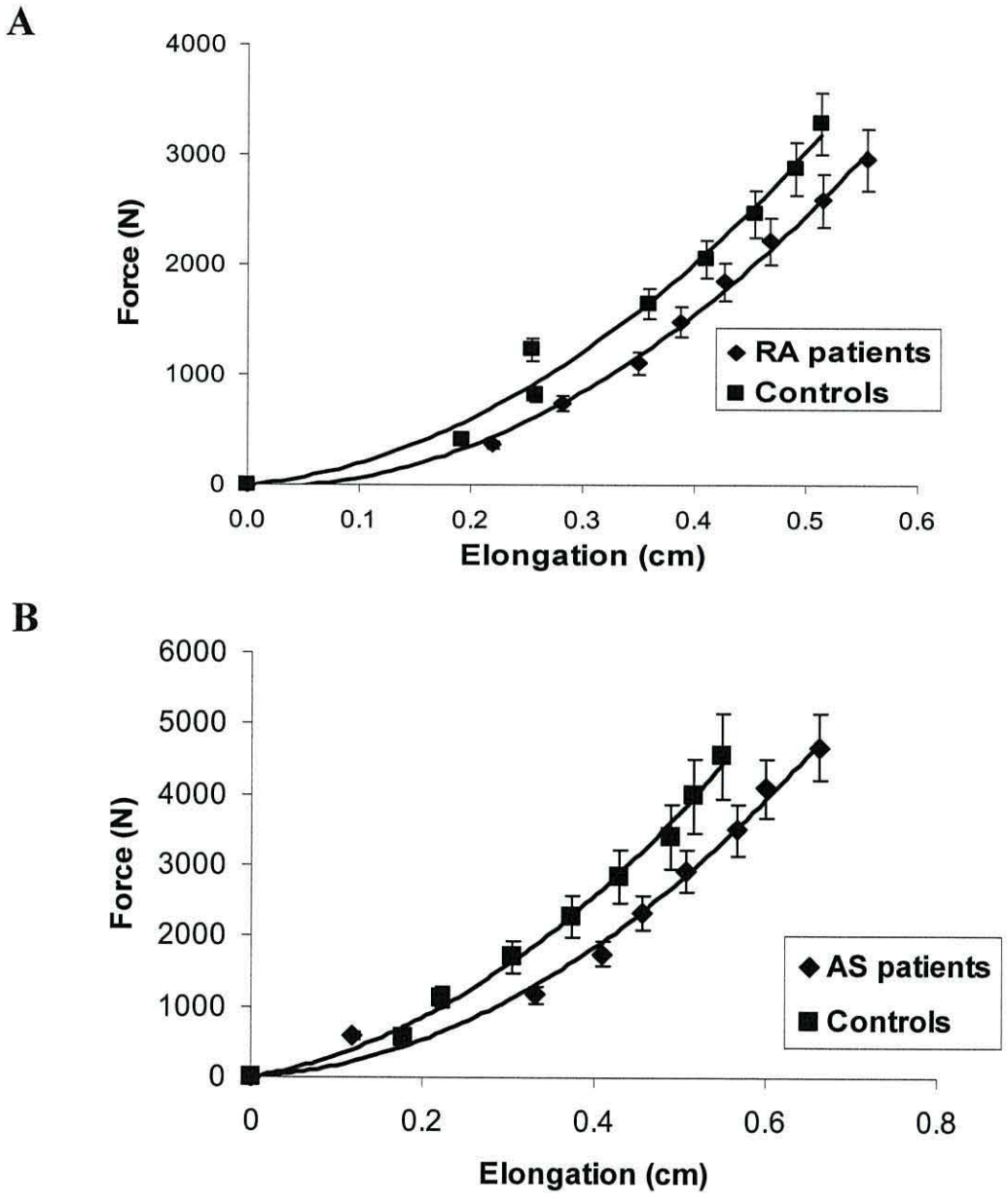

Figure 18. PT force-elongation relationship in RA patients (n=18) (A) and AS patients (n=12) (B) and their respective matched controls. Results are presented as means ± SEM

Tendon and muscle physiological data are presented in Table 14. Consistent with the interpretation of the force-elongation curves, the calculated PT stiffness was significantly reduced in both the RA and AS patients compared to controls. However, whereas the PT cross-sectional area was similar for the RA group and their healthy control group, the AS patients showed increased PT cross-sectional area compared to their controls. There were no differences in PT length between the patient groups and their controls. Young's modulus, which normalises PT stiffness to PT cross-sectional area, was therefore reduced in AS patients, but not in RA patients, relative to their controls.

Quadriceps muscle cross-sectional area and muscle specific force

There were no differences in quadriceps muscle force or CSA between RA and AS patients and their respective matched controls. Consequently, muscle specific force was not compromised for either patient group (Table 14).

	RA study			AS study		
	RA patients (n=18)	**Healthy controls (n=18)**	**P value**	**AS patients (n=12)**	**Healthy controls (n=12)**	**P value**
Quadriceps force (N)	3407 ± 301	3640 ± 244	0.44	4887 ± 492	4922 ± 494	0.93
Quadriceps ACSA (cm^2)	62.7 ± 3.6	60.3 ± 2.7	0.40	77.4 ± 5.3	74.6 ± 4.9	0.62
MQ (N/cm^2)	55.1 ± 4.0	60.9 ± 3.4	0.30	61.6 ± 3.3	65.4 ± 4.2	0.34
Patella tendon stiffness (N/mm)	1017 ± 122	1385 ± 158	**0.04**	1131 ± 133	1751 ± 212	**0.01**
Patella tendon CSA (mm^2)	91.4 ± 4.5	91.3 ± 2.6	0.89	111.8 ± 5.8	96.9 ± 3.9	**0.04**
Young's modulus (GPa)	0.59 ± 0.07	0.74 ± 0.08	0.13	0.49 ± 0.04	0.90 ± 0.10	**<0.001**

Table 14. Physiological data of RA (n = 18) and AS patients (n = 12) and their respective age- and sex-matched healthy controls. Results presented as mean ± SEM

Discussion

This study is to our knowledge the first to investigate the physiological properties of patellar tendons in patients with stable RA or AS. Interestingly, whereas the size of the PT was unchanged in RA, there was PT thickening in the AS group, resulting in pronounced reduction of YM. Both patient groups, however, showed reduced tendon stiffness compared to their healthy age- and sex-matched controls. These changes in tendon properties were accompanied by significant impairments in physical function despite preserved muscle force and size.

The reduction in PT stiffness is most likely due to local and systemic effects of cytokines on the tendon, since proinflammatory cytokines are known to alter tendon structural characteristics in inflammatory arthropathies. The main drivers of the local inflammatory process are TNF-α, IL-1 and IL-6, which produce proteolytic enzymes such as matrix metalloproteinases that lead to collagen destruction (Jain et al. 2001), and proangiogenic vascular endothelial growth factor which promotes synovial hyperplasia and infiltration of macrophages and T-cells into synovium (Kaibara et al. 2008). Thus, tendons are invaded by tenosynovial pannus and get frayed against eroded bone margins, all of which is conducive to tendon ruptures (McQueen et al. 2005). Systemically circulating cytokines could have an additional detrimental effect on the tendon in both RA and AS (Roubenoff et al. 1994). Although patellar and achilles tendons do not have a tendon sheath, they also display characteristics of tendinopathy in inflammatory arthropathies (McGonagle et al. 1998; Cesari et al. 2005; Emad et al. 2009; Falsetti et al. 2009).

In addition to the effects of inflammation, disuse can be a contributor to reduced PT stiffness. Reduced loading of tendons is a known cause of diminished tendon stiffness, and is associated with negative effects on the force output of the attached muscle due to the altered force-length relation of muscle (Kubo et al. 2004; Reeves et al. 2005). Especially in the acute stage of an inflammatory joint disease, patients usually reduce their physical activity because of pain, fatigue, and the fear of exacerbating joint damage by exercising. In our studies with controlled disease activity, however, there were no differences in habitual physical activity levels between the patient groups and their controls with all cohorts being predominantly sedentary. Thus, respective activity levels do not account for the differences we observed in PT stiffness.

The reduced PT stiffness observed for both our patient groups was accompanied by significantly impaired physical function, despite no differences in muscle strength or size. Tendon mechanical properties are essential for proprioception and for the reflex responses involved in the rapid adjustment of muscle tension to positional changes (Onambele et al. 2006), as well as the storing of elastic strain energy which is key to efficient locomotion. The underlying physiological explanation is that increased compliance of the tendon reduces muscle fascicle length changes in response to passive joint movements and thereby impairs recognition of small movements by the muscle spindle (Magnusson et al. 2008). This agrees with Onambele *et al.* who found that the decline in postural stability in the elderly correlated with reduced gastrocnemius tendon stiffness and YM (Onambele et al. 2006).

It was interesting that PT CSA was increased in the AS patients, but not in the RA patients, relative to their respective matched, healthy controls. This difference may be

due to the disparate pathologies of these conditions with the predominant role of enthesis pathology (Benjamin and McGonagle 2009), and increased bone formation in AS versus bone destruction in RA (Appel et al. 2009). With MRI imaging, McGonagle *et al.* (McGonagle et al. 1998) demonstrated characteristic entheseal inflammatory changes associated with knee synovitis in spondylarthropathies that are not seen in RA; in particular perienthesal oedema and bone marrow oedema adjacent to entheseal insertions.

Although PT CSA was increased in AS in our study, this did not attenuate loss of PT stiffness, thereby supporting the theory of a disorganised tendon repair process in AS (Benjamin and McGonagle 2009). In ageing, studies have shown variable results, demonstrating increases, decreases or no change in tendon CSA with age (Magnusson et al. 2003; Reeves et al. 2003; Maganaris et al. 2004). Increases in tendon stiffness in healthy individuals following exercise training have been primarily attributed to intrinsic adaptations of the tendon material properties (Reeves et al. 2003; Seynnes et al. 2009). Some training studies have also shown increases in PT CSA with exercise (Kongsgaard et al. 2007; Couppe et al. 2008; Seynnes et al. 2009), particularly at the enthesis, which is thought to provide protection to the tendon at high stress levels (Kongsgaard et al. 2007; Seynnes et al. 2009).

As the current study was cross-sectional in design, our results do not provide information on the time course of tendon changes. However, in a case report on unilateral inflammatory knee effusion in a patient with newly diagnosed RA (chapter 6) (Matschke et al. 2011), we found an early local reduction of PT stiffness only in the leg affected by knee joint effusion, and one year later a reduction in PT stiffness in both

legs despite having gained control of disease activity and the maintainance of regular physical activity. Loss of muscle specific force and muscle CSA in the affected leg in the active stage of knee effusion was also observed. Whilst muscle specific force and muscle size showed signs of partial recovery following resolution of the joint effusion by intraarticular corticosteroid injection and stabilisation of disease activity, there was no recovery of the PT biomechanics. This corresponds to the results now presented in moderately physically active patients with controlled, established RA and AS, where tendon stiffness is reduced. Previous publications showed that whereas stable RA patients are characterised by attenuated muscle mass and consequently reduced physical function (Giles et al. 2008a; Lemmey et al. 2009), their muscle specific force and activation capacity are preserved (chapters 3 and 4) (Matschke et al. 2010a; Matschke et al. 2010b).

In RA, high intensity exercise has been shown to restore muscle quantity, strength and function (Roubenoff et al. 1994; Hakkinen et al. 2005; Marcora et al. 2005; Giles et al. 2008a; Lemmey et al. 2009). Similarly, high intensity exercise training may be required to achieve beneficial adaptations of tendon properties. In healthy populations, high intensity resistance exercise is associated with increases in tendon stiffness and rate of force development (Reeves et al. 2003; Seynnes et al. 2009). In studies on immobility and on ageing, intensive exercise training has been shown to reverse loss of tendon stiffness (Reeves et al. 2003; Reeves et al. 2004; Reeves et al. 2005; Stevens et al. 2006). Eccentric exercise, which is characterised by high frequency fluctuations of force and transfers higher loads through the tendons than concentric exercise, has shown clinical effectiveness in several studies of overuse tendinopathies (Rees et al. 2009). Eccentric exercise is thought to promote tendon remodelling through increased cross-

linking of collagen fibres (Rompe et al. 2007). The so-called overuse tendinopathies differ from arthritis-linked tendinopathies in their degenerative, rather than inflammatory, pathogenesis (Benjamin et al. 2006). However, intermittent loading has been shown to reduce inflammation in tendon tissue *in vitro* (Yang et al. 2005). Therefore, it is possible that eccentric exercise with intermittent high loading of the tendons would also be beneficial in inflammatory arthropathies. Future studies should investigate the response of RA and AS to tendon-specific training.

There are several limitations to our study. Firstly, higher participant numbers would have been helpful to clarify if, in the context of the loss of tendon stiffness, the apparent reduction in YM in the RA group is significant. Secondly, although we assessed disease activity through patient questionnaires and inflammatory markers in the blood, we did not have an objective measure of the local inflammation of the PT or enthesis. Both MRI and US can provide a detailed assessment of tendinopathic features in different regions along the tendon and at the enthesis, however, a radiologist or clinician trained in clinical ultrasound and MRI evaluation of the tendinous structures was not available to this study. Similarly, histological data on the inflammatory processes in the tendon alongside our tests would enhance understanding of the association between inflammation of tendinous and peritendinous structures and their physiological properties. In our study this would have necessitated an unjustifiably invasive procedure. A future project could assess tendon physiological properties in patients awaiting surgery for conditions such as tenosynovectomy, whereby biopsy material could be gained without inconveniencing patients.

In summary, the present study reveals that patellar tendon properties are adversely affected in RA and AS and possibly contribute to the disability associated with these conditions. Tendinopathies can be asymptomatic and therefore go unnoticed in the context of inflammatory arthropathies (Genc et al. 2005). However, further research is needed to elucidate the role of tendon properties in the impact of chronic arthropathies, and to develop and evaluate treatments for preserving and restoring function of the muscle-tendon complex.

Chapter 8: Summary and conclusions

Thesis findings

Given that patients with RA have severely impaired physical function - despite modern medications that lead to better control of disease activity, and to an extent that cannot be fully attributed to known influences such as joint damage and loss of muscle mass - this thesis aimed to establish whether intrinsic qualitative properties of the tendon-muscle complex are adversely affected by the inflammatory process in RA and also contribute to RA disability. Figure 19 was developed from our studies and illustrates the factors affecting functional limitation, disability and loss of independence in RA, and their interaction (Cooney et al. 2011). The grey box highlights properties of muscle that are known to be altered in RA (muscle strength and mass) and properties of muscle that were thought to be potentially altered in RA (muscle morphology, neural activation, power, contractile properties, and tendon mechanical properties) These were therefore investigated in this thesis.

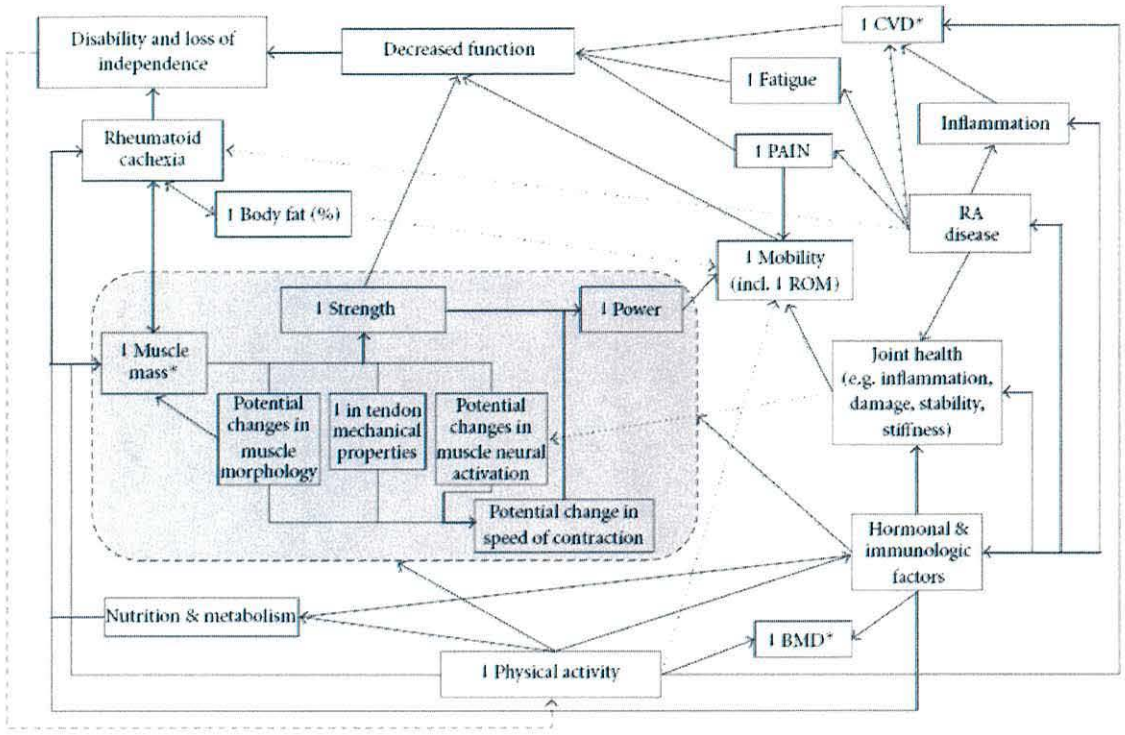

Figure 19. A summary of the potential influence of skeletal muscle properties on the factors affecting functional limitation, disability and loss of independence in RA. BMD: bone mineral density, CVD: cardiovascular disease, ROM: range of motion. Published in Cooney et al. (2011).

Several studies were conducted. Chapter 3 investigated muscle properties in patients with stable disease activity and compared them to age- and sex-matched healthy controls with similar habitual physical activity levels. Stable disease was chosen so that the confounding effects of pain and joint effusion on force production and muscle activation could be excluded. Adverse changes of muscle quality play a major role in the reduced physical function in ageing and disuse, and therefore it was expected that muscle quality would also be altered in RA. However, the results of this study showed that muscle specific force, architecture, voluntary activation capacity and antagonist

coactivation, contractile properties and velocity-specific power were all not significantly different in stable RA.

To further investigate this, in chapter 4 the subgroup of cachectic RA patients from chapter 3 was chosen and compared to their matched healthy controls. The prevalence of rheumatoid cachexia is high at >50% and indicates that this subgroup would have suffered more lifetime disease activity (Roubenoff 2000; Marcora et al. 2005; Lemmey et al. 2009). Therefore, if a change in muscle characteristics were taking place in RA then it should more likely be present in this group. Even in this analysis, no difference of qualitative properties of RA muscle was found, thereby confirming that these characteristics are preserved in this disease.

Another aspect influencing muscle quality is the MHC composition of muscle fibres. Therefore, biopsies were taken from the vastus lateralis muscle of patients with stable RA and healthy controls in chapter 5. Protein electrophoresis showed that there were no significant differences in MHC distribution between stable RA patients and controls.

As our earlier studies demonstrated that muscle quality is preserved in stable RA, Chapter 5 consequently concentrated on the mechanisms underlying the loss of muscle mass generally seen in RA patients. Accordingly, representative markers of the pathways of muscle atrophy, hypertrophy and apoptosis were explored. As loss of muscle mass is thought to occur early in the process of RA and during periods of high disease activity, patients with newly diagnosed acute RA and patients with chronic active RA were recruited and assessed in a longitudinal study before and 3 months after disease control with medication (MTX and anti-TNF agents, respectively). This

investigation showed that in chronic active RA, there is suppression of muscle hypertrophy as demonstrated by reduced levels of pAkt, and that pAkt is restored when disease activity is controlled by anti-TNF therapy. In parallel, atrogin-1, a marker of muscle atrophy, was also suppressed in these patients during disease flare and similarly restored when control of disease activity was achieved, perhaps indicating a reduction in muscle protein turnover during periods of uncontrolled disease activity in established RA patients. In newly diagnosed RA (albeit for an n of 3), this phenomenon was not observed, as no effects of disease activity were noted on either pAkt or atrogin-1 in these patients. In addition, caspase-3, a marker of apoptosis, and IκBα, a downstream effector protein of TNF-α promoting muscle atrophy, were not different in the longitudinal assessment of either group. Caspase-3 was also not different between patients with stable RA and healthy controls in the cross-sectional study. In conclusion, the analysis of muscle biopsies of RA patients gave an indication of alterations in muscle protein metabolism, however, the pathways of muscle hypertrophy and atrophy require further investigations.

The case of a woman with newly diagnosed RA and a unilateral knee effusion, who was physically active and had no pain in her swollen knee, presented the unique opportunity to compare the effects of a rheumatoid knee effusion on muscle quality to the unaffected knee joint in the same person, and possible changes over time (Chapter 6). Here, tendon properties were also assessed. This case demonstrated dramatic consequences of the inflammatory knee effusion on the physiology of the local tendon-muscle complex, with quadriceps wasting, reduced voluntary activation capacity and reduced muscle-specific force, as well as impaired lower body physical function. In addition, patellar tendon stiffness and Young's modulus were reduced in the affected leg. After treatment and

resolution of the knee joint effusion, the parameters of muscle quality had improved one year later. However, tendon stiffness and Young's modulus had further deteriorated over time and were now present also in the unaffected leg, suggesting a systemic effect of RA on tendon properties.

To investigate tendon properties in RA further, a group of patients with stable RA were compared with matched healthy controls in a cross-sectional study (chapter 7). Here, PT stiffness and Young's modulus were reduced as well, confirming the findings of the case study. In addition, a group of patients with AS, a condition primarily affecting the entheses, were investigated in comparison with matched healthy controls. The AS patients also had reduced PT stiffness and Young's modulus. In contrast to RA, however, the AS patients had increased PT CSA, highlighting the different pathologies of these conditions.

Implications and future directions

Though contrary to our expectations, the fact that muscle quality is not altered in stable RA is positive news to patients with RA and health professionals. The findings are consistent with, and provide an explanation for, the normal responses to resistance training (i.e. muscle hypertrophy and increased strength) observed in RA patients (Nordemar et al. 1976a; Van den Ende et al. 1998; Van Den Ende et al. 2000b; Hakkinen et al. 2001; de Jong et al. 2003; Hakkinen et al. 2005; Hambrecht et al. 2005; Marcora et al. 2005; Lemmey et al. 2009). Thus, the results of this thesis with regards to muscle quality are important for rheumatology health professionals and sports scientists

involved in designing exercise training for RA patients, as they help to demonstrate why patients with RA are not resistant to the anabolic effects of exercise as previously thought, and why rheumatoid muscle adapts to exercise training in a similar way as muscle from matched healthy individuals. Consequently, the same exercise programmes and recommendations can usually be given to RA patients that are provided for the general population (Lemmey et al. 2011).

This thesis attempted a comprehensive assessment of the characteristics of the tendon-muscle complex in RA. However, the results indicate the need for further research in a number of areas:

The results of the case study indicate that muscle quality is impaired locally in early active RA, and studies need to be conducted to assess this in a larger group of patients with active RA. However, this could be difficult to investigate due to the confounding factors of pain and fatigue in uncontrolled disease.

Tendon properties are adversely affected in patients with stable RA, with indication from the case study that tendon stiffness is already reduced locally at an early stage of disease. Again, studies should confirm tendon characteristics in active disease and the time course of tendon changes in a representative group of participants. In addition, longitudinal studies to assess the response of tendons in RA to high intensity training or involving tendon-focused eccentric exercise are required.

Finally, the pathways leading to rheumatoid cachexia need further exploration, on larger samples of biopsies, and including investigations on the myostatin-induced muscle

atrophy pathway via FOXO, and other downstream effector proteins of Akt such as the mTOR pathway.

In over 30 studies over the past years, exercise training has been shown to be an effective intervention to increase muscle strength and objective physical function. In particular high intensity training results in marked strength gain compared to low training intensity (Hakkinen et al. 1995; van den Ende et al. 1996; van den Ende et al. 2000a; Hakkinen et al. 2001; Hakkinen et al. 2004; Hakkinen et al. 2005; Lemmey et al. 2009). Several studies have shown the hypertrophic response of rheumatoid muscle to progressive resistance training (Hakkinen et al. 2004; Hakkinen et al. 2005; Marcora et al. 2005; Lemmey et al. 2009). Additionally reductions in appendicular and truncal fat mass were achieved by intensive exercise intervention (Marcora et al. 2005; Lemmey et al. 2009). Furthermore, high intensity exercise has been shown to be safe in RA without leading to an increase in disease activity or progression of joint damage (van den Ende et al. 2000a; Hakkinen et al. 2001; de Jong et al. 2003; Hakkinen et al. 2004; Hakkinen et al. 2005; Marcora et al. 2005; Lemmey et al. 2009). This information together with the results of this thesis on preserved muscle quality is also encouraging news to impart to people with RA. Patients not only experience physical barriers to exercise such as pain and fatigue, but often fear a lack of effect of exercise or even worry about a negative effect (Law et al. 2011). Knowing about muscle characteristics and the benefits of exercise can help improve their perception of exercise training and motivate them to persist with this essential part of rehabilitation in RA which is likely to lead to meaningful improvements in physical function and mortality for patients.

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Appendices

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