Rheumatoid cachexia: a review of the literature and a potential therapeutic avenue

‘...wasting occurs, in greater or lesser degree, in all muscles near joints that are inflamed...It is, I repeat, not a mere wasting from disuse.’
Sir James Paget, 1873
# Table of Contents:

List of abbreviations ................................................................................. ii
List of figures .............................................................................................. iii
Abstract ........................................................................................................ iv

Introduction ................................................................................................. 1

1. Background of Rheumatoid Cachexia – framing the burden .................. 2
   1.1 Historical Perspective and Current definition ..................................... 2
   1.2 Epidemiology ..................................................................................... 3
   1.3 Socioeconomic Consequences .......................................................... 5
   1.4 Clinical Picture – an under-recognised phenomenon ....................... 6
   1.5 Cardiovascular Disease in RA – does rheumatoid cachexia contribute? 8
   1.6 Consequences of Skeletal Muscle Depletion ..................................... 9
   1.7 Aetiology .......................................................................................... 10

2. Introduction to Pathogenesis and Management ....................................... 11
   2.1 Pathogenesis – a poorly understood positive feedback mechanism ...... 11
      2.1.1 Muscle-fat Crosstalk .................................................................... 13
   2.2 Management of Rheumatoid Cachexia ................................................ 15

3. Muscle Structure ..................................................................................... 17
   3.1 Normal Architecture .......................................................................... 17
   3.2 Structural Changes in Rheumatoid Cachexia ....................................... 18
   3.3 Structural Changes in Rheumatoid Cachexia ....................................... 19

4. Muscle Breakdown Pathways .................................................................. 21
   4.1 Ubiquitin-Proteasome System ......................................................... 21
   4.2 Myostatin Signalling .......................................................................... 23
   4.3 Myostatin in Rheumatoid Cachexia .................................................. 23

5. The Role of Muscle – not just a passive target? ....................................... 25

   6.1 Tofacitinib for Rheumatoid Arthritis ............................................... 28
   6.2 Janus Kinase in Health and Disease .................................................. 29
   6.3 Tofacitinib for Rheumatoid Cachexia – how could it work? ............... 30

Conclusion – the story so far ................................................................. 33

References .................................................................................................. 34
### List of Abbreviations:

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AiA</td>
<td>Antigen-induced arthritis</td>
</tr>
<tr>
<td>BIA</td>
<td>Bioelectrical impedance analysis</td>
</tr>
<tr>
<td>BMD</td>
<td>Bone mineral density</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>CHF</td>
<td>Chronic heart failure</td>
</tr>
<tr>
<td>CIA</td>
<td>Collagen-induced arthritis</td>
</tr>
<tr>
<td>COPD</td>
<td>Chronic obstructive pulmonary disease</td>
</tr>
<tr>
<td>CRP</td>
<td>C-reactive protein</td>
</tr>
<tr>
<td>CSA</td>
<td>Cross-sectional area</td>
</tr>
<tr>
<td>CSD</td>
<td>Cross-sectional diameter</td>
</tr>
<tr>
<td>CVD</td>
<td>Cardiovascular disease</td>
</tr>
<tr>
<td>CT</td>
<td>Computerised tomography</td>
</tr>
<tr>
<td>DMARD</td>
<td>Disease-modifying antirheumatic drug</td>
</tr>
<tr>
<td>DXA</td>
<td>Dual-energy x-ray absorptiometry</td>
</tr>
<tr>
<td>ECF</td>
<td>Extracellular fluid</td>
</tr>
<tr>
<td>ECS</td>
<td>Extracellular solids</td>
</tr>
<tr>
<td>ESR</td>
<td>Erythrocyte sedimentation rate</td>
</tr>
<tr>
<td>EULAR</td>
<td>European League Against Rheumatism</td>
</tr>
<tr>
<td>FDA</td>
<td>Food and Drug Administration</td>
</tr>
<tr>
<td>FFM</td>
<td>Fat-free mass</td>
</tr>
<tr>
<td>FFMI</td>
<td>Fat-free mass index</td>
</tr>
<tr>
<td>FMI</td>
<td>Fat mass index</td>
</tr>
<tr>
<td>ICM</td>
<td>Immune cell mass</td>
</tr>
<tr>
<td>IL-1β</td>
<td>Interleukin-1 beta</td>
</tr>
<tr>
<td>IL-6</td>
<td>Interleukin-6</td>
</tr>
<tr>
<td>JAK 1, 2, 3</td>
<td>Janus kinase 1, -2, -3</td>
</tr>
<tr>
<td>JAK/STAT</td>
<td>Janus kinase/signal transducer and activator of transcription</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
</tr>
<tr>
<td>MuRF-1</td>
<td>Muscle RING-finger protein-1</td>
</tr>
<tr>
<td>RA</td>
<td>Rheumatoid arthritis</td>
</tr>
<tr>
<td>RC</td>
<td>Rheumatoid cachexia</td>
</tr>
<tr>
<td>SCID</td>
<td>Severe combined immunodeficiency</td>
</tr>
<tr>
<td>STAT</td>
<td>Signal transducer and activator of transcription</td>
</tr>
<tr>
<td>STAT3</td>
<td>Signal transducer and activator of transcription 3</td>
</tr>
<tr>
<td>TNF</td>
<td>Tumour necrosis factor alpha</td>
</tr>
<tr>
<td>TYK 2</td>
<td>Tyrosine kinase 2</td>
</tr>
<tr>
<td>UPS</td>
<td>Ubiquitin-proteasome system</td>
</tr>
<tr>
<td>VCM</td>
<td>Visceral cell mass</td>
</tr>
</tbody>
</table>
List of Figures:

Figure 1.1: Calculation of fat-free mass index and fat mass index ........................................ 2
Figure 1.2: Prevalence of rheumatoid cachexia by age and gender, 2012 ................................. 3
Figure 1.3: Schematic of rheumatoid arthritis disease burden ............................................. 5
Figure 1.4: Representation of tissue and body compartments ............................................. 7

Figure 2.1: Possible pathways of cytokine-mediated muscle wasting ...................................... 11
Figure 2.2: Effects of reduced exercise on skeletal muscle and adipose .................................. 12
Figure 2.3: Metabolic crosstalk between skeletal muscle and adipose .................................. 13
Figure 2.4: Complete diagram of possible pathogenesis of rheumatoid cachexia ................. 14

Figure 3.1a: Diagram of components of skeletal muscle ..................................................... 17
Figure 3.1b: Cross-section of skeletal muscle with fibre-specific stain ................................. 17
Figure 3.2: Cross-sections of gastrocnemius in rat model of rheumatoid cachexia ............. 19
Figure 3.3: Cross-section of monkey gastrocnemius stained for ATPase ............................ 20

Figure 4.1: Diagram of the ubiquitin-proteasome system ..................................................... 21

Figure 5.1: Longitudinal gastrocnemius showing hyper-cellularity in arthritis ................... 26
Figure 5.2: TNF immunofluorescent staining in gastrocnemius in AIA ............................... 26
Figure 5.3: IL-1β immunohistochemistry in gastrocnemius cross-sections ....................... 27

Figure 6.1: Type I and II receptors and activation of JAK signalling ...................................... 29
Figure 6.2: Effects of JAK deficiency or inhibition ............................................................... 30
Figure 6.3: Immunofluorescent staining of wasted myofibres after JAK inhibition ............ 31
Abstract

Background: Rheumatoid arthritis (RA) is an inflammatory arthropathy affecting 0.5-1% of the population. In addition to progressive joint destruction, RA patients suffer a range of systemic consequences, including altered body composition. Rheumatoid cachexia (RC) is an advanced form of body tissue derangement in RA. Despite affecting between 10-20% of RA patients, RC is under-recognised in both a clinical and scientific context. Clinical recognition and the development of effective pharmacological treatment are impeded by poor understanding of the underlying pathogenic mechanisms.

Aims: Provide an up-to-date appraisal of the literature surrounding RC and identify remaining knowledge gaps. Consider the efficacy of tofacitinib, a broad-spectrum anti-inflammatory medication, as a potential therapeutic agent.

Methods: The literature search was conducted using Ovid MEDLINE® and Scopus® databases from their inception to May 2014. Medical Subject Headings (MeSH) included: rheumatoid cachexia, rheumatoid arthritis, muscle and tofacitinib. Reference lists of all full-text retrieved papers were also searched to identify additional eligible studies.

Findings: There are significant deficiencies in our understanding of the definition, prevalence, pathogenesis, consequences and management of RC. Circulating inflammatory cytokines appear crucial in the disease development, yet the exact causative mechanisms remain poorly understood. While there is a paucity of evidence, it is biologically plausible that alterations to pro-inflammatory cytokine expression within muscle may also contribute to the wasting process. Targeting these intra-muscular inflammatory pathways via tofacitinib may represent the first effective pharmacological agent for RC.

Key words: Rheumatoid cachexia; Rheumatoid arthritis; Muscle atrophy; Tofacitinib
Introduction

Rheumatoid arthritis (RA) is a common, autoimmune-mediated joint disease with potentially debilitating clinical outcomes. Untreated, chronic synovial inflammation leads to progressive cartilage and sub-articular bone destruction with subsequent functional decline. The treatment paradigm for RA now includes early initiation of disease-modifying antirheumatic drugs (DMARDs) and targeted biologic therapies. While not always effective, this strategy allows many patients to achieve clinical remission or a reduced disease activity. The once inevitable bone and joint destruction can now be partially prevented or controlled.

However, joint destruction is only one aspect of the burden of disease. The inflammatory properties of RA cause systemic bio-psycho-social consequences. Increased cardiovascular disease and accelerated osteoporosis in particular are attracting greater attention. Despite growing interest in the systemic manifestations of RA, many co-morbidities remain under-appreciated. Rheumatoid cachexia is one such phenomenon.

Rheumatoid cachexia is characterised by reduced skeletal muscle mass with either stable or increased fat mass. Such body tissue alterations are a common feature of RA. However, defining cut-off values used to diagnose rheumatoid cachexia in the scientific literature estimate that approximately one-fifth of RA patients are affected. The omission of reduced fat mass separates rheumatoid cachexia from the wasting seen in cancer, chronic heart failure and respiratory disease. As such, rheumatoid cachexia represents a distinct clinical entity with its own set of adverse outcomes. Preliminary evidence suggests that rheumatoid cachexia may contribute to the increased cardiovascular risk described in RA patients. Skeletal muscle wasting in particular may have deleterious effects on patients’ lives. Of concern, these changes to body composition occur even in patients with clinically well-controlled RA, suggesting conventional therapies are ineffective at preventing rheumatoid cachexia. However, there is a scarcity of literature addressing the clinical significance and diagnosis of this condition. As such, rheumatoid cachexia remains under-appreciated in RA.

While inflammatory activity appears critical, the pathogenetic mechanisms of muscle wasting and potential therapeutic agents to reverse these effects are not known. These two gaps in the literature require investigation in order to identify effective interventions for this potentially disabling condition.
1. Background of Rheumatoid Cachexia – *framing the burden*

### 1.1 Historical Perspective & Current Definition

From the Greek meaning ‘bad condition,’ cachexia in RA patients was first described in 1873 by Sir James Paget\(^\text{10}\). However, despite incidental discoveries concerning distorted muscle and nutritional status in RA patients\(^\text{11,12}\), the topic of rheumatoid cachexia (RC) remained largely quiescent until the late 20\(^{\text{th}}\) century.

In 1992, Roubenoff et al. demonstrated that RC affected two-thirds of RA patients\(^\text{13}\), a significant increase from prior estimates\(^\text{12}\). Counter-intuitively, the condition remained underappreciated and poorly studied until a relative boom in scientific interest over the last 10 years.

Current definitions of RC vary, however most authors appear to have reached consensus on the following definition: *a condition of reduced muscle mass and stable or increased fat mass associated with rheumatoid arthritis*\(^\text{6}\).

Despite a seemingly intuitive definition, there is a marked absence of guiding cut-off values to identify what degree of variance constitutes RC. The two most common numerical definitions used for research purposes are:

1. Fat-free mass index <10\(^{\text{th}}\) centile *plus* fat mass index >25\(^{\text{th}}\) centile\(^\text{14}\)
2. Fat-free mass index <25\(^{\text{th}}\) centile *plus* fat mass index >50\(^{\text{th}}\) centile\(^\text{15}\)

Fat-free mass (FFM) and fat mass indices are found via anthropometric or whole-body imaging techniques (Figure 1.1). Values can then be compared to percentile scores of a pre-defined reference population.

\[
\text{FFMI} = \frac{\text{fat-free mass}}{\text{height}^2} \left(\frac{\text{kg}}{\text{m}^2}\right) \quad \text{and} \quad \text{FMI} = \frac{\text{fat mass}}{\text{height}^2} \left(\frac{\text{kg}}{\text{m}^2}\right)
\]

*Figure 1.1 Calculation of fat-free mass index (FFMI) and fat mass index (FMI)*

Many older studies define RC solely on reduced fat-free mass\(^\text{13,16}\). However, due to differing disease characteristics\(^\text{17}\), this review will focus on the definition that also considers alterations in fat mass.
1.2 Epidemiology

Given the paucity of literature addressing RC, epidemiological data are based solely upon approximations from studies of RA patients. Therefore, before discussing the epidemiological patterns of RC, I must first frame the burden of RA.

In 2012, an estimated 500 000 Australians suffered from RA\textsuperscript{18}, a prevalence of 2.1\%. Of these patients, 63.5\% of cases were females, with the highest prevalence between the ages of 55-64 years (Figure 1.2).

Despite a modest decline in prevalence from 2.4\% in 1995\textsuperscript{21}, the number of people with RA is projected to increase by 40\% in 2032, reaching an unprecedented high of 0.7 million Australians\textsuperscript{18}.

As a relatively new area of research, the epidemiology of RC has been poorly studied and varies greatly depending on the study population and the definition used.

\textsuperscript{*} Estimates from the United Kingdom and North America, typically quote an RA prevalence of 0.5-1\%\textsuperscript{19,20}, between 25-50\% of the estimated Australian prevalence. The apparent doubling of prevalence may be due to the nature of Australian data acquisition with many statistics relying on self-reporting from the Australian Bureau of Statistics National Health Survey.
Most reviews state that RC occurs in up to two-thirds of RA patients – a figure based on Roubenoff et al.’s landmark study of RC from 1992\textsuperscript{13}. However, this finding was based on comparative arm muscle area, a surrogate marker for lean body mass that has low sensitivity and specificity when compared to dual-energy x-ray absorptiometry (DXA)\textsuperscript{22}.

Using more accurate measurement techniques, recent studies estimate RC to affect between 11-21% of RA patients\textsuperscript{8,14,23,24}, with the vast majority reporting a higher prevalence in women. If we apply these figures to the RA prevalence in Australia, we can crudely estimate that RC affects between 55 000 and 105 000 Australians.

However, the significance of the cut-off values used to define RC is yet to be evaluated. More subtle changes to body composition appear to affect the majority of RA patients\textsuperscript{7}, with unknown consequences.
1.3 Socioeconomic Consequences

As the most likely period of disease onset correlates with the most active years of workplace, familial and societal roles, RA can have dramatic socioeconomic consequences. A major reason for the significant impact of RA is its propensity for systemic, bio-psycho-social ramifications, including fatigue, depression, cognitive dysfunction, reduced work performance and a wide array of debilitating co-morbidities (Figure 1.3).

Figure 1.3. Schematic of rheumatoid arthritis disease burden. Illustrating bio-psycho-social consequences of rheumatoid arthritis
Taken from: Cutolo et al, Seminars in Arthritis and Rheumatism; 2014

In financial terms, the estimated direct health costs of RA reached $537 million in 2012\textsuperscript{18}. These figures encompass RA as a whole, including the cost of systemic consequences. However, while many of the widespread effects of RA are well characterised, RC remains under-appreciated. This makes determining the proportion of the total disease burden attributable to RC problematic.
1.4 Clinical Picture – an under-recognised phenomenon

Under appreciation of RC, coupled with changing definitions of the condition, contribute to diagnostic uncertainty. Disturbances in body composition occur in early RA\textsuperscript{24,25}, with alterations found within one year of diagnosis\textsuperscript{25}. Despite such early manifestations, RC often remains unnoticed on clinical examination\textsuperscript{26}.

Traditional anthropometric measures such as body mass index (BMI) and weight are also unhelpful in identifying changes in body composition\textsuperscript{24,27,28}. While decreased lean mass is the predominant feature of RC\textsuperscript{27,29-31}, BMI is often preserved by a relative increase in fat mass\textsuperscript{28}. Indeed, RA patients have been shown to exhibit 4.3\% more body fat for a given BMI when compared to healthy controls\textsuperscript{28}.

Rather than crude bedside assessment, diagnosis of RC requires the ability to partition the body into anatomical compartments. This is most commonly performed by either whole-body medical imaging such as computerised tomography (CT), magnetic resonance imaging (MRI) and DXA, or comparing electrical properties between tissues, such as bioelectrical impedance analysis (BIA)\textsuperscript{32}.

While CT and MRI can accurately measure skeletal muscle and adipose tissue, cost and accessibility limit widespread use\textsuperscript{32}. As a result, DXA plays a large role in the assessment of RA patients and has since been validated for measuring various parameters of body composition\textsuperscript{33,34} (Figure 4b). When DXA is unavailable, BIA has proven to be a useful second-line option that, although less accurate, has relatively good agreement with DXA\textsuperscript{8} (Figure 4a). However, neither technique can determine the exact tissue being altered. As such, their main utility is monitoring the longitudinal changes to body composition in RA.
The concept of fat-free mass index (FFMI) and fat mass index (FMI) provide numerical cut-offs for the diagnosis of RC. While the sum of these indexes is equivalent to BMI, when examined individually, they reflect the contribution of each compartment to the total body composition. Despite widespread use in studies of RC, the FFMI and FMI are poorly implemented in clinical practice. As well as preventing epidemiological and socioeconomic analysis, the under-recognition of RC limits the evaluation of its potential consequences.
1.5 Cardiovascular Disease in RA – does rheumatoid cachexia contribute?

RA patients suffer a reduced life expectancy\textsuperscript{37}, facing up to a 60% increase in all-cause mortality compared to the general population\textsuperscript{38}. Most RA-associated mortality appears to be related to cardiovascular disease (CVD)\textsuperscript{17}. Adverse cardiovascular events occur earlier and result in worse clinical outcomes when compared to healthy individuals\textsuperscript{39,40}. Whether RC contributes to this increased CVD risk is contentious, and requires further investigation.

In addition to increased total fat mass, patients with RC often exhibit copious central redistribution of their adipose tissue, resulting in central adiposity\textsuperscript{15,24}. The relationship between truncal obesity, the metabolic syndrome and subsequent CVD is well established\textsuperscript{41,42}. This association extends to the RA population, with data supporting early vascular changes in RA patients with central adiposity\textsuperscript{43}. Therefore, it is reasonable to assume that fat gain in RC may contribute to increased CVD in RA patients. An additional proposed mechanism for increased CVD in RA patients is the deleterious effects of systemic inflammation on cardiovascular health\textsuperscript{44-47}. By amalgamating these two observations, Summers et al.\textsuperscript{17} have argued that RC may represent ‘the worst of both worlds’. That is, RC patients face a dual mechanism of increased CVD: high inflammatory activity plus increased total and central adiposity.

Despite this intuitive theory, the two studies that have directly compared the mortality of RA patients with and without RC show conflicting results. Elkan et al.\textsuperscript{15} found that RC patients had a significantly worse CVD profile than RA patients with normal body composition. Total cholesterol, low-density lipoprotein and the frequency of hypertension and metabolic syndrome were significantly higher in the RC group compared to the non-cachectic RA group.

In contrast, Metsios et al.\textsuperscript{48} found a similar CVD risk factor profile between cachectic- and non-cachectic RA patients and no differences in 10-year CVD risk. However, the strict criteria used to define RC in this study resulted in an under-representation of obese patients in the RC group and therefore a possible underestimation of cardiovascular risk\textsuperscript{17}. This reflects the importance of identifying cut-off values that correlate with clinically significant consequences.

Current literature is insufficient to determine the influence of RC on the increased CVD experienced by RA patients. Muscle loss appears to have its own direct consequences, some of which further implicate RC in the development of CVD.
1.6 Consequences of Skeletal Muscle Depletion

Skeletal muscle is the largest organ in the human body. In addition to locomotion, muscle mediates a number of critical metabolic functions, including insulin sensitivity and energy metabolism. As a major target in RC, skeletal muscle depletion has numerous adverse effects.

Muscle loss has been associated with weakness and imbalance; in RC patients this translates to a reduced quality of life. Importantly, these associations remained after adjusting for the inflammatory activity and duration of RA, suggesting skeletal muscle loss is an independent risk factor for adverse clinical outcomes.

Although not yet demonstrated in RC, skeletal muscle depletion is also independently associated with increased mortality across a range of wasting diseases, including cancer, chronic obstructive pulmonary disease (COPD) and chronic kidney disease patients receiving dialysis.

Supporting a potential role for RC in CVD, wasting has been associated with a multitude of cardiovascular risk factors. In particular, low muscle mass has been linked to insulin resistance and type II diabetes. Results from bed-rest experiments indicate that this insulin resistance may then lead to dyslipidaemia. Combined with the common phenotype of central adiposity, insulin resistance and dyslipidaemia constitute the metabolic syndrome, a known contributor to CVD. Therefore, despite deficiencies in the literature, it is plausible that skeletal muscle loss in RC may contribute to increased CVD risk.

Decreased muscle mass and function has also been suggested to contribute to decreased bone mineral density. This suggests that muscle wasting in RC patients may directly influence osteoporosis and CVD, two key pathogenic consequences of RA. Given these diverse and dramatic consequences, there is a need for greater understanding of the aetiology and pathogenesis of this debilitating condition.
1.7 Aetiology

The development of rheumatoid arthritis has been intensely studied, with the current paradigm proposing a combination of genetic factors and environmental triggers. However, once RA has been diagnosed, there is insufficient literature to predict which subset of patients will develop RC. Currently, the evidence base suggests only two risk factors for progression to RC: inflammatory severity and disease duration.

There is an inverse correlation between inflammatory severity and FFMI. Surrogate measures of inflammation shown to correlate with altered body composition include: serum erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), tumour necrosis factor alpha (TNF) and interleukin-6 (IL-6). These results indicate that RC develops in the presence of systemic inflammation. Disappointingly, such alterations in body composition have been observed even when RA is clinically well controlled. This implies that RC may develop due to sub-clinical inflammation that remains unresponsive to conventional RA therapies. Disease duration has also been positively associated with changes to RC. In fact, Fukuda et al. hypothesise that, in terms of muscle protein depletion, the duration of inflammation may be more important than its severity.

Clearly, further research is required to quantify the role of the above influences in the development of RC, as well as identify additional aetiological factors. In particular, the identification of modifiable factors is critical to inform early risk avoidance.
2. Introduction to Pathogenesis & Management

2.1 Pathogenesis – a poorly understood positive feedback mechanism

There are numerous theories that attempt to describe the pathogenesis of RC. Although incompletely understood, elevated inflammatory cytokines and decreased exercise appear to be key initiating factors for muscle wasting and fat gain. Further progression may involve a positive feedback mechanism driven by secretory products of both muscle and adipose tissue.

The fundamental role of the inflammatory cytokines TNF, IL-6 and interleukin-1 beta (IL-1β) in RA also appear to be the driving mechanisms of RC. TNF in particular has been shown to play a major role in alterations of body composition and is implicated in multiple pathways of muscle wasting.

There is a dual mechanism contributing to muscle wasting in RC, decreased protein synthesis and increased protein breakdown. Elevated pro-inflammatory cytokines in RA patients drive both routes of muscle loss via three key pathways (Figure 2.1).

![Figure 2.1 Possible pathways of cytokine-mediated muscle wasting in rheumatoid cachexia.](image)

Rheumatoid arthritis patients have increased levels of pro-inflammatory cytokines. These cytokines have been shown to induce muscle wasting via at least three key pathways. TNF and IL-6 in particular have been associated with increased peripheral insulin resistance, impairing insulin-mediated muscle anabolism. Similarly, these cytokines are known to reduce muscle cell differentiation and repair via inhibition of two positive regulatory genes, MyoD and myogenin. The final pathway of muscle loss occurs via activation of the ubiquitin-proteasome system (UPS). The UPS mediates controlled proteolysis within cells throughout the body. In skeletal muscle, this correlates to protein breakdown and subsequent atrophy.
Firstly, enhanced TNF and IL-6 signalling are associated with peripheral insulin resistance\textsuperscript{63}. As insulin has an anabolic effect on muscle protein\textsuperscript{64}, resistance to this hormone further impairs skeletal muscle protein synthesis\textsuperscript{65}. Secondly, TNF inhibits MyoD and myogenin, key regulators of skeletal muscle differentiation and repair\textsuperscript{66}. Down-regulation of these genes directly reduces muscle protein anabolism. The third cytokine-mediated pathway of muscle loss occurs via increased protein catabolism. Both TNF and IL-6 stimulate a proteolytic pathway known as the ubiquitin-proteasome system (UPS)\textsuperscript{67,68}. The UPS functions by tagging target proteins with a regulatory peptide called ubiquitin. Ubiquitin binding marks the substrate for subsequent degradation by the cell proteasome, an organelle mediating proteolysis\textsuperscript{69}. By up-regulating this system, elevated cytokine signalling increases protein turnover. Furthermore, coupled with the aforementioned inhibition of MyoD, attempts at muscle protein repair may be suppressed.

Despite a predominant role, pro-inflammatory mediators are not the only mechanism of muscle wasting in RC. Joint pain, fatigue and lack of confidence all contribute to reductions in exercise levels with consequent muscle atrophy\textsuperscript{70,71}. Reduced exercise under-stimulates myocytes and impairs muscle protein synthesis\textsuperscript{72}. Additionally, reduced exercise introduces the first mechanism of fat gain in RA patients (Figure 2.2).

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure2.png}
\caption{The effects of reduced exercise on skeletal muscle and adipose tissue.}
\end{figure}

Increased fat mass occurs via multiple pathways and may promote further muscle wasting via communication between these two body tissues.
2.1.1 Muscle-fat Crosstalk

As previously discussed, muscle wasting in RC is often accompanied by increased adiposity. Unlike cachexia in cancer patients\textsuperscript{73}, RC does not appear to be associated with either decreased dietary intake\textsuperscript{15,29,31} or anorexia\textsuperscript{26}. In combination with markedly reduced physical activity in RA\textsuperscript{70,71}, normal dietary intake creates an overall positive energy balance and consequent tendency to store fat\textsuperscript{74,75}.

Secretory products of skeletal muscle may mediate an additional mechanism of fat retention in RC. Muscles secrete both cytokines, known as myokines, and positive and negative regulators of muscle growth\textsuperscript{76}. As well as autocrine action, these products allow communication with various tissues throughout the body\textsuperscript{77}.

Myostatin is a key muscle-derived hormone with widespread roles in energy and protein metabolism. In regards to muscle homeostasis, myostatin mediates numerous pathways of protein loss via autocrine signalling. Consequently, mice deficient in myostatin have increased muscle mass\textsuperscript{78,79}. Yet, remarkably, these mice exhibit simultaneous reductions in fat tissue, suggesting that myostatin has an important role in crosstalk between skeletal muscle and adipose\textsuperscript{78,79} (Figure 2.3). More recently, decreased myostatin levels have been shown to reduce adipocyte size and enhance fatty acid oxidation and thermogenesis, thereby reducing fat mass\textsuperscript{80,81}. If skeletal muscle of RC patients has increased myostatin secretion, this pathway may contribute to the parallel increase in adiposity. However, as will be discussed in a following section, the role of myostatin and myokines in the muscle wasting of RC is currently unknown.

![Figure 2.3 Metabolic crosstalk between skeletal muscle and adipose tissue.](image)

Myostatin has autocrine actions on skeletal muscle reducing both protein accretion and muscle cell development. It is also able to exert distant effects on adipose tissue where it acts to increases fat accumulation and adipogenesis – the development of adipocytes. These dual actions make myostatin an attractive candidate for involvement in rheumatoid cachexia. In addition, increased fat mass may decrease adiponectin secretion, thereby reducing its positive role in insulin sensitivity. Taken from: Argilés et al. Drug Discovery Today, 2012\textsuperscript{77}.
Increased fat mass may also directly contribute to muscle wasting via introducing a positive feedback loop. This highlights a reciprocal relationship between muscle and fat in RC, where their mass appears inversely associated. Increased adipose tissue leads to secretion of adipocytokines, including TNF and IL-6, thereby enhancing the three cytokine-dependent mechanisms of muscle loss\textsuperscript{62}. In addition, obesity decreases adiponectin expression\textsuperscript{83}, preventing its protective role in insulin sensitisation and further reducing insulin-mediated anabolism\textsuperscript{84}.

While the mechanisms of action remain incompletely understood, an amalgamation of the above theories is represented in the following diagram (Figure 2.4).

\begin{itemize}
  \item [\textbf{Rheumatoid Arthritis}]\hspace{1cm} \textbf{Joint Pain & Fatigue}
  \item [\textbf{Cytokines (TNF-\alpha, IL-1\beta, IL-6)}] \hspace{1cm} \textbf{Peripheral insulin resistance}
  \item [\textbf{\uparrow UPS}] \hspace{1cm} \textbf{\downarrow MyoD & Myogenin?}
  \item [\textbf{\uparrow Protein catabolism}] \hspace{1cm} \textbf{\downarrow Protein anabolism}
  \item [\textbf{\downarrow Muscle Mass}] \hspace{1cm} \textbf{Myostatin?}
  \item [\textbf{\downarrow Fat Mass}]
\end{itemize}

\textbf{RHEUMATOID CACHEXIA}

\textbf{Figure 2.4 Complete diagram of the possible pathogenesis of rheumatoid cachexia.} Rheumatoid arthritis patients exhibit increased levels of pro-inflammatory cytokines. These up-regulate the ubiquitin-proteasome system (UPS) of muscle catabolism as well as down-regulate two anabolic processes: insulin-mediated protein synthesis as well as expression of MyoD and myogenin – proteins involved in myocyte maturation. In addition, muscle disuse from decreased exercise leads to decreased protein synthesis and increased fat gain. Increased fat may directly contribute to skeletal muscle loss via enhanced pro-inflammatory cytokine signalling and decreased adiponectin-mediated insulin sensitisation. The location of these actions is represented with orange borders. In addition, muscle-derived pro-inflammatory cytokines (myokines) may contribute to systemic inflammation and cause direct autocrine muscle atrophy via up-regulation of the UPS, and down regulation of MyoD and myogenin (blue borders). Myostatin may also exert autocrine muscle atrophy and contribute to fat gain. However the role of both of myokines and myostatin in RC requires further investigation.
2.2 Management of Rheumatoid Cachexia

No effective pharmacological treatment has been identified for RC. The only proven treatment for reversing muscle wasting is exercise. Assessing treatment efficacy is complicated, as the management of RC must be considered from two perspectives: reversal of muscle loss and prevention of further wasting.

In a randomised control trial of 40 patients with early RA, Engvall et al. demonstrated that combination DMARD therapy with methotrexate, sulphasalazine and hydroxychloroquine, partially restored FFM over 12 and 24 months. In contrast, methotrexate plus anti-TNF therapy had no significant effect. While triple DMARD therapy is not the first-line treatment for RA, these findings indicate that there is a window for early, combination DMARD therapy to restore alterations to body tissue, potentially reducing the incidence of RC. However, these results require confirmation by further interventional studies in early RA.

In established RA, both DMARD and anti-cytokine therapy appear unable to reverse alterations in body composition. Four studies on anti-TNF agents in RA patients have failed to show significant improvements in fat-free mass. A control group receiving methotrexate, first-line therapy for RA, also exhibited no improvement. This precludes the ability of traditional DMARDs and biologic agents to reverse wasting. However, we are unable to comment on the ability of anti-TNF agents to prevent further muscle wasting, as neither treatment nor control groups demonstrated loss of FFM. Moreover, none of these studies evaluated participants according to body composition or RC status. As RC only affects around one-fifth of RA patients, any muscle gain may be rendered statistically insignificant when grouped with the more common non-cachectic RA patients.

Of concern, anti-TNF therapy has been associated with increased fat gain. Three of the above studies demonstrated significant increases in truncal or total fat mass after anti-TNF exposure. Therefore, as well as being ineffectual for muscle wasting, anti-TNF therapy appears to worsen fat gain in RA. These disappointing findings seem counter-intuitive, given the central role of TNF in the pathogenesis of muscle wasting.

The efficacy of other cytokine-directed therapies is inconclusive. While the blockade of IL-6 in RA patients has been associated with a significant BMI increase, which tissue is responsible for the weight gain is unknown. That is, due to the omission of body composition measurement, we are unable to conclude whether weight increase
occurred via increased skeletal muscle mass or increased fat mass, as was observed with anti-TNF therapy. Therefore, the role of anti-cytokine therapy in the treatment of RC requires further investigation.

In contrast, exercise programs may be an effective intervention for RC. Multiple studies have demonstrated reversal of muscle loss after exercise intervention\textsuperscript{7,92,93}. Indeed, a randomised control trial of 28 patients with established RA found that a 6-month resistance training program increased lean body mass (P <0.01) and decreased percentage body fat (P < 0.05), compared to controls prescribed non-muscle building exercises\textsuperscript{7}. Despite the effectiveness of resistance training for RC, exercise therapy has practical limitations. Factors such as disease activity, patient motivation and access to appropriate facilities reduce the efficacy of this promising therapy. In particular, exercise levels must be maintained long-term to retain improvements in FFM\textsuperscript{94}.

As such, there is a need for the development of new therapeutic agents to ameliorate the effects of RC. An agent that can both reverse muscle loss and prevent further deterioration while not increasing fat mass is yet to be identified. In order to develop novel therapeutic targets, we must first gain a more thorough understanding of pathogenic mechanisms of muscle wasting.
Rheumatoid cachexia requires extensive research to allow a greater understanding of its significance for RA patients. Poorly understood disease pathogenesis impedes clinical recognition. Therefore, this review will now focus on the structural and pathophysiological alterations of skeletal muscle in rheumatoid cachexia, and a potential treatment for these effects. In the absence of human data, experimental animal models are an effective method to investigate these features. Identifying and resolving the knowledge gaps within this field may aid in the development of an effective therapeutic agent for rheumatoid cachexia.

3. Muscle Structure

3.1 Normal architecture

A basic introduction of muscle structure is required to understand the varied effects of inflammatory cachexia. Unlike most other cell types, a muscle fibre is a multinucleated syncytium, formed by the fusion of individual immature muscle cells, called myoblasts. Each muscle fibre (myofibre) is filled with longitudinally arranged myofibrils. A collection of muscle fibres constitutes a fascicle which represents the functional unit of skeletal muscle. Connective tissue surrounds each individual fibre, bundle and whole muscle. This supportive network transmits the neurovascular supply of muscle and plays an important role in force transduction (Figure 3.1a).

![Diagram of components of skeletal muscle](image1)

**Figure 3.1** (a) Diagram of components of skeletal muscle and (b) Cross-section of skeletal muscle with fibre-specific stain. This photomicrograph demonstrates typical myofibre shape in cross-section and the surrounding connective tissue network. The deeply stained, smaller fibres are type I and the larger, lighter-staining fibres are type IIb fast fibres. Magnification x 500. Stain: NADH-tetrazoleum reductase. Taken from: Ross. *Histology: a text and atlas.* 6th edition; 2011.
There are three main types of fibre within skeletal muscle: type I, type IIa and type IIb. Type I fibres are small, slow and fatigue-resistant, type IIa fibres are medium-sized and capable of more rapid energy production. Type IIb fibres are the largest, fastest and most fatigue-prone of the three subtypes\textsuperscript{96} (Figure 3.1b).

The proportion of each type varies across muscles. In animal models of inflammatory cachexia, lower limb muscles are typically assessed. Most commonly, studies investigate gastrocnemius, composed of primarily type II fibres, tibialis anterior, with a greater balance of type I and type II fibres or the soleus muscles, predominantly type I fibres\textsuperscript{97}.

### 3.2 Structural Changes in Rheumatoid Cachexia

Animal models of RC consistently demonstrate reductions in macroscopic measures of muscle mass. Reductions in weight have been demonstrated in the following muscles: gastrocnemius\textsuperscript{98-100}, soleus\textsuperscript{101-103} tibialis anterior\textsuperscript{102}, rectus femoris\textsuperscript{101} and extensor digitorum longus\textsuperscript{102}. Of the above muscles, gastrocnemius has demonstrated the greatest atrophy with reported weight loss of up to 37\%\textsuperscript{99}, significantly greater than that seen in muscles such as the soleus\textsuperscript{103}, which contains few or no type II fibres\textsuperscript{97}.

Animal models typically use one of two methods of arthritis induction, collagen-induced arthritis (CIA) or antigen-induced arthritis (AIA). Both act to recreate the characteristic pathogenetic alterations of RA, however they have subtle differences regarding arthritis onset and suitable species\textsuperscript{104}. For example, AIA is generally only used in rats, while CIA has been used in mice, rats and monkeys\textsuperscript{104,105}.

Reduced muscle mass appears to occur via progressive atrophy. In their rat model of AIA, Castillero et al.\textsuperscript{106} found decreasing muscle mass across three time points: 10, 15 and 22 days post-arthritis induction. Filippin et al.\textsuperscript{107} confirmed the progression of wasting in their mouse model of CIA by analysing muscle changes at 25, 35 and 45 days post-CIA induction. Interestingly, Filipin et al. found no reduction in myofibre cross-sectional area (CSA) at day 25. Additionally, a previous pilot study reported by Filipin et al. described no increased wasting after day 45.

This shift in the onset of muscle wasting may be due to the use of separate species or the different method of arthritis induction between the two studies. However, the observation of wasting cessation is an attractive finding that may suggest a limit to
RC-induced muscle atrophy. However, given the heterogeneity observed even between mice and rats, extrapolating these findings to a human population is problematic. This theory requires clarification by additional long-term experiments of chronic cachexia in a wider range of animal species.

Consistent with our understanding of human RC, animal model atrophy is not solely due to decreased food intake or reduced exercise. Decreased muscle weight appears to be due to reduced muscle fibre CSA. Reduced myofibre size in turn, causes reductions in total muscle CSA of up to 50% (Figure 3.2).

**3.3 Fibre-specific Alterations**

Interestingly, it appears that there is an unequal contribution of each muscle fibre type to the overall decrease in muscle mass. Many studies measuring fibre-specific myocyte diameter have found that type II myofibres are more affected than their slow-twitch, type I counterparts (Figure 3.3). This is in accordance with the prior observation that muscles such as gastrocnemius, with a higher proportion of type II fibres exhibit greater atrophy than muscles such as the soleus.
However, this observation is not universal, with some authors reporting a similar degree of atrophy in both fibre types\textsuperscript{101,108}. Examining the methodology of these studies reveals potential causes for these contradictory findings. The staining technique used by Teixeira et al.\textsuperscript{108} is an inaccurate method of fibre-typing and as such, may have led to an under identification of type II fibres and subsequent exaggeration of type I wasting. Although Ozawa et al.\textsuperscript{101} employed the more precise fibre-typing method of immunohistochemistry, they studied the soleus and rectus femoris muscles, two tissues with a greater proportion of type I fibres\textsuperscript{111}. As such, they may have had insufficient type II fibres in which to observe selective atrophy.

In summary, animal models of RC have consistently demonstrated reductions in muscle weight, myofibre CSA and total CSA. However, more studies are needed to clarify the contribution of each fibre type in the wasting process. Furthermore, more evidence is required to determine the long-term effects of experimental RC. Future studies should include quantification of muscle wasting in new species to provide a greater depth of understanding before the advancement to human studies.

Figure 3.3 Cross-section of monkey gastrocnemius stained for ATPase
This slide demonstrates marked type II fibre atrophy (pink) with relative sparing of type I fibres (brown) in an animal model of collagen-induced arthritis (CIA).
Taken from: Horai et al. BMC Musculoskeletal Disorders; 2013\textsuperscript{110}
4. Muscle Breakdown Pathways

4.1 Ubiquitin-Proteasome System

Activation of the ubiquitin-proteasome system plays an integral role in muscle wasting. Ubiquitin is a small, abundant peptide that can be conjugated with specific target proteins to trigger their destruction by the cell proteasome. This process requires three enzymes (Figure 4.1): E1 for activation, E2 for transportation and E3 ligases for binding to the target protein.

Muscle RING-finger protein-1 (MuRF-1) and atrogin-1 are two muscle-specific E3 ubiquitin ligases commonly used in cachexia models to indicate UPS activity. They have since been termed 'atrogenes' and appear to be up-regulated in a variety of wasting conditions, including glucocorticoid-induced wasting, immobilisation, chronic heart failure (CHF) and human immunodeficiency virus infection. Consequently, the UPS appears to be a common final pathway in conditions characterised by muscle catabolism, including RA.

There are various mechanisms of UPS induction, many of which are stimulated by inflammatory cytokines such as TNF and IL-6. Therefore, the increased cytokine levels in RA activate the UPS in models of RC.
In experimental animal studies of RC, skeletal muscle expression of atrogene correlates with the degree of muscle wasting\(^{99,109}\). Increased atrogin-1 and MuRF-1 expression confirms the ability of atrogene to act as markers of muscle breakdown in inflammatory cachexia. Importantly, this enhanced atrogene expression in RC is likely to be independent of reduced mobility. Teixeria et al.\(^{108}\) found that arthritic rats exhibited significantly higher MuRF-1 secretion than immobilised controls, confirming that the inflammatory properties of RC activate the UPS independent of the known disuse-mediated induction.

However, the level of atrogene expression may not always correlate with muscle breakdown. Vary et al.\(^{120}\) demonstrated that although acute alcohol intoxication enhanced both MuRF-1 and atrogin-1 expression in rats, this did not correlate with increased muscle protein turnover. However, muscle breakdown was measured indirectly, by muscular expression of amino acids, thereby reducing the weight of this finding. In addition, the trial was investigating acute changes, and the duration of study may not have been sufficient to demonstrate wasting.

Despite the lack of correlation described above, enhanced atrogene expression is generally believed to signify muscle degradation. Therefore, atrogene expression serves as an additional measure of muscle breakdown to complement the more obvious changes to muscle structure. In addition, as they signify a specific pathway of muscle catabolism, alterations in atrogene expression may allow conclusions about the mechanism by which treatment and hormone levels affect muscle structure.
4.2 Myostatin Signalling

In addition to its aforementioned role in muscle-fat crosstalk, myostatin is a key regulatory hormone of muscle homeostasis\textsuperscript{121}. It is secreted primarily from skeletal muscle, and to a lesser extent adipose tissue, exerting direct autocrine, paracrine and endocrine effects\textsuperscript{77,122}.

Early observations of myostatin deficient cattle demonstrated two-fold increases in skeletal muscle mass\textsuperscript{123,124}. Myostatin loss-of-function mutations have also been identified in humans, with predictably increased muscle mass and decreased fat mass\textsuperscript{125}. These dramatic effects are mediated by multiple mechanisms including both decreased anabolism and increased catabolism.

The main mechanism of myostatin-induced atrophy culminates in the suppression of myogenesis, while the secondary pathway of muscle breakdown occurs via up regulation of the UPS\textsuperscript{126,127}. Myostatin has been shown to increase expression of MuRF1 and atrogin-1 in various models of wasting\textsuperscript{128,129}, however, this observation is not universal\textsuperscript{130}.

Despite a contentious role in UPS induction, myostatin has been implicated in the pathogenesis of several wasting conditions in both humans and animals, including: age-related sarcopaenia\textsuperscript{131}, starvation\textsuperscript{132}, cancer\textsuperscript{133}, CHF\textsuperscript{117,134} and COPD\textsuperscript{135}. Consequently, myostatin inhibition is currently being investigated for the prevention of muscle atrophy in a range of diseases\textsuperscript{136}. Despite diffuse involvement in these conditions, the literature is conflicting regarding the role of myostatin in RC.

4.3 Myostatin in Rheumatoid Cachexia

Studies investigating the role of myostatin in the development of RC produce conflicting results. Contention arises due to issues with comparing animal studies that use both different species and different methods of arthritis induction.

In three rat models of AIA, myostatin was found in similar levels across both wasted and control groups, indicating no active role in the pathogenesis of experimental RC\textsuperscript{99,106,137}. Conversely, Kim et al.\textsuperscript{138} found enhanced myostatin expression in their mouse model of CIA.
Aside from the differences in species and arthritis induction, evaluation of the above studies reveals distinct methodologies that may account for the varied findings.

In the three studies that found no enhanced myostatin signalling, the duration of arthritis was 15 days and the muscle under investigation was the gastrocnemius – a muscle rich in type II fibres. In contrast, Kim et al. allowed a 21-day study period and investigated the tibialis muscles – a group with a greater proportion of type I fibres.

While difficult to compare disease activity across species, the above observations may indicate two key explanations for the conflicting views on myostatin in RC. Firstly, myostatin may have a delayed-onset role in the pathogenesis of RC. Secondly, there may be a fibre-specific secretion of myostatin, with greater expression from type I muscle fibres. These two hypotheses require further investigation to assess their merit.

The above data suggests that myostatin expression varies according to the method of arthritis induction, the species used, the timing of measurement and the muscle under investigation. Therefore, the role of myostatin in animal models of RC requires clarification.
While the exact pathogenetic mechanisms are yet to be determined, the above two sections present convincing evidence that muscle is a target in RC. Yet, recent data implies that skeletal muscle may also have an active role in propagating the inflammatory process.

In their recent study of CIA in monkeys, Horai et al. concluded that ‘muscle wasting exacerbates joint swelling,’ and subsequently the ‘therapeutic target would not only be arthritic joints but also the skeletal muscle.’ The conclusion is remarkable as it represents an unprecedented shift in perspective and introduces a novel therapeutic avenue for the treatment of RA. However, cursory examination of the results reveals disappointing limitations. Firstly, serum creatinine was used as a surrogate marker of muscle mass, a test highly dependent on extraneous variables, such as kidney function. Subsequently, its reliability as a marker of lean body mass has been questioned.

Secondly, Horai et al. did indeed find an inverse correlation between serum creatinine and arthritis severity, as determined by clinical examination (R = -0.799). However, it is difficult to conclude that this is a causal relationship. Rather, the decreasing muscle mass may be due to worsened arthritis severity which likely coincides with a greater quantity of inflammatory cytokine secretion from synovial fibroblasts.

Despite the fallibility of the above conclusion, the theory itself may be biologically plausible, given the expression of inflammatory myokines and inflammatory cell infiltrate. A wide range of anti- and pro-inflammatory cytokines are secreted from muscle. Of interest in RC, TNF, IL-6 and IL-1β have all been detected from stress-induced skeletal muscle, suggesting that muscle may be able to contribute to systemic inflammation. However, there are limited studies that measure muscle expression of these cytokines within models of RC.

Available studies measuring TNF from skeletal muscle demonstrated elevations in cytokine expression. However, it is not known if muscle-derived TNF is able to act locally via autocrine induction of muscle atrophy or can be secreted into the systemic circulation. Nevertheless, distant functions of muscle-derived TNF are biologically plausible, given that muscle-derived IL-6 appears to correlate with serum IL-6 levels. Yet, even if muscle-derived TNF is released into the systemic circulation, it is unknown whether the quantity is sufficient to have a meaningful inflammatory impact.
Importantly, it is difficult to determine the cell of origin for muscle-derived inflammatory cytokines in animal models of RC. This is because three separate models have demonstrated hyper-cellularity of skeletal muscle in cachectic animals\textsuperscript{101,108,110}. Currently, no studies have attempted to identify the cellular subtype of this infiltrate. However, all three authors concluded that they were likely inflammatory in origin. Therefore, the inflammatory cell population may be responsible for the apparent elevations in TNF expression.

In muscle biopsies of healthy adults, TNF has been detected in the cytoplasm of type II muscle fibres\textsuperscript{146}. This suggests that myocytes are capable of TNF secretion independent of inflammatory cell infiltration. However, the only study localising TNF expression in an animal model of RC reports contradictory findings. Ramirez et al.\textsuperscript{144} performed immunofluorescent staining for TNF in cross-sections of the tibialis anterior muscle. Their results demonstrate lattice-like expression of TNF surrounding the myofibres (Figure 5.2).
As skeletal muscle contains resident inflammatory cells within its connective tissue network, we cannot conclude that the TNF is muscle-derived. As such, several key questions remain regarding the quantity, systemic effect and cellular origin of muscle-derived TNF.

Unlike TNF, mRNA and protein levels of muscle-derived IL-1β or IL-6 have not been previously measured in RC. However, increased IL-1β expression has been visualised via immunohistochemistry in muscles of arthritic rats (Figure 5.3). While IL-1β was primarily expressed around cells within the connective tissue matrix rather than myocytes, this finding suggests that muscle tissue may up-regulate inflammatory cytokine expression in RC. Similarly to TNF, we propose that increased muscle-derived IL-1β expression may contribute to the disease process in RC.

In summary, it is biologically plausible for catabolic muscle to contribute to systemic inflammation via up-regulation of myokine secretion. However, which myokines are enhanced in RC and the origin of these inflammatory mediators is unknown. Currently, both TNF and IL-1β have been detected in muscle from animal models of RC, but their cell of origin and distant effects remain unknown. Relying on three stand-alone projects, there is insufficient evidence to extinguish this uncertainty. Furthermore, no available data exists on muscle-derived IL-6 in inflammatory models of muscle wasting. As such, while skeletal muscle has the capacity to promote systemic inflammation in RC patients, further research is required to determine its contribution.

Figure 5.3 IL-1β immunohistochemistry in the gastrocnemius muscles of control (a) and CIA mice (c). The photomicrograph demonstrates IL-1β expression in the connective tissue of the CIA group. Scale = 50μm. Taken from: Teixeira et al. Experimental Biology and Medicine; 2013.
6. Tofacitinib – a novel therapy for rheumatoid cachexia?

Tofacitinib is an oral Janus kinase (JAK) inhibitor used for its immunosuppressant actions. Initially developed for transplant rejection\textsuperscript{148}, it is currently being investigated for a number of autoimmune inflammatory conditions. These include inflammatory bowel disease\textsuperscript{149}, plaque psoriasis\textsuperscript{150} and RA\textsuperscript{151}.

6.1 Tofacitinib for Rheumatoid Arthritis

A recent systematic review concluded that tofacitinib can provide sustained improvements in RA disease activity and may be an option for patients having failed anti-TNF biologic therapies\textsuperscript{152}. However, it must be kept in mind that while the authors of this review declare no industry-funding or conflicts of interest, the phase 3 trials on which they base their conclusions are funded by the producer of tofacitinib, Pfizer\textsuperscript{153-155}.

In November 2012, the United States Food and Drug Administration (FDA) approved tofacitinib ‘to treat adults with moderately to severely active rheumatoid arthritis who have had an inadequate response to, or are intolerant of, methotrexate’\textsuperscript{156}. In contrast, marketing authorization has been refused in Europe, due to an unfavourable risk-benefit ratio\textsuperscript{157}. The European League Against Rheumatism (EULAR) state that, given similar efficacy and cost to more established biologic DMARDs, tofacitinib requires more long-term safety data to determine an overall benefit/harm ratio for the treatment of joint destruction in RA\textsuperscript{86}.

However, the drug’s extra-articular actions have not yet been investigated. Due to its wide acting anti-inflammatory action, tofacitinib may prove efficacious in the treatment of RC. In particular, the diverse effects of JAK blockade may ameliorate muscle atrophy in RC patients.
6.2 Janus Kinases in Health and Disease

The JAKs are a family of tyrosine kinases that activate intracellular cascades for a number of ligands\(^{158}\). Currently, over 40 cytokines and growth factors are known to signal via JAK-mediated pathways. These include IL-2, -4, -7, -9, -15 and IL-21, cytokines integral for lymphocyte activation and function\(^{159,160}\). Of particular relevance to RC, IL-6 signalling is also mediated via JAK\(^{161}\).

After cytokines bind to their surface receptors, JAKs become activated, triggering phosphorylation and stimulation of the signal transducer and activator of transcription (STAT) pathway\(^{158}\). Phosphorylated STAT molecules are then able to translocate into the nucleus to regulate gene transcription, thereby mediating cytokine function\(^{162}\) (Figure 6.1).

![Diagram of JAK/STAT signaling](image)

Figure 6.1 Type I and II receptors and activation of JAKs signaling. JAK activation leads to JAK/STAT phosphorylation, nuclear translocation and subsequent gene transcription. JAK inhibitors therefore have the potential to block downstream signaling of all Type I and II receptor ligands. Taken from: Garber et al. Nature Biotechnology; 2011\(^{162}\)
The JAK family contains four members, JAK1, JAK2, JAK3 and tyrosine kinase 2 (TYK2). JAK1 and JAK3 are mainly responsible for stimulation of inflammatory pathways, while JAK2 plays a large role in haematopoiesis. Normal JAK activity is critical for the immune response and congenital defects lead to severe immunodeficiency or death (Figure 6.2). Originally thought to be JAK3 selective, tofacitinib is now known to exert pan-JAK inhibition, further expanding its spectrum of action. These broad anti-inflammatory effects may prove efficacious in reducing muscle wasting in RC patients.

<table>
<thead>
<tr>
<th></th>
<th>Knockout</th>
<th>Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>JAK1</td>
<td>Perinatal death</td>
<td>Increased infection</td>
</tr>
<tr>
<td>JAK2</td>
<td>Death in utero</td>
<td>Anaemia, neutropaenia</td>
</tr>
<tr>
<td>JAK3</td>
<td>SCID</td>
<td>Increased infection</td>
</tr>
<tr>
<td>TYK2</td>
<td>Immunodeficiency</td>
<td>Increased infection</td>
</tr>
</tbody>
</table>

Figure 6.2 Effects of JAK deficiency or inhibition.
JAK = janus kinase; TYK = tyrosine kinase; SCID = severe combined immunodeficiency

6.3 Tofacitinib for Rheumatoid Cachexia – how could it work?

The mechanisms of action of tofacitinib are incompletely understood. Furthermore, no studies have investigated the efficacy of tofacitinib to reduce the muscle wasting of RC. As such, the following discussion is based on extensive biological extrapolation.

By targeting a common downstream pathway, tofacitinib is able to block the function of multiple pro-inflammatory cytokines, reflecting a broad anti-inflammatory action. While critical in RA, many of these cytokines have an unknown role in the development of RC. Conversely, IL-6 induction is thought to be important in the development and progression of cachexia in RA.

Functional IL-6 inhibition may reflect the most promising mechanism of action for tofacitinib to ameliorate muscle wasting. In a large body of work by Bonetto et al., IL-6 has been implicated in both in vitro and in vivo models of muscle wasting.
Administration of murine IL-6 to cultured myocytes has been shown to reduce myofibre diameter by up to 36% compared with controls (P < 0.001). In vivo animal studies by the same author confirm this capacity for IL-6-induced muscle atrophy. Furthermore, reduced muscle weight in the animal model of RC coincided with marked elevations in the ubiquitin ligase, atrogin-1. This suggests that IL-6 activates the ubiquitin-proteasome system, highlighting the potential for IL-6 blockade to reduce muscle protein breakdown. Critically, skeletal muscle expression of STAT3, a major STAT family, was also increased, suggesting that IL-6-induced muscle atrophy occurs via JAK/STAT signalling. This introduces a potential therapeutic mechanism of action for tofacitinib in muscle wasting.

Indeed, myocytes with a constitutively active STAT3 gene exhibit reduced fibre diameter with simultaneously increased atrogin-1 expression. This indicates that STAT3 activation alone is sufficient for muscle cell atrophy in vitro. Furthermore, myocytes with negative STAT3 mutations are resistant to IL-6-induced atrophy. Therefore, we can conclude that in vitro IL-6-induced muscle atrophy is dependent on the action of STAT3.

Moreover, upstream pharmacological blockade with a dual JAK1/JAK2 inhibitor prevents IL-6-induced muscle wasting (Figure 6.2). As these two molecules are among those inhibited by tofacitinib, this brings further promise that tofacitinib may reduce muscle wasting in RC. However, as previously described, the pathogenesis of RC is likely to involve a multitude of inflammatory pathways. Therefore, the efficacy of pan-JAK inhibition to reduce muscle wasting of RC requires in vivo trials to complement the impressive results of in vitro experimentation outlined above.

![Figure 6.3](image-url) Immunoflourescent staining of longitudinal muscle fibres and graphical representation of myofibre diameter in control medium (PBS), with JAK inhibitor alone (INCB018424), IL-6 alone (IL-6) and IL-6 + JAK inhibitor (INCB018424 + IL-6). The JAK inhibitor induced modest hypertrophy in control myotubes and completely blocked IL-6-mediated myofibre atrophy after 48 hours of co-treatment. PBS = phosphate-buffered saline; IL-6 = interleukin-6; INCB018424 = JAK 1/JAK 2 inhibitor

As well as inhibiting IL-6 signalling via JAK/STAT blockade, tofacitinib may also reduce total available IL-6 levels. Meyer et al.\textsuperscript{160} demonstrated normalisation of serum IL-6 levels in experimental arthritis after administration of tofacitinib. Maeshima et al.\textsuperscript{164} also demonstrated reduced IL-6 production by human RA synovium implanted in mice. However, generalisation to human in vivo outcomes is limited by differences in rodent pharmacokinetics and dosage variance.

More recently, IL-6 levels were significantly reduced in a subset of RA patients after just 4 weeks of tofacitinib treatment\textsuperscript{165}. Yet, this trial demonstrated striking heterogeneity in the serum IL-6 levels, as only patients with markedly elevated baseline IL-6 showed dramatic reductions. Furthermore, half of the patients with the most impressive improvements were receiving a dose of 20mg per day, the maximum amount approved by the FDA\textsuperscript{156}. More evidence is required to determine if tofacitinib is able to reduce serum IL-6 levels, and at what dose this is achieved. This is of particular importance for understanding its role in reducing the muscle atrophy of RC, as it introduces a second mechanism of action for tofacitinib-mediated IL-6 inhibition.

Paradoxically, muscle-derived IL-6 may have a protective role in the regulation of adipose tissue\textsuperscript{166}. Indeed, IL-6 can be induced by exercise, leading to several positive effects on adipose in addition to suppression of TNF signalling\textsuperscript{167-169}. However, the relevance of this phenomenon for skeletal muscle mass is currently unknown.

Given the promising preliminary evidence, it is biologically plausible that pan-JAK inhibition by tofacitinib may limit the muscle wasting of RC. While tofacitinib has broad anti-inflammatory actions, IL-6 blockade in particular appears to be a promising therapeutic mechanism for the treatment of muscle atrophy. Therefore, amongst other effects, tofacitinib may ameliorate the contribution of IL-6 to muscle wasting. This may be achieved via reduced IL-6 levels plus a blockade of downstream IL-6 signalling. Yet, IL-6 is only one pathogenetic mechanism of RC, and this cytokine may exert both anti- and pro-inflammatory actions. Therefore, whether IL-6 blockade has a meaningful impact on the multifactorial in vivo muscle atrophy of RC remains to be seen.
Conclusion – the story so far

Currently, the assessment of RA patients focuses on joint destruction and the growing appreciation of the major systemic effects on cardiovascular and bone health. As a result, RC is a relatively neglected phenomenon in the disease process. However, alterations in muscle and adipose tissue are a common feature of RA.

RC is said to have occurred when these changes reach a pre-defined, yet perhaps arbitrary, cut-off value. Using these criteria, RC affects roughly 10-20% of RA patients. Despite its frequency, the condition remains under-appreciated in the scientific literature, and poorly recognised in clinical practice.

In order to gain acknowledgment, future research needs to clarify the potential consequences of RC. Available literature already suggests a potential association with the increased risk of cardiovascular disease and osteoporosis in RA. Furthermore, muscle wasting may contribute to insulin resistance, immune dysregulation, functional decline and a reduced quality of life.

Development of muscle tissue alterations is a complex, multifactorial process. While inactivity and enhanced inflammatory cytokines play a central role, the exact pathogenesis of muscle wasting in RA is unknown. In particular, the role of muscle-derived products such as myostatin and inflammatory myokines remains contentious. The occasionally described cellular infiltration of muscle is also poorly understood, with the cell-type currently unknown. In addition to quantifying the degree of muscle involvement, the mechanism of these effects requires clarification.

Incomplete understanding of various key pathways, hormones and cytokines prevents the identification of an effective pharmacological treatment. While the broad-spectrum, anti-inflammatory activity of tofacitinib may represent a promising therapeutic agent, there is no evidence to support its use in RC. Agents that maintain skeletal muscle in patients suffering from RA are critical to avoid deteriorations in quality of life, functional mobility and metabolic profile associated with RC.

Word count: 7518
References:


35. Summers GD, Deighton CM, Rennie MJ, Booth AH. Rheumatoid cachexia: a clinical


69. Chitra S, Nalini G, Rajasekhar G. The ubiquitin proteasome system and efficacy of


119. Moylan JS, Smith JD, Chambers MA, McLoughlin TJ, Reid MB. TNF induction of atrogin-1/MAFbx mRNA depends on Foxo4 expression but not Akt-Foxo1/3 signaling. Am J Physiol,


