Rheumatoid cachexia in rheumatoid arthritis patients treated in the current treat-to-target era: An exploration of the incidence, mechanisms, effects on physical function, and a potential nutritional treatment

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Summary

Rheumatoid cachexia (RC), i.e. muscle wasting and adiposity gain in rheumatoid arthritis (RA), is driven by inflammation and is a major contributor to reduced strength and physical function. Current treatment for RA is based on approaches that aim to tightly control inflammation from diagnosis onwards. This is best exemplified by 'treat-to-target' (T2T), which targets low disease activity, preferably 'clinical remission'. The success of T2T in controlling inflammation is well-established, but whether it has attenuated RC is unknown. In a cross-sectional trial, we demonstrated that, despite well controlled disease activity, RC and substantially impaired physical function was still present in patients treated by T2T.

Consequently, adjunct interventions are required to restore body composition and function. Nutritional creatine (Cr) supplementation may provide a safe and easy means of improving muscle mass and physical function. In a randomised control trial, 12 weeks Cr supplementation significantly increased muscle mass, but was unable to improve objective measures of physical function.

To investigate their associations with body composition, using serum from RA patients with: treated versus untreated disease; patients versus healthy controls; and patients before and after anabolic interventions (PRT or Cr supplementation), we were unable to identify serum biomarkers (i.e. tumor necrosis factor (TNF)-α, soluble TNF-α receptor-I, interleukin-6, insulin-like growth factor (IGF)-1, IGF-binding protein 3, myostatin, adiponectin, and leptin) for RC.

Following an unexpected, and substantial, loss of muscle mass in a patient in our Cr supplementation trial, we investigated and identified a probable catabolic effect of intramuscular (IM) corticosteroid (CS) injections used to treat active disease. Preliminary findings from an ongoing study revealed significant muscle loss in all five RA patients tested within 4 weeks of IM CS injection to treat a disease flare. These findings raise concerns about this routine and recommended treatment, and provide a further potential mechanism for RC.

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List of common abbreviations used

50'W	50-foot walk test
8'UG	8-foot up and go test
ACR	American College of Rheumatology
ALM	Appendicular lean mass
ATP	Adenosine triphosphate
BF%	Body fat percentage
BM	Body mass
BSR	The British Society of Rheumatology
CI	Confidence interval (95%)
Cr	Creatine monohydrate
CRP	C-reactive protein
CS	Corticosteroid
DAS28	Disease Activity Score (0-10) in 28 joints
DMARDs	Disease modifying anti-rheumatic drugs
DXA	Dual energy X-ray absorptiometry
ELISA	Enzyme-linked immunosorbent assay
ES	Effect size
EULAR	European League Against Rheumatism
FM	Fat mass
HAQ	Health Assessment Questionnaire
HC(s)	Healthy control(s)
HCQ	Hydroxychloroquine
HGS	Handgrip strength
IGFBP-3	Insulin-like growth factor-binding protein 3
IGF-I	Insulin-like growth factor-I
IKES	Isometric knee extensor strength
IL	Interleukin (e.g., IL-6)
IM	Intramuscular
LDA	Low disease activity
LFM	Leflunomide
LM	Lean mass
MDHAQ	Multi-dimensional Health Assessment Questionnaire
MTX	Methotrexate
MYF	Mycophenolate / Mycophenolic acid
n	Number (of participants)
NICE	National Institute for Health and Care Excellence
PCr	Phosphocreatine
PMRC	Peter Maddison Rheumatology Centre
RA	Rheumatoid arthritis
RC	Rheumatoid cachexia

RCT	Randomised controlled trial
SF-36	Medical Outcomes Study 36-Item Short Form
SSZ	Sulfasalazine
sTNF-RI	Soluble TNF receptor I
STS-30	Sit-to-stand in 30 second test
T2T	‘Treat-to-target’
TAC	Tacrolimus
TNF-RI	TNF-α receptor I
TNF-α	Tumor necrosis factor-α
VO_2max	Maximal oxygen uptake (i.e. aerobic capacity)

Acknowledgements

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Impact and achievements

Original publications

- **Wilkinson, T. J.,** O'Brien, T. D., & Lemmey, A. B. (2014). Oral creatine supplementation: A potential adjunct therapy for rheumatoid arthritis patients. World Journal of Rheumatology, 4(3), 22–34.

- **Wilkinson, T. J.,** Lemmey, A. B., O'Brien, T. D., & Jones, J. G. (2015). Significant muscle loss following intramuscular CS injection used to treat active rheumatoid arthritis; a case report. Journal of Rheumatology & Orthopedics, 2(2).

- **Wilkinson, T. J.,** Lemmey, A. B., Jones, J. G., Sheikh, F., Ahmad, Y., Chitale, S., Maddison, P. J., & O'Brien, T. D. (2016). Can creatine supplementation improve body composition and objective physical function in rheumatoid arthritis patients? A randomized controlled trial. Arthritis Care & Research*, Epub ahead of print in June 2016.*

- Lemmey, A. B., **Wilkinson, T. J.,** Clayton, R. J., Sheikh, F., Whale, J., Jones, H., Ahmad, Y., Chitale, S., Jones, J., Maddison, P. J., & O'Brien, T. D. (2016). Tight control of disease activity fails to improve body composition or physical function in rheumatoid arthritis patients. Rheumatology (Oxford), *In press*.

- **Wilkinson, T. J.,** Sheikh, F., Lemmey, A. B., Ahmad, Y., Chitale, S., O'Brien, T. D., & Jones, J. G. Does a single high dose intramuscular corticosteroid injection, used to treat disease flare, exacerbate muscle loss in rheumatoid arthritis? A quasi-experimental pilot trial. *On-going study – manuscript to be prepared.*

Conference papers

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Invited talks and presentations

- **Wilkinson, T. J.** Rheumatology multi-disciplinary team educational meeting, Ysbyty Gwynedd, 8th May and 7th June 2013. 'Can creatine supplementation improve body composition and physical function in rheumatoid arthritis patients? A randomised controlled pilot trial'. *(Oral presentations).*

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- **Wilkinson, T. J.** Rheumatology multi-disciplinary team educational meeting, *Ysbyty Gwynedd* – Bangor, 13th May 2015. 'An investigation into the prevalence, treatment and aetiology of rheumatoid cachexia in rheumatoid arthritis' *(Oral presentation).*

- **Wilkinson, T. J.** North West Rheumatology Club (NWRC) bi-annual conference. Wrexham, May 2015. 'Effect of oral creatine supplementation on body composition and objective physical function in rheumatoid arthritis patients? Double blind randomised controlled trial' *(Poster presentation).*

- **Wilkinson, T. J.** National Institute for Health Research Leicester-Loughborough Diet, Lifestyle and Physical Activity Biomedical Research Unit (ACES meeting), Loughborough University, November 2015. 'Rheumatoid cachexia: muscle wasting and obesity in rheumatoid arthritis' *(Oral presentation).*

- **Wilkinson, T. J.** Fifth Annual East Midlands Research Showcase, November 2015. 'Effect of oral creatine supplementation on body composition and objective physical function in rheumatoid arthritis patients? A double blind randomised controlled trial' *(Poster presentation).*

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Other impact

- **Medpage Today**
  - Article title: 'RA cachexia persists despite 'Treat-to-target'', available at: medpagetoday.com/MeetingCoverage/BSR/51392. May 2015.
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- **Healthline** (Article title: 'Despite successful treatment, many RA patients still experience low muscle mass', available at: healthline.com/health-news/despite-treatment-many-ra-patients-still-experience-low-muscle-mass-052015#1). May 2015.

- **A Women's Health** magazine (Article title: 'Treatment to control RA disease activity may not improve cachexia', available at: awomanshealth.com/treatment-to-control-ra-disease-activity-may-not-improve-cachexia/). May 2015.

- **'theRAconnection.com'** (Article title: 'Treatment to control RA disease activity may not improve cachexia', available at: theraconnection.com/news/treatment-to-control-ra-disease-activity-may-not-improve-cachexia/). June 2015.

- **Pharmastar** (Article title: 'Rheumatoid arthritis, 'treat-to-target' approach does not prevent rheumatoid cachexia', available at: pharmastar.it/index.html?cat=24&id=18395). May 2015.

- **‘MedicalResearch.com’** (Article title: ‘Creatine supplement increased muscle mass but not strength or function in RA patients’, available at: medicalresearch.com/author-interviews/creatine-supplement-increased-muscle-mass-but-not-strength-or-function-in-ra-patients/17943/). September 2015.

Awards & prizes

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General introduction

This general introduction aims to give a brief overview of rheumatoid arthritis (RA) and ‘rheumatoid cachexia’. Current therapy strategies for RA and their effectiveness in restoring body composition and physical function at the time my doctorate commenced will also be summarised. At the end of the chapter, an outline on how this thesis aims to investigate the key questions and issues raised is presented.

1.1. Rheumatoid arthritis

Rheumatoid arthritis (RA) is a chronic autoimmune disease that affects approximately 1% of the United Kingdom (UK) population (Symmons et al., 2002) and features persistent synovitis and systemic inflammation. Rheumatoid arthritis is estimated to cost the National Health Service (NHS) ~£560 million a year in health care costs, with an additional cost to the economy of ~£1.8 billion from sick leave and work-related disability (Zhang & Anis, 2011; Ruderman et al., 2012). The disease presents as swollen joints with consequent arthralgia (Heiberg & Kvien, 2002; Scott et al., 2010; Hetland, 2011), and is caused by a series of complex immune interactions that result in chronic synovial inflammation and a dysfunctional immunity response (Liu & Pope, 2003; Scott et al., 2010; Choy, 2012; Jung et al., 2012).

The inflammatory state in RA involves over-expression of pro-inflammatory cytokines such as tumor necrosis nuclear factor-alpha (TNF-α), interleukin (IL)-1β, and IL-6 (Bingham, 2002; Walsmith & Roubenoff, 2002; Shrivastava & Pandey, 2013) relative to concentrations of anti-inflammatory cytokines and natural cytokine antagonists such as soluble TNF-α receptor-1 (Arend, 2001). This chronic inflammation results in progressive joint destruction, specifically to the bone and cartilage around the synovium (Walsmith & Roubenoff, 2002; Le Goff et al., 2010; Choy, 2012; Jung et al., 2012; Shrivastava & Pandey, 2013).

Rheumatoid arthritis is also associated with a range of co-morbidities. For example, patients with RA are at a ~2-fold increased risk of cardiovascular disease (CVD) events (Kitas & Gabriel, 2011), an equivalent impact on CVD risk as diabetes mellitus (Peters et al., 2009; John et al., 2011). The increased CVD risk in RA is not fully explained by the presence of traditional cardiovascular risk factors such as dyslipidemia, hypertension, or obesity (Elkan et al., 2009; Van Halm et al., 2009; Summers et al., 2010; Boyer et al., 2011; Kitas & Gabriel, 2011), and appears, at least in part, to be attributable to the chronic inflammatory process integral to RA (Solomon et al., 2003; Kitas & Gabriel, 2011). Other co-morbidities include an increased risk of type II diabetes mellitus (Boyer et al., 2011), infections (Doran et al., 2002), osteoporosis (Haugeberg et al., 2000), and fatigue (Wolfe et al., 1996; Helal et al., 2012).

1.2. Rheumatoid cachexia

In addition to arthropathy, patients with RA experience substantial changes in body composition. In particular, significant loss of muscle in the region of ~8–15% occurs in patients with controlled RA disease compared to age- and sex-matched healthy controls (HCs) (Helliwell et al., 1984; Helliwell & Jackson, 1994; Roubenoff et al., 1994; Westhovens et al., 1997; Roubenoff et al., 2002; Walsmith et al., 2004; Toussirot et al., 2005; Arshad et al., 2007; Blackman et al., 2007; Engvall et al., 2008; Giles et al., 2008b; Book et al., 2009; Matschke et al., 2010a, 2010b; Binymin et al., 2011; Book et al., 2011; Dao et al., 2011).

Additionally, patients with RA are typically overweight and obese (Marcora et al., 2005a, 2005b, 2006; Stavropoulos-Kalinoglou et al., 2007; Elkan et al., 2009; Lemmey et al., 2009; Matschke et al., 2010a, 2010b; Stavropoulos-Kalinoglou et al., 2010; Santos et al., 2011), with excess fat mass (FM) ~12–18% greater than controls (Elkan et al., 2008; Giles et al., 2008b; Book et al., 2009; Matschke et al., 2010a, 2010b; Book et al., 2011; Dao et al., 2011; Santos et al., 2011). Worryingly, this increased FM occurs prominently on the truncal area with ~14–25% greater trunk adiposity reported (Giles et al., 2008b; Book et al., 2009; Elkan et al., 2009; Book et al., 2011; Dao et al., 2011; Santos et al., 2011).

This 'involuntary loss of muscle, coupled with elevated adiposity' is known as 'rheumatoid cachexia' (RC) (Roubenoff et al., 1992, 1994, 2004), and is a major contributor to the ~25–30% losses of strength (Helliwell & Jackson, 1994; Marcora et al., 2005a; Lemmey et al., 2009; Matschke et al., 2010a, 2010b; Chen et al., 2011) and physical function (Walsmith & Roubenoff, 2002; Giles et al., 2008a; Lemmey et al., 2009; Matschke et al., 2010a, 2010b; Dao et al., 2011; Dufour et al., 2012; Kramer et al., 2012) observed in RA. Additionally, the notable increase in truncal adiposity (Marcora et al., 2005a, 2005b, 2006; Inaba et al., 2007; Giles et al., 2008b; Giles et al., 2010; Elkan et al., 2009; Lemmey et al., 2009, Santos et al., 2011; Lemmey et al., 2012) exacerbates CVD risk (Abbasi et al., 2002; Dessein & Joffe, 2006; Inaba et al., 2007; Elkan et al., 2009; Giles et al., 2010; Stavropoulos-Kalinoglou et al., 2009). With muscle wasting noted in RA patients with early disease (Marcora et al., 2006, <6 months since symptom onset; Book et al., 2009, ≤12 months disease duration), it is conceivable RC may occur prior to disease onset in an inflammatory active 'pre-clinical' RA stage. More research into the temporal-course of RC is required.

1.3. Pathogenesis of rheumatoid cachexia

The pathological processes responsible for RC are multifactorial and appear to involve a collective series of complex processes (Walsmith & Roubenoff, 2002; Metsios, et al., 2006). Rheumatoid cachexia is predominantly attributed to inflammation, specifically elevated concentrations of circulating pro-inflammatory cytokines, with TNF-α thought to be the principal driver (Roubenoff et al., 1992; 1994; Metsios et al., 2006; Engvall et al., 2008). Excess inflammation is thought to disrupt protein synthesis by reducing the anabolic actions of insulin-like growth factor (IGF)-I in the muscle, as well as increasing the activity of transcription factors responsible for protein degradation (Broussard et al., 2004; Fanzani et al., 2012). Reduced peripheral insulin action (Walsmith & Roubenoff, 2002), increases in muscle protein suppressant hormones (e.g., myostatin (Roth & Walsh, 2004; Schiaffino & Mammucari, 2011)), reduced habitual physical activity (Metsios et al., 2006; Stavropoulos-Kalinoglou et al., 2010), and increased resting energy expenditure (REE) (Rall & Roubenoff, 2004; Metsios et al., 2007; Roubenoff, 2009) are all too thought to contribute.

1.4. Effects of pharmacological treatment for rheumatoid arthritis on rheumatoid cachexia

Fundamental to current RA treatment is a strategy involving early (i.e. prompt diagnosis and commencement of medication) and aggressive use of disease-modifying anti-rheumatic drugs (DMARDs) (Ruderman et al., 2012) to achieve 'tight control' of disease activity. Frequent evaluation of disease activity (e.g., Disease Activity Score in 28 joints, DAS28) and inflammation (erythrocyte sedimentation rate (ESR) and/or C-reactive protein (CRP)) allows assessment of treatment success and, if required, modification in DMARD therapy (Luqmani et al., 2006; Dale & Porter, 2010; Scott et al., 2010; Hetland, 2011; Upchurch & Kay, 2012)). In addition, a key development in RA treatment has been the wider use of biological agents designed to specifically target immune cells and cytokines (e.g., TNF-α) involved in disease pathology (Ding & Deighton, 2010; Upchurch & Kay, 2012).

More specifically, the 'treat-to-target' (T2T) recommendations have become one of the 'cornerstones' in contemporary RA management (Ruderman et al., 2012). Initially outlined by Smolen et al. (2010a), T2T exemplifies the principles of 'tight control' through the use of a 'target' – ideally 'clinical remission' (usually defined as DAS28 <2.6), or failing that, low disease activity (LDA; DAS28 <3.2) – to guide therapy adjustment accordingly. Following a T2T

approach has been shown to achieve superior clinical outcomes (reduced inflammation, disease activity, and progression of joint damage) than previous treatment regimens for RA (Saunders et al., 2008; Smolen et al., 2010a; Hetland et al., 2012; Jurgens et al., 2012; Wevers-de-Boer et al., 2012).

Since RC has been primarily attributed to TNF-α driven muscle catabolism, it was suggested that treatment with anti-TNF-α drugs may reverse the process of RC and restore lean mass (LM) in RA patients (Walsmith & Roubenoff, 2002; Rall & Roubenoff, 2004). However, research (Marcora et al., 2006; Metsios et al., 2007; Serelis et al., 2008; Engvall et al., 2010) has demonstrated that despite successful control of disease activity (i.e. inflammation), no favourable effects on LM are observed. Furthermore, relative to standard DMARDs, anti-TNFs may increase FM (Engvall et al., 2010), particularly trunk FM (Metsios et al., 2007). Worryingly, other treatments used to supress disease activity such as chronic high dose corticosteroid (CS) therapy exert a catabolic effect on the muscle mass of patients (Roubenoff et al., 1990).

Due to its favourable effect on disease activity it could be assumed that early 'tight control' of inflammation by a T2T approach, may avert, or at least attenuate, RC and the concurrent loss of physical function. When this doctorate commenced, the effect of current treatment strategies (specifically T2T), where 'clinical remission' and LDA are frequently attained, on body composition and objective physical function in RA was unknown, although assumed to be beneficial.

1.5. Anabolic interventions for rheumatoid cachexia

Owing to the ineffectiveness of conventional RA medication in reversing RC, the need for potential adjunct anabolic interventions that restore muscle mass and physical function in RA are required. Progressive resistance training (PRT) has been identified as an effective means of reversing RC and restoring physical function in RA patients (e.g., Häkkinen et al., 1999, 2005; Häkkinen et al., 2003; Häkkinen, 2004; Marcora et al., 2005a; Lemmey et al., 2009; Sharif et al., 2011; Lemmey, 2011). Nevertheless, despite the well-established benefits and safety of exercise training, RA patient uptake of exercise is generally poor (e.g., Sokka et al., 2008), particularly when supervision is withdrawn (Lemmey et al., 2012). Whilst adherence to exercise in RA patients is better in patients with well-controlled disease, superior functional ability, and a strong social structure (Munneke et al., 2003; Metsios et al., 2008), it seems that

high-intensity exercise training is unlikely to be widely adopted as a therapy for RC by the majority of RA patients.

Anabolic nutritional supplementation offers a potential adjunct treatment option that is easily administered, inexpensive, and makes limited demands of the patient. Whilst oral amino acid (i.e. protein) supplementation has been shown successful in increasing LM in the elderly with sarcopenia (Solerte et al., 2008) and patients with cancer cachexia (May et al., 2002), there is a distinct lack of published studies on the effect of nutritional supplementation on RC, with the majority of trials investigating whether diets or dietary supplements are able to moderate RA disease symptomology (for a review, see Stamp et al., 2005).

Our group (Marcora et al., 2005b) previously investigated the effects of 12 weeks daily protein supplementation in patients with RA, and demonstrated favourable effects on muscle mass and some measures of physical function. Another potential protein supplement, creatine (Cr) monohydrate, potentially offers even greater benefits than other protein supplements (Nissen & Sharp, 2003), and the solitary trial investigating its efficacy in RA patients (Willer et al., 2000) found that 3 weeks oral Cr supplementation significantly increased strength. Unfortunately, body composition and other measures of objective physical function were not assessed in this study. Thus, dietary Cr supplementation may provide a promising option, but a well-conducted investigation into its efficacy in RA is needed.

1.6. Thesis design and hypotheses

This thesis consists of four experimental studies (**Chapters 3, 5, 6, 8**), an invited review (**Chapter 4**), and a case report (**Chapter 7**). All chapters are written as stand-alone manuscripts that were/are to be submitted to international peer-reviewed medical journals. Author contributions to each chapter are shown in **Appendix A**. Tables and figures are imbedded within the text at appropriate locations. References are compiled at the back of the thesis and are written in accordance with the American Psychological Association (5th Ed.) referencing format.

- *Chapter 2*

In an extended literature review, **Chapter 2** expands upon the concepts introduced in **Chapter 1** and explores the effects of RC, along with its proposed pathogenesis and treatment options.

- *Chapter 3 (Study 1)*

The first experimental chapter (**3**) investigates whether RC and consequent physical disability remain features of RA in the current treatment era where LDA and 'clinical remission' is widely attained. **Chapter 3** presents a cross-sectional study in which body composition, physical function, and CVD risk in RA patients was compared with that of age- and sex-matched sedentary HCs. Findings from this study were compared with those previously reported by our group and others (i.e. studies performed either before local adoption of T2T strategies, or, if more recent, on patients who commenced treatment pre-T2T). Additionally, the RA patients were divided into 'recent-onset' (≤12 months since diagnosis) and 'established' (>12 months) cohorts to provide an insight into the temporal evolution of RC, disability, and CVD risk. **Chapter 3** is written in accordance with the 'STrengthening the Reporting of OBservational studies in Epidemiology' (STROBE) guidelines (von Elm et al., 2007).

- As we theorise RC occurs early in the disease process (i.e. in the 'pre-clinical' phase prior to the commencement of DMARD treatment), without a sufficient anabolic stimulus to improve body composition (e.g., physical activity or exercise), we hypothesised that RA patients, despite improved control of inflammation and disease activity, would still present with RC (i.e. significantly reduced LM and increased FM (particularly trunk FM)), poorer

objectively-assessed physical function, and exacerbated CVD risk, compared to age- and sex-matched healthy controls (HCs).

- We hypothesised that body composition (e.g., LM, FM, trunk FM), and physical function would be comparable to those previously reported by our group and others (i.e. studies performed either before local adoption of T2T strategies, or, if more recent, on patients who commenced treatment pre-T2T).

- We hypothesised that there would be no difference in measures between patients with 'recent-onset' disease (≤12 months since diagnosis) and those with more 'established' disease (>12 months since diagnosis); inferring that changes to body composition, and consequent reductions in function and exacerbation of CVD risk, occur early in the disease process (i.e. in the 'pre-clinical' phase prior to the commencement of DMARD treatment).

- *Chapter 4*

In a review, **Chapter 4** critically examines the relevant literature on Cr supplementation to evaluate whether this may provide an effective treatment option for RC.

- *Chapter 5 (Study 2)*

Chapter 5 describes the results of a double blind, randomised placebo-controlled study that investigated the effect of oral Cr supplementation on body composition, strength, and physical function in RA patients. **Chapter 5** is written in accordance with the 'Consolidated Standards of Reporting Trials' (CONSORT 2010) statement (Schulz et al., 2010).

- We hypothesised that 12 weeks of oral Cr supplementation would increase LM and improve measures of strength and objective physical function in patients with RA.

- *Chapter 6 (Study 3)*

Chapter 6 reports the results from biochemical assays conducted on samples from current (i.e. studies reported in this thesis) and previous studies performed by our group. This investigation sought to identify potential serum markers of inflammation and muscle anabolism/catabolism in a range of clinical RA scenarios including: RA versus HCs, 'treated' versus 'untreated' disease, and pre- and post-anabolic treatments (i.e. Cr supplementation (from **Chapter 5**), and PRT).

- *Rheumatoid arthritis patients versus sedentary healthy controls* – We hypothesised that RA patients would have higher serum concentrations of inflammatory (TNF-α, sTNF-RI, IL-6) and catabolic (myostatin) markers, lower concentrations of anabolic markers (IGF-I and IGFBP-3), and higher levels of adipokines (adiponectin and leptin) than age- and sex-matched HCs.

- *'Recent-onset' versus 'established' disease* – As there were no differences in demographic, disease activity (DAS28), systemic inflammation (CRP), or body composition measures between the full **Chapter 3** 'recent-onset' versus 'established' disease cohorts, we hypothesised no differences in any of the assessed serum biomarkers.

- *Untreated, uncontrolled disease versus treated, controlled disease* - We hypothesised that treatment with methotrexate (MTX) or etanercept (ETN) would reduce levels of the inflammatory markers CRP and IL-6. Whilst we expect MTX to reduce TNF-α concentrations, an increase in circulating TNF-α levels is expected following ETN treatment. Despite this hypothesised increase in TNF-α, as the TNF-α is made biologically inactive by ETN, we would anticipate no changes in sTNF-RI. Both treatments would have no effect on myostatin, or IGF-I and IGFBP-3. Adipokine, adiponectin, and leptin, concentrations may decrease and increase, respectively, due to elevated FM following treatment.

- *Etanercept versus methotrexate therapy* - We hypothesised that both treatments would decrease inflammatory markers to the same degree, and would have no effect on anabolic markers or myostatin. Adipokine (adiponectin and leptin) concentrations may decrease and increase, respectively, with treatment, especially with ETN, due to increases in adiposity.

- *Effect of progressive resistance training* - We hypothesised that PRT would have no effect on serum levels of pro-inflammatory, anabolic (IGF-I and IGFBP-3), or catabolic (myostatin) markers. In contrast, we anticipated reductions in adipokines (adiponectin and leptin) as a consequence of attenuated adiposity.

- *Effect of oral creatine supplementation* - We hypothesised that Cr supplementation would have no effect on any of the assessed serum biomarkers.

- *Chapter 7*

Chapter 7 describes the case of a participant, from the **Chapter 5** trial, who experienced substantial muscle loss following an intramuscular (IM) CS injection.

- *Chapter 8 (Study 4)*

To determine whether the response seen in **Chapter 7** (i.e. loss of muscle mass) to IM CS administration is typical, we are conducting an on-going exploratory non-randomised trial (**Chapter 8)**. This chapter presents the preliminary results from five patients who were administered an IM CS injection to treat active disease activity. **Chapter 8** is written in accordance with the 'Transparent Reporting of Evaluations with Nonrandomised Designs' (TREND) guidelines (Des Jarlais et al, 2004).

- We hypothesised that RA patients administered an IM CS injection to treat a disease flare (i.e. active disease) would experience significant loss of LM.

- *Chapter 9*

An overall discussion of the thesis findings is presented in **Chapter 9**. This chapter aims to answer the questions posed in the introduction by incorporating the results of the presented studies with evidence from the literature that has emerged since the commencement of my doctoral studies.

2

Extended literature review

This extended literature review expands on themes introduced in **Chapter 1**, and discusses, in greater depth, the rheumatoid cachexia (RC) research relevant to the studies in this thesis.

The literature review is divided into three sections:

- 2.1. Overview of RC, including its prevalence, its effect on physical function and co-morbidity risk, and the underlying pathogenesis.
- 2.2. Effect of current pharmacological treatment for rheumatoid arthritis (RA) on RC.
- 2.3. Potential adjunct treatments that may attenuate or reverse RC and subsequently improve physical function in RA patients.

2.1. What is rheumatoid cachexia?

'I can't stay seated because I'm so thin. Forty six kilos, that can't be called fat. My bones are sticking through my skin and this despite a good appetite….'

Pierre-Auguste Renoir (**Figure 2.1.**)

Figure 2.1. Impressionist painter Pierre-Auguste Renoir in 1915 when 'rheumatoid cachexia was clearly visible'. Source: 'How Renoir coped with RA (Boonen et al., 1997), image reproduced under the CC-BY-AT 2.0 licence.

In addition to arthropathy (Scott et al., 2010), significant loss of muscle mass frequently occurs in patients with rheumatoid arthritis (RA) (Summers et al., 2008). Muscle loss caused by inflammatory disease is not a new observation as Sir James Paget noted in 1873 that:

'…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: it is far more rapid than that…'

Cachexia (Greek. *Kachexi 'a; kako's* bad*; 'e' xis* condition) implies a state of advanced malnutrition and muscle wasting, and has been used to denote the loss of body cell mass (BCM), mostly muscle mass, which occurs in illness (Roubenoff, 2009). The accelerated and 'involuntary loss of muscle, coupled with elevated adiposity' seen in patients with RA was termed 'rheumatoid cachexia' (RC) by Roubenoff and colleagues in the early 1990's

(Roubenoff et al., 1992, 1994). In RA, muscle loss is typically unrecognised in clinical practice (Summers et al., 2008) as it is masked by an increase in adiposity (i.e. fat mass (FM)) which often makes it undetectable if only body mass (BM) or 'body mass index' (BMI) is assessed as 85% of patients have a 'normal' BMI (Summers et al., 2008). Due to the inaccuracy of BMI in RA, Stavropoulos-Kalinoglou et al. (2007) suggest that traditional BMI cut-offs should be reduced by 2 kg/m^2 (i.e. 23 kg/m^2 for overweight, 28 kg/m^2 for obesity). Thus, RC is dissimilar from the cachexia seen in cancer or cardiac diseases which typically manifests in significant weight loss (Morley et al., 2011). Due to the regular concurrence of muscle loss and obesity, RC typically presents as 'sarcopenic-obesity' (Baumgartner et al., 2004).

2.1.1. The prevalence of rheumatoid cachexia

Currently no universally accepted definition of RC exists, and the prevalence varies depending upon the measurement method and definition of significant muscle loss employed (Summers et al., 2008). Using <50th percentile for arm muscle area or circumference of a reference population as their definition of RC, Roubenoff and colleagues identified 67% of RA patients as muscle wasted (Roubenoff et al., 1992). Using similar anthropometric measures (upper arm muscle circumference) to Roubenoff, and taking the <20th percentile of a reference population as the cut-off point, muscle wasting was observed in 14% of patients by Helliwell et al. (1984) and in 29% of patients by Fukuda et al. (2005). Employing the same measurement method but a more stringent criteria (<10th percentile of the reference population), muscle wasting was observed in 50% of patients by Munro and Capell (1997) and 24% by Hernandez-Beriain et al. (1996).

Body composition assessments by dual energy x-ray absorptiometry (DXA) identified RC in 18% of women and 26% of men using the definition of fat-free mass index (FFMI) <25th percentile of a matched healthy population (Elkan et al., 2009). Whilst, with a definition of FFMI <10th percentile, 38% of patients were categorized as having RC by Engvall et al. (2008), 18% and 21% of women and men by Elkan et al. (2009), and 10% by Metsios et al. (2009). Using DXA-derived skeletal muscle index (cachexia defined as ≤5.45 kg (of appendicular lean mass[1] (ALM))/height (m^2) for women, and ≤7.26 kg/m^2 for men) (Baumgartner et al., 1998)), a series of studies from our research group (Marcora et al., 2005a, 2005b, 2006; Lemmey et al., 2009, 2012) have identified that 57–67% of RA patients with controlled disease are significantly

[1] Appendicular LM (ALM) can be used as a surrogate measure of muscle mass (Kim, Wang, Heymsfield, Baumgartner, & Gallagher, 2002).

muscle depleted. When compared to non-RA controls, the overall loss of LM in RA patients ranges between ~8–15% (Helliwell et al., 1984; Helliwell & Jackson, 1994; Roubenoff et al., 1994; Westhovens et al., 1997; Roubenoff et al., 2002; Walsmith et al., 2004; Toussirot et al., 2005; Arshad et al., 2007; Blackman et al., 2007; Elkan et al., 2008; Giles et al., 2008b; Book et al., 2009; Matschke et al., 2010a, 2010b; Binymin et al., 2011; Book et al., 2011; Dao et al., 2011; for an excellent review, the reader is directed to Summers et al., 2008).

Aside from LM loss, RA is also characterised by elevated adiposity (Roubenoff et al., 1992; Stavropoulos-Kalinoglou et al., 2007; Stavropoulos-Kalinoglou et al., 2010). Elkan et al. (2009) identified that 31% of women and 53% of RA males had a DXA-assessed FMI >90th percentile for healthy adults (reference population from Schutz et al. (2002)). Whilst using the same criteria, Engvall et al. (2008) found the prevalence of obesity was 40% amongst females and 67% amongst males with RA. Santos et al. (2011) identified 35% of RA patients as 'overfat' (defined as a BIA-assessed BF% ≥40%), whilst Dao et al. (2011) found 42% of their patients were obese using DXA cut-off points of BF% (derived by age, sex, and race from a large cohort of healthy adults (Gallagher et al., 2000)). Disturbingly, Marcora et al. (2005a, 2005b, 2006) and Lemmey et al. (2009) found that ~80% of patients were obese when defined as a DXA-derived BF% ≥38% and ≥27% for females and males, respectively (Baumgartner et al., 1998). When compared to matched non-RA controls, levels of total FM have been shown to be ~12–18% greater in RA patients (Elkan et al., 2008; Giles et al., 2008b; Book et al., 2009; Matschke et al., 2010a, 2010b; Book et al., 2011; Dao et al., 2011; Santos et al., 2011), with elevations in trunk adiposity of ~14–25% (Giles et al., 2008b; Book et al., 2009; 2011; Elkan et al., 2009; Dao et al., 2011; Santos et al., 2011).

Some researchers have found that females experience RC more than males. Giles et al. (2008b) found that female RA patients displayed lower LM and greater adiposity (i.e. higher FM, trunk FM, and BF%) relative to age- and sex-matched controls than male RA patients. Similar results were reported by Book et al. (2009) who also found deficits in LM and increases in FM (particularly trunk FM) were more pronounced in female than male patients with early RA. Conversely, sex-based disparities in the prevalence or degree of RC has not been observed in studies conducted by our group (Marcora et al., 2005a, 2005b, 2006; Lemmey et al., 2009, 2012; Matschke et al., 2010a, 2010b).

2.1.2. When does rheumatoid cachexia occur?

There is evidence to suggest that RC is established early in the course of the disease. In a cohort of patients with very earlier RA (<6 months since symptom onset), Marcora et al. (2006) found a prevalence of low ALM (~63% of patients) and obesity (~80%) (unpublished observations) which were similar to those of established RA patients from previous studies (Marcora et al., 2005a, 2005b). Similarly, Book et al. (2009) found that RA patients with a disease duration of ≤12 months had low ALM relative to matched HCs. In this study, female patients also had higher total FM and trunk fat distribution than the HCs.

The importance of identifying 'pre-clinical' stages (i.e. before symptoms appear) of RA has been recognized by the European League Against Rheumatism (EULAR) (Gerlag et al., 2012). Research (Rantapää-Dahlqvist et al., 2003; Nielen et al., 2004; Sokolove et al., 2012) has indicated that RA disease processes, including elevated concentrations of pro-inflammatory cytokines such as TNF-α (i.e. one of the key purported drivers of RC) may already be apparent in the 'pre-clinical' phase (Sokolove et al., 2012). Both studies from Marcora et al. (2006) and Book et al. (2009) suggest that body composition changes occur early in the disease, conceivably, as hypothesised by these authors, before disease development and commencement of treatment.

Interestingly, it appears that after this 'initial' accelerated loss of muscle (presumably during the pre-treatment period of uncontrolled disease activity/inflammation), once control of disease activity is achieved the rate of muscle decline in RA appears to be similar to that of sedentary non-RA individuals (i.e. annual muscle decline rates in the general population after the age of 30 years = ~0.10 kg/1% per year (Guo et al., 1999; Frontera et al., 2000; Morley et al., 2011)). Only two studies have looked at muscle decline in RA; each reporting a similar rate of loss (Westhovens, 1999[2]; Lemmey et al., 2012) to non-RA controls. In contrast, the accumulation of FM may be 'chronically elevated' (Lemmey et al., 2012), as the rate of FM increases in a small sample (n = 9) of patients with established controlled RA was 0.8 kg/year, double that of sedentary healthy individuals (~0.4 kg/year, Guo et al., 1999). Overall, however, evidence concerning the time course (commencement and rate) of RC is scant and remains unclear.

[2] Observations from doctoral studies.

2.1.3. Consequences of rheumatoid cachexia

Rheumatoid cachexia is associated with impaired physical function and disability (Engvall et al., 2008; Giles et al., 2008a; Dao et al., 2011; Kramer et al., 2012). Just a 5% decrease in LM can lead to significant muscle weakness and loss of functional capacity (Walsmith & Roubenoff, 2002), which reduces a patient's self-reported independence and quality of life (Bazzichi et al., 2005; Benitha et al., 2007; Engvall et al., 2008; Summers et al., 2008). Conversely, increasing muscle mass and reducing FM has been shown to have beneficial effects on physical function and strength in RA patients (Marcora et al., 2005a; Lemmey et al., 2009). As the qualitative properties (e.g., muscle-specific force and architecture, activation capacity, and recruitment) of muscle that determine specific force do not appear to be compromised in patients with stable RA (Matschke et al., 2010a, 2010b), it appears it is loss of muscle *quantity* (i.e. muscle mass), rather than muscle *quality*, that contributes to the reduced function and strength.

2.1.3.1. Effect on functional capacity

Prior to the advent of the current DMARDs (e.g., the introduction of methotrexate (MTX) in the late 1980's, and biologic agents in the early 2000's (Upchurch & Kay, 2012)), the physical function of RA patients was found to be markedly reduced (up to 60%, Ekblom et al., 1974) compared to matched controls.

However, despite substantial advancements in treatment, objectively measured physical function of patients with RA still appeared reduced by ~20–25%. The evidence for this includes studies conducted by our group, for example, Lemmey et al. (2009) found walking time (assessed by a 50'foot walk (50'W)) and the number of chair stands (assessed by the sit-to-stand in 30 second test (STS-30)) were 21% and 25% poorer, respectively, compared to age- and sex-matched population norms (calculated using the 50th percentile performance level for healthy 60–64 year olds for the relevant tests; from the Senior Fitness Test Manual (Rikli & Jones, 2012)); and Matschke et al. (2010a, 2010b) who showed that RA patients, relative to matched non-RA individuals, demonstrated reductions of ~11% in the STS-30 test, and ~17% and ~25% slower 8'foot up and go (8'UG) and 50'W times, respectively. Kramer et al. (2012) found that, in RA patients, low thigh muscle mass was associated with higher reported disability and limitations in performance assessed by the Short Physical Performance Battery (e.g., single and repeated chair stands, walking tests).

Loss of handgrip strength (HGS) and hand function in particular is a major cause of disability in RA (Fraser et al., 1999). Bodur et al. (2006) found that up to 81% of patients reported experiencing some form of hand disability when compared to controls, and HGS has been found to be ~20–30% deficient in RA patients (Nordenskiöld & Grimby, 1993; Helliwell & Jackson, 1994; Häkkinen et al., 1995; Fraser et al., 1999), with Brorsson et al. (2012) reporting a 34% loss in the (Hand) Grip Ability Test. Consistent with these findings, Van Bokhorst-de van der Schueren et al. (2012) reported that 95% of their RA patients were below the 33rd percentile of an age- and sex-matched reference population for HGS.

2.1.3.2. Effect on strength

Muscle mass is a major determinant of strength in RA patients (Helliwell & Jackson, 1994; Roubenoff, 2001; Marcora et al., 2005a; Giles et al., 2008a; Matschke et al., 2010a, 2010b; Kramer et al., 2012). In earlier studies of strength, the lower extremity muscle function (using the Index of Muscle Function) of RA patients was 23% less than a matched control group (Ekdahl et al., 1989), and research revealed significant reductions in maximum isometric strength of both knee extensors (~35%) and flexors (~70%) in RA patients compared to controls (Nordesjö et al., 1983; Danneskiold-Samsøe & Grimby, 1986; Ekdahl & Broman, 1992; Madsen et al., 1997). More recently, Lemmey et al. (2009) revealed that isometric knee extensor strength (IKES) was deficient by ~25% in stable RA patients relative to matched HCs.

2.1.3.3. The role of adiposity on strength and physical function

Whilst loss of LM significantly contributes to poor physical function, the excess adiposity characteristic of RC may exacerbate this (Stavropoulos-Kalinoglou et al., 2009; Kramer et al., 2012). Some research (Giles et al., 2008a; Kramer et al., 2012) even suggests that FM may have a greater effect on disability in RA than loss of muscle, and that efforts to improve physical function in RA require a focus on fat reduction with at ‘least as much emphasis, if not more, than increasing LM’ (Giles et al., 2008a). Mechanistically, increased adiposity acts as ‘dead weight’, ‘increasing the load faced by the limited muscle mass’ (Rolland et al., 2009), as well as obstructing range of motion and impairing movement (Giles et al., 2008a).

Further, fat infiltration *into* the muscle is associated with decreased physical function in the elderly (Visser et al., 2003, 2005; Goodpaster et al., 2008; Marcus et al., 2012). Whilst it remains unclear what mechanism(s) explain the association between fat infiltration and physical function (Visser et al., 2002, 2005), intramuscular (IM) fat may be a marker of functional aspects of the muscle other than strength, such as muscle contractibility (cellular function), muscle metabolism (energy utilisation), neural factors (nerve function), or reduced

blood flow (Visser et al., 2002, 2005; Goodpaster et al., 2008). Further research into these mechanisms are needed.

In general, while both low muscle mass and excess FM are independently associated with disability and loss of function (Kramer et al., 2012), research suggests that a synergistic relationship exists i.e. the co-existence of both conditions (sarcopenic-obesity), which regularly occurs in RA, markedly increases (~2.5–12.0 fold) disability risk (Baumgartner, 2000; Baumgartner et al., 2004; Dufour et al., 2012) relative to sarcopenia only or obese only participants.

2.1.3.4. Rheumatoid cachexia, mortality, and co-morbidity

In addition to loss of strength and physical functioning, RC has other serious health implications. The effect of RC on mortality rate has not been investigated in RA, in older adults, low muscle mass has been associated with increased all-cause mortality (Metter et al., 2002; Wannamethee et al., 2007). Whilst reduced LM may indicate poorer cardiorespiratory fitness, as muscle is the primary store of glucose, loss of muscle can also increase insulin resistance and its associated CVD risk (Srikanthan et al., 2010). Severe loss of LM also diminishes the body's ability to fight infection as muscle is the primary store of body protein, and depletion of this store impairs adaptation to metabolic stress (Roubenoff & Rall, 1993; Walsmith & Roubenoff, 2002; Summers et al., 2008). In fact, the ~8 to 15% reduction in LM often described in RA patients represents 1/3–1/4 of the maximum survivable loss of total BCM (~40%, Roubenoff, 2001).

The elevated adiposity seen in RA, particularly the accumulation of truncal fat, may contribute to insulin resistance (Dessein & Joffe, 2006) and increased risk of CVD (Walsmith & Roubenoff, 2002; Inaba et al., 2007; Book et al., 2009; Elkan et al., 2009; Stavropoulos-Kalinoglou et al., 2009; Giles et al., 2010; Summers et al., 2010; Solomon et al., 2012). Although contrastingly, Metsios et al. (2009) found that presence of RC was not predictive of a worse CVD profile. Furthermore, as adipose tissue is a source of inflammation, reductions in pro-inflammatory cytokine activity have been associated with loss of FM in obese men (Sharman & Volek, 2004) and women (Ziccardi et al., 2002). As such, improving body composition, specifically reducing adiposity, may also reduce RA disease burden (i.e. inflammation).

Rheumatoid cachexia is also associated with an increased risk of osteopenia and osteoporosis as depletion of LM is significantly correlated with bone mineral density of the spine and hip, and is a strong independent predictor of bone mass in RA (Shibuya et al., 2002).

2.1.3.5. Other factors that effect functional disability in rheumatoid arthritis

Whilst loss of LM and excess adiposity are substantial predictors of impaired strength and physical function in RA, other factors may also contribute. Rheumatoid arthritis is characterised by chronic fatigue, joint damage, and pain; all of which have been associated with reductions in physical functioning (Scott et al., 2000; Heiberg & Kvien, 2002; Ormseth et al., 2015). Univariate correlations of patient characteristics with subjective physical functioning (HAQ) by Giles et al. (2008a) found that, alongside body composition (i.e. ALM and FM), RA disease activity (DAS28), disease duration, pain, morning stiffness, and radiographical damage were all associated with increased disability. Further, Lusa et al. (2015) found that significant indicators of slower walking speed (over 400m) were: older age, higher depression scores, higher reported pain and fatigue, higher swollen and replaced joint counts, higher cumulative prednisone exposure (possibly indicative of more active disease), non-treatment with DMARDs, and worse body composition (i.e. low muscle mass and increased FM).

2.1.4. Pathogenesis of rheumatoid cachexia

2.1.4.1. Inflammation and cytokines

The pathological processes responsible for RC are multifactorial (Walsmith & Roubenoff, 2002). Originally proposed in the early 1990's, Roubenoff's group and others have demonstrated that reductions in BCM or LM in RA are inversely associated with systemic inflammation and 'sarco-active' (i.e. muscle-active) pro-inflammatory cytokines, principally TNF-α, IL-1β, and IL-6 (Roubenoff et al., 1992, 1994; Rall et al., 1996; Munro & Cappell, 1997; Walsmith & Roubenoff, 2002; Walsmith et al., 2004; Metsios et al., 2006; Engvall et al., 2008).

Although it is not precisely understood how these cytokines exert their effect on LM (Walsmith & Roubenoff, 2002, 2004; Rall & Roubenoff, 2004), several potential mechanisms of action in RA, and other muscle wasting conditions, have been proposed. The 'IGF-Akt' pathway has been identified as the key molecular pathway in controlling muscle growth (Fanzani et al., 2012). Simply, IGF-1 increases protein synthesis by activating Akt (protein kinase B). Activated Akt *inhibits* protein degradation by phosphorylating, and thus repressing, the FoxO

family of transcription factors, specifically reducing the activity of the muscle-specific ubiquitin ligases 'muscle atrophy F-box' (MAFbx) and 'muscle ring finger-1' (MuRF1); key proteins responsible for ubiquitylation (i.e. the degradation) of myosin and other muscle proteins (Wang & Maldonado, 2006; Fanzani et al., 2012; Schiaffino & Mammucari, 2011).

Activated Akt also *stimulates* mammalian target of rapamycin (mTOR) activity which in turn phosphorylates the ribosomal protein S6 (S6K) and other factors involved in translation initiation and elongation, and consequently, protein synthesis (Fanzani et al., 2012) (**Figure 2.2.**).

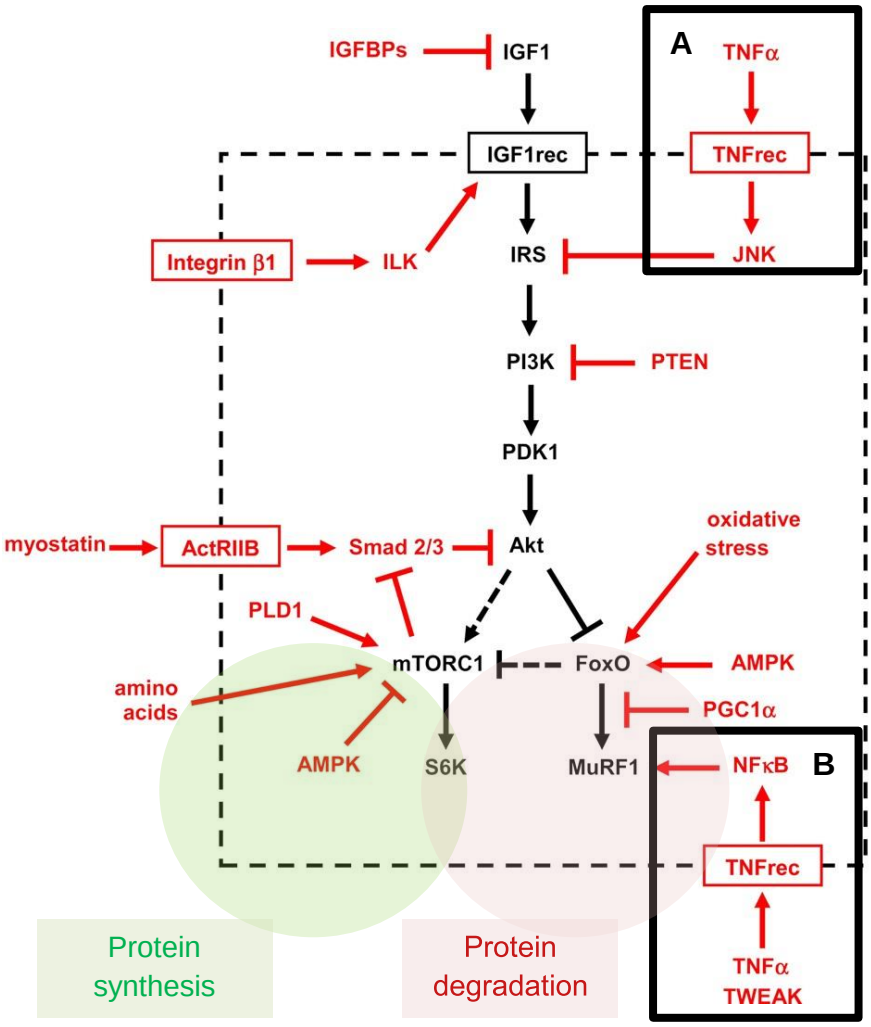


Figure 2.2. The IGF-Akt pathway and how cytokines such as TNF-α can disrupt key stages in protein synthesis. Source: Schiaffino and Mammucari (2011) and reproduced under the CC-BY-AT 2.0 licence.

In RA, elevated pro-inflammatory cytokines, in particular TNF-α, may promote proteolysis (i.e. muscle protein degradation) through several mechanisms:

1) By activating Jun N-terminal kinase (JNK), pro-inflammatory cytokines (e.g., TNF-α, IL-1β, and IL-6) can impair activation of Akt by inhibiting insulin receptor substrate (IRS) – an important step in the 'IGF-Akt' pathway (Lang et al., 2002; Broussard et al., 2003, 2004; Strle et al., 2004) (*box **A** in **Figure 2.2.***).

2) Pro-inflammatory cytokines can activate the transcription factor 'nuclear factor kappa-β' (NF-κB) (Lang et al., 2002). Activation of NF-κB increases the expression and accumulation of ubiquitinated proteins such as MuRF1 and MAFbx (Granado et al., 2005; Wang & Maldonado, 2006; Schiaffino & Mammucari, 2011; Fanzani et al., 2012). Activated NF-κB also inhibits skeletal muscle differentiation by suppressing the mRNA expression of myogenic differentiation 1 (MyoD) - a transcription factor that modulates signalling pathways involved in muscle development (Rall & Roubenoff, 2004) (*box **B** in **Figure 2.2.***).

3) Inflammatory cytokines can also block the action of IGF-I, either by reducing its expression or impairing its ability to bind to its receptor (Engvall et al., 2008; Fanzani et al., 2012).

2.1.4.2. Adipokines

Adipose tissue, especially visceral fat, is a recognised source of inflammation, producing catabolic pro-inflammatory cytokines such as TNF- α (Berg & Scherer, 2005; Metsios et al., 2006; Garcia-Poma et al., 2007; Stavropoulos-Kalinoglou et al., 2010). Adipose tissue also expresses adipokines such as adiponectin and leptin (Popa et al., 2005; Otero et al., 2006; Engvall et al., 2010), two proteins that can modulate the inflammatory environment in RA (Otero et al., 2006).

The exact inflammatory role of adiponectin is unclear. Some research in RA patients has shown it to exert an anti-inflammatory action, attenuating the immune response by reducing the activity of TNF- α and IL-6 (Wulster-Radcliffe et al., 2004; Chen et al., 2006; Toussirot et al., 2007; Targońska-Stępniak et al., 2010). However, conflicting findings suggest that adiponectin has a pro-inflammatory role in RA (Ebina et al., 2009; Rho et al., 2009; Oranskiy et al., 2012), with both Choi et al. (2009) and Giles et al. (2009, 2011) proposing that elevated serum adiponectin is associated with increased radiographical joint damage in RA.

Whilst serum-based levels of adiponectin in RA are inversely correlated with FM (Giles et al., 2009; Engvall et al., 2010), no research to date has identified an association with muscle loss in RA. However, elevated adiponectin concentrations have been associated with an increased

cachectic state in elderly patients with chronic heart disease (McEntegart et al., 2007; Paulo Araújo et al., 2009) and cancer (Wolf et al., 2006). Although the mechanisms behind adiponectin's role in cachexia require further investigation, it has been hypothesised that adiponectin may increase REE (i.e. hypermetabolism) (Paulo Araújo et al., 2009), as well as increasing the expression of IL-6 (a key catabolic cytokine in RC) in RA (Ehling et al., 2006).

Leptin plays a key role in appetite regulation and satiety. Leptin is expressed as adipocyte size increases, and generally acts as a negative feedback signal, decreasing appetite and increasing energy expenditure (Wislowska et al., 2007), although these effects in obese individuals are compromised due to leptin-resistance (Zhang & Scarpace, 2006). Additionally, it has been demonstrated that circulating levels of leptin increase in RA patients during inflammation, suggesting a contribution to the immune response (Otero et al., 2006; Seven et al., 2009). The exact inflammatory role of leptin is equivocal (Otero et al., 2006); leptin may increase the expression of pro-inflammatory cytokines in RA (Popa et al., 2005; Wislowska et al., 2007), although no relationship between RA disease activity (Bokarewa et al., 2003; Gunaydin et al., 2006; Allam & Radwan, 2012) and inflammation (Popa et al., 2005) have also been observed. Like adiponectin, leptin is primarily associated with adipose tissue (Wislowska et al., 2007), and no evidence, to date, has emerged to suggest a link with muscle loss in RA.

2.1.4.3. Muscle-protein suppressant hormones

Muscle wasting can be initiated by increased concentrations of muscle-protein suppressant hormones (Roth & Walsh, 2004; Schiaffino & Mammucari, 2011). One such hormone, myostatin, mediates catabolic signalling and is a potent negative regulator of skeletal muscle mass (Lee & McPherron, 2001; Roth & Walsh, 2004; Elkina et al., 2011; Schiaffino & Mammucari, 2011; Elliot et al., 2012). Circulating serum concentrations of myostatin have been found to be inversely related with muscle mass in the elderly (Schulte & Yarasheski, 2001; Yarasheski et al., 2002, Léger et al., 2008) and in other conditions featuring muscle wasting such as cardiac cachexia (Hoenig, 2008) and COPD (Ju & Chen, 2012). Whilst myostatin has been found to be highly expressed in the synovial tissue of RA (Dankbar et al., 2011), no research has investigated its role in RC.

2.2. Pharmacological treatment of rheumatoid arthritis and its effect on rheumatoid cachexia

2.2.1. Fundamental principles of modern treatment

Over the last decades there have been significant developments in both medication and strategy in RA treatment (Emery, 2006; Scott et al., 2010; Dale & Porter, 2010; Hetland, 2011; Ruderman et al., 2012). Although different RA management guidelines are available (e.g., from the American College of Rheumatology (ACR), British Society of Rheumatology (BSR), EULAR, National Institute for Health and Care Excellence (NICE)), several core principles exist:

- *Core principle 1: The importance of early diagnosis and treatment initiation*

Delayed therapy initiation is associated with poorer long-term prognostic outcomes in RA (Dale & Porter, 2010). Consequently, a 'window of opportunity' exists in which prescribed treatment is most effective (Möttönen et al., 2002; O'Dell, 2002; Goldbach-Mansky & Lipsky, 2003; Luqmani et al., 2006; Ding & Deighton, 2010), and results in superior prospective clinical outcomes (i.e. reduced inflammation, pain, and joint damage) (Goldbach-Mansky & Lipsky, 2003; Dale & Porter, 2010). Therefore, the earlier the diagnosis and initiation of treatment, the better the clinical outcomes.

- *Core principle 2: 'Tight control' of disease activity*

'Tight control' involves adjusting RA treatment until disease activity is suppressed below a predefined level of LDA or, preferably, 'remission' (often defined as DAS28 <2.6) (Emery, 2006; Luqmani et al., 2006, 2009; Bakker et al., 2007; Schipper et al., 2010; Scott et al., 2010; Hetland, 2011). 'Tight control' is maintained by frequent re-evaluation of disease severity (Scott et al., 2010), with aggressive DMARD therapy, including combination therapy involving ≥2 DMARDs (Goldbach-Mansky & Lipsky, 2003; Luqmani et al., 2006; Dale & Porter, 2010), and addition of biological agents if required. Corticosteroids (CS) may also be used to bridge the gap between DMARDs, or to provide rapid suppression of active disease (Ding & Deighton, 2010; Hetland et al., 2012). Overall, 'tight control' of RA has been shown to result

in significantly better clinical outcomes than previous treatment regimens (Grigor et al., 2004[3]; Bakker et al., 2007; Verstappen et al., 2007; Goekoop-Ruiterman et al., 2010; Schipper et al., 2010; Hetland et al. 2012), including greater rate of achievement of LDA and 'remission', and reduced radiographic progression.

- *Core principle 3: 'Treat-to-target'*

In 2010, a set of 'treat-to-target' (T2T) recommendations were formulated (Smolen et al., 2010a). These guidelines, entitled: 'Treating rheumatoid arthritis to target: recommendations of an international task force', were developed by a multinational steering committee of eminent rheumatologists as an 'international initiative to help define RA treatment targets and recommendations to measure disease severity and encourage earlier diagnosis and optimize treatment' (Smolen et al., 2010a). The principle of 'tight control' is a fundamental feature of T2T, which comprises four overarching principles and ten recommendations **(Figure 2.3.)**. The T2T recommendations are now one of the 'cornerstones of current RA management' (Ruderman et al., 2012).

Randomized controlled trials have demonstrated that a T2T approach achieves superior clinical outcomes compared to previous 'non-targeted' treatment regimens when various response ('target') criteria such as the 'EULAR good response' (Grigor et al., 2004), 'Boolean criteria' (Hetland et al., 2012; Wevers-de-Boer et al., 2012), DAS28 (Fransen et al., 2005; Verstappen et al., 2007; Saunders et al., 2008; Goekoop-Ruiterman et al., 2010), and 'ACR remission criteria' (Möttönen et al., 1999) are employed. A meta-analysis by Jurgens et al. (2012), entitled 'Overview and analysis of treat-to-target trials in rheumatoid arthritis reporting on remission', on four trials which featured tight disease control by T2T showed that applying this strategy approximately doubles the remission rate relative to usual carein patients with early RA.

The four overarching principles of T2T

A. The treatment of RA must be based on a shared decision between patient and rheumatologist.

B. The primary goal of treating the patient with RA is to maximise long term health-related quality of life through control of symptoms, prevention of structural damage, normalisation of function and social participation.

C. Abrogation of inflammation is the most important way to achieve these goals.

D. Treatment to target by measuring disease activity and adjusting therapy accordingly optimises outcomes in RA.

[3] The principle of 'tight control' of RA disease was first, and most thoroughly, investigated in the 'TIght COntrol for Rheumatoid Arthritis' (TICORA) study (Grigor et al., 2004).

The ten recommendations of T2T

1. The primary target for treatment of RA should be a state of clinical remission.

2. Clinical remission is defined as the absence of signs and symptoms of significant inflammatory disease activity.

3. While remission should be a clear target, based on available evidence LDA may be an acceptable alternative therapeutic goal, particularly in established long-standing disease.

4. Until the desired treatment target is reached, drug therapy should be adjusted at least every 3 months.

5. Measures of disease activity must be obtained and documented regularly, as frequently as monthly for patients with high/moderate disease activity or less frequently (such as every 3–6 months) for patients in sustained LDA or remission.

6. The use of validated composite measures of disease activity, which include joint assessments, is needed in routine clinical practice to guide treatment decisions.

7. Structural changes and functional impairment should be considered when making clinical decisions, in addition to assessing composite measures of disease activity.

8. The desired treatment target should be maintained throughout the remaining course of the disease.

9. The choice of the (composite) measure of disease activity and the level of the target value may be influenced by consideration of co-morbidities, patient factors and drug-related risks.

10. The patient has to be appropriately informed about the treatment target and the strategy planned to reach this target under the supervision of the rheumatologist.

Figure 2.3. The 'treat-to-target' (T2T) principles and recommendations developed in 2008. The principle of 'tight control' is fundamental to T2T, with emphasis on the use of a 'target' to guide therapy. Source: Smolen et al. (2010a), for more information see https://www.t2t-ra.com, available online March 2016).

2.2.2. Existing treatment guidelines for rheumatoid arthritis

The current ACR, BSR, EULAR, and NICE guidelines all encourage and incorporate the principles of 'tight control' and T2T. This includes early initiation of DMARDs, with combination DMARD therapy if required, and frequent assessment of disease activity and damage until treatment targets have been achieved (Deighton et al., 2009 (NICE); Luqmani et al., 2009 (BSR); Smolen et al., 2010b (EULAR); Singh et al., 2012 (ACR)). The primary aim for rheumatologists is to slow disease progression and relieve symptoms of inflammation and pain (Luqmani et al., 2006, 2009; Deighton et al., 2009; Scott et al., 2010; Smolen et al.,

2010a, 2010b; Ruderman et al., 2012; Singh et al., 2012; Upchurch & Kay, 2012) by targeting 'clinical remission' or, failing that, at least achieving LDA (Luqmani et al., 2006, 2009; Dale & Porter, 2010; Scott et al., 2010; Smolen et al., 2010a, 2010b; Singh et al., 2012; Upchurch & Kay, 2012).

2.2.2.1. Assessment of physical function in rheumatoid arthritis patients

Physical function is an important outcome measure in RA treatment. Both the ACR (Singh et al., 2012) and EULAR (Smolen et al., 2010b) state that the normalisation and maintenance of physical function is an important goal of T2T treatment. The current NICE (Deighton et al., 2009) guidelines advocate measuring 'functional ability' (NICE Guidelines 79, section 1.5.1.4), whilst the BSR (Luqmani et al., 2009) state that disability should be assessed. Although no method of assessing functional limitation is suggested by EULAR (Smolen et al., 2010a/b) (**Figure 2.2.**): the ACR (Singh et al., 2012), BSR (Luqmani et al., 2009), and NICE (Guidelines 79, section 1.5.1.4; Deighton et al., 2009) advocate the use of the Health Assessment Questionnaire (HAQ) – the 'most widely used functional outcome measure' in rheumatology (Ødegård et al., 2006).

When assessed by the HAQ, initiation of treatment has favourable effects on physical function (e.g., Hallert et al., 2003; Marcora et al., 2006; Metsios et al., 2007; Book et al., 2011). However, this subjective, patient-reported measure, that is influenced considerably by the patient's expectations, lack of recall, mood, general health status (Spiegel et al., 1988; Ødegård et al., 2006; Kingsley, Scott, & Scott, 2011), and, in particular, pain (which is generally reduced by 'tight control' of disease activity (e.g., Marcora et al., 2006; Kingsley et al., 2011)) has short-comings when used to assess function in patients with controlled RA. The HAQ (Bruce & Fries, 2005) generally fails to detect even substantial improvements in objectively measured physical function in stable RA patients who complete exercise training interventions (Van den Ende et al., 1997). For example, in Lemmey et al. (2009), significant improvements of 17–30% in objective function measures (50'W, 30 second arm curl test, 8'UG, IKES) in established RA patients following 6 months PRT, which normalised their function relative to age- and sex-matched population norms, were undetected by the Multi-dimensional HAQ (MDHAQ).

Thus, for accurate evaluation of disability, objective function tests should be used in clinical practice (Arvidson et al., 2002). Objective physical function tests, such as walking tests, chair tests, or HGS, have been found preferable to the HAQ in the assessment of physical disability

in RA (Arvidson et al., 2002), although they (i.e. objective tests) are not encouraged or employed by any current treatment guideline (i.e. ACR, BSR, EULAR, NICE).

2.2.3. If treatment suppresses disease activity and inflammation in rheumatoid arthritis, does it reverse rheumatoid cachexia?

2.2.3.1. Pharmaceutical therapy has no favourable effect on rheumatoid cachexia

It was proposed that treatment with anti-TNF-α drugs could reverse RC and restore muscle mass in RA patients (Walsmith & Roubenoff, 2002; Rall & Roubenoff, 2004; Marcora et al., 2006; Metsios et al., 2006). The physiological basis behind this hypothesis was that blocking TNF-α activity (using pharmalogical treatment) would reduce TNF-α-driven muscle catabolism (*see section* ***2.1.4.***) (Metsios et al., 2006). Further, suppression of disease activity (and symptoms) may also increase physical activity and appetite levels, as well as reducing REE; possible components of RC atiology (Stavropoulos-Kalinoglou et al., 2010).

However, Marcora et al. (2006) found that despite control of systemic inflammation, 6 months of treatment with anti-TNF therapy (etanercept (ETN)) had no effect on DXA-assessed LM relative to standard DMARD (MTX) therapy in treatment-naive early RA patients. Similarly, in patients with established RA, Metsios et al. (2007) found that whilst 3 months of treatment with ETN increased protein intake and physical activity levels, it had no effect on LM, and disturbingly increased truncal FM relative to standard DMARDs. Over a longer treatment period, in an open trial in female RA patients with established disease, Serelis et al. (2008) found no changes in either LM or FM following 12 months of infliximab treatment. Whilst in a 21 month trial, Engvall et al. (2010) found patients treated with anti-TNFs (predominantly infliximab) in combination with MTX had a significant increase in FM and BF%, and no increase in LM, relative to patients treated with standard DMARDs (triple therapy: MTX, sulfasalazine (SSZ), and hydroxychloroquine (HCQ)). Whilst longer term (i.e. over a period of several years) studies are required, it appears that anti-TNF-α therapy, despite its clinical efficacy, fails to restore LM in RA patients, and may exacerbate adiposity, particularly trunk adiposity.

2.2.3.2. Effect of corticosteroid therapy on body composition

Along with DMARDs and biologics, an important pharmacological tool in the treatment of RA, particularly active RA, are CS. Corticosteroids can be administered orally, via IM injection, intravenous infusion, or injected directly into an inflamed joint (NICE Guidelines 79, 2009), and

rapidly suppress inflammation in patients with high disease activity i.e. early untreated RA or those experiencing a disease flare (Ding & Deighton, 2010). However, despite providing rapid and effective relief of inflammation and pain, chronic exposure to high dose CS has unfavourable effects on body composition including muscle loss (Horber et al., 1985; Roubenoff et al., 1990; Gibson et al., 1991; Short et al., 2004; Schakman et al., 2008), and can result in general myopathy which exacerbates muscle weakness (Owczarek et al., 2005; Pereira & de Carvalho, 2011).

Another notable effect of chronic high and low dose CS use is an increase and redistribution of FM, particularly to the trunk (Horber et al., 1985; Da Silva et al., 2006; Mok et al., 2008). Whilst much research has investigated the effects of oral CS administration on body composition, the effects of a single high dose IM CS injection, as recommended for patients with active RA disease (i.e. at initial diagnosis or during a disease flare), is currently unknown.

2.2.3.3. Does rheumatoid cachexia still exist in the modern treatment era?

As discussed, current treatment, which follow the principles of 'tight control' and T2T generally successfully controls inflammation (Goldbach-Mansky & Lipsky, 2003; Bakker et al., 2007; Dale & Porter, 2010; Schipper et al., 2010; Emery et al., 2011), reduces the progression of radiographic damage, diminishes pain, and (as assessed subjectively by the HAQ) appears to attenuate functional disability (Molenaar et al., 2002; Cohen et al., 2007; Mäkinen et al., 2007; Smolen et al., 2009).

It has been suggested that achievement of disease 'remission' and the reduction in pro-inflammatory cytokines may increase the potential for muscle synthesis, thus increasing physical activity, and optimising body composition (Stavropoulos-Kalinoglou et al., 2010). When this doctorate began, whether the relative success of a T2T treatment strategy in achieving LDA or 'clinical remission' has resulted in the attenuation of RC and subsequent improvements in objective physical function in RA patients had not been investigated.

2.3. Potential adjunct interventions for attenuating rheumatoid cachexia and improving physical function

2.3.1. Is there still a need for adjunct interventions that may restore muscle mass and physical function?

Pharmaceutical treatments for RA (i.e. DMARDs) fail to significantly improve body composition or restore physical function (Marcora et al., 2006; Metsios et al., 2007; Serelis et al., 2008; Engvall et al., 2010) in RA. Therefore, when this doctorate commenced potential interventions that focus on restoring muscle mass and physical function needed to be identified. Indeed, if current treatment for RA (specifically T2T) proves ineffective in reversing RC and restoring normal function, then this necessity to identify adjunct treatments that do remains.

2.3.1.1. Exercise and progressive resistance training

Aerobic-based exercise has been shown to have beneficial effects on cardiorespiratory fitness, quality of life, and physical function (Cooney et al., 2011), and whilst RA patients should be encouraged to include both aerobic and strength exercise training as part of routine care, due to its substantial effects on muscle mass, resistance training appears to be the most beneficial means of reversing the consequences of RC (Lemmey, 2011).

Two Cochrane Reviews (Van den Ende et al., 2000; Hurkmans et al., 2009) have supported the inclusion of PRT in the routine management of RA patients, and numerous studies have demonstrated strength training results in both functional and strength gains, and favourable changes to body composition (e.g., Häkkinen et al., 1999, 2003, 2004, 2005; Van den Ende et al., 2000). This also comprises work by our group including Marcora et al. (2005) who, in a pilot study, showed that 12 weeks of high-intensity progressive resistance training (PRT; 3 x's/week) increased total LM and ALM, decreased FM, trunk FM, and BF%, and substantially improved objectively-assessed functional capacity (including IKES and the STS-30) in RA patients.

To further confirm the efficacy of PRT, Lemmey et al. (2009), in a randomised controlled trial, demonstrated that 24 weeks of PRT (2 x's/week) significantly increased total LM and ALM,

decreased FM (particularly trunk FM), and was able to restore normal levels of physical function as measured by objective functional tests (training-specific strength was improved by 119%, chair stands by 30%, knee extensor strength by 25%, arm curls by 23%, and 50'W time by 17%) in patients with established RA. Notably in this trial, low intensity range-of-movement exercises (i.e. the form of exercise most commonly prescribed for RA patients), which were performed by the control group, had no effect on measures of body composition or objective physical function.

Unfortunately, RA patients are generally very sedentary (Sokka et al., 2008, 2010; Lee et al., 2012) and despite its clear benefits and safety, patient uptake of exercise is poor. In the UK, ~68% of RA patients perform no regular weekly exercise, and in some countries such as Italy and France, this figure is above 85% (Sokka et al., 2008). In the UK, only 18% of patients reported being physical active at least 3 x's/week (Sokka et al., 2008).

Remarkably, some research has shown that even patients who experience the benefits of exercise cease training once supervision is withdrawn. In a three year follow-up study to their 2009 PRT trial, Lemmey et al. (2012) found that, despite the normalisation of objective physical function and the improvements in body composition, none of the patients in the PRT group had maintained this activity or any other form of regular high intensity exercise. As a result, the gains in ALM were completely lost and much of the losses in FM, trunk FM, and BF% were regained. Similarly, the training-induced gains in IKES, and the chair and arm curl tests were completely lost during detraining, although most (66%) of the improvement in the 50'W was retained. Lack of adherence to exercise programmes once supervision is withdrawn in RA has been reported elsewhere (e.g., Hsieh et al., 2009), and loss of strength and functional gains from PRT is inevitable once training ceases (Lemmer et al., 2000; De Jong et al., 2009).

Conversely, it should be noted that some studies have reported 'high' adherence rates to exercise, including high-intensity PRT. In the 'Rheumatoid Arthritis Patients in Training' (RAPIT) trial, the median percentage of sessions attended over 2 years, of 309 RA patients, was 74% (De Jong et al., 2003; Munneke et al., 2003). Further, in patients who had continued exercising once per week for the subsequent 18 months after the trail ended, strength (knee extension) gains were maintained (De Jong et al., 2009). Whilst it is beyond the scope of this thesis to discuss all the factors influencing exercise adherence, it appears that adherence is improved in patients with well-controlled disease, better functional ability, and a strong social structure (Munneke et al., 2003; Metsios et al., 2008). Owing to the substantial benefits on

body composition and physical function in RA, interventions aimed at improving exercise (or physical activity), particularly high-intensity PRT, adherence should be conducted.

Overall, it seems that although highly beneficial, sustained high intensity exercise training is unlikely to be widely adopted by every RA patient as a therapy for reversing RC and restoring physical function. Thus, the challenge is to develop a treatment option that is easily administered, inexpensive, makes limited demands of the patient, and consequently would be widely acceptable.

2.3.1.2. Nutritional supplementation

Anabolic nutritional supplementation offers a treatment option that, if efficacious, should be widely acceptable to patients. Whilst oral amino acid supplementation has been shown successful in increasing LM in the elderly with sarcopenia (Solerte et al., 2008) and patients with cancer cachexia (May et al., 2002), interestingly, there is a striking lack of published studies on the effect of nutrition on RC, with the majority of trials investigating whether diets or dietary supplements are able to moderate RA disease symptomology (for a review, see Stamp, James, & Cleland, 2005).

Our group (Marcora et al., 2005) previously investigated the effects of ß-hydroxy-ß-methylbutyrate, glutamine, and arginine (HMB/GLN/ARG) amino acid supplementation as a treatment for RC in a randomised controlled trial involving forty RA patients. The results showed that 12-weeks daily protein supplementation (both HMB/GLN/ARG and a control mixture of non-essential amino acids (alanine, glutamic acid, glycine, serine)) was effective in increasing muscle mass (~0.4 kg ALM) and improving some (STS-30, IKES), but not all (HGS, elbow flexor strength), measures of physical function and strength.

Creatine (Cr) monohydrate supplementation (methylguanidine-acetic acid; a naturally occurring compound made from three amino acids: arginine, glycine, and methionine) has in athletes and the general population generally been shown to be more effective in increasing LM and physical performance than other anabolic supplements including HMB/GLN/ARG (for a review, see Nissen & Sharp, 2003; Cribb et al., 2007).

However, the findings from the only trial to investigate the effects of oral Cr supplementation in RA patients (Willer et al., 2000) are inconclusive. In this uncontrolled, un-blinded trial, twelve RA patients were orally supplemented with Cr supplementation for 21 days using recommended doses (20 g/day for 5 days followed by 2 g/day for 16 days), and the effects on

muscle strength, subjectively assessed function (HAQ), and disease activity were examined. It was found that Cr supplementation increased composite strength, as determined by the muscle strength index, in 8/12 patients by an average of 14%, although this improvement was not associated with changes in muscle Cr or PCr levels. The authors attributed the limited effectiveness of Cr to alterations in the kinetics of Cr in patients with RA (e.g., reduced transport into muscle, and increased metabolism and/or excretion).

Whilst the findings of the Willer et al. trial are compromised by methodological limitations and a failure to assess body composition, the study does provide some indication that Cr supplementation may be safe, and also effective in improving strength in RA patients. Clearly, well-conducted investigations are needed to establish the efficacy of Cr supplementation in improving body composition and function in RA.

2.4. Literature review summary

Rheumatoid arthritis is characterised by adverse changes in body composition (reduced muscle mass and increased adiposity) termed RC, which is a key contributor to the reduced physical function and strength seen in these patients. Since RC is attributed to inflammation, successful control of disease activity (i.e. inflammation) may attenuate RC, and the resulting decrements in physical function. The effect of current treatment strategy (i.e. 'tight control' and T2T), with much more frequent achievement of LDA or 'remission', on body composition and objectively-measured physical function in RA is yet to be investigated.

Although high-intensity PRT has been shown to help restore muscle mass and normalise physical function in RA patients, the lack of uptake and adherence to high intensity exercise is poor. If RC is still a feature of modern day RA then adjunct anabolic treatments, such as nutritional oral Cr supplementation, may provide a specific treatment for improving body composition and physical function. The effects of oral Cr supplementation in RA are currently unclear.

To advance the understanding of RC, additional investigations into its mechanisms are also warranted. In particular, the role of circulating pro-inflammatory cytokines, adipokines, catabolic proteins such as myostatin, and anabolic factors such as IGF-I should be further explored. Specifically, what is not fully understood is how these markers change in response to different pharmacological treatments of RA, or interventions designed to attenuate or reverse RC such as PRT or protein supplementation.

Whilst the effect of current RA treatment strategies on RC is unclear, it is known that the pharmacological intervention initially thought most likely to succeed (anti-TNFs) does not recover lost LM, may exacerbate adiposity, and fails to restore normal physical function. Treatment by chronic high dose CS, although highly effective in suppressing disease activity, has deleterious effects on body composition in patients including muscle loss and increased adiposity, particularly centrally. The effects of a single bolus IM injection of CS, a routine and recommended treatment in active and early RA, on RC are unknown.

3

Has ‘treat-to-target’ therapy attenuated rheumatoid cachexia and improved physical function in patients with rheumatoid arthritis? A cross-sectional study

This study has been accepted for publication in Rheumatology (Oxford).

3.1. Introduction

Rheumatoid arthritis (RA) is characterised by adverse changes in body composition (i.e. substantial loss of lean mass (LM) and increased adiposity), termed 'rheumatoid cachexia' (RC) (Roubenoff et al., 1992). Previously, a loss of ~8 to 15% in LM has been observed compared to matched non-RA healthy controls (HCs) (e.g., Roubenoff et al., 1994; Rall et al., 2002; Roubenoff et al., 2002; Walsmith & Roubenoff, 2002; Giles et al., 2008b; Book et al., 2009; Matschke et al., 2010a, 2010b; Dao et al., 2011; Baker et al., 2014), with total fat mass (FM) reportedly ~12 to 18% greater in RA (e.g., Giles et al., 2008b; Book et al., 2009; Elkan et al., 2009; Matschke et al., 2010b; Dao et al., 2011; Santos et al., 2011). Disturbingly, the majority of this excess FM occurs on the truncal area (Marcora et al., 2005a, 2005b; Marcora et al., 2006; Lemmey et al., 2009; Book et al., 2009, Book et al., 2011; Dao et al., 2011), and results in a large majority (up to 80%, Lemmey et al., 2009) of patients classified as obese and overweight (Elkan et al., 2009; Engvall et al., 2010; Santos et al., 2011). Examination into the prevalence and differences in RC between males and females in equivocal (Giles et al., 2008b; Book et al 2009; Baker et al., 2015).

This undesirable change in body composition can exacerbate mortality risk (Summers et al., 2008), and is cited as a major contributor to the ~25% reduction in physical function and strength observed in RA (Roubenoff, 2001; Marcora et al., 2005a, 2005b, 2006; Giles et al., 2008a; Lemmey et al., 2009; Stavropoulos-Kalinoglou et al., 2009; Matschke et al., 2010a, 2010b; Chen et al., 2011; Kramer et al., 2012; Van Bokhorst-de van der Schueren et al., 2012; Lusa et al., 2015).

In general, RA is associated with a greater risk of co-morbidity, most notably a two-fold increased risk (Solomon et al., 2003; Kitas & Gabriel, 2011) of cardiovascular disease (CVD) which accounts for the majority of deaths in patients (Humphreys et al., 2014; Bag-Ozbek & Giles, 2015). The increase in CVD in RA is not fully explained by the presence of traditional cardiovascular risk factors (Elkan et al., 2009; Kitas & Gabriel, 2011; Amaya-Amaya et al., 2013) and is thought to be due to the inflammatory process inherent of the disease (Solomon et al., 2003). Inflammation is an important component of atherosclerosis (Kitas & Gabriel, 2011), whilst TNF-α increases insulin resistance and the release of free fatty acids into the blood; both of which increase CVD risk (Dessein et al., 2006; Kitas & Gabriel, 2011).

As RC is driven by inflammation (i.e. pro-inflammatory cytokines e.g., TNF-α) (Roubenoff et al., 1992, 1994; Rall & Roubenoff, 2004; Walsmith et al., 2004; Engvall et al., 2008; Chen et al., 2011; Maghraoui et al., 2015), it was reasoned that pharmacological reduction of inflammation, specifically TNF-α, could reverse RC (Walsmith & Roubenoff, 2002; Summers et al., 2008). However, evidence suggests that anti-TNF-α therapy has no beneficial effect on body composition (i.e. does not reverse RC) (Marcora et al., 2006; Metsios et al., 2007; Serelis et al., 2008; Engvall et al., 2010; Toussirot et al., 2014).

The primary aim of current RA treatment is to achieve a state of low disease activity (LDA), or if possible, 'clinical remission' (i.e. defined as a Disease Activity Score in 28 joints (DAS28) score of <2.6) (Luqmani et al., 2009; Dale & Porter 2010; Scott et al., 2010; Smolen et al., 2010a, 2010b, 2014; Ruderman et al., 2012). To achieve this, management of RA is based around early diagnosis, and 'tight control' of disease activity by regular assessment, and adjustment of treatment when such control is not achieved (Luqmani et al., 2009; Scott et al., 2010; Smolen et al., 2010a, 2010b, 2014). This approach is exemplified by the 'treat-to-target' (T2T) recommendations outlined by Smolen et al. (2010a, 2015).

Since their formulation, the T2T recommendations have become one of the 'cornerstones of current RA management' (Ruderman et al., 2012), with the UK National Institute of Clinical Excellence (NICE) (Deighton et al., 2009), British Society of Rheumatology (BSR) (Luqmani et al., 2009), European League Against Rheumatism (EULAR) (Dale & Porter, 2010; Smolen et al., 2010b, 2014), and American College of Rheumatology (ACR) (Singh et al., 2012) guidelines for the management of RA all encouraging the use of early, tailored and targeted treatment, with achievement of LDA or 'remission' the primary objective. Several clinical trials have demonstrated that using a T2T (Fransen et al., 2005; Verstappen et al., 2007; Van Tuyl et al., 2008; Schoels et al., 2010; Jurgens et al., 2012; Farman et al., 2015; Stoffer et al., 2015) or 'tight control' (Grigor et al., 2004; Bakker et al., 2007; Schipper et al., 2010) approach achieves superior clinical outcomes (i.e. lower DAS28, reduced pain, higher rate of remission, and radiographic joint (cartilage) damage) compared to usual care.

Restoration of functional ability is an explicit aim of the ACR, BSR, EULAR, and T2T recommendations (Deighton et al., 2009; Luqmani et al., 2009; Smolen et al., 2010a, 2010b, 2014; Singh et al., 2012) with the 'abrogation of inflammation' proposed to be a potential solution (Smolen et al., 2010a). The few investigations which have assessed the effect of T2T on functional impairment in RA, to date, have only used subjective self-report instruments such

as the Health Assessment Questionnaire (HAQ) (e.g., Sakellariou et al., 2013; Seto et al., 2013; Vermeer et al., 2013; Solomon et al., 2014; Sugihara et al., 2015). The HAQ is strongly influenced by symptomatological features, such as pain (Arvidson et al., 2002; Marcora et al., 2006; Kingsley et al., 2011), that is often reduced following treatment. Further, the HAQ fails to detect substantial improvments in objective physical function in RA patients (Van de Ende et al., 1997; Lemmey et al., 2009). Therefore, objective measures of physical function are the most valid means of 'true' functional assessment (Arvidson et al., 2002).

It would be anticipated that the relative success of current treatment (e.g., T2T) in effectively reducing patients' inflammation and disease activity would also benefit body composition and objective physical function, particularly in those with well treated early disease. This stance is taken by Binymen et al. (2011) who stated that the most effective means to promote anabolism in RA is control of inflammatory disease activity. A similar suggestion was made by Stavropoulos-Kalinoglou et al. (2010), who stated that achievement of disease 'remission' and the reduction in pro-inflammatory cytokines may increase the 'potential for muscle synthesis'. Whether the success of current treatment strategies, specifically a T2T approach, in achieving much lower disease activity (or clinical 'remission') has resulted in attenuated RC, and subsequent improvements in physical function in RA patients has not been investigated.

Research suggests that the RA disease process may already be active before symptoms become clinically detectable, and the importance of identifying 'pre-clinical' stages of RA has been recognized by EULAR (Gerlag et al., 2012). In particular, there is evidence to suggest that inflammation (Kraan et al., 1998; Van de Sande et al., 2011), such as elevated concentrations of cytokines such as TNF-α, IL-1β, and IL-6 (Sokolove et al., 2012), exist in the 'pre-clinical' phase of RA; consequently, it may be that these same processes initiate RC in the very early stage of the disease, possibly prior to the appearance of detectable RA symptoms. Although there is some evidence to suggest that RC is established early in the course of RA (within 12 months of diagnosis) (Marcora et al., 2005a; Book et al., 2009, 2011), the question surrounding the temporal-course of RC requires further investigation.

3.1.1. Aims and hypothesises

The aim of this cross-sectional study was to investigate body composition, objectively-assessed physical function, and CVD risk in RA patients with stable and well-controlled disease activity resultant of the contemporary treatment era. Additionally, the investigation

sought to further examine the time-course of RC, disability, and elevated CVD risk in RA patients.

The study hypothesised that, despite improved control of inflammation and disease activity, RA patients would present with reduced muscle mass, increased FM (particularly trunk FM), poor objectively-assessed physical function, and exacerbated CVD risk, compared to age- and sex-matched HCs. We hypothesised that these findings would be similar to those previously reported by our group and others (i.e. studies performed either before local adoption of T2T strategies, or, if more recent, on patients who commenced treatment pre-T2T). Further, we predicted that there would be no difference in these measures between patients with 'recent-onset' disease (≤12 months since diagnosis) and those with more 'established' disease (>12 months since diagnosis); inferring that changes to body composition, and consequent reductions in function and exacerbation of CVD risk, occur early in the disease process.

As achieving 'remission' is a fundamental goal of T2T, we also investigated the differences in body composition and objective physical function in patients whom had achieved clinical 'remission' (i.e. defined as a DAS28 score <2.6) versus those who had not. We also compared patients 'in remission' with the HC group.

Due to our hypothesis that RC possibly occurs prior to disease diagnosis and treatment inititation, we hypothesised that there would be no difference in body composition between patients 'in remission' and those not. We hypothesised that whilst patients 'in remission' will have better objective physical function (due to better control of pain and symptoms), their body composition and objective physical function will still remain significantly deficient compared to the HC. As research into RC prevalence between males and females is equivocal, we also investigated the sex differences in measures of body composition and objective physical function.

3.2. Patients and methods

This cross-sectional study was conducted at the School of Sport, Health and Exercise Science, Bangor University, UK between February 2013 and March 2015. The study was approved by the North Wales Research Ethics Committee (REC) – West (12/WA/0323).

3.2.1. Study population

3.2.1.1. Inclusion/exclusion criteria

Eighty-two (*n* = 82) RA patients with stable disease (as assessed by their rheumatologist) were recruited from outpatient clinics of the North West Wales Rheumatology Department (Peter Maddison Rheumatology Centre (PMRC), Llandudno, North Wales). For inclusion, participants had to: (a) fulfil the American Rheumatism Association 1987/2010 revised criteria for the diagnosis of RA (Aletaha et al., 2010); (b) be aged ≥18 years; (c) not be cognitively impaired; (d) be free of other cachectic diseases or conditions preventing safe participation; (e) not be taking anabolic drugs or nutritional supplements; (f) not be currently participating in a regular, intense exercise training; and (g) not be pregnant. Once recruited, participants were categorised into either 'recent-onset'[4] (disease duration of ≤12 months) or 'established' (>12 months) groups. For comparison, *n* = 85 age- and sex-matched HCs were recruited from local community groups. To be eligible for the study, HCs must have satisfied all of the inclusion criteria for RA patients, except for the diagnosis of RA.

3.2.1.2. Local patient care and treatment strategy

In order to assess patients treated wholly using contemporary treatment approaches (e.g., treating to LDA or 'remission' characteristic of 'tight control' and T2T), to be eligible for the study, patients must have had commenced treatment post 1/1/2008. This date was chosen to approximately represent the time when PMRC adopted the T2T treatment strategies subsequently outlined by Smolen et al. (2010a). Whilst we acknowledge that patients locally

[4] Patients in the 'recent-onset' group are being re-assessed annually for a duration of 8 years. All methods and outcome measures described in this section are repeated, and this longitudinal data will provide information on how patients' relative body composition, physical function, and CVD risk change over time. This data is not presented in this thesis.

may have been treated by a ‘tight control’/T2T approach prior to this date, we believe patients, under the care of the PMRC, recruited after this date would provide a representative depiction of patients treated wholly with these approaches.

3.2.2. Outcome measures

Participants presented for assessments in an overnight fasted state, having refrained from strenuous exercise, caffeine, and alcohol over the preceding 24 hours. Relevant information (age, disease duration, medication) was collected by structured interview and from review of medical records.

3.2.2.1. Anthropometric measures

Routine anthropometric measures (body mass, height, and waist and hip circumferences) were recorded in accordance with standard procedures (Eston & Reilly, 2009). Body mass was measured to the nearest 0.1 kg using digital floor scales (SECA 635, Birmingham, UK), and height to the nearest 0.5 cm using a wall-mounted stadiometer (Body Care, Warwickshire, UK).

3.2.2.2. Body composition measures

Total and regional lean, fat, and bone masses were estimated using a whole body fan-beam dual energy X-ray absorptiometry (DXA) scanner (Hologic, QDR Discovery 45615, software V12.4). Appendicular lean mass (ALM; the summed LM of the arm and leg regions, i.e. left arm LM + right arm LM + left leg LM + right leg LM = ALM) was estimated using the method described by Heymsfield et al. (1990) (**Appendix B**) and acted as a surrogate measure of total body muscle mass (Kim et al., 2002). Manufacturer DXA examination procedures (daily calibration, subject preparation, positioning, and analysis) were followed for each scan. The radiation exposure was 3.6 μSv per scan. In-house assessment revealed a DXA co-efficient of variation (CV) of 1.4%, which corresponds with both manufacture guidelines and other studies (Kim et al., 2002).

3.2.2.3. Strength and objective physical function measures

Isometric maximal voluntary knee extensor strength (IKES) was measured using an isokinetic dynamometer (Humac Cybex Norm 2004, Computer Sports Medicine Inc, Massachusetts, USA). Participants were seated upright in the dynamometer with the hip and knee flexed to 90° (0° = full extension in both), the dynamometer arm attached to the lower leg just above

the ankle and the axis of rotation aligned with the lateral condyle of the femur during contraction. After a submaximal warm-up and practice attempts, participants were asked to exert maximum force for ~3–5 seconds on three occasions for each leg with a one minute rest between trials. Peak force (Newtons, N) during each trial was recorded, and the largest force from the left and right leg were averaged and used for analysis. The dynamometer and its accuracy were verified periodically over the course of the study by loading the dynamometer with weights and recording the force produced. The repeatability was 'good' (CV = 0.3%) and compares well with other fixed dynamometers (Impellizzeri et al., 2008).

Maximal voluntary hand-grip strength (HGS) was measured using a Grip-A dynamometer (Takei Kiki Kogyo, Japan). Participants were asked to stand upright holding the dynamometer parallel to their side, and to squeeze the hand-grip maximally whilst simultaneously adducting the arm. After a practice trial, both hands were tested alternatively three times with the best overall score in kilograms (kg) recorded. These strength tests have been routinely used by our group to assess RA patients (Marcora et al., 2005a, 2005b, 2006; Lemmey et al., 2009, 2012; Matschke et al., 2010a, 2010b; Matschke et al., 2013).

Participants completed three objective assessments of whole body physical function, developed specifically for assessing the capacity to perform activities of daily living in older adults (Rikli & Jones, 2012), and used routinely by our group to determine physical capacity in RA patients (Marcora et al., 2005a, 2005b, 2006; Lemmey et al., 2009, 2012; Matschke et al., 2010a, 2010b, 2013):

- The 'sit-to-stand in 30 second' test (STS-30), which measures lower-body strength, involves participants rising from a seated position on a fixed straight-back chair (seat height 43.2 cm / 17 inches), while keeping their arms folded across the chest, as many times as possible in 30 seconds. The number of full repetitions completed was used for analysis.

- The '8-foot up and go test' (8'UG), which assesses dynamic balance, requires participants to rise from the same seated position as for the STS-30 , walk forward around a cone 8 feet (2.44 metre) away, and return to the seated position as quickly as possible. The best time out of two attempts was used for analysis.

- The ‘50-foot walk test’ (50’W) assesses walking speed, and is the time taken in seconds to walk 50 feet unaided along a single straight line as quickly as possible. Participants had one practice walk before performing the test.

3.2.2.4. Aerobic capacity

The ‘Siconolfi’ step test (Siconolfi et al., 1985) was used as a predictive, sub-maximal measure of aerobic capacity (VO_2max). This test consists of stepping up and down from a portable 10 inch (25.4 cm) step for 3 minutes per stage, for a maximum of three stages. The stepping rates increased progressively from stage to stage (i.e. the stepping rates for stages 1, 2, and 3 are 17, 26, and 34 steps per minute, respectively), and were maintained using a metronome (Metronome 3.0, ONYX). Each stage was separated by 1 minute of rest, and participants only progressed to the next stage if their heart rate, measured by telemetry (Polar Electro OY, Finland), at the end of the previous stage was less than 65% of their predicted maximal heart rate (i.e. 220–age).

Steady state absolute oxygen consumption and a predicted relative VO_2max were calculated using established equations. Patients taking beta (β)-adrenergic blocking agents (β-blockers) were excluded from performing this assessment. This test has been validated by our group for estimating aerobic capacity in RA (Cooney et al., 2013), systemic lupus erythematosus (Marcora et al., 2007), and ankylosing spondylitis patients (Thompson et al., 2015).

3.2.2.5. Clinical measures

Disease activity of each patient was assessed by the Disease Activity Score in 28 joints (DAS28), and systemic inflammation by serum C-reactive protein (CRP) level. The DAS28 combines a 28 tender and swollen joint count with circulating CRP level and a subjective assessment of general health status (using a visual analogue (0–100) scale (VAS)). The DAS28, which is extensively used in clinical trials and routine RA management (Fransen et al., 2003), defines ‘clinical remission’ as a score <2.6 (Smolen et al., 2010a, 2010b, 2015).

Subjective physical disability was assessed using the Multi-dimensional Health Assessment Questionnaire (MDHAQ) (Pincus et al., 2007). The MDHAQ is designed to improve the ability to detect improvements in function at the lower end of the scale as compared to the HAQ (Maska et al., 2011). The functional scale of the MDHAQ, the ‘disability index’ is rated from 0 (best) to 3 (worse) with scores of 0 to 1 generally considered to represent mild to moderate difficulty, 1 to 2 moderate to severe disability, and 2 to 3 severe to very severe disability (Bruce & Fries, 2005; Pincus et al., 2007). The MDHAQ also contains a 21-point general pain VAS,

the RADAI (Rheumatoid Arthritis Disease Activity Index), a self-report joint count where patients indicate the current amount of pain (0 = none, 1 = mild, 2 = moderate, 3 = severe) being experienced in 16 different joints, and a 10-point fatigue VAS.

Health-related quality of life was assessed using the Medical Outcomes Study 36-Item Short Form survey (SF-36) (Ware & Sherbourne, 1992) which is divided into physical and mental components. For this instrument, lower scores represent poorer patient-perceived mental and/or physical wellbeing.

3.2.2.6. Physical activity

A surrogate 'physical activity' measure was taken from the MDHAQ 'exercise frequency' question. For this question, participants indicated how many times they engaged in exercise (defined as 'shortness of breath, sweating, and increased heart rate'). Participants were discouraged for including activities that were part of their daily routine (e.g., walking to the shop).

3.2.2.7. Cardiovascular risk profile

To assess CVD risk in the RA patients, blood samples were harvested by venipuncture at the median cubital vein following an overnight fast. The venipuncture procedure was performed by a trained investigator, or by a phlebotomist at the patients' general practitioner (GP) surgery (a referral to GP for blood sampling was requested by the rheumatologist if necessary). The blood variables measured included serum lipids: total cholesterol (TC, mmol/L), low-density lipoprotein cholesterol (LDL-C, mmol/L), high-density lipoprotein cholesterol (HDL-C, mmol/L), triglycerides (TG, mmol/L), TC: HDL-C ratio; fasting plasma glucose (mmol/L); and CRP (mg/L). These variables were determined by analysis at the Department of Clinical Biochemistry's laboratory (Blood Sciences group at Ysbyty Gwynedd, i.e. Gwynedd Hospital) in line with standard analysis procedures.

Cardiovascular disease risk was predicted using the QRISK2 algorithm. Validated in different ethnic groups across England and Wales, the QRISK2 has been identified as a better discriminator of CVD than the modified Framingham score as it recognises ethnicity and the presence of RA as independent risk factors (Hippisley-Cox et al., 2008). The QRISK2 algorithm also accounts for traditional CVD risk factors including: participant's height (cm), weight (kg), systolic blood pressure (mmHg), TC: HDL-C ratio, current smoking status, diabetes status, family history (angina or heart attack in a first degree relative ≤60 years of age), presence of any renal impairment, and if anti-hypertensive medication is necessary.

Participants' rested blood pressure reading was obtained manually, whilst seated, using a stethoscope (Littmann, 3M Health Care, St. Paul, USA) and sphygmomanometer (Welch Allyn, New York, USA).

3.2.3. Statistical analysis

No previous comparable research was available to calculate an a-priori power calculation; a target of 100 participants was planned in each group (RA and HC). The primary outcome of the study was ALM, and secondary outcomes included other measures of body composition (total LM, FM, trunk FM, ALM/BM% (ALM%), and total LM/BM% (LM%)), objective physical function, and CVD risk factors. Proportional body composition measures (in accordance with Janssen et al., 2002) were used (i.e. measures normalised for bodyweight, e.g., ALM%, LM%) as they more accurately interpret a participant's relative muscle to fat composition when BM is dissimilar (Giles et al., 2008a; Schautz et al., 2012). Due to the effects of age and sex on body composition and physical function, appropriately matched groups were generated by excluding the oldest male and female HCs alternatively until the groups achieved similarity in terms of mean age (≤1 year) and gender distribution (equal % of females).

Primary analysis included the comparison of the RA group versus the HC group; followed by a sub-analysis of 'recent-onset' versus 'established', 'remission' versus 'not in remission', and male versus female RA patients. All data is presented as mean (±SD) unless otherwise stated. Variables were checked for multi-collinearity, uni- and multi-variate outliers (Mahalanobis Distance), and normal distribution using Shapiro-Wilk tests. Where necessary, data (hip circumference, BMI, total FM, MDHAQ function, 8'UG, 50'W, VO_2max) was logarithm transformed to obtain normally distributed data, and to assess its relative effect on associated significance values.

Data analysis involved multiple (MANOVA) or univariate analysis of variance (ANOVA) where appropriate. Chi-squared tests were used for comparison of dichotomous variables. Significance was set at $P < .05$ and a trend was recognised as $P = .05–.10$, unless corrected by Bonferroni adjustment. As recommended by the STROBE (STrengthening the Reporting of OBservational studies in Epidemiology) 2007 guidelines (von Elm et al., 2007), confidence intervals (95% CI) for the between-group difference was reported. Pearson product–moment correlation (bivariate and partial when appropriate) was used to test the significance of relationships (*r*) of interest. Effect size was calculated (for body composition, objective physical

function, and lipid profile) data analysed by (M)ANOVA) as η^2: small ≥.01; medium ≥.08; large ≥.26; very large ≥.50. Participants with missing data (e.g., did not complete a particular test) were included the subsequent analysis of other measures; the number of missing data is shown in **Appendix C**.

Over the study period, four researchers were involved in data collection (TJW, BJC, JW, and HJ). Intra-rater reliability assessment of anthropometric and physical function measurements revealed infraclass correlation coefficients (ICC) between .704 and .996[5] ('good' to 'excellent' (based on commonly-cited cut-offs by Cicchetti, 1994)). All assessors were trained to perform DAS28 assessments by PMRC rheumatologists. All data was analysed using the Statistical Package for the Social Sciences 22.0 (SPSS) (Chicago, USA).

[5] An ICC is measured on a scale of 0 to 1; 1 represents perfect reliability with no measurement error, whereas 0 indicates no reliability: height, .996, excellent; waist circumference, ICC =.977, excellent; hip circumference, ICC =.735, good; STS-30, ICC =.700, good; 8'UG, ICC =.972, excellent; 50'W, ICC = .704, good; HGS, ICC =.934, excellent, IKES, ICC =.928, excellent.

3.3. Results

One hundred and ninety-seven (*n* = 197) patients with RA were considered to be eligible by the rheumatologists and were approached to take part in the study. Of these, 115 (58%) declined participation. Thus, 82 patients with RA were recruited and tested; and of these patients, at the time of assessment, 33 had been diagnosed less than 12 months previously ('recent-onset' group; mean duration of 7.1 months), whilst the other 49 ('established' group) had a disease duration of between 12–61 months (mean duration of 34.7 months). Eighty five (*n* = 85) age- and sex-matched, sedentary HC participants served as controls. All participants were Caucasian.

3.3.1. Primary analysis: Rheumatoid arthritis versus healthy control group

3.3.1.1. Descriptive data and participants

Table 3.1. shows the demographic and clinical characteristics of the 82 RA patients and 85 HCs. The groups were precisely matched for mean age (*P* = .962) and gender distribution (*P* = .992). In regard to disease activity, the mean DAS28 score was 2.8, and 49% of patients were in a state of 'clinical remission' (DAS28 score <2.6). Most of the patients were receiving standard DMARD treatment, with 61% of patients treated by monotherapy and 37% by combination therapy (i.e. ≥2 DMARDs). The majority (83%) of patients were prescribed MTX (dose range: 10–25 mg). Seven patients were receiving low dose corticosteroid therapy (dose range: 2.5–10.0 mg); a dosage not thought to affect LM (Da Silva et al., 2006). No patients were on biological (e.g., anti-cytokine) therapy. Self-reported physical function (i.e. MDHAQ) in the RA group was 0.6 (defined as 'mildly disabled' by Bruce & Fries, 2005). There was a significantly greater number of current (*P* <.001) and ex-smokers (*P* = .016) in the RA group.

Table 3.1. Participant demographics for rheumatoid arthritis patients and sedentary, age- and sex-matched health controls

	RA (*n* = 82)	HC (*n* = 85)	*P*
Age (years)	60.9 (±11.7)	60.9 (±8.1)	.962
Sex (*n* female) (%)	53 (65)	55 (65)	.992
Height (cm)	165.1 (±7.9)	168.1 (±8.6)	**.019***
Disease duration (months)	23.8 (±19.0)		
Rheumatoid factor positive; *n* (%)	46 (56)		
SPRA; *n* (%)	67 (85)		
DAS28 (0-10)	2.8 (±1.0)		
Systolic blood pressure (mmHg)	127.5 (±15.1)	120.5 (±9.5)	**.004***
Diastolic blood pressure (mmHg)	72.6 (±9.9)	70.9 (±6.9)	.304
Medications, *n* (%)			
NSAIDS	22 (27)	5 (6)	**<.001***
MTX [a]	68 (83)		
SSZ	5 (6)		
LFM	7 (9)		
HCQ	26 (32)		
TAC	3 (4)		
MYF	1 (1)		
Mono DMARD therapy	50 (61)		
Combo DMARDs (double or triple) [b]	30 (37)		
No current DMARDs	2 (2)		
Current corticosteroid use [c]	7 (9)	1 (1)	**.026***
Blood pressure medications	21 (26)	11 (13)	**.031***
Cholesterol medications	18 (22)	7 (8)	**.013***
Analgesics	35 (43)	3 (4)	**<.001***
Calcium supplements	11 (13)	3 (4)	**.021***
Smoking status, *n* (%)			
Current smokers	18 (22)	3 (5)	**<.001***
Ex-smokers	39 (48)	25 (31)	**.016***
Never smokers	25 (30)	52 (61)	**<.001***
Exercise frequency [d], *n* (%)			
Do not exercise	40 (50)	7 (8)	**<.001***
1-2 times a month	6 (8)	7 (8)	.825
1-2 times a week	11 (14)	27 (32)	**.005***
3+ times a week	20 (25)	41 (49)	**.001***
Cannot exercise due to disability	3 (4)	2 (2)	.621

Unless stated, data presented as mean (±SD). Differences at baseline were assessed using analyses of variance, or Chi-square test as appropriate. RA = Rheumatoid arthritis; HC = Healthy control group; SPRA = Sero-positive RA; DAS28 = Disease activity score in 28 joints; NSAIDS = Non-steroidal anti-inflammatory drugs; MTX = Methotrexate; SSZ = Sulfasalazine; LFM = Leflunomide; HCQ = hydroxychloroquine; TAC = Tacrolimus; MYF =

Myfenax; DMARDs = Disease modifying anti-rheumatic drugs. [a] = Additional folate supplement; [b] = Combination therapy (i.e. two or more DMARDs); [c] = Current corticosteroid range 5.0–10.0 mg; [d] = self-reported exercise frequency taken from Multi-dimensional Health Assessment Questionnaire (MDHAQ) (not reported: RA = 2, HC = 1); * significant ($P < .05$).

3.3.1.2. Anthropometry and body composition

Despite being shorter (3.0 cm, $P = .019$, $\eta^2 = .06$ (small), **Table 3.1.**), RA patients were considerably heavier (BM: +4.8 kg, $P = .093$, $\eta^2 = .03$ (small), **Table 3.2.**), which resulted in a significantly higher BMI (+2.6 kg/m^2, $P = .002$, $\eta^2 = .07$ (small)). Patients with RA had greater waist circumference (+7.7 cm, $P = .001$, $\eta^2 = .07$ (small)), and subsequently a higher waist: hip ratio ($P < .001$, $\eta^2 = .08$ (medium)), than HCs. Body composition assessed by DXA revealed that, when adjusted for BM (i.e. % of), RA patients had 10% proportionally less muscle (ALM%, $P < .001$, $\eta^2 = .10$ (medium)) than HCs (**Table 3.2.** and **Figure 3.1.**). Compared to HCs, patients with RA also exhibited lower levels of absolute (kg) ALM (-1.1 kg) and total LM (-0.8 kg), although statistically these differences were non-significant (*P's* = .158 ($\eta^2 = .01$, small), and .578 ($\eta^2 = .00$), respectively).

The RA group had considerably greater FM than the HC group (+5.4 kg; 27%, $P < .001$, $\eta^2 = .09$ (medium)) with the majority (3.2 kg, 32%, $P = .001$, $\eta^2 = .08$ (medium)) of this FM distributed on the trunk. Body fat % was also higher in the RA patients ($P < .001$, $\eta^2 = .08$ (medium)). Overall, almost a half of RA patients were obese[6] (49%; $n = 40/82$), compared to just 15% ($n = 13/85$) in the HC group ($P < .001$). When taking into account participants who were also overweight, 74% ($n = 61$) of RA patients could be classified as either overweight or obese, compared to 46% ($n = 39$, $P < .001$) of the HCs.

3.3.1.3. Strength and objective physical function

Patients with RA were much poorer than matched, sedentary HCs for each of the objective measures of strength and physical function (**Table 3.3.**): IKES was 24% less ($P < .001$, $\eta^2 = .08$ (medium)); HGS was 25% less ($P < .001$, $\eta^2 = .13$ (medium)); STS-30 was 34% poorer ($P < .001$, $\eta^2 = .17$ (medium)); 8'UG was 31% slower ($P < .001$, $\eta^2 = .20$ (medium)); and 50'W was 28% slower ($P < .001$, $\eta^2 = .22$ (medium)). Estimated relative VO_2max (ml/kg/min) from the Siconolfi step test revealed that RA patients had an 11% reduced aerobic capacity ($P = .017$, $\eta^2 = .04$ (small)) compared to the HCs. Due to self-reported joint pain, problems with balance,

[6] Based on definitions from Baumgartner et al. (1998): obesity was defined as ≥27% in males, and ≥38% in females. Overweight was defined as ≥24% in males, and ≥31% in females. Note: obesity was also calculated in accordance to the adjusted BMI cut-offs proposed by Stavropoulos-Kalinoglou et al. (2007), that is, BMI cut-off points should be reduced by 2 kg/m^2 (23 kg/m^2 for overweight and 28 kg/m^2 for obesity). When using this definition, there was absolutely no difference in obesity prevalence (i.e. it remained 49% in the RA group) supporting the validity of these cut-offs in RA.

or effects of mediations (i.e. β-blockers), 20 RA patients (24%) were unable to complete (or attempt) the test, compared to just 4 HCs (5%).

Table 3.2. Body composition measures for rheumatoid arthritis patients and sedentary, age- and sex-matched health controls

	RA (*n* = 82)	HC (*n* = 85)	Absolute difference (CI) (*% difference*)	*P*	η^2
Waist circ. (cm)	91.6 (±17.9)	83.9 (±10.8)	↑ **7.7 (3.2–12.2)** ***(8)***	**.001***	**.07**
Hip circ. (cm)	101.9 (±12.7)	99.1 (±7.8)	↑ 2.8 (-0.4–6.1) *(3)*	.128	.02
Waist: hip ratio	0.90 (±0.10)	0.85 (±0.08)	↑ **0.05 (0.03–0.08)** ***(6)***	**<.001***	**.08**
BM (kg)	76.5 (±17.9)	71.7 (±11.1)	↑ 4.8 (0.2–9.3) *(6)*	.093[#]	.03
BMI (kg/m^2)	28.0 (±6.0)	25.4 (±3.4)	↑ **2.6 (1.2–4.1)** ***(9)***	**.002***	**.07**
DXA-assessed body composition					
ALM (kg)	19.8 (±4.6)	20.9 (±5.2)	↓ 1.1 (-0.4–2.6) *(6)*	.158	.01
ALM% (ALM/BM%)	26.2 (±4.0)	28.8 (±4.2)	↓ **2.6 (1.4–3.9)** ***(10)***	**<.001***	**.10**
Total LM (kg)	48.7 (±9.8)	49.5 (±10.0)	↓ 0.8 (-2.2–3.9) *(2)*	.578	.00
Total LM% (LM/BM%)	64.4 (±7.5)	68.6 (±6.8)	↓ **4.2 (1.9–6.3)** ***(7)***	**<.001***	**.08**
Total FM (kg)	25.8 (±10.4)	20.4 (±6.2)	↑ **5.4 (2.7–7.9)** ***(27)***	**<.001***	**.09**
BF (%)	32.7 (±7.8)	28.3 (±7.2)	↑ **4.4 (2.1–6.7)** ***(16)***	**<.001***	**.08**
Trunk FM (kg)	13.1 (±6.3)	9.9 (±3.7)	↑ **3.2 (1.6–4.8)** ***(32)***	**.001***	**.08**
Trunk FM% (trFM/FM%)	49.4 (±7.3)	47.8 (±7.7)	↑ 1.6 (-0.6–4.0) *(3)*	.146	.01

Data presented as mean (±SD). CI = 95% confidence interval; RA = Rheumatoid arthritis; HC = Healthy control group; BM = Total body mass (scales); BMI = Body mass index; DXA = Dual x-ray absorptiometry; ALM = Appendicular lean mass; LM = Lean mass; FM = Fat mass; BF = Body fat; unless adjusted by Bonferroni adjustment: * significant ($P < .05$); [#] trend ($P \geq .05$–.10). Effect size (η^2): small ≥.01; medium ≥.08; large ≥.26; very large ≥.50.

3.3.1.4. Subjective measures of disability and health

As expected, patients with RA had a higher MDHAQ scores ($P = .001$, $\eta^2 = .30$ (large)), and ~75% higher general pain ($P = .010$, $\eta^2 = .30$ (large)) and fatigue ($P = .004$, $\eta^2 = .30$ (large)) scores than the HC group. Joint pain (RADAI) was also higher ($P < .001$, $\eta^2 = .29$ (large)) in the RA group. Additionally, RA patients reported 30% poorer physical ($P < .001$, $\eta^2 = .36$ (large)) and 9% mental components ($P = .003$, $\eta^2 = .03$ (small)) of the SF-36 questionnaire compared to controls (**Table 3.3.**).

Table 3.3. Objective physical function and self-reported disability for rheumatoid arthritis patients and sedentary, age- and sex-matched health controls

	RA (*n* = 82)	HC (*n* = 85)	Absolute difference (CI) (*% difference*)	*P*	η^2
Objective function measures					
IKES (N)	380 (±140)	472 (±152)	↓ **92 (46–138)** ***(24)***	**<.001***	**.08**
HGS (kg)	26.5 (±8.8)	33.2 (±9.9)	↓ **6.7 (3.8–9.7)** ***(25)***	**<.001***	**.13**
STS-30 test (reps)	12.0 (±3.6)	16.1 (±4.3)	↓ **4.1 (2.8–5.3)** ***(34)***	**<.001***	**.17**
8'UG (secs)	7.4 (±3.9)	5.1 (±1.0)	↑ **2.3 (1.4–3.1)** ***(31)***	**<.001***	**.20**
50'W (secs)	10.7 (±5.3)	7.7 (±1.8)	↑ **3.0 (1.8–4.3)** ***(28)***	**<.001***	**.22**
Estimated VO_2max § (ml/kg/min)	22.9 (±6.2)	25.3 (±6.4)	↓ **2.4 (0.3–4.5)** ***(11)***	**.017***	**.04**
Subjective measures of disability and health					
MDHAQ score (/3)	0.6 (±0.5)	0.1 (±0.2)	↑ **0.5 (0.4–0.6)** ***(83)***	**.001***	**.30**
MDHAQ pain (/10)	3.6 (±2.5)	0.9 (±1.4)	↑ **2.7 (2.0–3.3)** ***(75)***	**.010***	**.30**
MDHAQ fatigue (/10)	3.7 (±3.1)	0.9 (±2.1)	↑ **2.8 (2.0–3.7)** ***(76)***	**.004***	**.30**
RADAI (/48)	8.1 (±6.9)	2.1 (±3.7)	↑ **6.0 (4.4–7.7)** ***(74)***	**<.001***	**.29**
SF-36 (physical) (/100)	42.5 (±10.2)	55.3 (±7.6)	↓ **12.8 (10.0–15.5)** ***(30)***	**<.001***	**.36**
SF-36 (mental) (/100)	45.2 (±10.6)	49.3 (±6.9)	↓ **4.1 (1.3–6.8)** ***(9)***	**.003***	**.03**

Data presented as mean (±SD). CI = 95% confidence interval; RA = Rheumatoid arthritis; HC = Healthy control group; IKES = Isometric knee extensor strength; HGS = Handgrip strength; STS-30 = Sit-to-stand in 30 second test; 8'UG = 8-foot up and go; 50'W = 50-foot walk; VO_2max = Estimated VO_2max from Siconolfi step test (§ data only for RA = 62/82 (*n* = 20 unable to complete), HC = 81/85 (*n* = 4 unable to complete)); MDHAQ = Multi-Dimensional Health Assessment Questionnaire; RADAI = Rheumatoid Arthritis Disease Activity Index; SF-36 = Short-form 36 questionnaire; unless adjusted by Bonferroni adjustment: * significant (*P* < .05). Effect size (η^2): small ≥.01; medium ≥.08; large ≥.26; very large ≥.50.

Table 3.4. Correlation matrix between strength and physical function in rheumatoid arthritis patients

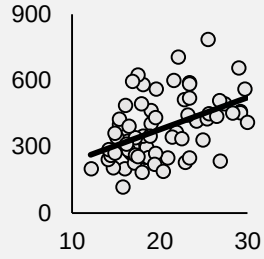
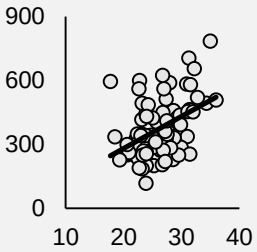
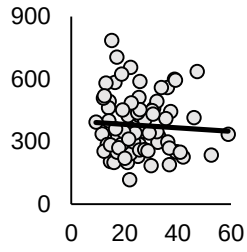
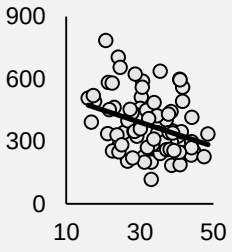
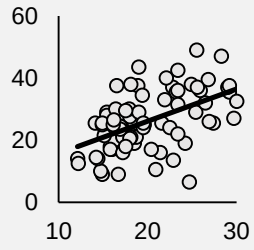
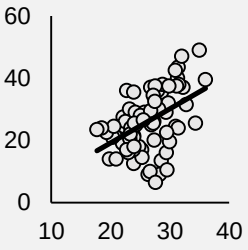
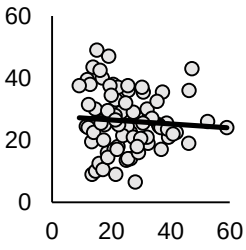
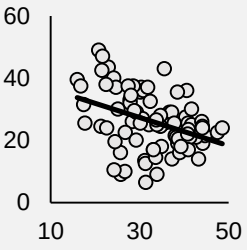
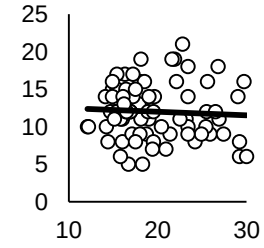
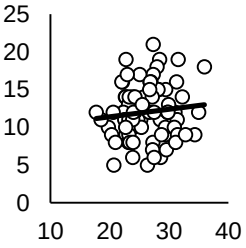
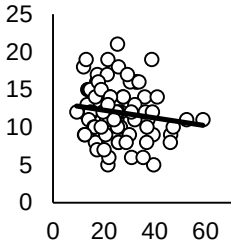
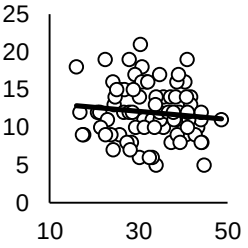
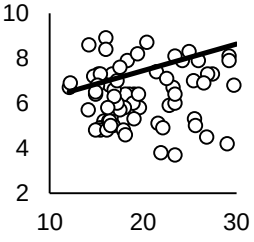
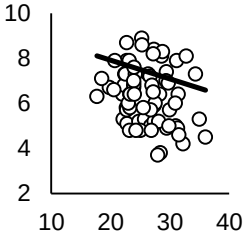
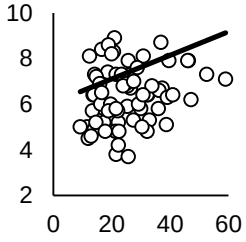
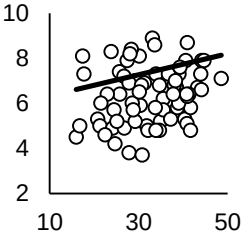
	STS-30 (reps)	8'UG (secs)	50'W (secs)	VO_2max (ml/kg/min)
IKES (N)	***r* = .378,** ***P* = .001***	***r* = -.456,** ***P* < .001***	***r* = -.553,** ***P* < .001***	***r* = .289,** ***P* = .022***
HGS (kg)	***r* = .228,** ***P* = .043***	***r* = -.388,** ***P* < .001***	***r* = -.363,** ***P* = .001***	***r* = .515,** ***P* < .001***

Pearson-product correlation (bivariate) (*r*). IKES = Isometric knee extensor strength; HGS = Handgrip strength; STS-30 = Sit-to-stand in 30 second test; 8'UG = 8-foot up and go; 50'W = 50-foot walk; VO_2max = Estimated VO_{2MAX} from Siconolfi step test. * significant (*P* < .05).

3.3.1.5. Correlational analysis

Pearson correlational analysis was used to investigate the relationships between muscle (i.e. ALM and ALM%) and fat (total FM and BF%) and the objective measures of physical function (**Figure 3.1.**). In the RA group, ALM and ALM% were positively correlated with strength measures: IKES (*r* = .480, *P* <.001; *r* = .437, *P* <.001, respectively), and HGS (*r* = .518, *P* <.001; *r* = .472, *P* <.001, respectively), but not STS-30, 8'UG, or 50'W performance. Only ALM% was correlated with VO_2max (*r* = .477, *P* <.001). Total FM was only correlated (negatively) with VO_2max (*r* = -.405, *P* = .001), and BF% correlated (negatively) with IKES (*r* = -.329, *P* = .003) and HGS (*r* = -.379, *P* <.001). Neither fat measure was significantly correlated with STS-30, 8'UG, or 50'W performance. In the RA group, strength (IKES and HGS) were highly significantly correlated with all the other measures of physical function (STS-30, 8'UG, 50'W, VO_2max) (**Table 3.4.**). Interestingly, both STS-30 (*r* = -.263, *P* = .044) and 8'UG (*r* = .349, *P* = .007), but not 50'W (*r* = .168, *P* = .203), became significant when IKES was used a co-variant (**Figure 3.1.**, values marked with ϫ represent this partial correlation).

In the HC participants, ALM and ALM% were positively correlated with strength measures (IKES; *r* = .684, *P* <.001; *r* = .596, *P* <.001, respectively, and HGS; *r* = .843, *P* <.001; *r* = .787, *P* <.001, respectively), as well as VO_2max (*r* = .309, *P* = .005; *r* = .613, *P* <.001, respectively) but not with any of the function tests (STS-30, 8'UG, 50'W). Total FM and BF% were negatively correlated with IKES (*r* = -.256, *P* =.019; *r* = -.544, *P* <.001, respectively), HGS (*r* = -.352, *P* = .001; *r* = -.695, *P* <.001, respectively), STS-30 (*r* = -373, *P* =.001; *r* = -.349, *P* = .001, respectively), and VO_2max (*r* = -.628, *P* <.001; *r* = -.659, *P* <.001, respectively), but not with 8'UG or 50'W performance.

	ALM (kg)	ALM%	FM (kg)	BF%
IKES (N)				
	r* = .480, *P* <.001	***r* = .437, *P* <.001***	*r* = -.063, *P* = .583	***r* = -.329, *P* = .003***
HGS (kg)				
	r* = .518, *P* <.001	***r* = .472, *P* <.001***	*r* = -0.71, *P* = .526	***r* = -.379, *P* <.001***
STS-30 (reps)				
	r = -.061, *P* = .596	*r* = .108, *P* = .347	*r* = -.149, *P* = .190	*r* = -.113, *P* = .320
	χr = -.263, P = .044*	*χr* = -.139, *P* = .295	*χr* = -.051, *P* = .701	*χr* = .064, *P* = .631
8'UG (secs)				
	r = .138, *P* = .222	*r* = -.085, *P* = .456	*r* = .140, *P* = .212	*r* = .094, *P* = .402
	χr = .349, P = .007*	*χr* = .105, *P* = .428	*χr* = .113, *P* = .393	*χr* = -.064, *P* = .629

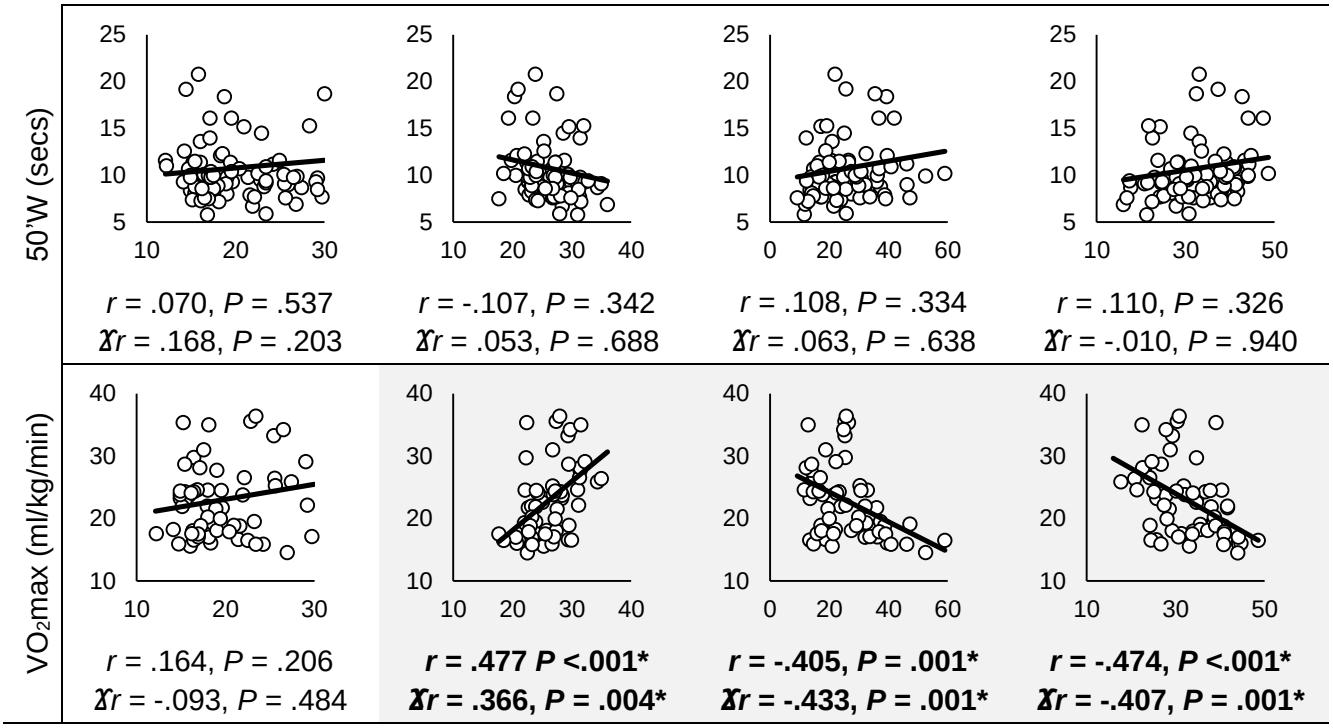


Figure 3.1. Correlation matrix for body composition and physical function in rheumatoid arthritis patients. Pearson-product correlation (*r*) matrix with R^2 linear trend line. ALM = Appendicular lean mass; FM = Total fat mass; IKES = Isometric knee extensor strength; HGS = Handgrip strength; STS-30 = Sit-to-stand in 30 second test; 8'UG = 8-foot up and go; 50'W = 50-foot walk; VO_2max = Estimated VO_2max from Siconolfi step test; χ = adjusted *r* and *P* values when IKES strength is used a co-variant in a Partial correlational analysis. * significant ($P \leq .05$); # trend ($P > .05–.10$).

3.3.2. Sub-analysis: 'Recent-onset' versus 'established' cohorts

3.3.2.1 Descriptive data and participants

Table 3.5. displays the demographic and clinical characteristics of the RA patients in regard to their disease duration ('recent-onset' versus 'established'). There were no significant group differences in gender (*P* = .531) or height (*P* = .452) between the two groups. Although not significant, the 'recent-onset' patients were 4.2 years older than the 'established' patients (*P* = .106).

From a disease perspective, there was no difference in the proportion of sero-positive RA (SPRA) patients (*P* = .407) between the groups. Overall, disease activity was typically 'low' for both patient cohorts, although the 'recent-onset' patients had a marginally higher mean DAS28 score (3.0; 42% were 'in remission'), than the 'established' patients (DAS28 of 2.7; 53% in remission, *P* = .345 for the group difference in achieving 'remission'. Nearly all (94%)

of the ‘recent-onset' patients were prescribed MTX, compared to 76% in the ‘established’ group (*P* = .030). Nearly half of the ‘recent onset’ patients (48%) were receiving combination therapy compared to 29% of the ‘established’ group (*P* = .066).

Table 3.5. Participant demographics ‘recent-onset’ (<12 months) and ‘established’ (1–7 years) rheumatoid arthritis patients

	‘Recent-onset’ (*n* = 33)	‘Established’ (*n* = 49)	*P*
Age (years)	63.4 (±12.0)	59.2 (±11.3)	.106
Sex (*n* female) (%)	20 (61)	33 (67)	.531
Height (cm)	165.9 (±8.4)	164.5 (±7.6)	.452
Disease duration (months)	7.1 (±3.0)	34.7 (±17.0)	**>.001***
Rheumatoid factor positive; *n* (%)	19 (58)	27 (55)	.601
SPRA; *n* (%)	25 (76)	42 (86)	.407
DAS28 (0–10)	3.0 (±1.1)	2.7 (±0.9)	.275
No. in remission (DAS28 <2.6)	14 (42)	26 (53)	.345
C-reactive protein (mg/L)	11.9 (±15.3)	9.2 (±8.9)	.305
Medications, n (%)			
NSAIDS	8 (24)	14 (29)	.664
MTX [a]	31 (94)	37 (76)	**.030***
SSZ	2 (6)	3 (6)	.991
LFM	2 (6)	5 (10)	.510
HCQ	13 (39)	13 (27)	.220
TAC	0 (0)	3 (6)	.148
MYF	0 (0)	1 (2)	.409
Mono DMARD therapy	16 (48)	34 (69)	.057[#]
Combo DMARDs (double or triple) [b]	16 (48)	14 (29)	.066[#]
No current DMARDs	1 (3)	1 (7)	.776
Current corticosteroids [c]	2 (6)	5 (10)	.573
Blood pressure medications	9 (27)	12 (25)	.862
Cholesterol medications	4 (12)	14 (29)	.078[#]
Analgesics	12 (36)	23 (47)	.342
Calcium supplements	5 (15)	6 (12)	.705
Smoking status, n (%)			
Current smokers	6 (18)	12 (24)	.499
Ex-smokers	15 (45)	24 (49)	.754
Never smokers	12 (36)	13 (27)	.343

Exercise frequency [d], *n* (%)			
Do not exercise	16 (50)	24 (50)	.965
1-2 times a month	2 (6)	4 (8)	.720
1-2 times a week	4 (13)	7 (15)	.778
3+ times a week	9 (28)	11 (23)	.618
Cannot exercise due to disability	1 (3)	2 (4)	.804

Unless stated, data presented as mean (±SD). Differences at baseline were assessed using analyses of variance, or Chi-square test as appropriate. SPRA = Sero-positive RA; DAS28 = Disease activity score in 28 joints; NSAIDS = Non-steroidal anti-inflammatory drugs; MTX = Methotrexate; SSZ = Sulfasalazine; LFM = Leflunomide; HCQ = hydroxychloroquine; TAC = Tacrolimus; MYF = Myfenax; DMARDs = Disease modifying anti-rheumatic drugs. [a] = Additional folate supplement; [b] = Combination therapy (two or more DMARDs); [c] = Current corticosteroid range 5.0–10.0 mg; [d] = self-reported exercise frequency taken from Multi-dimensional Health Assessment Questionnaire (MDHAQ) (not reported: recent = 1, established = 1). * significant ($P < .05$); [#] trend ($P \geq .05$–.10).

3.3.2.2. Anthropometry and body composition

There was no differences in DXA-assessed body composition measures between 'recent-onset' and 'established' patients (P's = .747–.998, all η^2 values = .00; **Table 3.6.**). There was also no difference between waist (P = .654, η^2 = .00) and hip (P = .960, η^2 = .00) circumferences, or waist: hip ratio (P = .726, η^2 = .00).

Table 3.6. Body composition measures for 'recent-onset' (<12 months) and 'established' (1-7 years) rheumatoid arthritis patients

	'Recent-onset' (n = 33)	'Established' (n = 49)	Absolute difference (CI) (*% difference*)	P	η^2
Waist circ. (cm)	92.7 (±16.3)	90.9 (±19.0)	1.8 (-6.2–9.9) *(2)*	.654	.00
Hip circ. (cm)	101.8 (±11.3)	102.0 (±13.7)	0.1 (-5.9–5.6) *(0)*	.960	.00
Waist: hip ratio	0.91 (±0.10)	0.90 (±0.11)	0.01 (-0.04–0.06) *(1)*	.726	.00
BM (kg)	77.2 (±19.5)	76.0 (±17.0)	1.2 (-9.2–6.9) *(2)*	.777	.00
BMI (kg/m^2)	27.9 ±(5.7)	28.1 (±6.3)	0.3 (-2.4–3.0) *(1)*	.838	.00
DXA-assessed body composition					
ALM (kg)	19.8 (±4.6)	19.8 (±4.7)	0.0 (-2.2–2.0) *(0)*	.951	.00
ALM% (ALM/BM%)	26.2 (±4.0)	26.1 (±4.0)	0.0 (-1.8–1.8) *(0)*	.970	.00
Total LM (kg)	49.6 (±11.1)	48.8 (±10.0)	0.8 (-3.9–5.5) *(2)*	.747	.00
Total LM% (LM/BM%)	64.4 (±7.2)	64.4 (±7.7)	0.0 (-3.3–3.4) *(0)*	.998	.00
Total FM (kg)	26.0 (±10.5)	25.6 (±10.4)	0.4 (-4.3–5.1) *(2)*	.878	.00
BF (%)	32.6 (±7.6)	32.7 (±8.1)	0.1 (-3.5–3.6) *(0)*	.965	.00
Trunk FM (kg)	13.3 (±6.7)	12.9 (±6.0)	0.4 (-2.5–3.2) *(3)*	.943	.00
Trunk FM% (trFM/FM%)	49.3 (±8.0)	49.5 (±6.9)	0.2 (-3.5–3.2) *(0)*	.927	.00

Data presented as mean (±SD). CI = 95% confidence interval; RA = Rheumatoid arthritis; BM = Total body mass (scales); BMI = Body mass index; DXA = Dual x-ray absorptiometry; ALM = Appendicular lean mass; LM = Lean mass; FM = Fat mass; BF = Body fat; trFM = Trunk fat mass. Effect size (η^2): small ≥.01; medium ≥.08; large ≥.26; very large ≥.50.

3.3.2.3. Strength and objective physical function

There were no differences between the 'recent-onset' and 'established' patients for any of the objective measures of strength or physical function (*P's* = .435–.778, η^2 = .00–.01 (no effect to small); **Table 3.7.**).

Table 3.7. Objective physical function and self-reported disability for 'recent-onset' (<12 months) and 'established' (1-7 years) rheumatoid arthritis patients

	'Recent-onset' (*n* = 33)	'Established' (*n* = 49)	Absolute difference (CI) (*% difference*)	*P*	η^2
Objective measures					
IKES (N)	385 (±146)	376 (±137)	9 (-56–75) *(2)*	.778	.00
HGS (kg)	25.9 (±10.3)	26.8 (±7.7)	0.9 (-5.0–3.2) *(4)*	.669	.01
STS-30 test (reps)	11.7 (±4.0)	12.2 (±3.3)	0.5 (-2.2–1.2) *(4)*	.546	.01
8'UG (secs)	7.0 (±2.3)	7.7 (±4.7)	0.6 (-1.1–2.4) *(9)*	.685	.00
50'W (secs)	10.0 (±2.5)	11.2 (±6.5)	1.2 (-1.2–3.5) *(12)*	.435	.00
Estimated VO_2max (ml/kg/min)	22.7 (±6.5)	23.0 (±6.0)	0.4 (-2.8–3.6) *(2)*	.745	.01
Subjective measures					
MDHAQ score (/3)	0.5 (±0.4)	0.6 (±0.6)	0.1 (-0.1–0.3) *(20)*	.088#	.01
MDHAQ pain (/10)	3.5 (±2.5)	3.6 (±2.6)	0.2 (-1.4–1.0) *(6)*	.130	.00
MDHAQ fatigue (/10)	3.8 (±3.1)	3.6 (±3.0)	0.2 (-1.2–1.6) *(5)*	.446	.01
RADAI (/48)	7.7 (±4.8)	8.4 (±8.0)	0.7 (-2.3–3.8) *(9)*	.634	.01
SF-36 (physical) (/100)	44.7 (±7.5)	41.1 (±11.5)	3.6 (-1.0–8.2) *(8)*	.065#	.03
SF-36 (mental) (/100)	44.4 (±11.0)	45.8 (±10.4)	1.4 (-6.2–3.4) *(3)*	.586	.00

Data presented as mean (±SD). CI = 95% confidence interval; RA = Rheumatoid arthritis; IKES = Isometric knee extensor strength; HGS = Handgrip strength; STS-30 = Sit-to-stand in 30 second test; 8'UG = 8-foot up and go; 50'W = 50-foot walk; VO_2max = Estimated VO_2max from Siconolfi step test; MDHAQ = Multi-dimensional Health Assessment Questionnaire; RADAI = Rheumatoid Arthritis Disease Activity Index; SF-36 = Short-form 36 questionnaire; unless adjusted by Bonferroni adjustment: * significant (*P* < .05); # trend (*P* ≥ .05–.10). Effect size (η^2): small ≥.01; medium ≥.08; large ≥.26; very large ≥.50.

3.3.2.4. Subjective measures of disability and health

There were no significant differences in any subjective measure of health and disability (**Table 3.7.**) including the MDHAQ (*P* = .088, η^2 = .00 (small)). There were no differences in the amount of general pain (*P* = .130, η^2 = .00), fatigue (*P* = .446, η^2 = .001 (small)), or in the mental (*P* = .586, η^2 = .00) component of the SF-36 questionnaire, although the difference of 3.6 in the physical component approached significance (*P* = .065, η^2 = .03 (small)).

3.3.2.5. Relative cardiovascular risk and lipid profile

Table 3.8. shows the lipid profiles of the two RA sub-groups, along with their relative cardiovascular risk (as determined by the QRISK2). There were no differences in fasting glucose (*P* = .290, η^2 = .01 (small)), LDL-C (*P* = .892, η^2 = .00), TG (*P* = .175, η^2 = .08 (medium)), or TC levels (*P* = .803, η^2 = .00). However, the 'recent-onset' group had a marginally higher HDL-C level (*P* = .017, η^2 = .05 (small)) and TC: HDL-C ratio (*P* = .030, η^2 = .08 (medium)). Patients with 'established' disease did tend to take a greater number of prescribed cholesterol-controlling medications (i.e. statins) (29% versus 12%, *P* = .078).

Whilst there were no group differences in 10-year QRISK2 score (*P* = .164, η^2 = .08 (medium)), relative risk (*P* = .675, η^2 = .01 (small)), or estimated heart age (*P* = .237, η^2 = .06 (medium)), 52% of the 'recent-onset' patients were classified as being at 'high risk' (≥20% probability) of having a cardiovascular event within 10 years, compared to 37% of the 'established' group (*P* = .024). Overall, 43% of RA patients were classed as being at 'high risk', 18% at 'moderate risk' (≥10–20% probability), and 39% at low risk (≤10% probability) of a suffering a cardiovascular event.

Table 3.8. Cardiovascular risk and lipid profile for 'recent-onset' (<12 months) and 'established' (1-7 years) patients

	'Recent-onset' (*n* = 33)	'Established' (*n* = 49)	Absolute difference (CI) (*% difference*)	*P*	η^2
Lipid profile (normal range)					
Glucose (mmol/L) (3.9–5.0)	5.1 (±0.8)	4.9 (±0.8)	0.2 (-0.2–0.6) *(4)*	.290	.01
HDL-C (mmol/L) (≥1.2)	1.6 (±0.5)	1.4 (±0.3)	**0.2 (0.0–0.4) *(13)***	**.017***	**.05**
LDL-C (mmol/L) (≤3.0)	3.0 (±0.9)	3.0 (±1.0)	0.0 (-0.5–0.4) *(0)*	.892	.00
TC:HDL-C ratio (≤4.5)	3.3 (±0.6)	3.8 (±1.1)	**0.5 (0.1–0.9) *(15)***	**.030***	**.08**
Cholesterol (mmol/L) (≤5.0)	5.1 (±1.2)	5.0 (±1.3)	0.1 (-0.7–0.6) *(2)*	.803	.00
Triglycerides (mmol/L) (≤1.5)	1.2 (±0.6)	1.4 (±0.7)	0.4 (-0.1–0.7) *(33)*	.175	.08
Cardiovascular risk profile					
Systolic blood pressure (mmHg)	128.1 (±14.3)	127.1 (±15.9)	1.0 (-6.1–8.1) *(1)*	.787	.00
Diastolic blood pressure (mmHg)	74.8 (±10.6)	71.1 (±9.3)	3.8 (-0.8–8.3) *(5)*	.106	.06
Hypertension; *n* (%)	7 (23)	14 (31)		.414	-
Diabetes; *n* (%)	2 (6)	1 (2)		.347	-
First degree relative CVD; *n* (%)	4 (12)	10 (20)		.296	-
Chronic kidney disease; *n* (%)	2 (6)	1 (2)		.347	-
Atrial fibrillation; *n* (%)	4 (12)	4 (8)		.586	-
History of CVD	3 (9)	4 (8)		.597	-
10-year QRISK2 score	21.2 (±15.5)	16.3 (±13.9)	4.9 (-2.1–11.9) *(23)*	.164†	.08
Typical person's risk score [a]	15.2 (±10.3)	10.8 (±9.8)	4.4 (0.4–9.2) *(29)*	.069#	.19
Relative risk [b]	1.7 (±1.3)	1.9 (±1.7)	0.2 (-0.6–0.9) *(12)*	.675	.01
QRISK2 heart age [c]	68.4 (±12.2)	64.9 (±12.6)	3.6 (-2.4–9.5) *(5)*	.237	.06
Difference from actual age (years)	5.2 (±7.0)	6.5 (±6.6)	1.3 (-1.9–4.5) *(25)*	.428	.00

Data presented as mean (±SD). CI = 95% confidence interval; RA = Rheumatoid arthritis; HDL = High-density lipoprotein - cholesterol; LDL-C = Low-density lipoprotein - cholesterol; TC = Triglycerides; CVD = Cardiovascular disease; [a] = taken from QRISK2, matched score for age, sex and ethnic group; [b] = patient risk divided by typical risk; [c] = age at which a typical person has the same 10-year QRISK2 score as patient; † = if age is a co-variant, $P = .781$: * significant ($P < .05$); [#] trend ($P \geq .05–.10$). Effect size (η^2): small ≥.01; medium ≥.08; large ≥.26; very large ≥.50.

3.3.3. Sub-analysis: ‘In remission’ versus ‘not in remission’

Table 3.9. shows the differences in demographics between RA patients ‘in remission’ (*n* = 40) (defined as a DAS28 score <2.6; mean DAS28 = 2.0 (±0.4)) and those ‘not in remission’ (*n* = 42) (mean DAS28 = 3.6 (±0.8), *P* <.001, η^2 = .62 (very large)). Those ‘in remission’ had lower inflammation (CRP, *P* = .024, η^2 = .06 (small)), and fewer tender (*P* <.001) and swollen joints (*P* = .002). Whilst there were no other meaningful differences, there were more males in the ‘in remission’ group (*P* = .187). To that end, in an ANCOVA with sex included as a co-variant, an adjusted significance value was calculated to remove this gender difference.

No significant differences in body composition were observed between those ‘in remission’ and those ‘not in remission’ (**Table 3.10.**). The majority of body composition measures were significantly different between the ‘in remission’ and HC groups, apart from BM (*P* = .397, η^2 = .00) and BMI (*P* = .084, η^2 = .03 (small)). Strength, and objective and subjective physical function scores were superior in the ‘in remission’ group compared to those ‘not in remission’ (*P*’s = .001–.057), with the exception of the STS-30 test (*P* = .459, η^2 = .00) (**Table 3.11.**). These differences remained constant even when sex was included as co-variant (*P’s* = .002–.052, η^2 = .00–.14). Apart from estimated VO_2max and the SF-36 mental score (*P’s* = .187 (η^2 = .02 (small)), and .647 (η^2 = .00), respectively), despite being ‘in remission’, patients were significantly poorer than the HC group (*P’s* < .001–.026).

3.3.4. Sub-analysis: Sex differences

Demographically, there appeared to be no differences between males and females (**Table 3.12.**). The difference in systolic blood pressure between female RA patients and female HCs was significant (*P* = .003), but no difference was observed between males (*P* = .351). The deficiency in ALM (-2.5 kg, a relative difference of 11%, *P* = .005) between RA males and HC males was significant; conversly, no difference was seen between female RA and HCs (-0.2 kg, 1%, *P* = .597). Further, whilst trunk FM% between female RA and HCs was significant (*P* = .032), the difference between male RA and HCs was not (*P* = .763) (**Table 3.13.**). Apart from VO_2max, which was significant between RA and HC females (*P* = .046) but not between males, *P* = .201, all other objective physical function measures between RA and HCs remained significantly different (**Table 3.14.**).

Table 3.9. Participant demographics for rheumatoid arthritis in 'remission' (DAS28 <2.6) or 'not in remission' (DAS28 ≥2.6)

	'In remission' versus 'Not in remission'			HC versus 'In remission'	
	'In remission' (*n* = 40)	*'Not* in remission' (*n* = 42)	*P*	HC (*n* = 85)	*P*
Age (years)	60.4 (±12.2)	61.4 (±11.3)	.706	60.9 (±8.1)	.764
Sex (*n* female) (%)	23 (58)	30 (71)	.187	55 (65)	.438
Height (cm)	166.0 (±8.2)	164.2 (±7.5)	.287	168.1 (±8.6)	.195
Disease duration (months)	23.1 (±17.5)	24.5 (±20.6)	.740		
Rheumatoid factor positive; *n* (%)	21 (53)	25 (60)	.395		
SPRA; *n* (%)	32 (80)	35 (83)	.886		
DAS28 (0-10)	2.0 (±0.4)	3.6 (±0.8)	**<.001***		
CRP (mg/L)	7.3 (±7.7)	13.1 (±14.4)	**.024***		
Systolic blood pressure (mmHg)	126.2 (±15.4)	128.9 (±15.0)	.444	120.5 (±9.5)	**.048***
Diastolic blood pressure (mmHg)	71.0 (±8.9)	74.3 (±10.7)	.149	70.9 (±6.9)	.985
Medications, *n* (%)					
NSAIDS	6 (15)	16 (38)	**.018***	5 (6)	.093[#]
MTX [a]	34 (85)	34 (81)	.626		
SSZ	3 (8)	2 (5)	.604		
LFM	3 (8)	4 (10)	.743		
HCQ	13 (33)	13 (31)	.880		
TAC	1 (3)	2 (5)	.586		
MYF	0 (0)	1 (2)	.326		
Biological agents	0 (0)	0 (0)	-		
Mono DMARD therapy	24 (60)	26 (62)	.860		

Combo DMARDs (double or triple) [b]	15 (38)	15 (36)	.867		
No current DMARDs	1 (3)	1 (2)	.972		
Current corticosteroid use [c]	3 (8)	4 (10)	.743	1 (1)	.061[#]
Blood pressure medications	5 (13)	16 (38)	**.005***	11 (13)	.945
Cholesterol medications	8 (20)	10 (24)	.677	7 (8)	.059[#]
Analgesics	14 (35)	21 (50)	.170	3 (4)	**<.001***
Calcium supplements	5 (13)	6 (14)	.813	3 (4)	.056[#]
Smoking status, *n* (%)					
Current smokers	7 (18)	11 (26)	.180	3 (5)	**.014***
Ex-smokers	19 (48)	20 (48)	.493	25 (31)	**.007***
Never smokers	14 (35)	11 (26)	.542	52 (61)	**.001***
Exercise frequency [d], *n* (%)					
Do not exercise	22 (55)	18 (45)	.272	7 (8)	**<.001***
1-2 times a month	4 (10)	2 (5)	.363	7 (8)	.745
1-2 times a week	4 (10)	7 (18)	.376	27 (32)	**.009***
3+ times a week	10 (25)	10 (25)	.900	41 (49)	**.014***
Cannot exercise due to disability	0 (0)	3 (8)	.085[#]	2 (2)	.328

Unless stated, data presented as mean (±SD). Differences at baseline were assessed using analyses of variance, or Chi-square test as appropriate. RA = Rheumatoid arthritis; SPRA = Sero-positive RA; DAS28 = Disease activity score in 28 joints; NSAIDS = Non-steroidal anti-inflammatory drugs; MTX = Methotrexate; SSZ = Sulfasalazine; LFM = Leflunomide; HCQ = hydroxychloroquine; TAC = Tacrolimus; MYF = Myfenax; DMARDs = Disease modifying anti-rheumatic drugs. [a] = Additional folate supplement; [b] = Combination therapy (i.e. two or more DMARDs); [c] = Current corticosteroid range 5.0–10.0 mg; [d] = self-reported exercise frequency taken from Multi-dimensional Health Assessment Questionnaire (MDHAQ) (not reported: not in remission = 2, HC = 1). * significant ($P < .05$); [#] trend ($P \geq .05$–.10).

Table 3.10. Body composition measures for rheumatoid arthritis in 'remission' (DAS28 <2.6) or 'not in remission' (DAS28 ≥2.6)

	'In remission' versus 'Not in remission'					HC versus 'In remission'		
	'In remission' (*n* = 40)	'Not in remission' (*n* = 42)	Absolute difference (CI)	*P*	*P*¥ (*η*²)	HC (*n* = 85)	Absolute difference (CI)	*P*¥ (*η*²)
Waist circ. (cm)	90.3 (±16.5)	92.9 (±19.2)	-2.6 (-10.5–5.3)	.514	.258 *(.01)*	83.9 (±10.8)	**-6.4 (-10.7– - 0.3)**	**.039* *(.04)***
Hip circ. (cm)	100.0 (±10.0)	103.8 (±14.7)	-3.9 (-9.4–1.7)	.169	.246 *(.02)*	99.1 (±7.8)	-0.9 (-5.1–2.9)	.592 *(.00)*
Waist: hip ratio	0.90 (±0.12)	0.90 (±0.09)	0.00 (-0.05–0.04)	.949	.139 *(.00)*	0.85 (±0.08)	**-0.05 (-0.07– -0.02)**	**<.001* *(.06)***
BM (kg)	74.9 (±17.7)	78.0 (±18.2)	-3.2 (-11.1–4.7)	.425	.183 *(.01)*	71.7 (±11.1)	-3.2 (-7.3–2.9)	.397 *(.00)*
BMI (kg/m^2)	27.0 (±5.1)	29.0 (±6.7)	-2.0 (-4.6–0.7)	.143	.133 *(.03)*	25.4 (±3.4)	-1.6 (-3.4–0.2)	.084# *(.03)*
DXA-assessed body composition								
ALM (kg)	19.7 (±4.6)	19.9 (±4.6)	-0.1 (-2.2–1.9)	.905	.148 *(.00)*	20.9 (±5.2)	**1.2 (0.6–2.8)**	**.003* *(.01)***
ALM% (ALM/BM%)	26.9 (±3.9)	25.5 (±3.9)	1.3 (-0.4–3.1)	.122	.347 *(.03)*	28.8 (±4.2)	**1.9 (1.2–3.5)**	**<.001* *(.05)***
Total LM (kg)	48.2 (±9.4)	49.2 (±10.3)	-1.0 (-5.4–3.4)	.650	.071# *(.00)*	49.5 (±10.0)	1.3 (-0.2–4.6)	.052# *(.00)*
TLM% (LM/BM%)	65.5 (±6.6)	63.3 (±8.0)	2.2 (-1.0–5.5)	.179	.458 *(.02)*	68.6 (±6.8)	**3.1 (1.5–5.8)**	**.001* *(.04)***
Total FM (kg)	24.2 (±9.2)	27.3 (±11.3)	-3.1 (-7.7–1.4)	.176	.241 *(.03)*	20.4 (±6.2)	**-3.8 (-7.1– -0.8)**	**.014* *(.04)***
BF (%)	31.5 (±7.0)	33.8 (±8.5)	-2.4 (-5.8–1.0)	.170	.434 *(.03)*	28.3 (±7.2)	**-3.2 (-6.1– -1.5)**	**.001* *(.04)***
Trunk FM (kg)	12.2 (±6.1)	13.9 (±6.4)	-1.6 (-4.4–1.1)	.242	.252 *(.03)*	9.9 (±3.7)	**-2.3 (-4.3– -0.4)**	**.017* *(.04)***
Trunk FM% (trFM/FM%)	49.0 (±7.9)	49.8 (±6.8)	-0.8 (-4.1–2.4)	.611	.281 *(.00)*	47.8 (±7.7)	-1.2 (-3.2–1.8)	.581 *(.00)*

Data presented as unadjusted mean (±SD). CI = 95% confidence interval; RA = Rheumatoid arthritis; HC = Healthy control group; BM = Total body mass (scales); BMI = Body mass index; DXA = Dual x-ray absorptiometry; ALM = Appendicular lean mass; LM = Lean mass; FM = Fat mass; BF = Body fat; unless adjusted by Bonferroni adjustment: * significant ($P < .05$); # trend ($P \geq .05$–$.10$); $P^{¥}$ = adjusted significance value when sex included as co-variant due to difference in proportion of male to females. Effect size (η^2): small ≥.01; medium ≥.08; large ≥.26; very large ≥.50.

Table 3.11. Objective physical function and self-reported disability for rheumatoid arthritis in 'remission' (DAS28 <2.6) or 'not in remission' (DAS28 ≥2.6)

	'In remission' versus 'Not in remission'					HC versus 'In remission'		
	'In remission' (*n* = 40)	*'Not* in remission' (*n* = 42)	Absolute difference (CI)	*P*	*P*¥ (*η*²)	HC (*n* = 85)	Absolute difference (CI)	*P*¥ (*η*²)
Objective function measures								
IKES (N)	414 (±141)	343 (±130)	**71 (10–132)**	**.023***	.052# *(.03)*	477 (±155)	**62 (26–117)**	**.002* *(.06)***
HGS (kg)	29.6 (±8.3)	22.9 (±9.3)	**6.6 (2.7–10.5)**	**.001***	**.002* *(.14)***	33.4 (±10.0)	**3.8 (2.4–7.4)**	**<.001* *(.12)***
STS-30 test (reps)	12.3 (±3.3)	11.7 (±3.9)	0.5 (-1.1–2.1)	.513	.459 *(.00)*	16.1 (±4.3)	**3.8 (2.3–5.3)**	**<.001* *(.16)***
8'UG (secs)	6.6 (±2.1)	8.2 (±4.9)	-1.6 (-3.3–0.1)	.057#	**.042* *(.02)***	5.1 (±1.0)	**-1.5 (-2.5– -0.4)**	**.008* *(.16)***
50'W (secs)	9.5 (±2.4)	11.9 (±6.8)	**-2.3 (-4.6– -0.1)**	**.042***	**.037* *(.01)***	7.7 (±1.8)	**-1.8 (-3.3– -0.4)**	**.014* *(.18)***
VO_2max §(ml/kg/min)	23.8 (±5.4)	21.8 (±7.0)	2.0 (-1.1–5.2)	.199	.368 *(.02)*	25.3 (±6.4)	1.5 (-0.8–4.0)	.187 *(.02)*
Subjective measures of disability and health								
MDHAQ score (/3)	0.3 (±0.3)	0.8 (±0.6)	**-0.5 (-0.7– -0.3)**	**<.001***	**<.001* *(.22)***	0.1 (±0.2)	**-0.2 (-0.4– -0.1)**	**.001* *(.17)***
MDHAQ pain (/10)	2.4 (±2.2)	4.6 (±2.3)	**-2.2 (-3.2– -1.2)**	**<.001***	**<.001* *(.21)***	0.9 (±1.4)	**-1.5 (-2.2– -0.8)**	**<.001* *(.14)***
MDHAQ fatigue (/10)	2.3 (±2.6)	5.1 (±3.0)	**-2.8 (-4.0– -1.5)**	**<.001***	**<.001* *(.10)***	0.9 (±2.1)	**-1.4 (-2.4– -0.5)**	**.002* *(.20)***
RADAI (/48)	4.2 (±4.1)	11.8 (±6.9)	**-7.6 (-10.1– -5.1)**	**<.001***	**<.001* *(.35)***	2.1 (±3.7)	**-2.1 (-3.9– -0.2)**	**.026* *(.06)***
SF-36 (physical) (/100)	46.1 (±9.2)	39.2 (±10.0)	**6.9 (2.6–11.1)**	**.002***	**.002* *(.11)***	55.3 (±7.6)	**9.2 (5.9–12.5)**	**<.001* *(.22)***
SF-36 (mental) (/100)	48.6 (±7.7)	42.0 (±12.0)	**6.5 (2.3–11.0)**	**.005***	**.007* *(.09)***	49.3 (±6.9)	0.7 (-2.5–4.1)	.647 *(.00)*

Data presented as unadjusted mean (±SD). CI = 95% confidence interval; RA = Rheumatoid arthritis; HC = Healthy control group; IKES = Isometric knee extensor strength; HGS = Handgrip strength; STS-30 = Sit-to-stand in 30 second test; 8'UG = 8-foot up and go; 50'W = 50-foot walk; VO_2max = Estimated VO_2max from Siconolfi step test (§ data only for RA = 62/82 (*n* = 20 (*n* = 7 in 'remission'; *n* = 13 not in 'remission', *P* = .156) unable to complete), HC = 81/85 (*n* = 4 unable to complete)); MDHAQ = Multi-dimensional Health Assessment Questionnaire; RADAI = Rheumatoid Arthritis Disease Activity Index; SF-36 = Short-form 36 questionnaire; unless adjusted by Bonferroni adjustment: * significant (*P* < .05); # trend (*P* ≥ .05–.10); *P*¥ = adjusted significance value when sex included as co-variant due to difference in proportion of male to females. Effect size (η^2): small ≥.01; medium ≥.08; large ≥.26; very large ≥.50.

Table 3.12. Participant demographics for male and female rheumatoid arthritis patients and sedentary, age- and sex-matched healthy controls

	Male			Female		
	RA (*n* = 29)	HC (*n* = 30)	*P*	RA (*n* = 53)	HC (*n* = 55)	*P* (η^2)
Age (years)	65.0 (±7.8)	63.8 (±9.3)	.593	58.6 (±12.9)	59.4 (±6.9)	.695
Height (cm)	172.9 (±6.0)	176.3 (±5.4)	**.026***	160.8 (±4.9)	163.6 (±6.3)	**.011***
Disease duration (months)	22.7 (±18.3)			24.4 (±19.5)		
Rheumatoid factor positive; *n* (%)	18 (62)			28 (53)		
SPRA; *n* (%)	23 (79)			44 (83)		
DAS28 (0-10)	2.7 (±1.2)			2.9 (±0.9)		
Systolic blood pressure (mmHg)	127.9 (±16.2)	124.1 (±8.1)	.351	127.3 (±14.8)	118.1 (±9.8)	**.003***
Diastolic blood pressure (mmHg)	72.5 (±8.4)	73.8 (±6.5)	.591	72.7 (±10.7)	69.1 (±6.7)	.109
Medications, n (%)						
NSAIDS	10 (34)	2 (7)	**.008***	12 (23)	3 (5)	**.010***
Corticosteroids [a]	5 (17)	0 (0)	**.024***	2 (4)	1 (2)	.536
Blood pressure medications	5 (17)	4 (13)	.676	16 (30)	7 (13)	**.020***
Cholesterol medications	8 (28)	5 (17)	.312	10 (19)	2 (4)	**.012***
Analgesics	12 (41)	0 (0)	**<.001***	21 (40)	3 (5)	**<.001***
Calcium supplements	7 (24)	0 (0)	**.004***	3 (6)	3 (5)	.659
Smoking status, n (%)						
Current smokers	3 (10)	2 (7)	.612	15 (28)	1 (2)	**<.001***
Ex-smokers	20 (69)	13 (43)	**.047***	19 (36)	12 (22)	.107
Never smokers	6 (21)	15 (50)	**.019***	19 (36)	42 (76)	**<.001***

Exercise frequency [b], *n* (%)						
Do not exercise	14	2	**<.001***	26	5	**<.001***
1-2 times a month	3	2	.612	3	5	.496
1-2 times a week	3	13	**.004***	8	14	.181
3+ times a week	8	13	.207	12	28	**.002***
Cannot exercise due to disability	1	0	.305	2	2	.970

Unless stated, data presented as mean (±SD). Differences at baseline were assessed using analyses of variance, or Chi-square test as appropriate. RA = Rheumatoid arthritis; HC = Healthy control group; SPRA = Sero-positive RA; DAS28 = Disease activity score in 28 joints; NSAIDS = Non-steroidal anti-inflammatory drugs; MTX = Methotrexate; SSZ = Sulfasalazine; LFM = Leflunomide; DMARDs = Disease modifying anti-rheumatic drugs. [a] = corticosteroid range 5.0–10.0 mg; [b] = self-reported exercise frequency taken from Multi-dimensional Health Assessment Questionnaire (MDHAQ) (not reported: RA female = 2, HC female = 1) * significant ($P < .05$).

Table 3.13. Body composition measures between male and females in rheumatoid arthritis patients and sedentary, age- and sex-matched healthy controls

	Male			Female		
	RA (*n* = 29)	HC (*n* = 30)	Absolute difference[a] (CI) *(% difference)*[b]	RA (*n* = 53)	HC (*n* = 55)	Absolute difference[a] (CI) *(% difference)*[b]
Waist circ. (cm)	99.2 (±13.7)	91.0 (±9.7)	**8.2 (2.0–14.4) *(8)****	87.5 (±18.6)	80.1 (±9.4)	**7.4 (1.7–13.0) *(8)****
Hip circ. (cm)	98.8 (±9.1)	98.3 (±6.2)	0.5 (-3.6–4.6) *(1)*	103.6 (±14.1)	99.5 (±8.6)	4.1 (-0.3–8.6) *(4)*[#]
Waist: hip ratio	1.00 (±0.07)	0.92 ±(0.06)	**0.08 (0.04–0.11) *(8)*****	0.85 (±0.07)	0.80 (±0.05)	**0.04 (0.02–0.07) *(5)****
BM (kg)	84.7 (±17.1)	80.1 (±9.3)	4.6 (-2.5–11.7) *(6)*	72.0 (±16.9)	67.1 (±9.2)	4.9 (-0.3–10.0) *(7)*[#]
BMI (kg/m^2)	28.2 (±4.8)	25.8 (±2.9)	**2.4 (0.4–4.5) *(9)****	27.9 (±6.7)	25.1 (±3.6)	**2.8 (0.8–4.8) *(11)****
DXA-assessed body composition						
ALM (kg)	24.4 (±3.6)	27.0 (±2.9)	**-2.6 (-4.3– -0.8) *(11)****	17.4 (±2.9)	17.6 (±2.3)	-0.2 (-1.3–0.7) *(1)*
ALM% (ALM/BM%)	29.5 (±3.0)	33.0 (±2.9)	**-3.5 (-5.1– -2.0) *(12)*****	24.4 (±3.2)	26.6 (±2.8)	**-2.2 (-3.3– -1.0) *(9)*****
Total LM (kg)	58.1 (±7.7)	61.2 (±5.9)	**-3.1 (-6.6–0.6) *(5)*[#]**	43.7 (±6.7)	43.2 (±4.5)	0.5 (-1.6–2.7) *(1)*
Total LM% (LM/BM%)	70.0 (±5.9)	74.8 (±4.7)	**-4.8 (-7.6– -2.1) *(7)****	61.3 (±6.4)	65.2 (±5.2)	**-3.9 (-6.0– -1.6) *(6)****
Total FM (kg)	23.6 (±9.6)	18.2 (±5.5)	**5.4 (1.4–9.5) *(30)****	26.9 (±10.7)	21.6 (±6.2)	**5.3 (2.0–8.6) *(25)****
BF (%)	26.9 (±6.2)	21.9 (±5.0)	**5.0 (2.0–7.9) *(23)****	35.8 (±6.8)	31.8 (±5.6)	**4.0 (1.7–6.4) *(13)****
Trunk FM (kg)	12.9 (±6.3)	9.9 (±3.7)	**3.0 (0.3–5.6) *(30)****	13.2 (±6.3)	9.8 (±3.7)	**3.4 (1.4–5.3) *(35)****
Trunk FM% (trFM/FM%)	53.1 (±6.5)	53.6 (±6.0)	-0.5 (-3.8–2.8) *(1)*	47.4 (±7.0)	44.6 (±6.6)	**2.8 (0.3–5.4) *(6)****

Data presented as mean (±SD). CI = 95% confidence interval for absolute difference; RA = Rheumatoid arthritis; HC = Healthy control group; BM = Total body mass (scales); BMI = Body mass index; DXA = Dual x-ray absorptiometry; ALM = Appendicular lean mass; LM = Lean mass; FM = Fat mass; BF = Body fat; [a] = between group (RA versus HC) difference; [b] = relative percentage (%) difference; ** = significant (P <.001); * significant (P < .05); [#] trend (P ≥ .05–.10).

Table 3.14. Objective physical function and self-reported disability between male and females in rheumatoid arthritis patients and sedentary, age- and sex-matched healthy controls

	Male			Female		
	RA (*n* = 29)	HC (*n* = 30)	Absolute difference[a] (CI) *(% difference)*[b]	RA (*n* = 53)	HC (*n* = 55)	Absolute difference [a] (CI) *(% difference)*[b]
Objective physical function						
IKES (N)	474 (±131)	610 (±136)	**-136 (-207– -66) *(29)*****	328 (±116)	402 (±108)	**-74 (-118– -31) *(23)****
HGS (kg)	34.0 (±9.6)	44.4 (±7.2)	**-10.4 (-14.8– -6.0) *(31)*****	21.9 (±5.8)	27.4 (±4.8)	**-5.5 (-7.6– -3.5) *(25)*****
STS-30 test (reps)	11.7 (±4.2)	16.6 (±5.0)	**-4.8 (-7.3– -2.4) *(41)*****	12.1 (±3.3)	15.8 (±3.9)	**-3.7 (-5.1– -2.3) *(31)*****
8'UG (secs)	7.8 (±5.6)	5.2 (±1.1)	**2.6 (0.5–4.7) *(33)****	7.2 (±2.6)	5.1 (±0.9)	**2.1 (1.4–2.9) *(29)*****
50'W (secs)	11.0 (±7.9)	7.5 (±1.9)	**3.5 (0.6–6.5) *(32)****	10.6 (±3.1)	9.2 (±2.9)	**2.8 (1.8–3.7) *(26)*****
Estimated VO_2max § (ml/kg/min)	26.0 (±7.0)	28.3 (±6.8)	-2.4 (-6.4–1.7) *(9)*	21.4 (±5.2)	23.6 (±5.4)	**-2.2 (-4.4–0.0) *(10)****
Subjective measures of disability and health						
MDHAQ score (/3)	0.6 (±0.6)	0.0 (±0.2)	**0.6 (0.4–0.8) *(100)*****	0.5 (±0.5)	0.1 (±0.3)	**0.4 (0.3–0.6) *(80)*****
MDHAQ pain (/10)	3.9 (±2.5)	0.5 (±0.9)	**3.4 (2.4–4.4) *(87)*****	3.4 (±2.5)	1.1 (±1.6)	**2.2 (1.4–3.0) *(65)*****
MDHAQ fatigue (/10)	3.7 (±2.9)	0.5 (±1.5)	**3.2 (2.0–4.4) *(86)*****	3.7 (±3.2)	1.1 (±2.4)	**2.6 (1.5–3.7) *(70)*****
RADAI (/48)	9.7 (±7.9)	1.3 (±2.2)	**8.4 (5.4–11.4) *(81)*****	7.3 (±6.1)	2.5 (±4.2)	**4.8 (2.8–6.8) *(66)*****
SF-36 (physical) (/100)	42.0 (±10.6)	56.7 (±6.6)	**-14.7 (-19.3– -10.1) *(35)*****	42.8 (±10.0)	54.4 (±8.0)	**-11.6 (-15.1– -8.1) *(27)*****
SF-36 (mental) (/100)	46.8 (±9.2)	49.7 (±7.0)	-2.9 (-7.2–1.4) *(6)*	44.4 (±11.2)	49.1 (±6.8)	**-4.7 (-8.3– -1.1) *(11)****

Data presented as mean (±SD). CI = 95% confidence interval for absolute difference; RA = Rheumatoid arthritis; HC = Healthy control group; IKES = Isometric knee extensor strength; HGS = Handgrip strength; STS-30 = Sit-to-stand in 30 second test; 8'UG = 8-foot up and go; 50'W = 50-foot walk; VO_2max = Estimated VO_2max from Siconolfi step test (§ data only for RA = 62/82 (*n* = 20 unable to complete: male (9), female (11)), HC = 81/85 (*n* = 3 unable to complete: male (1), female (3)); MDHAQ = Multi-Dimensional Health Assessment Questionnaire; RADAI = Rheumatoid Arthritis Disease Activity Index; SF-36 = Short-form 36 questionnaire; [a] = between group (RA versus HC) difference; [b] = relative percentage (%) difference; ** = significant (*P* <.001); * significant (*P* < .50); [#] trend (*P* ≥ .50–.10).

3.4. Discussion

3.4.1. Summary of key results

Despite successful treatment of disease activity, RA patients treated exclusively with T2T demonstrated substantial deficits in muscle mass (~10%) and significantly greater levels of FM (~27%) when compared to age- and sex -matched sedentary HCs, along with functional and strength impairments in the region of ~24–34%. Both these incidences are extremely similar to those previously reported. Additionally, there were no differences in any measure of either body composition or physical function between patients with 'recent-onset' or 'established disease' indicating changes occur early in the course of the disease. There was no difference in body composition between patients whom had achieved clinical 'remission' and those 'not in remission'. Whilst 'remission' patients had superior physical function than those 'not in remission', it remained significantly poorer than the HC group.

3.4.2. Interpretation of results

A key goal of contemporary RA treatment (i.e. T2T) is achieving LDA (usually defined as DAS28 <3.2), preferably 'clinical remission' (usually defined as a DAS28 score <2.6; Smolen et al., 2010a, 2010b, 2015). In our cohort, disease activity was seemingly well controlled (mean DAS28 score was 2.8) with almost half (49%) of patients achieving 'remission'. This success in controlling disease activity, due to early treatment instigation and 'tight control' of disease, supports evidence of the superior efficacy of current treatment approaches such as T2T (Bakker et al., 2007; Verstappen et al., 2007; Schipper et al., 2010; Schoels et al., 2010; Jurgens et al., 2012; Ruderman et al., 2012; Stoffer et al., 2015).

However, despite these improvements in clinical markers of disease, abnormal body composition was still evident with a relative loss of muscle mass (adjusted for BM) of 10% and an increased in total FM of 27% relative to age- and sex-matched sedentary HCs. The loss of muscle in patients exclusively treated by T2T strategies is similar to that reported for patients treated by previous, less effective strategies i.e. 8–15% compared to non-RA controls (e.g., Roubenoff et al., 1994; Roubenoff et al., 2002; Walsmith & Roubenoff, 2002; Toussirot et al.,

2005; Giles et al., 2008b; Book et al., 2009, 2011; Matschke et al., 2010a, 2010b; Dao et al., 2011). Remarkably, the 10% deficiency in muscle mass observed here is comparable to the 13% deficiency originally reported by Roubenoff et al. (1994) some 20 years previously. Further, as with our current findings, total FM (and trunk FM) values have previously been reported to be ~20% greater in RA patients relative to HCs (Giles et al., 2008b; Book et al., 2009; Elkan et al., 2009; Matschke et al., 2010b; Dao et al., 2011; Santos et al., 2011).

In our cohort, the deficiency in muscle (i.e. ALM) between RA males and HC males (a relative difference of 11%) appeared greater than the deficiency in female RA versus female HCs (1%). This supports the findings of Baker et al. (2015) but not those of others (Giles et al., 2008b; Book et al., 2009), and may occur as female patients have less LM to lose. Similar to Book et al. (2009) and Giles et al (2008b), we found female RA patients, relative to HCs, had greater trunk adiposity % than male patients with RA.

Despite 'mild' self-reported disability (an MDHAQ score of 0.6) and LDA, objectively-assessed physical function was greatly impaired in RA patients compared to the HC group. This included 24–25% deficits in strength (IKES and HGS), and even larger relative reductions in the STS-30 (34%), 8'UG (31%), and 50'W (28%) tests. Remarkably, like body composition, these deficits in performance are identical or, in the case of the 8'UG, actually worse than those previously observed by our group (Marcora et al., 2005a, 2005b, 2006; Lemmey et al., 2009, 2012; Matschke et al., 2010a, 2010b, 2013). When compared to matched-HC function, Lemmey et al. (2009) found IKES (25%), number of chair stands (STS-30) (30%), and walking time (50'W) (17%) were reduced compared to a population normative values (Rikli & Jones, 2012); whilst Matschke et al. (2010a, 2010b, 2013), using the same tests, showed when compared to matched HCs, RA patients demonstrated a 11–12% reduction in the STS-30 test, 17% slower times in the 8'UG and 25–26% slower 50'W times.

In 2013, Rikli and Jones published 'minimal fitness standards' compatible with living independently until late in life using a battery of five objective tests (including the STS-30 and 8'UG). Comparing the scores observed in our RA patients to the values provided by Rikli and Jones further empathises just how poor the physical function of our patients is. In our RA cohort, the females (mean age 58.6 years) achieved a STS-30 score appropriate for healthy 'moderate functioning' females aged 80–84 years, and the RA males (mean age 65.0 years) a score in line with healthy 'moderate functioning' males of 85–89 years. For the 8'UG test, the respective equivalents were 85–89 years for the females, whilst the males actually failed to achieve the standard of 90–94 year old healthy males. Hence on average, both the female and male RA patients had the function of healthy sex-matched individuals ~25 years older.

As well as poor physical function, patients in the current study also displayed poor levels of aerobic capacity (estimated VO_2max = 22.9 ml/kg/min), which was 11% worse than the HC group mean. Worryingly, this value may actually be an overestimation of the whole RA population as it only represents the more functionally able three quarters of the RA group as 20 patients could not perform or complete the test. Furthermore, such low aerobic fitness (i.e. a VO_2max <28 ml/kg/min (Kodama et al., 2009)) is associated with substantially higher rates of all-cause mortality and CVD risk in healthy individuals (Franklin & McCullough, 2009) and RA patients (Metsios et al., 2015).

A primary goal of current treatment for RA (Smolen et al., 2010a; 2015) is 'normalisation' of function. Since our successfully treated RA patients objective function is considerably aberrant relative to sedentary matched HCs, then this aspiration of 'normalising' physical function is some way off being realised, and in this regard, current treatment approaches are failing. Body composition (i.e. LM and FM) has a key role in a patient's strength and objective physical function (Giles et al., 2008a, Kramer et al., 2012; Lusa et al., 2015), therefore, it is unsurprising that without any improvements in body composition as a result of T2T, that physical function is still reduced. Consequently, the identification and promotion of potential adjunct anabolic treatment interventions in a clinical setting, such as exercise (Marcora et al., 2005a; Lemmey et al., 2009) and nutritional supplements (Marcora et al., 2005b), that improve body composition and restore strength and physical function should be an important aspect of RA care and management.

Whilst loss of LM has been identified as a key contributor to the disability and impaired physical functioning (Walsmith & Roubenoff, 2002; Giles et al., 2008a, 2008b; Chen et al., 2011; Dao et al., 2011; Kramer et al., 2012; Lusa et al., 2015) seen in RA. In our RA patients, a greater level of muscle mass (i.e. both relative (ALM%) and absolute ALM) was positively correlated with increased strength. Interestingly, muscle mass was not associated with performance of any of the other objective functional tests (i.e. STS-30, 8'UG, 50'W). However, because muscle mass is highly correlated with muscle strength (Landers et al., 2001; Marcora et al., 2005a; Baker et al., 2014), the relationship between muscle mass and physical function that is often cited could simply be a mediating function of muscle strength (Visser et al., 2005). Whether low muscle mass itself contributes to decline in physical function, or whether this association is mediated by muscle strength remains unclear (Visser et al., 2005). In our study, the correlation between LM and both STS-30 and 8'UG became significant when lower limb strength (i.e. IKES) was used a co-variant.

This finding supports previous research, in SLE (Andrews et al., 2015) and the elderly (Visser et al., 2005; Manini & Clark, 2012), that weakness (i.e. low muscle strength) is a key predictor of disability. Consequently, interventions aimed at increasing muscular *strength* could be employed above those that focus on muscle *mass*. For example, in the promotion of exercise, a 'strength protocol' of heavy weight (80–100% of maximum) and low reps (1–3 reps) (Campos et al., 2002) could be used to emphasise strength gains over muscle hypertrophy. However, as muscle mass is significantly associated with strength, increasing muscle mass could also improve physical function by increasing strength.

The excess adiposity observed in our patients is also likely to have a negative influence on their physical function (Giles et al., 2008a; Stavropoulos-Kalinoglou et al., 2009; Rolland et al., 2009; Kramer et al., 2012). The 5.4 kg of additional FM (essentially 'dead weight') found in the RA patients, compared to the matched HCs, could have increased the load faced by the limited muscle mass (Rolland et al., 2009) (i.e. they lacked appropriate muscle mass for their BM), along with reducing the range of motion of the limbs (Giles et al., 2008a). In our trial, higher FM and BF% were significantly associated with poorer strength (IKES, HGS) and aerobic capacity. Like muscle, FM was not correlated with the performance of the other function tests. It appears a 'synergistic relationship' between muscle mass, FM, weakness, and impaired physical function exists, supporting evidence that individuals with both conditions (i.e. sarcopenic-obesity) are more disabled that those with either condition alone (Baumgartner, 2000; Morley et al., 2001; Baumgartner et al., 2004; Dufour et al., 2012).

Whilst there were no significant differences in body composition between the groups (i.e. 'in remission' versus 'not'), RA patients 'in remission' (i.e. lower inflammation, tender, and swollen joints) generally had superior physical function measures (both objective and subjectively measured) than those 'not in remission'. However, it is unclear whether better response to treatment is the reason for these patients 'in remission' having better function. Although the cross-sectional nature of the current study is unable to ascertain changes in body composition and physical function, it may be that as a result of milder disease, the function of the 'remission' patients has been relatively better than the 'not in remission' patients throughout the respective courses of disease. Further research is needed to assess longitudinal changes across disease duration.

Nonetheless, apart from aerobic capacity in the 'in remission' group, body composition and physical function still remained considerably poorer than those of the HCs which makes clear that successful suppression of disease activity alone, even when 'clinical remission' is attained, does not reverse RC or normalise physical function.

It is important to state that the majority of patients in our cohort were treated using MTX monotherapy, in contrast with the more 'aggressive' combination DMARD or biologic therapies often advocated in current treatment guidelines. Although this may appear atypical on appearance, our patients were successfully controlled with a relatively simple and conservative treatment strategy (i.e. MTX monotherapy). This may infer that only patients with the mildest, most responsive, and best controlled disease agreed to take part. If this was the case, then our RA participants were at the higher end of functional capacity and the large deficits in function we observed constitute an underestimation of the disability of the broader RA population.

3.4.3. Differences in 'recent-onset' versus 'established' rheumatoid arthritis

There were no differences in disease activity and systemic inflammation between the 'recent-onset' and 'established' RA groups, indicating that treatment was successfully able to attain early control of the disease. Although there was no difference in the dosage, the proportion of 'recent-onset' patients (48%) on a combination of DMARDs (generally MTX plus HCQ) was higher than for the 'established' group (29%). This follows both the NICE and ACR guidelines which state that in patients with newly diagnosed RA, a combination of DMARDs should be used. No patients in either group were being treated by biological agents, seemingly as good disease control had generally been achieved by initial DMARD monotherapy.

No disparities were seen between the two RA groups in any of the anthropometric, body composition, or physical function measures. Notably, this suggests that even prompt control of disease activity (i.e. within <12 months of diagnosis) by contemporary treatment regimens (42% of the 'recent-onset' group were in remission) does not arrest RC or the resultant loss in objective-physical function. As suggested by both Book et al. (2009) and Marcora et al. (2006), these results support the idea that the perturbations in body composition (i.e. RC) occur early, certainly within 12 months, but conceivably prior to diagnosis and initiation of treatment in the 'pre-clinical' stage of RA (Gerlag et al., 2012). Pertinently, inflammation (Kraan et al., 1998; Van de Sande et al., 2011) and elevated levels of cytokines such as TNF-α, IL-1β, and IL-6 (Sokolove et al., 2012) have been observed pre-RA diagnosis; these pro-inflammatory cytokines are thought to be the principle drivers of RC ((Roubenoff et al., 1994; Walsmith et al., 2004; Engvall et al., 2008; Giles et al., 2010)). If body composition changes do occur in

the ‘pre-clinical’ stage of RA, this explains why even prompt and successful control of disease and inflammation is unable to prevent RC.

Interestingly, the ‘recent-onset’ group had an 8% (relative to ‘established’; absolute difference = 3.6%) greater physical component score from the SF-36. This difference, albeit not significant, can be classified as a minimally clinically significant difference (≥2.5%) (Strand & Singh, 2008). Greater self-reported physical function in early disease, followed by a subsequent decline as disease duration increases, is not an uncommon finding (e.g., Welsing et al., 2001; Hallert et al., 2003; Book et al., 2011) and has been attributed to the recent adaptation of treatment (Welsing et al., 2011), and early overestimation of functional ability (Drossaers-Baker et al., 1999). Patients with ‘established’ disease may have a better understanding of their physical limitations.

Both the ‘recent-onset’ and ‘established’ RA patient groups displayed normal and well-controlled levels of HDL-C, TG, and TC: HDL-C ratio. High levels (i.e. ≥ normal range) of TC and LDL-C were found in both groups indicating better clinical control of ‘bad cholesterol’ (e.g., LDL-C) is needed in these patients. The ‘recent-onset’ patients had significantly higher levels of HDL-C, a lower TC: HDL-C ratio, and tended to have lower (albeit, non-significantly) levels of TG, than the ‘established’ patients. In RA, systemic inflammation leads to pro-atherogenic changes of the lipoprotein metabolism (Nurmohamed, 2007; Bag-Ozbek & Giles, 2015) making lipid profiles often difficult to interpret in clinical practice. Further, the literature surrounding these changes is contradictory, reporting either increased, decreased, or similar levels for TC, LDL-C and HDL-C in comparison to HCs (for review, see Nurmohamed, 2007).

Further, the association between lipid measures and the risk of CVD in RA appears to be paradoxical, whereby lower levels of TC, LDL-C, and atherogenic ratios are actually associated with higher CVD risk (Kitas & Gabriel, 2011; Myasoedova et al., 2011; Bag-Ozbek & Giles, 2015). This may be due to the lipid-lowering effects of RA-related systemic inflammation (Kotler, 2000; Nurmohamed, 2007; Popa et al., 2007; Kitas & Gabriel, 2011; Myasoedova et al., 2011; Bag-Ozbek & Giles, 2015). Successful immunotherapy in RA (i.e. controlling disease activity and reducing inflammation) has been shown to increase these serum lipid values (Myasoedova et al., 2011; Bag-Ozbek & Giles, 2015). In our results, interestingly, it was the ‘recent-onset’ group only who displayed characteristics of the ‘lipid paradox’ (i.e. a lower CVD risk (via QRISK2 score) was correlated with higher levels of TC (r = -.467, P = .016), and LDL-C (r = -.517, P = .007)). In the ‘established’ group, higher inflammation (CRP) was associated with higher CVD risk (i.e. QRISK2 score) (r = .560, P < .001).

Additionally, the role of HDL-C in RA in particular is difficult to interpret. Normally, HDL-C exerts an athero-protective, anti-atherogenic, anti-inflammatory effect. For example, protecting LDL from oxidation and preventing atherosclerosis (Ansell et al., 2004). However in RA, and in the presence of inflammation, changes in HDL-C composition, including displacement of the anti-atherogenic, anti-inflammatory components apolipoprotein A1 (ApoA-1) and paraoxonase 1 (PON-1) by pro-inflammatory proteins such as serum amyloid A (SAA) and complement component 3, may exert pro-atherogenic, pro-inflammatory effects that can increase atherosclerotic and CVD risk (Van Lenten et al., 2006; Eren et al., 2013; Bag-Ozbek & Giles, 2015). Therefore, the levels of HDL-C in our cohort should be viewed with caution.

Despite no differences in gender, smoking, hypertension, and obesity, the 'recent-onset' group trended towards a higher CVD risk (i.e. QRISK2 score). This could be an artefact of the age-difference observed between the two groups (4.2 years). Indeed, when age was included as a co-variant in an ANCOVA, there was no significant difference between the groups 10 year QRISK2 score (adjusted means; recent: 18.6 (±1.9) versus established: 18.0 (±1.6), $P = .781$); i.e. age explained approximately 80% of the pre-adjusted difference[7]. Overall, the mean 10 year QRISK2 score of all the RA patients was 19%, with 43% of patients classified as 'high' risk. This 1.9 times increased CVD risk seen in our patients is similar to that widely stated for RA patients (e.g., Solomon et al., 2003; Boyer, et al., 2011; Kitas & Gabriel, 2011; Humphreys et al., 2014; Bag-Ozbek & Giles, 2015).

3.4.4. Study strengths and limitations

The study benefits from having well matched RA patient and HC cohorts for comparison, and the use of DXA is considered the 'gold-standard' for body composition assessment in research (Provyn, et al., 2008). Furthermore, we investigated and favoured the use of objectively assessed physical function, over subjective measures, such as the HAQ, which has previously been used to assess favourable effects of RA treatments on functional capacity (Sakellariou et al., 2013; Seto et al., 2013; Vermeer et al., 2013; Solomon et al., 2014; Sugihara et al.,

[7] In the QRISK2, the relative effect of age is amplified as it used to multiply BMI, systolic blood pressure, family history, smoking, hypertension, type 2 diabetes and atrial fibrillation. Using the QRISK2 algorithm, a difference of 4 years (59 to 63) can equate to 4% change in QRISK2 estimated 10 year risk of CVD (manual manipulation of calculation using the following criteria representative of our sample: female with RA, white, light smoker, no diabetes, no chronic kidney disease, no atrial fibrillation, current blood pressure treatment, TC: HDL-C ratio of 3.5, systolic blood pressure of 125 mmHg, height of 165.0 cm, weight of 75.0 kg). This 4% change equates to 80% of the 5% difference in QRISK2 scores.

2015). Although the HAQ (and its variations e.g., MDHAQ) can be valuable in measuring the initial self-reported functional improvements following treatment initiation in uncontrolled RA (Young et al., 2000, Welsing et al., 2001, Hallert et al., 2003; Kingsley et al., 2011; Marcora et al., 2006), in patients with stable, well-controlled disease activity, the HAQ can be an insensitive measure of functional change (e.g., Van den Ende et al., 1997; Lemmey et al., 2009). The lack of association between the HAQ and objective function in patients with controlled disease may occur due to a 'ceiling effect'. In patients who can already perform some of the basic functional tasks described the HAQ without any difficulty (e.g., '1b. Get in and out of bed' or '1c. Lift a cup or glass to your mouth'), then no matter how effective an intervention in improving strength or objective functional measures is (e.g., exercise), the ability to perform these tasks remains the same. Therefore, the HAQ may not reflect 'true' physical capacity (Arvidson et al., 2002).

Indeed, in our study, RA patients reported 'mild' functional disability (via the MDHAQ) despite having objectively measured physical function significantly inferior than the HCs (i.e. an equivalent function of healthy sex-matched individuals ~25 years older). Further, despite the 'recent-onset' RA group self-reporting an 8% greater SF-36 physical component and a 20% greater MDHAQ score (both trends), no differences were observed in objective functional measures. Whilst the importance of measuring function (albeit, self-reported by HAQ) in RA management has been recognised by both NICE and ACR, it seems that an objective measure of physical function would be the most valid means of assessment (Arvidson et al., 2002). Currently, the outcome metric for evaluating the efficacy of RA treatment is usually a composite measure of disease activity (e.g., DAS28, Simple Disease Activity Index (SDAI), or Combined Disease Assessment (CDA) index), but none of these measures includes any objective test of function, and consequently none is accounting for patient disability using the most valid assessment tools.

The principle limitation to the study is its cross-sectional design, preventing identification of causality. Whilst the pathogenesis of RA (i.e. inflammation) has been associated with RC (Roubenoff et al., 1994, 2002), other factors may also contribute to the aberrant body composition and physical function observed. Whilst the RA patients in the current study were less sedentary than the HC, the between-group difference only amounted to ~30 minutes of 'exercise' per week, and both groups fell well short of the minimum recommendation for long-term loss of FM of 250 minutes per week of moderate intensity physical activity (PA) advocated by the American College of Sports Medicine (2010). This ~30 minute disparity in low-moderate intensity PA is unlikely to account for the difference in muscle mass, as higher-intensity exercise is required to elicit hypertrophy (e.g., Marcora et al., 2005; Lemmey et al., 2009). In

our trial, RA patients reported more analgesia and fatigue than the HCs; both of which can conceivably result in poorer physical function.

We acknowledge that RA treatment is a constantly evolving process and as such, no single 'modern/current era' of treatment may exist. However, by recruiting patients from within the last 7 years (post 1/1/2008), we feel we were able to assess a group of patients diagnosed and treated using the most contemporary treatment regimens (i.e. early recognition of disease, prompt and aggressive treatment when required, and 'tight control' of disease through a T2T approach to ensure attainment of LDA or 'remission'). A limitation of the present study is the lack of quantitative assessment (using the Smolen et al. (2010a) guidelines) of whether patients were treated by a T2T approach (i.e. adherence to T2T). However overall, our trial highlights that this approach, despite being successful in bringing about LDA, and in about half of patients, 'remission', does not reverse RC.

An additional limitation was our lack of an a-priori power (sample size) calculation. Although we did not achieve our initial target of 100 RA patients, a post hoc calculation using measures of relative ALM% revealed adequate power had been achieved (d = 0.63, 1-β error probability = 0.98).

3.5. Conclusion

This study has shown that RA patients only exposed to contemporary, enhanced treatment remain significantly deficient in muscle mass, fatter, particularly around the trunk, and functionally much poorer than sedentary, age- and sex-matched HCs. Thus, although current treatment, observing the principles of 'tight control' and T2T, is successful in the control of inflammation and disease activity, it has failed to preserve body composition or physical function any more than previous treatment regimes. A primary ACR, EULAR, and NICE goal of current treatment for RA is normalisation of function. Since our successfully treated RA patients objective function is considerably aberrant relative to sedentary matched HCs, then this aspiration of normalising physical function is some way off being realised, and in this regard, current treatment approaches are failing.

Consequently, the identification and promotion of potential adjunct anabolic treatment interventions, such as exercise (specifically PRT) and nutritional supplements that restore

body composition (not just increase LM, but also reduce FM), strength, and physical function still remain an important aspect of future RA care and research. Further, assessment of objective physical function should feature or be, at least, considered when making clinical decisions. Objective physical function could be used as a 'target' in the evaluation of treatment therapy; in addition to assessing composite measures of disease activity and structural changes, and ideally body composition.

We observed no differences between patients with 'recent-onset' and 'established' disease duration suggesting that RC occurs early in the disease process, possibly before symptom presentation. These 'pre-clinical' changes to body composition may explain why even successful and prompt control of disease in the present does not prevent RC (i.e. it has already taken place). Hence, effective adjunct anabolic treatments need to be advocated, and hopefully initiated, at diagnosis of RA.

4

Oral creatine supplementation: a potential adjunct intervention for rheumatoid arthritis patients. A review

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4.1. Introduction

Patients with rheumatoid arthritis (RA) often experience a substantial loss of muscle mass ('rheumatoid cachexia' (RC)) (Roubenoff et al., 1992; Walsmith & Roubenoff, 2002), which results in significant adverse consequences such as decreased strength (Van Bokhorst-de van der Schueren et al., 2012), impaired physical function (Giles et al., 2008a; Summers et al., 2008), and a reduction in quality of life (Giles et al., 2008a). Unfortunately, current pharameutical treatments for RA do not attenuate this muscle loss, nor fully restore physical function (Marcora et al., 2006; Metsios et al., 2007; Engvall et al., 2010). Whilst progressive resistance training (PRT) has been shown to be effective in restoring both muscle mass and function in RA patients (e.g., Marcora et al., 2005a; Lemmey et al., 2009; Sharif et al., 2011), the high-intensity (i.e. 80% of 1RM) of this training means that this form of exercise is unlikely to be widely adopted by all RA patients.

Consequently, anabolic nutritional supplementation offers a potential treatment option that is easily administered, inexpensive, and makes limited demands of the patient. It has been reported that up to 75% of RA patients believe that food and nutrition may play an important role in their symptom severity, with up to 50% of RA patients reportedly trying some form of dietary manipulation in an attempt to attenuate symptomology (Stamp et al., 2005). Scientific evidence continues to suggest that diet should be part of routine care in those with wasting disorders (for review, see Stamp et al., 2005).

Interestingly, there appears to be a lack of research into the use of diet to improve body composition or functional outcomes in RA. Our group previously investigated the effects of 12 weeks of a mixture of ß-hydroxy-ß-methylbutyrate, glutamine and arginine (HMB/GLN/ARG) protein supplementation in 40 RA patients (Marcora et al., 2005b). The results showed that both HMB/GLN/ARG and a control mixture of other, non-essential, amino acids (alanine, glutamic acid, glycine, and serine) were effective in increasing muscle mass and improving physical function in RA patients. Thus it appears that protein per se is capable of significantly improving LM, total body protein, and objective measures of physical function, such as the sit-to-stand in 30 second test, which reflect the ability to perform activities of daily living in RA patients (i.e. getting in and out of a chair).

Another potential nutritional supplement that could be used to restore muscle mass and functional capacity in RA patients is oral administration of creatine (Cr). This article reviews the evidence regarding the potential of Cr as an adjunct treatment to improve muscle mass and function in RA patients. In the course of doing this, the mechanisms and effectiveness of Cr in athletic populations will be described before we present a review of the existing evidence regarding the efficacy of Cr in RA-relevant clinical trials.

4.1.1. What is creatine?

Creatine, or methylguanidine-acetic acid, is a naturally occurring compound made from three amino acids; arginine, glycine, and methionine (Casey & Greenhaff, 2000), and is synthesized within the body, primarily in the liver, kidney, and pancreas (American College of Sports Medicine, 2010). Most (~95%) of the total Cr pool is contained in skeletal muscle, with ~60% (75 mmol· kg dry weight $(dw)^{-1}$) in the phosphorylated form, phosphocreatine (PCr) (Harris et al., 1974; Casey et al., 1996), and the remaining 40% (50 mmol· kg dw^{-1}) existing as free Cr (Bogdanis et al., 2007). Muscle does not synthesize Cr itself but is dependent on Cr uptake through specific membrane sodium dependent transporters (Longo et al., 2011).

Creatine has generally been shown to be more effective than other protein-based supplements in increasing LM. For example, Cribb et al. (2007) showed that Cr (1.5 g/kg per day for 11 weeks) was able to significantly improve LM by 6%, compared to whey protein (4%; $P < 0.05$) in 33 trained males. Further to this, in a meta-analysis (Nissen & Sharp, 2003) of 48 studies, both LM and strength gain were unaffected by whey protein and other supplementation such as androstenedione when compared to a placebo treatment, and only supplementation with either Cr or HMB resulted in a significant gains (**Table 4.1.**). The superior gains in LM and strength from Cr relative to HMB, combined with the additional benefits of Cr to energy production and recovery identifies Cr as a potentially highly effective adjunct treatment for improving RC and physical function.

4.1.2. What does creatine do?

4.1.2.1. Changes in adenosine triphosphate energy synthesis

Creatine performs many roles in the body, the most important of which is in generating energy for the muscles. Muscle relaxation and contraction, and therefore the movement of the body, is fuelled by energy liberated from the dephosphorylation of adenosine triphosphate (ATP).

ATP ↔ adenosine diphosphate (ADP) + phosphate (P) + energy

(catalysed by the enzyme ATPase)

The ATP stores in the body are limited (concentration in skeletal muscle is approximately 24 mmol· kg/dw (Harris et al., 1974)), and without a means of resynthesizing ATP at an equally rapid rate, maximal exercise exhausts these stores within 1–2 seconds (Burton et al., 2004). To overcome this storage limitation, the body is able to maintain a continuous ATP supply through different metabolic re-synthesis pathways: either anaerobically in the cytosol, or aerobically in the mitochondrion.

As stated previously, Cr is primarily stored in the body in a phosphorylated form as PCr, with the muscle content of PCr 3–4 times higher than that of ATP (Bogdanis et al., 2007). In a process called dephosphorylation, some energy for ATP re-synthesis comes directly from the hydrolysis (splitting) of phosphate from PCr (Bogdanis et al., 2007).

PCr ↔ Cr + P + Energy

(catalysed by the enzyme creatine kinase (CK))

In this process, the liberated phosphate group can then combine with ADP in a reaction catalysed by CK to restore ATP levels (Kreider et al., 1998) and maintain high cellular ATP/ADP ratios (Bemben et al., 2001):

ADP + P ↔ ATP + Cr

(catalysed by CK)

As a consequence, it would be anticipated that increasing initial Cr stores and thereby delaying PCr depletion would enhance re-synthesis of ATP and augment performance (Greenhaff, 1995; Wyss & Kaddurah-Daouk, 2000). Ingestion of Cr supplements (20 g a day for 5 days) has been shown to increase the total Cr and PCr concentration of human skeletal (**Table 4.2.**),

and indeed, reduced blood lactate concentrations have been observed after high-intensity (Balsom et al., 1995) and endurance exercise (Tang et al., 2013); although these findings are not universal (Engelhardt et al., 1998).

4.1.2.2. Changes in muscle mass and protein synthesis

Creatine is an osmotically active substance. Thus, as skeletal muscle cell Cr and PCr concentrations rise, the cell will rapidly draw in extracellular water (ECW) via osmosis in order to maintain equilibrium (Lang et al., 1998). The uptake of Cr and water into the muscle accounts for the increases in body mass (approximately 1–2 kg) usually observed after a few days of supplementation (e.g., Powers et al., 2003). Total body water has been reported to increase up to 3 litres (9%) (Bemben et al., 2001); of which intracellular water (ICW) has been shown to increase by between 0.8-3.0 litres (an increase of 3–9% from baseline values) (e.g., Ziegenfuss et al., 1998; Poortmans & Francaux, 1999; Gotshalk et al., 2002; Chrusch et al., 2001) in the absence of changes in ECW (Ziegenfuss et al., 1998).

The intramuscular uptake of Cr and the associated increase in ICW increases osmotic pressure, which in turn stimulates protein synthesis. Cellular hydration state is an important factor in controlling cellular protein turnover, i.e. an increase in cellular hydration inhibits proteolysis and stimulates protein synthesis (Ingwall et al, 1974), whereas cell shrinkage has opposite effects (Sipilä et al., 1981; Bessman & Savabi, 1988; Häussinger et al., 1993; Balsom et al., 1993; Lang et al., 1998). However, it is unclear whether acute Cr supplementation augments muscle protein by this mechanism (Parise et al., 2001; Louis et al., 2003).

Creatine has also been shown to stimulate muscle hypertrophy by inducing expression of muscle myogenic factors such as MRF4, MyoD, and myogenin (Hespel et al., 2001). Deldicque et al. (2005, 2008) showed that the muscle gene expression of insulin-like growth factor (IGF)-I was raised following Cr supplementation. This finding was corroborated by Burke et al. (2008) who found increased muscle content of IGF-I as a result of Cr supplementation combined with 8 weeks of PRT. These findings are highly relevant to Cr's anabolic potency as IGF-I produced locally in the muscle (mIGF-I) is thought to regulate adult skeletal muscle maintenance and hypertrophy (Adams, 2002).

Table 4.1. Summary of the results from the meta-analysis by Nissen and Sharp (2003)

Supplement (*n* = studies)	Average dosage (maintenance dose)	Duration (weeks)	Net lean mass change	Net strength change
Creatine (*n* = 18)	19.4 g/day for 5.3 days (6.7 g/day)	7.5	**+0.36%/week***	**+1.09%/week***
HMB (*n* = 9)	3 g/day	8	**+0.28%/week***	**+1.40%/week***
Chromium (*n* = 12)	485 ug/day	11.2	+0.08%/week	+0.25%/week
Androstenedione (*n* = 3)	200 mg/day	10.7	+0.05%/week	-0.06%/week
Protein (*n* = 4)	1.15 g/kg per day	6.3	+0.12%/week	-0.18%/week
DHEA (*n* = 2)	125 mg/day	10	+0.12%/week	+0.06%/week

The net change is expressed as % change per week. Only Cr and HMB resulted in significant changes; * = $P < .05$. HMB: ß-hydroxy-ß-methylbutyrate; DHEA: Dehydroepiandrosterone

Table 4.2. Changes in total creatine and phosphocreatine levels in the body following Cr supplementation

	Mean baseline total Cr levels in the body[1]	Increase after 20 g/day for 5 days
Total creatine	Approximately 125 mmol· kg/dw (Harris et al., 1992) (90 to 160 mmol· kg/dw) (Casey & Greenhaff, 2000)	+ 25 mmol· kg/dw (approximately 20%) (Hultman et al., 1996)
Phosphocreatine	Approximately 75 mmol· kg/dw (Bogdanis et al., 2007)	+ 8 mmol· kg/dw (approximately 11%) (Stec & Rawson, 2010)

[1]Typical values for an average 70 kg male.

Conversely, Cr supplementation in conjunction with PRT has been shown to lower serum levels of myostatin (Saremi et al., 2009; Schiaffino et al., 2011), a hormone that is highly expressed in RA synovial tissues and inhibits muscle growth by reducing myoblast (muscle) proliferation (Zimmers et al., 2002; Schiaffinio et al., 2011) and thus is associated with muscle atrophy (Zimmers et al., 2002) and joint destruction (Dankbar et al., 2011). The anabolic response to Cr supplementation is particularly evident in type II muscle fibres (Sipila et al., 1981; Soderlund et al., 1992), which is particularly important because RA patients present with preferential atrophy of type II fibres (Wortmann, 1993).

4.1.2.3. Reduction in inflammatory cytokines

Patients with RA exhibit high synovial levels and serum concentration of the cytokines TNF-α and IL-1ß (Walsmith & Roubenoff, 2002). These cytokines, in addition to causing synovial inflammation (Choy 2012), also modulate the expression of enzymes controlling muscle protein degradation (Fanzani et al., 2012). Bassit et al. (2008) investigated the effects of Cr supplementation (20 g/day for 5 days prior to competition) on plasma levels of pro-inflammatory cytokines, TNF-α, IL-1ß, and prostaglandin E2 (PGE2), in triathletes after a half-ironman competition. These cytokines are typically raised following prolonged strenuous exercise (Morley et al., 2001), but Cr supplementation attenuated the increases in TNF-α by 42% and 64%, IL-1ß by 72% and 71%, and PGE2 by 86% and 91%, 24 and 48 hours post exercise, respectively. Similar results by the same group were reported in Santos et al. (2004).

The exact mechanisms behind Cr supplementations apparent 'anti-inflammatory' effects are unclear. Santos et al. (2004) postulated that Cr's ability to increase muscle cell volume may increase its integrity and resistance to injury and tissue damage, thus reducing the production of inflammatory cytokines such as TNF-α. Deminince et al. (2013) hypothesised that Cr supplementation increases total ATP stores in the cell, this reduces adhesion of neutrophils as well as increasing the activity of the adenosine A_{2A} receptor (which has potent anti-inflammatory effects).

4.1.2.4. Creatine and bone degradation

RA patients are at 2-fold increased risk of having osteoporosis and ~28% of patients develop this condition (Haugeberg et al., 2000; Engvall et al., 2008). In wheelchair-independent patients experiencing Duchenne dystrophy, Cr supplementation was able to enhance bone mineral density (3%) and reduce urinary cross-linked N telopeptides of type I collagen (NTx) excretion, a marker for bone resorption (Louis et al., 2003). In addition, Candow et al. (2008) also reported a reduction in NTx (-27%) versus placebo (13%; $P < .05$), and similar findings

were reported by Chilibeck et al. (2005) who showed that in elderly men, Cr was able to improve arm bone mineral density by 3% ($P < .001$) versus placebo (-1%) However, more research is needed in this area to understand the mechanisms behind this action.

4.1.2.5. Athletic performance

Creatine has repeatedly demonstrated efficacy in improving high-intensity short-term exercise performance and subsequent recovery. For example, in cycling, Cr supplementation has been shown to significantly enhance peak power output (Balsom et al., 1995; Tarnopolsky, 2000; Wiroth et al., 2001) and maximal work (Casey et al., 1996) during repetitive sprints. Similarly, runners who supplemented with Cr decreased their 100 metre sprint time and total time for 6 × 60 metre sprint intervals (Skare et al., 2001), and highly trained football players improved their repeated sprint performance (6 × 15 metre sprints with 30 seconds recovery) and attenuated fatigue-induced decline in jumping ability following Cr supplementation (Mujika et al., 2000).

Creatine supplementation has also been found to be effective in improving performance of a variety of sustained high-intensity activities (e.g., kayaking for 5 minutes (McNaughton et al., 1998); 1000 metre rowing (Rossiter et al., 1996); and running 300 and 1000 metre intervals (3–4 minute rest) (Harris et al., 1993)). These functional benefits are attributed to increased ATP re-synthesis, heightened availability of PCr in type II fibres, and increased total Cr stores (Bogdanis et al., 2007). These effects may be particularly beneficial to older adults or clinical populations who experience difficulty performing short-term, relative high intensity activities such as hurrying for a bus, crossing roads, climbing stairs, or digging in the garden.

Creatine has also been shown to improve strength related measures. In an analysis of 22 studies, athletes supplementing with Cr had an average 8% greater increase in muscle strength than placebo (for a review, see Rawson and Volek, 2003). Furthermore, Cr supplementation when combined with PRT has been shown to be more effective at increasing strength and weightlifting performance than PRT alone (Volek et al., 1997; Larson-Meyer et al., 2000). Improvements in strength translate into increased work capacity, and thus improved ability to perform activities of daily living such as walking, carrying shopping, doing housework etc. (Baumgartner et al., 2004; Marcora et al., 2005a; Giles et al., 2008a).

Although ~70% (Kreider, 2003) of short-term studies on Cr supplementation report some ergogenic benefit, the responses are often variable amongst individuals (Rawson and Volek, 2003), and supplementation generally does not result in improvements in endurance

performance (e.g., repeated 6 km treadmill and terrain run performance) (Balsom et al., 1993; Stroud et al., 1994; Balsom et al., 1995; Chilibeck et al., 2007).

4.2. Critical review of relevant clinical literature

4.2.1. Aim

The aim of this review is to examine existing evidence assessing the efficacy of Cr supplementation in improving muscle mass and physical function, with particular reference to its potential use in treating RC and its consequences. To achieve this we searched for, and extracted relevant data from published research papers in RA and other conditions for which findings are likely transferable to the RA population, e.g., aging population and other musculoskeletal and wasting diseases.

4.2.2. Search methods

Peer-reviewed research articles were included in this review provided they: (1) investigated the effects of Cr supplementation in RA patients or other populations deemed relevant to RA (i.e. elderly populations (>60 years) or musculoskeletal disorders featuring loss of muscle and physical function); (2) included body composition (muscle and/or FM) and/or physical function as outcome measures; and (3) conducted an intervention of any design in RA patients; or undertook a blinded placebo-controlled trial for non-RA populations, to ensure only evidence of higher certainty of evidence was included. As the purpose of this review is to investigate alternative treatments to high-intensity exercise for restoring muscle mass and physical function, data on the additive effects of Cr supplementation and PRT were excluded. Publications were also excluded if they were a literature review, thesis, abstract, or a letter or comment, and the search was limited to English language citations.

PubMed and Google Scholar were searched for literature until May 2014 using the search term ‘creatine supplementation’ combined with ‘cachexia’; ‘clinical’; ‘patient’; ‘older adults’; ‘elderly’; ‘sarcopenia’, and ‘rheumatoid arthritis’. In addition, the reference sections of the selected papers were hand-searched for relevant ancestral references. The title and abstract of each search result was first screened for relevance according to the inclusion criteria above, before full articles were obtained. Full-text articles were then screened before final inclusion in this review.

4.2.3. Search results

The initial search returned 758 articles, excluding duplicates, of which 21 met the inclusion criteria and were selected for this review. One trail investigating Cr supplementation of RA patients was found (Willer et al., 2000). This study was not controlled in any way so is considered to provide evidence of low certainty. The body composition and physical function data extracted from trials in older adults are presented in **Table 4.3.**, and data extracted from trials in other relevant clinical populations appear in **Table 4.4**.

4.2.3.1. Rheumatoid arthritis

Willer et al. (2000) was the only study identified that completed a trial of Cr in an RA population. Twelve RA patients were un-blinded to the Cr supplementation and no placebo group or control arm existed. Participants were given oral Cr supplementation for 21 days using recommended doses (day 1-5: 20 g/day; day 6-21: 2 g/day) and the effects on muscle strength, subjectively assessed function during activities of daily living (Health Assessment Questionnaire; HAQ), and disease activity were examined. It was found that Cr supplementation increased muscle strength by an average of 14% (P = .020), as determined by the muscle strength index (the mean of eight strength measurements during flexion and extension of the knee and elbow/max sample strength*100 (Stucki et al., 1994). This increase in muscle strength was not associated with changes in skeletal muscle Cr or PCr levels. Routine clinical measures of disease activity and subjectively evaluated physical function showed no changes.

The authors attributed the limited effectiveness of Cr to alterations in the kinetics of Cr in patients with RA (e.g., reduced transport into the muscle, increased metabolism, and/or excretion). However, this interpretation places emphasis on the subjectively assessed function, which was unchanged, rather than the objectively measured strength, which did improve significantly. It is known that the HAQ is weakly associated with objective measures of physical condition such as strength (r = -.35) and joint mobility (r = .27) (Van den Ende et al., 1997), and is often insensitive to even large changes in objective function in patients with controlled disease (e.g., Van den Ende et al., 1997; Lemmey et al., 2009). Additionally, only 12 patients were included in the study. Given that Cr supplementation reported to be ineffective in approximately 30% of individuals (Greenhaff et al., 1995), it would be anticipated that only 8 of the RA patients in this investigation would see any benefit. Consistent with this prediction, strength increases were noted in 8 patients. Moreover, the study supplementation

period only lasted three weeks, much less than the 8–12 weeks recommended by manufacturers and used by other studies. Thus, whilst the findings of Willer et al's trial are inconclusive, they do provide some indications that Cr may be effective in the RA population. Clearly more research is needed in this area.

4.2.3.2. Aging and sarcopenia

Nine studies (Bermon et al., 1998; Rawson et al., 1999; Rawson & Clarkson, 2000; Jakobi et al., 2001; Gotshalk et al., 2002; Cañte et al., 2006; Stout et al., 2007; Gotshalk et al., 2008; Gualano et al., 2014) were identified that investigated the effects of Cr supplementation in older adults and met the inclusion criteria. Four of these studies, reported that Cr increased body mass by 0.5–1.9 kg (Rawson et al., 2000; Jakobi et al., 2001; Gotshalk et al., 2002; Gotshalk et al., 2008) and that this gain was predominantly LM, with increases in muscle mass of up to 2.2 kg (Gotshalk et al., 2002). In contrast, no significant changes in body mass or LM were found in the remaining five studies (Bermon et al., 1998; Rawson et al., 1999; Rawson et al., 2000; Stout et al., 2007; Gualano et al., 2014), although a trend of increased LM following Cr supplementation relative to placebo (P = .062) was found by one of these (Gualano et al., 2014). As expected, no significant changes in body fat % (BF%) subsequent to Cr supplementation in older participants were reported (Rawson et al., 1999; Rawson et al., 2000; Gotshalk et al., 2002).

Three of the six studies that measured muscle strength changes reported improvements following Cr supplementation. Gotshalk et al. (2002) reported strength increases of both maximal leg press (7–8%), knee extensor (9%), and knee flexor muscles (15%) in older males, whilst in females increases in leg press (3% or 5.2 kg) and bench press (4% or 1.7 kg) were found (Gotshalk et al., 2008). In a cross-over design, Stout et al. (2007) found that Cr significantly increased maximal isometric grip strength by 7%. Conversely, Jakobi et al. (2001) found that 5 days of Cr supplementation was unable to increase elbow flexor maximal voluntary strength or any other muscle contractile properties (twitch and tetanic recordings from electrical stimulation of the muscles). Similar findings were reported by Rawson (2000) who found no significant effect on isometric elbow flexor strength after 5 days supplementation, and Bermon et al. (1998) who found no increase in chest strength compared to a placebo (P > .05).

All studies assessing short-term physical function reported significant improvements in lower-extremity functional tests such as the STS test by up to 12% (Gotshalk et al., 2002; Canete et al., 2006; Gotshalk et al., 2008; Neves et al., 2011), and a tandem gait test by 6% (Gotshalk

et al., 2002) to 9% (Gotshalk et al., 2008) following Cr supplementation. Lower body power (as assessed by a 10 second Wingate test) was shown to improve by 11% (Gotshalk et al., 2002) and Rawson et al. (1999) reported that leg fatigue (as expressed as a % change in the total peak torque generate and assessed by 5 × 30 second knee extensions at 180° on an isokinetic dynamometer) was reduced by 9% (compared to a 5% increase in the placebo group, $P < .05$). Similar findings by Stout et al. (2007) showed lower body muscle endurance (cycling capacity at fatigue threshold) was improved by 16% compared to the placebo group. However, owing to Cr supplementation's ability to predominantly increase ATP/PCr re-synthesis, as expected, assessments of endurance capacity (i.e. 1-mile walk test; and gross mechanical efficiency, ventilatory threshold, and peak oxygen intake determined during cycle ergometry) were not significantly improved following Cr supplementation (Cante et al., 2006).

Overall, these studies demonstrate that Cr supplementation appears beneficial in improving measures of short-term objective physical functioning in older adults, but not tasks with an endurance component. Improvements in such tasks (e.g., improving the number of chair stands) may translate into an improved ability to perform more practical activities of daily living such as getting out of a car, or in and out of a chair.

4.2.3.3. Trials in other clinical populations

One study (Roy et al., 2005) trialled Cr supplementation in osteoarthritis (OA). Osteoarthritis is the most common form of arthritis, and as with RA, is characterised by joint damage, muscle weakness, poor physical function (Slemenda et al., 1997), and predominantly affects females (Lawerence et al., 2008). In this investigation, Roy et al. reported limited effects of Cr supplementation in OA patients recovering from total knee arthroplasty, despite a significant increase in serum Cr concentration, with no improvements in muscle strength (handgrip, dorsiflexion, and quadriceps strength, 30-foot timed walk, and 4-step climb) observed after 40 days (10 days pre-surgery and 30 days post-surgery) of Cr supplementation relative to placebo.

One trial (Alves et al., 2013) reported the use of Cr supplementation in fibromyalgia, another chronic syndrome of unknown etiology, characterized by some similarities in symptomology to RA, including pain, muscle dysfunction, disability, and fatigue (Leader et al., 2009). Some of the fibromyalgia symptoms such as muscle dysfunction and fatigue could, in theory, be due to low muscle levels of ATP and PCr (Alves et al., 2013). A randomised controlled trial of Cr supplementation in fibromyalgia patients (Alves et al., 2013) found that muscle PCr content increased and muscle strength improved relative to the placebo group (leg-press by 10%, $P =$

.020; chest-press by 1%, *P* = .020; and isometric handgrip strength by 6%, *P* = .070) in the Cr group.

Myopathy is a muscle wasting disorder which primarily affects skeletal muscle. Much like RC, this can cause a variety of complaints including progressive weakness and wasting of skeletal muscle, and fatigue (for a review, see Kley et al., 2011). Seven trials of Cr supplementation in populations with myopathies were found, with these investigations reporting mixed results on the efficacy of oral Cr. In a cross-over design trial in 30 Duchenne muscular dystrophy (DMD) adolescents (Tarnopolsky et al., 2004), the Cr supplementation phase increased LM by 0.7 kg and grip strength by ~20% compared to the placebo phase. In a similar design, Cr supplementation improved maximal strength and fatigue resistance in 15 other patients with DMD (Louis et al., 2003). Further to these trials, improvements in muscle PCr/P ratio and preservation of calf muscle strength were also reported by Banerjee et al. (2010) in 18 DMD patients.

In contrast, in cross-over design trials of patients with Myotonic muscular dystrophy 1 (DM1), Cr failed to induce any changes in muscle strength, LM, or disease symptoms (Walter et al., 2002; Tarnopolsky et al., 2004), or improve function or strength in DMD patients (Escolar et al., 2005) or patients with myotonic dystrophy type 2 (DM2) (Schneider-Gold et al., 2003).

Two studies (Norman et al., 2006; Bourgeois et al., 2008) were found that reported trials of Cr supplementation in cancer patients. Up to 80% of cancer patients have associated muscle wasting which is termed cancer cachexia (Tan & Fearon, 2008). Like other forms of cachexia, this is characterised by a preferential loss of skeletal muscle mass (with or without a loss of FM) which cannot be reversed through conventional methods of nutrition (Fearon et al., 2011). In patients with cancer, Cr supplementation improved handgrip strength by 6% (*P* = .019) (Norman et al., 2006) and reduced BF% (-4%; *P* < .05) relative to a placebo group (Bourgeosis et al., 2008).

Overall, results in clinical trials investigating the use of Cr supplementation are equivicol, of the twelve clinical trials identified, six showed positive effects of Cr on muscle mass and/or strength and function measures.

Table 4.3. Summary of studies investigating the effects of creatine supplementation on body composition and function in adults over 60 years

Study	Treatment arm (mean age ±SD)	Supplementation period	Study design	Body composition changes	Physical function changes
(Rawson et al., 1999)	10 males (66.7 ±1.9 years)	20 g/d for 10 days followed by 4 g/day for 20 days	*vs* PL group (dextrose) (*n* = 10)	↔Body density, ↔LM, ↔% BF	↑Leg fatigue performance
(Rawson et al., 2000)	9 males (65.0 ±2.1 years)	20 g/day for 5 days	*vs* PL group (sucrose) (*n* = 8)	↑BM, ↔LM	↔Strength
(Cante et al., 2006)	10 females (67.0 ±6.0 years)	0.3 g per kg/day for 7 days	*vs* PL group (*n* = 6)	No details	↑Objective function tests, ↑Endurance capacity
(Gotshalk et al., 2002)	10 males (65.4 ±1.5 years)	0.3 g per kg/day for 7 days	*vs* PL group (powdered cellulose) (*n* = 8)	↑BM, ↑LM	↑Strength, ↑Power, ↑Objective function tests
(Gotshalk et al., 2008)	15 females (63.3 ±1.2 years)	0.3 g per kg/day for 7 days	*vs* PL group (powdered cellulose) (*n* = 12)	↑BM, ↑LM, ↔%BF	↑Strength, ↑Objective function tests
(Stout et al., 2007)	7 males and 8 females (74.5 ±6.4 years)	20 g/day for 7 days followed by 10 g/day for 7 days	Cross-over design	↔BM	↑Strength, ↑Endurance (cycling capacity at fatigue threshold), ↔Objective function tests
(Jakobi et al., 2001)	7 males (72.5 ±2.5 years	20 g/day for 5 days	*vs* PL group (maltodrextin) (*n* = 5)	↑BM, ↔LM	↔MVC or contractile force
(Bermon et al., 1998)	4 males and 4 females (71.0 ±1.9 years)	20 g/day for 5 days followed by 3 g/day for 8 weeks	*vs* PL (glucose) (*n* = 8)	↔Lower limb volume, ↔BM, ↔%BF	↔Strength ↔Endurance
(Gualano et al., 2014)	15 females (66.1 ±4.8 years)	20 g/day for 5 days followed by 5 g/day for 23 weeks	*vs* PL (dextrose) (*n* = 15)	↑LM, ↔FM	↑Strength ↑Objective function tests

↑Significant increase/improvement; ↔No significant change ([a]*P* < .050 for interaction between placebo and Cr group). Cr: Creatine; PL: Placebo; BM: Body mass; BF%: Body fat %; FM: Fat mass; LM: Lean mass; MVC: Maximal voluntary contraction; No details: No details are specified or this measure was not made.

4.2.4. Review conclusions

Around 2/3rds of RA patients are middle-aged or elderly females (Symmons et al., 2002), and the existing evidence indicates that Cr can be successful in countering the effects of sarcopenia in older populations independent of exercise training (Rawson & Venezia, 2011), specifically in older females (Brose et al., 2003; Aguiar et al., 2013). Of the nine included trials that have supplemented the elderly with Cr, only three (Bermon et al., 1998; Rawson, 2000; Jakobi et al., 2001) found no beneficial effect on LM, strength, or physical function. However, the magnitude of effect appears to be reduced relative to that observed in young healthy individuals (Moon et al., 2013), and the limited number of studies indicates that further work is needed to fully evaluate the role of Cr supplementation (Devries & Phillips, 2014).

Creatine has generally been shown to be effective in a range of clinical conditions (Chung et al., 2007) including muscle wasting disorders (Tarnopolsky et al., 2004; Banerjee et al., 2010), and cancer cachexia (Norman et al., 2006). Despite the inconclusive findings of the solitary RA study (Willer et al., 2000), of the twelve clinical trials identified, six showed positive effects of Cr on muscle mass and/or strength and function measures.

Whilst this review has sought to evaluate studies investigating the use of Cr supplementation in populations with similar presentation to RA, it is limited by the lack of bias and/or quality assessment. Future reviews should ultilise an assessment of study quality and bias to help further determine the effects of Cr supplementation in these groups.

Table 4.4. Summary of clinical trials investigating the effects of creatine supplementation on body composition and physical function

Study	Condition	Treatment arm	Supplementation period	Control arm	Body composition changes	Physical function changes	Other effects
(Roy et al., 2005)	Osteoarthritis	*n* = 18	10 g/day pre surgery; 5 g/day for 30 day post-surgery	*vs* PL (*n* = 19) (dextrose)	↓%BF, ↓FM, ↔LM (CSA), ↔BW	↔Strength	↔PCr
(Alves et al., 2013)	Fibromyalgia	*n* = 16	20 g/day for 5 days followed by 5 g/day for 16 weeks	*vs* PL (*n* = 16) (dextrose)	Not measured	↑Strength	↔QoL scores, ↔Pain, ↔Cognition, ↑PCr
(Norman et al., 2006)	Cancer (cachexia)	*n* = 16 (colorectal cancer)	20 g/day for 5 day followed by 5 g/day for 8 weeks	*vs* PL (*n* = 15) (cellulose)	↔LM	↑Strength	↔QoL scores
(Bourgeosis et al., 2008)		*n* = 9 (adolescents with leukaemia (acute lymphoblastic)	2 sets of 8 weeks (with a 6 weeks wash out in-between)	*vs* control 'natural history' group (*n* = 50)	↔LM, ↓%BF	No details	↔Bone mineral content
(Banerjee et al., 2010)	Duchenne muscular dystrophy (DMD)	*n* = 18 (adolescents)	5 g/day for 8 weeks	*vs* PL (*n* = 15) (vitamin C)	No details	↑Strength	↑PCr
(Escolar et al., 2005)		*n* = 15 (adolescents)	5 g/day for 24 weeks	*vs* PL (*n* = 16) (cocoa powder)	No details	↔Strength, ↔Objective function tests	
(Tarnopolsky et al., 2004)		*n* = 30 (adolescents)	0.10 g per kg/day for 16 weeks	Cross-over design (PL group dextrose)	↑LM	↑Strength	↓Bone breakdown markers

(Louis et al., 2003)		*n* = 15 (adolescents) (12 with DMD and 3 with Becker dystrophy)	3 g/day for 13 weeks	Cross-over design (PL group maltodextrin)	No details	↑Strength (MVC), ↑Fatigue resistance	
(Tarnopolsky et al., 2004)	Mytonic muscular dystrophy 1 (DM1)	*n* = 34	5 g/day for 36 weeks	Cross-over design (PL group dextrose)	↔LM	↔Strength, ↔Objective function tests	
(Walter et al., 2002)		*n* = 34	11 g/day for 10 days followed by 5 g/day for 45 days	Cross-over design (PL group cellulose)	↔LM	↔Strength	↔ADL, ↔QoL scores
(Schneider-Gold et al., 2003)	Mytonic muscular dystrophy 2 (DM2)	*n* = 10	10 g/day for 13 weeks	*vs* PL (*n* = 10)	No details	↔Strength	↔QoL scores

↑Significant increase/improvement; ↓Significant decrease/reduction; ↔No significant change ([a]*P* < .05 for interaction between placebo and Cr group). Cr: Creatine; PL: Placebo; CSA: Cross sectional area; ADL: Activities of daily living; PCr: Phosphocreatine; QoL: Quality of life; MVC: Maximal voluntary contraction; BM: Body mass; BF%: Body fat %; FM: Fat mass; LM: Lean mass; PRT = Progressive resistance training; No details: No details are specified or this measure was not made.

4.2.5. Factors affecting creatine effectiveness in certain individuals or populations

Apart from inadequate supplement duration or dose, various other factors influence Cr effectiveness. It has been reported that 20–30% of individuals do not respond to Cr supplementation; when 'non-responsiveness' is defined as an increase in resting total muscle Cr of <10 mmol· kg/dw following 5 days loading at 20 g per day (Greenhaff et al., 1995). Syrotuik et al. (2004) found that based on pre-existing biological and physiological factors, 'responders' (defined in that study as ≥20 mmol· kg/dw increase in intramuscular Cr) possessed a biological profile of: (i) low initial levels of total Cr or PCr (~<110 mmol· kg/dw); (ii) higher percentage of type II fibres (>63%); and (iii) a higher preload muscle fibre cross-sectional area (CSA) (~>1500 μm^2). For individuals whose initial muscle Cr concentrations reach near or above 150 mmol· kg/dw, Cr supplementation does not appear to augment muscle Cr uptake, increase PCr re-synthesis, or improve performance (Harris et al., 1992; Casey et al., 2000; Syrotuik et al., 2004). Not surprisingly, optimal responses to Cr supplementation are generally observed in groups with reduced serum and muscle levels of Cr such as vegetarians and low meat eaters, which include many older individuals.

Although the majority of the studies reviewed found benefits of Cr supplementation in the elderly, it has been suggested that uptake of Cr into muscle is reduced in older adults (>60 years) relative to younger participants (Rawson et al., 1999; Stec & Rawson, 2010), and that subsequently older adults may require a longer Cr treatment period (Chrusch et al., 2001).

4.2.6. Safety of creatine

Concerns about possible side effects of Cr supplementation have been raised in lay publications, mailing lists, and online forums. However, none of the studies included in this review, including those in clinical trials, reported any adverse incidents during the trials ranging from 5 days to 36 weeks. This is consistent with other studies of long term (10 months to 5 years) (e.g., Poortmans et al., 1997; Poortmans & Francaux, 1998) or high dose Cr supplementation (10 g/day) (e.g., Earnest et al., 1996; Gualano et al., 2008) that have reported no adverse side effects. Further, a review of by Persky and Rawson (2007) found no increased incidence of side effects in clinical studies supplementing with Cr. Pertinent to RA, study by Chung et al. (2007) found that Cr supplementation had no effect on conventional anti-inflammatory and immunosuppressive medical treatment, including steroid and MTX

treatment, for patients with chronic idiopathic inflammatory myopathies. Overall, current evidence does not hint towards any negative health effects of Cr (Walliman, 2013). Therefore, the anecdotal reports remain unsubstantiated and may be unrelated to Cr supplementation (Kreider et al., 1998).

Concerns about the long-term safety of Cr have specifically been related to kidney function. Theoretically, the high nitrogen content (~32%) of Cr could place additional strain on the kidney if taken in large excess for a long period of time (Poortmans et al., 1997). Estimated glomerular filtration rate (eGFR) is widely accepted as the best overall measure of kidney function, with elevated serum and urine creatinine levels the most commonly used markers for estimating eGFR (Gualano et al., 2008). However, since Cr is converted to creatinine (Wyss & Kaddurah-Daouk, 2000), it is normal for individuals who take Cr supplements to have elevated creatinine levels (Shao & Hathcock, 2006), thus falsely suggesting renal function impairment. In 18 young healthy sedentary males, use of alternative eGFR markers such as cystatin C has shown that Cr supplementation does not promote renal dysfunction (Gualano et al., 2008). Whether cystatin C is useful marker of renal function following Cr supplementation in clinical populations or older adults requires further study.

There is currently limited research on the effects of Cr supplementation in patients with exiting low eGFR. A prospective report (Gualano et al., 2009) suggests that short-term (35 days) Cr supplementation (5 days of 20 g/day followed by 5 g/day) does not affect kidney function in individuals with a single kidney and mildly decreased eGFR. However, more research is needed in this area. Similarly, no evidence has emerged that Cr supplementation results in impaired liver function or liver damage (Mayhew & Mayhew, 2002; Schroder et al., 2005).

4.2.7. Prescription of creatine to patients

4.2.7.1. Type of creatine

Creatine supplements are usually taken as a tablet or powder (mixed with water), and exist in a variety of forms including Cr ethyl ester, Cr hydrochloride, and the most commonly available, Cr monohydrate (Cr complexed with a molecule of water). No differences in effectiveness have been found between these different Cr forms (Spillane et al., 2009).

4.2.7.2. 'Loading' dosage

Creatine should be 'loaded' into the muscle (using a high dose) for the first few days followed by a lower maintenance dose (Bogdanis et al., 2007). The most common 'loading' dosage recommendation for Cr supplementation is 20 g/day (in four 5 g doses) for 5 days, as stores appear to be maximised within 5 to 6 days at this dose (Harris et al., 1992). Alternate loading phases exist including daily doses based on body mass such as 0.25 g/kg (Bogdanis et al., 2007) or 0.15 g/kg (Vorgerd et al., 2000). However, a constant dose of 3 g/day, without an intensive loading phase, achieved an increase in total Cr levels equal to a standard 5 day loading protocol and subsequent maintenance phase after 28 days (Hultman et al., 1996).

4.2.7.3. 'Maintenance' and frequency

Total muscle Cr can be maintained after the initial loading phase by the ingestion of small daily Cr doses of 2–5 g (Hultman et al., 1996). This period of low dosage is called the 'maintenance phase'. Here, Cr is usually taken in 8 to 12 week cycles, with a 4 to 5 weeks 'washout' period in between to allow serum Cr to return to baseline levels.

4.3. Conclusion

Oral Cr supplementation works primarily by enhancing the re-synthesis of ATP via increased stores of PCr in the muscle, and thus improving recovery during and after physical activity. Creatine also augments muscle protein synthesis thereby increasing muscle mass. This review found only one study in which RA patients were supplemented with Cr and its findings, whilst promising, were inconclusive. However, trials in populations with similar presentation to RA (i.e. reduced muscle mass and impaired physical function), including older females, indicate that Cr is an efficacious way to improve muscle mass, strength, and physical function. Therefore, additional studies in RA populations are advocated, as confirmation of the efficacy of Cr supplementation would provide an easy, safe, and effective means of reversing the effects of RC in the majority of the RA population.

5

Can oral creatine supplementation improve body composition, strength and objective physical function in rheumatoid arthritis patients? A randomised controlled trial

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5.1. Introduction

Patients with rheumatoid arthritis (RA) usually experience substantial loss of lean mass (LM) (known as 'rheumatoid cachexia' (RC) (Roubenoff et al., 1992; Roubenoff, 2009). This loss of LM is a major contributor to the decreased strength (Van Bokhorst-de van der Schueren et al., 2012) and impaired physical function (Giles et al., 2008a; Summers et al., 2008; Lemmey et al., 2009; Kramer et al., 2012; Lusa et al., 2015) observed in RA.

Contemporary pharmacologic treatments for RA, exemplified by the 'treat-to-target (T2T)' strategy, do not ameliorate this LM loss, nor fully restore physical function (**Chapter 3**; Marcora et al., 2006; Metsios et al., 2007; Engvall et al., 2010; Toussirot et al., 2014). Whilst exercise (specifically, progressive resistance training (PRT)) has been shown to be highly effective in restoring both LM and function in RA patients (e.g., Marcora et al., 2005a; Lemmey et al., 2009), the lack of adherence to sufficiently intense training means this form of therapy is unlikely to be widely adopted (Lemmey et al., 2012). Due to its relative ease and simplicity, anabolic nutritional supplementation offers a potential adjunct treatment intervention for improving LM and function that could be widely accepted. Indeed, our group (Marcora et al., 2005b) has previously demonstrated that daily oral protein supplementation, without additional exercise, for 12 weeks improved LM and some measures of objectively-assessed physical function in RA patients.

Creatine (Cr), a combination of essential amino acids, is a popular dietary supplement generally shown to have greater benefits on both LM and physical function than generic protein supplementation (Nissen & Sharp, 2003; Cribb et al., 2007). Oral Cr supplementation is able to enhance adenosine triphosphate (ATP) re-synthesis by increasing initial stores of phosphocreatine (PCr) in the muscle, and thereby aid recovery during and after physical activity (Greenhaff et al., 1994). Creatine supplementation also increases LM (Nissen & Sharp, 2003). Following Cr uptake, extracellular water (ECW) is absorbed by muscle via osmosis in order to restore intramuscular protein levels (Greenhaff et al., 1994; Ziegenfuss et al., 1998; Francaux & Poortmans, 2006), and the resulting increase in mechanical stress caused by the expansion in intracellular water (ICW) has been proposed to act as an anabolic signal for protein synthesis (Ingwall et al., 1974; Powers et al., 2003; Francaux & Poortmans, 2006).

The majority of trials investigating Cr usage have shown it to be effective in improving LM and performance measures in a range of athletic (e.g., Skare et al., 2001; Kreider, 2003; Kreider et al., 2010) and clinical populations (e.g., Rawson & Volek, 2003; Gualano et al., 2012; Alves et al., 2013; Gualano et al., 2014) including those with similar presentation to RA (i.e. reduced muscle mass and impaired physical function) such as muscular dystrophy and the elderly (Gotshalk et al., 2002, 2008). However, results are not completely unequivocal with several studies in older adults (Rawson et al., 1999; Rawson & Clarkson, 2000; Jakobi et al., 2001) and clinical populations (Walter et al., 2002; Tarnopolsky et al., 2004a, 2004b; Sakkas et al., 2009) showing no effect on either LM or physical function (for review, see **Chapter 4**).

To date, only one study (Willer et al., 2000) has investigated the efficacy of oral Cr supplementation in RA patients. In this short uncontrolled trial, twelve patients underwent three weeks of supplementation, and although strength increased, no changes in subjectively-assessed physical function or muscle Cr levels were found, and body composition changes were not investigated. Thus, the findings of the trial are inconclusive, although they do provide some indication that Cr supplementation may be efficacious in RA patients.

The current study aimed to investigate the effects of 12 weeks of oral Cr supplementation on body composition, strength, and objectively-assessed physical function in patients with RA. We hypothesised that Cr supplementation would: (1) increase LM; and (2) improve strength and objective physical function.

5.2. Patients and methods

A 24-week, double-blind randomised, placebo-controlled trial was conducted between April 2013 and August 2014 at the School of Sport, Health, and Exercise Science, Bangor University, UK. The study was approved by the North Wales Research Ethics Committee - West, and registered on the International Standard Randomised Controlled Trial Number Register (ISRCTN: 37558313). The full trial protocol can be found at http://clinicaltrials.gov/show/NCT01767844.

5.2.1. Study population

Rheumatoid arthritis patients with stable disease (i.e. no change in medications in the preceding 3 months) were recruited from outpatient clinics of the North West Wales Rheumatology department (Peter Maddison Rheumatology Centre, Llandudno General Hospital, North Wales). For inclusion, participants had to: (a) fulfil the American College of Rheumatology/European League Against Rheumatism 2010 revised criteria for the diagnosis of RA (Aletaha et al., 2010); (b) be aged ≥18 years; (c) not be cognitively impaired; (d) be free of other cachectic diseases or conditions preventing safe participation in the study; (e) have a glomerular filtration rate (GFR) ≥60 mL/min/1.73m^2; (f) not be taking anabolic drugs or nutritional supplements; (g) not be currently participating in a regular, intense exercise training; and (h) not be pregnant. Participants were withdrawn if they experienced a change in medication, including the delivery of a corticosteroid (CS) injection, to treat active disease (i.e. disease flare).

5.2.2. Supplementation and randomisation protocol

Participants were randomised to receive either supplementary Cr (treatment) or placebo (control) drinks for 12 weeks. Randomisation was performed using a secure online system independently from the research team by the North Wales Organisation for Randomised Trials in Health (NWORTH), a registered clinical trials unit. Patients were stratified into two groups (i.e. Cr and placebo) based on age and sex (stratification variables: 18–44, 45–59, 60+ years).

Both the principle researcher (TJW) and participants were blinded to supplement assignment until trial completion.

In accordance with manufacture recommendations, and previous strategies (e.g., Greenhaff et al., 1994; Willer et al., 2000), the Cr group received 20 g of Cr monohydrate (myprotein.co.uk, Cheshire, UK) (4 x 5 g/day) for a 5-day 'loading period' followed by 3 g/day for the remainder of the 12 week supplementation period ('maintenance dose'). The Cr was mixed with a mango-flavoured drink powder (Foster Clarks Ltd, Malta, EU) to improve taste. The placebo group received only the mango-flavoured drink powder. Both groups received their supplements in individually portioned packets, which they were instructed to dilute with water to produce a mango-flavoured drink. The appearance of the different treatment packets were indistinguishable, as were the flavouring and colouring of the drinks (**Figure 5.1.**). Adherence was monitored through return of the empty packets, and participants were asked to maintain their routine physical activity and dietary habits and notify the investigators of any substantial lifestyle changes.

Figure 5.1. Image shows placebo (left) and creatine (Cr) supplementation (right) treatments. Both treatments were indistinguishable in appearance, taste and smell. Image shows both treatments in their individually prepared bags (front), and mixed with water (back).

5.2.3. Assessments and outcome measures

Participants were assessed at baseline (pre-supplementation), day 6 (post-loading phase), week 12 (immediately after cessation of supplementation), and week 24 (follow-up 12 weeks after cessation of supplementation). For each assessment, participants presented fasted, and having refrained from strenuous exercise, caffeine, and alcohol in the preceding 24 hours. Relevant information (age, disease duration, medication) was collected by interview and review of medical records. Throughout the study participants were questioned about any adverse events or side effects related to taking the supplement.

5.2.3.1. Anthropometric and body composition measures

As previously described in **Chapter 3**, body mass (BM) and height were recorded in accordance with standard procedures (Eston & Reilly, 2009). Body mass index (BMI) was calculated as BM (kg)/height (m^2). Total and regional lean, fat, and bone masses were estimated using a whole body fan-beam dual energy X-ray absorptiometry (DXA) scanner (Hologic, QDR Discovery 45615, software V12.4). Appendicular lean mass (ALM; i.e. the summed LM of the arms and legs) was used as a surrogate measure of total body muscle mass (Kim et al., 2002). The radiation exposure was 3.6 μSv per scan and 14.4 μSv for the total study (i.e. four scans per person).

Immediately after the DXA scan, and whilst still supine, total body water (TBW), intracellular (ICW), and extracellular (ECW) were estimated using bioelectrical impedance spectroscopy (BIS; Hydra 4200, Xitron Technologies, San Diego, USA). Measurements were taken on the right side of the body using disposable electrodes (Kendall Q-Trace Gold 5500, Mansfield, USA): two attached at the wrist and two at the ankle in accordance with the manufacturer's wrist-to-ankle protocol. Quality control procedures were performed periodically (all measurements satisfied the manufacturer's parameters for ICW and ECW; CV's = 0.05% and 0.02%, respectively) and the proximity of the scanner had no effect on the BIS (unpublished observations by our group).

5.2.3.2. Strength and objective physical function measures

Isometric maximal voluntary knee extensor strength (IKES) was measured using an isokinetic dynamometer (Humac Cybex Norm 2004, Computer Sports Medicine Inc, Massachusetts, USA) and maximal voluntary handgrip strength (HGS) was measured using a Grip-A dynamometer (Takei Kiki Kogyo, Japan). Three objective assessments of whole body physical

function were completed; the ‘sit-to-stand in 30 second’ test (STS-30), the ‘8-foot up and go test’ (8’UG), and the ’50-foot walk test’ (50’W). These tests were specifically developed for assessing the capacity of older adults to perform activities of daily living (Rikli & Jones, 2012), and are routinely used by our group (e.g., Marcora et al., 2005a, 2005b, 2006; Lemmey et al., 2009, 2012; Matschke et al., 2010a, 2010b).

5.2.3.3. Aerobic capacity

The submaximal ‘Siconolfi’ step test (Siconolfi et al., 1985) was used to estimate aerobic capacity (VO_2max).

5.2.3.4. Clinical measures and self-reported physical disability

Disease activity was assessed by the Disease Activity Score in 28 joints (DAS28), and systemic inflammation by C-reactive protein (CRP). To determine subject eligibility, and examine the effect on renal function, estimated glomerular filtration rate (eGFR) was monitored at baseline and then periodically over the course of the treatment period from review of patients’ regular blood chemistry screenings. Self-reported physical disability was subjectively assessed using the physical function component of the Multi-dimensional Health Assessment Questionnaire (MDHAQ) (Pincus et al., 2007).

5.2.4. Statistical analysis

An a-priori power calculation using the muscle strength index (MSI) data[8] of Willer et al. indicated that a minimum sample of 12 per group was required (mean Δ = 7.4 MSI units (%), SD Δ = 9.8, effect size (ES) = 0.8, *P* = .050, power = 0.80). This estimate was confirmed using the maximal work improvements during sprint cycling observed in elderly men following Cr supplementation (Wiroth et al., 2001) (mean Δ 3.1, SD Δ = 2.7, ES = 1.1, *P* = .050, power = 0.85). To allow for dropouts we aimed to recruit 20 patients per group.

Unless otherwise stated, data is presented as mean (±SE). Significance was set at $P < .05$ and a trend was recognised as $P = .05–.10$, for analysis conducted on participants who completed supplementation. The primary outcome of the study was ALM (i.e. ‘muscle mass’), and secondary outcomes included measures of objective physical function; DXA-measures of

[8] Ideally we would have powered the trial using body composition data from previous trials investigating Cr use in RA, however, no such studies exist. Although trials investigating body composition change in the elderly were available, we felt that powering our study using data from RA patients was more appropriate. Consequently, we chose the Willer et al. strength (i.e. MSI) changes to calculate required sample size.

total LM, fat mass (FM) and BF%; bioelectrical impendence measures of TBW, ICW, and ECW; anthropometric measures; self-reported health and disability (SF-36 and HAQ); and disease activity (DAS28 score). Chi-squared tests were used for comparison of dichotomous variables. Differences between groups for outcome variables at each assessment point were tested by analysis of variance (ANCOVA), with baseline values controlled as a co-variant.

Variables were checked for univariate outliers, and normal distribution using Shapiro-Wilk tests. Where necessary, data (STS-30, 8'UG, 50'W) was logarithm transformed to obtain normally distributed data, and to assess its relative effect on associated significance values. Confidence intervals (CI) (95%) and ES (η^2: small ≥.01; medium ≥.08; large ≥.26; very large ≥.50) were calculated, and Pearson product–moment correlation assessed relationships (*r*) of interest. Statistical guidance was provided by the NWORTH trials unit, and data was analysed using the Statistical Package for the Social Sciences 22 (SPSS) (Chicago, USA).

5.2.4.1. Missing data

Where appropriate, the expectation-maximization algorithm (EM) was used to impute missing DXA (8% of data points missing; 12/140), IKES (6%; 9/140), HGS (5%; 7/140), STS-30 (5%; 7/140), 8'UG (5%; 7/140), 50'W (7%; 10/140)) values and restore sample size. The same eight participants (Cr: *n* = 5; placebo: *n* = 3) were unable (not requested), due to problems with pain or balance, to perform the step test throughout the trial (i.e. at each time point). As such, VO_2max data was also imputed.

In these instances of missing data, the EM algorithm was used to impute missing values (Schafer, 1997), restore sample size and ensure that missing outcome measures were unlikely to bias the results. Expectation-maximization is based on two iterating (50 iterations were used) steps – expectation and maximization – which generate means and variances for missing data based on known values for that variable. Data was estimated from other variables (i.e. based on the *r* in each group for that particular variable). Little's MCAR test (to assess if data was 'missing completely at random') and Separate Variance *t*-tests (to assess if data was 'missing at random') indicated that EM was an appropriate method to use. Expectation-maximization was employed above 'multiple imputation'[9] which does not permit necessary analysis in SPSS on multiple datasets (Van Ginkel & Kroonenberg, 2014), and above other imputation methods such as 'last observation carried forward' which is now largely discouraged in clinical trials (e.g., Molnar et al., 2008; Blankers et al., 2010). Sensitivity

[9] No differences in accuracy have been reported between methods (Lin, 2010), and for reference we compared both methods and considered the outputs (group means) to be near identical.

analysis using only measured ('raw' or 'complete case') data was used to confirm our observations with EM data[10].

[10] Sensitivity analysis using 'raw'/'complete case' data confirmed results from EM data. In terms of between group differences, in the Cr group statistical significance (i.e. $P < .05$) was lost in some variables that had previously demonstrated (possibly due to insufficient *n*): (baseline to week 12): ALM (primary outcome measure) (0.50 kg, $P = .005$*, i.e. maintained significance); total LM (0.90 kg, $P = .097$); FM (0.26 kg, $P = .674$); TBW (1.10 L, $P = .064$§); ICW (0.76 L, $P = .064$§); ECW (0.35 L, $P = .251$§); (B-24): ALM (0.08 kg, $P = .778$); total LM (-0.12 kg, $P = .832$); FM (0.01 kg, $P = .893$); TBW (0.28 L, $P = .678$); ICW (-0.14 L, $P = .794$); ECW (0.19 L, $P = .491$).

5.3. Results

5.3.1. Baseline demographics

Forty patients were randomised and commenced treatment with either Cr (*n* = 18) or placebo (*n* = 22). The flow of patients through the study is shown in **Figure 5.2**. For patients who completed the trial (Cr: *n* = 15; placebo: *n* = 20), there were no statistically significant differences in demographic, disease, treatment, body composition, strength, or objective physical function variables between the groups at baseline; although the placebo group were somewhat larger (BM, LM, and FM) and consequently tended to be stronger (**Table 5.1.**).

5.3.2. Treatment safety and compliance

Five patients withdrew from the trial. In the Cr group, one female (64 years) withdrew complaining of lethargy and aching muscles (this was not considered treatment related, and was attributed to fatigue following function testing due to poor physical fitness, obesity, being a smoker, and having moderate disease activity), and a female (70 years) and a male (44 years)[11] were both withdrawn after being administered IM CS injection to treat a disease flare druing the study. In the placebo group, one male (62 years) suffered from a reoccurrence of angina (prior history), and one female (74 years) was withdrawn due to receiving an IM CS injection for a disease flare.

Over the 12 week treatment period, no changes in DAS28 were observed in either group (Cr Δ= -0.1 ±0.2; placebo Δ= -0.1 ±0.2; between-group difference: 0.0 (95% CI: -0.6–0.6), P = .990, η^2 = .00)). No treatment-related adverse side effects were reported in the Cr group, and all patients' eGFR remained ≥60 mL/min/1.73m^2. The supplementary drinks were well received, with no differences in compliance (P = .896; mean consumption of 99% of provided supplement consumed, range 87–100%; and mean of 99%, range 80–100%, for Cr and placebo, respectively). All participants declared no substantial changes in diet, medication, and lifestyle during the study.

[11] See **Chapter 7** for the effect of this CS injection on body composition.

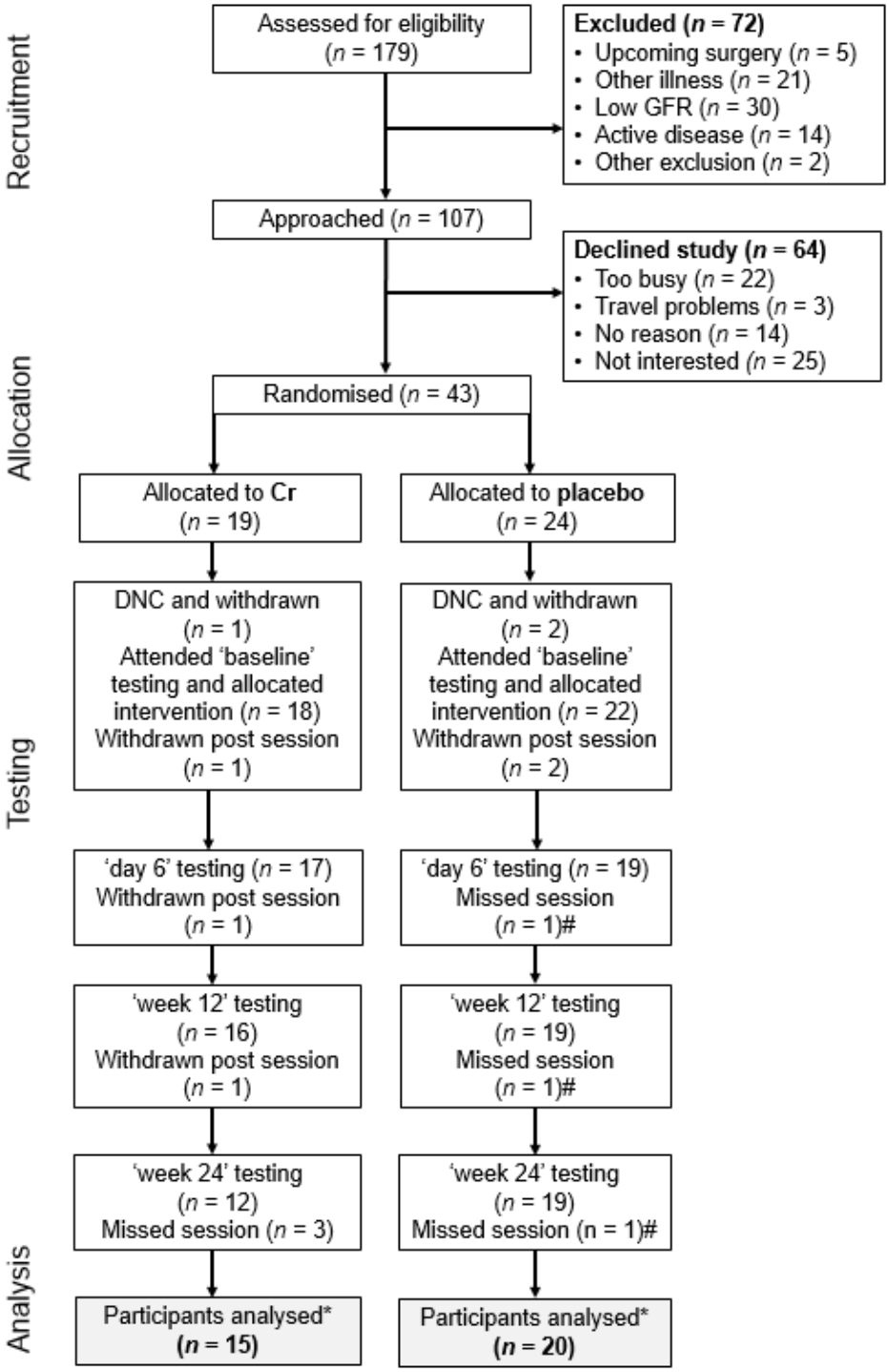

Figure 5.2. CONSORT diagram showing recruitment and path of patients through the study. GFR = (estimated) glomerular filtration rate; Cr = Creatine supplementation group; DNC = randomised but did not commence treatment (i.e. did not attend baseline and were subsequently withdrawn); * = due to missing data, final analysis for body composition data included values using expectation-maximization imputed data; # = missed sessions (placebo) at day 6, week 12 and week 24 were not the same participant.

Table 5.1. Baseline demographics of rheumatoid arthritis patients who underwent 12 weeks of oral creatine or placebo supplementation

	Creatine (*n* = 15)	Placebo (*n* = 20)	*P*
Age (years)	63.0 (±10.0)	57.2 (±10.4)	.104
Sex (female) (%)	10 (67)	14 (70)	.833
Disease duration (months)	112.4 (±82.8)	141.4 (±160.1)	.493
Rheumatoid factor +, *n* (%)	8 (53)	13 (65)	.376
Height (cm)	165.1 (±7.9)	166.1 (±9.1)	.734
BM (kg)	67.3 (±10.3)	76.7 (±19.0)	.092[#]
BMI (kg/m^2)	24.7 (±3.6)	27.8 (±6.6)	.113
ALM (kg)	18.4 (±4.2)	20.6 (±5.7)	.227
Total LM (kg)	45.9 (±8.5)	50.1 (±12.4)	.274
Total FM (kg)	19.8 (±7.2)	24.9 (±10.5)	.113
DAS28	2.8 (±0.8)	2.6 (±0.9)	.608
Medications, n (%)			
NSAIDS	4 (27)	10 (50)	.163
Methotrexate	9 (60)	12 (60)	1.00
Other DMARDs	6 (40)	7 (35)	.889
Biologics	1 (7)	4 (20)	.617
Current corticosteroids [a]	2 (13)	2 (10)	.759
Strength and physical function measures			
IKES (N)	348 (±156)	417 (±127)	.159
HGS (N)	236.6 (±92.8)	237.9 (±99.8)	.969
STS-30 (reps)	11.7 (±4.0)	13.2 (±2.9)	.206
8'UG (secs)	8.2 (±3.3)	6.6 (±1.7)	.119
50'W (secs)	11.0 (±4.0)	9.8 (±2.2)	.300
VO_2max (L/min)	1.8 (±0.4)	1.7 (±0.5)	.918
MDHAQ	0.5 (±0.5)	0.5 (±0.4)	.917

BM = body mass; BMI = body mass index; ALM = appendicular lean mass; FM = fat mass; DAS28 = disease activity score in 28 joints; NSAIDS = non-steroidal anti-inflammatory drugs; DMARDs = disease modifying anti-rheumatic drugs; IKES = isometric knee extensor strength; HGS = handgrip strength; STS-30 = sit-to- stand in 30 second test; 8'UG = 8-foot up and go; 50'W = 50-foot walk; VO_2max = estimated VO_2max from Siconolfi step test; MDHAQ = Multi-dimentional Health Assessment Questionnaire. [a] = current corticosteroid use, range 2.5–5.0 mg. Unless stated, data presented as mean (±SD). * *P* < .05; [#] *P* = .05–.10.

5.3.3. Treatment effectiveness

5.3.3.1. Body composition measures

Twelve weeks of Cr supplementation resulted in a significant increase in ALM of 0.52 (±0.13) kg in the Cr group, with no change in the placebo group (0.05 (±0.13) kg; between-group *P* = .004, η^2 = .23 (medium)) (**Table 5.2**). Similarly, total LM increased by 0.60 (±0.37) kg) following

Cr supplementation, with no change in the placebo group over the same period (-0.06 (±0.29) kg), albeit the between-group change was not significant (P = .158, η^2 = .06 (small)). The increase in LM accounted for most of the 1.10 (±0.58) kg BM gain observed in these patients from baseline to week 12 (P = .195, η^2 = .06 (small)). In the Cr group there was an increase in ICW from baseline to week 12 (0.64 ±0.22 L, P = .035, η^2 = .13 (medium)), however this change was only weakly correlated with the ALM increase (r = .481, P = .082).

At week 24, the increases from baseline values for ALM (P = .293, η^2 = .03 (small)) and total LM (P = .977, η^2 = .00) were comparable for both groups. This indicates a regression back to baseline for ALM and total LM in the Cr group following supplementation cessation and further supports a treatment effect. From weeks 12 to 24, the decline in ALM in the Cr group corresponded with reductions in water compartments (TBW (r = .801, P = .001) and, more pertinently, ICW (r = .711, P = .004). No changes in FM or body fat % were observed at any time point, and, similarly, no significant changes in any aspect of body composition were detected at day 6, for either group.

5.3.3.2. Strength and physical function measures

The effects of Cr supplementation on strength and objective physical function measures are displayed in **Table 5.3**. There was no change in IKES over the 12 week treatment period with the increase over time between the groups comparable (P = .408, η^2 = .02 (small)). Following 12 weeks cessation of Cr supplementation, IKES was seemingly increased in the Cr group, as evidenced by a 34 (±14) N increase from baseline to week 24 (P = .075, η^2 = .10 (medium)) relative to the placebo group. However, this trend was the result of one participant who improved by 143 N from baseline to week 24. Removing this individual resulted in the loss of this trend (adjusted means, baseline to week 24 change: Cr = 25 (±14) N, placebo = 2 (±11) N, between-group difference: 23 (95% CI: -14–60) P = .215, η^2 = .05 (small)). Similarly, there were no differences between the two groups in changes in HGS from baseline to week 12 (P = .833, η^2 = .00), or to week 24 (P = .969, η^2 = .00).

Consistent with the lack of effect on strength measures, there were no meaningful changes in any of the objective physical function measures, as both groups improved their STS-30, 8'UG, and 50'W test performances comparably (between-group *P's* = .764, .555, and .335, respectively, for baseline to week 12 between-group changes). Creatine supplementation also had no effect on estimated VO_2max (L/min) (between-group P = .762, η^2 = .00), or self-reported physical disability (MDHAQ) (Cr = -0.1 ±0.1, placebo = -0.1 ±0.1; between-group difference, 0.0 (95% CI: -0.3–0.4), P = .836, η^2 = .06 (small)) over the 12 week supplementation period.

Table 5.2. Changes in body composition in rheumatoid arthritis patients following 12 weeks oral creatine supplementation

		Creatine (*n* = 15)	Placebo (*n* = 20)	Differences between-group for Δ		
		Mean	Mean	Mean (CI)	*P*	η^2
ALM (kg)	Δ B–12	+0.52 (±0.13)	+0.01 (±0.11)	**0.52 (0.18–0.86)**	**.004***	**.23**
	Δ B–24	+0.40 (±0.18)	+0.15 (±0.15)	0.25 (-0.23–0.73)	.293	.03
Total LM (kg)	Δ B–12	+0.60 (±0.37)	-0.06 (±0.29)	0.65 (-0.27–1.57)	.158	.06
	Δ B–24	+0.21 (±0.37)	+0.19 (±0.32)	0.01 (-0.99–1.01)	.977	.00
BM (kg)	Δ B–12	+1.10 (±0.58)	+0.11 (±0.46)	0.99 (-0.54–2.52)	.195	.06
	Δ B–24	+0.61 (±0.70)	+0.92 (±0.55)	-0.31 (-2.15–1.53)	.736	.00
Total FM (kg)	Δ B–12	+0.41 (±0.45)	+0.18 (±0.37)	0.23 (-0.94–1.40)	.693	.01
	Δ B–24	+0.65 (±0.52)	+0.48 (±0.45)	0.17 (-1.26–1.60)	.810	.00
Body fat (%)	Δ B–12	+0.1 (±0.4)	+0.5 (±0.3)	-0.3 (-1.4–0.8)	.595	.01
	Δ B–24	+0.3 (±0.5)	+0.6 (±0.4)	-0.3 (-1.6–1.0)	.608	.01
Water compartments						
TBW (L)	Δ B–12	+1.08 (±0.27)	-0.01 (±0.23)	**1.07 (0.34–1.80)**	**.005***	**.22**
	Δ B–24	+0.42 (±0.31)	-0.11 (±0.27)	0.53 (-0.32–1.37)	.213	.05
ICW (L)	Δ B–12	+0.64 (±0.22)	-0.01 (±0.19)	**0.65 (-0.05–1.24)**	**.035***	**.13**
	Δ B–24	+0.12 (±0.24)	-0.10 (±0.20)	0.22 (-0.41–0.85)	.481	.02
ECW (L)	Δ B–12	+0.44 (±0.11)	0.0 (±0.09)	**0.44 (-0.15–0.73)**	**.004***	**.23**
	Δ B–24	+0.36 (±0.12)	+0.03 (±0.11)	**0.36 (0.03–0.68)**	**.035***	**.13**

ALM = appendicular lean mass; BM = body mass (scales); FM = fat mass; TBW = total body water; ICW = intracellular water; ECW = extracellular water. Changes (Δ) between time points (B = baseline, 12 = week 12 (immediately post-supplementation); 24 = week 24 (12 weeks post-supplementation)) are presented as the adjusted mean (±SE) from ANCOVA. The between-group difference for each Δ is displayed with 95% confidence interval (CI) along and effect size, η^2: small = .01; medium = .08; large = .26; very large = .50. * *P* < .05.

Table 5.3. Changes in strength and objective physical function measures in rheumatoid arthritis patients following 12 weeks oral creatine supplementation

		Creatine (*n* = 15)	Placebo (*n* = 20)	Differences between-group for Δ		
		Mean	Mean	Mean (CI)	*P*	η^2
Strength measures						
IKES (N)	Δ B–12	+26 (±12)	+13 (±10)	13 (-19–45)	.408	.02
	Δ B–24	+34.3 (±13.7)	+0.7 (±11.8)	33.6 (-3.6–70.9)	.075[#]	.10
HGS (N)	Δ B–12	+11.0 (±6.8)	+9.1 (±5.9)	1.9 (-16.3–20.1)	.833	.00
	Δ B–24	+9.5 (±6.0)	+9.2 (±5.2)	0.3 (-15.9–16.6)	.969	.00
Objective physical function measures						
STS-30 (reps)	Δ B–12	+2.0 (±0.7)	+1.8 (±0.5)	0.2 (-1.6–1.9)	.764	.02
	Δ B–24	+2.1 (±0.7)	+2.3 (±0.6)	-0.2 (-1.9–1.4)	.856	.01
8'UG (secs)	Δ B–12	-0.44 (±0.24)	-0.25 (±0.21)	-0.19 (-0.85–0.46)	.555	.01
	Δ B–24	-0.29 (±0.30)	-0.32 (±0.26)	0.03 (-0.80–0.86)	.943	.00
50'W (secs)	Δ B–12	-0.31 (±0.23)	-0.61 (±0.20)	0.30 (-0.32–0.91)	.335	.03
	Δ B–24	-0.23 (±0.25)	-0.40 (±0.22)	0.17 (-0.50–0.85)	.606	.08
VO_2max (L/min)	Δ B–12	0.0 (±0.0)	0.0 (±0.0)	0.0 (-0.1–0.1)	.762	.00
	Δ B–24	0.0 (±0.1)	+0.1 (±0.0)	-0.1 (-0.2–0.1)	.219	.06

IKES = isometric knee extensor strength; HGS = handgrip strength; STS-30 = sit-to- stand in 30 second test; 8'UG = 8-foot up and go; 50'W = 50-foot walk; VO_2max = estimated $V0_2$max from Siconolfi step test. Changes (Δ) between time points (B = baseline, 12 = week 12 (immediately post-supplementation); 24 = week 24 (12 weeks post-supplementation)) are presented as the adjusted mean (±SE) from ANCOVA. The between-group difference for each Δ is displayed with 95% confidence interval (CI) and effect size, η^2: small = .01; medium = .08; large = .26; very large = .50. * $P < .05$; [#] $P = .05–.10$.

5.4. Discussion

Our results indicate that Cr supplementation improves body composition, specifically muscle mass, but not strength or objective physical function in patients with RA. In the current study, both ALM (+0.52 kg) and total LM, (+0.60 kg) increased following 12 weeks of Cr supplementation. Whilst there was a small and non-significant increase in FM as a consequence of Cr supplementation (0.41 ±0.45 kg), the greater gain in ALM meant that proportional muscle mass (ALM/BM%) was not diminished (27% to 28%, respectively) from baseline to week 12. The addition of LM observed in the Cr group cannot be attributed to supplementation-induced increased calorie intake. Twelve weeks of Cr supplementation resulted in an additional calorie intake of approximately 1348kcal (based on ~4kcal/g protein). Given that 1 kg FM ≈ 7700 kcal, this over nutrition would equate to a FM gain of ~0.18 kg. The difference observed in FM gain between the Cr and placebo groups was 0.23g, therefore whilst the additional calories account for the majority of the difference in FM gain, they do not account for the difference in LM (a 0.60 kg increase in the Cr group).

The magnitude of LM increase we observed is comparable to that seen previously in older men (Gotshalk et al., 2002), older women (Gotshalk et al., 2008; Gualano et al., 2014), and patients with muscle dystrophy (Tarnopolsky et al., 2004a) following Cr supplementation. The body composition changes are also similar to those we previously observed following 12 weeks of protein supplementation in RA patients (i.e. increases of 0.40 kg in ALM and 0.73 kg in total LM, whilst FM remained unchanged (Marcora et al., 2005b)). These results, together with the response to PRT (Marcora et al., 2005a; Lemmey et al., 2009), and the finding that muscle quality (i.e. maximal force exerted per unit muscle) is not impaired in RA patients (Matschke et al., 2010a, 2010b), further emphasise that RA patients are not, as once believed (Rall et al., 1996), resistant to muscle anabolic stimuli.

The changes in ALM following 12 weeks Cr supplementation were reflected in changes in body water, specifically a significant 1.08 L increase in TBW due to expansion of both ICW (0.64 L), and ECW (0.44 L) during this period. Similar changes in body water were observed in younger adults following Cr supplementation (Ziegenfuss et al., 1998; Powers et al., 2003; Francaux & Poortmans, 2006). The mechanisms by which Cr supplementation increases TBW and shifts fluid into the intracellular space are unclear (Ziegenfuss et al., 1998). However, it

has been suggested that as skeletal muscle cell Cr and PCr concentrations rise, ECW is drawn into the cell by osmosis to maintain intracellular protein concentration (Lang et al., 1998; Ziegenfuss et al., 1998; Francaux & Poortmans, 2006). The uptake of Cr into the muscle following supplementation (Greenhaff et al., 1994), and subsequent increases in mechanical stress caused by the rise in ICW have been postulated to stimulate protein synthesis (Ingwall et al., 1974), although it is unclear if Cr augments muscle protein by this mechanism (Francaux & Poortmans, 2006).

In our trial, at week 24 (i.e. 12 weeks after Cr supplementation ceased), ICW returned towards its baseline level and, over the same 'washout' period, 0.12 kg ALM and 0.38 kg total LM were lost. These reversions to, or toward, baseline over the 12 week withdrawal period, provide further evidence that the changes seen at week 12 are due to Cr supplementation. Interestingly, at week 24, despite the losses due to withdrawal of Cr, ALM and total LM were still 0.40 kg and 0.21 kg, respectively, above baseline values, suggesting some longer term retention of body composition changes following Cr supplementation.

The lack of a Cr-induced improvement in either strength or function that we observed in this study contrasts with the 14% gain in composite strength reported by Willer et al. (2000) following short-term Cr supplementation in RA patients. Similarly, improvements in both strength (IKES and HGS) and objective physical function measures, such as the 5-repetition STS and 6 metre tandem walk test, following Cr supplementation have been observed in older adults (Gotshalk et al., 2002; Brose et al., 2003; Stout et al., 2007; Gotshalk et al., 2008; Gualano et al., 2014), as well as other clinical groups such as patients with fibromyalgia (Alves et al., 2013) and muscle dystophy (Tarnopolsky et al., 2004).

However, the reported effects of Cr supplementation on measures of strength and function are equivocal. Creatine supplementation had no effect on HGS, IKES, timed 30ft walk (30'W), and a timed four step climb test (SCT) in osteoarthritic patients following surgery (Roy et al., 2005), whilst in patients with muscular dystrophy, supplementation with Cr failed to improve HGS or IKES (Walter et al., 2002; Schneider–Gold et al., 2003; Tarnopolsky et al., 2004b), or function: SCT, 30'W, and time taken to stand from supine (Tarnopolsky et al., 2004a, 2004b; Escolar et al., 2005). Furthermore, despite eliciting an increase in LM, 2 weeks of Cr supplementation did not improve ankle dorsiflexion strength in 20 HIV–positive men (Sakkas et al., 2009). Additionally, several studies in older adults (Rawson et al., 1999; Rawson & Clarkson, 2000; Jakobi et al., 2001; for review, see **Chapter 4**) found no benefit of Cr supplementation on either strength or function. Consistent with the literature, Cr

supplementation in our investigation had no effect on aerobic capacity (Kreider, 2003; Kreider et al., 2010; Alves et al., 2013).

The explanations for the lack of change in strength and objective physical function following Cr supplementation in our trial is unclear, but may be due to several factors:

- Since both groups in our trial had comparable improvements in the function tests, it suggests that, despite prior practice, performance was enhanced by a learning effect.

- The increase in LM over the 12 weeks may simply reflect an increase in ICW (i.e. muscle water content), and, as hypothesised by Sakkas et al. (2009), may not be of 'functional benefit' as protein synthesis has not yet occurred.

- As Cr supplementation primarily increases the performance of high-intensity activity lasting ~2-5 seconds (i.e. those using the ATP/PCr system), it may that the objective physical function tests used in the current trial were unable to noticeably benefit from an improvement in this system. For example, the STS-30 lasts for a period of 30 seconds. However, the one test expected to benefit most from improvments in the ATP/PCr system, the IKES which lasts for ~3 seconds, also did not improve.

Responsiveness to Cr supplementation is reported to vary, with only ~70–75% of individuals, irrespective of age, deemed to be 'responders' (Greenhaff et al., 1994; Syrotuik & Bell, 2004). The main determinant of 'responsiveness' is thought to be initial muscle Cr concentrations, as when this is high (~150 mmol· kg/dw) supplementation does not appear to augment muscle Cr stores further. (Syrotuik & Bell, 2004). Consistent with this estimation, strength increases were noted in 67% of RA patients in the Willer et al. study, and in our study, 80% of participants 'responded', when 'response'[12] was defined by an increase in ALM (range: 0.24–1.47 kg).

As the current study was a 'proof of principle' (efficacy) trial, we did not consider it appropriate to use an intention-to-treat approach (i.e. include data from patients withdrawn or whom dropped out). However, it should be noted that dropouts from the study were not treatment-related, and therefore, exclusion of these participants was unlikely to have biased our sample heavily.

[12] The remaining ~20% 'non-responders' change in ALM between Baseline and Week 12 ranged from -.43 to .05 kg.

In the current study, oral Cr supplementation was well tolerated, with high compliance and no adverse side effects. Additionally, supplementation had no effects on RA disease activity or renal function (eGFR), thus providing further evidence that supplementing with Cr is safe (Willer et al., 2000; Gotshalk et al., 2002; Francaux & Poortmans, 2006). Although the lack of effects on strength and physical function are disappointing, the increase in LM suggests that Cr supplementation may be beneficial in patients with severe RC, since a marked loss of LM both impairs the body's ability to fight infection due to limited expendable protein reserve for immune cell production, and increases the risk of mortality (Summers et al., 2008). The lack of efficacy demonstrated on physical function in this study further emphasises that sustained PRT (Marcora et al., 2005a; Lemmey et al., 2009) should be performed by RA patients wishing to substantially increase LM, and, subsequently, restore their strength and physical functioning.

5.5. Conclusion

In patients with RA, 12 weeks of oral Cr supplementation had beneficial effects on muscle mass, but not on strength or objectively-assessed physical function. Given compliance to Cr was high, and no adverse treatment related effects were observed, Cr may offer an acceptable, safe, low-cost, and reasonably effective means for RA patients with severe RC to help restore muscle mass. However, for patients wishing to improve their muscle mass, strength and physical function, PRT should be performed as an adjunct intervention option.

6

Serum biomarkers of muscle anabolism and catabolism, and systemic inflammation in rheumatoid arthritis patients: Potential markers of rheumatoid cachexia?

In collaboration with Professor Claire Stewart at Liverpool John Moore's University.

6.1. Introduction

'Rheumatoid cachexia' (RC) is the term given for the loss of lean mass (LM) and increased fat mass (FM) seen in patients with rheumatoid arthritis (RA) (Roubenoff et al., 1992; Roubenoff, 2009). The pathogenesis of this condition, and in particular the muscle loss, is complex and unresolved (Walsmith et al., 2004; Fanzani et al., 2012). Investigations into these mechanisms are often cost-intensive and invasive (e.g., muscle biopsies), creating a major difficulty in developing research-informed anti-muscle wasting therapy strategies (Palus et al., 2014). Specifically, there is a need for the identification of reliable serum-based biological markers (biomarkers) that can be easily attained and reliably detected at low cost to guide diagnosis and therapy in conditions characterised by muscle wasting (Zoico & Roubenoff, 2002; Cesari et al., 2012; Palus et al., 2014; Hofmann et al., 2015).

In this study, we assessed a variety of serum-based biomarkers potentially implicated in the pathogenesis of RA and RC. Subsequently, and in order to provide insight into disease adaptation, we conducted exploratory investigations of these biomarkers in a range of clinical scenarios including: RA patients versus matched healthy controls (HC); RA patients with untreated, uncontrolled disease versus patients with disease-modifying anti-rheumatic drug (DMARD) treated, controlled disease; 'recent-onset' versus 'established' RA; anti-TNF therapy versus standard DMARD therapy; and following two non-pharmaceutical anabolic interventions (i.e. high intensity PRT, and nutritional supplementation) used to attenuate the effects of RC. To our knowledge this is the first study to investigate a range of potential biomarkers of RC to see if consistent patterns or models emerge across a variety of clinical and anabolic scenarios.

6.2. Potential biomarkers of interest: a review

6.2.1. Overview and justification of biomarkers selected

Rheumatoid arthritis is characterised by over-expression and, subsequently, elevated concentrations of pro-inflammatory cytokines (e.g., tumor necrosis factor-α (TNF-α), interleukin (IL)-1, and IL-6) locally in the joint and systemically in the blood (Walsmith & Roubenoff, 2002; Choy, 2012; Jung et al., 2012; Shrivastava & Pandey, 2013). Aside from arthropathy, these cytokines are thought to be central to the inflammation-driven muscle loss seen in RC (Roubenoff et al., 1994; Walsmith & Roubenoff, 2002). Consequently, measurement of inflammatory markers are important in determining markers of RC.

As a result of interference from cytokines, in particular TNF-α (Roubenoff et al., 1994; Walsmith & Roubenoff, 2002; Engvall et al., 2008), RC is attributed to an imbalance of anabolic (e.g., insulin growth factor-I (IGF-I)) and catabolic (e.g., myostatin) factors (e.g., Lee & McPherron, 2001; Blackman et al., 2007; Engvall et al., 2008; Palus et al., 2014). As such, we assessed both IGF-I and myostatin concentrations. As RC is characterised by excessive adiposity, we also explored the role of two key adipokines (adiponectin and leptin) which are principally expressed by adipocytes. In recent times, these adipokines have attracted increasing interest in rheumatology due to their roles in inflammation and immune regulation (Giles et al., 2009; Neumann et al., 2011).

A figurative summary of our proposed markers and their potential roles in RC is shown in **Figure 6.1**. In the next section, these biomarkers and their response to pharmacological and non-pharmacological anabolic interventions are discussed in greater detail.

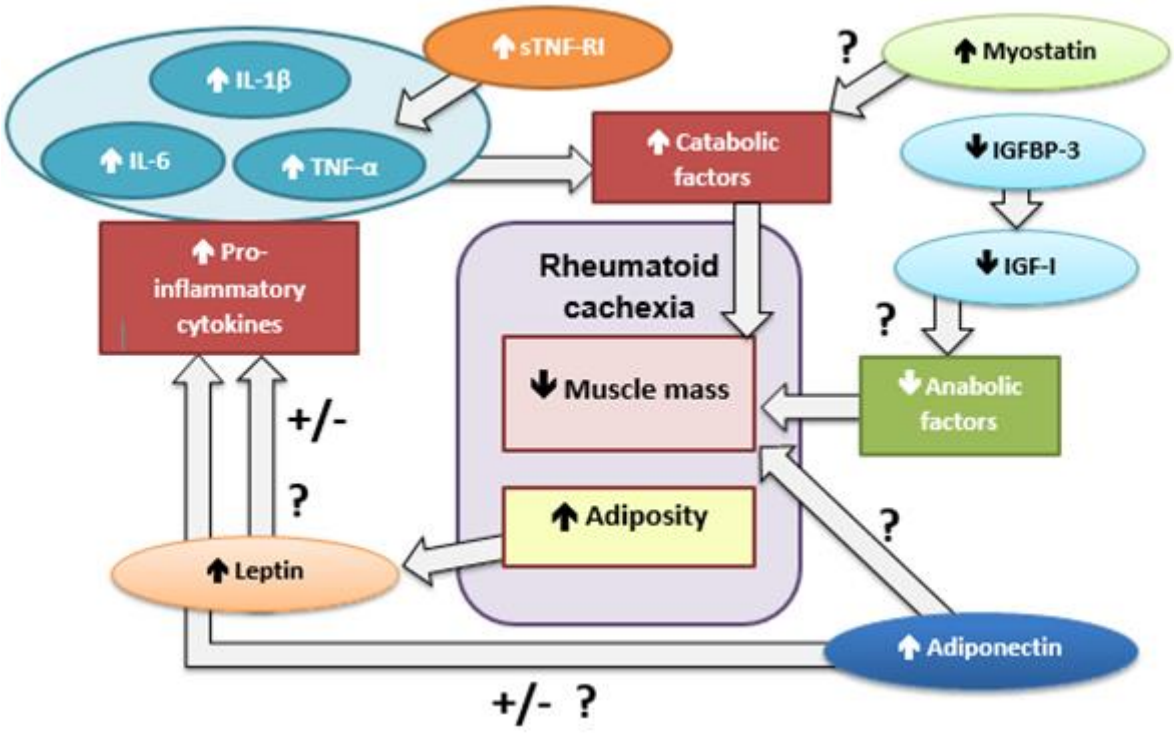

Figure 6.1. A summative figure of the complex interaction of potential cytokines and hormones in RC. ? = unknown/clear relationship in RA; +/- = inconsistent findings in regard to direction of effect.

6.2.2. Inflammatory biomarkers

6.2.2.1. Tumor necrosis factor-α (TNF-α)

The cytokine TNF-α is produced primarily by monocytes and macrophages (Shrivastava & Pandey, 2013), in addition to adipocytes and skeletal muscle (Pedersen & Febbraio, 2012). Whilst beneficial at low concentrations in RA (Feldmann & Steinman, 2005), at high concentrations, TNF-α causes excess inflammation, overproduction of other cytokines, and articular damage (Jung et al., 2012; Shrivastava & Pandey, 2013). Further, TNF-α, formerly known as 'cachectin' (because it causes cachexia (Beutler et al., 1985)), has been implicated in the muscle wasting found in chronic disease (Sherry & Cerami, 1988), and although the exact mechanisms are unclear, is believed to be the principal driver of RC (Roubenoff et al., 1992, 1994; Walsmith & Roubenoff, 2002; Walsmith et al., 2004).

6.2.2.2 Soluble tumor necrosis factor-alpha receptor-I (sTNFR-I)

Tumor necrosis factor-α binds to two high affinity cell surface receptors: TNF-RI (p55) and -RII (p75). Soluble TNF receptors I and II (sTNF-RI and II) are released by proteolytic cleavage of the extracellular domains of these transmembrane receptors (Rooney et al., 2000; Spoettl

et al., 2007). These sTNF-Rs, also known as 'TNF-binding proteins' (Björnberg et al., 1994), act as TNF-α antagonists and can inhibit TNF-α mediated pro-inflammatory effects (Hawari et al., 2004; Spoettl et al., 2007). For example, sTNF-RI binds with circulating TNF-α, competing with cell surface receptors and inhibiting its biological activity (Rooney et al., 2000). As such, in an effort to attenuate its effects, increased sTNF-RI expression often occurs in the presence of elevated TNF-α (Olsson et al., 1992; Rooney et al., 2000), and sTNF-RI have been found elevated in the serum of RA patients compared to HCs (Cope et al., 1992).

6.2.2.3. Interleukin-6 (IL-6)

Interleukin-6 is another pro-inflammatory cytokine involved in RA (Shrivastava & Pandey, 2013; Blüml et al., 2014) and potentially RC pathogenesis. Increased circulating IL-6 has been shown to be negatively correlated with LM in RA (Engvall et al., 2008) with research suggesting a role in muscle protein turnover (Zoico & Roubenoff, 2002; Bowen et al., 2015); although findings are equivocal (García-Martínez et al., 1994; Zoico & Roubenoff, 2002). When interpreting the role of IL-6, it is important to consider that it can also have anti-inflammatory effects, including TNF-α down-regulation (Dinarello & Moldawer, 2000).

6.2.3 Anabolic biomarkers

6.2.3.1. Insulin-like growth factor-I (IGF-I) and insulin-like growth factor-binding protein 3 (IGFBP-3)

Progressive decline in the secreation of growth hormone (GH) and its principal circulating and tissue mediator, insulin-like growth factor-I (IGF-1) have been identified as one of the the key pathophysiological mechanisms of muscle loss in aging (Corpus et al., 1993). Interestingly, total GH production in RA is comparable to matched non-RA controls (Rall et al., 2002; Blackman et al., 2007); thus GH deficiency is 'not the cause of RC' (Rall et al., 2002; Rall & Roubenoff, 2004).

As the anabolic mediator of GH, IGF-1 is considered the primary factor in maintaining adult skeletal muscle mass and inhibiting muscle protein degradation (Adams, 2002; Engvall et al., 2008; Schiaffino & Mammucari, 2011; Fanzani et al., 2012; Bowen et al., 2015; Sharples et al., 2015). Low circulating IGF-I concentrations have been associated with sarcopenic-related muscle loss (Corpus et al., 1993) and cachexia in malnourished older adults (Ponzer et al., 1999).

Although impaired, or reduced, systemic IGF-I function would be anticipated to exacerbate the catabolism in RA (Lemmey et al., 2001), investigations into circulating IGF-I concentrations have yielded contradictory and inconsistent results, partly due to the demographic and clinical differences in patients studied (Blackman et al., 2007). Compared to HC, both reduced (Lemmey et al., 2001; Matsumoto & Tsurumoto, 2002; Häkkinen et al., 2005; Blackman et al., 2007; Engvall et al., 2008) and normal (Rall et al., 2002; Toussirot et al., 2005) IGF-I concentrations have been reported. As GH production seems to be unaffected in RA, the reductions in IGF-1 occasionally reported (e.g., Lemmey et al., 2001) may suggest that RA patients are GH resistant or insensitive (Blackman et al., 2007).

Insulin-like growth factor-binding protein 3 (IGFBP-3) is a multifunctional protein that can mediate the effects of IGF-I on a variety of cellular functions (Baxter, 2001). As its principle carrier protein (Baxter, 2001), the bioavailability of IGF-I is moderated by IGFBP-3 concentrations (Neidel et al., 1997). Like IGF-I, contrasting levels of IGFBP-3 have been identified in the serum of RA patients (e.g., lower: Lemmey et al., 2001; Blackman et al., 2007; normal: Rall et al., 2002; Toussirot et al., 2005; and elevated: Matsumoto & Tsurumoto, 2002).

Few studies have measured the potential involvement of IGF dysregulation on muscle deficiency in RA. A recent trial by Baker et al. (2015) found low LM was associated with low serum IGF-I concentrations in RA patients. In contrast, Engvall et al. (2008) found no such association with circulating IGF-I, but found that the IGF-I/IGFBP-1 ratio (a surrogate measure of bioavailable IGF-I) was correlated with reduced LM. Given the findings of Baker et al. (2015) and Engvall et al. (2008), and the strong anabolic function of IGF-I (Adams, 2002; Hofmann et al., 2015), investigation into the utility of serum IGFs as blood-based biomarkers of RC is justified.

6.2.4. Catabolic biomarkers

6.2.4.1. Myostatin

Myostatin is a protein expressed in developing skeletal muscle (Lee & McPherron, 2001; Han et al., 2013). It is a natural candidate for a muscle atrophy biomarker (Palus et al., 2014) as it mediates catabolic signalling and is a potent negative regulator of muscle mass (Lee & McPherron, 2001; Zimmers et al., 2002; Roth & Walsh, 2004; Dankbar et al., 2011; Elkina et al., 2011; Schiaffino & Mammucari, 2011; Han et al., 2013). Circulating myostatin concentrations are inversely related with LM in the elderly (Schulte & Yarasheski, 2001;

Yarasheski et al., 2002; Léger et al., 2008) and in other conditions associated with muscle wasting including cardiac cachexia (Hoenig, 2008) and chronic obstructive pulmonary disease (Ju & Chen, 2012). Whilst myostatin has been found to be highly expressed in RA synovial tissue (Dankbar et al., 2011), no research has investigated whether systemic myostatin associates with RC.

6.2.5. Adipokines

6.2.5.1. Adiponectin

Adiponectin is predominantly involved in lipid and carbohydrate metabolism (Serelis et al., 2008; Oranskiy et al., 2012). Although adiponectin may exert an anti-inflammatory action by attenuating the immune response and reducing the secretion and activity of TNF- α and IL-6 (Wulster-Radcliffe et al., 2004; Toussirot et al., 2007; Targońska-Stępniak et al., 2010), a pro-inflammatory role has also been identified (Ebina et al., 2009; Giles et al., 2009; Oranskiy et al., 2012; Meyer et al., 2013). In RA specifically, serum (Giles et al., 2009; Giles et al., 2011; Klein-Wieringa et al., 2011; Oranskiy et al., 2012; Meyer et al., 2013) and synovial (Choi et al., 2009) concentrations of adiponectin have been associated with greater radiographic joint damage and may be related to a more aggressive disease phenotype (Baker et al., 2015).

Interestingly, elevated serum adiponectin levels have been considered a biomarker of a cachectic state in starvation (Szabó et al., 2014), elderly patients with chronic heart failure (i.e. cardiac cachexia) (McEntegart et al., 2007; Paulo Araújo et al., 2009; Loncar et al., 2013), and in patients with cancer cachexia (Wolf et al., 2006). Further, research by Baker et al. (2015) recently reported that serum adiponectin was associated with low muscle mass in RA. Whether the increased concentrations of adiponectin represent a more active, inflammatory (and thus, catabolic) disease state, or if adiponectin can directly influence the catabolic pathways (McEntegart et al., 2007; Paulo Araújo et al., 2009) responsible for RC is unclear.

6.2.5.2. Leptin

Leptin primarily regulates adipose tissue mass and energy balance (Wislowska et al., 2007) by controlling satiety (Myers, 2015). Leptin is expressed when adipocyte size increases; acting centrally as a negative feedback signal, decreasing appetite and increasing energy expenditure (Wislowska et al., 2007). As such, leptin is highly correlated with adiposity (Popa et al., 2005; Wislowska et al., 2007). Leptin release from adipose tissue is stimulated by pro-inflammatory cytokines such as TNF-α and IL-1β (Härle et al., 2006). Acting in opposition to

adiponectin (Otero et al., 2006), leptin may in turn stimulate proliferation and activation of monocytes (Härle et al., 2006) and the expression of pro-inflammatory cytokines in RA (Popa et al., 2005; Wislowska et al., 2007; Seven et al., 2009). However, findings are inconsistent with no association between leptin and disease activity reported by others (Anders et al., 1999; Bokarewa et al., 2003; Popa et al., 2005; Gunaydin et al., 2006; Allam & Radwan, 2012; Abdalla et al., 2014). Whilst the association with adiposity is well recognised, leptin's role in inflammation, and potentially muscle loss in RA, requires further investigation.

6.2.6. Effect of current pharmaceutical therapy

Rheumatoid arthritis is treated with a combination of DMARDs, primarily methotrexate (MTX); a widely-functioning immunosuppressant (Fransen et al., 2003). In patients who fail to respond to traditional DMARDs, biological agents that target specific immunological processes (e.g., TNF-α) are used (Dale & Porter, 2010; Ruderman et al., 2012). The agent etanercept (ETN), a genetically recombinant soluble TNF-α receptor protein (Fox, 2000), binds to TNF-α forming a complex that is unable to interact with the TNF receptor (Choy, 2012). Whilst ETN renders TNF-α immunologically inactive (Fox, 2000), it does not eradicate it from the tissue fluid and may even lengthen its half-life (Feldmann & Maini, 2001; Bhatia & Kast, 2007). Consequently, TNF-α concentrations are often increased following ETN treatment (e.g., Bhatia & Kast, 2007).

6.2.6.1. Effect of current pharmaceutical treatment on body composition

It was originally thought that inhibiting TNF-α activity, a proposed driver of RC, would potentially reverse, or at least attenuate, muscle loss in RA (Rall & Roubenoff, 2004). However, although successful in reducing inflammation and disease activity (Fransen et al., 2003; Nishina et al., 2013), anti-TNF-α therapy does not restore muscle (Marcora et al., 2006; Metsios et al., 2007; Serelis et al., 2008; Engvall et al., 2010; Toussirot et al., 2014), and appears to contribute to RC by exacerbating FM (Engvall et al., 2010), particularly trunk FM (Metsios et al., 2007; Toussirot et al., 2014). Recent research by our group (**Chapter 3**) found that, despite early well-controlled disease activity and inflammation characteristic of a 'treat-to-target' (T2T)) strategy, RC is still a feature of modern RA.

6.2.6.2. Effect of pharmaceutical treatment on anabolic factors

In regard to IGF status, Marcora et al. (2006) found that although 12 weeks of treatment with either ETN or MTX caused an initial increase in serum IGF-I and IGFBP-3, these returned to baseline concentrations at 24 weeks. Sarzi-Puttini et al. (2006) also found that 12 weeks of

anti-TNF-α therapy increased serum IGF-I values in corticosteroid (CS) treated RA patients, whilst Engvall et al. (2010) reported no changes in IGF-I levels following anti-TNF-α treatment or triple DMARD therapy (MTX+HCQ+SSZ) over a longer 21 month period.

6.2.6.3. Effect of pharmaceutical treatment on catabolic factors

As TNF-α primarily exerts its inflammatory and catabolic effects independent of myostatin, anti-TNF-α therapy is unlikely to affect its concentrations. A literature search for the effects of anti-TNF-α or traditional DMARD therapy (e.g., MTX) on myostatin concentrations yielded no results.

6.2.6.4. Effect of pharmaceutical treatment on adipokines (adiponectin and leptin)

Suppression of inflammation has been thought to have no effect on serum adiponectin levels (Neumann et al., 2011), however results are inconsistent. Methotrexate administration has been shown to increase (Laurberg et al., 2009) and decrease (Manrique-Arija et al., 2016) serum adiponectin levels, whilst both stable (Härle et al., 2006; Derdemezis et al., 2009; Popa et al., 2009; Gonzalez-Gay et al., 2011; Toussirot et al., 2014) and increased adiponectin levels (Komai et al., 2007; Nagashima et al., 2008; Serelis et al., 2008; Engvall et al., 2010; Cansu et al., 2011) have been reported in RA patients receiving anti-TNF-α therapy.

Manrique-Arija et al. (2016) found that 6 months of MTX treatment significantly increased blood leptin concentrations, although adiposity was also increased; it is not reported if leptin changes were associated with FM changes as would be expected. No changes in serum leptin was observed following 3–24 months of anti-TNF-α therapy (Derdemezis et al., 2009; Gonzalez-Gay et al., 2009; Popa et al., 2009; Toussirot et al., 2014), although Engvall et al. (2010) reported increased levels following 21 months treatment of infliximab.

6.2.7. Adjunct treatments that improve muscle mass in rheumatoid arthritis

Without a pharmacological means of reversing RC, potential adjunct treatments that focus on restoring muscle mass are needed (Rall & Roubenoff, 2004). Below, two different interventions and their potential effect on the biomarkers selected are outlined.

6.2.7.1. Effect of progressive resistance training

Progressive resistance training (PRT) is undoubtedly the most effective means of reversing the effects of RC (e.g., Häkkinen et al., 2005; Marcora et al., 2005a; Lemmey et al., 2009; Lemmey, 2011). It has been suggested that exercise and PRT may have an anti-inflammatory effects via the reduction of pro-inflammatory cytokine activity (e.g., Petersen & Pedersen, 2005; Kadoglou et al., 2007). However, the exact effects are unclear, with studies, including one in RA (Rall et al., 1996), finding no effect of exercise on TNF- α, IL-1, or IL-6 concentrations (e.g., Conraads et al., 2002; Bruunsgaard et al., 2004; Bautmans et al., 2005; Kelley & Kelley, 2006; Olson et al., 2007; De Salles et al., 2010).

Despite significant LM increases in RA patients following PRT, both Lemmey et al. (2009) and Häkkinen et al. (2005) demonstrated no change in serum IGF-I or IGFBP-3 concentrations. This absence of a change in circulating IGFs is consistent with obsrevations in healthy elderly participants following PRT (e.g., Kraemer et al., 1999; Häkkinen et al., 2005). Conversely, intramuscular levels (mIGF-I and mIGFBP-3) are significantly increased following PRT (Lemmey et al., 2009). The absence of serum IGF-I change with training suggests circulating, predominantly liver-derived, IGF-I in the blood may be of 'minor or only transitory, noncumulative importance' in muscle hypertrophy (Walker et al., 2004) and, as such, may not be an appropriate marker of muscle metabolism (Kraemer et al., 1999; Adams, 2002), particularly following anabolic stimuli such as PRT.

The effects of PRT on serum myostatin levels are inconsistent. Whilst serum myostatin concentrations were found to be decreased following 8 weeks (Saremi et al., 2010) and 10 weeks PRT (Walker et al., 2004) in healthy untrained males, they were increased after 12 weeks of PRT by two studies by Willoughby (2004a; Willoughby & Taylor, 2004b).

Although some researchers have found that PRT increased serum adiponectin concentrations in healthy individuals (Olson et al., 2007; De Salles et al., 2010), including the elderly (Fatouros et al., 2005; Brooks et al., 2007), findings are variable (Lee & Kwak, 2014) and are influenced by training duration and intensity (De Salles et al., 2010). Adiponectin increases following PRT have also been attributed to an enhancement of insulin sensitivity (Fatouros et al., 2005), as well as a reduction in FM that often accompanies training (Giles et al., 2009; Oranskiy et al., 2012)). Reductions in serum leptin have been reported following exercise training in the elderly (Kohrt et al., 1996; Fatouros et al., 2005; De Salles et al., 2010), although these changes also appear to be largely a function of reduced adiposity (Kohrt et al., 1996).

6.2.7.2. Effect of oral creatine supplementation

Anabolic nutritional supplementation offers a potential adjunct treatment that is easily administered, inexpensive, and, compared to PRT, makes limited demands of the patient. Creatine monohydrate (Cr) supplementation has favourable effects on both LM and physical function in healthy adults (Nissen & Sharp, 2003), the elderly (e.g., Rawson et al., 1999; Rawson & Clarkson, 2000; Gotshalk et al., 2002; 2008), and clinical populations (e.g., Louis et al., 2003; Leader et al., 2009). However, its efficacy in RA is inconclusive (Willer et al., 2000; **Chapters 4** and **5**).

Although limited, some research has found Cr supplementation reduces post-exercise inflammation (Santos et al., 2004; Bassit et al., 2008). Specifically, Cr supplementation attenuated increases in TNF-α (Santos et al., 2004; Bassit et al., 2008), IL-1β (Bassit et al., 2008), and CRP (Deminince et al., 2013) in trained athletes after long and short distance running. Whilst these effects were identified in healthy athletes, if confirmed to be generalisable, these findings potentially have favourable implications for RA where elevated levels of these cytokines may drive RC (Roubenoff et al., 1994, 2009).

Although studies have shown that Cr may increase expression of mIGF-I (Deldicque et al., 2005, 2008; Burke et al., 2008), these trials included concurrent PRT making it difficult to isolate the effect of Cr *per se*. Whether these IGF-I increases are observable in serum concentrations is unknown. Similarly, whilst Cr supplementation following PRT has been shown to reduce myostatin concentrations (Saremi et al., 2010; Schiaffino et al., 2013), it is unknown if Cr *per se* is responsible. A literature search for the effects of Cr supplementation on adiponectin and leptin generated no results.

6.2.8. Aims and hypothesis

The aim of the following study was to investigate a comprehensive range of serum-based markers of RC in order to identify key biomarkers for future application in RA. Principally, we set out to explore putative muscle anabolism and catabolism, and inflammatory biomarkers in a range of clinical scenarios.

6.2.8.1. Disease states and the effects of pharmaceutical DMARD treatment

- ***(1a) Rheumatoid arthritis patients versus sedentary healthy controls*** – We hypothesised that RA patients would have higher serum concentrations of inflammatory (TNF-α, sTNF-RI, IL-6) and catabolic (myostatin) markers, lower concentrations of anabolic markers (IGF-I and IGFBP-3), and higher levels of adipokines (adiponectin and leptin) than age- and sex-matched HC.

- ***(1b) 'Recent-onset' versus 'established' disease*** – In this sub-analysis of **Chapter 3** RA patients described in (1a), as disease activity will be well controlled, and that body composition changes have already occurred prior to disease diagnosis (see **Chapter 3** for discussion), we hypothesised no differences in demographic, disease activity (DAS28), systemic inflammation (CRP), or body composition between 'recent-onset' versus 'established' disease cohorts. Accordingly, we hypothesised no differences in any of the assessed serum biomarkers.

- ***(2a) Untreated, uncontrolled disease versus treated, controlled disease*** – We hypothesised that:

- Treatment initiation with MTX or ETN would reduce levels of the inflammatory markers of CRP and IL-6. Whilst we expect MTX to reduce TNF-α concentrations, an increase is expected following ETN treatment. Despite this hypothesised increase in TNF-α, as the TNF-α is made biologically inactive by ETN, we would anticipate no changes in sTNF-RI.

- Treatment would have no effect on myostatin, or IGF-I and IGFBP-3. Adipokine, adiponectin and leptin, concentrations may decrease and increase, respectively, due to elevated FM following treatment.

- ***(2b) Etanercept versus methotrexate therapy*** – We hypothesised that:

- Both treatments would decrease inflammatory markers, but would have no effect on anabolic markers or myostatin.

- Adiponectin and leptin concentrations would decrease and increase, respectively, consistent with concurrent treatment-induced increases in adiposity.

6.2.8.2. Effects of non-pharmaceutical anabolic interventions

- ***(3) Effect of progressive resistance training*** – As serum markers may not be a true indicator of intramuscular metabolism, we hypothesised that PRT would have no effect on serum levels of pro-inflammatory[13], anabolic (IGF-I and IGFBP-3), or catabolic (myostatin) markers. In contrast, we anticipated adiponectin and leptin concentrations would increase and decrease, respectively, consistent with concurrent exercise-induced decreases in adiposity.

- ***(4) Effect of oral creatine supplementation*** – Owing to limited research on Cr supplementation and inflammatory processes, and the insensitivity of biomarkers in assessing intramuscular changes in muscle metabolism, we hypothesised that Cr supplementation would have no effect on any of the inflammatory, anabolic, or catabolic markers. As Cr supplementation does not affect adipose tissue, no changes are hypothesised in adiponectin and leptin concentrations.

[13] Unfortunately, CRP was not measured in Lemmey et al. (2009) and therefore is not presented for this analysis.

6.3. Patients and methods

6.3.1. Participants

Data was derived from trials conducted by the Rehabilitation of Musculoskeletal Disorders with Exercise Sciences (ReMeDES) group over an approximate ten-year period (~2005–2015). All participants were 18 years and older, not pregnant, and were not taking part in regular high-intense physical activity when recruited. All patients fulfilled the ACR/EULAR 1987 and/or 2010 criteria for RA (Aletaha et al., 2010). Eligible age- and sex-matched HC participants with no history of chronic disease were recruited from the local community. Each study was granted approval by the North West Wales Research Ethics Committee, and written informed consent was obtained from all participants (*n* = 134[14]). In order to investigate the effects of different treatment strategies, disease states, and anabolic interventions on the range of biomarkers, several scenarios were investigated:

6.3.1.1. Disease states and the effects of pharmaceutical DMARD treatment

- ***(1a) Rheumatoid arthritis patients (n = 32) versus sedentary healthy controls (n = 39)***

Samples from 32 RA patients and 39 HC were taken during the **Chapter 3** cross-sectional trial. As a sub-analysis ***(1b)***, the differences between 'recent-onset' (≤12 months diagnosis; *n* = 13) and 'established' (1-7 years diagnosis; *n* = 19) disease cohorts were also investigated.

- ***(2a) Untreated, uncontrolled disease versus treated, controlled disease (n = 24)***

Baseline samples from the Marcora et al. (2006) investigation into the comparative effects of 12 weeks ETN or MTX treatment on body composition in treatment-naïve RA patients were used to compare the serum biomarker status of RA patients before and after initiation of treatment. As a sub-analysis ***(2b)***, we investigated the relative effects of MTX (*n* = 12) and ETN (*n* = 12) treatment.

[14] All (*n = X)* refers to the number of samples that were available to be analysed from each study, and not the total number of participants that were recruited and participated in each of the trials.

6.3.1.2 Effects of non-pharmaceutical anabolic interventions

- ***(3) Effect of progressive resistance training (n = 19)***

To investigate the effects of 24 weeks PRT on serum biomarkers, pre- and post-intervention samples were taken from RA patients who were part of the Lemmey et al. study (2009). Samples included patients randomised into PRT (*n* = 10) or low-intensity range-of-movement home exercise (control) (*n* = 9) groups.

- ***(4) Effect of oral creatine monohydrate supplementation (n = 20)***

To investigate the effects of nutritional Cr supplementation on serum biomarkers, pre- and post-intervention samples were taken from the trial described in **Chapter 5**. Samples included patients randomised into Cr (*n* = 9) or placebo (control) (*n* = 11) groups.

6.3.2. Serum preparation

For each of the studies, fasted overnight blood samples were harvested by venipuncture from the median cubital vein. After collection, blood was allowed to clot by leaving it undisturbed on ice for ~30 minutes. Once clotted, blood was spun at 3000 rpm for 10 minutes at 4°C (Eppendorf centrifuge 5810 R, Germany). The resulting liquid supernatant (serum) was aliquoted into Eppendorf tubes using a Pasteur pipette. The serum was apportioned into 3 x 1.0 ml and 2 x 0.5 ml quantities, with any remaining sample distributed as 200 µl samples. Samples were frozen at –80°C prior to delivery to Liverpool John Moore's University for analysis.

6.3.3. Assay outcome measures and methods

6.3.3.1. Serum biomarkers and assay procedure

Eight biomarkers (TNF-α, sTNF-RI, IL-6, myostatin, IGF-I, IGFBP-3, adiponectin, and leptin) were analysed using a 'sandwich' enzyme-linked immunosorbent assay (ELISA) technique (R&D Systems, Minneapolis, USA). In a 'sandwich' ELISA, a monoclonal antibody specific for the biomarker is pre-coated onto a microplate[15]. Standards and samples were pipetted into the wells of the microplate and any biomarker present was bound by the pre-coated

[15] See **Appendix D** for full individual detailed assay procedures including specific diluent, sample quantities, and intra-assay co-efficient of variations.

immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for the biomarker was added to the wells – this 'sandwiched' the biomarker between the antibodies. Following another wash to remove any unbound antibody-enzyme reagent, a substrate solution was added which resulted in a colour development in proportion to the amount of biomarker bound in the initial step. The colour development was stopped and the intensity (i.e. optical density; OD) was measured (**Figure 6.2.**).

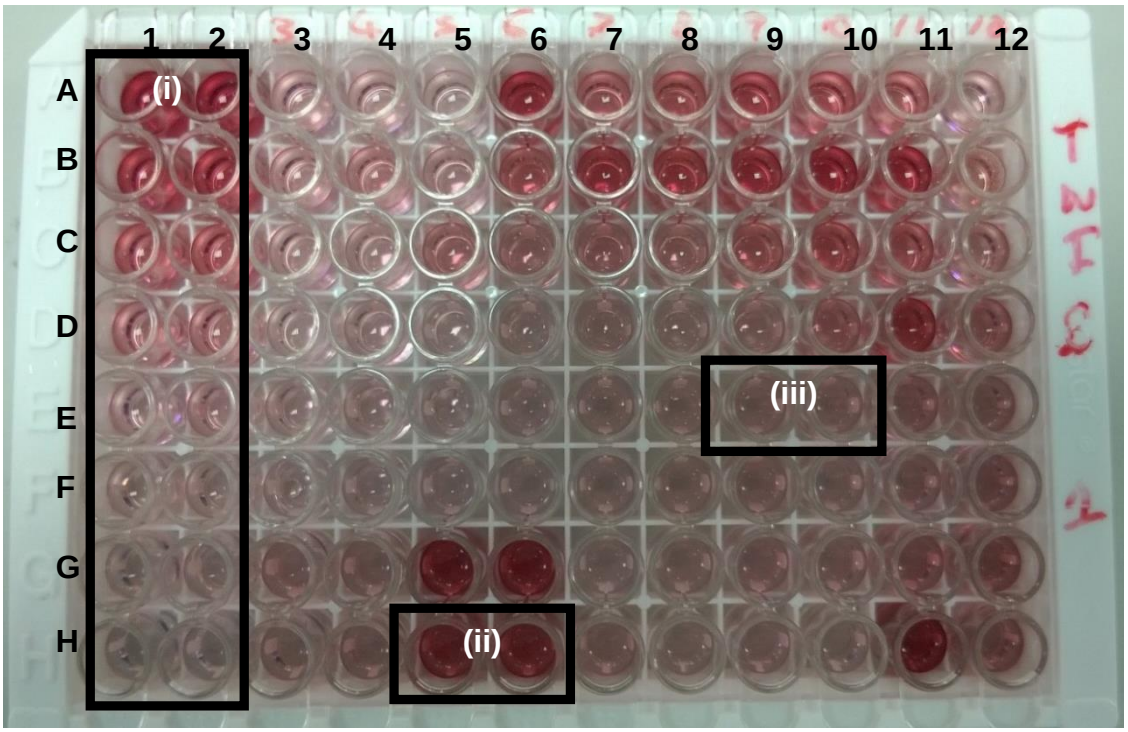

Figure 6.2. A fully developed microplate (TNF-α shown as an example). Standards are shown in **box i**. The highest (32.00 pg/ml) (and darkest) standard is at the top (A1-2), and the lowest (0.00 pg/ml) standard at the bottom (H1-2). Columns 3-12 show the participant samples in duplicate. For example, H5-6 **(box ii)** shows relatively high TNF-α levels (darker colour), and E9-10 **(box iii)** show a relatively low TNF-α concentration (clearer colour).

6.3.3.2. Assay preparation and equipment

Reagents, samples, and working standards were prepared in line with the manufacturer's protocols detailed in the instructional inserts. All samples, including standards, were analysed in duplicate in a 96-well microplate. To read the samples, the microplate reader (CLARIOstar, BMG LABTECH, Germany) was set to the appropriate wavelength (typically 450 nm, with a λ correction of 540 or 570 nm, although this varied per marker (see **Appendix D**)). The OD was determined <30 minutes of assay completion, and a curve was generated (using four parameter log/log regression) from the standards. From this curve, biomarker values were ascertained by the microplate reader's data analysis software (MARS, V3.10 R2, BMG LABTECH, Germany).

6.3.3.3. Routine clinical disease activity measures

Routine disease activity measures (Disease Activity Score in 28 joints (DAS28) and CRP) are presented. Elevated serum CRP is part of the acute phase response and is used as a standard marker of systemic inflammation (Shrivastava & Pandey, 2013).

6.3.3.4. Anthropometric measurements and body composition measures

Body mass (BM) was recorded in accordance with routine procedures (Eston & Reilly, 2009). To facilitate understanding of participant's relative anabolic and catabolic state, body composition (dual energy X-ray absorptiometry (DXA)) data is presented. These measures include: appendicular LM (ALM; a surrogate measure of muscle mass (Kim et al., 2002)), ALM% (ALM/BM%), total FM, and body fat percentage (BF%). For the purpose of this investigation, RC refers to measures of muscle mass and FM. A Hologic QDR Discovery 45615 (software V12.4) DXA scanner was used in the cross-sectional **(Chapter 3)** and Cr supplementation trials **(Chapter 5)**, whilst a Hologic QDR1500 (software V5.72) scanner was employed in the Lemmey et al. (2009) and Marcora et al. (2006) studies.

6.3.4. Statistical analysis

All data are presented as mean (±SD) unless otherwise stated. Chi-squared tests were used for dichotomous variable comparison. Variables were checked for multi-collinearity, uni- and multi-variate outliers (Mahalanobis Distance), and normal distribution using Shapiro-Wilk tests. Where necessary, data was logarithm transformed to obtain normal distribution (RA versus HC: TNF-α, IL-6, leptin; ETN versus MTX: sTNF-α, IL-6, leptin). Data analyses to compare two distinct groups (e.g., RA versus HC, 'recent' versus 'established') involved univariate analysis of variance (ANOVA). Consistent with the original analysis from Marcora et al., a 2 x 2 repeated measures ANOVA was used to detect an effect of treatment (ETN versus MTX) (treatment x time interaction). If an interaction was identified, post-hoc tests were used to identify within group differences. Due to its similar pre-post nature, this analysis method was used for the PRT and Cr supplementation data. Subsequently, using the 'time effect' output from the 2 x 2 ANOVA analysis (ETN versus MTX), we were able to ascertain the difference between untreated and treated states as this 'time effect' provides pooled data from both treatment arms at time point 1 and time point 2 (i.e. untreated and treated).

Significance was set at $P < .05$ and a trend was recognised as $P = .05–.10$. An effect size (ES) (η^2: small ≥.01; medium ≥.08; large ≥.26; very large ≥.50) was calculated to support interpretation of differences. Confidence intervals (95% CI) are reported when appropriate, and Pearson product–moment correlation (bivariate and partial) was used to test relationships (*r*) considered of interest by the researchers.

6.3.4.1. Treatment of missing or unknown data

During analysis, TNF-α plates 2, 4, and 5 standards were distorted. With no procedure (known to the researchers) to manually alter the standards once in the data analysis software, these values were unattainable. To overcome this, we used an online application (elisaanalysis.com, LTG Ventures Pty, 2015) to fit an ELISA curve to our raw OD values. By averaging the standards from plates 1 and 3, we were able to attain an appropriate mean set of standards. Using the application, missing TNF-α values could be determined. In order to assess the accuracy, we compared plate 1 TNF-α values from the data analysis software to the application values using a paired samples *t*-test and Pearson correlation. The values were comparable and deemed acceptable by the researchers (*t*-test: data analysis software TNF-α mean: 5.74 (±5.82) pg/mL; online application mean: 5.95 (±6.15) pg/mL; $P = .468$; correlation: $r = .956$, $P < .001$).

In patients being treated with ETN, all 'post-treatment' TNF-α values increased above the concentration of the top standard. When we applied the raw data to the online analysis tool, this too was unable to determine these exceedingly high values. Subsequently, a curve was created on Microsoft Excel using the standards from plates 1 and 3 (as previously described). Using a standard linear regression line ($y = 25.082x–8.4573$, $R^2 = 0.9506$), we were able to determine these missing values.

6.4. Results

6.4.1. Disease states and the effects pharmaceutical DMARD treatment

6.4.1.1. Rheumatoid arthritis patients versus sedentary healthy controls (1a)

Consistent with **Chapter 3**, there were no differences in age (*P* = .242) or gender (*P* = .658) between the RA patients and HC (**Table 6.1.**). Overall mean disease duration for the patients was 28.0 (±21.7) months; ~2.3 years. Compared to HC, RA patients had aberrant body composition with relative muscle mass (i.e. ALM%) deficient by 8% (*P* = .121, η^2 = .03, small), and total FM (21%) and BF% (13%) significantly higher (*P* = .016, η^2 = .08, medium; and *P* = .040, η^2 = .07, small, respectively) (body composition data for this subset of **Chapter 3** cohorts can be found in **Appendix E**).

Disease activity and serum biomarker data is presented in **Table 6.1**. Compared to HC, serum markers of inflammation, TNF-α (*P* = .002, η^2 = .13, medium) and IL-6 (*P* < .001, η^2 = .28, large), were elevated in the RA group by 44 and 81%, respectively. Consistent with the elevation in TNF-α, concentrations of sTNF-RI were also raised in the RA group (by 26%, *P* = .001, η^2 = .20, medium). There were no differences in myostatin, IGF-I, IGFBP-3, or adiponectin concentrations[16] (*P*'s = .284 –.854). Patients with RA had greater leptin levels (*P* = .047, η^2 = .06, small), although when FM was used a co-variant, the estimated difference was negligible (*P* = .971).

Full correlational analysis for RA and HC groups is shown in **Table 6.2.** With regard to the association between serum biomarkers, and body composition: in the RA group there were no correlations between TNF-α and measures of muscle mass: ALM (*r* = -.202, *P* = .268) and ALM% (*r* = -.266, *P* = .141). There were also no correlations between CRP and ALM (*r* = .168, *P* = .388) or ALM% (*r* = -.205, *P* = .278), or between IL-6 and ALM (*r* = .230, *P* = .206) or ALM% (*r* = -.040, *P* = .829).

[16] Difference in adiponectin between RA and HC remained insignificant (*P* = .593) when FM was used as co-variant.

Table 6.1. Basic demographics, disease activity, and serum biomarkers of rheumatoid arthritis patients and sedentary age- and sex-matched healthy controls

	Normal range [a]	RA (*n* = 32)	HC (*n* = 41)	Absolute difference (CI) *(%)*	*P*	η^2
Demographics						
Age (years)	-	62.1 (±7.2)	59.6 (±10.2)	↔ 2.5 (-1.6–6.6) *(4)*	.242	-
Sex (female *n*;%)	-	20 (62.5)	27 (65.8)	-	.658	-
Disease activity						
DAS28 (1-10)	-	2.9 (±0.8)	-	-	-	-
CRP (mg/L)	-	8.5 (±7.0)	-	-	-	-
Biomarkers						
TNF-α (pg/mL)	0.55–2.82 (Δ 1.21)	3.66 (±2.34)	2.06 (±2.26)	↑ **1.60 (0.50–2.69)** ***(44)***	**.002***	**.13**
sTNF-RI (pg/mL)	749–1966 (Δ 1198)	1467.0 (±472.1)	1079.7 (±306.7)	↑ **387.3 (200.8–573.8)** ***(26)***	**.001***	**.20**
IL-6 (pg/mL)	<3.13	6.73 (±6.57)	1.29 (±1.31)	↑ **5.45 (3.30–7.60)** ***(81)***	**<.001***	**.28**
Myostatin (pg/mL)	1264–8588 (Δ 4206)	1913.6 (±784.9)	2095.6 (±788.4)	↔ 181.9 (-199.5–563.3) *(10)*	.344	.02
IGF-I (ng/mL)	40–258 (Δ 105)	107.1 (±36.6)	105.6 (±32.5)	↔ 1.5 (-14.6–17.6) *(1)*	.854	.00
IGFBP-3 (ng/mL)	835–3778 (Δ 2375)	3072.9 (±836.9)	2873.6 (±739.0)	↔ 199.3 (-169.1–567.7) *(7)*	.284	.02
Adiponectin (ng/mL)	865–21424 (Δ 6641)	13303.0 (±6850.7)	14694.4 (±6284.2)	↔ 1292.3 (-4435.4–1850.8) *(9)*	.415	.01
Leptin (pg/mL)	2205–11149 (Δ 4760)	18840.4 (±25758.28)	9861.1 (±9304.8)	↑ **8979.3 (-326.3–17632.3)** ***(48)***	**.042***	**.06**

Group means (±SD) with 95% confidence intervals (CI) reported for the difference. Data was analysed using analysis of variance. RA = Rheumatoid arthritis; HC = Healthy controls; TNF-α = Tumor necrosis factor-α; sTNF-RI = Soluble tumor necrosis factor-α receptor-1; IL-6 = Interleukin-6; IGF = Insulin-like growth factor; IGFBP-3 = Insulin-like growth factor binding protein-3. [a] = as provided by manufacturer. * $P < .05$; Effect size (η^2), small = ≥ .01; medium = ≥ .08; large = ≥ .26; very large = ≥ .50.

Table 6.2. Correlations between primary outcome measures: rheumatoid arthritis patients versus sedentary age- and sex-matched healthy controls

Rheumatoid arthritis (*n* = 32)

	sTNF-RI	IL-6	Myostatin	IGF-I	IGFBP-3	Leptin	Adiponectin	DAS28	CRP	ALM	ALM%	FM	BF%	BM
TNF-α	-.206	.003	.037	-.286	-.067	.005	.284	-.122	-.144	-.202	-.266	.069	.146	-.048
sTNF-RI		**.384***	.220	.209	-.161	**.481***	-.242	-.009	.340#	.300#	-.104	.319#	-.094	**.396***
IL-6			-.141	-.099	-.346	-.008	-.318#	.202	.230	.116	-.040	.191	.232	-.040
Myostatin				.198	-.141	**.517***	-.051	-.240	-.107	**.360***	.058	.328#	-.127	**.401***
IGF-I					**.533***	.049	-.127	.244	.072	.120	.289	-.170	-.399#	-.065
IGFBP-3						-.060	.063	.335#	-.096	**-.463***	-.049	-.232	.047	**-.417***
Leptin							-.269	.016	.237	.193	**-.557***	**.829***	**.440***	**.698***
Adiponectin								**-.388***	-.240	**-.503***	-.028	-.278	-.053	**-.489***
DAS28									.195	-.120	-.005	-.103	.074	-.116
CRP										.164	-.205	.299	.157	.315#
ALM											**.411***	.259	-.319	**.716***
ALM%												**-.737***	**-.823***	-.325#
FM													**.700***	**.854***
BF%														.301

Healthy control (*n* = 39)

TNF-α	-.125	.050	-.132	-.102	.103	-.185	.188			-.134	-.233	-.071	.027	.189
sTNF-RI		.137	.103	-.125	-.203	-.112	**-.326***			.173	.039	-.031	-.136	.137
IL-6			-.126	**-.351***	-.243	-.115	-.032			.057	-.012	-.004	-.055	.119
Myostatin				.270#	.116	.023	.014			.310#	.115	.013	-.188	.284#
IGF-I					**.480***	.230	.160			.234	.241	.216	.064	.007
IGFBP-3						.226	.252			-.205	.119	.057	.151	**-.442***
Leptin							.055			**-.331***	-.071	**.801***	**.788***	**-.348***
Adiponectin										-.305#	-.232	.058	.233	-.093
ALM											**.752***	-.142	**-.628***	**.362***
ALM%												.054	**-.334***	**-.330***
FM													**.848***	-.238
BF%														**-.395***

Data presented as *r* value. TNF-α = Tumor necrosis factor-α; sTNF-RI = Soluble tumor necrosis factor-α receptor-1; IL = Interleukin; IGF = Insulin-like growth factor; IGFBP-3 = Insulin-like growth factor binding protein 3; DAS28 = Disease activity score in 28 joints; CRP = C-reactive protein (both DAS28 and CRP data only available for RA group; ALM

= Appendicular lean mass; FM = Fat mass; BF% = Body fat percentage; BM = Total body mass (on scales). * $P < .05$; # trend ($P \geq .05–.10$) (significant positive and negative relationship).

There was a positive correlation between myostatin and ALM in both the RA (r = .360, P = .047) and HC (r = .310, P = .051). Conversely, adiponectin was inversely correlated with ALM (r = -.503, P = .003) and BM (r = -.489, P = .005) in the RA group. Interestingly, adiponectin was also moderately and inversely associated with ALM in the HC (r = -.305, P = .055) (however BM was not; r = -.093, P = .570). Higher concentrations of IGFBP-3 were also correlated with lower levels of ALM (r = -.463, P = .008) and BM (r = -.417, P = .017) in the RA group. Whereas only BM (r = -.442, P = .004), and not ALM (r = -.205, P = .206), was correlated with IGFBP-3 in the HC. Leptin was highly correlated with adiposity in the RA patients (FM: r = .829, P <.001, and BF%: r = .440, P = .035), and as a consequence, BM (r = .698, P <.001). Similar significant correlations were seen in the HC (**Table 6.2.**).

From a disease perspective, there was a negative correlation between adiponectin and disease activity (DAS28) (r = -.388, P = .034), and a weak association between adiponectin and IL-6 (r = -.318, P = .076). No correlation was seen between adiponectin and TNF-α (r = .284, P = .116). No correlations were observed in the RA patients between leptin (with FM controlled for in a partial correlation) and DAS28 (r = .184, P = .340); IL-6 (r = -.207, P = .273); TNF-α (r = -.090, P = .637), or disease duration (r = .023, P = .905). There was a weak relationship between IGFBP-3 and DAS28 (r = .335, P = .071).

6.4.1.2. 'Recent-onset' and 'established' disease (1b)

Mean disease duration for the 'recent-onset' RA cohort was 6.9 (±2.6) months, and 41.3 (±17.4) months for the 'established' RA group (P <.001). There were no differences in age, sex (*P's* = .801–.926, **Table 6.3.**), or body composition (*P's* = .422–.926, **Appendix E**). Differences in disease activity and biomarkers are shown in **Table 6.3**. Compared to the 'recent-onset' group, patients with 'established' disease had a lower DAS28 score (P = .043, η^2 = .14, medium) but similar CRP levels (P = .911, η^2 = .00). There were no differences in TNF-α, sTNF-RI, IL-6, IGF-I, IGFBP-3, adiponectin, or leptin measures (*P's* = .130–.744). Myostatin appeared elevated in the 'established' patients (P = .077, η^2 = .10, medium), but remained within the 'normal range' provided by the manufacture.

6.4.1.3. Untreated, uncontrolled disease versus treated, controlled disease (2a)

The collective group (n = 24) consisted of 18 (75%) females, and the mean age was 51.8 (±13.0) years. Whilst there was no change in ALM (P = .102, η^2 = .12, medium), measures of BM, FM, and BF% (*P's* = .050–.096) were moderately increased following treatment (**Appendix F**).

Table 6.4. shows the effects of treatment on disease activity and serum biomarkers. Both DAS28 (P <.001, η^2 = .75, very large) and CRP (P = .002, η^2 = .36, large) decreased significantly by 52 and 47%, respectively. Although overall TNF-α concentration was increased following treatment (P = .037, η^2 = .18, medium), further examination revealed that this response was isolated to the ETN group (see **6.4.1.4.**). Increases in myostatin (P = .030, η^2 = .21, medium), IGFBP-3 (P = .034, η^2 = .19, medium), and leptin (P = .012, η^2 = 26, large) with control of disease were also observed. In contrast, IL-6 level was reduced (P = .021, η^2 = .22, medium), with change correlated with the concurrent reduction in CRP (r = .596, P = .002). The increase in leptin was correlated with the increase in FM (r = .554, P = .005), and as previously noted, this became non-significant when FM was used as co-variant (data not shown). Increases in IGFBP-3 were not correlated with TNF-α (r = 0.13, P = .952). No changes were seen in the other biomarkers (sTNF-RI, IGF-I, or adiponectin, P's = .223–.734).

In a state of high disease activity (i.e. untreated disease) (mean DAS28: 6.0 (±0.9)), similar to the cross-sectional RA patients (see **6.3.1.1.**), there was no correlation between TNF-α and measures of muscle mass including ALM (r = .042, P = .854) and ALM% (r = -.281, P = .206). There were also no correlations between CRP and ALM (r = -.180, P = .401) or ALM% (r = -.280, P = .185), or IL-6 and ALM (r = -.229, P = .282) or ALM% (r = -.090, P = .676). Again, there was a significant positive correlation with myostatin and ALM (r = .705, P < .001) in the RA group.

6.4.1.4. Etanercept versus methotrexate therapy (2b)

There was no difference in age between the two treatment groups (ETN: 53.7 (±10.9) versus MTX: 49.8 (±15.0) years, P = .482, η^2 = .02, small), with an equal female distribution (n = 9/12, 75% in each). No significant treatment x time interactions were observed for any body composition variables (**Appendix G**), however, there were moderate time main effects for BM (P = .093, η^2 = .12, medium), FM (P = .053, η^2 = .16, medium), and BF% (P = .069, η^2 = .14, medium). Post hoc tests revealed moderate FM (P = .099) and BF% (P = .065) increases in the MTX group.

Whilst no significant treatment x time interactions were observed for DAS28 and CRP (**Table 6.5.**), there were large main effects for time (**Table 6.4.**). As well as a time main effect (P = .037, η^2 = .18, medium), there was also a significant treatment x time interaction (P = .039, η^2 = .18, medium) for TNF-α, with post hoc tests revealing a 67% increase (P = .035) following ETN treatment. Time main effects were observed for IL-6 and myostatin, with post hoc tests revealing decreases in IL-6 for both the MTX (P = .011) and ETN groups (P = .095). Myostatin was reduced in the MTX group (P = .046).

A time main effect was identified for IGFBP-3 (P = .034, η^2 =.19, medium), with post hoc analysis showing a non-significant increase in the ETN group (P = .085). There was a time main effect for leptin (P = .012, η^2 = .26, medium), and post hoc tests revealed, although leptin concentrations increased in both groups, a trend in the MTX group only (P = .085). No other main effects were seen (sTNF-RI, IGF-I, or adiponectin).

Table 6.3. Basic demographics, disease activity, and serum biomarkers between 'recent-onset' (<12 months disease duration) and 'established' (≥12 months disease duration) rheumatoid arthritis patients

	'Recent-onset' (*n* = 13)	'Established' (*n* = 19)	Absolute difference (CI) *(%)*	*P*	η^2
Demographics					
Age (years)	59.0 (±10.8)	60.0 (±10.0)	↔1.0 (-6.6–8.5) *(2)*	.801	-
Sex (female *n*;%)	8 (61.5)	12 (63.2)	-	.926	-
Disease activity					
DAS28 (1-10)	3.2 (±0.9)	2.6 (±0.6)	↑ **0.6 (0.0–1.1)** ***(19)***	**.043***	**.14**
CRP (mg/L)	8.3 (±5.8)	8.6 (±8.0)	↔ 0.3 (-5.1–5.7) *(4)*	.911	.00
Biomarkers					
TNF-α (pg/mL)	3.11 (±2.07)	5.20 (±5.55)	↔ 2.09 (-1.21–5.39) *(40)*	.205	.05
sTNF-RI (pg/mL)	1610.5 (±554.1)	1355.0 (±377.4)	↔ 255.6 (-79.8–591.1) *(16)*	.130	.08
IL-6 (pg/mL)	8.69 (±7.25)	5.47 (±5.71)	↔ 3.22 (-1.47–7.90) *(37)*	.171	.06
Myostatin (pg/mL)	1661.3 (±773.6)	2241.4 (±932.7)	↓ 580.1 (-67.9–1228.1) *(27)*	.077[#]	.10
IGF-I (ng/mL)	113.0 (±38.2)	103.1 (±35.9)	↔ 9.9 (-17.2–36.9) *(9)*	.462	.02
IGFBP-3 (ng/mL)	3186.3 (±969.3)	2995.3 (±751.1)	↔ 191.0 (-430.3–812.2) *(6)*	.535	.01
Adiponectin (ng/mL)	11255.8 (±5850.2)	14654.7 (±7106.0)	↔ 3398.9 (-1476.5–8274.2) *(23)*	.165	.06
Leptin (pg/mL)	17418.0 (±14278.3)	19813.6 (±31690.7)	↔ 2395.6 (-16831.3–21622.5) *(12)*	.744	.00

Group means (±SD) with 95% confidence intervals (CI) reported for the difference. Data was analysed using analysis of variance. DAS28 = Disease activity score in 28 joints; CRP = C-reactive protein; TNF-α = Tumor necrosis factor-α; sTNF-RI = Soluble tumor necrosis factor-α receptor-1; IL-6 = Interleukin-6; IGF = Insulin-like growth factor; IGFBP-3 = Insulin-like growth factor binding protein-3. * $P < .05$; [#] trend ($P \geq .05$–.10); Effect size (η^2), small = ≥ .01; medium = ≥ .08; large = ≥ .26; very large = ≥ .50.

Table 6.4. Basic demographics, disease activity, and serum biomarkers of untreated and treated disease in rheumatoid arthritis patients (*n* = 24)

	Untreated disease	Treated disease	Absolute difference (CI) *(%)*	*P*	η^2
Disease activity					
DAS28 (1-10)	6.0 (±0.9)	3.2 (±1.5)	↓ **2.8 (2.1–3.5) *(47)***	**<.001***	**.75**
CRP (mg/L)	36.8 (±33.3)	15.9 (±21.7)	↓ **20.9 (8.8–33.0) *(57)***	**.002***	**.36**
Biomarkers					
TNF-α (pg/mL)	23.64 (±25.42)	35.85 (±27.14)	↑ **12.21 (-24.51–0.09) *(52)***	**.037***	**.18**
sTNF-RI (pg/mL)	1490.8 (±732.7)	1457.9 (±520.7)	↔ 32.9 (-148.6–214.4) *(2)*	.734	.01
IL-6 (pg/mL)	22.90 (±24.01)	12.20 (±23.71)	↓ **10.70 (1.34–20.05) *(47)***	**.021***	**.22**
Myostatin (pg/mL)	1848.0 (±1146.4)	2126.7 (±1150.7)	↑ **278.7 (39.9–517.4) *(15)***	**.030***	**.21**
IGF-I (ng/mL)	96.7 (±41.7)	103.0 (±42.5)	↔ 6.3 (-8.0–0.5) *(7)*	.367	.04
IGFBP-3 (ng/mL)	3288.1 (±834.6)	3525.5 (±784.1)	↑ **237.4 (18.4–456.5) *(7)***	**.034***	**.19**
Adiponectin (ng/mL)	12879.8 (±6768.5)	13288.4 (±6955.4)	↔ 408.6 (-787.76–1604.9) *(3)*	.497	.02
Leptin (pg/mL)	14957.4 (±12643.5)	19985.0 (±15,622.3)	↑ **5027.5 (2282.7–7772.4) *(34)***	**.012***	**.26**

Untreated and treated data is presented as means (±SD) with 95% confidence intervals (CI) reported for the difference. Data was analysed using a 2x2 analysis of variance for the effects of ETN vs MTX (see **Table 6.5.**), with the main effect for time (i.e. pooled group data) used as the relative untreated versus treated states. DAS28 = Disease activity score in 28 joints; CRP = C-reactive protein; TNF-α = Tumor necrosis factor-α; sTNF-RI = Soluble tumor necrosis factor-α receptor-1; IL-6 = Interleukin-6; IGF = Insulin-like growth factor; IGFBP-3 = Insulin-like growth factor binding protein-3. * $P < .05$; Effect size (η^2), small = ≥ .01; medium = ≥ .08; large = ≥ .26; very large = ≥ .50.

Table 6.5. Disease activity and serum biomarkers changes in etanercept and methotrexate treated rheumatoid arthritis patients

Measure	ETN (*n* = 12)	MTX (*n* = 12)	*P* (η^2)	
			Treatment x time	Time
Disease activity				
DAS28				
Pre	6.1 (±0.7)	5.8 (±1.1)	.793 (.00)	**<.001*** **(.75)**
Post	3.2 (±1.6)	3.1 (±1.4)		
Change	**-2.9 (±0.5)***	**-2.7 (±0.5)***		
CRP (mg/L)				
Pre	46.0 (±41.8)	27.7 (±19.6)	.471 (.02)	**.002*** **(.36)**
Post	20.8 (±30.5)	11.1 (±2.9)		
Change	**-25.3 (±10.4)***	**-16.6 (±5.6)***		
Biomarkers				
TNF-α (pg/mL)				
Pre	36.11 (±28.75)	11.17 (±13.62)	**.039*** **(.18)**	**.037*** **(.18)**
Post	60.40 (±10.13)	11.30 (±11.08)		
Change	**+24.29 (±10.10)***	+0.14 (±4.38)		
sTNF-RI (pg/mL)				
Pre	1766.85 (±946.29)	1237.8 (±337.1)	.555 (.02)	.734 (.01)
Post	1789.56 (±513.32)	1154.0 (±303.2)		
Change	+22.71 (±155.91)	-83.83 (±92.75)		
IL-6 (pg/mL)				
Pre	33.16 (±28.26)	19.12 (±27.93)	.766 (.00)	**.021*** **(.22)**
Post	20.57 (±30.99)	3.06 (±2.21)		
Change	-12.59 (±8.30)[#]	**-16.07 (±8.00)***		
Myostatin (pg/mL)				
Pre	1623.7 (±1095.0)	2034.9 (±1201.6)	.276 (.06)	**.030*** **(.21)**
Post	1762.5 (±946.0)	2430.2 (±1254.8)		
Change	+138.7 (±135.7)	**+395.3 (±175.7)***		
IGF-I (ng/mL)				
Pre	94.6 (±33.9)	98.8 (±49.7)	.213 (.07)	.367 (.04)
Post	92.2 (±29.9)	113.8 (±51.3)		
Change	-2.5 (±8.8)	+15.0 (±10.3)		
IGFBP-3 (ng/mL)				
Pre	3067.1 (±751.1)	3509.1 (±886.5)	.276 (.05)	**.034*** **(.19)**
Post	3422.3 (±833.9)	3628.8 (±752.9)		
Change	+335.2 (±187.4)[#]	+119.7 (±96.2)		
Adiponectin (ng/mL)				
Pre	12080.6 (±7309.4)	13679.0 (±6400.7)	.880 (.00)	.497 (.02)
Post	12579.1 (±6913.8)	13997.7 (±7228.8)		
Change	+498.5 (±735.3)	+318.6 (±925.4)		

Leptin (pg/mL)				
Pre	17892.1 (±17439.9)	16578.6 (±16583.7)	.235 (.06)	**.012* (.26)**
Post	21069.7 (±9602.7)	20457.6 (±20642.8)		
Change	+3177.6 (±3446.4)	+3879.1 (±2052.9)[#]		

Pre-and post-treatment scores are presented as means (±SD). Changes are presented as means (±SE). Both the treatment x time interaction and main effect for time significance values are presented from analysis of variance (2 x 2 repeated measures design). If an interaction was detected, post-hoc tests were used to identify where the difference lay at within group level. Note: time effect donates untreated versus treated states, and therefore is also presented in **Table 6.4.** DAS28 = Disease activity score in 28 joints; CRP = C-reactive protein; TNF-α = Tumor necrosis factor-α; sTNF-RI = Soluble tumor necrosis factor-α receptor-1; IL-6 = Interleukin-6; IGF = Insulin-like growth factor; IGFBP-3 = Insulin-like growth factor binding protein-3. * $P < .05$; Effect size (η^2), small = ≥ .01; medium = ≥ .08; large = ≥ .26; very large = ≥ .50.

6.4.2. Non-pharmaceutical anabolic interventions

6.4.2.1. Effect of progressive resistance training (3)

At baseline, the groups were not significantly different for age (PRT: 56.0 (±7.3); control: 62.7 (±12.7) years, $P = .169$, $\eta^2 = .11$, small), sex (PRT: female *n* = 9/10, 90%; control: female *n* = 6/9, 67%, $P = .210$), or disease duration (PRT: 69.2 (±82.0) months; control: 148.9 (±114.2) months, $P = .096$, $\eta^2 = .39$, large)). Twenty four weeks PRT resulted in a significant 1.0 (±0.2) kg increase in ALM ($P = .001$), and a 2.7 (±1.3)% reduction in BF% ($P = .071$) (**Appendix H**). Whilst no interaction was detected ($P = .304$, $\eta^2 = .07$, small), a main effect for time was observed for sTNF-RI ($P = .033$, $\eta^2 = .27$, large). No other changes were observed in any measure (TNF-α, IL-6, IGF-I, IGFBP-3, adiponectin, and leptin) (**Table 6.6.**).

Table 6.6. Disease activity and serum biomarkers changes in rheumatoid arthritis patients undergoing 24 weeks of high-intensity progressive resistance training or low-intensity range-of-movement home exercise.

Measure	PRT (*n* = 10)	Control (*n* = 9)	*P* (η^2)	
			Group x time	Time
Disease activity				
DAS28				
Pre	3.2 (±1.2)	3.3 (±1.0)	.383 (.05)	.840 (.00)
Post	2.9 (±1.2)	3.5 (±0.7)		
Change	-0.3 (±0.3)	+0.2 (±0.3)		
Biomarkers				
TNF-α (pg/mL)				
Pre	2.69 (±1.35)	3.45 (±2.06)	.829 (.00)	.582 (.02)
Post	2.26 (±1.50)	3.26 (±2.29)		
Change	-0.43 (±0.52)	-0.19 (±0.97)		
sTNF-RI (pg/mL)				
Pre	1278.4 (±335.6)	1998.8 (±909.1)	.304 (.07)	**.033* (.27)**
Post	1162.5 (±315.7)	1689.9 (±1174.7)		
Change	-116.0 (±109.5)	-308.9 (±147.7)[#]		
IL-6 (pg/mL)				
Pre	8.04 (±11.03)	9.32 (±6.70)	.539 (.02)	.904 (.00)
Post	6.48 (±6.69)	10.37 (±7.70)		
Change	-1.56 (±1.97)	+1.05 (±3.97)		
Myostatin (pg/mL)				
Pre	2090.7 (±845.01)	2038.0 (±509.1)	.816 (.01)	.895 (.00)
Post	2063.7 (±1291.2)	2135.3 (±1190.8)		
Change	-27.0 (±427.0)	+97.3 (±314.1)		
IGF-I (ng/mL)				
Pre	94.4 (±27.1)	115.6 (±42.5)	.397 (.05)	.637 (.01)
Post	98.0 (±27.6)	109.2 (±42.6)		
Change	+3.6 (±10.2)	-12.5 (±15.4)		
IGFBP-3 (ng/mL)				
Pre	2970.5 (±798.4)	3450.2 (±936.6)	.342 (.06)	.373 (.05)
Post	2979.2 (±945.8)	3181.9 (±641.0))		
Change	+8.8 (±144.2)	-268.3 (±261.3)		
Adiponectin (ng/mL)				
Pre	11131.9 (±7527.5)	12253.6 (±7310.5)	.159 (.12)	.877 (.00)
Post	12864.4 (±8721.7)	10853.7 (±7409.7)		
Change	+1732.5 (±1558.8)	-1399.9 (±1342.4)		

Leptin (pg/mL)				
Pre	10883.4 (±8041.5)	44694.7 (±39353.3)	.595 (.02)	.363 (.05)
Post	9316.9 (±6880.9)	38819.1 (±30937.7)		
Change	-1566.5 (±1235.0)	-5875.6 (±8818.6)		

Pre-and post-exercise scores are presented as means (±SD). Changes are presented as means (±SE). Treatment x time, and time main effects are presented from analysis of variance (2 x 2 repeated measures design). If a main effect was detected, post-hoc tests were used to identify where the difference lay at within group level. PRT = Progressive resistance training; Control = Home exercise group; DAS28 = Disease activity score in 28 joints; TNF-α = Tumor necrosis factor-α; sTNF-RI = Soluble tumor necrosis factor-α receptor-1; IL-6 = Interleukin-6; IGF = Insulin-like growth factor; IGFBP-3 = Insulin-like growth factor binding protein-3. * $P < .05$; Effect size (η^2), small = ≥ .01; medium = ≥ .08; large = ≥ .26; very large = ≥ .50.

6.4.2.2. Effect of oral creatine monohydrate supplementation (4)

No differences between the Cr and placebo groups were identified for age (Cr: 56.6 (±11.3); placebo: 55.3 (±10.8) years, $P = .800$, $\eta^2 = .00$), sex (Cr: *n* females = 5/8, 63%; placebo: *n* females = 9/12, 75%, $P = .550$)), or disease duration (Cr: 91.5 (±54.4) months; placebo: 80.6 (±95.5) months, $P = .774$, $\eta^2 = .09$, medium)). In this smaller subset of patients (*n* of 8) (compared to the whole population reported in the **Chapter 5**), Cr supplementation increased ALM by 0.5 (±0.3) kg, with a moderate group x time effect ($P = .078$, $\eta^2 = .18$, medium) but no main effect for time ($P = .165$, $\eta^2 = .12$, medium). No main effects were seen for BM or FM, although there was a main effect for time for BF% ($P = .064$, $\eta^2 = .22$, medium). Post hoc analysis revealed an unexplainable 1.3 (±0.4) BF% increase in the placebo group ($P = .039$) (**Appendix I**)**.**

Whilst no main effects were seen in DAS28, there was a main effect for time in CRP ($P = .029$, $\eta^2 = .25$, medium) (**Table 6.7.**). Post hoc tests showed a CRP reduction of 2.5 (±1.1) mg/L in the placebo group ($P = .052$). There was a moderate group x time interaction for TNF-α ($P = .073$, $\eta^2 = .188$, medium), but no main effect for time ($P = .405$, $\eta^2 = .00$). Subsequent analysis showed a 34% reduction in TNF-α in the Cr group ($P = .083$). There was a group x time interaction for sTNF-RI ($P = .025$, $\eta^2 = .25$, medium), with post hoc analysis revealing a decrease in the placebo group ($P = .006$). No main effects were observed in any other biomarker (IL-6, myostatin, IGF-I, IGFBP-3, adiponectin, or leptin).

Table 6.7. Disease activity and serum biomarkers changes of rheumatoid arthritis patients orally supplementing for 12 weeks with either creatine or placebo.

Measure	Creatine (*n* = 8)	Placebo (*n* = 12)	*P* (η^2)	
			Group x time	Time
Disease activity				
DAS28				
Pre	3.0 (±0.8)	2.5 (±0.6)	.371 (.05)	.185 (.10)
Post	2.6 (±0.9)	2.4 (±0.8)		
Change	-0.4 (±0.2)	-0.1 (±0.2)		
CRP (mg/L)				
Pre	6.1 (±3.3)	8.5 (±6.2)	.449 (.03)	**.029* (.25)**
Post	4.9 (±1.6)	6.0 (±4.0)		
Change	-1.3 (±1.0)	-2.5 (±1.1)#		
Biomarkers				
TNF-α (pg/mL)				
Pre	7.02 (±1.92)	4.18 (±1.64)	.073# (.19)	.405 (.00)
Post	4.65 (±1.72)	5.09 (±1.47)		
Change	-2.37 (±1.17)#	+0.90 (±1.18)		
sTNF-RI (pg/mL)				
Pre	896.5 (±123.3)	1383.7 (±100.7)	**.025*** (.25)	.952 (.00)
Post	1044.9 (±123.4)	1227.7 (±100.8)		
Change	+148.5 (±138.1)	**-156.0 (±45.5)***		
IL-6 (pg/mL)				
Pre	11.12 (±6.14)	14.79 (±4.69)	.686 (.01)	.244 (.07)
Post	6.02 (±4.55)	11.12 (±3.47)		
Change	-5.10 (±1.34)	-3.67 (±4.07)		
Myostatin (pg/mL)				
Pre	2058.6 (±293.5)	2111.3 (±245.5)	.662 (.01)	.535 (.03)
Post	1919.1 (±279.2)	2086.9 (±233.6)		
Change	-139.5 (±134.5)	-24.4 (±193.4)		
IGF-I (ng/mL)				
Pre	84.4 (±11.3)	103.0 (±9.2)	.476 (.03)	.059# (.19)
Post	64.5 (±10.3)	93.6 (±8.4)		
Change	-19.9 (±15.3)	-9.4 (±6.2)		
IGFBP-3 (ng/mL)				
Pre	2902.8 (±176.4)	2719.1 (±150.4)	.091# (.15)	.431 (.04)
Post	3020.3 (±160.9)	2569.8 (±137.2)		
Change	+117.4 (±100.8)	-149.3 (±93.3)		
Adiponectin (ng/mL)				
Pre	13990.2 (±3325.8)	12534.7 (±2836.2)	.966 (.00)	.102 (.14)
Post	11893.9 (±2834.6)	11471.8 (±2417.4)		

Change	-2096.4 (±1906.1)	-1062.9 (±1215.2)		
Leptin (pg/mL)				
Pre	9029.3 (±6687.7)	19734.2 (±8424.7)	.455 (.03)	.711 (.01)
Post	9859.9 (±8197.5)	24766.5 (±6539.3)		
Change	+830.7 (±833.5)	-5032.2 (±3906.1)		

Pre-test and post-test scores are presented as means (±SD). Changes are presented as means (±SE). Treatment x time, and time main effects are presented from analysis of variance (2 x 2 repeated measures design). If a main effect was detected, post-hoc tests were used to identify where the difference lay at within group level. DAS28 = Disease activity score in 28 joints; CRP = C-reactive protein; TNF-α = Tumor necrosis factor-α; sTNF-RI = Soluble tumor necrosis factor-α receptor-1; IL-6 = Interleukin-6; IGF = Insulin-like growth factor; IGFBP-3 = Insulin-like growth factor binding protein-3. * $P < .05$; # trend ($P \geq .05$–$.10$); Effect size (η^2), small = ≥ .01; medium = ≥ .08; large = ≥ .26; very large = ≥ .50.

6.5. Discussion

6.5.1. Summary of key findings

The aim of this exploratory study was to investigate a comprehensive range of serum-based biomarkers of RC (i.e. body composition, specifically LM and FM). As hypothesised, patients with well-controlled stable RA disease activity had elevated levels of circulating pro-inflammatory cytokines (TNF-α and IL-6) compared to HCs. However, no differences in anabolic, catabolic, and markers of adiponectin and leptin were observed. Adiponectin and IGFBP-3 were inversely associated with muscle mass, although serum markers of inflammation were not. Myostatin was positively correlated with muscle mass.

When investigating the effects of either ETN, a recombinant sTNF-receptor, or MTX, a traditional DMARD, on RC in treatment-naive patients with high disease activity, initiation of either treatment reduced DAS28 and inflammation (i.e. CRP). As hypothesised, levels of TNF-α were increased following ETN use. Treatment had no effect on anabolic markers or adipokines. Anabolic interventions (both PRT and oral Cr supplementation), despite favourable effects on body composition, resulted in negligible changes in the biomarkers analysed including serum concentrations of IGF-I. Interestingly, TNF-α concentrations were reduced following Cr supplementation, and may potentially warrant further study in RA where chronic inflammation exists.

Mechanistically, whilst no single biomarker was consistently associated with the LM deficits characteristic of RC, the role of adiponectin, along with the relationship between myostatin, IGFBP-3, and body composition, may warrant further study. Despite its association with RC in the literature, it appears that current systemic inflammation in RA patients with controlled disease is not a good indicator of muscle mass or the mechanism behind muscle deficiency. Similarly, serum IGF status is a poor indicator of body composition, and in particularly, muscle anabolism.

Overall, in a range of serum biomarkers in various disease and treatment conditions, including two interventions used to attenuate RC, disappointingly, no consistent roles for these biomarkers in RC could be identified.

6.5.2. Disease states and the effects pharmaceutical DMARD treatment

6.5.2.1. Rheumatoid arthritis patients versus sedentary healthy controls (1a)

Unsurprisingly, serum pro-inflammatory cytokine (TNF-α and IL-6) concentrations were elevated in RA patients compared to HC. These cytokines play a key role in RA path ogenesis (Fox, 2000; Jung et al., 2012), and research has consistently shown raised systemic concentrations in RA patients (Roubenoff et al., 1994; Arend, 2001; Walsmith & Roubenoff, 2002; Shrivastava & Pandey, 2013; Blüml et al., 2014; Manrique-Arija et al., 2016). However, although elevated in comparison to our HCs, the observed mean serum TNF-α value of 3.7 pg/mL suggests relatively mild disease activity, with concentrations falling below the 10.6 pg/mL reported in early (<12 months) active RA, defined as DAS28 ≥3.2 (Manrique-Arija et al., 2016)[17]. The elevated sTNF-RI concentrations observed in our RA group suggest an effort to counter the effects of TNF-α (Fox, 2000; Hawari et al., 2004). This soluble receptor has an anti-inflammatory role as it competes with cell surface TNF receptors to bind with TNF-α (Rooney et al., 2000; Spoettl et al., 2007).

Along with arthropathy, pro-inflammatory cytokines are considered the main drivers of RC (Roubenoff et al., 1994; Walsmith & Roubenoff, 2002; Rall & Roubenoff, 2004; Engvall et al., 2008). Although the exact mechanisms by which these cytokines exert their catabolic effect are unclear (Walsmith & Roubenoff, 2002; Rall & Roubenoff, 2004), it is thought they can disrupt key protein synthesis pathways including down regulating IGF-I (Broussard et al., 2003, 2004; Strle et al., 2004; Engvall et al., 2008), and increasing the rate of muscle degradation (Granado, Priego, Martín, Villanúa, & López-Calderón, 2005; Dogra et al., 2007; Schiaffino & Mammucari, 2011; Fanzani et al., 2012). However, we observed no association between inflammatory markers (i.e. serum TNF-α, IL-6, and CRP) and measures of muscle mass. Pertinently, early research by Roubenoff's group also found no direct association between circulating TNF-α and loss of body cell mass (essentially, skeletal muscle mass) (Roubenoff et al., 1994). In addition, no association between serum TNF-α or CRP and LM was reported in two studies in RA by Engvall et al. (2008, 2010), and in cancer cachexia by Moldawer et al. (1988) and Maltoni et al. (1997). Conversely, Engvall et al. (2008) found IL-6 levels to be inversely related with LM, a result not seen in our data.

[17] Our mean RA IL-6 concentration (6.7 pg/mL) was also less than the 20.1 pg/mL reported in this study.

The absence of any relationship between circulating inflammatory markers and LM may be explained by several factors. Firstly, the effects of cytokines often occur in a paracrine or autocrine fashion (Roubenoff et al., 1994; Maltoni et al., 1997), influencing pathways that downstream are responsible for protein synthesis/degradation (Reid & Li, 2001; personal communication, Dr Emma Watson, University of Leicester). Thus, serum concentrations may not be representative of intramuscular activity (Roubenoff et al., 1994; Engvall et al., 2008) or cachexia (Moldawer et al., 1988; Maltoni et al., 1997). In the future, treatment of cachexia should focus on the identification of downstream signals that are specific to skeletal muscle (Reid & Li, 2001).

Secondly, identification of casual relationships between inflammation and RC is difficult due to the different 'timelines' involved; whereas inflammatory cytokine production can vary day by day, changes in body composition can take much longer (Walsmith and Roubenoff, 2002). Loss of LM is greatest during active disease (Roubenoff et al., 1994). Therefore, in well-controlled RA, such as in our cross-sectional patients, concentrations of pro-inflammatory cytokines will be relatively low when obtained, whilst changes in muscle mass may have already occured. Interestingly, no association between muscle mass and systemic inflammation was noted in our highly active disease, treatment-naïve, patients, although these patients were already presenting with low ALM and obesity (i.e. RC) (Marcora et al., 2006). These findings strengthen our belief, postulated in **Chapter 3**, that RC, principally muscle loss, primarily occurs in the 'pre-clinical stage', before DMARD treatment has been initiated and gained control over inflammation. Additionally, preliminary evidence from our group (see **Chapter 7** and **8**) suggests intramuscular CS injections given to treat active RA (e.g., at diagnosis) may also contribute to RC.

No differences in serum IGF-I or IGFBP-3 concentrations were observed between the RA and HC groups. This supports findings by Rall et al. (2002), and Toussirot et al. (2005), but not others (Lemmey et al., 2001; Matsumoto & Tsurumoto, 2002; Blackman et al., 2007; Baker et al., 2015). Whilst Engvall et al. (2008) reported no differences in absolute serum IGF-I concentrations between RA and controls, they postulated that increased IGFBP-1 levels in RA reduce bioavailable IGF-I. Although considered a key factor in muscle anabolism (Bowen et al., 2015), we observed no relationship between serum IGF-I and LM. This finding confirms previous research (Kraemer et al., 1999; Adams, 2002; Walker et al., 2004; Engvall et al., 2010) that, unlike local muscle IGF-I (mIGF-I), circulating, predominantly liver-derived, serum IGF-I is not an accurate marker of anabolic processes. Longitudinal studies are needed to clarify the involvement of systemic IGF-I in RC (Baker et al., 2015).

Interestingly, in the RA group, higher levels of IGFBP-3 were correlated with reduced ALM and BM (the latter association was also observed in the HC). Although processes are unclear, research by Foulstone et al. (2003) suggests that excessive IGFBP-3 during early differentiation reduces proliferation of mononucleated muscle cells, and their fusion into multinucleated, contractile muscle fibres. However, this effect requires further clarification (personal communication, Professor Claire Stewart, co-investigator in the Foulstone et al. study).

Contrary to our hypothesis, we observed no difference in systemic myostatin levels between RA and HC. Serum myostatin has previously been associated with low muscle mass in the elderly (Schulte & Yarasheski, 2001; Yarasheski et al., 2002; Léger et al., 2008) and other clinical conditions such as COPD (e.g., Hoenig, 2008; Ju & Chen, 2012). As such, it was purported to be a promising biomarker of cachexia (Palus et al., 2014). We unexpectedly observed a positive relationship between ALM and serum myostatin in the RA group[18]. As a similar correlation was also seen in the HC, we assume that this is a non-disease specific effect.

The most likely explanation for this positive correlation is that since myostatin is mainly synthesised and excreted into circulation by skeletal muscle (Lee & McPherron, 2001; Bergen et al., 2015), it is conceivable that total muscle mass determines serum myostatin level. Positive correlations between LM and myostatin have been reported by Bergen et al. (2015) in 240 participants (including young (~33 years, *n* = 80), elderly (~75 years, *n* = 80), and sarcopenic[19] (~79 years, *n* = 80) participants), and Yamada et al. (2016) in *n* = 69 adult (~56 years) outpatients undergoing peritoneal dialysis. Thus, circulating concentrations of myostatin may provide a significant, albeit weak, biomarker of muscle mass, (Bergen et al., 2015), and when investigating changes in serum myostatin, it may be useful to take individual muscle mass into account (Yamada et al., 2016).

Interestingly, myostatin was also associated with increased FM and overall BM, and this is consistent with the suggestion that myostatin may be involved in regulating adiposity (Allen et al., 2011; Zhu et al., 2014). In support of this, Zhu et al. (2014) and Hittel et al. (2009) found that serum myostatin concentrations were increased in overweight patients compared with normal-weight controls and, similar to our results, positive correlations between BM and myostatin were observed (Hittel et al., 2009). In RA patients, who are characteristically obese

[18] This relationship (i.e. myostatin concentrations positively correlated with muscle mass) was also observed in patients with untreated disease activity from Marcora et al. (2006).
[19] Based on ALM cut-offs (male: ≤5.67 and female: ≤7.23 kg/m^2).

(e.g., Summers et al., 2008; Lemmey et al., 2009), there may be an association between myostatin and adiposity, although mechanisms are undefined (Allen et al., 2011). Myostatin is expressed by adipose tissue in low concentrations (Han et al., 2013), thus increased myostatin levels, like muscle mass, may reflect increased FM. Further, as LM often increases as an anatomical response to the stress imposed by increased body weight (i.e. obesity) (Baumgartner et al., 2004), it may be that elevated myostatin concentrations with FM are actually a result of the increased muscle mass seen in these patients. Overall, we observed no evidence for myostatin as a biomarker of muscle wasting mechanisms, although owing to extensive previous literature (e.g., Schulte & Yarasheski, 2001; Yarasheski et al., 2002; Léger et al., 2008) further research should be conducted into its role in RA-related cachexia.

Patients with RA had a ~2-fold increase in serum leptin concentration compared to controls. As leptin is produced by adipocytes (Wislowska et al., 2007), these elevated levels are consistent with the excess FM in RA patients. Indeed, when FM was controlled for, no differences were seen. Research into leptin differences between RA patients and HC have yielded contrasting findings, with comparable (Anders et al., 1999; Popa et al., 2005, 2009; Manrique-Arija et al., 2016), lower (Härle et al., 2004), and higher (Bokarewa et al., 2003; Toussirot et al., 2005; Gunaydin et al., 2006; Seven et al., 2009; Abdalla et al., 2014) levels reported in RA. The correlation between leptin and relative FM (i.e. BF%) may explain the inverse relationship with relative LM (i.e. ALM%) in the RA group.

Leptin has been implicated in the pathogenesis of RA (Härle et al., 2006; Otero et al., 2006; Rho et al., 2009; Seven et al., 2009; Targońska-Stępniak et al., 2010; Toussirot et al., 2015) by increasing pro-inflammatory cytokine expression (Härle et al., 2006; Wislowska et al., 2007). However we, like others, found no association between leptin and DAS28 (Anders et al., 1999; Bokarewa et al., 2003; Toussirot et al., 2005; Gunaydin et al., 2006; Allam & Radwan, 2012; Abdalla et al., 2014), TNF-α, IL-6, or disease duration (Popa et al., 2005; Abdalla et al., 2014; Manrique-Arija et al., 2016).

Some research suggests that adiponectin concentrations are elevated in RA patients compared to controls (Choi et al., 2009; Laurberg et al., 2009; Popa et al., 2009; Ozgen et al., 2010), however, we found no difference (Nagashima et al., 2008; Targońska-Stępniak et al., 2010; Manrique-Arija et al., 2016). Although adiponectin may reduce pro-inflammatory cytokine expression in RA (Wulster-Radcliffe et al., 2004; Toussirot et al., 2007; Targońska-Stępniak et al., 2010), elevated serum adiponectin has also been found to be associated with increased radiographical damage and disease activity (Giles et al., 2009, 2011; Klein-Wieringa

et al., 2011; Oranskiy et al., 2012; Meyer et al., 2013). Supporting an anti-inflammatory action, we observed a significant negative correlation between adiponectin and DAS28.

Interestingly, we found an inverse relationship between adiponectin and muscle mass in the RA (significant) and HC (trend) groups. Whilst this suggests a non-disease specific mechanism, this result supports observations by Baker et al. (2015) who found that elevated serum adiponectin levels were associated with reduced muscle mass in RA patients. Although the precise role of adiponectin on muscle metabolism is unclear (Mourtzakis & Bedbrook, 2009), some research suggests that adiponectin can directly influence the inflammatory (and catabolic) pathways responsible for cachexia (McEntegart et al., 2007), including IL-6 up-regulation (Ehling et al., 2006) and increased energy expenditure (Paulo Araújo et al., 2009). As adiponectin can be associated with active disease (Giles et al., 2009; Rho et al., 2009; Meyer et al., 2013), Baker et al. postulated that adiponectin may represent a 'biological marker of a catabolic state in RA', and that high circulating adiponectin levels may identify those who have undergone, or have ongoing, catabolism (Baker et al., 2015). Conversely, we observed a negative association between adiponectin and IL-6 in our RA patients, again supporting an anti-inflammatory role for adiponectin.

6.5.2.2. Untreated versus treated disease / (2b) etanercept versus methotrexate (2a)

Treatment with ETN or MTX moderately increased adiposity and BM. These changes have subsequently been observed by others (Metsios et al., 2007; Engvall et al., 2010; Chen et al., 2013; Toussirot et al., 2014), and may occur due to increased well-being and appetite due to reductions in inflammation and symptoms (Marcora et al., 2006; Metsios et al., 2007), or metabolic and/or hormonal changes (Toussirot et al., 2014). Metsios et al. (2007) and Engvall et al. (2010) both reported that, despite similar disease activity reductions, anti-TNF's increased FM and trunk FM, respectively, more than traditional DMARDs. Therefore, FM gain following anti-TNF-α treatment may be a drug specific effect, although further study is necessary (Engvall et al., 2010; Toussirot et al., 2014).

Unsurprisingly, disease activity (DAS28) and CRP were reduced following treatment (Catrina et al., 2002; Kotyla et al., 2015). The comparable reductions indicate MTX was equally effective as ETN in reducing disease activity in our patients. Whilst we hypothesised that other inflammatory markers would also decrease upon treatment initiation, we observed a large 67% increase in serum TNF-α concentrations in the ETN group. This effect has been observed previously (Zou et al., 2002; Zou et al., 2003; Madhusudan et al., 2004; Bhatia & Kast, 2007; Kotyla et al., 2015) with TNF-α concentrations reported to increase ~7-fold in a dose-related manner after ETN administration (Barrera et al., 2001). This action occurs as ETN

competitively binds to TNF-α, rendering it biologically and immunologically inactive (Fox, 2000; Bhatia & Kast, 2007) but not eradicating it from the circulation (Fox, 2000; Feldmann & Maini, 2001). As others (Barrera et al., 1995; Aggarwal & Misra, 2003; Nishina et al., 2013) have found, no meaningful change in TNF-α occurred following MTX therapy, although this is not a universal finding (Majumar & Aggarwal, 2001; Manrique-Arija et al., 2016).

In an effort to regulate its effects, the binding of TNF-α with its membrane-bound receptor (i.e. TNF-RI) induces the release of sTNF-RI (Olsson et al., 1992). As TNF-α was unable to interact with its transmembrane receptor due to the competitive binding action of ETN (a recombinant human sTNF-receptor with a similar anti-inflammatory action to natural sTNF-RI (Fox, 2000)), as anticipated, and as previously reported (Sato et al., 2011), we saw no changes in sTNF-RI concentrations following treatment.

As hypothesised, treatment resulted in a large reduction in IL-6. Whilst MTX is a known inhibiter of IL-6 expression (e.g., Aggarwal & Misra, 2003; Halilova et al., 2012; Nishina et al., 2013; Manrique-Arija et al., 2016), IL-6 concentrations were also partially reduced in the ETN group, most likely due to the decrease in bioactive TNF-α; a recognised inducer of IL-6 (Fox, 2000). Like others, we observed a correlation between the reductions in IL-6 and CRP (e.g., Madhok et al., 1993; Lacki et al., 1997). The acute-phase response (which includes CRP expression) follows IL-6 changes (Madhok et al., 1993), thus the ability of MTX to reduce CRP concentration (Kapral et al., 2006) may be in part due to a reduction in IL-6 expression.

As expected, we observed no change in serum IGF-I concentrations in either treatment arm. No change in IGFBP-3 was seen in the MTX group, which supports findings by Özden et al. (2008) in patients with psoriatic arthritis, although we did observe a moderate increase in IGFBP-3 following ETN treatment. Research in juvenile idiopathic arthritis patients suggested that intensified anti-inflammatory treatment with ETN may increase serum IGF-I and IGFBP-3 by attenuating the inhibitory effect of pro-inflammatory cytokines (Schemling et al., 2002). However, unlike Schemling et al. we observed no change in serum IGF-I, and therefore why we observed an IGFBP-3 increase is unclear. For reasons also unknown, myostatin was increased in the MTX group. Owing to the associations between IGFBP-3 and myostatin with LM in previous scenarios, we tested whether changes in myostatin and IGFBP-3 were associated with treatment-induced body composition improvements, however, no correlations existed. Increases in myostatin and IGFBP-3 were not associated with any other biomarker changes.

Although there is evidence that MTX treatment (Laurberg et al., 2009; Manrique-Arija et al., 2016) and anti-TNF-α therapy (Komai et al., 2007; Nagashima et al., 2008; Serelis et al., 2008; Cansu et al., 2011) may increase adiponectin concentrations, like others (e.g., Härle et al., 2006; Derdemezis et al., 2009; Gonzalez-Gay et al., 2011; Toussirot et al., 2014) we observed no changes. Leptin was non-significantly elevated following treatment, with increases correlated with elevated adiposity. Manrique-Arija et al. (2016) also found that 6 months of MTX treatment significantly increased leptin concentrations, although it was not reported if the changes in leptin were due to changes in FM.

6.5.3. Non-pharmaceutical anabolic interventions

6.5.3.1. Effect of progressive resistance training (3)

Twenty four weeks PRT significantly increased muscle mass along with reducing adiposity in RA patients. This supports the role of PRT as an effective anabolic intervention that can be used to reverse the effects of RC (e.g., Häkkinen et al., 2005; Marcora et al., 2005a; Lemmey, 2011). No exacerbation of inflammation (i.e. increase in TNF-α or IL-6) was observed. This lack of an inflammatory effect of PRT supports previous research in both RA (Rall et al., 1996) and healthy individuals (e.g., Conraads et al., 2002; Bruunsgaard et al., 2004; Bautmans et al., 2005; Kelley & Kelley, 2006; Olson et al., 2007; De Salles et al., 2010; Libardi et al., 2012).

As with the original analysis (Lemmey et al., 2009), and research by Häkkinen et al. (2005) in RA, we observed no changes in serum IGF-I or IGFBP-3 following PRT. However, Lemmey et al. (2009) did observe increases in mIGF-I, supporting the stance by Walker et al. (2004) that growth factor responses local to the muscle are more important than circulating factors in contributing to muscle hypertrophy with resistance training, and that circulating serum IGF-I may not be a meaningful marker of mIGF-I activity (Kraemer et al., 1999; Adams, 2002; Engvall et al., 2010).

No changes were seen in myostatin concentrations following PRT. The literature on the effect of PRT on serum myostatin is contradictory with both increases and decreases reported (Walker et al., 2004; Willoughby, 2004a; Willoughby & Taylor, 2004b; Saremi et al., 2010). These differences have been partly attributed to the sampling time with acute myostatin increases reported immediately post-exercise (Willoughby, 2004a; Willoughby & Taylor, 2004b;) but then returning to baseline levels ~48 hours later (Walker et al., 2004). In our trial,

samples were harvested 3–7 days after the intervention period (Lemmey et al., 2009), thus acute myostatin changes resultant of PRT may have gone undetected.

Despite eliciting a reduction in adiposity, PRT resulted in no changes in serum adiponectin or leptin concentrations. Whilst this contrasts with research in the elderly where increases in adiponectin (Fatouros et al., 2005; Brooks et al., 2007; Olson et al., 2007; De Salles et al., 2010; Lee & Kwak, 2014) and reductions in leptin (Kohrt et al., 1996; Fatouros et al., 2005; De Salles et al., 2010) were observed (primarily due to exercise-induced FM reductions), results are inconsistent (see review by Golbidi & Laher, 2014).

The explanations for this lack of change are unclear. In type 2 diabetic males (mean age: ~50 years), Boudou et al. (2003) found no differences in adiponectin and leptin levels, despite significant exercise-induced reductions in FM (8 weeks of exercise training). The authors identified that ≥10% loss of FM is required to elicit changes in these adipokines concentrations; pertinently this threshold was not achieved in our study (i.e. reduction in FM following PRT was ~7%). Further, the effects on adipokines concentrations can also be influenced by training protocols (intensity, volume, duration), as well as participants initial conditioning status, energy balance conditions, and baseline levels of FM (Fatouros et al., 2005; Golbidi & Laher, 2014).

6.5.3.2. Effect of oral creatine monohydrate supplementation (4)

Like PRT, Cr supplementation increased LM. Increased muscle protein synthesis following Cr supplementation is thought to be stimulated by an increase in mechanical stress from a Cr-induced osmotic rise in intracellular water (Ingwall et al., 1974; Francaux & Poortmans, 2006). Whilst research has found that Cr supplementation with (Burke et al., 2008) and without exercise training (Deldicque et al., 2005) can increase mIGF-I concentrations, we observed no change in serum IGF-I following supplementation. The lack of effect on serum IGF status following Cr supplementation is not surprising following the lack of change following PRT; a much greater anabolic stimulus than Cr. Thus, this finding provides further support that serum IGF-I levels may not be an accurate marker of local muscle activity following anabolic interventions (Adams, 2002; Lemmey et al., 2009).

Research has suggested that Cr supplementation in trained athletes may attenuate increases in systemic inflammation such as TNF-α (Santos et al., 2004; Bassit et al., 2008), IL-1β (Bassit et al., 2008), and CRP (Deminince et al., 2013), but not IL-6 (Bassit et al., 2008). Interestingly, and in support of these findings, the Cr group in our trial showed a 34% reduction in serum TNF-α, and no change in IL-6, following 12 weeks of supplementation. This finding potentially has favourable implications for RA patients as elevated concentrations of TNF-α have been

associated with arthropathy (Scott et al., 2010) and RC (e.g., Roubenoff et al., 1994, 2009), although we observed no such relationship between TNF-α and measures of muscle mass.

Two studies (Saremi et al., 2010; Schiaffino & Mammucari, 2011) have reported that reductions in serum myostatin after PRT can be augmented by Cr supplementation. Whilst the specific physiological processes are unknown (Saremi et al., 2010), it is thought by some that the anabolic actions of Cr may involve a down regulation of myostatin (Willoughby & Rosene, 2003). However, we saw no change in myostatin following 12 weeks Cr supplementation, possibly due to a lack of concurrent exercise (Saremi et al., 2010; Schiaffino & Mammucari, 2011). As expected, due to its lack of effect on adiposity, we saw no change in circulating adiponectin or leptin levels.

6.5.5. Similarities in biomarker changes in the scenarios investigated

In a variety of clinical scenarios, this study investigated a range of serum biomarkers in the hope of enhancing understanding of RC. Whilst some markers possibly warrant additional investigation, disappointingly, across the different scenarios explored, no consistent roles for the serum biomarkers in RC could be identified.

- *Inflammatory markers*

As expected, compared to controls, RA patients have elevated levels of systemic markers of inflammation (TNF-α, sTNF-RI, and IL-6), although these are substantially reduced by drug treatment. Although ETN therapy increased TNF-α concentrations, as evident by substantial reductions in disease activity, the circulating TNF-α was rendered biologically and immunologically inactive by the treatment. Whilst Cr supplementation resulted in a reduction in TNF-α, it caused an unexplained increase in sTNF-α. In contrast, PRT had no effect on any inflammatory marker (TNF-α, sTNF-RI, or IL-6). Systemic inflammation was not associated with LM measures in any scenario, including in patients with active RA disease.

- *Catabolic markers*

Serum myostatin concentrations were comparable between RA and HC cohorts. Surprisingly, serum myostatin, generally thought as a catabolic marker, was positively correlated with muscle mass and adiposity in both the cross-sectional and untreated RA cohorts, as well as the HC group. The most likely explanation for this is that myostatin is produced by muscle, so those with more LM express more myostatin. Circulating myostatin was increased following

DMARD initiation, although this was not related to improvements in body composition, whilst myostatin was unchanged by Cr supplementation and PRT, even though these interventions significantly increased LM.

- *Anabolic markers*

No differences or changes in serum IGF-I were seen in any scenario, and muscle mass was repeatedly not correlated with serum IGF-I. There was no difference in IGFBP-3 between RA and HC cohorts, and interestingly, serum IGFBP-3 was inversely correlated with LM in these groups. Changes in IGFBP-3 were inconsistent across the intervention studies with a significant increase following treatment initiation (but no change in LM) and a moderate increase following Cr supplementation (which increased LM), but no change following PRT (which had a much greater anabolic effect than Cr supplementation).

- *Adipokines*

Whilst no changes or differences in adiponectin were observed in any scenario, serum adiponectin was inversely associated with LM in both the RA and HC participants. Serum leptin was greater in RA patients than controls, and was increased by treatment and suppression of disease activity in previously treatment-naïve patients. These differences in leptin were seemingly due to increased adiposity. Conversely, despite a decrease in FM following PRT, no reductions in leptin were observed. Creatine supplementation had no effect on leptin (or adiposity).

6.5.6. Study limitations

6.5.6.1. Intramuscular activity

Biomarkers derived from serum samples were chosen as they provided a relatively non-invasive and cost-effective means to assess the mechanisms of RC. However, it appears circulating biomarkers are not accurate reflections of further downstream mechanisms or local muscle activity (Roubenoff et al., 1994; Kraemer et al., 1999; Reid & Li, 2001; Adams, 2002; Walker et al., 2004; Engvall et al., 2008, 2010). Therefore, although more invasive and expensive to acquire by biopsy, intramuscular concentrations are likely to provide better markers of RC, especially in response to anabolic stimuli. Unfortunately, no muscle biopsy samples were available for this study. In future research, to gain a better understanding of the complex cascades involved in RC, and the direction of any metabolic effects, studies should

investigate our biomarkers and their subsequent influences on different receptors, hormones, cytokines, or molecular responses downstream in the signalling pathway(s).

6.5.6.2. Assay methodology

Biochemistry data reported in the Lemmey et al. (2009) and Marcora et al. (2006) studies were generated by radioimmunoassays (RIA) rather than ELISA which was used in the present investigation. Radioimmunoassay's are regarded as being more specific and sensitive than ELISA (Fowler & Cheng, 1983), e.g., RIA often give higher (~3–4 fold) IGF-I values than ELISA (Clemmons, 2007). In our study, we were able to perform a comparative analysis of original baseline IGF-I/BP-3 data from Lemmey et al. and Marcora et al. to the recent (ELISA) analysis[20]. Overall, whilst showing similar patterns of data, RIA generated data was ~25% higher than our current ELISA generated values.

Inconsistent myostatin results may have occurred to the technical limitations and low sensitivity (Lakshman et al., 2009; Bergen et al., 2015; Hofmann et al., 2015; Yamada et al., 2016) associated with enzyme-linked immunosorbent assay-based approaches (e.g., ELISA) to measure serum myostatin.

6.5.6.3. Sample condition

Whilst in the current study serum was immediately frozen upon harvesting and stored at –80°C, potential sample decay needs to be considered (de Jager et al., 2009). Samples from both the cross-sectional study and the Cr supplementation trial were obtained in the previous ~2 years, whereas samples from Lemmey et al. and Marcora et al. were collected ~7–10 years ago. Research has shown that in the absence of repeated freeze-thaw cycle, serum samples, including TNF-α, IL-6 (Kenis et al., 2002; Ho et al., 2005), IGF-I, and IGFBP-3 (Ito et al., 2005), stored at -80°C are stable (i.e. lack of sample degradation) for a period of up to ~10 years (Arts et al., 2014).

6.5.6.4. Low sample sizes

Sample numbers in each scenario ranged from 8 to 41, and in some cases, despite well-matched groups, the relatively small *n* made interpretation of the data problematic. Although

[20] For example, pooled data from the original PRT manuscript (Lemmey et al., 2009) showed a mean IGF-I concentration of 123.5 ±41.5 ng/mL, this is compared to an IGF-I concentration of 105.0 ±36.3 ng/mL ('recently analysed') (Δ 15%, $P = .007$). The 'older' IGFBP-3 mean concentration of 4069.5 ±739.16 ng/mL was significantly different (Δ 22%, $P < .001$) than the 'recently analysed' IGFBP-3 mean of 3176.21 ±847.16 ng/mL. In the ETN/MTX study (Marcora et al., 2006), the 'older' IGF-I concentrations were significantly greater than the 'recently analysed' levels (139.71 ±55.29 versus 96.73 ±41.66, Δ 31%, $P < .001$). Similar results were seen for IGFBP-3 (5013.75 ±1142.50 versus 3288.10 ±834.63, Δ 34%, $P < .001$).

a significant value did generally indicate a large change or group difference in the data, some effects may have been missed due to small sample sizes. Whilst the use of 95% CI's and ES enhance data interpretation, larger *n*'s would have aided analysis.

6.5.6.5. Other potential biomarkers of rheumatoid cachexia

The biomarkers chosen in the current study aimed to represent a wide range of different inflammatory, anabolic, and catabolic pathways. Whilst the majority of RA research attributes RC to either: 1) increased pro-inflammatory cytokine activity; 2) reduced physical activity; or 3) increased resting energy expenditure (e.g., Metsios et al., 2006), other potential mediators of RC may exist and merit future study. In particular, testosterone (anabolic factor) and cortisol (catabolic factor) could be investigated further.

Testosterone is a hormone that promotes protein synthesis and efficient repair of muscle damage, as well as inhibiting the release of pro-inflammatory (and catabolic) cytokines such as TNF-α and IL-6 (Morley et al., 2006). Research suggests that, compared to age-matched controls, male RA patients have lower levels of testosterone (Cutolo et al., 1988; Tengstrand et al., 2002), however these reduced values may still be high enough to afford some protection against LM loss (Munro & Capell, 1997). Overall, the contribution of testosterone to RC requires further investigation.

Cortisol is a steroid hormone involved in different metabolic processes in the body including inhibiting inflammation (in particular, TNF-α and IL-6) (Jessop & Harbuz, 2005). However, reports suggest that in RA circulating levels of cortisol are 'normal' (Rall et al., 1996; Jessop & Harbuz, 2005; Blackman et al., 2007), and not elevated as would be expected in the setting of increased pro-inflammatory activity (e.g., IL-6). This abnormal 'normal' level suggests a relative hypoadrenalism and hypocortisolism (i.e. underactivity of adrenal glands and insufficient cortisol production) in patients with RA (Jessop & Harbuz, 2005; Blackman et al., 2007) and an overall inadequate anti-inflammatory response to inflammation.

Hypercortisolemia (excessive levels of cortisol) can lead to loss of LM (Thomas, 2007) by increasing skeletal muscle proteolysis (Paddon-Jones et al., 2003; 2006). Whilst elevated levels of cortisol have been associated with cachectic patients with chronic heart failure (Anker et al., 1997), there is limited research into the role of cortisol and RC. Study by Rall et al. (1996) and Blackman et al. (2007) both reported no differences in cortisol levels between RA and HC participants, despite the presence of RC (i.e. significantly reduced LM) in these patients.

6.6. Conclusion

The aim of this study was to investigate a comprehensive range of serum-based markers of RC in order to identify key biomarkers for future application in RA. However, despite studying a range of easily attainable and cost-effective serum-biomarkers in various disease and treatment conditions, including two efficacious interventions used to attenuate RC, disappointingly we observed no consistent themes for circulating inflammatory, anabolic, catabolic, and adipokine (i.e. adiponectin and leptin) biomarkers.

7

Significant muscle loss following intramuscular corticosteroid injection used to treat active rheumatoid arthritis; a case report

This case report was published in the Journal of Rheumatology and Orthopedics, 2015

7.1. Background

Intramuscular (IM) injection of corticosteroids (CS) is the recommended treatment, routinely used, to suppress inflammation and relieve pain during an acute episode or exacerbation of rheumatoid arthritis (RA) (National Institute for Health and Care Excellence (NICE) Guidelines 79, 2009). Although potential adverse events such as increased blood pressure and infection have been reported following IM CS injection (Choy et al., 2005; Da Silva et al., 2006; Berthelot et al., 2013), the effects on muscle mass are less well documented. We present the case of a patient with RA who developed significant muscle loss following an IM CS (triamcinolone acetonide, 40 mg) injection given for a disease flare.

7.2. Case presentation

A 44-year-old male, with established RA (disease duration ~10 years), controlled with leflunomide (20 mg daily), was participating in a clinical trial (**Chapter 5**) assessing the effects of oral creatine (Cr) monohydrate supplementation (a form of protein supplementation taken to increase muscle mass). The primary outcome measure of this study was dual x-ray absorptiometry (DXA)-assessed appendicular lean mass (ALM; a surrogate measure of muscle mass (Kim et al., 2002)), and the inclusion criteria were stable disease and medication, with no recent history (<3 months) of CS injection. At the start of the trial, disease activity was controlled and 'low' (Disease Activity Score in 28 joints; DAS28 score = 2.9). Seven weeks later, his disease 'flared'. Consequently, he consulted his GP who confirmed a flare (but did not record a DAS28), and gave an IM CS injection (triamcinolone acetonide, 40 mg) to the outer upper quadrant of the gluteal muscle. The rheumatology and research team were not notified of the CS injection at the time.

Twenty eight days after the injection, the patient was followed up as per the Cr supplementation trial protocol. He reported a dramatic improvement in his flare symptoms, and his DAS28 was 1.5. Body composition was re-assessed by DXA (see **Table 7.1.**), and revealed a 2.4 kg (4%) loss of total lean mass (LM) in the 12 weeks between baseline and follow-up measures, mostly from the arms and legs (2.0 kg, 7% loss of ALM), indicating general rather than localised muscle loss. Notably, the patient in question was randomised into the Cr supplementation group, consequently his muscle mass was expected to increase

during this period. Over the same period, other patients who had been administered Cr supplementation increased their ALM and LM by 0.5 and 0.6 kg, respectively. It should be noted that neither total LM nor ALM were restored in the ensuing ~2 months.

This loss of muscle is substantial. To put it into context, a loss of 5% total LM results in muscle weakness and loss of functional capacity (Walsmith & Roubenoff, 2002). Further, a recent study performed by our group (**Chapter 3**) comparing body composition and physical function in RA (*n* = 82) patients with well-controlled disease and healthy age- and sex-matched controls (*n* = 85) revealed that RA patients have ~10% less muscle mass when ALM is normalised to bodyweight, and this loss coincides with substantial deficits in function (i.e. 25–35% poorer performance in objective function tests).

7.3. Discussion

Possible explanations for the LM loss observed following IM CS injection are: i) variance in the DXA measurement; ii) the effect of inflammation during the RA flare (i.e. due to elevated levels of pro-inflammatory cytokines, principally TNF-α (Walsmith & Roubenoff, 2002)); or iii) an effect of the IM CS injection. The effect is unlikely to be related the DXA-measurement. Using data from our trial's placebo arm (*n* = 20), in-house assessment of our DXA revealed a co-efficient of variation (CV) of 1.4%, which is within the manufacturers recommendation of ≤1.5%. Whilst it is possible that the depletion of LM occurred between the onset of flare and the CS injection, this single case report raises the possibility that the IM CS injection, which is regularly used as treatment of active RA, may be contributing to the substantial and sudden loss of muscle mass observed.

Previous evidence on muscular atrophy induced by IM CS is scarce. A search of the literature revealed only a single case report that subjectively-assessed CS injection induced local muscle atrophy (Park et al., 2013). Additionally, although the exact mechanisms are unclear, stimulation by CS of the ubiquitin-proteasome system through the increased expression of atrogenes (atrophy genes such as MuRF-1), as well as inhibition of anabolic pathways (e.g., mTOR/S6 kinase 1 and insulin-like growth factor (IGF)-I), may result in muscle atrophy (Schakman et al., 2013). If muscle loss is a common iatrogenic effect of IM CS treatment, this is of particular concern for RA patients, as 'rheumatoid cachexia' (Walsmith & Roubenoff, 2002) characterises the disease and contributes to the reductions in strength and physical function seen in these patients (Giles et al., 2008a).

Table 7.1. Change in body composition and disease activity following disease flare and subsequent treatment with intra-muscular injection of corticosteroid (triamcinolone acetonide)

	Assessment 1 (56 days *pre-CS*)	Assessment 2 (28 days *post-CS*)	Δ 1–2 *(%)*	Assessment 3 (86 days *post-CS*)	Δ 2–3 *(%)*
ALM (kg)	29.4	27.4	-2.0 *(-7)*	27.8	+0.4 *(2)*
Total lean mass (kg)	66.1	63.7	-2.4 *(-4)*	64.0	+0.3 *(1)*
Fat mass (kg)	22.0	21.6	-0.4 *(-2)*	21.6	0 *(0)*
Body fat (%)	24.2	24.5	+0.3 *(1)*	24.5	0 *(0)*
DAS28 (0–10):	2.9	1.5	-1.4 *(-47)*	*NA*	*NA*
▪ Tender joints (*n*)	1	0	-1 *(-100)*	*NA*	*NA*
▪ Swollen joints (*n*)	1	0	-1 *(-100)*	*NA*	*NA*
▪ VAS global health (1-100)	23	0	-23 *(-100)*	*NA*	*NA*
▪ CRP (mg/L)	8	<5[a]	*NA*	*NA*	*NA*

CS: Corticosteroid injection (40 mg triamcinolone acetonide); Δ = change; ALM = Appendicular lean mass (muscle of arms and legs); DAS28 = Disease Activity Score in 28 joints (not measured at Assessment 3); CRP = C-reactive protein level; VAS = Visual analogue scale for global health; *NA* = Not assessed. [a] = CRP at 'Assessment 2' fell below detectable range (<5 mg/L); [b] = no measure of DAS28 was made at 'Assessment 3'.

7.4. Conclusion

In an era in which single high-dose CS injections are, in accordance with NICE and European League against Rheumatism (EULAR) (Smolen et al., 2010b) recommendations, routinely used to treat active disease (Da Silva et al., 2006), this incident gives rise to concerns that this standard treatment may be contributing to the deficiency of muscle mass commonly observed in RA patients (Walsmith & Roubenoff, 2002; Giles et al., 2008a; Summers et al., 2008). Thus, it is important to investigate whether the apparent effect of acute IM CS injection that we observed is a common response. If this adverse effect is confirmed by an on-going observational study (**Chapter 8**) by our group, this raises important concerns about the use of IM CS injection in the treatment of active RA.

8

Does a single high dose intramuscular corticosteroid injection, used to treat disease flare, exacerbate muscle loss in rheumatoid arthritis? A pilot trial

This chapter contains preliminary results from an on-going trial.

8.1. Introduction

Corticosteroids (CS) are anti-inflammatory agents routinely used in the treatment of active rheumatoid arthritis (RA) (Da Silva et al., 2006; Ding & Deighton, 2010). Administration of CS by intramuscular (IM) injection has been used since the late 1980's, and is recommended to suppress inflammation and relieve pain in the management of early disease (i.e. recently diagnosed) ('European League Against Rheumatism (EULAR) recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying anti-rheumatic drug', Smolen et al., 2010b; Luqmani et al., 2006), and during an acute episode or exacerbation of RA (i.e. a disease flare) (National Institute for Health and Care Excellence (NICE) Guidelines 79, Section 1.4.2.1, 2009). Intramuscular CS injections have been shown to provide significant short-term benefits in relieving inflammation and pain in RA (e.g., Corkill et al., 1990; Choy et al., 1993; Gough et al., 1994; Choy et al., 2005; Luqmani et al., 2006).

Despite being effective in controlling disease activity, chronic high dose CS treatment is known to have detrimental effects on body composition including loss of lean mass (LM) and an increase in fat mass (FM) (e.g., Horber et al., 1986, Roubenoff et al., 1990; Dekhuijzen & Decramer, 1992; Natsui et al., 2006; Pereira & de Carvalho, 2011; Mok et al., 2008). Whilst even at relatively low doses, chronic CS use reduces bone mass and increases the risk of osteoporosis (Ding & Deighton, 2010).

Although potential adverse events such as hypertension and infection have been reported following IM CS injection (Choy et al., 2005; Da Silva et al., 2006; Berthelot et al., 2013), the effects on body composition of this treatment are unclear. Our research group recently observed a substantial loss of dual-energy x-ray absorptiometry (DXA)-assessed muscle mass (-2.0 kg in appendicular LM, ALM; i.e. ~7% of total ALM) in an RA patient following a single CS injection given to treat a disease flare (**Chapter 7**). However, it is possible that the depletion of LM occurred prior to the CS injection, due to the catabolic effects of active disease (i.e. elevated pro-inflammatory cytokines, principally TNF-α (Roubenoff et al., 1994)).

A search of the literature revealed one other case report of local muscle loss following CS injection (Park et al., 2013); although this assessment was only made subjectively (i.e. by visual observation). Nonetheless, these reported cases raise concerns that CS treatment may

be contributing to the loss of muscle mass, known as ‘rheumatoid cachexia’ (RC) (Roubenoff et al., 1992; Walsmith & Roubenoff, 2002; **Chapter 3**), and physical function (Marcora et al., 2005a; Giles et al., 2008a; Summers et al., 2008; Lemmey et al., 2009; Kramer et al., 2012; Lusa et al., 2015), observed in RA patients. Thus, it is important to determine whether the possible iatrogenic effects of acute CS injection on muscle loss is a common response.

To our knowledge, this pilot study is the first to investigate the effects on body composition of a single high dose IM CS injection (120 mg depomedrone; methylprednisolone acetate aqueous solution) given to treat disease flare in RA patients. We hypothesised that acute loss of muscle mass (assessed by ALM) occurs as a result of high dose IM CS injection in RA patients. The findings from this study will provide insights into whether the routine, and recommended, treatment of RA ‘flares’ with IM injection of CS exacerbates muscle loss and, thus, contributes to impaired physical function in RA patients.

8.2. Methods

This quasi-experimental (non-randomised, single group, pre-post intervention) pilot study was conducted at the School of Sport, Health and Exercise Science (SSHES), Bangor University between March 2015 and August 2015. The study was approved by the North Wales Research Ethics Committee – West (15/WA/0013).

8.2.1. Study population

Rheumatoid arthritis patients presenting with active disease (disease flare), and treated with an IM injection of CS, were recruited from outpatient clinics of the North West Wales Rheumatology department (Peter Maddison Rheumatology Centre, Llandudno, North Wales). For inclusion, participants had to: (a) fulfil the American College of Rheumatology/EULAR 2010 revised classification criteria for the diagnosis of RA *(*Aletaha et al*.,* 2010); (b) be aged ≥18 years; (c) not be cognitively impaired; (d) be free of other cachectic diseases or conditions preventing safe participation; (e) not be pregnant; and (f) not have any contraindication to a high dose IM CS injection (e.g., uncontrolled diabetes mellitus, active infection, previous hypersensitivity to CS injections, idiopathic thrombocytopenia, acute heart failure, or active peptic ulcer).

Active disease was determined by the attending rheumatologist and was appraised by clinical assessment of overall disease activity (i.e. worsening of signs and symptoms of sufficient intensity and duration to lead to change in therapy), and not necessary based solely on the Disease Activity Score in 28 joints (DAS28). If considered appropriate, a standard CS injection: 120 mg of depomedrone (methylprednisolone acetate aqueous solution) was given as an IM injection. The injection was administered deep into the lateral upper quadrant of the gluteal muscle. Potential adverse effects of a single CS IM injection were explained to the patient including risk of infection, injection site reaction, and raised blood pressure or blood sugar levels (taken from manufacture guidelines; Pfizer Limited, Kent, 2014 (reference: PL 00057/0963)).

8.2.2. Assessments and outcome measures

Due to ethical considerations (i.e. delaying treatment), we could not assess patient's body composition prior to administration of an IM CS injection. Therefore, patients were DXA-scanned within four hours after their IM CS injection having refrained from food, exercise, caffeine, and alcohol during the interim period ('Visit 1', baseline). Approximately four weeks (~27-32 days) later, participants returned to Bangor University for a follow-up testing session and reassessment ('Visit 2', post-CS), having followed a similar pre-DXA dietary and fluid intake to 'Visit 1'. Relevant clinical and demographic information was collected by structured interview and from review of medical records.

8.2.2.1. Anthropometric and body composition measures

Routine anthropometric measures (body mass (BM), height, and waist: hip ratio) were recorded in accordance with standard procedures (Eston & Reilly, 2009). Total and regional lean and fat masses, along with bone mineral content (BMC) and density (BMD), were estimated using a whole body fan-beam DXA scanner (Hologic, QDR Discovery 45615, software V12.4). Appendicular lean mass (ALM) (the summed LM of the arms and legs) was estimated using the method described by Heymsfield et al. (1990) (**Appendix B**) and acted as a surrogate measure of total body muscle mass (Kim et al., 2002).

8.2.2.2. Clinical measures

Disease activity (DAS28) of each patient was assessed at both visits to SSHES by the same investigator (TJW). The 'EULAR response criteria' was used to determine response to treatment (Fransen et al., 2005). The venipuncture procedure (for CRP assessment) was performed by a phlebotomy-trained investigator (TJW) and CRP was determined by analysis at the Department of Clinical Biochemistry's laboratory (Ysbyty Gwynedd, Gwynedd Hospital) in line with routine procedures.

8.2.3. Statistical analysis

Inclusion of a control group was not possible for this pilot study as denying treatment to patients with uncontrolled disease activity ('flare') would be unethical. As a prospective study, and due to the absence of appropriate comparable studies, it was also not possible to perform

an a-priori power calculation to determine sample size. Whilst the preliminary results presented here are taken from patients during the first 5 months of recruitment, increased power for the full dataset will be achieved with an overall target of 12–15 patients over a 52 week recruitment period.

All data is presented as mean (±SD) unless otherwise stated. Significance was set at $P < .05$ and a trend was recognised as $P = .05–.10$. The primary outcome measure was DXA-assessed ALM, and secondary outcome measures included disease activity (DAS28), and other body composition variables (i.e., LM, relative measures of LM (i.e. ALM%, LM%), FM and relative measures of FM (i.e. body fat % (BF%), trunk FM, trunk FM%), bone mineral content (BMC), bone mineral density (BMD), and anthropometric measures (i.e. waist and hip circumferences, and waist: hip ratio)). A CRP test that returned below the detectable range (i.e. <5.0 mg/L) was recorded as 4.0 mg/L as per standard PMRC clinical practice.

Data analysis involved paired samples *t*-tests (pre- and post-measures), with confidence intervals (95% CI) and effect size (η^2: small = .01; medium = .08; large = .26; very large = .50) reported for each variable. Patients who did not attend both sessions (i.e. 'Visit 1' and 'Visit 2', baseline and post-CS injection, respectively) were not included in the final analysis. Data was analysed using the Statistical Package for the Social Sciences 22 (SPSS) (Chicago, USA).

8.3. Results (preliminary)

8.3.1. Recruitment and participant flow

Over the trial's preliminary 5 month recruitment period, a total of 10 patients were administered an IM CS injection for active disease and were eligible for the trial. Of these 10 patients, *n* = 6 consented to take part and attended the initial assessment (Visit 1; baseline). Of the 6 patients recruited who attended baseline assessment, one patient, female aged 61 years, was withdrawn following admittance to hospital for suspected meningitis. This was not deemed treatment-related by the attending rheumatologist. Five patients completed the trial and were analysed. The mean duration from injection to measurement at Visit 1 was 0.7 (range: 0.3–1.0) hours (~40 minutes), whilst the mean duration from 'Visit 1' (baseline) to 'Visit 2' (post-CS) was 30 days (range: 27–32 days).

8.3.2. Descriptive data and participants

Table 8.1. shows the baseline demographic data for the five patients who were followed up ~four weeks following the IM CS injection. The mean age of participants was 59.0 (±7.1) years, with a disease duration of 198.4 (±169.3) months (approximately 17 years). All patients were on standard disease modifying anti-rheumatic drug (DMARD) therapy, with no use of biologic agents reported. One patient was taking oral CS treatment (5.0 mg/day); a dose not thought to cause muscle atrophy (Da Silva et al., 2006). No patients reported any substantial changes to lifestyle (e.g., diet or exercise) over the 4 week trial period. No adverse events were reported.

8.3.3. Body composition changes

Mean body composition changes are shown in **Table 8.2.**, with individual changes in BM, ALM, total LM, and FM shown in **Figure 8.1**. Four weeks following a CS IM injection, an average of 1.1 (-1.9– -0.4) kg ALM (i.e. muscle mass) was lost (P = .015 (η^2 = .81, very large)), whilst total LM was reduced by 1.9 (-3.3– -0.5) kg (P = .020 (η^2 = .78, very large)). Muscle

mass corrected for BM (ALM%) showed a reduction of 3% (P = .046 (η^2 = .67, very large)) relative to baseline measures.

Table 8.1. Participant demographics at baseline

	(n = 5)
Age (years)	59.0 (±7.1)
Sex (n female) (%)	3 (60)
Height (cm)	166.7 (±12.7)
Disease duration (months)	198.4 (±169.3)
Medications, *n* (%)	
NSAIDS	2 (40)
MTX [a]	4 (80)
HCQ	2 (40)
Combination therapy (≥2 DMARDs)	1 (20)
Biological agents	0 (0)
Current oral corticosteroid use [b]	1 (20)
Analgesics	3 (60)
Calcium supplements	0 (0)

Data presented as mean (±SD). DAS28 = Disease Activity Score in 28 joints; NSAIDS = Non-steroidal anti-inflammatory drugs; MTX = Methotrexate; HCQ = Hydroxychloroquine; DMARDs = Disease modifying anti-rheumatic drugs. [a] = Additional folate supplement; [b] = Current corticosteroid dose of 5.0 mg.

Whilst no significant changes were seen in total FM (P = .725 (η^2 = .03, small)), BF% (P = .258 (η^2 = .30, large)), or trunk FM (P = .343 (η^2 = .22, medium)), there was a 3% increase in trunk FM% (P = .069 (η^2 = .60, very large)). The concurrent reduction in LM and small increase in FM resulted in a mean BM loss of 1.5 (-3.7–0.6) kg (P = .119 (η^2 = .50, very large)), and a 0.7 (-2.0–0.6) kg/m^2 reduction in BMI (P = .205 (η^2 = .36, large)). No significant changes were seen in mean BMD (P = .620 (η^2 = .07, small)) or BMC (P = .664 (η^2 = .05, small)).

8.3.4. Disease activity changes

Mean DAS28 was significantly reduced by 40% (P = .007 (η^2 = .87, very large)) (**Table 8.3.** and **Figure 8.2.**). There were large reductions in the number of tender joints (-50%, P = .205 (η^2 = .36, large)), number of swollen joints (-80%, P = .066 (η^2 = .61, very large)), and the VAS global health (-63%, P = .008 (η^2 = .86, very large)). All five patients showed a ‘moderate’

DAS28 response based on the 'EULAR response criteria'[21] (Fransen et al., 2005), with three patients attaining 'clinical remission' (DAS28 score <2.6) (Smolen et al., 2010a, 2010b; Pincus et al., 2011) post-IM injection. Thus, all patients were deemed to be responsive to the IM CS injection.

8.3.4.1. Patient 03, F, 67

For mean CRP, we observed a non-significant increase of 5.0 mg/L (P = .702 (η^2 = .04, small)), but this was due to an increase of 52.0 mg/L in one patient (female, aged 67 years). Despite the apparent increase in inflammation, this patient showed an overall DAS28 reduction of 1.6, with decreases in the number of tender (-3) and swollen joints (-1), and the VAS (-48). Based on her initial DAS28 score of 4.4, the 1.6 reduction in her score indicates a 'moderate' response (EULAR response criteria). Excluding the CRP rise in this patient, the mean CRP in the remaining four patients was reduced by 6.7 mg/L (-59%, CI: -19.3–5.8, P = .186 (η^2 = .04, small)).

[21] Due to all patients presenting with an initial DAS28 score >3.2, only a 'moderate' or 'none' EULAR response could be attained. To achieve a 'good' response, patients must have an initial DAS28 score of <3.2.

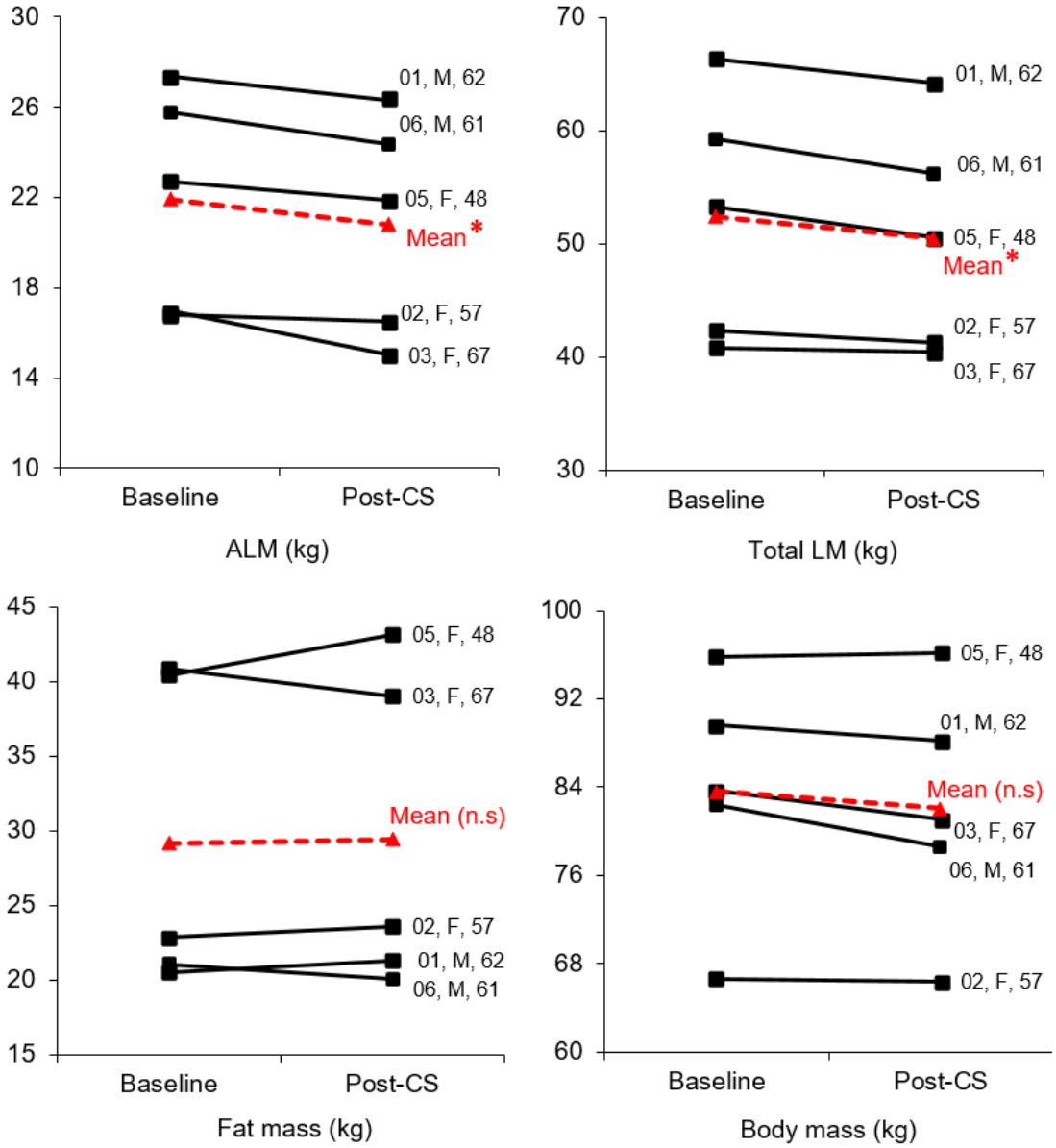

Figure 8.1. Individual, and mean, absolute body composition changes following an intramuscular corticosteroid injection to treat a rheumatoid arthritis disease flare. Data presented as individual plots (labelled as study number, male (M) or female (F), and age in years, e.g., 01, M, 62) (pre (baseline) - and post- corticosteroid (CS) injection) with mean line (▲- - -▲). ALM = appendicular lean mass; LM = lean mass; * *P* < .05; n.s = non-significant.

Table 8.2. Body composition changes following an intramuscular corticosteroid injection to treat a rheumatoid arthritis disease flare

	Baseline	Post-CS	Absolute difference (CI)	*% difference*	*P*	η^2
Waist circumference (cm)	94.8 (±15.3)	94.4(±13.7)	-0.4 (-8.6–7.8)	*0*	.898	.01
Hip circumference (cm)	106.0 (±9.5)	106.8 (±10.3)	0.8 (-9.0–10.6)	*1*	.831	.01
Waist: hip ratio	0.86 (±0.10)	0.88 (±0.08)	0.02 (-0.05–0.09)	*2*	.388	.19
BM (kg)	83.6 (±10.9)	82.1 (±11.1)	-1.5 (-3.7–0.6)	*-2*	.119	.50
BMI (kg/m^2)	30.5 (±6.0)	29.8 (±6.2)	-0.7 (-2.0–0.6)	*-2*	.205	.36
Body composition by DXA						
ALM (kg)	21.9 (±4.9)	20.8 (±4.9)	**-1.1 (-1.9– -0.4)**	***-5***	**.015***	**.81**
ALM% (ALM/BM%)	26.1 (±4.5)	25.2 (±5.0)	**-0.8 (-1.7–0.0)**	***-3***	**.046***	**.67**
Total LM (kg)	52.4 (±10.9)	50.5 (±10.1)	**-1.9 (-3.3– -0.5)**	***-4***	**.020***	**.78**
Total LM% (total LM/BM%)	62.3 (±10.3)	61.4 (±10.2)	-1.0 (-2.6–0.7)	*-2*	.183	.39
Fat mass (kg)	29.2 (±10.5)	29.5 (±10.8)	0.3 (-1.9–2.5)	*1*	.725	.03
Body fat (%)	34.6 (±11.0)	35.5 (±11.0)	0.8 (-0.9–2.6)	*2*	.258	.30
Trunk FM (kg)	14.5 (±5.9)	15.1 (±6.2)	0.6 (-1.0–2.3)	*4*	.343	.22
Trunk FM%	49.2 (±7.3)	50.8 (±6.8)	1.6 (-0.2–3.4)	*3*	.069$^{\#}$	.60
BMD (g/cm^2)	1.22 (±0.14)	1.21 (±0.16)	-0.01 (-0.07–0.04)	*-1*	.620	.07
BMC (g)	2590.9 (±689.9)	2573.5 (±720.9)	-17.5 (-120.8–85.9)	*-1*	.664	.05

Data presented as mean (±SD). CI = 95% confidence interval; CS = Corticosteroid injection; BM = Body mass (scales); BMI = Body mass index; DXA = Dual x-ray absorptiometry; ALM = Appendicular lean mass; LM = Lean mass; BMD = Bone mineral density; BMC = Bone mineral content; * $P < .05$; $^{\#}$ trend ($P = .05–.10$); Effect size (η^2), small = ≥ .01; medium = ≥ .08; large = ≥ .26; very large = ≥ .50.

Table 8.3. Change in disease activity (DAS28 and sub-components) following an intramuscular corticosteroid injection to treat a rheumatoid arthritis disease flare

	Baseline	Post-CS	Absolute difference (CI)	*% difference*	*P*	η^2
DAS28 (0–10):	4.2 (±0.7)	2.6 (±1.2)	**-1.7 (-2.6– -0.8)**	***-40***	**.007***	**.87**
▪ Tender joints (*n*)	4 (±3)	2 (±3)	-2 (-7–2)	*-50*	.205	.36
▪ Swollen joints (*n*)	5 (±5)	1 (±1)	-4 (-8–0)	*-80*	.066[#]	.61
▪ VAS global health (1-100)	57 (±23)	21 (±21)	**-36 (-57– -16)**	***-63***	**.008***	**.86**
▪ CRP (mg/L)	12.2 (±7.3)	17.2 (±27.8)	5.0 (-28.7–38.7)†	*41*	.702	.04

Data presented as mean (±SD). CI = 95% confidence interval; CS = Corticosteroid injection; DAS28 = Disease Activity Score in 28 joints; VAS = Visual analogue scale for global health; CRP = C-reactive protein; * $P < .05$; [#] trend ($P = .05–.10$); Effect size (η^2), small = ≥ .01; medium = ≥ .08; large = ≥ .26; very large = ≥ .50. † = CRP includes the increase of 52.0 mg/L in patient 03, F, 67.

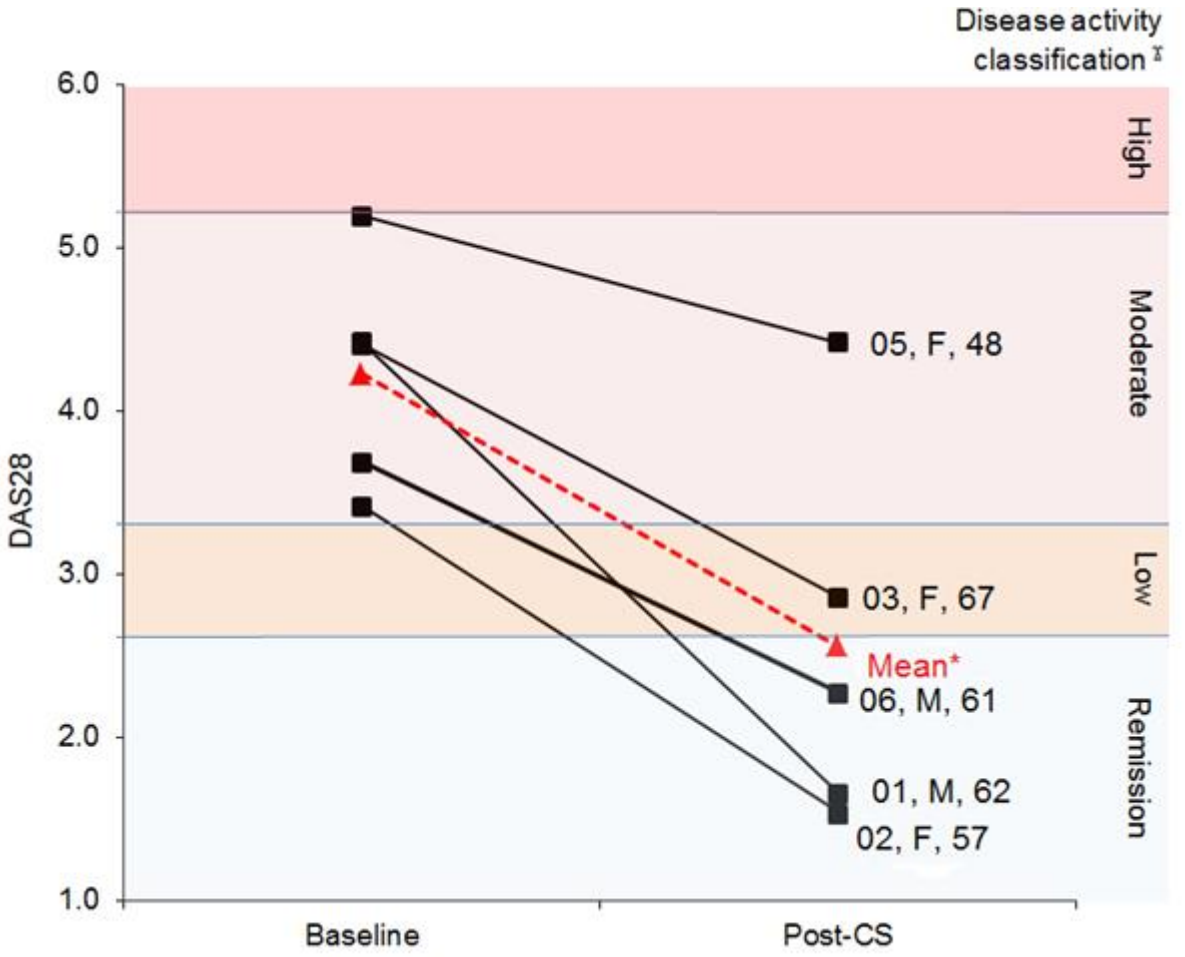

Figure 8.2. Individual, and mean, disease activity scores change following an intramuscular corticosteroid injection to treat a rheumatoid arthritis disease flare. Data presented as individual plots (labelled as study number, male (M) or female (F), and age in years, e.g., 01, M, 62 = participant 01, male, aged 62 years) (pre (baseline) - and post-corticosteroid (CS) injection) with mean line (▲- - -▲). DAS28 = Disease Activity Score in 28 joints; *P < .05. ϫ = Disease activity classification: high = >5.1; moderate = >3.2–≤5.1; low = ≥2.6–≤3.2; remission = <2.6; taken from Pincus et al. (2011).

8.4. Discussion

8.4.1. Main findings

Although only preliminary, results from these five patients and our case study (**Chapter 7**), suggest that a bolus IM CS injection used to treat active RA disease results in significant reductions in LM, specifically ALM. As this loss of LM is likely to have clinically significant adverse effects on physical function, these findings raise important concerns about the routine use of this treatment in RA patients with active disease.

Patients presenting in clinic with active or uncontrolled RA are usually administered an IM CS injection. Such injections are recommended by the national guidelines for the management of active RA ('EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying anti-rheumatic drugs', Smolen et al., 2010b; NICE Guidelines 79, section 1.4.2.1, 2009; 'British Society of Rheumatology (BSR) and British Health Professionals in Rheumatology (BHPR) guidelines for the management of polymyalgia rheumatica', Dasgupta et al., 2010), and provide significant short-term benefits by rapidly attenuating inflammation and pain (e.g., Corkill et al., 1990; Choy et al., 1993; Gough et al., 1994; Choy et al., 2005). Indeed, in our five patients, the CS injection was extremely successful in controlling disease activity, with DAS28 significantly reduced by 40% and 3/5 of patients going from 'moderate' disease activity to 'remission' (DAS28 of <2.6).

However, despite control of the disease, approximately four weeks later, 1.1 kg of ALM (i.e. muscle mass) (~5%) was lost while total LM decreased by 1.9 kg (~4%). Whilst the literature on IM CS-driven muscle loss is scant, our group previously observed a loss of 2.0 kg ALM (~7%) in an RA patient following a single CS injection given to treat a disease flare (**Chapter 7**). In this case, we were uncertain if this loss of muscle had occurred prior to the CS injection (i.e. as a result of active inflammation). However, the preliminary results from this current trail support the proposal that this loss was primarily a result of the IM CS injection.

8.4.2. Interpretation of findings

Our findings are consistent with the reported effects of chronic high dose CS treatment which is known to have detrimental effects on body composition including loss of LM (e.g., Horber et al., 1986, Roubenoff et al., 1990; Dekhuijzen & Decramer, 1992; Natsui et al., 2006; Mok et al., 2008; Pereira & de Carvalho, 2011). The exact mechanism underlying the changes to muscle following CS use is unclear. It is thought that the stimulation of the ubiquitin-proteasome system by CS is mediated through the increased expression of several atrogenes (i.e. genes involved in atrophy), such as MuRF-1; a ubiquitin ligase involved in the identification of protein to be degraded by the proteasome system. Corticosteroids may also exert an anti-anabolic action by inhibiting anabolic pathways (e.g., mTOR/S6 kinase 1 and insulin-like growth factor (IGF)-I), thus blunting muscle protein synthesis (Short et al., 2004; Schakman et al., 2013).

Chronic low (Da Silva et al., 2006) and high dose CS use (Mok et al., 2008) has also been implicated in the redistribution of fat to the truncal area. Although we saw no change in the total FM of our patients, we did observe a moderate 3% increase in trunk FM% suggesting a shift of FM to the trunk may also occur following acute administration of high dose CS. Whilst the precise mechanisms are unknown, hyperinsulinemia, changes in expression and activity of adipocyte derived hormones and cytokines such as leptin and TNF-α, and increased food intake (CS increases appetite) are all thought to contribute to this effect (Da Silva et al., 2006). Trunk obesity is a distinctive feature of RA body composition (Giles et al., 2008b; Elkan et al., 2009; Dao et al., 2011) and exacerbates the risk of CVD (Inaba et al., 2007; Stavropoulos-Kalinoglou et al., 2009; Giles et al., 2010; Summers et al., 2010).

Although chronic (Da Silva et al., 2006; Natsui et al., 2006; Mok et al., 2008; Ding & Deighton 2010) and acute IM CS (Choy et al., 2005) use have also been recognised to increase the risk of osteoporosis, we saw no changes to bone measures (BMD or BMC).

8.4.3. Significance

A loss of just 5% LM (as seen in this trial) can result in muscle weakness and loss of functional capacity (Walsmith & Roubenoff, 2002), and reductions in muscle mass are a major contributor to the decreased strength (Marcora et al., 2005a; Van Bokhorst – de van der Schueren et al., 2012; **Chapter 3**) and impaired physical function seen in RA (Giles et al.,

2008a; Summers et al., 2008; Lemmey et al., 2009; Lusa et al., 2015). Further, loss of LM (and therefore loss of expendable protein) also impairs the immune system's ability to respond to infection and trauma (Roubenoff, 2001; Summers et al., 2008).

Significantly, the 1.1 kg loss of ALM seen in our patients following the IM CS injection is identical to that (i.e. 1.1 kg) we observed in **Chapter 3** comparing RA patients with age- and sex-matched healthy controls. Considering the majority of newly diagnosed patients, and patients undergoing active disease 'flares', experience an IM CS injection to supress disease activity, it may be that the treatment is contributing significantly to the discrepancy in LM observed in RA patients. On appearance there appeared to be no difference in the magnitude of loss between males (*n* = 2) and females (*n* = 3), although gender-differences in treatment response need to be confirmed in a larger study.

Without some form of anabolic stimuli, it is unlikely that the body is able to spontaneously restore this lost LM. Certainty, in our case patient (**Chapter 7**), the lost LM had not been restored 12 weeks later. This further emphasises the importance for adjunct interventions designed to increase muscle mass in RA. Primarily, progressive resistance training (PRT) (Marcora et al., 2005a; Lemmey et al., 2009) appears most beneficial for patients wishing to increase LM and improve their physical functioning.

Interestingly, it appears muscle loss following IM CS injections may be a generic treatment class effect. In our case report (**Chapter 7**) the patient received *triamcinolone acetonide* (40 mg), whilst in the current study, 120mg of *methylprednisolone* was administered. Both CS forms resulted in comparable reductions in muscle mass (~5%).

8.4.4. Limitations

We acknowledge several limitations of the preliminary findings presented here. First, the low *n* of our sample makes it difficult to generalise the effect of IM CS injection to all RA patients, and results should be interpreted conservatively. However, we feel that even with the small sample, the large effect sizes (η^2 = .67–.81) and consistent pattern of muscle loss supports concerns that CS IM injection can contribute to muscle loss in RA. This will be confirmed once more patients are recruited in the full trial.

Due to ethical considerations we were unable to perform a randomised, placebo-controlled trial, as denying treatment to patients with active RA would not be ethically justifiable. The lack of a control group is a weakness of our quasi-experimental study design. However, even without a control/placebo arm, associations identified in quasi-experiments do meet some requirements of causality because the intervention precedes the outcome measurement (Harris et al., 2006).

8.5. Conclusion

The preliminary results from this study indicate that bolus IM injection of high-dose CS, a recommended and routine treatment for uncontrolled disease activity in RA, causes substantial loss muscle mass. If this effect is confirmed by our full study ($n \approx 12–15$), then risk: benefit analyses of this treatment should be conducted, as should investigations into potential alternative treatments for rapidly dealing with the inflammation and pain of uncontrolled RA.

9

General discussion

This general discussion reviews the key findings of this thesis, proposes potential future research and recommendations, and outlines the strengths and limitations of the work completed during my doctoral research.

9.1. Summary of key findings

9.1.1. 'Treat-to-target', despite providing effective control of disease activity, does not prevent rheumatoid cachexia

Patients with rheumatoid arthritis (RA) typically experience significant loss of muscle mass and increased adiposity, a condition known as 'rheumatoid cachexia' (RC) (Roubenoff et al., 1992; Roubenoff et al., 1994; Summers et al., 2008). As RC has been attributed to inflammation (especially tumor necrosis factor (TNF)-α)-driven muscle catabolism (Roubenoff et al., 1992, 1994; Metsios et al., 2008), it was proposed that successful control of inflammation/disease activity may attenuate the effects of RC. Current RA treatment, exemplyfied by the 'treat-to-target' (T2T) strategy (Verstappen et al., 2007; Saunders et al., 2008; Goekoop-Ruiterman et al., 2010; Smolen et al., 2010a, 2010b; Jurgens et al., 2012; Stoffer et al., 2015), emphasises tight control of inflammation, with achievement 'clinical remission' (usually defined as a disease activity score in 28 joints (DAS28) <2.6 (Smolen et al., 2010a, 2010b)), or failing that achievement of low disease activity (LDA), the goal. Whether T2T, specifically the achievement of 'clinical remission' or LDA, has resulted in the attenuation of RC and subsequent improvements in objective physical function in RA patients had not previously been investigated.

In **study 1** (**Chapter 3**), the disease activity of 82 RA patients exclusively treated by a T2T approach was well-controlled with the mean DAS28 score of the group (= 2.8) indicating LDA and approximately half (49%) achieving 'remission'. However, despite successful control of disease activity we found that compared to age- and sex-matched healthy controls (HCs) aberrant body composition was still evident in RA patients, with significant loss of muscle mass (10%; adjusted for body mass (BM)), and increased total (27%) and trunk (32%) adiposity observed. These values are remarkably similar to those reported for patients who were treated, or commenced treatment, prior to T2T (prior to ~2008), i.e. muscle mass loss of ~8–15% and total (and trunk) fat mass (FM) ~12–18% greater relative to HCs (see **Chapters 2** and **3**). Consequently, **study 1** is the first to show that despite tightly controlling disease activity, T2T has not prevented, or even attenuated RC.

9.1.2. Strength and objective physical function remains significantly poorer compared to sedentary controls

Despite only self-reporting their disability as 'mild' (Multi-dimensional Health Assessment Questionnaire (MDHAQ) score = 0.6), RA patients in **study 1** displayed greatly impaired strength (reduced by 24–25%) and objective physical function (reduced by 28–34%) relative to age- and sex-matched HCs. Consistent with the absence of improvement in body composition, these deficits in performance are similar to, and certainly not better, than those previously observed by our group (Marcora et al., 2005a, 2005b, Marcora et al., 2006; Lemmey et al., 2009, 2012; Matschke et al., 2010a, 2010b; Matschke et al., 2013) and others (e.g., Brorsson et al., 2012; Kramer et al., 2012). This finding indicates that even aggressive treatment to suppress disease activity leaves RA patients with significant functional deficiencies equivalent to a healthy individual of the same sex who is 25 years older.

9.1.3. Rheumatoid cachexia may occur prior to disease diagnosis

In **study 1**, there was no difference in body composition between the 'recent-onset' (<12 months from diagnosis) and 'established' (1–7 years from diagnosis) disease groups. This finding supports previous suggestions (Marcora et al., 2006; Book et al., 2009) that RC is established early in the course of RA, possibly in a 'pre-clinical' phase. Research has shown that disease processes may be active before RA symptoms become clinically detectable (Gerlag et al., 2012). Specifically, elevated systemic inflammation has been found to exist in a 'pre-clinical' phase of RA (Kraan et al., 1998; Van de Stade et al., 2011; Sokolove et al., 2012). Using stored serum (1–12 years prior to disease diagnosis[22]) samples from RA patients, Sokolove et al. (2012) found elevated concentrations of pro-inflammatory cytokines (TNF-α, interleukin (IL)-1β, and IL-6) prior to diagnosis, with concentrations appearing to peak ~0–2 years prior to diagnosis (**Figure 9.1.**).

[22] In this study, the median time of onset of symptoms was ~6 months prior to diagnosis, and in no instance where it could be assessed did symptoms precede the presence of cytokines.

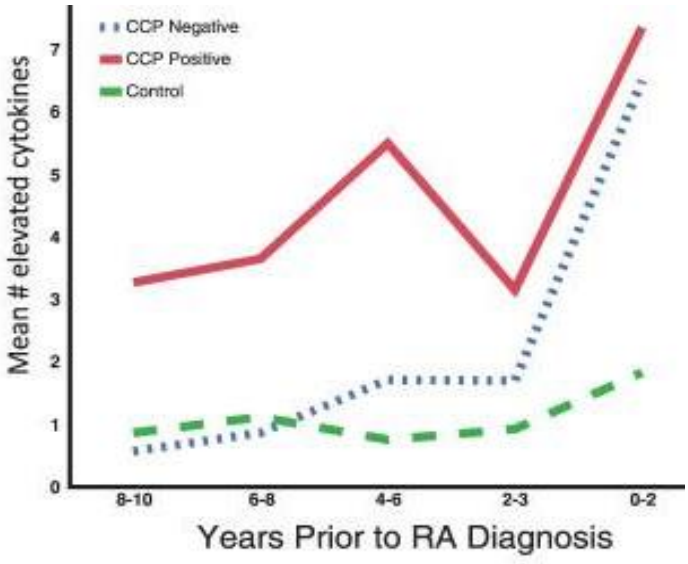

Figure 9.1. Graph showing the mean number of elevated cytokines (TNF-α, IL-1β, and IL-6) evaluated in a 'pre-clinical' stage. Source: Sokolove et al. (2012) and reproduced under the CC-O licence.

Pertinently, these specific pro-inflammatory cytokines (i.e. TNF-α, IL-1β, and IL-6) have been associated with the pathophysiological processes behind RC (Roubenoff et al., 1992; Roubenoff et al., 1994; Metsios et al., 2006). Thus, it is possible that uncontrolled inflammation initiates muscle wasting and adiposity gain prior to the appearance of detectable joint symptoms, subsequent diagnosis, and the commencement of disease modifying anti-rheumatic drugs (DMARD) treatment.

Interestingly, it appears that after this 'initial' loss of muscle, and once RA has been stabilised, the rate of muscle mass decline is comparable to that of normal sedentary individuals of the same sex and similar age (Westhovens, 1999; Lemmey et al., 2012) (i.e. annual muscle decline rates after 30 years of age in the general population = ~1.0% per year (Frontera et al., 2000; Morley et al., 2011; Von Haehling et al., 2012)). This finding is supported by preliminary data from the longitudinal arm of **study 1** which shows in (n = 10) RA patients the mean decline of muscle (adjusted for BM; ALM%) over 12 months is 0.7%. However, only a full data set will allow a conclusive interpretation of this finding.

9.1.4. Adjunct anabolic treatments are still needed to reverse rheumatoid cachexia and normalise physical function

Study 1 revealed that RC still exists despite tightly-controlled disease activity through T2T. Together with evidence that DMARD and anti-TNF-α therapy is unable to reverse RC (Marcora et al., 2006; Metsios et al., 2007; Serelis et al., 2008; Engvall et al., 2010; Toussirot et al., 2014) and preliminary evidence from our group (**Chapter 7** and **8**) that intramuscular (IM) corticosteroid (CS) injections are contributing to RC, the identification and promotion of adjunct anabolic interventions such as exercise (specifically progressive resistance training (PRT) (Marcora et al., 2005a; Lemmey et al., 2009)), or nutritional supplements (Willer et al., 2000; Marcora et al., 2005b) should be an important aspect of RA care and research. Since the beneficial effects of exercise such as high-intensity PRT are well-established, and the uptake of this form of treatment is generally poor (e.g., Lemmey et al., 2012), in this thesis, a less demanding, more acceptable, nutritional treatment option was investigated.

9.1.5. Nutritional creatine supplementation can be effective in reversing muscle mass loss from rheumatoid cachexia

A nutritional supplement considered likely to be effective in reversing the effects of RC was creatine (Cr) monohydrate. Creatine is a popular form of protein supplementation shown to improve physical function via enhanced energy (i.e. ATP) regeneration, and increased muscle mass (e.g., Casey & Greenhaff, 2000). In **Chapter 4,** we reviewed the mechanisms of Cr supplementation and its potential as an anabolic therapy for RA patients. Our review identified only one study in which RA patients supplemented with Cr (Willer et al., 2000), and its findings, whilst promising, were inconclusive. Conversely, trials in populations with similar presentation to RA patients (i.e. reduced muscle mass and impaired physical function), including older adults (e.g., Rawson & Clarkson, 2000; Tarnopolsky, 2000; Brose et al., 2003; Aguiar et al., 2013) and those with muscle wasting conditions (e.g., Tarnopolsky et al., 2004; Norman et al.. 2006; Chung et al., 2007; Banerjee et al., 2010), indicated that Cr may offer an easy, safe, and effective means to improve muscle mass, strength, and physical function.

Accordingly, we conducted a randomised controlled trial (RCT) (**Chapter 5**) that found 12 weeks of oral Cr supplementation was able to significantly increase LM (primarily skeletal muscle mass) (~0.5 kg), but not strength or objective physical function. Whilst this lack of effect on physical function is disappointing, the increase in LM observed suggests that Cr may

be beneficial in patients with severe RC since a marked loss of LM impairs the body's ability to fight infection due to limited expendable protein reserve for immune cell production (Summers et al., 2008). Nonetheless, in patients wishing to increase muscle *and* strength and physical function, high intensity exercise such as PRT (e.g., Hakkinen et al., 1999; 2005; Marcora et al., 2005a; Lemmey et al., 2009) remains easily the most efficacious treatment option.

Overall it appears that exercise remains the most important and clinically relevant countermeasure against RC (Walsmith & Roubenoff, 2002). However, the challenge now is to increase adherence and uptake of physical activity in patients with RA. It has been shown that patients whom participate in regular exercise have lower disease activity and better functional ability (Munneke et al., 2003; Metsios et al., 2008). Thus, the first step to increasing physical activity appears to be controlling RA disease activity.

Whilst other barriers such as facility access and social support exist (Metsios et al., 2008), another important aspect is clinician knowledge and the lack of time invested in exercise advice during a typical ~15 minute consultation. Research by Iversen et al. (2004) found that just 42% of rheumatologists believe strengthening and aerobic exercises are useful in the management of RA, with only ~20% (Iversen et al., 2004) confident they could instruct patients to exercise effectively. Clearly, there is a need for better rheumatologist-based knowledge of exercise prescription in RA, and for health care professionals to recognise the pivotal role they play in disseminating information about exercise to their patients. Whilst the latest 'ACR-EULAR current care guidelines' (2015) recommend exercise training in the management of RA, it is yet to be seen whether patient uptake to exercise is improved.

9.1.6. Serum-based markers of rheumatoid cachexia

In order to enhance the understanding of markers of RC, **Chapter 6** investigated a range of anabolic, catabolic, and inflammatory serum biomarkers in different disease and treatment conditions. These conditions included a sub-sample of RA and HC participants from **study 1**, untreated and subsequently treated patients, and the effects of two types of anabolic interventions used to attenuate RC (PRT and oral Cr supplementation (**study 2**)). As would be predicted, patients with RA, even when disease activity was stable and well-controlled, had elevated levels of circulating pro-inflammatory cytokines (TNF-α and IL-6) compared to HCs (Walsmith & Roubenoff, 2002; Shrivastava & Pandey, 2013). However, no differences in

anabolic, catabolic, and markers of adiponectin and leptin were observed between the groups (Rall et al., 2002; Toussirot et al., 2005).

Interestingly, adiponectin and insulin-like growth factor binding protein 3 (IGFBP-3) were significantly inversely associated with muscle mass in the RA group, whilst weaker correlations were also observed in the HC group suggesting a non-disease specific mechanism. Elevated adiponectin may directly stimulate inflammatory (and catabolic) pathways responsible for cachexia (McEntegart et al., 2007), including increased IL-6 expression (Ehling et al., 2006), whilst increased IGFBP-3 may prevent muscle cell proliferation (Foulstone et al., 2003). Markers of inflammation (TNF-α and IL-6) were not associated with reduced muscle mass. A possible explanation for this is that RC probably occurs during active inflammation (i.e. in early RA prior to commencement of treatment, or during disease flares), but our samples generally only included patients with controlled disease (see section ***9.1.3. Does rheumatoid cachexia occur prior to disease diagnosis?***). Despite having a role in muscle wasting (Schulte & Yarasheski, 2001; Ju & Chen, 2012), our data showed that myostatin was actually positively correlated with muscle mass. This finding is supported by other studies (Lee & McPherron, 2001; Bergen et al., 2015; Yamada et al., 2016), and most probably occurs as myostatin is excreted into circulation by skeletal muscle (Lee & McPherron, 2001), and therefore serum myostatin level may reflect total muscle mass.

When investigating the effects of etanercept (ETN) or methotrexate (MTX) on RC in treatment-naive patients with high disease activity, initiation of either treatment (i.e. ETN or MTX) reduced DAS28 and C-reactive protein (CRP) levels by ~50%. Levels of TNF-α were increased by 67% following ETN use. This occurred as ETN binds with TNF-α rendering it biologically and immunologically inactive (Fox, 2000; Bhatia & Kast, 2007), but not eradicating it from the blood. No change in TNF-α was seen following MTX therapy, which predominantly reduces IL-6 expression (Aggarwal & Misra, 2003; Nishina et al., 2013). Neither ETN nor MTX had any effect on anabolic markers or adipokines (e.g., Gonzalez-Gay et al., 2011; Toussirot et al., 2014).

Anabolic interventions (both PRT and oral Cr supplementation) resulted in negligible changes in the serum biomarkers analysed, with the exception of TNF-α levels, which were reduced by 34% following Cr supplementation. Interestingly, similar results have been reported in athletes following post-exercise Cr supplementation (Santos et al., 2004; Bassit et al., 2008) and this effect may warrant further study in RA as elevated TNF-α is believed to drive RC (Roubenoff et al., 1994).

The overarching conclusion from this set of experiments (**Chapter 6**) was that no single serum biomarker was consistently associated with the LM deficits characteristic of RC. Despite its association with RC in the literature, it appears that current low-level systemic inflammation in RA patients with controlled disease is not a good indicator of muscle mass or of any mechanism of muscle catabolism (Roubenoff et al., 1994; Engvall et al., 2008). Similarly, serum IGF status is a poor indicator of body composition and muscle anabolism (Adams, 2002). The role of adiponectin and IGFBP-3 with body composition may warrant further investigation.

9.1.7. Intramuscular corticosteroid injections may contribute to rheumatoid cachexia

Whilst numerous potential mechanisms of RC, including inflammation and physical inactivity, have been identified (Roubenoff et al., 1992, 1994; Walsmith & Roubenoff, 2002; Metsios et al., 2006; Engvall et al., 2008), in **Chapter 7** and **8** we explored whether an IM CS injection, a National Institute for Health and Care Excellence (NICE) recommended (NICE Guidelines 79, section 1.5.1.4) and routinely used treatment to rapidly suppress inflammation and relieve pain during an acute episode or exacerbation of disease activity, may also contribute to muscle wasting in RA.

During the analysis of **study 2**, we identified a 2.4 kg (4%) loss of total LM and a 2.0 kg (7%) loss of ALM in an RA patient whom had previously been administered an IM CS injection by his GP to control a disease flare. This novel finding was highly concerning as a possible contributor to RC. However, the duration between the original and follow-up measurements meant we could not exclude the effect of active inflammation on muscle mass during the RA flare (i.e. prior to the CS injection). To that end, in **Chapter 8** we found that, in a preliminary sample (n = 5), a bolus IM CS injection used to treat active RA disease (i.e. a disease flare) resulted in substantial reductions in LM (-1.9 kg, ~4%), specifically ALM (-1.1 kg, ~5%). Coincidently, the 1.1 kg loss of ALM seen in these patients following an IM CS injection is identical to the difference (1.1 kg) between RA patients and age- and sex-matched HC we observed in **Chapter 3**.

In patients with recently diagnosed RA, an IM CS injection (either Methylprednisolone acetate or Triamcinolone acetonide) is recommended by NICE to supress disease activity. Therefore, it may be that this treatment is partly responsible for the discrepancy in LM reported in early

RA. This finding emphasises the importance for adjunct interventions designed to increase muscle mass, such as PRT (e.g., Marcora et al., 2005a; Lemmey et al., 2009), to be delivered as early in the disease process as possible.

9.2. Future direction

9.2.1. Assessing physical function and body composition in clinic

Whether using DAS28 or EULAR response criteria, research has shown that applying a 'treat-to-*any* target' strategy results in greater RA remission rates (Jurgens et al., 2012). Given that, as we have shown, even RA patients considered to be in 'remission' have substantial deficits in strength and physical functioning, assessment of function should be considered as a 'target' of treatment alongside composite measures based on disease activity (e.g., DAS28).

Pertinently, a fundamental objective of T2T is normalisation of function (Smolen et al., 2010a; Smolen et al., 2015), although no recommendation on its measurement is provided. Whilst a subjective patient-reported measures of function (i.e. the HAQ) has been used in rheumatology for decades, it is influenced substantially by pain (which is often reduced by initiation and 'tight control' of disease activity (e.g., Marcora et al., 2006; Kingsley et al., 2011)). Further, the HAQ generally fails to detect substantial, and clinically significant improvements in objectively measured physical function in RA patients with controlled disease (Van den Ende et al., 1997; Lemmey et al., 2009).

To that end, in the future management of RA, the evaluation of treatment success should include *objective* assessments of physical function. Perhaps the two most appropriate assessments are the STS-30 and handgrip strength (HGS). Both of these tests are quick (<60 seconds), easy, and safe to administer in clinics, and together measure upper and lower body functioning. The STS-30 correlates well with walking ability (as assessed by the 50'W test (r = -.553, P = .001; **study 1**), a test that, due to space limitations, may be difficult to implement in a busy hospital environment).

Measuring HGS in RA may be particularly favourable as the 'British Society of Rheumatology (BSR) Guidelines for the Management of RA' (Luqmani et al., 2006) state hand function should be maintained or improved following treatment. Further, research in older adults from the general population (Newman et al. 2006; Gale et al., 2007) and RA patients (Pincus et al., 2001; Wolfe et al., 2003) shows that HGS is a strong predictor of independence and mortality. Like all objective assessments of function, these tests could be influenced by joint

involvement, nevertheless they provide a superior indication of functional status in RA patients than subjective measures such as the HAQ (**Chapter 3**).

As RC is a key determinant of strength and physical function (Giles et al., 2008a; Stavropoulos-Kalinoglou et al., 2009; Kramer et al., 2012; Lusa et al., 2015), there is also a benefit in assessing body composition in an outpatient setting (Chen et al., 2011; Lemmey, 2016). In all of the studies in this thesis, body composition was assessed via dual x-ray absorptiometry (DXA) – the 'gold standard' for research body composition assessment (Ellis, 2000; Provyn et al., 2008). However, DXA is not readily available to rheumatologists, and usually requires a scheduled appointment with the hospital imaging department. Due to its relatively low cost, accuracy, easy operation, and high portability (Ellis, 2000), bioelectrical impedance analysis (BIA) may be the most favourable method of evaluating body composition in a clinical setting (Chen et al., 2011; Androutsos et al., 2014). Research indicates BIA has moderate to strong agreement with DXA in the assessment of body composition in the elderly (e.g., Moon et al., 2013; Bosaeus et al., 2014), and has been successfully used to investigate RC by others, including the group of Kitas et al. (e.g., Stavropoulos-Kalinoglou et al., 2007; Metsios et al., 2007; Metsios et al., 2009).

Whilst participants in our studies were assessed following an overnight fast, Androutsos et al. (2014) found that food and drink consumption prior to BIA results in only minor, non-clinically significant changes in body composition (i.e. changes are within the precision limits of the device). Therefore, assessments in clinical settings do not require strict adherence to fasting, and this should increase the opportunities for clinical application. A pragmatic trial could investigate the feasibility of performing these tests (i.e. strength, physical function, and body composition) in clinic, with potential research focusing on their use as a treatment goal (the 'target') to guide a T2T strategy.

9.2.2. Adiposity and other factors may also affect physical function

Excess adiposity contributes to self-reported disability, weakness, and poor functional performance in RA (Giles et al 2008b; Stavropoulos-Kalinoglou et al., 2009; Kramer et al., 2012). In **study 1**, our RA patients were significantly 'fatter' (5.4 kg) than the matched HCs, and this increased adiposity was correlated with poorer strength and aerobic fitness. Several mechanisms may account for the role of FM on physical function, including the increased load

on the muscle (Rolland et al., 2009), and interference with limb mechanical kinematics and range of motion (Giles et al., 2008a; Runhaar et al., 2011).

Excess adiposity can also influence physical function via 'fat infiltration' in skeletal muscle (Visser et al., 2002, 2005; Goodpaster et al., 2008; Kramer et al., 2012; Addison et al., 2014). Intramuscular adipose tissue (IMAT) is an ectopic fat depot found beneath the fascia and within the muscles (Addison et al., 2014), and although the mechanism(s) are unclear, may impair local muscle contractility (cellular function), muscle fibre recruitment (nerve function), or muscle metabolism (energy utilisation) (Visser et al., 2002, 2005). Fat infiltration into the muscle is positively correlated with body fat percentage (BF%) (Visser et al., 2002, 2005; Baker et al., 2014) suggesting that those with high BF% (such as the majority of RA patients) also typically have high levels of IMAT. No study to date has investigated the role of IMAT on physical function in RA patients. Whilst loss of LM and excess adiposity are substantial predictors of impaired strength and physical function in RA, other factors such as joint damage, fatigue (Scott et al., 2000; Giles et al., 2008a; Ormseth et al., 2015), and pain (**Appendix J**; Heiberg & Kvien, 2002; Lusa et al., 2015) should not be overlooked.

9.2.3. Corticosteroid injections and rheumatoid cachexia

Study 4 raises concerns about the routine use of IM CS treatment in RA, and if the effect of iatrogenic muscle loss is confirmed by our full study (expected $n \approx 12–15$), then since loss of muscle mass has substantial adverse functional (Walsmith & Roubenoff, 2002) and immunological (Roubenoff, 2001; Summers et al., 2008) consequences, careful consideration needs to be given to administration of this treatment.

9.2.3.1. A potential role for creatine supplementation following corticosteroid injection

Whilst the effects on muscle mass are concerning, there are currently no other alternative treatments for uncontrolled RA that can provide such rapid suppression of inflammation and pain as IM CS injections. Other anti-inflammatory treatments (e.g., MTX), including biologics, take several weeks to take full effect (Lambert, 2012), in which time significant loss of muscle as a result of untreated active disease would have taken place. If muscle loss is a common effect of IM CS injections, a short period of oral Cr supplementation, commenced on the day of treatment, may maintain muscle mass. This cheap and convenient intervention, if successful, would allow RA patients to continue to benefit from the potent and rapid anti-

inflammatory effects afforded by an IM CS injection, without the adverse effect on body composition.

9.3. Thesis strengths and limitations[23]

9.3.1. Study design

Study 1 involved a large cross-sectional design which allowed a comparison of body composition and physical function in RA patients and matched HCs. Whilst cross-sectional trials are useful in estimating the prevalence and magnitude of a condition (e.g., RC, disability), they make it difficult to determine the cause or time-course of these conditions (Sedgwick, 2014). In our study, to determine temporal changes in body composition and physical function, patients are being followed-up for 8 years as part of a longitudinal design. However, to investigate if, as we hypothesise, RC does occur in a 'pre-clinical' stage, a very large longitudinal prospective study would need to track body composition changes in the general population and then explore the (~1%) who develop RA.

Chapter 4 involved a review of the Cr supplementation literature with specific consideration to populations with similar losses of muscle mass and function as RA. A systematic search method allowed the identification of all relevant publications, specifically placebo-controlled trials. Further investigation could utilise a meta-analysis approach which would allow for better statistical assessment (e.g., error, bias, risk, and effect size) of the clinical effectiveness of Cr supplementation on a particular outcome measure (e.g., LM) in these groups (e.g., the elderly, muscular dystrophy).

Study 2 involved a double blind placebo-controlled RCT which investigated the effects of Cr supplementation in RA patients. A RCT design is considered the 'gold standard' in clinical research (Misra, 2012), as they are largely untainted by bias (Kaptchuk, 2001) (see ***9.3.3. Internal validity***) and allow demonstration of causality. In **study 4** (i.e. the effects of IM CS), we used a single group in a non-randomised, pre-post intervention design. Methodologically, it would have been ideal to use a randomised control group who did not receive an IM CS injection, however, denying treatment to patients experiencing active disease would be unethical, thus this approach was not an option. It is important to acknowledge that

[23] As **study 3** (biochemical analysis of various disease scenarios) contains experimental studies from this thesis, the relative limitations (i.e. participant bias and study design) of these individual studies (**1, 2** and **4**) are described separately. The relative limitations of the assay analysis for **study 3** is described in section ***9.3.2.3. Detection and measurement bias***.

associations identified in quasi-experiments, such as **study 4**, do meet some requirements of causality because the intervention often precedes the outcome measurement (Harris et al., 2006).

9.3.2. Internal validity

Internal validity refers to the certainty that results and findings are true for the study population, and indicates the control over potential confounding variables to reduce alternative explanations for the effects of any intervention (Jüni et al., 2001; Halperin et al., 2015).

9.3.2.1. Selection and non-response bias

'Selection bias' refers to biased allocation of comparison groups. A strength of **study 2**'s (and the trials of Marcora et al. (2006) and Lemmey et al. (2009) analysed in **Chapter 8**) RCT design was the use of randomised groups, thus reducing 'selection bias'. In **study 2**, participants were randomised (stratified for age and sex) independently from the research team by the North Wales Organisation for Randomised Trials in Health (NWORTH; a registered clinical trials unit) using a secure online system.

In all trials, there was a risk of 'non-response bias' if participants who consented to take part in the study differed from those who did not, which may have resulted in a sample that is not representative of the population (Sedgwick, 2014). In clinical trials, 'study *non*-participants' often have higher disease and mortality rates, poorer health status, and lower levels of functioning than 'study participants' (Gelea & Tracy, 2007). In RA specifically, patients who participate in research trials typically have less fatigue, pain, and stiffness, and are more motivated (Nordgren et al., 2013). Consequently, it may be that the RA patients who agreed to participate were at the greater end of functional capacity. If this is the case for our studies, then the alarming deficiencies in function and body composition (e.g., **study 1**) we observed in our RA cohort would be underestimations of even greater deficits in the general RA population.

9.3.2.2. Performance bias

Participant 'performance bias' refers to unequal provision of care, apart from treatment, under evaluation, and may occur if additional treatment interventions or attention are provided preferentially to one group (Jüni et al., 2001). To remove 'performance bias' in **study 2**, both the patient and myself (researcher) were blinded to treatment allocation (i.e. Cr or placebo)

until after trial termination and initial data analysis. Blinding both patients and experimenters prevents 'performance bias' and safeguards against differences in placebo responses between the group (Jüni et al., 2001).

In **study 2**, our inability to determine an improvement in objective physical function tests following Cr supplementation (i.e. both groups improving at similar rates) may, in part, be due to a learning effect. This apparent learning effect occurred despite participants performing one practice beforehand. However, in previous studies when our group has employed the same practice routine (e.g., Lemmey et al., 2009), we have observed no such learning effect. In future trials, a familiarisation session (~1–2 weeks pre-randomisation) could be used to reduce the chance of such effect from occurring.

In order to further remove potential sources of 'performance bias', in **studies 1** and **2** all participants were exposed to the same tests of strength and function. The whole body objective physical function assessments, specifically developed and validated for assessing the capacity of older adults to perform activities of daily living, were taken from the Senior Fitness Manual (Rikli & Jones, 2012). The protocols for strength and other functional tests used have been extensively employed by our group (e.g., Marcora et al., 2005a, 2005b, 2006; Lemmey et al., 2009, 2012; Matschke et al., 2010a, 2010b, 2013). Additionally, in **studies 1** and **2**, all instructions and encouragement were pre-prepared and standardised, although in some cases participants' required additional instruction.

The 'Siconolfi' step test (Siconolfi et al., 1985) was used as a predictive, sub-maximal measure of aerobic capacity (VO_2max). This test has been validated by our group in RA (Cooney et al., 2013), systemic lupus erythematosus (SLE; Marcora et al., 2007), and ankylosing spondylitis (Thompson et al., 2015) patients. In additional support of this test, direct VO_2max measures (i.e. treadmill-based using a calibrated online breath-by-breath system) in 144 RA patients (mean: 20.9 (±5.7) ml/kg/min) by Metsios et al. (2015) yielded similar, albeit smaller, values to those from the step test (**study 1** mean VO_2max: 22.9 (±6.2) ml/kg/min; **study 2** mean: 25.1 (±6.8) ml/kg/min).

9.3.2.3. Detection and measurement bias

The assessment and measurement of an outcome is exposed to potential bias and error. In all the experimental studies, the primary outcome variable was body composition, specifically measures of LM by DXA. Dual-energy x-ray absorptiometry has little measurement error, and is considered the 'gold-standard' for body composition assessment in research (Ellis, 2000;

Provyn et al., 2008). Appendicular LM was estimated using the validated method described by Heymsfield et al. (1992) (**Appendix B**), and acted as a surrogate measure of total muscle mass (Kim et al., 2002). For blood sample collection (**studies 1** and **2**), participants presented for assessment following an overnight fast (i.e. no food upon waking, and only water allowed to help medication intake), therefore food and drink consumption was relatively comparable for all participants prior to each scan. In **study 4**, as patients presented immediately after their IM CS injection and non-fasted, at visit 2 they were asked to consume a comparable diet to that at visit 1. Manufacturer DXA examination procedures (daily calibration, subject preparation (i.e. clothes, jewellery), positioning, and analysis) were followed for every scan, and an in-house assessment revealed a DXA co-efficient of variation (CV) of 1.4%. This value corresponds with both manufacture guidelines and other studies (e.g., Scafoglieri et al., 2011).

In **study 1**, four researchers were involved in data collection. Intra-rater reliability assessment of anthropometric and physical function measurements revealed intra-class correlation coefficients (ICC) between .704 and .996 ('good' to 'excellent', based on based on commonly-cited cut-offs by Cicchetti (1994)).

In **studies 1, 2,** and **4**, a DAS28 score is reported. In **study 1**, this was performed by either myself or another researcher (BJC). In **study 2** and **4**, this was performed by myself. Prior to data collection, both experimenters were taught how to perform a DAS28 evaluation by rheumatologists from the PMRC. In several patients attending rheumatology outpatient clinics the difference in DAS28 values between the rheumatologists and experimenters were negligible (unfortunately, no quantitative data was collected). The rheumatologists were satisfied with our competence to perform this assessment during data collection. Research has shown that following appropriate training, DAS28 scores between non-medically trained professionals (i.e. health care assistants) and rheumatologists are comparable (Toms et al., 2015).

9.3.2.4. Attrition bias

Attrition bias is the biased occurrence and handling of deviations from protocol and loss to follow up. A frequently reported reason for a deviation from protocol is 'non-adherence to treatment' (Jüni et al., 2001). In **study 2**, self-reported adherence to the treatment drinks was excellent (i.e. 99% in both Cr and placebo groups). As part of **studies 2** and **4**, participants were asked not to change their lifestyle (e.g., start exercising intensely) to prevent confounding the treatment effects. In both trials, participants declared no substantial changes in lifestyle, although this was not quantitatively measured.

Loss to follow up relates to participants unavailability for assessments during the study period because they: (i) refuse to participate further ('drop outs'); (ii) cannot be contacted; or (iii) clinical decisions are made to stop the assigned interventions ('withdrawn') (Jüni, Altman, & Egger, 2001). In **study 2**, five patients were 'withdrawn' or 'dropped out' (Cr group; *n* = 3, and placebo group; *n* = 2). Over the course of the trial, there were also several 'missed sessions' in both groups, primarily due to patients being uncontactable. In line with CONSORT, the proportion of patients not included in the analysis, along with the number of missing data was reported. In **study 4**, one patient was withdrawn due to hospital admittance (suspected meningitis) and this was deemed non-treatment (i.e. IM CS injection) related.

9.3.3. External validity

External validity concerns the generalisability of the results of a clinical trial to other patient populations, settings, treatment variables, and measurement variables (Jüni et al., 2001).

9.3.3.1. Patient selection

All patients were recruited from rheumatology outpatient clinics in North West Wales. Patients in **study 1** and **2** were ineligible if they had active RA (stable disease was quantified by no change in medication in the preceding three months, or by the expert opinion of the rheumatologist in care), or were participating in regular high-intense exercise/taking anabolic supplements.

In **study 1**, the reduced use of combination DMARD therapy and complete lack of biological agents in our RA patients suggests an apparent 'selection bias'. In the general UK RA population, ~5% of patients are prescribed biological agents (Ding & Deighton, 2010), thus we would expect ~3–4 patients to be on biologics in **study 1.** Although our RA sample may appear atypical on appearance, it seems our patients were successfully controlled with a relatively simple and conservative treatment strategy (predominantly MTX monotherapy). However, as discussed, this may further infer that only patients with the mildest and best controlled disease agreed to take part. Whilst, this is perhaps the largest threat to external validity in this thesis, if correct, then the differences observed would have underestimated the already alarming body composition and functional deficiencies of the RA patient population.

For safety purposes, in the Cr supplementation study (**study 2**), 30 patients screened with an estimated glomerular filtration rate (eGFR) of <60 ml/min/1.73m^2 were excluded (17% of the total *n* = 179 screened). This reduces the external validity to this group of patients (i.e. those with renal impairment). Overall, RA patients in our samples may not be completely representative of the patient population (Rothwell, 2005), and the use of larger, more inclusive multi-centre trials should be encouraged. Further investigation could explore the safety of Cr in patients with renal impairment.

9.3.3.2. Outcome measures

All the objective physical function tests used in this thesis have been validated in a comparable population (e.g., the elderly; Rikli & Jones, 2012), and have been extensively used in testing RA patients. Surrogate outcome measures can affect external validity (Rothwell, 2005). The main surrogate measure used was ALM (as a proxy measure of muscle mass), although this has been validated by Fuller et al. (1992). Aerobic capacity (VO_2max) was predicted using heart rate changes during an indirect, sub-maximal 'Siconolfi' step test. This test has been extensively validated in appropriate populations including RA (see ***9.3.2.2. Performance bias***), and shows good agreement with direct online VO_2max measures (Metsios et al., 2015). In **Chapter 3**, a surrogate self-reported 'physical activity' measure was taken from the MDHAQ 'exercise frequency' question. Whilst giving a good overall indication of the physical activity of our patients, as far as the author is aware, this question has not been validated against objective measures of physical activity (e.g., accelerometers). Future research could utilise objective measures of physical activity or validated physical activity questionnaires (e.g., the International Physical Activity Questionnaire (IPAQ)) to accurately measure the physical activity of participants with RA.

9.3.3.3. Follow up duration

A risk to external validity in some intervention trials is inadequate treatment and/or follow-up duration (Rothwell, 2005). In **study 2**, a Cr supplementation period of 12 weeks was used. This time period was recommended by the manufacturer, but has also been used in other clinical trials (for a review, see **Chapter 4**). We subsequently followed patients up 12 weeks after cessation of Cr supplementation. This is longer than the 4 week 'wash-out' period often reported, and provided a good indication of how long treatment benefits were maintained.

In **study 4**, we re-assessed patients 4 weeks following their IM CS injection. Four weeks was chosen as this was the same amount of time, following an IM CS injection, that a substantial change in body composition (i.e. loss of LM) was noted in our case patient (**Chapter 7**). An

extension (granted in November 2015) to **study 4** allows our group to assess body composition in the participants 6–9 months after their IM CS injection, thus providing long term data into whether any lost muscle is regained. However, without any anabolic stimulus (e.g., exercise, PRT), restoration in muscle mass seems unlikely.

9.3.3.4. Application to other conditions

As RC has been attributed primarily to elevated pro-inflammatory cytokines (Roubenoff et al., 1992, 1994; Walsmith & Roubenoff, 2002; Metsios et al., 2006), it can serve as a model of chronic inflammatory disease-driven muscle wasting. As such, the findings of this thesis may be applicable to sarcopenia (Morley et al., 2011) and other diseases characterised by inflammatory drive-muscle wasting (for a review, see Tan & Fearon, 2008). **Study 2** adds to the existing literature surrounding Cr supplementation and its use in RA and other clinical conditions characterised by muscle wasting (e.g., cancer cachexia, COPD, HIV, muscular dystrophy).

Study 4, and the findings from the case report in **Chapter 7**, describe a potentially serious consequence of using IM CS injections in the treatment of active RA (i.e. disease flare). Due to logistical and ethical reasons, we did not investigate the use of IM CS injection at point of disease diagnosis where an IM CS injection is regularly given (Ding & Deighton, 2010), consequently we cannot generalise to these patients. Worryingly, IM depots of CS are used in other conditions, not just RA, to treat acute uncontrolled inflammation (e.g., SLE, leukaemia, Crohn's disease, acute interstitial nephritis, asthma (Shatsky, 2009)). The adverse effect on body composition from this type of treatment should be investigated in these populations to fully ascertain its relative risk benefit.

9.3.4.5. Effect versus efficacy analysis

As a 'treatment intervention' focused trial, **study 2** used a 'per-protocol' analysis (i.e. efficacy among those who are adherent and able to tolerate the treatment, Del Re et al., 2013) over an 'intention-to-treat' (ITT) analysis which compares all patients or groups as initially randomised (including data from those whom were withdrawn or dropped out). An ITT analysis was not deemed appropriate as the aim of **study 2** was exploratory, and the primary aim was to investigate the physiological *efficacy* (i.e. to determine whether the intervention produces the expected result under ideal circumstances (Gartlehner et al., 2006)) of Cr rather than its *effect* (i.e. the degree of beneficial effect under a more 'real world' clinical setting (Gartlehner et al., 2006)) in medical practice. A 'per protocol' approach was used as it tests the 'true efficacy of the intervention when used as directed' (Del Re et al., 2013) (i.e. the efficacy of the

patients whom consumed Cr). Regardless of analysis approach (ITT or ‘per protocol’, according to The European Agency for the Evaluation of Medicinal Products (CPMP/EWP/1776/99, 2001), if appropriate, imputation (as performed in **study 2**) of missing data is acceptable.

As another treatment intervention-based trial, **study 4** also employed an efficacy analysis of IM CS on patients who had both pre- and post-data only. As one patient was withdrawn, and not included in the final analysis, this too was a ‘per protocol’ analysis.

9.4. Final conclusion and recommendations

9.4.1. Summary

In summation, despite a tightly controlled T2T approach which results in low disease activity and ‘clinical remission’ for the majority of patients with RA, RC (i.e. muscle wasting and adiposity gain) remains a major contributor to reduced physical function and strength. As high intensity exercise is unlikely to be universally adopted as treatment of RC, nutritional Cr supplementation may be beneficial in patients with severe muscle wasting. Physiologically, no consistent biomarkers for RC were identified in the serum of RA patients in a range of scenarios including treated versus untreated disease, versus healthy controls, and following exercise and Cr supplementation. Disturbingly, an IM CS injection, a routine and recommended treatment given to supress active disease (i.e. flare), may contribute to RC.

9.4.2. Recommendations

- Abnormal body composition and physical function (i.e. RC) should be investigated at RA disease diagnosis, and used alongside measures of inflammation and disease activity to evaluate treatment efficacy and to help guide treatment. Ideally, quick and easy assessment of both objective physical function (by STS-30 and HGS) and body composition (by BIA) should be made in an outpatient setting.

- Adjunct anabolic therapies should be prescribed. High intensity PRT (exercise) remains the most efficacious tool to help reverse RC and restore lost physical function in those willing and able to do it, however, in patients with extreme muscle wasting, nutritional Cr supplementation may be beneficial in increasing muscle mass.

- Mechanistically, the individual roles of adiponectin and IGFBP-3 in RC warrant further investigation. Additionally, the anti-inflammatory effects (reductions in TNF-α) of Cr supplementation should also be explored further.

- If consistent muscle loss following IM CS injections is confirmed by our full study, then research into the efficacy of oral Cr supplementation, commenced immediately after IM CS injection, in preventing CS-induced muscle loss should be performed.

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Appendices

Appendix A - Author contributions to thesis chapters

Contributions based on the International Committee of Medical Journal Editors criteria[24]:

- 1a) study conception and design; and/or 1b) acquisition of data; and/or 1c) analysis and interpretation of data;
- 2: Drafting the article or revising it critically for important intellectual content;
- 3: Final approval of the version of the article to be published.

Chapter 3

▪ Lemmey, AB – 1a, 1c, 2, 3; ▪ **Wilkinson, TJ – 1b (49% of RA sample, 42% of HC sample), 1c, 2, 3**; ▪ Clayton, RJ – 1b (51% of RA, 19% of HC); ▪ Sheikh, F – 1b, 1c, 3; ▪ Whale, J – 1b (19.5% of HC); ▪ Jones, H – 1b (19.5% of HC); ▪ Ahmad, Y- 1a, 1c, 3; ▪ Chitale, S - 1a, 1c, 3; ▪ Jones, JG - 1a, 1c, 2, 3; ▪ Maddison, PJ - 1a, 3; ▪ O'Brien, TD - 1a, 1c, 2, 3

Chapter 4

▪ **Wilkinson, TJ – 1a, 1b, 1c, 2, 3**; ▪ O'Brien, TD – 1a, 2, 3; ▪ Lemmey, AB - 1a, 2, 3

Chapter 5

▪ **Wilkinson, TJ – 1b, 1c, 2, 3**; ▪ Lemmey, AB - 1a, 1c, 2, 3; ▪ Ahmad, Y – 3; ▪ Chitale, S – 3; ▪ Sheikh, F – 3; ▪ Jones, JG - 2, 3; ▪ O'Brien, TD - 1a, 1c, 2, 3

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Chapter 6

▪ **Wilkinson, TJ – 1a, 1b, 1c, 2**; ▪ Stewart, C – 1a, 1b, 1c; ▪ Lemmey, AB - 1a, 1b, 1c, 2; ▪ O'Brien, TD - 1a, 1c, 2

Other acknowledgments:

- Authors and researchers involved in the studies from which serum was collected.

Chapter 7

▪ **Wilkinson, TJ – 1a, 1b, 1c, 2, 3**; ▪ O'Brien, TD- 1c, 2, 3; ▪ Lemmey, AB- 1a, 1c, 2, 3; ▪ Jones, JG – 1a, 1c, 2, 3

Chapter 8

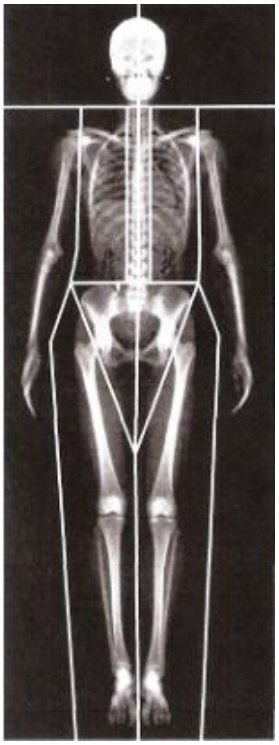
▪ **Wilkinson, TJ – 1a, 1b, 1c, 2**; ▪ Sheikh, F- 1a, 2; ▪ Jones, JG – 1a, 2; ▪ Lemmey, AB- 1a, 2; ▪ Ahmad, Y- 1a; ▪ Chitale, S- 1a; ▪ O'Brien, TD- 1a, 2

[24] International Committee of Medical Journal Editors authorship guidelines available at www.icmje.org/.

Appendix B - Appendicular muscle estimated using the method described in Heymsfield et al. (1992)

Heymsfield et al. (1992) evaluated the potential of DXA to isolate appendages of human participants and to quantify extremity skeletal muscle mass. The post-scan skeleton is subdivided into several regions:

1. The neck cut is made just below the chin.
2. The rib cuts are made as close to, but not touching, the spine.
3. The arms are isolated by running a line through the humeral head.
4. The pelvis cut is placed just above the pelvic brim and the system computer automatically draws the lower pelvic lines.
5. The spine cut is placed just below the last pair of ribs coming out of T12



Appendicular lean mass (ALM) is calculated by summing the lean mass (LM) (as measured by DXA) of two arm regions (#3) with the two leg regions (#4) (i.e. L Arm + R Arm + L Leg + R Leg).

See example below for a DXA-assessed body composition table and the four regions summed to create ALM.

DXA Results Summary:

Region	BMC (g)	Fat Mass (g)	Lean Mass (g)	Lean+ BMC (g)	Total Mass Mass (g)	% Fat
L Arm	142.88	1723.6	1711.5	1854.4	3578.0	48.2
R Arm	162.16	1559.1	1764.3	1926.4	3485.6	44.7
Trunk	576.13	13157.8	24272.5	24848.7	38006.5	34.6
L Leg	407.72	4285.5	5929.1	6336.8	10622.3	40.3
R Leg	397.16	4239.0	5913.2	6310.4	10549.4	40.2
Subtotal	1686.05	24965.1	39590.6	41276.7	66241.8	37.7
Head	444.22	1033.2	2759.6	3203.9	4237.0	24.4
Total	**2130.27**	**25998.3**	**42350.3**	**44480.5**	**70478.8**	**36.9**

Appendix C - Missing data for each variable of interest in Chapter 3 from STROBE 2007 guidelines

	RA *n*/82 *(%)*	HC *n*/85 *(%)*
Body composition measures		
Waist circ. (cm)	82 *(100%)*	83 *(98%)*
Hip circ. (cm)	82 *(100%)*	83 *(98%)*
Waist: hip ratio	82 *(100%)*	83 *(98%)*
BM (kg)	82 *(100%)*	85 *(100%)*
BMI (kg/m^2)	82 *(100%)*	85 *(100%)*
ALM (kg)	81 *(99%)*	85 *(100%)*
ALM% (ALM/BM%)	81 *(99%)*	85 *(100%)*
Total LM (kg)	81 *(99%)*	85 *(100%)*
Total LM% (LM/BM%)	81 *(99%)*	85 *(100%)*
FM (kg)	82 *(100%)*	85 *(100%)*
BF (%)	82 *(100%)*	85 *(100%)*
Trunk FM (kg)	82 *(100%)*	85 *(100%)*
Trunk FM% (trFM/FM%)	82 *(100%)*	85 *(100%)*
Objective physical function		
IKES (N)	78 *(95%)*	84 *(99%)*
HGS (kg)	82 *(100%)*	85 *(100%)*
STS-30 test (reps)	79 *(96%)*	83 *(98%)*
8'UG (secs)	81 *(99%)*	83 *(98%)*
50'W (secs)	82 *(100%)*	84 *(99%)*
VO_2max (ml/kg/min)	62 *(76%)*	81 *(95%)*
Self-reported disability		
HAQ score (/3)	82 *(100%)*	84 *(99%)*
MDHAQ pain (/10)	80 *(98%)*	84 *(99%)*
MDHAQ fatigue (/10)	79 *(96%)*	84 *(99%)*
RADAI (/48)	82 *(100%)*	84 *(99%)*
SF-36 (physical) (/100)	80 *(98%)*	84 *(99%)*
SF-36 (mental) (/100)	80 *(98%)*	84 *(99%)*

RA = Rheumatoid arthritis; HC = Healthy control; BM = Total body mass (on scales); BMI = Body mass index; ALM = Appendicular lean mass; ALM% = ALM/BM%; FM = Fat mass; BF% = Body fat percentage ; IKES = Isometric knee extensor strength; HGS = Handgrip strength; STS-30 = Sit-to-stand in 30 second test; 8'UG = 8-foot up and go; 50'W = 50-foot walk; HAQ = Health Assessment Questionnaire; MDHAQ = Multi-dimensional Health Assessment Questionnaire; RADAI = Rheumatoid Arthritis Disease Activity Index; SF-36 = Short-form 36 questionnaire.

Appendix D - Full assay procedures for each biomarker tested

Tumor Necrosis Factor-α (TNF-α)

Human TNF-α was measured using a quantitative 'sandwich' ELISA technique. 50 µl of Assay Diluent RD1F was added to each well of the 96-well ELISA plate, before 200 µl of Standard, sample, or control, was added per well. After being covered by an adhesive strip, the wells were left to incubate for 3 hours at room temperature. Liquid was removed by the wells by aspirating or inverting the plate and decanting the contents. Excess liquid was removed by rapping the inverted plate on a clean paper towel several times. Each well was filled with 400 µl of 'Wash Buffer', before the liquid was removed again. These 'washing' steps were repeated 6 times, before all excess liquid was removed. 200 µl of Human TNF-α HS Conjugate was added to each well, covered and incubated for 2 hours at room temperature. The plate was then washed again before 50 µl of 'Substrate Solution' was added to each well. After covering, this was incubated for 1 hour at room temperature. 50 µl of 'Amplifier Solution' was added to each well, covered and incubated for 30 minutes, before finally, 50 µl of 'Stop Solution (SS)' was then added to each well. To read the samples, the microplate reader (CLARIOstar, BMG LABTECH, Germany) was set to a wavelength of 490 nm (λ correction of 540 or 570 nm). The optical density (OD) was determined within 30 minutes of the Stop Solution being added. The normal range of TNF-α according to manufactures data was between 0.550 and 2.816 pg/mL (mean 1.206 pg/mL). The manufactures minimum detectable dose (MDD) of TNF-α ranged from 0.038-0.191 pg/mL. The mean MDD was 0.106 pg/mL. The mean intra-assay co-efficient of variation (CV) between TNF-α duplicates was 18.8%. Although this is greater than reported by the manufacture (CV = 7.2 to 10.4), this is comparable to those reported by Aris et al. (2000) whom found CV of 14.3% in 17 adult cystic fibrosis patients.

Soluble Tumor Necrosis Factor-alpha Receptor-1 (*sTNF-RI*)

Soluble TNF receptor was measured using a quantitative 'sandwich' ELISA technique. 50 µl of Assay Diluent RD1-7 was added to each well before 200 µl of Standard, control or sample was added (the serum sample was diluted using the manufactures suggested 10-fold dilution of 50 µl of sample plus 450 µl of Calibrator Diluent RD5-5). The plate was incubated for 2 hours at room temperature. Each well was aspirated and washed using 'Wash Buffer' (400 µl), with the process being repeated a total of four washes. Excess water was removed by inverting the plate and blotting against a paper towel. 200 µl of sTNF-RI Conjugate was added to each well, covered and incubated for 2 hours (serum only). The plate was washed again, before 200 µl of 'Substrate Solution' was added to each well, and the plate was covered and incubated for 20 minutes at room temperature. Attention was given to protecting the plate from

light. 50 µl of SS was added, and the wells changed from blue to yellow in colour. To read the samples, the microplate reader was set to a wavelength of 450 nm (λ correction of 540 or 570 nm), and the OD was determined within 30 minutes of the SS being added. The normal range of sTNF-RI according to manufactures data was between 749 and 1966 pg/mL (mean 1198 pg/mL). The manufactures MDD of sTNF-RI ranges from 0.43-1.20 pg/mL. The mean MDD was 0.77 pg/mL. The mean intra-assay CV between sTNF-RI duplicates was 4.5%. This is comparable to those reported by the manufacture (CV = 4.4 to 5.2%).

Interleukin-6 (IL-6)

Human IL-6 was measured using a quantitative 'sandwich' ELISA technique. 100 µl of Assay Diluent RD1W was added to each well before 100 µl of Standard, control or sample was added, and the plate was incubated for 2 hours at room temperature. Each well was aspirated and washed using 'Wash Buffer' (400 µl), with the process being repeated a total of four washes. Excess water was removed by inverting the plate and blotting against a paper towel. 200 µl of Human IL-6 Conjugate was added to each well, covered and incubated for 2 hours. The plate was washed again, before 200 µl of 'Substrate Solution' was added to each well, and the plate was covered and incubated for 20 minutes at room temperature. Attention was given to protecting the plate from light. 50 µl of SS was added, and the wells changed from blue to yellow in colour. To read the samples, the microplate reader was set to a wavelength of 450 nm (λ correction of 540 or 570 nm), and the OD was determined within 30 minutes of the SS being added. The normal range of IL-6 according to manufactures data is typically below the lowest IL-6 standard (3.13 pg/ml). The manufactures MDD of human IL-6 is typically less than 0.70 pg/mL. The mean intra-assay CV between IL-6 duplicates was 15.2%. Although this is greater than reported by the manufacture (CV = 1.7 to 4.4), this is comparable to those reported by Knudsen et al. (2007) whom found CV of 10.5% in 10 adult RA patients.

Insulin-like Growth Factor-1 (IGF-I)

Serum IGF-I was measured using a quantitative 'sandwich' ELISA technique. To begin, the serum sample was pre-treated to release the IGF-I from binding proteins. This was achieved by adding 20 µl of serum to 380 µl of 'Pretreatment *A*' (21 mL of acidic dissociation solution) in a polypropylene tube. Following vortex, this was incubated for 10 minutes at room temperature. 50 µl of the resultant sample was then added to 200 µl of reconstituted 'Pretreatment *B*' (buffered protein with blue dye and preservatives) and mixed. 150 µl of Assay Diluent RD1-53 was added to each well before 50 µl of Standard, control or sample was added. The plate was then incubated for 2 hours at 2-8 °C. Each well was aspirated and washed using Wash Buffer (400 µl), with the process being repeated a total of four washes.

Excess water was removed by inverting the plate and blotting against a paper towel. 200 µl of cold IGF-I Conjugate was added to each well, covered and incubated for 1 hours at 2-8°C. The plate was washed again, before 200 µl of 'Substrate Solution' was added to each well, and the plate was covered and incubated for 30 minutes at room temperature. Attention was given to protecting the plate from light. 50 µl of SS was added, and the wells changed from blue to yellow in colour. To read the samples, the microplate reader was set to a wavelength of 450 nm (λ correction of 540 or 570 nm), and the OD was determined within 30 minutes of the SS being added. As the sample was pre-treated, the concentration read from the standard curve was multiplied by a dilution factor of 100. The normal range of IGF-I according to manufactures data was between 40 and 258 ng/mL (mean 105 ng/mL). The manufactures MDD of IGF-I ranges from 0.007-0.056 ng/mL. The mean MDD was 0.026 ng/mL. The mean intra-assay CV between IGF-1 duplicates was 4.6%. This is comparable to those reported by the manufacture (CV = 3.5 to 4.3%).

Insulin-like Growth Factor Binding Protein-3 (IGFBP-3)

IGFBP-3 was measured using a quantitative 'sandwich' ELISA technique. 100 µl of Assay Diluent RD1-62 was added to each well before 100 µl of Standard, control or sample was added (the serum sample was diluted using the manufactures suggested 100-fold dilution of 10 µl of sample plus 990 µl of Calibrator Diluent RD5P-1X). The plate was incubated for 2 hours at 2-8 °C. Each well was aspirated and washed using 'Wash Buffer' (400 µl), with the process being repeated a total of four washes. Excess water was removed by inverting the plate and blotting against a paper towel. 200 µl of IGFBP-3 Conjugate was added to each well, covered and incubated for 2 hours at 2-8 °C. The plate was washed again, before 200 µl of 'Substrate Solution' was added to each well, and the plate was covered and incubated for 30 minutes at room temperature. Attention was given to protecting the plate from light. 50 µl of SS was added, and the wells changed from blue to yellow in colour. To read the samples, the microplate reader was set to a wavelength of 450 nm (λ correction of 540 or 570 nm), and the OD was determined within 30 minutes of the SS being added. The normal range of IGFBP-3 according to manufactures data was between 835 and 3778 ng/mL (mean 2375 ng/mL). The manufactures MDD of IGFBP-3 ranges between 0.02-0.14 ng/mL. The mean MDD is 0.05 ng/mL. The mean intra-assay CV between IGFBP-3 duplicates was 8.3%. Although this is greater than reported by the manufacture (CV = 2.3 to 5.0), this is comparable to those reported by Toussirot et al. (2005) whom found CV of 6.2% in 38 adult RA patients.

Myostatin

Myostatin (or GDF-8) was measured using a quantitative 'sandwich' ELISA technique. Prior to assay analysis, to remove the pro-peptide from GDF-8, solutions of 1 N HCI (Hydrochloric acid solution; 100 mL: 91.67 mL of deionized water added slowly to 8.22 ml of 12 N HCI), and 1.2 N Sodium hydroxide (NaOH)/0.5 M 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES; 100 mL: 75 mL of deionized water added slowly to 12 mL of 10 N NaOH, along with 11.9 g of HEPES – final volume made up of deionized water) were created. To activate GDF-8 to immunoreactive GDF-8 detectable by the Quantikine GDF-8 immunoassay, the following activation procedure was used: 1 N HCI was added to the sample before being incubated for 10 minutes at room temperature. Following this, 1.2 N NaOH/0.5 M HEPES was added and mixed. Finally, Calibrator Diluent RD5-26 (1X) was mixed in preparation of the assay.For the remaining assay, 50 µl of Assay Diluent RD1-17 was added to each well before 50 µl of Standard, control or sample was added. The plate was incubated for 2 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ±50 rpm. Each well was then aspirated and washed using 'Wash Buffer' (400 µl), with the process being repeated a total of four washes. Excess water was removed by inverting the plate and blotting against a paper towel. 200 µl of GDF-8 Conjugate was added to each well, covered and incubated for 2 hours. The plate was washed again, before 200 µl of 'Substrate Solution' was added to each well, and the plate was covered and incubated for 30 minutes at room temperature *on the benchtop*. Attention was given to protecting the plate from light. 50 µl of SS was added, and the wells changed from blue to yellow in colour. To read the samples, the microplate reader was set to a wavelength of 450 nm (λ correction of 540 or 570 nm), and the OD was determined within 30 minutes of the SS being added. The normal range of GDF-8 according to manufactures data was between 1264 and 8588 pg/mL (mean 4206 pg/mL). The manufactures MDD of GDF-8 ranges from, 0.922–5.32 pg/mL. The mean MDD is 2.25 pg/mL. The mean intra-assay CV between myostatin duplicates was 4.6%. This is comparable to those reported by the manufacture (CV = 3.5 to 4.3%).

Adiponectin

Total Adiponectin was measured using a quantitative 'sandwich' ELISA technique. 100 µl of Assay Diluent RD1Wwas added to each well before 50 µl of Standard, control or sample was added (the serum sample was diluted using the manufactures suggested 100-fold dilution of 10 µl of sample plus 990 µl of Calibrator Diluent RD6-39). The plate was incubated for 2 hours at room temperature. Each well was aspirated and washed using 'Wash Buffer' (400 µl), with the process being repeated a total of four washes. Excess water was removed by inverting the plate and blotting against a paper towel. 200 µl of adiponectin conjugate was added to

each well, covered and incubated for 2 hours at room temperature. The plate was washed again, before 200 µl of 'Substrate Solution' was added to each well, and the plate was covered and incubated for 30 minutes (serum only) at room temperature. Attention was given to protecting the plate from light. 50 µl of SS was added, and the wells changed from blue to yellow in colour. To read the samples, the microplate reader was set to a wavelength of 450 nm (λ correction of 540 or 570 nm), and the OD was determined within 30 minutes of the SS being added. The normal range of adiponectin according to manufactures data was between 865 and 21,424 ng/mL (mean 6641 ng/mL). The manufactures MDD of adiponectin ranges between 0.079–0.891 ng/mL. The mean MDD is 0.246 ng/mL. The mean MDD is 0.05 ng/mL. The mean intra-assay CV between adiponectin duplicates was 7.6%. Although this is greater than reported by the manufacture (CV = 2.5 to 4.7), this is comparable to those reported by Harle et al. (2006) whom found CV of 10% in 16 adult RA patients.

Leptin

Human Leptin was measured using a quantitative 'sandwich' ELISA technique. 100 µl of Assay Diluent RD1-19 was added to each well before 100 µl of Standard, control or sample was added (the serum sample was diluted using the manufactures suggested 100-fold dilution of 10 µl of sample plus 990 µl of Calibrator Diluent RD5P-1X). The plate was incubated for 2 hours at room temperature. Each well was aspirated and washed using 'Wash Buffer' (400 µl), with the process being repeated a total of four washes. Excess water was removed by inverting the plate and blotting against a paper towel. 200 µl of leptin conjugate was added to each well, covered and incubated for 1 hour. The plate was washed again, before 200 µl of 'Substrate Solution' was added to each well, and the plate was covered and incubated for 30 minutes at room temperature. Attention was given to protecting the plate from light. 50 µl of SS was added, and the wells changed from blue to yellow in colour. To read the samples, the microplate reader was set to a wavelength of 450 nm (λ correction of 540 or 570 nm), and the OD was determined within 30 minutes of the SS being added. The normal range of leptin according to manufactures data was between 2205 and 11,149 pg/mL (mean 4760 pg/mL). The manufactures MDD of leptin is less than 7.8 pg/mL. The mean intra-assay CV between leptin duplicates was 7.8%. Although this is greater than reported by the manufacture (CV = 3.0 to 3.3), this is comparable to those reported by Harle et al. (2006) whom found CV of 10% in 16 adult RA patients.

Appendix E - Body composition of (A) subset Chapter 3 rheumatoid arthritis patients and sedentary age- and sex-matched healthy controls; and (B) between 'recent-onset' (<12 months disease duration) and 'established' (≥12 months disease duration) rheumatoid arthritis patients

(A)

	RA (*n* = 32)	HC (*n* = 41)	Absolute difference (CI) *(%)*	*P*	η^2
ALM (kg)	20.4 (±4.9)	20.6 (±4.7)	↓ 0.2 (-2.5–2.1) *(1)*	.867	.00
ALM%	27.5 (±4.4)	29.7 (±7.2)	↓ 2.3 (-5.2–0.6) (*8*)	.121	.03
BM (kg)	75.2 (±18.3)	70.1 (±10.5)	↑ 5.1 (-1.7–12.0) *(7)*	.139	.03
FM (kg)	23.8 (±11.3)	18.8 (±5.4)	↑ **5.0 (1.0–9.0) *(21)***	**.016***	**.08**
BF% (%)	31.2 (±8.4)	27.2 (±6.7)	↑ **4.0 (0.2–7.9) *(13)***	**.040***	**.07**

(B)

	'Recent-onset' (*n* = 13)	'Established' (*n* = 19)	Absolute difference (CI) *(%)*	*P*	η^2
ALM (kg)	20.0 (±5.0)	20.6 (±4.9)	0.6 (-3.0–4.2) *(3)*	.738	.00
ALM%	27.2 (±4.6)	27.6 (±4.4)	0.4 (-2.8–3.7) *(1)*	.786	.00
BM (kg)	74.1 (±15.4)	76.0 (±20.4)	1.9 (-11.7–15.5) *(3)*	.780	.00
FM (kg)	23.3 (±8.5)	24.1 (±13.1)	0.8 (-7.6–9.3) *(3)*	.845	.00
BF% (%)	32.5 (±8.0)	29.6 (±9.0)	2.9 (-4.5–10.3) *(9)*	.422	.03

Group means (±SD) with 95% confidence intervals (CI) reported for the difference. Data was analysed using analysis of variance. RA = Rheumatoid arthritis; HC = Healthy controls; ALM = Appendicular lean mass; ALM% = ALM/BM%; BM = Total body mass (on scales); FM = Fat mass; BF% = Body fat percentage. * = $P < .05$; # = trend ($P \geq .05$–.10); Effect size (η^2), small = .01; medium = .08; large = .26; very large = .50.

Appendix F - Body composition of untreated and treated disease in rheumatoid arthritis patients from Marcora et al. (2006)

	Untreated disease (*n* = 24)	Treated disease (*n* = 24)	Absolute difference (CI) *(%)*	*P*	η^2
ALM (kg)	15.8 (±4.3)	16.1 (±4.4)	↑ 0.3 (-0.1–0.7) *(2)*	.102	.12
BM (kg)	73.9 (±16.4)	75.1 (±16.9)	↑ 1.2 (-0.2–2.6) *(2)*	.093[#]	.12
FM (kg)	30.3 (±11.2)	31.1 (±10.9)	↑ 0.8 (-1.7–0.0) *(3)*	.053[#]	.16
BF% (%)	40.3 (±8.1)	40.9 (±7.8)	↑ 0.5 (-0.1–1.1) *(1)*	.069[#]	.14

Pre-test post-treatment scores are presented as means (±SD). Changes are presented as means (±SE). Treatment x time, and time main effects are presented from analysis of variance (2 x 2 repeated measures design). If a main effect was detected, post-hoc tests were used to identify where the difference lay at within group level. ALM = Appendicular lean mass; BM = Total body mass (on scales); FM = Fat mass; BF% = Body fat percentage. * = $P < .05$; [#] = trend ($P \geq .05$–.10); Effect size (η^2), small = .01; medium = .08; large = .26; very large = .50.

Appendix G - Body composition changes of etanercept (ETN) and methotrexate (MTX) treated rheumatoid arthritis patients from Marcora et al. (2006)

Measure	ETN (*n* = 12)	MTX (*n* = 12)	*P* (η^2)	
			Treatment x time	Time
ALM (kg)				
Pre	15.8 (±4.2)	15.8 (±4.2)	.583 (.01)	.102 (.12)
Post	16.2 (±4.9)	15.9 (±4.2)		
Change	+0.4 (±0.3)	+0.2 (±0.2)		
BM (kg)				
Pre	76.4 (±14.4)	72.4 (±18.7)	.991 (.00)	.093[#] (.12)
Post	77.5 (±16.1)	73.6 (±18.3)		
Change	+1.1 (±1.1)	+1.2 (±0.7)		
FM (kg)				
Pre	31.7 (±8.2)	28.9 (±13.8)	.567 (.02)	.053[#] (.16)
Post	32.3 (±8.5)	30.0 (±13.2)		
Change	+0.6 (±0.6)	+1.1 (±0.6)[#]		
BF% (%)				
Pre	42.0 (±6.8)	38.6 (±9.2)	.196 (.08)	.069[#] (.14)
Post	42.2 (±6.5)	39.5 (±8.9)		
Change	+0.2 (±0.3)	+0.9 (±0.4)[#]		

Pre- and post-treatment scores are presented as means (±SD). Changes are presented as means (±SE). Both the treatment x time interaction, and main effect for time significance values are presented from analysis of variance (2 x 2 repeated measures design). If an interaction was detected, post-hoc tests were used to identify where the difference lay at within group level. Note: time effect donates untreated versus treated states, and therefore is also presented in **Appendix F.** ALM = Appendicular lean mass; BM = Total body mass (on scales); FM = Fat mass; BF% = Body fat percentage. * = *P* <.05; [#] = trend (*P* ≥ .05–.10); Effect size (η^2), small = .01; medium = .08; large = .26; very large = 0.50.

Appendix H - Body composition changes in rheumatoid arthritis patients undergoing 24 weeks of progressed resistance training or home exercise from Lemmey et al. (2009)

Measure	PRT (*n* = 10)	Control (*n* = 9)	*P* (η^2)	
			Group x time	Time
ALM (kg)				
Pre	14.1 (±2.0)	15.7 (±4.0)	**.011***	**.045***
Post	15.0 (±1.9)	15.6 (±3.6)	(.32)	(.22)
Change	+1.0 (±0.2)*	-0.1 (±0.3)		
BM (kg)				
Pre	63.3 (±7.1)	75.8 (±13.2)	.266	.403
Post	63.8 (±6.2)	72.4 (±11.1)	(.07)	(.04)
Change	0.5 (±0.9)	-3.4 (±3.4)		
FM (kg)				
Pre	23.6 (±6.6)	31.8 (±9.8)	.678	.133
Post	21.9 (±6.8)	28.9 (±12.2)	(.01)	(.13)
Change	-1.7 (±1.1)	-3.0 (±2.9)		
BF% (%)				
Pre	36.9 (±7.8)	41.7 (±9.0)	.939	**.048***
Post	34.2 (±9.3)	39.1 (±12.6)	(.00)	(.21)
Change	-2.7 (±1.3)#	-2.5 (±2.1)		

Pre-test and post-test scores are presented as means (±SD). Changes are presented as means (±SE). Treatment x time, and time main effects are presented from analysis of variance (2 x 2 repeated measures design). If a main effect was detected, post-hoc tests were used to identify where the difference lay at within group level. PRT = Progressive resistance training; ALM = Appendicular lean mass; BM = Total body mass (on scales); FM = Fat mass; BF% = Body fat percentage. * = $P < .05$; # = trend ($P \geq .05$–.10); Effect size (η^2), small = .01; medium = .08; large = .26; very large = 0.50.

Appendix I - Body composition changes between the subset of Chapter 5 rheumatoid arthritis patients supplementing with 12 weeks of oral creatine or placebo

Measure	Creatine (*n* = 8)	Placebo (*n* = 12)	*P* (η^2)	
			Group x time	Time
ALM (kg)				
Pre	19.1 (±3.3)	21.9 (±6.2)	.078[#]	.165
Post	19.5 (±3.7)	21.9 (±6.0)	(.18)	(.12)
Change	0.5 (±0.3)	-0.1 (±0.2)		
BM (kg)				
Pre	71.5 (±13.4)	79.6 (±23.1)	.224	.134
Post	73.1 (±14.7)	79.8 (±21.9)	(.02)	(.01)
Change	1.6 (±1.0)	0.2 (±0.6)		
FM (kg)				
Pre	21.6 (±9.0)	24.8 (±13.4)	.680	.202
Post	22.4 (±9.8)	25.2 (±13.3)	(.01)	(.10)
Change	0.8 (±0.3)	0.4 (±0.5)		
BF% (%)				
Pre	28.1 (±6.1)	34.1 (±11.2)	.292	.064[#]
Post	28.6 (±6.4)	35.4 (±11.9)	(.00)	(.22)
Change	0.4 (±0.7)	**1.3 (±0.4)***		

Pre-test and post-test scores are presented as means (±SD). Changes are presented as means (±SE). Treatment x time, and time main effects are presented from analysis of variance (2 x 2 repeated measures design). If a main effect was detected, post-hoc tests were used to identify where the difference lay at within group level. ALM = Appendicular lean mass; BM = Total body mass (on scales); FM = Fat mass; BF% = Body fat percentage. * $P < .05$; [#] trend ($P \geq .05$–.10); Effect size (η^2), small = .01; medium = .08; large = .26; very large = 0.50.

Appendix J - The association of pain with strength and physical function in rheumatoid arthritis (Chapter 3)

Physical function measure	Subjective pain measures		
	MDHAQ general pain (Q2) φ	MDHAQ joint pain (Q3) φ	SF-36 'bodily pain' component †
IKES (N)	*r* = -.198, *P* = .086#	*r* = -.202, *P* = .077#	***r* = .286, *P* = .012***
HGS (N)	***r* = -.239, *P* = .032***	*r* = -.208, *P* = .061#	***r* = .226, *P* = .044***
STS-30 (reps)	***r* = -.250, *P* = .028***	*r* = -.217, *P* = .055#	***r* = .262, *P* = .021***
8'UG (secs)∞	***r* = .460, *P* < .001***	***r* = .476, *P* < .001***	***r* = -.380, *P* = .001***
50'W (secs)∞	***r* = .414, *P* < .001***	***r* = .514, *P* < .001***	***r* = -.380, *P* = .001***
VO_2max (ml/kg/min)	*r* = .044, *P* = .736	*r* = -.067, *P* = .606	*r* = -.007, *P* = .959

MDHAQ = Multi-dimensional Health Assessment Questionnaire; SF-36 = Short-form 36 questionnaire; IKES = Isometric knee extensor strength; HGS = Handgrip strength; STS-30 = Sit-to-stand in 30 second test; 8'UG = 8-foot up and go; 50'W = 50-foot walk; VO_2max = Estimated VO_2max from Siconolfi step test; ∞ = higher score denotes poorer performance; φ = higher score on MDHAQ denotes more pain; † = higher score on SF-36 denotes less pain. * *P* < .05; # trend (*P* ≥ .05–.10).