DOCTOR OF PHILOSOPHY

Anabolic and anticatabolic interventions for the treatment of cachexia in rheumatoid arthritis patients

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ANABOLIC AND ANTICATABOLIC INTERVENTIONS FOR THE TREATMENT OF CACHEXIA IN RHEUMATOID ARTHRITIS PATIENTS

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To mum, dad and Caroline

SUMMARY

Rheumatoid arthritis is a chronic disease characterised by synovial joint inflammation and erosion. However, it is also a systemic disease featuring alterations in protein and energy metabolism with consequent accelerated loss of skeletal muscle. This syndrome, called rheumatoid cachexia, affects >50% of patients and is associated with disability and increased risk of death. Therefore, interventions aimed at stimulating muscle growth or preventing hypercatabolism have the potential to improve the long-term outcome of rheumatoid arthritis. In this thesis the results of three different controlled clinical trials are presented. In the first study, 10 rheumatoid arthritis patients underwent a high intensity progressive resistance training program. Compared to 10 age and sex matched patients who did not exercise, the subjects in the training group had a significant increase in muscle mass and strength and a reduction in disability with no exacerbation of disease activity. In the second study, 40 rheumatoid arthritis patients were randomly assigned to dietary supplementation with a mixture of anabolic amino acids (β-hydroxy-β-methylbutyrate, glutamine and arginine) or a nitrogen-balanced (7.19 g/day) mixture of other "metabolically inert" amino acids (placebo). Contrary to our hypothesis, subjects in both groups experienced the same significant increase in total body protein and some improvements in physical function. As habitual protein intake was normal in these patients, these results suggest that rheumatoid arthritis increases nitrogen requirements. The last study was a randomised controlled trial of anti-tumor necrosis factor therapy with etanercept (n = 12) compared to standard therapy with methotrexate (n = 12). Although both agents were equally effective in preventing muscle wasting in patients with early rheumatoid arthritis, etanercept improved the anabolic response to overfeeding in those patients who gained weight over the six month follow-up period. In conclusion, rheumatoid cachexia is reversible and anabolic/anticatabolic interventions should be studied further and implemented in clinical practice.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

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CHAPTER 1

GENERAL INTRODUCTION

BACKGROUND

What is Rheumatoid Arthritis?

Rheumatoid arthritis (RA) is an autoimmune disease affecting between 0.5 and 1.0 % of the adult population worldwide (Kvien 2004). Rheumatoid arthritis is three times more common in women than men and usually begins between the ages of 40 and 60. Although its aetiology is unknown, various genetic (e.g., human leukocyte antigen DRB1 alleles) and environmental (e.g. sex hormones, pregnancy, infectious agents, smoking) risk factors for RA have been identified (Silman and Pearson 2002).

Regardless of the exact cause, the result is an activation of fibroblasts, macrophages, and lymphocytes in the affected joints with subsequent overexpression of proinflammatory cytokines such as tumor necrosis factor (TNF) and interleukin-1 (IL-1). These cytokines stimulate synovial lining cells to proliferate (the so called "pannus") and produce catabolic enzymes which degrade articular cartilage, bone, tendons and ligaments. Increased osteoclast activity and decreased synthetic activity of chondrocytes are also involved in this catabolic process (Ritchlin 2004). Clinically, erosive synovitis manifests itself as swelling, pain, stiffness, redness and deformity of the affected joints, usually the metacarpophalangeal, proximal interphalangeal, and metatarsophalangeal joints, wrists, shoulders, knees, ankles and elbows, often in a symmetrical manner. However, RA is a truly systemic disease and organs other than the synovial joints can be directly involved in the rheumatoid process including the skin, heart and blood vessels (Lee and Weinblatt 2001). The course of RA varies from patient to patient, but periods of increased disease activity ("flares") and remission are typical.

Rheumatoid arthritis is not a benign disease. In many patients, chronic inflammation leads to joint damage which, together with pain and other psychosocial factors, causes significant disability, poor quality of life, and considerable medical and

non-medical costs to the patients, their families, and society at large (Escalante and Del Rincon 2002; Kvien 2004). In addition, RA patients have an increased risk of death from various causes including infection and cardiovascular disease (Doran, Crowson et al. 2002; Watson, Rhodes et al. 2003; Sihvonen, Korpela et al. 2004).

There is no known cure for RA and the management of this disease consists of a combination of medical therapy, patient education, balancing rest and exercise, joint protection, and, occasionally, joint replacement surgery. The drugs currently used, alone or in various combinations, to treat RA are steroidal and non-steroidal anti-inflammatory drugs, disease-modifying anti-rheumatic drugs (e.g., methotrexate, cyclosporine, sulfasalazine, gold salts, and leflunomide) and biological agents (anti-cytokine therapy targeting either TNF or IL-1) (Lee and Weinblatt 2001).

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" (Boonen, van de Rest et al. 1997). With these words, the famous French impressionist painter Pierre-Auguste Renoir eloquently described to a friend the profound catabolic effects of the severe RA he suffered for the last 25 years of his life. Although modern anti-rheumatic therapies have made this kind of profound involuntary weight loss less common (Munro and Capell 1997), many RA patients still suffer from a syndrome called "rheumatoid cachexia" (Figure 1) (Walsmith and Roubenoff 2002). This is characterized by lower-than-normal body cell mass, elevated resting energy expenditure and accelerated whole-body protein breakdown, and is principally driven by inflammatory cytokines such as tumor necrosis factor. In most RA patients, loss of body cell mass, predominantly skeletal muscle mass,

occurs with little or no weight loss because of a concomitant increase in fat mass consequent to low levels of physical activity (Roubenoff, Walsmith et al. 2002).

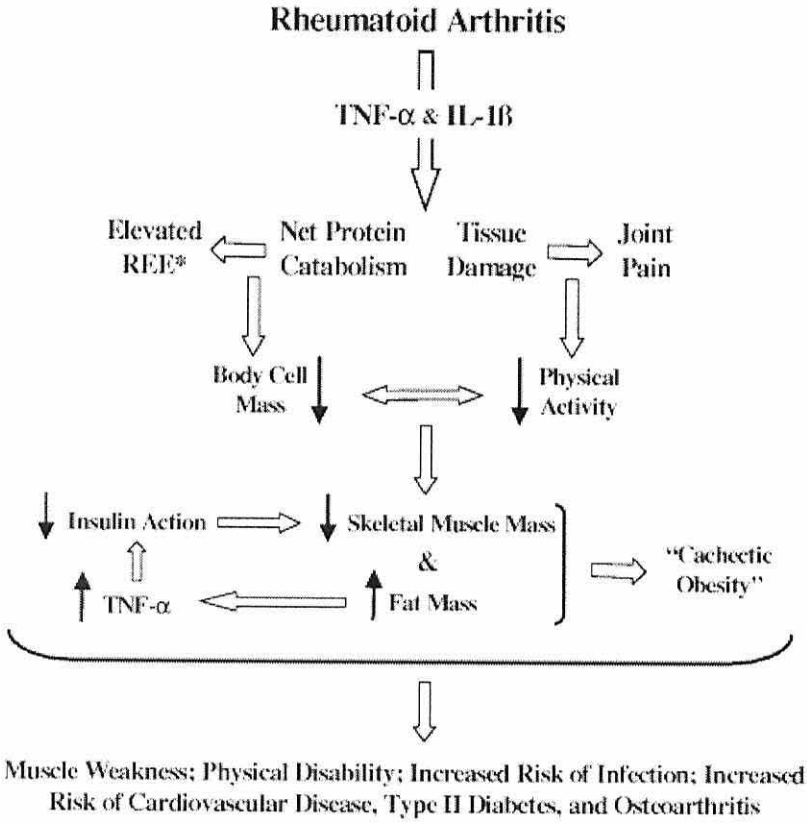

Figure 1. Summary of the metabolic consequences of rheumatoid arthritis. Rheumatoid arthritis is an autoimmune disease characterized by excess production of the inflammatory cytokines tumor necrosis factor-α (TNF- α) and interleukin-1 (IL-1). TNF-α and IL-1 act synergistically to: shift protein metabolism toward net catabolism, mediate joint and bone degradation, and cause joint pain and stiffness. This leads to a loss of body cell mass, predominantly skeletal muscle mass, and reduced physical activity, which reinforce each other and lead to further losses of skeletal muscle mass and predispose to fat gain. Fat gain increases circulating tumor necrosis factor-α levels (produced by adipocytes) and predisposes to insulin resistance and further muscle loss. This reinforces the negative cycle of muscle loss and fat gain, and causes cachectic obesity. These metabolic alterations cause muscle weakness and physical disability, and increase the risk of infection, cardiovascular disease, type II diabetes, and osteoarthritis. *Resting energy expenditure (REE) is elevated in active rheumatoid arthritis. Extracted from Joseph Walsmith and Ronenn Roubenoff: Cachexia in Rheumatoid Arthritis. International Journal of Cardiology, Volume 85, Issue 1, September 2002, Pages 89-99.

This combination of muscle atrophy and increased adiposity is strongly associated with physical disability (Baumgartner 2000) and other adverse health consequences (Walsmith and Roubenoff 2002). Therefore, prevention and treatment of rheumatoid cachexia has the potential to improve the long-term outcome of RA which is characterised by joint damage, decline in physical function, frequent work disability, high levels of co-morbidities, and premature death (Pincus and Callahan 1993).

AIMS AND OUTLINE OF THE THESIS

Although the work of Roubenoff, illustrated in Figure 1, has been critical in characterizing this syndrome and understanding its pathophysiology, there is very little research on the treatment of rheumatoid cachexia (reviewed in detail in **Chapter 2**). Therefore, the general aim of my doctoral research program was to investigate whether currently available anabolic and anti-catabolic interventions are safe and effective for the treatment of muscle wasting secondary to RA.

Progressive resistance training (PRT) is the most effective method to stimulate muscle hypertrophy in healthy adults (Kraemer, Adams et al. 2002) and some anabolic effects have also been demonstrated in patients with cachectic diseases such as chronic renal insufficiency (Castaneda, Gordon et al. 2001), HIV infection (Roubenoff and Wilson 2001), and chronic heart failure (Pu, Johnson et al. 2001). However, in the only previously published investigation on the effect of PRT on body composition in a well defined group of RA patients, no changes were observed despite a normalization of whole-body protein breakdown and significant improvements in fatigue, pain, muscle strength and other measures of physical function (Rall, Meydani et al. 1996; Rall, Rosen et al. 1996). I believe that the absence of an anabolic response in this study was due to the rather low volume of the PRT program which was prescribed to avoid possible

exacerbation of disease activity and joint damage in RA patients. Therefore, in **Chapter 3** I present the results of a Phase II trial aimed at testing, in RA patients, the efficacy, feasibility and safety of a PRT program prescribed for optimal stimulation of muscle hypertrophy in healthy adults (Study 1).

As profound anorexia and weight loss is not common in RA patients, treatment of rheumatoid cachexia with hypercaloric feeding or appetite stimulants is not recommended as it would lead to even greater accumulation of body fat. However, high doses of specific amino acids have been used to stimulate anabolism/reduce catabolism and obtain other "pharmacological" effects in metabolically stressed patients (De Bandt and Cynober 1998). I proposed a similar approach for the nutritional treatment of rheumatoid cachexia and conducted a randomized controlled trial of an oral mixture of β-hydroxy-β-methylbutyrate, glutamine and arginine (HMB/GLN/ARG) in RA patients (Study 2). This commercially available dietary supplement has been proven safe and effective in stimulating muscle growth in cachectic patients with advanced malignancy and HIV (Clark, Feleke et al. 2000; May, Barber et al. 2002; Rathmacher, Nissen et al. 2004) and the results of my study are presented in **Chapter 4**.

The working model of rheumatoid cachexia proposed by Roubenoff and colleagues (Figure 1) suggests that TNF blockade should be a very effective therapy not only for controlling pain and joint damage, but also to reduce hypercatabolism and consequent muscle wasting in RA patients. In recent years biological agents capable of blocking TNF actions have been approved for the treatment of RA but clinical trials of these new anti-rheumatic drugs focused on disease activity, joint damage, self-reported physical function (i.e., disability), and safety whereas body composition was not assessed. This is unfortunate as "an increase in non-fat cellular body mass" is the recommended primary endpoint of clinical trials for the treatment of cachexia (Bone 1999). The aim of

my third original study (**Chapter 5**) was to address this important question by investigating the effects of anti-TNF therapy with etanercept (ETN) on body composition in patients with early and active RA (Study 3). A secondary aim of this study was to assess its effects on objective measures of physical function. A general discussion (**Chapter 6**) containing a summary of the main findings as they apply to the research question detailed in this general introduction (**Chapter 1**), their critical discussion and directions for future research concludes my thesis.

Eventually, the three original studies included in my doctoral research program should advance our knowledge-base and motivate clinicians to prescribe safe and effective anabolic interventions for stimulating muscle growth in the large number of RA patients (~30%) suffering from severe muscle atrophy (Roubenoff, Roubenoff et al. 1992; Munro and Capell 1997), and better inform the current debate about the use of expensive TNF-blocking agents in patients with early RA who have not previously failed other, less costly, disease-modifying anti-rheumatic drugs (DMARDs) such as methotrexate (MTX) or sulphasalazine (Moots, Taggart et al. 2003). As anti-TNF therapy have been proposed, but not tested, for the treatment of cachexia secondary to other chronic diseases (Sharma and Anker 2002; Argiles, Moore-Carrasco et al. 2003; Guarnieri, Antonione et al. 2003; Berry and Baum 2004), RA could also serve as a convenient human model for testing the possible anti-catabolic effects of ETN in these other chronic diseases in which TNF is thought to be a major mediator of muscle catabolism.

HYPOTHESES

Study 1: There will be an increase in total/appendicular lean mass and muscle strength, a decrease in disability, and no change in disease activity in the PRT group compared to the control group.

Study 2: There will be an increase in total/appendicular lean mass and muscle strength, a decrease in disability, and no change in disease activity in the HMB/GLN/ARG group compared to the placebo group.

Study 3: There will be a decrease in disease activity and improvement in physical function and quality of life in both groups. The ETN group, unlike the MTX group, will not loose total and appendicular lean mass.

THESIS FORMAT

This thesis consists of one qualitative review of the literature and three clinical trials of which I am the principal investigator. All four manuscripts are written as stand-alone papers that have been invited/submitted to relevant biomedical international journals. For consistency I wrote all manuscripts according to the American Psychological Association Publication Manual (2001) and the Consolidated Standards of Reporting Trials statement for improving the quality of reports of parallel-group randomised trials (Moher, Schulz et al. 2001). For the same reason I have included all cited references in a single list at the end of the thesis and numbered illustrations consecutively. However, to facilitate reading, I defined abbreviations at their first appearance within each chapter of the thesis. The contribution of the co-authors of each original manuscript is detailed in the acknowledgments. As all the manuscripts included in this thesis are independent but linked, at times there is a necessary overlap between chapters. Additional methods and results are included as an appendix.

CHAPTER 2

RHEUMATOID CACHEXIA:

MECHANISMS AND THERAPEUTIC IMPLICATIONS

THIS LITERATURE REVIEW HAS BEEN INVITED BY THE EDITOR OF RHEUMATOLOGY

SAMUELE MARCORA

INTRODUCTION

Rheumatoid arthritis (RA), like other systemic diseases, is accompanied by several biochemical, metabolic, physiological, and behavioural changes in organ systems distant from the site or sites of inflammation, the so-called acute-phase response (Gabay and Kushner 1999). Of these, rheumatologists have focused most of their attention on the haematological and biochemical alterations, such as elevated erythrocyte sedimentation rate (ESR) and increased serum concentration of C-reactive protein (CRP) which serve as convenient laboratory markers of disease activity (Wollheim 2000). Additionally, they are attentive to anaemia (Wilson, Yu et al. 2004), generalised bone loss (Haugeberg, Orstavik et al. 2003), and accelerated arteriosclerosis (Sattar, McCarey et al. 2003). Most rheumatologists, however, are unaware of another important systemic complication of RA, cachexia. Cachexia has been defined by Kotler as "accelerated loss of skeletal muscle in the context of a chronic inflammatory response" and it is common in many chronic organ diseases (e.g., chronic heart failure, uraemia, chronic obstructive pulmonary disease, liver cirrhosis), malignancy, and chronic infection (Kotler 2000). After providing evidence for the presence and adverse consequences of cachexia in RA patients, I will review the literature on its pathogenesis and treatment, and provide suggestions for clinical practice and future research.

CACHEXIA IN RHEUMATOID ARTHRITIS PATIENTS

Accelerated loss of skeletal muscle in RA patients (i.e., rheumatoid cachexia) was first described by Sir James Paget in 1873 (Paget 1873). More recently, rheumatoid cachexia has been demonstrated, with few exceptions (Kalla, Brown et al. 1992; Madsen, Egsmose et al. 1998), by numerous cross-sectional studies in which patients with long standing RA (average disease duration 10 years) treated with standard therapy (i.e., no biologics) were

compared to healthy controls using different measurement techniques including anthropometry (Helliwell, Coombes et al. 1984; Helliwell and Jackson 1994; Hernandez-Beriain, Segura-Garcia et al. 1996; Gomez-Vaquero, Nolla et al. 2001), dual energy X-ray absorptiometry (DXA) (Sambrook, Spector et al. 1995; Westhovens, Nijs et al. 1997; Moriguchi, Terai et al. 1999), whole-body counting (Roubenoff, Roubenoff et al. 1994; Rall, Walsmith et al. 2002; Roubenoff, Walsmith et al. 2002; Walsmith, Abad et al. 2004), and creatinine excretion (Roubenoff, Roubenoff et al. 1990; Krahenbuhl, Willer et al. 1999; Moriguchi, Terai et al. 1999). The average difference in muscle mass between RA patients and age-, gender-, and body size-matched controls found in these investigations was -12%, which is clinically significant (Roubenoff and Kehayias 1991). Importantly, anthropometric and DXA studies found significant atrophy in both the lower and upper limbs suggesting the presence of systemic, rather than local, muscle wasting.

The findings of these cross-sectional studies have been corroborated by a longitudinal study in which 39 patients with very early RA treated with standard therapy were compared to an age- and sex-matched group of healthy controls (mean age in both groups, 60 years) (Westhovens 1999). At baseline the two groups had a similar total lean mass as measured by DXA. A gradual reduction of skeletal muscle mass and strength with advancing age (i.e., sarcopenia) is a well known phenomenon (Gallagher, Ruts et al. 2000), so it is not surprising that the controls lost 0.9% of their initial total lean mass over 56 week. It is striking, however, that over the same period of time patients experienced a total lean mass loss of 2.5%, which provides further evidence of accelerated loss of skeletal muscle in RA.

The direct consequence of this cachectic process is generalised muscle atrophy and two separate studies found that, in RA patients, the prevalence of severe muscle atrophy (defined as an upper arm muscle area below the lowest 5th percentile of the age-

and sex-matched reference population) is more than 30% (Roubenoff, Roubenoff et al. 1992; Munro and Capell 1997). Despite its high prevalence, rheumatoid cachexia usually goes undetected during routine physical examination because in RA, contrary to other conditions in which cachexia is often complicated by anorexia and weight loss (e.g., cancer), muscle wasting is masked by a concomitant gain in body fat (Westhovens, Nijs et al. 1997) and most patients present with normal or even excessive body weight (Morgan, Anderson et al. 1997; Munro and Capell 1997). Fortunately, simple techniques such as arm anthropometry or bioelectrical impedance analysis (Kyle, Piccoli et al. 2003) can be used in clinical practice to identify RA patients with severe muscle atrophy who might benefit from adjunct therapies aimed at stimulating muscle growth.

These body composition studies have generally been confirmed by histological investigations demonstrating muscle fibre atrophy, particularly of the type II fibres (Magyar, Talerman et al. 1977; Fiori, Andreola et al. 1983; Gibson, Poyser et al. 1991; Miro, Pedrol et al. 1996; de Palma, Chillemi et al. 2000). This is not surprising as these muscle fibres seem to be more susceptible to catabolic stimuli (Hasselgren 2000). The higher concentration of DNA in muscle tissue of RA patients also suggests that muscle atrophy in this population is mainly due to muscle fibre shrinkage rather than loss of necrotic/apoptotic muscle fibres (Gibson, Poyser et al. 1991).

ADVERSE CONSEQUENCES OF RHEUMATOID CACHEXIA

In healthy individuals skeletal muscle is the most abundant tissue in the body and includes ~60% of the metabolically active body cell mass (Wang, Zhu et al. 2003). The main function of skeletal muscles is to produce force for respiration and locomotion. However, they also contribute to postprandial glucose disposal (Kelley, Mitrakou et al. 1988), produce heat (van Marken Lichtenbelt and Daanen 2003) and act as reservoir of

amino acids mobilisable during periods of acute metabolic stress for tissue repair, gluconeogenesis (particularly alanine), increased synthesis of certain acute-phase proteins, and for providing energy substrates (glutamine) to activated immune cells and enterocytes (Fischer and Hasselgren 1991). For these reasons, it is not surprising that, in every condition in which it has been investigated so far, muscle atrophy has been consistently associated with a variety of poor outcomes including muscle weakness (Frontera, Hughes et al. 1991), reduced exercise capacity (Yoshikawa, Yoneda et al. 2001), slow gait speed (Wollheim 2000), physical disability (Janssen, Baumgartner et al. 2004), poor respiratory function (Arora and Rochester 1982; Arora and Rochester 1982), risk of infection (Cegielski and McMurray 2004), fasting hypoglycaemia (Orngreen, Zacho et al. 2003), hypothermia (Yokoyama, Noto et al. 2000), reduced absolute resting energy expenditure (REE) and glucose intolerance (Toth, Sites et al. 1999; Natali, Toschi et al. 2000), premature death (Kotler, Tierney et al. 1989), increased length of stay in hospital (Pichard, Kyle et al. 2004), complications after surgery (Howard and Ashley 2003), and osteoporosis (Hedstrom 1999).

Although both severe muscle atrophy and many of these adverse outcomes are highly prevalent in RA, only a few studies have investigated their association in this population. In a cross-sectional study of 100 patients, the main predictor of grip strength was forearm muscle cross-sectional area, which accounted for 33.4% of the variance, with joint deformity and pain explaining only an additional 4.5% when entered subsequently in a multiple regression model (Helliwell and Jackson 1994). Upper arm muscle area is also significantly associated with grip strength in RA patients (Collins, Dunn et al. 1987) and vastus lateralis type II fibre size strongly correlates with knee extensors strength (Nordemar, Berg et al. 1976; Nordemar, Edstrom et al. 1976). Even with no or minimal knee joint involvement, knee extensors strength is significantly

reduced compared to healthy controls (Hsieh, Didenko et al. 1987; Meireles, Oliveira et al. 2002) which suggests alterations in muscle mass and/or quality in RA patients. These findings are clinically relevant because muscle weakness is an important and independent determinant of physical disability in this population (Stucki, Bruhlmann et al. 1998). Moreover, both Roubenoff et al. (Roubenoff, Roubenoff et al. 1994) and Hernandez-Beriain et al. (Hernandez-Beriain, Segura-Garcia et al. 1996) found a direct association between anthropometric measures of muscle mass and physical disability, with patients in the lower functional classes presenting with more severe muscle atrophy. Similarly, Gomez-Vaquero et al. (Gomez-Vaquero, Nolla et al. 2001) and Roubenoff and colleagues (Roubenoff, Roubenoff et al. 1992) found that arm muscle mass is directly associated with disability as measured by the Health Assessment Questionnaire (HAQ) even when disease activity and duration are controlled for. Lastly, patients with overt rheumatoid cachexia (involuntary weight loss > 15%) are significantly more disabled than other RA patients (Munro and Capell 1997). Overall, these findings demonstrate that arthrogenous inhibition (Young 1993) is not the only determinant of muscle weakness in RA patients, and that rheumatoid cachexia, in addition to pain, joint damage and psychosocial factors (Escalante and Del Rincon 2002), is likely to be an important cause of disability in this population. Additionally, muscle atrophy has also been associated with increased mortality in a 2-year prospective study of 30 RA patients (Collins, Dunn et al. 1987) and is one of the major risk factors for generalised osteoporosis in RA patients (Shibuya, Hagino et al. 2002).

Overall, the results of these initial studies confirm the hypothesis that muscle atrophy, as in other chronic diseases, is clinically relevant and may be an important therapeutic target in the management of RA. It is therefore essential to understand its

pathogenesis and to develop safe and effective interventions aimed at preventing or reversing cachexia in RA patients.

PATHOGENESIS OF RHEUMATOID CACHEXIA

Alterations in Protein and Energy Metabolism

Rheumatoid cachexia is not a primary, degenerative muscular or neuromuscular disease like rheumatoid myositis or peripheral neuromyopathy. On the contrary, it should be considered an atrophic myopathy secondary to cytokine-induced metabolic alterations. Of particular pathophysiological interest are alterations in protein homeostasis which is lost at two levels in RA patients. Firstly, in healthy adults nitrogen intake and output are well matched so that over 24 hours the balance is zero and no net loss or gain of total body protein occurs. In RA patients with active disease, however, a state of negative nitrogen balance (nitrogen output > nitrogen intake) has been described, and this is exacerbated by corticosteroids therapy (Ruchelman and Ford 1962; Roubenoff, Roubenoff et al. 1990). As dietary protein intake was adequate in these studies, negative nitrogen balance in RA is clearly due to excessive proteolysis. These metabolic studies have been confirmed by neutron activation analysis which demonstrated a significant 12.5% reduction in total body nitrogen in 32 female RA patients compared to a group of healthy females with similar age, height and weight (Kennedy, Boddy et al. 1979). Although this loss of body protein occurred over a much longer period of time (average disease duration 8 years), its extent is very similar to that experienced over 21 days by patients with severe sepsis and major blunt trauma undergoing intensive therapy (Plank and Hill 2000). As in critically ill patients, body proteins are mainly lost from skeletal muscle tissue in RA (Gibson, Poyser et al. 1991).

Altered protein homeostasis in RA patients is also evident in terms of balance between protein synthesis and breakdown. Because proteins (at whole-body, organ, tissue or cellular level) are in continuous turnover, important losses occur when protein breakdown even slightly exceeds protein synthesis. In the only study published so far on whole-body protein turnover in RA patients, protein breakdown in the postabsorptive state was 19% higher relative to three groups of healthy controls ($p < 0.05$) when normalised by the metabolically active body cell mass (Rall, Rosen et al. 1996). Since protein synthesis was not affected by RA, this study clearly demonstrates that net postabsorptive protein catabolism is abnormally high in these patients. The effect of RA on the anabolic response to a meal (i.e., postprandial protein metabolism) is not known and needs to be investigated to obtain a full picture, as the first-pass metabolism of dietary amino acids and the endocrine response to meal ingestion could be altered in these patients.

Although protein synthesis is normal at whole-body level, this might result from divergent changes at organ level, i.e. the increased synthesis of some acute-phase proteins in the liver might be offset by decreased skeletal muscle protein synthesis. Indeed, Walsmith et al. (Walsmith, Vannier et al. 2003) reported a significant 25% reduction in skeletal muscle protein synthesis in eight RA patients compared to age-, sex- and body mass index (BMI)-matched healthy controls. Five of these patients were treated with low-dose oral corticosteroids but a similarly depressed muscle protein synthesis was found in the patients not treated with corticosteroids (Joseph Walsmith, personal communication). This finding is in contrast with that of Gibson et al. (Gibson, Poyser et al. 1991) who found a significant 30% reduction in skeletal muscle protein synthesis only in RA patients treated with corticosteroids. These authors suggested that in RA patients not treated with corticosteroids the main cause of reduced muscle protein content is accelerated muscle

proteolysis and this is supported by the greatly increased protein breakdown observed at whole-body level. However, in five patients with active RA the ratio between daily excretion of 3-methylhistidine and creatinine, a marker of myofibrillar protein breakdown, was not significantly different from normal (Elia, Carter et al. 1981). More and larger studies on skeletal muscle protein breakdown and its regulation are crucial in RA patients as this is now recognised as the main pathophysiological process in other cachectic conditions (Costelli and Baccino 2003).

Another distinctive feature of cachexia is hypermetabolism and this has been demonstrated in RA patients with active disease or when resting energy expenditure (REE) is normalised by the metabolically active body cell mass (Kvapil 1993; Roubenoff, Roubenoff et al. 1994; Walsmith, Abad et al. 2004). Hypermetabolism could exacerbate the protein catabolism induced by RA by determining a state of negative energy balance (Wechsler, Wenzel et al. 1984), however, total energy expenditure is reduced in these patients compared to healthy controls because of low levels of physical activity (Roubenoff, Walsmith et al. 2002). As energy intake is normal in RA patients, energy balance is actually positive and causes accumulation of body fat with no effect on protein catabolism (Westhovens, Nijs et al. 1997; Hart, Wolf et al. 2002). This refutes a role for hypermetabolism in the pathogenesis of rheumatoid cachexia, and it is likely to be simply a reflection of increased muscle protein breakdown through the ATP-dependent proteasome systems (Costelli and Baccino 2003) and increased energy requirements of activated immune cells (Kuhnke, Burmester et al. 2003).

Systemic Inflammation

The primary mediators of cachexia and the other systemic responses to local inflammation are cytokines such as tumor necrosis factor (TNF) (Kotler 2000). Several

lines of evidence exist for the catabolic properties of TNF which was originally designated as "cachectin" (Beutler, Greenwald et al. 1985).

First of all, it can be found in high concentrations not only in the synovial fluid of inflamed joints (where its acts in a paracrine/autocrine fashion) but also in the serum of patients with active disease (Saxne, Palladino et al. 1988; Tetta, Camussi et al. 1990; Roubenoff, Roubenoff et al. 1992; Hasselgren 2000; Straub, Paimela et al. 2002). Systemic TNF probably leaks from inflamed joints and other involved organs (i.e., rheumatoid nodules) but is also produced by activated peripheral blood monocytes (Roubenoff, Roubenoff et al. 1994; Walsmith, Abad et al. 2004). Once in the circulation, TNF can act as a classical hormone and influence the metabolism and function of many organs distant from the site or sites of inflammation, including skeletal muscles (Li and Reid 2001) which are known to express TNF receptors and TNF itself (Zhang, Pilon et al. 2000; Alvarez, Quinn et al. 2002; Lang, Silvis et al. 2003).

Importantly, prolonged exposure of skeletal muscle cells to concentrations of TNF (3 ng/ml) similar to those found in RA patients with active disease accelerates ubiquitin-dependent muscle protein breakdown with subsequent net loss of myosin and other proteins with no reduction of total DNA content (Li, Schwartz et al. 1998), a metabolic scenario which resembles the catabolic process described in skeletal muscle tissue of RA patients (Gibson, Poyser et al. 1991). This study proves that TNF can directly affect skeletal muscle protein metabolism. *In vivo*, however, TNF can also induce skeletal muscle loss indirectly by reducing the systemic and local production of, bioavailability, and tissue sensitivity to anabolic mediators such as growth hormone (GH) insulin-like growth factor-I (IGF-I) (Lang and Frost 2002), and stimulating the production of catabolic hormones such as cortisol (Michie, Spriggs et al. 1988).

For all these reasons, it is not surprising that animals treated with exogenous TNF (Hoshino, Pichard et al. 1991; Llovera, Lopez-Soriano et al. 1993; Lang, Frost et al. 2002), or with conditions that increase endogenous TNF production such as sepsis (Zamir, Hasselgren et al. 1992; Breuille, Voisin et al. 1999), adjuvant arthritis (Roubenoff, Freeman et al. 1997) and TNF transgene (Li and Schwarz 2003), develop the classical features of cachexia even when control animals are pair-fed to control for the acute anorectic effect of TNF (Laviano, Russo et al. 2002). Similarly, therapeutic administration of TNF to cancer patients increases REE and doubles forearm (i.e., skeletal muscle) efflux of total amino acids, particularly alanine and glutamine, with a concomitant decrease in their arterial levels, indicating that TNF also stimulates the uptake of amino acids in other organs (Warren, Starnes et al. 1987).

Similar experimental studies on the effects of TNF in RA patients have not been performed, but in four different cross-sectional studies, Roubenoff and colleagues demonstrated that systemic TNF production by circulating monocytes is significantly associated with the most important features of cachexia such as elevated REE (Roubenoff, Roubenoff et al. 1994), increased whole-body protein breakdown (Rall, Rosen et al. 1996), and skeletal muscle mass (Roubenoff, Roubenoff et al. 1992; Walsmith, Abad et al. 2004).

Although TNF is the prime suspect in the etiology of rheumatoid cachexia, many other cytokines, particularly IL-1, can affect skeletal muscle protein metabolism and function, and the interested reader is referred to the review of Zoico and Roubenoff (Zoico and Roubenoff 2002). I reviewed TNF in some detail as drugs capable of blocking its biological actions are now available in the UK for the clinical management of RA and they have been proposed for the treatment of cachexia in other chronic diseases (Kotler 2000).

Endocrine Abnormalities

Inflammatory cytokines have profound effects on both hypothalamic-pituitary function (McCann, Kimura et al. 2000) and that of other endocrine glands/organs (von Laue and Ross 2000; Bornstein, Rutkowski et al. 2004). In addition, a sedentary lifestyle (Lemmey, Maddison et al. 2001) and pain (Holte and Kehlet 2002) can also influence the endocrine system. Therefore, it is not surprising that RA patients present with several hormonal abnormalities which, in turn, can affect skeletal muscle protein metabolism (Rooyackers and Nair 1997). The anabolic hormones testosterone, GH/IGF-I, and insulin stimulate muscle growth by increasing protein synthesis, decreasing proteolysis or via a combination of these two processes (Sheffield-Moore and Urban 2004). On the other hand, catabolic hormones like cortisol induce loss of myofibrillar proteins by inhibiting protein synthesis and upregulating both ubiquitin-proteasome-dependent and calcium-dependent proteolysis in catabolic conditions (Hasselgren 1999).

Men with RA have significantly lower serum levels of bioavailable testosterone and a much higher prevalence of hypogonadism (32% vs. 7%) compared to age-matched controls (Tengstrand, Carlstrom et al. 2002). Testosterone is also reduced in female RA patients (Cutolo, Villaggio et al. 2002). Rheumatoid arthritis patients of both sexes also have low serum levels of dehydroepiandrosterone (DHEA) and its sulphate (DHEAS) (Cutolo, Villaggio et al. 2002), but these adrenal androgens are, at best, only mildly anabolic (Dhatariya and Nair 2003). Similarly, the role of ovarian steroids in controlling skeletal muscle metabolism is not clear and more research is warranted (Sheffield-Moore and Urban 2004). Overall these findings suggest that sex hormones deficiency, particularly testosterone in male patients (Bhasin, Woodhouse et al. 2003), is likely to be a cause of muscle wasting in RA and could be an important target for the treatment of cachexia in this population.

In addition, there seem to be significant alterations in the GH/IGF-I axis. Although either low (Templ, Koeller et al. 1996; Demir, Kelestimur et al. 1999), normal (Rall, Walsmith et al. 2002; Otero, Nogueiras et al. 2004) or increased (Rovensky, Bakosova et al. 2002; Denko and Malemud 2004) spontaneous or stimulated GH production has been found in RA patients, most studies are in agreement on the reduction in circulating IGF-I compared to healthy controls of similar age and sex (Johansson, Baylink et al. 1994; Denko, Boja et al. 1996; Lemmey, Maddison et al. 2001; Neidel 2001; Matsumoto and Tsurumoto 2002; Rall, Walsmith et al. 2002; Otero, Nogueiras et al. 2004). Some (Neidel 2001; Matsumoto and Tsurumoto 2002) but not all (Lemmey, Maddison et al. 2001) studies also shown increased serum levels of inhibitory IGF-I binding proteins such as IGFBP-3. As IGF-I is a powerful anabolic hormone (Carroll 2001), its reduced systemic bioavailability might contribute to muscle atrophy in RA patients. More studies are necessary to characterise the IGF system at muscle level, where it has important autocrine/paracrine actions (Adams 2002), and to ascertain whether serum IGF-I deficiency in RA patients is secondary to partial GH deficiency or GH resistance as this might have important implications for the dosing and safety of therapy with recombinant human GH (Van den Berghe 2002). The clinical relevance of the recently observed reduction in plasma ghrelin (a gastric hormone with stimulates GH production and appetite) in RA patients compared to healthy controls is unknown at present as anorexia is not widespread in this population and low circulating ghrelin seems to be independent from alterations of the GH/IGF-I axis in RA patients (Otero, Nogueiras et al. 2004). Studies in patients with other cachectic diseases have also failed to clearly demonstrate any anabolic effect of ghrelin and further research is necessary (Janssen, van der Lely et al. 2004).

Insulin, especially when exogenous amino acids are available, is also a potent stimulator of skeletal muscle protein accretion (Prod'homme, Rieu et al. 2004) and a state of insulin resistance has been described in RA patients with active disease (Svenson, Pollare et al. 1988). However, all studies conducted so far have concentrated on glucose metabolism and it is unknown if a concomitant resistance to the anabolic actions of insulin is present in RA patients. An answer to this important question is necessary before insulin therapy (Hadley and Hinds 2002) or insulin-sensitising agents (Zierath, Ryder et al. 1998) can be proposed and tested for the treatment of muscle wasting in RA patients.

Traditionally, all stress hormones (catecholamines, glucagon and cortisol) have been considered catabolic (Gelfand, Matthews et al. 1984). However, recent evidence suggests that both epinephrine and norepinephrine may increase protein synthesis and reduce protein degradation in skeletal muscles, and anabolic beta-2 agonists have been successfully used to counteract muscle wasting in a variety of catabolic conditions (Navegantes, Migliorini et al. 2002). These drugs could also be a potential therapy for cachexia in RA patients especially considering that impairment of the hypothalamic-pituitary-adrenal axis and sympathetic nervous system have been demonstrated in this population (Wilder 2002).

Although glucagon is negatively correlated with whole-body protein synthesis in a group of RA patients and healthy young and elderly individuals (Rall, Rosen et al. 1996), serum levels of this hormone are actually below normal in RA patients (Svenson, Pollare et al. 1988). This leaves cortisol as the only catabolic hormone likely to contribute to rheumatoid cachexia, but even in patients with active disease serum cortisol levels are only slightly elevated (~16 microgram/dl) (Mirone, Altomonte et al. 1996; Straub, Paimela et al. 2002) and much lower than the levels known to have cachectic effects (>30 microg/dl) (Darmaun, Matthews et al. 1988; Ferrando, Stuart et al. 1999; Paddon-Jones,

Sheffield-Moore et al. 2003). However, in rats corticosterone potentiates the catabolic effect of TNF in amounts that by itself do not influence muscle catabolism (Raina and Jeejeebhoy 1998). As such, a synergy between increased endogenous cortisol production and systemic inflammation in the pathogenesis of cachexia in RA patients cannot be excluded.

Local Mediators

Although an inbalance between humoral anabolic and catabolic factors has been clearly demonstrated in RA patients, very little research exists at skeletal muscle level. This is unfortunate as it is now clear that in other cachectic diseases tissue-specific expression of inflammatory cytokines and growth factors can be greatly altered (Spate and Schulze 2004).

In RA, Gibson et al. (Gibson, Poyser et al. 1991) demonstrated significant alterations in intra-muscular prostaglandins and proposed that diminution of muscle protein synthesis in patients treated with corticosteroids is consequent to a lower concentration of PGF2α. Similarly, an elevation in PGE2 concentration might mediate increased muscle protein breakdown in patients not treated with corticosteroids. Based on these findings, the authors suggested that nonsteroidal anti-inflammatory drugs (NSAIDs) which target specific prostaglandins could be used for the treatment of muscle atrophy in RA patients.

More recently, Walsmith et al. (Walsmith, Vannier et al. 2003) analysed skeletal muscle tissue taken from eight RA patients and eight age-, sex- and body size-matched healthy controls, and demonstrated a significant overexpression of TNF (3.0x) and myostatin (4.0x), a potent and locally-produced negative regulator of skeletal muscle mass (Gonzalez-Cadavid and Bhasin 2004). Interestingly, these authors also found an

increased expression of interleukin-15, a cytokine which is now being investigated for its anabolic and anti-catabolic properties (Quinn, Haugk et al. 1995; Quinn, Haugk et al. 1997; Carbo, Lopez-Soriano et al. 2000; Carbo, Lopez-Soriano et al. 2001; Quinn, Anderson et al. 2002; Furmanczyk and Quinn 2003; Figueras, Busquets et al. 2004) and that is present in high concentration in serum of RA patients with long-standing disease (Gonzalez-Alvaro, Ortiz et al. 2003). This suggests a counter-regulatory role of this cytokine in the pathophysiology of rheumatoid cachexia. The presence of a counter-regulatory response in skeletal muscle of cachectic RA patients is also supported by the significant overexpression (4.2x) of a skeletal muscle-specific gene-regulating protein known as MyoD which is crucial in stimulating myogenesis (Walsmith, Vannier et al. 2003). In this study, all these sarcoactive factors, with the exception of MyoD, were significantly correlated with the *in vivo* rate of mixed skeletal muscle protein synthesis. This suggests that altered gene expression is relevant to the pathophysiology of muscle wasting in RA and more of these studies are necessary to elucidate the cellular and molecular basis of rheumatoid cachexia and to identify possible pharmacological targets.

Disuse

Even though Sir James Paget, in the late XIX century, noted that muscle wasting in RA patients exceeds that expected from disuse alone (Paget 1873), most modern rheumatologists still think that reduced mobility is the most important cause of muscle atrophy in this population. I believe this is not the case because advances in arthroplasty mean that the number of bedridden RA patients has been greatly reduced, and most doctors no longer prescribe prolonged bed rest to patients with active RA (Allen, Glasziou et al. 1999). Moreover, rheumatoid cachexia occurs even in ambulant patients. However, even these RA patients have a significant 27% reduction in habitual physical

activity (Roubenoff, Walsmith et al. 2002) and this might cause some muscle atrophy in the long term. Nonetheless, more research in this area is necessary as significant muscle atrophy has been empirically demonstrated only in conditions of severe disuse such as strict bed rest (Abe, Kawakami et al. 1997), limb casting (Narici and Cerretelli 1998) and microgravity (LeBlanc, Lin et al. 2000).

The sedentary lifestyle adopted by most RA patients is, however, the main determinant of the positive energy balance and consequent increase in body fat characteristic of this disease (Westhovens, Nijs et al. 1997; Roubenoff, Walsmith et al. 2002). Increased adiposity not only makes the diagnosis of cachexia more difficult, but can increases cardiovascular risk (Ridker, Buring et al. 2003) and greatly exacerbates the effect of muscle atrophy on physical disability (Baumgartner 2000). Therefore, an increase in habitual physical activity is recommended to all RA patients to improve their functional status and general health. Patients with rheumatoid cachexia, however, might benefit from a more intense and specific (i.e., "pharmacological") mode of exercise such as progressive resistance training (PRT).

Corticosteroid Therapy

Although endogenous cortisol production is only slightly increased in RA patients, they are often treated with exogenous corticosteroids. Although some histological and functional studies suggest otherwise (Danneskiold-Samsoe and Grimby 1986; Danneskiold-Samsoe and Grimby 1986; Danneskiold-Samsoe and Grimby 1986; Danneskiold-Samsoe and Grimby 1986), many body composition studies suggest that chronic treatment with oral corticosteroids does not exacerbate rheumatoid cachexia (Kennedy, Boddy et al. 1979; Roubenoff, Roubenoff et al. 1994; Westhovens, Nijs et al. 1997; Westhovens 1999; Gomez-Vaquero, Nolla et al. 2001; Walsmith, Abad et al.

2004), possibly because its catabolic effects are counterbalanced by a reduction in disease activity and an increase in protein-energy intake. A study in which both approaches (histological and body composition analysis) are combined is necessary to clarify this important issue. On the contrary, it is quite clear that short-term (3 days), pulse, high-dose (1000 mg/day), intravenous methylprednisolone therapy induces a significant and sustained negative nitrogen balance despite clinical resolution and improved appetite (Roubenoff, Roubenoff et al. 1990), and that strategies should be implemented to minimise this catabolic response in RA patients hospitalised for a flare-up of the disease.

Another drug commonly used for the treatment of RA which might have catabolic effects is leflunomide, which has been associated with significant involuntary weight loss in ~ 7% of patients (Coblyn, Shadick et al. 2001). The composition of weight lost in these patients is, however, unknown and it could be adipose rather than skeletal muscle tissue as this agent could increase fat oxidation and REE (Coblyn, Shadick et al. 2001).

Other Pathogenic Factors

Several other factors might be involved in the pathogenesis of muscle atrophy in RA patients. Of particular interest is oxidative stress which is significantly increased in RA patients compared to healthy controls of similar age and sex distribution (Jikimoto, Nishikubo et al. 2002) and increases protein degradation in skeletal muscle through the ubiquitin-proteasome pathway (Gomes-Marcondes and Tisdale 2002). However, serum biomarkers of oxidative stress can originate from the inflamed joints and oxidative stress has not been investigated in skeletal muscle tissue of RA patients. This is crucial has reactive oxygen species generated by skeletal muscle mitochondria seem to be a crucial step in the post-receptor signal transduction of TNF-induced catabolism and contractile

dysfunction (Reid and Li 2001) and could be manipulated with antioxidant therapy in RA patients (Jaswal, Mehta et al. 2003).

In contrast to other cachectic diseases complicated by profound anorexia (e.g., advanced malignancy), protein and energy intake is normal in most RA patients and, therefore, does not contribute to muscle wasting in this population (Roubenoff, Roubenoff et al. 1992; Roubenoff, Roubenoff et al. 1994; Morgan, Anderson et al. 1997; Gomez-Vaquero, Nolla et al. 2001; Roubenoff, Walsmith et al. 2002). Some patients, however, voluntarily reduce their animal proteins and energy intake in an attempt to reduce joint symptoms and excessive weight, and these diets might exacerbate rheumatoid cachexia (Podenphant, Gotfredsen et al. 1996) by reducing circulating IGF-I (Fraser, Thoen et al. 2000). It is also possible that RA patients, because of altered protein metabolism, have increased protein requirements and this should be further investigated. Similarly, micronutrients intake, including vitamin D and antioxidants, is not adequate in RA patients (Rennie, Hughes et al. 2003) and might contribute to accelerated loss of skeletal muscle (Gomes-Marcondes and Tisdale 2002; Demay 2003; Li, Chen et al. 2003; Visser, Deeg et al. 2003). However, energy requirements are not increased by RA despite hypermetabolism because of a concomitant reduction in physical activity (Roubenoff, Walsmith et al. 2002).

Reconstructive surgical treatment is relatively common in RA patients (Massardo, Gabriel et al. 2002) and causes temporary protein catabolism (Michelsen, Askanazi et al. 1979; Michelsen, Askanazi et al. 1982) thus contributing to the development of muscle atrophy in this population. However, in a group of RA patients undergoing total knee or hip replacement, anthropometric assessment one day before and 10 days after surgery did not reveal any significant change in muscle mass possibly because these patients were already cachectic (Haugen, Homme et al. 1999). Studies using more sensitive body

composition techniques are warranted as biochemical markers suggest a catabolic response to surgery in RA patients (Hall, Peerbhoy et al. 2000)

Frequent infections in RA patients (Doran, Crowson et al. 2002) might also aggravate cachexia in this population (Powanda and Beisel 2003). Similarly, translocation of bacterial products (i.e. endotoxin) across a more permeable gastrointestinal tract (Mielants, De Vos et al. 1991) could stimulate systemic cytokine production in RA patients (Roubenoff, Roubenoff et al. 1994; Walsmith, Abad et al. 2004) and a catabolic response in skeletal muscle (Chai, Wu et al. 2003).

Last but not least, muscle atrophy could also be caused by reduced neural stimulation secondary to reflex effects of joint damage, swelling and pain (Young, Stokes et al. 1987; Bearne, Scott et al. 2002). However, arthrogenous muscle wasting is selective (e.g. only quadriceps muscle in knee pathology) and can not fully explain generalised cachexia in RA patients (Young 1993).

TREATMENT OF RHEUMATOID CACHEXIA

Endpoints in Clinical Trials for the Treatment of Cachexia

In 1997 experts from a variety of biomedical sciences and clinical specialties agreed that the major endpoint in clinical trials for the treatment of cachexia should be "an increase in nonfat cellular body mass" (Bone 1999). They also agreed that biochemical and metabolic markers should be used in Phase I and II trials or as explanatory variables, and that body composition measurements should be corroborated by an improvement in muscle strength or other performance measures. Additionally, the need to look at clinical endpoints or outcomes independently of body mass and composition (e.g. quality of life or length of stay in hospital) has also been emphasised. In this section, I will review studies in which body composition and other relevant endpoints have been investigated in

RA patients in response to a variety of anabolic or anti-catabolic interventions and make recommendations for future clinical trials.

Anti-TNF Therapy

Although analgesia (Kehlet 2000), NSAIDs (Kennedy, Boddy et al. 1979; Zhou, Jiang et al. 2003; McCarthy, Whitney et al. 2004) and a variety of standard DMARDs (Rofe, Whitehouse et al. 1990; Ferraccioli, Guerra et al. 1995; Rall, Rosen et al. 1996; Soto, Martin et al. 2001; Goodson, Morgan et al. 2003) seem to reduce catabolism, these agents, alone or in combination, do not completely halt rheumatoid cachexia, as patients with well-controlled but long-standing RA still present with reduced muscle mass compared to healthy controls. The introduction of biological agents capable of specifically blocking TNF activity for the treatment of RA, however, gives rheumatologists a potentially more powerful tool to reduce not only disease activity and joint damage, but also muscle wasting in RA patients.

In several animal studies, anti-TNF therapy has been proved to be effective in attenuating or even reversing cachexia (Costelli, Carbo et al. 1993; Siegel, Shealy et al. 1995; Truyens, Torrico et al. 1995; Llovera, Carbo et al. 1996). However, in the only human study published so far, a 300-mg single dose of monoclonal antibody to TNF given intravenously within 12 hours of the onset of severe sepsis did not prevent the loss of total body protein, expansion of extracellular fluid, hypermetabolism and mortality typical of critical illness (Clark, Plank et al. 1998). The highly catabolic nature of severe sepsis and the limited duration of treatment might explain these disappointing results.

Studies in animal models of RA (Feige, Hu et al. 2000; Shealy, Wooley et al. 2002; Fathalla, Hamada et al. 2004) and RA patients (Fonseca, Canhao et al. 2002) have shown significant weight gain in response to TNF antagonists. However, this might be

simply due to increased appetite and well-being and body composition studies are necessary to examine whether this weight gain is secondary to an anabolic response in skeletal muscle tissue and not a mere increase in body fat. Clinical trials of anti-TNF therapy for the treatment of rheumatoid cachexia might also prove to be an interesting human model for the use of this strategy in other chronic diseases complicated by cytokine-driven muscle catabolism such as cancer (Ramos, Suzuki et al. 2004).

Although anti-IL1 therapy is also available and potentially useful for reducing catabolism in RA patients (Bendele, Sennello et al. 1999; Bendele, Chlipala et al. 2000; Feige, Hu et al. 2000; Fathalla, Hamada et al. 2004), this biological agent is not recommended for the treatment of RA in UK and more clinical trials are needed (Clark, Jobanputra et al. 2004).

Physical Training

Intense and regular physical training, particularly PRT, is a powerful stimulus for muscle hypertrophy in healthy individuals (Kraemer, Adams et al. 2002) and both Walsmith and Roubenoff (Walsmith and Roubenoff 2002) and Zinna and Yarasheski (Zinna and Yarasheski 2003) have recently recommended it as the most effective treatment for rheumatoid cachexia. However, the evidence for these recommendations is weak as it is based on the positive effects of physical training on secondary endpoints such as muscle strength and other performance measures in RA patients (Van Den Ende, Vliet Vlieland et al. 2000). There is also evidence that 12 weeks of PRT normalises whole-body protein breakdown (Rall, Rosen et al. 1996) and that short-term, high intensity (6 week) but not long-term, moderate intensity (7 months) mixed aerobic and resistance training increases muscle fibre size in RA patients (Nordemar, Berg et al. 1976; Nordemar, Edstrom et al. 1976). Moreover, Hakkinen and colleagues (Hakkinen, Hakkinen et al. 1994), found a

significant enlargement (5.5%) of the quadriceps femoris after six months of PRT in patients with early inflammatory arthritis. However, the pooling of an unknown number of rheumatoid and psoriatic arthritis patients prevents definitive conclusions being made on the anabolic effect of PRT in RA patients or on muscles other than the quadriceps femoris.

In the only study in which body composition was measured in a group ($N = 8$) of patients with well-defined RA before and after a PRT program, Rall et al. (Rall, Meydani et al. 1996) did not find any increase in total body potassium, a proxy measure of total body skeletal muscle mass (Wang, Zhu et al. 2003), despite large gains in strength of all major muscle groups. It is likely that the relatively high intensity (80% of one maximal repetition) PRT program was adequate to induce neural adaptations but its volume (five resistance exercises, three sets of eight repetitions each) and frequency (less than twice a week) was not enough to stimulate muscle growth. Therefore, at present, there is no solid evidence for recommending this mode of physical training as adjunct therapy for cachexia in RA patients and future studies should urgently investigate the efficacy and safety of a PRT program prescribed for optimal stimulation of muscle hypertrophy according to the guidelines of the American College of Sports Medicine for healthy adults (Kraemer, Adams et al. 2002).

However, there is some evidence that, in obese female RA patients on a hypocaloric, high-protein diet for 12 weeks, mixed aerobic and resistance training can prevent the loss of body cell mass commonly observed during weight loss programs (Engelhart, Kondrup et al. 1996). Future randomised controlled trials should investigate further this anti-catabolic effect of physical training as many RA patients are overweight or obese and would benefit from regimes that would reduce in body fat without exacerbating skeletal muscle loss.

Nutritional Treatment

Although there is much interest on the role of diet and nutritional supplementation in the management of joint symptoms and damage in RA (Rennie, Hughes et al. 2003), Akner and Cederholm (Akner and Cederholm 2001) recently highlighted the fact that there are no published clinical trials for the nutritional treatment of cachexia in RA. This lack of interest might be explained by the well-nourished appearance of most RA patients, and the fact that generally they are not anorectic and have normal protein and energy intake (Roubenoff, Roubenoff et al. 1992; Roubenoff, Roubenoff et al. 1994; Morgan, Anderson et al. 1997; Gomez-Vaquero, Nolla et al. 2001; Roubenoff, Walsmith et al. 2002). Therefore, hypercaloric feeding and appetite stimulants would be inappropriate for the treatment of rheumatoid cachexia.

However, RA patients might benefit from dietary supplementation with high doses of specific nutrients, i.e. "pharmacological nutrition". Possible strategies shown to have some positive effects in other catabolic conditions include nutritional therapy with anabolic amino acids (Clark, Feleke et al. 2000; May, Barber et al. 2002; Rathmacher, Nissen et al. 2004), fish oil (Fearon, Von Meyenfeldt et al. 2003; Burns, Halabi et al. 2004; Moses, Slater et al. 2004), antioxidants (Mantovani, Maccio et al. 2003) and vitamin D (Kenny, Biskup et al. 2003). A small trial on the effect of creatine supplementation on muscle strength in RA patients gave disappointing results (Willer, Stucki et al. 2000) but further and larger clinical trials on the effects of this and other dietary supplements are important as they could provide a safer and better tolerated alternative to anabolic/anti-catabolic drugs.

Hormonal Therapy

The use of anabolic steroids in clinical practice has been seriously limited by the "bad press" these agents have received in the attempt to discourage the abuse common among competitive athletes and bodybuilders. However, an open minded review of the scientific literature suggests that anabolic steroids could play a significant role in the management of muscle wasting in a variety of chronic diseases (Basaria, Wahlstrom et al. 2001; Orr and Fiatarone Singh 2004). A number of clinical trials of testosterone and other anabolic steroids as natural immunosuppressors (Cutolo, Villaggio et al. 2002) or for the treatment of osteoporosis (Joffe and Epstein 1991) have been conducted in RA patients, but only in few of them endpoints relevant for the treatment of rheumatoid cachexia have been included.

Bird et al. (Bird, Burkinshaw et al. 1987) randomly assigned postmenopausal women with RA to either nandrolone decanoate (50 mg intramuscular every third week) (n = 24) or placebo (n = 23) for two years. Body composition by neutron activation analysis revealed significant increases in total body nitrogen and potassium (i.e., fat-free mass) in the group treated with nandrolone decanoate. As body weight did not change over the intervention period, a significant loss of fat mass must have occurred concomitantly in this group. These changes in body composition, however, did not result in a differential improvement in grip strength which increased slightly in both groups. Nevertheless, there was a striking improvement of anaemia and only minor androgenic side effects in the group treated with nandrolone decanoate. Even when using pure testosterone, a much more androgenic anabolic steroid, for a year in postmenopausal patients with active RA, the androgenic side effects were limited and none of the subjects considered them to be a serious problem and stopped treatment for this reason (Booji,

Biewenga-Booji et al. 1996). This good tolerability is clinically important as most RA patients are middle-age and old women.

In men with RA, a small metabolic study suggests that high doses of methandrostenolone, an anabolic steroid, can reverse the negative nitrogen balance caused by corticosteroid therapy with dexamethasone (Ruchelman and Ford 1962). Very recently, the beneficial effects of anabolic steroids (testosterone and nandrolone decanoate) in male patients with chronic inflammatory diseases requiring corticosteroid therapy (including RA) has been confirmed by a randomised, placebo-controlled clinical trial in which significant increases in muscle mass and strength were measured after 12 months of treatment (Crawford, Liu et al. 2003).

In all these studies no major adverse events have been reported and overall these findings suggest that therapy with supraphysiological doses of anabolic steroids might be safe and effective in reversing cachexia in both male and female RA patients as it is in other catabolic conditions (Bross, Casaburi et al. 1998). More research is necessary to confirm and extend these preliminary results, and on the effects of testosterone in the large number of male RA patients suffering from hypogonadism (Tengstrand, Carlstrom et al. 2002) as this form of hormone replacement therapy seems to be beneficial in men with primary hypogonadism (Bhasin, Woodhouse et al. 2003) or the elderly (Allan and McLachlan 2004). As safety of supraphysiological anabolic steroids and testosterone replacement therapy is an important consideration, before their implementation in clinical practice, future trials should thoroughly assess possible long-term consequences of these hormonal therapies.

An alternative to anabolic steroids and testosterone, especially in young female patients concerned about the possible masculinising effects and patients with reduced activity of the somatotropic axis, is administration of exogenous GH. This form of

hormonal therapy has not been tested in adults with RA, but studies in an animal model of RA (Ibanez De Caceres, Villanua et al. 2000) and juvenile RA patients (Kenny, Biskup et al. 2003) suggest that recombinant human GH administration might normalise the somatotropic axis and body composition in this population. Similarly, exogenous insulin might overcome resistance and reverse catabolism in conditions such as RA characterised by elevated levels of TNF (Fraker, Merino et al. 1989). I feel, however, that more basic research is necessary before clinical trials of recombinant human GH, GH secretagogues (including ghrelin), IGF-I, insulin, and insulin-sensitising agents can be ethically conducted in RA patients.

Other forms of endocrine treatment include standard hormone replacement therapy for postmenopausal women (HRT) and DHEA(S). A recent study suggests that 2-year HRT in postmenopausal women with active RA can significantly increase circulating IGF-I (D'Elia, Mattsson et al. 2003) but its effects on body composition in this population are unknown. Dehydroepiandrosterone supplementation have been proposed to stimulate androgen-dependent anabolism especially in female RA patients treated with corticosteroids (Robinzon and Cutolo 1999), but again no clinical trials with appropriate endpoints have been published to date. Therefore, neither HRT nor DHEA(S) can be recommended for the treatment of rheumatoid cachexia at present.

Experimental Therapies

Several new and exciting therapies for muscle wasting are being investigated in animals and, in the future, might also prove to be useful in the management of cachexia in RA patients. The most promising interventions are modulation of myostatin activity using the JA16 antibody or inhibitory proteins such as myostatin propeptide and follistatin (Roth and Walsh 2004) and IGF-I gene therapy (Rosenthal and Musaro 2002). So far the

efficacy and safety of these agents has been tested in healthy young and old mice and a mouse model of Duchenne muscular dystrophy. Future studies should investigate the potential of these and other experimental therapies in animal models of inflammatory cachexia such as adjuvant arthritis (Roubenoff, Freeman et al. 1997).

SUMMARY AND CONCLUSIONS

Rheumatoid cachexia is a common and clinically relevant extra-articular feature of RA, and all patients should be regularly screened for it using simple and readily available body composition techniques. Good control of disease activity with the least possible use of corticosteroids and promotion of a healthy lifestyle (i.e., regular physical activity and consumption of an adequate and balanced diet) are essential to minimize cachexia and improve functional status and general health. However, this generic approach is clearly not enough to stimulate muscle growth, and RA patients with severe muscle atrophy ought to receive specific anabolic therapies.

Many different pharmacological (hormonal therapy) and non-pharmacological (nutritional treatment and physical training) strategies have proven safe and effective in reversing cachexia in other conditions but a solid evidence base for the prescription of these anabolic therapies in RA patients does not exist, and hence specific clinical trials should conducted in this population. It is also necessary to assess the effects of anti-TNF therapy on body composition as this anti-catabolic treatment could prove to be superior to standard therapy in the prevention of rheumatoid cachexia. Alongside clinical trials, continuous effort is required to understand the basic pathophysiological, cellular, molecular and genetic mechanisms of rheumatoid cachexia, particularly at skeletal muscle level, as such insight should provide new therapeutic targets for the future.

CHAPTER 3

CAN PROGRESSIVE RESISTANCE TRAINING REVERSE CACHEXIA IN RHEUMATOID ARTHRITIS PATIENTS?

A PAPER ACCEPTED BY THE JOURNAL OF RHEUMATOLOGY

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INTRODUCTION

Rheumatoid arthritis (RA), like many other chronic systemic diseases, is often complicated by cachexia, a syndrome characterised by cytokine-induced hypermetabolism, high protein turnover (i.e., increased protein synthesis in the liver and accelerated muscle protein breakdown), negative nitrogen balance, muscle wasting, anorexia and weight loss (Kotler 2000). In most RA patients, however, weight loss is not apparent as protein and energy intake is normal and the decrease in body cell mass, predominantly in skeletal muscle, is masked by a concomitant increase in fat mass consequent to reduced physical activity and resultant low levels of total energy expenditure (Roubenoff, Walsmith et al. 2002). Rheumatoid cachexia affects more than 50% of RA patients (Munro and Capell 1997) and is thought to contribute to weakness, fatigue, disability, morbidity and mortality in RA (Walsmith and Roubenoff 2002). Therefore, interventions aimed at preventing/reversing muscle loss, and the usually associated fat gain, have the potential to significantly improve outcome in these patients.

Progressive resistance training (PRT) is safe and effective in stimulating muscle hypertrophy in healthy adults (Kraemer, Adams et al. 2002) and in patients with other cachectic diseases such as chronic renal insufficiency (Castaneda, Gordon et al. 2001), HIV infection (Roubenoff and Wilson 2001), and chronic heart failure (Pu, Johnson et al. 2001). Progressive resistance training can also decrease body fat in old men and women (Hunter, Bryan et al. 2002). However, there has only been one previously published investigation on the effect of PRT on body composition in a well defined group of RA patients (Rall, Meydani et al. 1996; Rall, Rosen et al. 1996).

In this study a group of eight RA patients trained at high intensity (three sets of eight repetitions with 80% of one repetition maximum [1-RM]) for 12 weeks but despite a normalization of whole-body protein breakdown and significant improvements in fatigue,

pain, muscle strength and other measures of physical function, there were no changes in total body potassium (a proxy measure of body cell mass (Wang, St-Onge et al. 2004) and skeletal muscle mass (Wang, Zhu et al. 2003)), fat-free mass (FFM), fat mass, and total body water (TBW). While it is possible that the humoral state characteristic of RA (Lemmey, Maddison et al. 2001) prevented the normal increase in FFM in response to PRT (Frost, Lang et al. 1997), it is more likely that the low "dose" prescribed (five dynamic resistance exercises per session for an average of ~ 1.7 sessions per week) was not adequate to stimulate muscle hypertrophy in these patients. This hypothesis is supported by the absence of a significant anabolic response in two separate groups of eight young and eight old healthy subjects that took part in identical PRT programs within the same investigation. The low weekly volume of PRT, together with a significant increase in caloric intake and no change in resting energy expenditure, could also explain why no reduction in fat mass was found in this study. Unfortunately, regional body composition data were not reported, thus the differential effects of PRT on limbs and trunk morphology can not be assessed.

Despite this negative evidence, both Roubenoff (Roubenoff 2003) and Zinna and Yarasheski (Zinna and Yarasheski 2003) have recently recommended PRT as adjunct treatment for rheumatoid cachexia on the basis of its beneficial effects on physical function and protein metabolism in RA patients. However, these can only be considered secondary endpoints in clinical trials for cachexia (Bone 1999) and more research on body composition is necessary to securely reject the hypothesis that RA patients are resistant to the anabolic effect of PRT. Experts at the 2002 Exercise and Physical Activity Conference in St. Louis, Missouri, went a step further and recommended that researchers should "study the molecular/cellular mechanisms by which exercise reverses the cachexia of chronic inflammation" (Chang, Roubenoff et al. 2003). However, we believe that

before embarking on such research program and Phase III and IV trials, a proof-of-concept trial is required. In this paper we present the results of a Phase II Trial aimed at (a) testing, in RA patients with stable and moderate disease, the feasibility and safety of a PRT program prescribed for optimal stimulation of muscle hypertrophy according to the guidelines of the American College of Sports Medicine for healthy adults (Kraemer, Adams et al. 2002), (b) providing preliminary evidence of its efficacy as adjunct treatment for rheumatoid cachexia and (c) quantifying the effect of PRT on body composition in RA patients for sample size calculations of future Phase III trials.

MATERIALS AND METHODS

Study Design and Ethics

This was a two-group, matched, parallel, controlled, pretest-posttest study which was conducted between July 2000 and August 2001. The study protocol and informed consent were approved by the North Wales Health Authority Research Ethics Committee.

Subject Recruitment and Eligibility

Ten patients willing and able to participate in a supervised PRT program (training group) were recruited during outpatient clinics at the Rheumatology Department of Gwynedd Hospital. Ten age (within five years) and sex matched patients willing to take part in the study, but not able to train regularly under supervision because of logistic reasons, were selected to serve as control group. In order to be included into the study, all volunteers had to: (a) fulfil the American Rheumatism Association 1987 revised criteria for the diagnosis of RA (Arnett, Edworthy et al. 1988); (b) be functional class I or II; (c) be age 18 or over; (d) not be cognitively impaired; (e) have been on stable drug therapy during

the previous three months; (f) be free of other cachectic diseases and any condition preventing safe participation in the study; (g) not be taking drugs or nutritional supplements known to increase muscle mass; (h) not be participating in another regular and intense physical training program. Before commencing the study, all subjects gave their written informed consent to participate.

Outcome Measures

Outcome measures were taken at baseline (pretest) and follow-up (posttest) by the same investigator (SM) in the Bone Densitometry Laboratory in the School of Sport, Health and Exercise Sciences, University of Wales-Bangor, at approximately at the same time of the day. Subjects presented fasted and were asked to void and remove all metallic objects. Subjects were instructed beforehand to avoid strenuous exercise in the 24 hours preceding testing and were questioned as to whether they had orthopaedic metal and silicone implants. During testing subjects were allowed to wear only socks, shorts, underwear (no bra) and a t-shirt. With respect to efficacy, the primary outcome measure was an increase in *both* arms *and* legs (i.e., appendicular) lean mass by DXA, a proxy measure of total body skeletal muscle mass (Kim, Wang et al. 2002). The primary outcome measure for safety was the disease activity index (RADAI), a patient questionnaire which is sensitive to flare-ups of disease activity in RA patients (Fransen, Hauselmann et al. 2001).

Body Mass and Composition

Body mass was measured to the nearest 0.1 kg using a calibrated balance scale (Seca, Hamburg, Germany). Total and regional (left and right arm, left and right leg, trunk, head) body composition was assessed by DXA using a pencil-beam scanner (QDR1500, Hologic, Bedford, Massachusetts) which calculates the masses (g) of three different

compartments: bone mineral content (BMC), fat mass and lean mass. The sum of total BMC and total lean mass corresponds to FFM. Percent body fat was calculated as (total fat mass/total body mass by DXA) · 100. The procedures recommended by the manufacturer for whole-body examination (subject positioning, scanning and analysis with software version V5.72) were followed and the quality control procedure was performed daily.

Immediately after the DXA scan, intracellular (ICW) and extracellular (ECW) water volumes (L) were estimated using bioelectrical impedance spectroscopy (BIS) (Hydra 4200, Xitron Technologies, San Diego, California). Total body water (TBW) is the sum of ICW and ECW. Extracellular water to ICW ratio was also calculated. Bioelectrical impedance measurements were taken on the left side of the body, with the use of disposable electrodes and in accordance with a standard wrist-to-ankle protocol (Van Loan, Withers et al. 1993). At the time subjects were measured, they had been supine for approximately 20 min. The quality control procedure recommended by the manufacturer was performed before each measurement and the proximity of the DXA scanner had no effect on the validity and reliability of BIS (unpublished observations).

By combining DXA and BIS data, we calculated FFM hydration (TBW/FFM), total BMC to TBW ratio, and total body protein estimated as total lean mass – 0.2302 · total BMC – TBW according to the model proposed by Fuller et al. (Fuller, Wells et al. 2001). For each of these calculations, TBW volume was converted into mass by assuming a water density of 0.99336 kg/L at a normal body temperature of 37°C.

Muscle Strength

After body composition assessment, subjects performed callisthenics for 5 min under the direction and supervision of one of the authors (SM). The following generic muscle

strength tests were then performed. Maximal voluntary hand-grip strength was measured using a Grip-A dynamometer (Takey Kiki Kogyo, Japan). Subjects were asked to stand erect holding the dynamometer parallel to their side, dial facing away from the body and to squeeze the hand-grip without moving the arm. Maximal voluntary strength of the knee extensors and elbow flexors was measured using a CSD300 hand-held dynamometer (Chatillon-Ametek, Largo, Florida). For the measurement of knee extensors strength, subjects were sitting on a medical table with hips and knees flexed 90° and arms across the chest. The curved push attachment was positioned over the tibia just proximal to the two malleoli and subjects were instructed to attempt to straighten the leg. For the measurement of elbow flexors strength, subjects were lying on a medical table with shoulder neutral, elbow flexed 90° and forearm neutral. The curved push attachment was positioned just below the styloid process of the radius and subjects were instructed to attempt to flex the arm. All these static strength tests followed the same protocol: after two submaximal warm-up and familiarisation trials (50% and 75% of maximum effort), subjects were asked three times to exert force maximally for approximately 5 s. Between all five trials 1 min rest was observed. Peak force produced during each of the three maximal trials was recorded in newtons and the best score noted. Both the right and left side of the body were tested and the average of the two best scores used for statistical analysis. The force of the tester (male, body mass 100 kg, height 1.79 m) was sufficient to fix the hand-held dynamometer against the forces produced by all subjects. None of the subjects approached the upper limit of the dynamometers and their accuracy was verified periodically over the course of the study by vertically loading the dynamometers with certified calibration weights. Lower body functional strength was assessed with the 30-s maximal sit-to-stand test (SST-30) validated by Jones et al. in older adults (Jones, Rikli et al. 1999). After a practice trial, subjects were asked to sit-to-stand from a fixed chair (seat

height 43.2 cm) as many times as possible in 30 s while keeping their arms folded across the chest. As suggested by McNair et al. (McNair, Depledge et al. 1996), subjects were verbally encouraged during testing in order to attain maximum performance.

Disability and Psychological Status

Disability was quantified by the modified health assessment questionnaire (HAQ) and advanced activities of daily living (ADLs) items included in the multidimensional HAQ developed by Pincus et al. (Pincus, Swearingen et al. 1999). Problems with sleep, stress, anxiety and depression (i.e., psychological status) were also assessed with this questionnaire.

Disease Activity

Disease activity was assessed using the RADAI (Stucki, Liang et al. 1995). With the exception of fatigue, this patient questionnaire quantifies the most apparent signs and symptoms of RA: (a) global disease activity over the past six months, (b) current disease activity in terms of swollen and tender joints, (c) arthritis pain, (d) duration of morning stiffness, and (e) amount of pain in several joint areas. These five items are then combined to provide a single index of disease activity on a 0 to 10 scale where higher scores indicate more active disease. Fatigue over the past week was measured with a numerical rating scale ranging from 0 (*fatigue is no problem*) to 10 (*fatigue is a major problem*). The erythrocyte sedimentation rate (ESR) was also assessed using the Westergren's method in the Biochemistry and Haematology Department of Gwynedd Hospital to provide an objective measure of disease activity.

Other Measures

At baseline these other measures were taken. Height was measured to the nearest 0.5 cm using a wall-mounted stadiometer (Body Care, Warwickshire, UK). Body mass index (BMI) was calculated as body mass/height squared. Typical dietary intake was estimated using a semiquantitative food frequency questionnaire (Willett, Sampson et al. 1985). Habitual physical activity both at work and during leisure time was ranked from mainly sedentary (score = 1) to heavy and regular (score = 4) using the questionnaire proposed by Saltin et al. (Saltin and Grimby 1968). The degree of muscle atrophy was calculated as the percentage of actual total body skeletal muscle mass estimated from appendicular (arms and legs) lean mass, age, and sex (Kim, Wang et al. 2002) compared to normal total body skeletal muscle mass estimated from body mass, height, age, sex, and ethnicity (Lee, Wang et al. 2000). Other baseline information (e.g., disease duration, current therapy etc.) were collected by a structured interview and review of medical records. At follow-up, all subjects were questioned if changes in habitual physical activity, diet, medications, and other aspects of their lifestyle occurred during the study.

Intervention

After baseline assessment and an introductory week (Week 0) for familiarisation with exercise equipment and technique, subjects in the training group underwent 12 weeks of PRT consisting of eight resistance exercises per session (Leg Press, Chest Press, Leg Extension, Seated Row, Leg Curl, Triceps Pushdown, Standing Calf Raise, Biceps Curl) with the following characteristics: (a) dynamic muscle action at moderate repetition velocity (1-2 s concentric, 1-2 s eccentric); (b) three sets of eight repetitions with a load corresponding to 80% of 1-RM; (c) 1- to 2-min rest periods between sets and exercises. Subjects trained three times a week with at least 48 h between training sessions (Monday,

Wednesday and Friday, or Tuesday, Thursday and Saturday). One repetition maximum was assessed at the end of Week 0 and every two weeks thereafter to adjust exercise load in proportion to changes in specific muscle strength. Each training session lasted approximately 75 min including warm-up (5 min low intensity aerobic exercise plus one set of 15 reps with half the load before each exercise) and cool-down (10 min of core stability and static stretching exercises) phases. A record of each session was kept and each training session workload calculated as the total weight lifted (i.e., the sum of each exercise load in kg· number of reps· number of sets). The occurrence of adverse events was also recorded. Subjects in the training group exercised in small groups on Powersport resistance exercise machines (Bridgend, United Kingdom) at Maes Glas Sports Centre under the supervision of one of the authors (SM).

After baseline assessment, subjects in the control group continued their usual care (medications and joint mobility exercises) with no additional PRT. All subjects were instructed to maintain their habitual physical activity and dietary habits throughout the study. Follow-up assessment of all subjects was conducted on the Monday or Tuesday of Week 13.

Statistical Analysis

We tested the null hypothesis of no difference between groups in all baseline measures using multiple paired *t* tests for continuous variables and Fisher's exact probability tests for categorical variables. We used multiple one-way analyses of covariance (ANCOVAs) to test the null hypothesis of no difference between groups (independent variable) on each outcome measure with the posttest score as the dependent variable and the pretest score as the covariate. However, because the assumption of homogeneity of regression slopes was violated for ESR, a one way analysis of variance (ANOVA) on change (posttest minus

pretest score) was used in this instance. For these analyses, the between subjects effect size for group was calculated as eta squared (η^2), a measure of strength of relationship between the independent and independent variable. Thresholds for small, moderate, large and very large effects were set at 0.01, 0.08, 0.26 and 0.50 respectively. We used a one-way repeated measure ANOVA to test the null hypothesis of no change in the average weekly training load within the training group. Pearson product-moment correlation was used to express and test the significance of the relationships between measures of interest (body mass by scale with body mass by DXA; changes in arms and legs lean mass with changes in muscle strength and disability) after pooling the data of both groups. Significance was set at 0.05 (two-tailed) for all analyses with a P level > 0.05 and < 0.10 being considered a trend. Data were analysed using the Statistical Package for the Social Sciences Version 11.

RESULTS

Subjects Characteristics

All subjects (six women and four men in each group) completed the study. Their baseline characteristics are shown in Tables 1, 2, 3 and 4. In terms of age, sex distribution, disease characteristics and therapy, our subjects are representative of functional class I and II patients (Stucki, Liang et al. 1995; Pincus, Swearingen et al. 1999). Despite a BMI in the overweight category and normal protein-energy intake, all but one of the patients presented with muscle atrophy. Actual total body skeletal muscle mass was, on average, 79% (range 62-118%) of normal muscle mass in the training group and 74% (range 64-83%) in the control group ($P = 0.29$). A minority of patients were taking low doses of vitamin and mineral complexes, calcium, cod liver oil, and glucosamine, with no

significant differences between groups (all $P \geq 0.47$). Four women in the training group and five women in the control group were postmenopausal ($P = 1.00$). All other women had regular menstruations. None of the postmenopausal women in the training group was on hormone replacement therapy while two in the control group were ($P = 0.44$). With the exception of sleep duration, there were no significant differences between groups at baseline in any of the measured characteristics. We did not include sleep duration as additional covariate as it was not significantly correlated with any of the outcome measures (data not shown) and because both groups reporting sufficient sleep. None of the subjects had silicone implants but one subject in the training group had bilateral hip replacement. Because the influence of orthopaedic metal on DXA measures of body composition is reproducible and relatively small when computerized high-density detection analysis is employed (Madsen, Egsmose et al. 1999; Giangregorio and Webber 2003), we decided to include this subject in the analysis. All subjects were Caucasians and sedentary.

Compliance and Adverse Events

Compliance to PRT was, on average, 85% (range 67 - 94%). This corresponds to a mean training frequency of 2.5 sessions per week (range 2.0 - 2.8 sessions per week). The progressive nature of the proposed resistance training program was confirmed by a significant increase in the average weekly training load throughout the intervention period (Figure 2). There were no flare-ups of disease activity, training-related injuries or any other adverse event during the study in either group. All subjects declared that habitual physical activity, diet, medications, and other aspects of their lifestyle remained unchanged during the study.

Effects on Body Mass and Composition

Body mass and composition at baseline and follow-up, and the results of ANCOVA are shown in Table 2 and 3. Progressive resistance training did not have a significant effect on body mass. The change in body mass measured by scale was highly correlated to the change in body mass measured by DXA (Figure 3), thus confirming the internal validity of our measurements.

Progressive resistance training had a large and significant effect on total lean mass (1242 g) and, as a result, FFM (1253 g). This increase in FFM mostly reflects a significant and moderate increase in total body protein (1063 g) as PRT did not have a significant effect on TBW and total BMC. The significant increase in lean mass was concentrated in the arms (280 g) and legs (839 g) suggesting a very large effect on total body skeletal muscle mass. Progressive resistance training did not significantly affect total fat mass but there was a trend for a moderate loss of fat mass in the trunk (-752 g) and a significant albeit moderate reduction in percent body fat (-1.1%). None of the other measures of body composition was significantly affected by PRT but there was a trend for a moderate increase in arms BMC (200 g).

Effect on Muscle Strength

Measures of muscle strength at baseline and follow-up, and the results of ANCOVA are shown in Table 4. Progressive resistance training caused a moderate increase in hand-grip strength (53 N), a large increase in elbow flexors strength (54 N), and a very large increase in lower body functional strength (3.6 repetitions). All these effects were statistically significant. The moderate increase in knee extensors strength (39 N) approached statistical significance.

Effects on Disability and Psychological Status

The scores of the specific components of the multidimensional HAQ at baseline and follow-up, and the results of ANCOVA are shown in Table 4. Although PRT did not affect MHAQ scores, at follow-up the training group reported, on average, less difficulty in advanced ADLs compared to the control group when adjusting for baseline scores. This effect (-0.25) was very large and statistically significant. Progressive resistance training did not have any significant effect on psychological status as measured by the multidimensional HAQ, but most patients in the training group anecdotally reported an improvement in mood.

Effect on Disease activity

Self-reported and laboratory measures of disease activity at baseline and follow-up, and the results of ANCOVA are shown in Table 4. Despite its relatively high intensity, volume and frequency, the proposed PRT program did not significantly affect RADAI, fatigue or ESR.

Correlations between Changes in Appendicular Lean Mass and Changes in Muscle Strength and Disability

There was a moderate and significant correlation between change in arms lean mass and change in handgrip strength ($r = 0.44$, $P = 0.05$), and change in elbow flexors strength ($r = 0.51$, $p = 0.02$) but not with change in advance ADLs score ($r = -0.20$, $P = 0.39$). On the other hand, change in legs lean mass correlated moderately and significantly with change in advance ADLs score ($r = -0.50$, $P = 0.03$). There was also a strong and significant association between change in legs lean mass and change in lower body functional strength ($r = 0.75$, $P < 0.01$).

DISCUSSION

This study demonstrates for the first time that a high intensity and volume PRT program is feasible, and can safely reverse cachexia in mildly disabled RA patients with moderate disease activity. Contrary to Rall et al. (Rall, Meydani et al. 1996), we found a significant increase in FFM which was secondary to a considerable increase in appendicular lean mass, a proxy measure of total body skeletal muscle mass. As relative exercise intensity, number of sets and repetitions per exercise, muscle action and repetition velocity, rest periods, and training duration were identical, we believe that the higher training frequency and the higher number of resistance exercises per session (i.e., an higher weekly volume) of our PRT program account for the different outcomes of the two studies.

By combining DXA and BIS, changes within the three main subcompartments of FFM were examined in our study. Like Rall et al., we did not observe any significant change in total BMC or TBW in response to PRT but, on the other hand, a significant increase in total body protein was evident in our study. As changes in body composition reflect the "area under the curve" of the organism metabolic state (Roubenoff 1997), this finding suggests that PRT is able to overturn the negative nitrogen balance characteristic of RA (Roubenoff, Roubenoff et al. 1990). This is not surprising as a similar resistance exercise protocol stimulates net mixed muscle proteins synthesis in healthy humans (Phillips, Tipton et al. 1997).

Although some authors consider ICW a sensitive proxy measure of body cell mass (Earthman, Matthie et al. 2000), the absence of any significant change in this compartment does not contradict our findings. In fact, an increase in body cell mass can result from an increase in intracellular solids (e.g., proteins) without any change in ICW provided that cellular hydration stays within the limits compatible with proper cell

function. According to the model and reference data of Wang et al. (Wang, Deurenberg et al. 1999; Wang, Heshka et al. 2003), and by making the reasonable assumption that PRT did not have a significant effect on the hydration of the extracellular fluid, the normal FFM hydration, ECW:ICW and total BMC:TBW we observed before and after intervention would indeed suggest that cellular hydration remained normal in our subjects.

Overall, these results suggest that PRT can reverse protein catabolism and stimulate skeletal muscle hypertrophy in RA patients and reject the hypothesis of resistance to the anabolic effect of PRT in this population. Together with the recent findings of Häkkinen et al. (Hakkinen, Hannonen et al. 2003), our data suggest that RA patients can adapt to physical training as well as healthy subjects when the proper exercise mode, intensity, duration, frequency and progression is prescribed and adhered to.

The positive effect of our PRT program on appendicular lean mass was corroborated by a significant increase in three out of four measures of muscle strength. The smaller overall increase in muscle strength found in our study (+25%) compared to that observed by Rall et al. (+57%) can be explained by the fact that our measures of muscle strength were chosen to minimize training specificity whereas Rall et al. used the same resistance exercises for both testing and training (Morrissey, Harman et al. 1995).

Although other neuromuscular adaptations occur in RA patients in response to PRT (Bearne, Scott et al. 2002), we believe that the increase in muscle strength measured in our study is, at least in part, secondary to muscle hypertrophy. In support of this hypothesis, we found significant correlations between changes in appendicular lean mass and changes in muscle strength. We also found an association between the increase in legs lean mass and the reduction in disability. This finding is very important because the

change in advanced ADLs score was larger than that considered clinically relevant (Bruce and Fries 2003) and provides support to the hypothesis that interventions aimed at preventing/reversing muscle wasting secondary to RA can have a positive influence on the poor outcome of this disease.

Previous studies on the effects of physical training in RA patients, despite proving its efficacy in improving aerobic capacity, muscle strength and joint mobility, have not convincingly demonstrated a positive effect on disability (Van Den Ende, Vliet Vlieland et al. 2000). Van den Ende et al. suggested that this discrepancy might be explained by the low sensitivity of questionnaires commonly used in RA to measure difficulty in ADLs, particularly in patients with mild disability, i.e. those usually recruited for physical training studies. Our results support this conclusion as we measured a substantial decrease in disability when using the advanced ADLs scale (designed by Pincus and colleagues to reduce the floor effects of other HAQ questionnaires when administered to RA patients with mild disability), but not when using the modified HAQ. Similarly, De Jong et al. (de Jong, Munneke et al. 2003) observed a significant improvement in functional ability in response to a long term intensive physical training program when disability was assessed with the McMaster Toronto Arthritis Patient Preference Disability Questionnaire (which covers a broader number of activities and measures changes in only those that are important to the individual patient) but not with the standard HAQ.

In addition to the significant anabolic response and improvements in both objective and subjective measures of physical function and disability, we measured a moderate loss of fat mass in the trunk in response to PRT. Although this effect was only a trend, its size and potential impact on outcome in RA patients are of great interest. In fact, RA predisposes to central obesity even in patients not taking corticosteroids (Westhovens, Nijs et al. 1997) and its combination with insulin resistance, blood lipids abnormalities,

hypertension and systemic inflammation (the so-called Metabolic Syndrome) greatly increases the probability of developing cardiovascular disease (Ridker, Buring et al. 2003) which is common in RA patients (Watson, Rhodes et al. 2003). Therefore, PRT might reduce mortality not only by augmenting the body protein disposable in situations of acute stress to provide vital amino acids to the liver (Rosenblatt, Clowes et al. 1983), but also by reducing cardiovascular risk in RA patients.

Importantly, we have also demonstrated that our PRT program, despite its relatively high intensity and volume, is feasible and does not exacerbate disease activity in patients with well-controlled RA as previously feared by Rall et al. who did not train their subjects more frequently to avoid potentially excessive articular stress (Jawed, Gaffney et al. 1997). This is not surprising as many other studies on PRT found no deleterious effects on pain and other signs and symptoms of RA in patients with low-to-moderate disease activity (Hakkinen 2004). The safety of intensive physical training, including frequent resistance exercises, has also been demonstrated in RA patients hospitalised because of very active disease (van den Ende, Breedveld et al. 2000). However, the recent results of de Jong et al. (de Jong, Munneke et al. 2003) suggest that the safety of PRT should be considered over a much longer period time and by taking additional measures of radiological progression, particularly in RA patients with considerable damage to the large joints. Nevertheless, unlike some of the activities included in de Jong and colleagues' physical training program, PRT is a low impact activity and does not accelerate damage of the small joints in patients with recent-onset rheumatoid or psoriatic arthritis trained for six months (Hakkinen, Hakkinen et al. 1994).

Although we matched the subjects by age and sex and demonstrated non significant differences between groups at baseline in all known factors likely to affect outcome, the study's main limitation remains the non-random assignment to treatment.

Other limitations of this study are the low power for detection of a significant change in trunk fat mass, the assessor being aware of subjects' allocation, the simple measures of knee extensors strength, fatigue and psychological status, and the absence of a "placebo" treatment. Although the mildly disabled RA patients with moderate disease activity included in our study are representative of the majority of patients with RA, our findings should not be generalised to patients more severely affected by the disease. Nevertheless, our study is the first to give empirical support to the prevalent opinion that PRT is the most effective treatment of cachexia in RA patients (Roubenoff 2003; Zinna and Yarasheski 2003). Pending confirmation of our results in a well-designed randomized, double-blind, placebo-controlled Phase III trial also including patients with severe disability and high disease activity, a similar PRT program should be included in the management of RA and its adverse metabolic consequences.

Table 1
Demographic, Anthropometric, Lifestyle, and Disease Characteristics of Rheumatoid Arthritis Patients Participating in the Study

Variable	Training group (n = 10)	Control group (n = 10)	P
Age, years	53 ± 13	54 ± 10	0.79
Height, m	1.67 ± 0.08	1.64 ± 0.08	0.21
BMI, kg/m^2	27.9 ± 4.6	29.1 ± 2.2	0.53
Energy intake, kcal/day	1908 ± 430	1981 ± 469	0.72
Protein intake, g/day	78.2 ± 23.4	81.8 ± 24.1	0.74
Activity at work, 1-4	1.6 ± 0.7	1.8 ± 0.6	0.44
Leisure-time activity, 1-4	2.1 ± 0.6	2.3 ± 0.5	0.44
Current smokers	2	4	0.63
Cigarettes per day	8.0 ± 9.9	6.0 ± 6.2	0.77
Alcohol measures per week	5.8 ± 4.6	4.6 ± 8.6	0.65
Sleep duration, hours	8.2 ± 1.0	6.3 ± 1.2	< 0.01
RF positive	6	8	0.63
Disease duration, years	8.9 ± 5.7	7.3 ± 5.3	0.56
Methotrexate[a]	5	5	1.00
Other DMARDs	6	9	0.63
NSAIDs	6	5	1.00
Corticosteroids	2	3	1.00
Dose, mg/day	6.2 ± 1.8	8.3 ± 5.8	0.67

Note. Data are presented as means ± *SD* or frequency. Differences between groups were tested by paired t tests or Fisher's exact probability tests as appropriate. BMI = body mass index; RF = rheumatoid factor; DMARDs = disease-modifying antirheumatic drugs; NSAIDs = nonsteroidal anti-inflammatory drugs.
[a]All subjects treated with methotrexate were also receiving folate supplementation.

Table 2
Effects of 12 Weeks of Progressive Resistance Training on Body Mass and Total Body Composition in Rheumatoid Arthritis Patients

Variable	Training group (n = 10)	Control group (n = 10)	P	η^2
Body mass, kg				
Pre	78.2 ± 14.6	79.0 ± 11.3	0.87	
Post	78.6 ± 13.0	78.8 ± 11.9		
Adjusted	79.0 ± 0.7	78.5 ± 0.7	0.59	0.02
Total lean mass[a], kg				
Pre	44.5 ± 9.4	43.8 ± 10.2	0.58	
Post	45.4 ± 8.9	43.5 ± 10.1		
Adjusted	45.0 ± 0.3	43.8 ± 0.3	< 0.01	0.38
Total BMC[a], kg				
Pre	2.58 ± 0.40	2.47 ± 0.52	0.38	
Post	2.59 ± 0.42	2.48 ± 0.53		
Adjusted	2.54 ± 0.01	2.53 ± 0.01	0.73	0.01
FFM, kg				
Pre	47.1 ± 9.7	46.3 ± 10.7	0.54	
Post	47.9 ± 9.2	45.9 ± 10.5		
Adjusted	47.6 ± 0.3	46.3 ± 0.3	< 0.01	0.39
Total fat mass[a], kg				
Pre	30.1 ± 9.9	31.6 ± 6.1	0.71	
Post	29.5 ± 9.6	31.7 ± 6.4		
Adjusted	30.2 ± 0.5	30.9 ± 0.5	0.35	0.05

Table 2 cont.

Variable	Training group (n = 10)	Control group (n = 10)	P	η^2
Percent body fat, %				
Pre	38.6 ± 9.9	40.9 ± 7.8	0.45	
Post	37.7 ± 10.1	41.1 ± 7.6		
Adjusted	38.9 ± 0.4	40.0 ± 0.4	0.05	0.21
ECW, L				
Pre	15.5 ± 2.2	15.7 ± 2.9	0.76	
Post	15.5 ± 1.9	15.7 ± 2.8		
Adjusted	15.6 ± 0.1	15.6 ± 0.1	0.89	0.00
ICW, L				
Pre	18.6 ± 3.3	19.3 ± 4.5	0.48	
Post	18.8 ± 3.5	19.0 ± 4.8		
Adjusted	19.1 ± 0.3	18.7 ± 0.3	0.32	0.06
TBW, L				
Pre	34.1 ± 5.1	35.0 ± 7.2	0.56	
Post	34.3 ± 5.2	34.7 ± 7.5		
Adjusted	34.8 ± 0.3	34.2 ± 0.3	0.26	0.07
ECW:ICW				
Pre	0.84 ± 0.11	0.83 ± 0.08	0.63	
Post	0.84 ± 0.09	0.84 ± 0.08		
Adjusted	0.83 ± 0.02	0.85 ± 0.02	0.55	0.02

Table 2 cont.

Variable	Training group (n = 10)	Control group (n = 10)	P	η^2
TBW:FFM				
Pre	0.73 ± 0.07	0.76 ± 0.05	0.23	
Post	0.72 ± 0.06	0.76 ± 0.03		
Adjusted	0.73 ± 0.01	0.75 ± 0.01	0.12	0.14
Total BMC:TBW				
Pre	0.076 ± 0.012	0.071 ± 0.008	0.13	
Post	0.076 ± 0.011	0.072 ± 0.007		
Adjusted	0.074 ± 0.001	0.074 ± 0.001	0.63	0.01
Total body protein, kg				
Pre	9.79 ± 5.29	8.27 ± 3.61	0.20	
Post	10.42 ± 4.53	8.18 ± 3.12		
Adjusted	9.78 ± 0.31	8.81 ± 0.31	0.04	0.22

Note. Pretest and posttest scores are presented as means ± *SD*. Adjusted scores (posttest scores adjusted for pretest scores) are presented as means ± *SEM*. Differences between groups at baseline were tested by paired t tests. Differences between groups in the adjusted scores were tested by ANCOVA. BMC = bone mineral content; FFM = fat-free mass; ECW = extracellular water; ICW = intracellular water; TBW = total body water.
[a]Including head.

Table 3
Effect of 12 Weeks of Progressive Resistance Training on Regional Body Composition in Rheumatoid Arthritis Patients

Variable	Training group (n = 10)	Control group (n = 10)	P	η^2
Arms lean mass, kg				
Pre	4.26 ± 1.51	4.24 ± 1.77	0.90	
Post	4.52 ± 1.59	4.22 ± 1.72		
Adjusted	4.51 ± 0.06	4.23 ± 0.06	< 0.01	0.39
Trunk lean mass, kg				
Pre	23.3 ± 4.7	23.3 ± 4.7	0.99	
Post	23.2 ± 4.0	22.8 ± 4.7		
Adjusted	23.2 ± 0.2	22.8 ± 0.2	0.26	0.08
Legs lean mass, kg				
Pre	13.3 ± 3.2	12.9 ± 3.4	0.48	
Post	14.1 ± 3.1	12.9 ± 3.3		
Adjusted	13.9 ± 0.1	13.1 ± 0.1	< 0.01	0.76
Arms fat mass, kg				
Pre	3.75 ± 1.11	3.85 ± 0.99	0.85	
Post	3.99 ± 1.34	3.89 ± 1.04		
Adjusted	4.04 ± 0.14	3.84 ± 0.14	0.32	0.06
Trunk fat mass, kg				
Pre	14.7 ± 6.5	16.9 ± 4.4	0.49	
Post	14.0 ± 5.9	16.8 ± 4.7		
Adjusted	15.0 ± 0.3	15.8 ± 0.3	0.08	0.17

Table 3 cont.

Variable	Training group (n = 10)	Control group (n = 10)	P	η^2
Legs fat mass, kg				
Pre	10.8 ± 3.5	10.2 ± 1.9	0.60	
Post	10.7 ± 3.6	10.2 ± 1.7		
Adjusted	10.4 ± 0.2	10.5 ± 0.2	0.63	0.01
Arms BMC, g				
Pre	345 ± 82	344 ± 107	0.96	
Post	354 ± 90	339 ± 102		
Adjusted	353 ± 5	339 ± 5	0.07	0.18
Trunk BMC, g				
Pre	682 ± 91	687 ± 161	0.89	
Post	682 ± 99	679 ± 169		
Adjusted	685 ± 8	676 ± 8	0.46	0.03
Legs BMC, g				
Pre	1064 ± 227	996 ± 223	0.26	
Post	1066 ± 231	1007 ± 226		
Adjusted	1031 ± 6	1041 ± 6	0.24	0.08

Note. Pretest and posttest scores are presented as means ± *SD*. Adjusted scores (posttest scores adjusted for pretest scores) are presented as means ± *SEM*. Differences between groups at baseline were tested by paired *t* tests. Differences between groups in the adjusted scores were tested by ANCOVA. BMC = bone mineral content.

Table 4
Effects of 12 Weeks of Progressive Resistance Training on Muscle Strength, Disability, Psychological Status, and Disease Activity in Rheumatoid Arthritis Patients

Variable	Training group (n = 10)	Control group (n = 10)	P	η^2
Hand-grip strength, N				
Pre	187 ± 108	223 ± 133	0.42	
Post	224 ± 115	204 ± 135		
Adjusted	241 ± 17	187 ± 17	0.04	0.22
Elbow flexors strength, N				
Pre	171 ± 43	196 ± 87	0.30	
Post	222 ± 63	194 ± 97		
Adjusted	235 ± 14	181 ± 14	0.02	0.30
Knee extensors strength, N				
Pre	297 ± 85	312 ± 154	0.75	
Post	347 ± 46	319 ± 147		
Adjusted	352 ± 15	313 ± 15	0.07	0.18
SST-30, repetitions				
Pre	11.3 ± 2.8	12.2 ± 3.2	0.45	
Post	15.7 ± 3.3	12.8 ± 2.7		
Adjusted	16.1 ± 0.6	12.4 ± 0.6	< 0.01	0.52
Modified HAQ, 1-4				
Pre	1.3 ± 0.3	1.5 ± 0.6	0.32	
Post	1.3 ± 0.2	1.3 ± 0.4		
Adjusted	1.2 ± 0.1	1.2 ± 0.1	0.90	0.00

Table 4 cont.

Variable	Training group (n = 10)	Control group (n = 10)	P	η^2
Advanced ADLs, 1-4				
Pre	2.1 ± 0.4	2.4 ± 0.6	0.09	
Post	1.8 ± 0.3	2.3 ± 0.6		
Adjusted	1.9 ± 0.1	2.2 ± 0.1	0.01	0.35
Psychological status, 1-4				
Pre	1.4 ± 0.3	1.6 ± 0.6	0.20	
Post	1.4 ± 0.3	1.5 ± 0.3		
Adjusted	1.4 ± 0.1	1.5 ± 0.1	0.54	0.02
RADAI, 0-10				
Pre	2.5 ± 1.1	2.8 ± 1.7	0.72	
Post	2.0 ± 1.4	2.3 ± 1.5		
Adjusted	2.1 ± 0.3	2.2 ± 0.3	0.89	0.00
Fatigue, 0-10				
Pre	4.4 ± 1.8	4.9 ± 3.2	0.60	
Post	3.1 ± 2.1	4.4 ± 3.3		
Adjusted	3.2 ± 0.8	4.3 ± 0.8	0.36	0.05
ESR, mm/hour				
Pre	18.8 ± 16.6	22.5 ± 17.6	0.64	
Post	16.7 ± 8.9	20.9 ± 18.1		
Change	-2.1 ± 11.0	-1.6 ± 7.0	0.91	0.00

Note. Pretest and posttest scores are presented as means ± *SD*. Adjusted scores (posttest scores adjusted for pretest scores) are presented as means ± *SEM*. Differences between groups at baseline were tested by paired t tests. Differences between groups in the adjusted scores were tested by ANCOVA. The difference between groups in erythrocyte sedimentation rate (ESR) change score (posttest score – pretest score) was tested by ANOVA. SST-30 = 30-s maximal sit-to-stand test; HAQ = health assessment

questionnaire; ADLs = activities of daily living; RADAI = rheumatoid arthritis disease activity index.

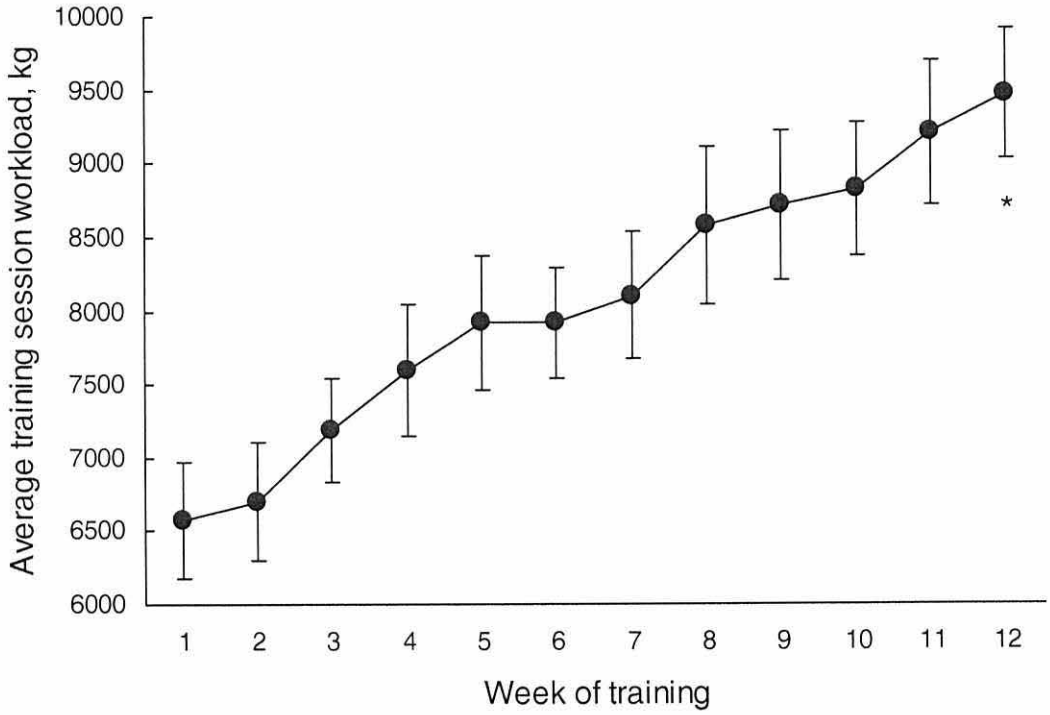

Figure 2. Weekly increase in average training session workload in ten RA patients participating in 12 weeks of progressive resistance training. Data are presented as means ± SEM.
* Main effect of time by within subjects ANOVA, $P < 0.01$.

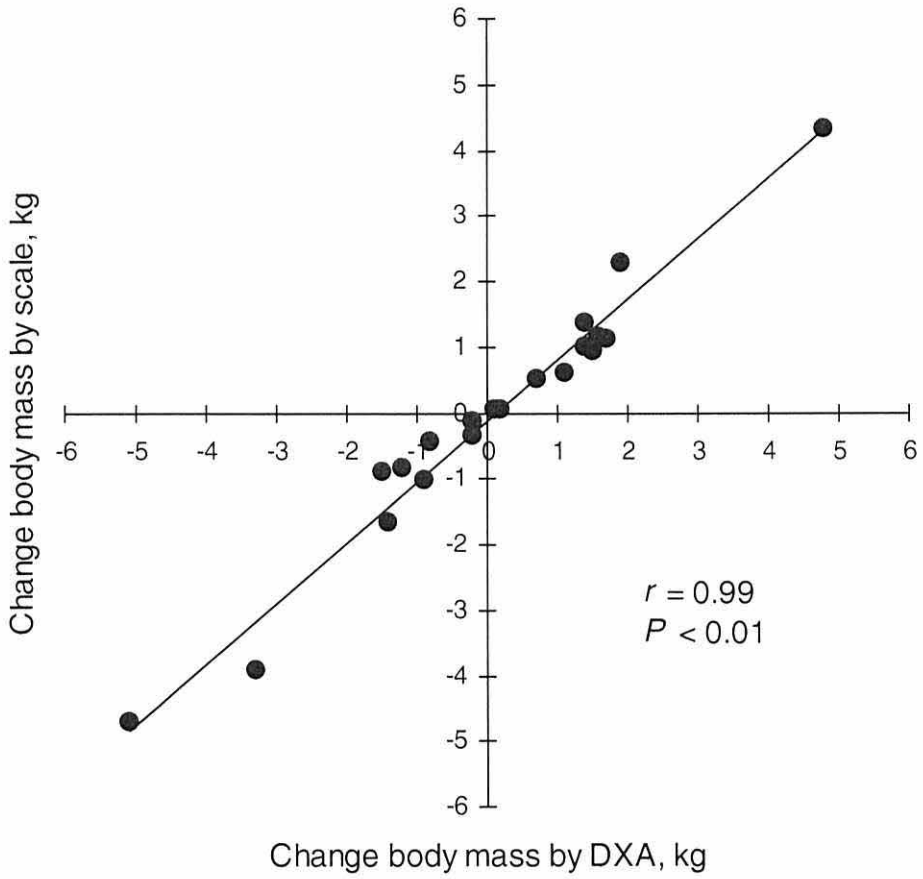

Figure 3. Association between change (posttest score – pretest score) in body mass by scale and by dual energy X-ray absorptiometry (DXA) in rheumatoid arthritis patients participating in the study.

CHAPTER 4

DIETARY SUPPLEMENTATION WITH β-HYDROXY-β-METHYLBUTYRATE, GLUTAMINE AND ARGININE IN RHEUMATOID ARTHRITIS PATIENTS: A RANDOMISED CONTROLLED TRIAL

A PAPER ACCEPTED BY CLINICAL NUTRITION

SAMUELE MARCORA, ANDREW LEMMEY, PETER MADDISON

INTRODUCTION

Rheumatoid arthritis (RA) is a common, often progressive, chronic inflammatory joint disease leading to erosion of articular cartilage and bone, disability and shortened life span (Hulsmans, Jacobs et al. 2000; Minaur, Jacoby et al. 2004). However, RA is also a truly systemic disease with several extra-articular features including cytokine-driven alterations in protein and energy metabolism, negative nitrogen balance, muscle wasting and, in some patients, anorexia and weight loss (Roubenoff, Roubenoff et al. 1990; Roubenoff, Roubenoff et al. 1994; Rall, Rosen et al. 1996; Munro and Capell 1997). In most RA patients, however, wasting is not apparent as energy intake is normal and the decrease in skeletal muscle mass is masked by an increase in fat mass consequent to reduced physical activity and resultant low levels of total energy expenditure (Roubenoff, Walsmith et al. 2002). This form of secondary malnutrition, named rheumatoid cachexia, affects more than 50% of RA patients (Munro and Capell 1997) and is thought to contribute to the poor outcome of this disease (Walsmith and Roubenoff 2002).

Although the efficacy and safety of dietary therapy for the treatment of protein-energy malnutrition has been investigated in cancer (Fearon, Von Meyenfeldt et al. 2003) and many other chronic non-malignant conditions, there is a striking lack of published studies on this topic in RA patients (Akner and Cederholm 2001). Most studies on the nutritional management of RA have focused on special diets and/or dietary supplements to reduce joint symptoms (Rennie, Hughes et al. 2003). The only exception being the pilot study of Willer et al. (Willer, Stucki et al. 2000) which investigated the effect of short term creatine supplementation on muscle weakness. Importantly, some dietary manipulations popular among RA patients such as fasting and elimination diets worsen malnutrition despite a reduction in disease activity (Kjeldsen-Kragh 1999). Clearly, more

research is necessary before comprehensive nutritional recommendations for RA patients can be made.

In this paper we report the results of a randomised controlled trial of dietary supplementation with β-hydroxy-β-methylbutyrate, glutamine and arginine (HMB/GLN/ARG) for the treatment of rheumatoid cachexia. β-hydroxy-β-methylbutyrate, a metabolite of leucine, is safe for human consumption (Nissen, Sharp et al. 2000) and it is one of the few dietary supplements proven to have anabolic and functional effects in humans undergoing resistance training (Nissen and Sharp 2003). Interestingly, there is evidence that HMB reduces muscle proteolysis in type II fibres (Ostaszewksi, Kostiuk et al. 2000), the fibre type most affected by catabolism in RA patients (Fiori, Andreola et al. 1983). The important role of glutamine in skeletal muscle protein turnover is well established (Rennie, Ahmed et al. 1996) and several studies have demonstrated that increasing glutamine intake is beneficial in catabolic patients (Boelens, Nijveldt et al. 2001). Dietary supplementation with arginine has also been shown to safely induce positive nitrogen balance in both healthy elderly humans (Hurson, Regan et al. 1995) and surgical patients (Daly, Reynolds et al. 1988), possibly by increasing muscle protein synthesis (Cui, Iwasa et al. 1999) and inhibiting muscle proteasome activity (Hamel, Upward et al. 2003). These amino acids have been combined in a dietary supplement (HMB/GLN/ARG) specifically designed for patients with catabolic diseases and tested in wasted HIV (Clark, Feleke et al. 2000) and cancer (May, Barber et al. 2002) patients. In both these randomised, double-blind, placebo-controlled trials, HMB/GLN/ARG proved to be safe and superior to placebo in reversing weight loss and increasing fat-free mass (FFM). Therefore, the primary aim of this study was to investigate whether HMB/GLN/ARG is also effective in improving nutritional status (i.e., body composition) in RA patients. As suggested by Akner and Cederholm (Akner and

Cederholm 2001), we also investigated its effects on clinically relevant outcomes such as muscle strength and disability (Stucki, Bruhlmann et al. 1998). As HMB, glutamine, and arginine have been shown to affect the immune system (Suchner, Kuhn et al. 2000), disease activity was monitored in order to establish the specific safety of this dietary supplement in RA patients.

SUBJECTS AND METHODS

Study Population

Adult patients fulfilling the American Rheumatism Association 1987 revised criteria for the diagnosis of RA (Arnett, Edworthy et al. 1988), and with stable disease activity (defined as no changes in medications in the previous three months), were recruited from outpatient clinics in the Department of Rheumatology of Gwynedd Hospital. Patients were excluded if they had any condition preventing safe participation in the required muscle strength tests (i.e., uncontrolled hypertension or musculoskeletal injury) or for which an increase in nitrogen intake is contraindicated (i.e., liver or kidney dysfunction). Additional exclusion criteria were cognitive impairment, the presence of any other cachectic disease (i.e., cancer or chronic heart failure), taking drugs or nutritional supplements known to affect skeletal muscle mass, and participation in a regular and intense physical training program. All volunteers gave their written informed consent to participate before commencing the study.

A sample size of 30 patients in each group (total 60 patients) was calculated assuming a large effect size (Cohen's $d > 0.8$), an alpha level equals to 0.05 and a power of 0.80. After the first 30 patients, an interim analysis was conducted and we decided to recruit only 10 further patients.

Study Design and Ethics

This was a randomised, two-group, parallel, pretest-posttest, placebo-controlled, double-blind trial conducted between October 2000 and September 2003 and approved by the North Wales Health Authority Research Ethics Committee. After baseline assessment (pretest), participants were randomised to receive HMB/GLN/ARG or an isonitrogenous and isocaloric mixture of other, non essential amino acids (placebo) for 12 weeks. Treatment was assigned to participants by a technician of the University of Wales-Bangor's School of Sport, Health and Exercise Sciences (SSHES) based on computer-generated permuted blocks of 10 with a one-to-one allocation ratio. This technician concealed allocation until the interim analysis (after the first three blocks of patients completed the study) and again until the final analysis (after the fourth block), and was not involved in any other aspect of the study. Both investigators and participants were blinded to treatment assignment until after follow-up (posttest) which occurred on the Monday or Tuesday of Week 13.

Dietary Supplementation

The HMB/GLN/ARG group received 3 g HMB (calcium salt), 14 g L-arginine and 14 g L-glutamine per day. Patients allocated to placebo received daily a mixture of L-alanine (11 g), L-glutamic acid (1.75 g), L-glycine (6.10 g) and L-serine (4.22 g). Both formulas provided the same amount of nitrogen (7.19 g/day) and calories (180 kcal/day) and were designed and provided by Metabolic Technologies Inc., Ames, Iowa. The HMB/GLN/ARG and placebo mixtures were identically presented as crystalline powders packed in plain white foil sachets. Subjects were instructed to mix the entire content of the packet of either HMB/GLN/ARG or placebo with 240 ml of water to produce drinks with an indistinguishable tangy orange flavour. This drink was taken twice a day, in the

morning immediately after breakfast and in the evening immediately after dinner. Compliance with treatment was ascertained monthly by asking each participant to maintain a log book and to return unpacked sachets. All participants were instructed to maintain their habitual physical activity and dietary habits throughout the study.

Outcome Measures

Outcome measures were taken at baseline and follow-up by the same investigator (SM) in the Bone Densitometry Laboratory in the School of Sport, Health and Exercise Sciences, University of Wales-Bangor, at approximately at the same time of the day. Subjects presented fasted and were asked to void and remove all metallic objects. Subjects were instructed beforehand to avoid strenuous exercise in the 24 hours preceding testing and were questioned as to whether they had orthopaedic metal and silicone implants. During testing subjects were allowed to wear only socks, shorts, underwear (no bra) and a t-shirt. With respect to efficacy, the primary outcome measure was arms and legs (i.e., appendicular) lean mass by DXA, a proxy measure of total body skeletal muscle mass (Kim, Wang et al. 2002). The primary outcome measure for safety was the disease activity index (RADAI), a patient questionnaire which is sensitive to flare-ups of disease activity in RA patients (Fransen, Hauselmann et al. 2001).

Body Mass and Composition

Body mass was measured to the nearest 0.1 kg using a calibrated balance scale (Seca, Hamburg, Germany). Total and regional (left and right arm, left and right leg, trunk, head) body composition was assessed by DXA using a pencil-beam scanner (QDR1500, Hologic, Bedford, Massachusetts) which calculates the masses (g) of three different compartments: bone mineral content (BMC), fat mass and lean mass. The sum of total

BMC and total lean mass corresponds to fat-free mass (FFM). Percent body fat was calculated as (total fat mass/total body mass by DXA) · 100. The procedures recommended by the manufacturer for whole-body examination (subject positioning, scanning and analysis with software version V5.72) were followed and the quality control procedure was performed daily.

Immediately after the DXA scan, intracellular (ICW) and extracellular (ECW) water volumes (L) were estimated using bioelectrical impedance spectroscopy (BIS) (Hydra 4200, Xitron Technologies, San Diego, California), with total body water (TBW) being the sum of ICW and ECW. Bioelectrical impedance measurements were taken on the left side of the body, with the use of disposable electrodes and in accordance with a standard wrist-to-ankle protocol (Van Loan, Withers et al. 1993). At the time subjects were measured, they had been supine for approximately 20 min. The quality control procedure recommended by the manufacturer was performed before each measurement and the proximity of the DXA scanner had no effect on the validity and reliability of BIS (unpublished observations).

By combining DXA and BIS data, we were able to calculate FFM hydration (TBW/FFM) and total body protein, the latter estimated as total lean mass – TBW – 0.2305 · total BMC according to the model proposed by Fuller et al. (Fuller, Wells et al. 2001). For each of these calculations, TBW volume was converted into mass by assuming a water density of 0.99336 kg/L at a normal body temperature of 37°C.

Muscle Strength

After body composition assessment, subjects performed callisthenics for 5 min under the direction and supervision of one of the authors (SM). The following generic muscle strength tests were then performed. Maximal voluntary hand-grip strength was measured

using a Grip-A dynamometer (Takey Kiki Kogyo, Japan). For this test, subjects were asked to stand erect holding the dynamometer parallel to their side, dial facing away from the body and to squeeze the hand-grip without moving the arm. Maximal voluntary strength of the knee extensors and elbow flexors was measured using a CSD300 hand-held dynamometer (Chatillon-Ametek, Largo, Florida). For the measurement of knee extensors strength, subjects were sitting on a medical table with hips and knees flexed 90° and arms across the chest. The curved push attachment of the dynamometer was positioned over the tibia just proximal to the two malleoli and subjects were instructed to attempt to straighten the leg. For the measurement of elbow flexors strength, subjects were lying on a medical table with shoulder neutral, elbow flexed 90° and forearm neutral. The curved push attachment of the dynamometer was positioned just below the styloid process of the radius and subjects were instructed to attempt to flex the arm. Each of these static strength tests observed the following protocol: after two submaximal warm-up and familiarisation trials (50% and 75% of maximum effort), subjects were asked to perform three trials in which they exerted force maximally for approximately 5 s each time. Between all five trials (i.e., submaximal and maximal), 1 min rest was provided. Peak force produced during each of the three maximal trials was recorded in newtons and the best score noted. Both the right and left side of the body were tested and the average of the two best scores used for statistical analysis. The force exerted by the tester (male, body mass 100 kg, height 1.79 m) was sufficient to fix the hand-held dynamometer against the forces produced by all subjects. None of the subjects approached the upper limit of the dynamometers, and their accuracy was verified periodically over the course of the study by vertically loading the dynamometers with certified calibration weights. Lower body functional strength was assessed with the 30-s maximal sit-to-stand test (SST-30) validated by Jones et al. in older adults (Jones, Rikli et

al. 1999). After a practice trial, subjects were asked to sit-to-stand from a fixed chair (seat height 43.2 cm) as many times as possible in 30 s while keeping their arms folded across the chest. As suggested by McNair et al. (McNair, Depledge et al. 1996), subjects were verbally encouraged during testing in order to attain maximum performance.

Disability and Psychological Status

Disability (i.e., the degree of difficulty experienced by the patient when performing activities of daily living [ADLs]), was quantified by the modified health assessment questionnaire (HAQ) together with the advanced ADLs items included in the multidimensional HAQ developed by Pincus et al (Pincus, Swearingen et al. 1999). Problems with sleep, stress, anxiety and depression (i.e., psychological status) were also assessed with this questionnaire. All items were scored on a scale of 1 (*without any difficulty*), 2 (*with some difficulty*), 3 (*with much difficulty*), and 4 (*unable to do*).

Disease Activity

Disease activity was assessed using the RADAI (Stucki, Liang et al. 1995). With the exception of fatigue, this patient questionnaire quantifies the most apparent signs and symptoms of RA: (a) global disease activity over the past six months, (b) current disease activity in terms of swollen and tender joints, (c) arthritis pain, (d) duration of morning stiffness, and (e) amount of pain in several joint areas. These five items are then combined to provide a single index of disease activity on a 0 to 10 scale where higher scores indicate more active disease. Fatigue over the past week was measured with a numerical rating scale ranging from 0 (*fatigue is no problem*) to 10 (*fatigue is a major problem*). To provide an objective measure of disease activity, the erythrocyte

sedimentation rate (ESR) was also assessed using the Westergren's method in the Department of Biochemistry and Haematology of Gwynedd Hospital.

Adverse Events

An adverse events questionnaire examining common signs and symptoms related to the major organ systems (Nissen, Sharp et al. 2000) was administered during the baseline assessment and every four weeks thereafter. Patients were also questioned monthly about any other side effects. Liver function tests (bilirubin, alkaline phosphatase [ALP], aspartate aminotransferase [AST], alanine aminotransferase [ALT]) were performed in the Department of Biochemistry and Haematology of Gwynedd Hospital using standard techniques.

Other Measures

Height was measured to the nearest 0.5 cm using a wall-mounted stadiometer (Body Care, Warwickshire, UK). Body mass index (BMI) was calculated as body mass (kg)/height squared (m^2). All anthropometric measures were taken following standard procedures by the same trained investigator (SM). The degree of somatic protein depletion was calculated as the percentage of actual total body skeletal muscle mass estimated from appendicular (arms and legs) lean mass, age, and sex (Kim, Wang et al. 2002) compared to normal total body skeletal muscle mass estimated from body mass, height, age, sex, and ethnicity (Lee, Wang et al. 2000). Habitual physical activity both at work and during leisure time was ranked from mainly sedentary (score = 1) to heavy and regular (score = 4) using the questionnaire proposed by Saltin et al. (Saltin and Grimby 1968). Typical dietary intake was estimated using a semiquantitative food frequency questionnaire (Willett, Sampson et al. 1985). Other required information (age, disease

duration, current medications, etc.) was collected at baseline by a structured interview and review of medical records. At follow-up, all subjects were questioned if changes in habitual physical activity, diet, medications, and other aspects of their lifestyle had occurred during the study, and if they would choose to continue taking the allocated dietary supplement (acceptability).

Statistical Analysis

Unless otherwise noted, all data are presented as means ± *SD*. A two-way mixed analysis of variance (ANOVA) was used on each continuous outcome measure (dependent variable). The independent variables included one between subjects factor, treatment, with two levels (HMB/GLN/ARG and placebo), and one within subjects factor, time, with two levels (pretest and posttest). We used these ANOVAs to test the null hypothesis of no difference in change over time between the HMB/GLN/ARG and placebo group (treatment x time interaction), and the null hypothesis of no change over time in response to nitrogen and calorie supplementation (main effect for time). Effect size for both the main effect for time and the treatment x time interaction was calculated as eta squared (η^2), a measure of strength of relationship between the independent and dependent variable. Thresholds for small, moderate, large and very large effects were set at 0.01, 0.08, 0.26 and 0.50 respectively. The adverse events questionnaires were analysed as categorical data and the difference between treatment groups was tested using the Cochran-Mantel-Haenszel statistic. Pearson product-moment correlation coefficient was used to express and test the significance of the relationships between measures of interest (body mass by scale with body mass by DXA; changes in arms and legs lean mass with changes in muscle strength and disability) after pooling the data of both groups. Because data were highly skewed, the null hypothesis of no difference between treatment groups

in compliance was tested with a Mann-Whitney U test. The results of the posttest interview about acceptability and changes in habitual physical activity, diet and medications were analysed with multiple Chi-Square tests. Significance was set at 0.05 (two-tailed) for all analyses, which were conducted on all participants who completed follow-up in an intention-to-treat manner. Data were analysed using the Statistical Package for the Social Sciences Version 11.

RESULTS

Retention, Subjects Characteristics and Compliance

Fifty-nine RA patients were recruited and screened. Of these patients, 11 refused to participate and eight were excluded for various reasons. The remaining 40 patients were allocated to treatment with either HMB/GLN/ARG (12 females and 8 males) or placebo (13 females and 7 males). Four subjects were lost to follow-up because of relocation to a different area (one female in the HMB/GLN/ARG group), sickness (one female in the HMB/GLN/ARG group), or because they withdraw themselves from the study after suffering gastrointestinal discomfort from the supplement (two females in the placebo group). Flow of participants is represented in Figure 4. Subjects lost to follow-up were not included in the analyses so the results presented hereafter refer only to the subjects who completed the study. With the exception of one subject who stopped taking the placebo formula after one week of treatment as she developed a rash she attributed to intervention, there were no serious protocol violations among subjects who completed the study. However, 39% of subjects in the HMB/GLN/ARG group and 33% of subjects in the placebo group reported an increase in habitual physical activity over the intervention period, the difference between groups being not significant ($P = 0.73$). All subjects

declared that no other substantial change in diet, regular medications and lifestyle occurred during the study.

The mean age, height and BMI of the HMB/GLN/ARG group was 54 ± 10 years, 1.64 ± 0.10 m, 25.2 ± 4.1 kg/m^2 respectively, while the placebo group was 57 ± 8 years old, 1.66 ± 0.07 m tall and had a BMI of 27.2 ± 4.8 kg/m^2. Habitual daily consumption of energy and protein were 1740 ± 398 kcal and 70 ± 16 g, and 1934 ± 325 kcal and 78 ± 14 g, for the HMB/GLN/ARG and placebo groups, respectively. Despite a mean BMI in the overweight category and normal protein and energy intakes, all patients presented with somatic protein depletion. Actual total body skeletal muscle mass was, on average, 75% (range 61-89%) of normal muscle mass in the HMB/GLN/ARG group and 75% (range 57-89%) in the placebo group. All subjects were receiving standard medical treatment, a combination of disease-modifying anti-rheumatic drugs, non-steroideal anti-inflammatory drugs and joint mobility exercises. Three patients in the HMB/GLN/ARG group (daily dose 6.3 ± 1.3 mg) and four in the placebo group (10.1 ± 3.4 mg/day) were also treated with oral corticosteroids. Four patients in each group regularly consumed cod liver oil supplements. Patients on oral corticosteroids and cod liver oil supplements were included in the study as there is no evidence that these agents, at the relatively low dosages taken by our patients, affect FFM (Kennedy, Boddy et al. 1979; Westhovens, Nijs et al. 1997; Fearon, Von Meyenfeldt et al. 2003). None of the subjects had silicone implants or orthopaedic metal. All subjects were Caucasian and ambulant with low-to-moderate levels of total (work plus leisure) habitual physical activity (HMB/GLN/ARG group: 3.7 ± 0.8; Placebo group: 3.8 ± 1.1).

There was no significant difference in compliance between the subjects who received HMB/GLN/ARG (median 160 drinks, range 100-168) and placebo (median 155 drinks, range 14-168), $P = 0.31$. However, a significantly higher proportion of subjects in

the HMB/GLN/ARG group (89%) considered continuing nutritional supplementation after the study compared to subjects in the placebo group (50%), $P = 0.01$.

Efficacy

Body mass and composition at baseline and follow-up are shown in Table 5 and Figure 5. There were no statistically significant group x time interactions in body mass or any of the measured body compartments (Table 5 and Figure 5). However, there were some moderate to large differences between pretest and posttest in both groups combined (main effect of time). Arms ($P < 0.01$, $\eta^2 = 0.28$) and legs ($P < 0.01$, $\eta^2 = 0.22$) lean mass, total lean mass ($P < 0.01$, $\eta^2 = 0.28$), FFM ($P < 0.01$, $\eta^2 = 0.28$) and total body protein (Figure 5) all increased significantly. Changes in total fat mass ($P = 0.98$, $\eta^2 = 0.00$), trunk fat mass ($P = 0.17$, $\eta^2 = 0.05$), % body fat ($P = 0.12$, $\eta^2 = 0.07$), total BMC ($P = 0.76$, $\eta^2 = 0.00$), ECW ($P = 0.85$, $\eta^2 = 0.00$), ICW ($P = 0.88$, $\eta^2 = 0.00$), TBW ($P = 0.99$, $\eta^2 = 0.00$) and FFM hydration ($P = 0.09$, $\eta^2 = 0.08$) were absent or small, and not significant. The moderate increase in body mass approached statistical significance ($P = 0.06$, $\eta^2 = 0.10$).

Muscle strength at baseline and follow-up is shown in Table 6. There were no statistically significant group x time interactions in any of the measures of muscle strength (Table 6), and no significant main effects for time in hand-grip strength ($P = 0.77$, $\eta^2 = 0.00$) and elbow flexors strength ($P = 0.87$, $\eta^2 = 0.00$). However, there was a moderate and significant increase in knee extensors strength ($P = 0.03$, $\eta^2 = 0.13$) and a very large and significant increase in lower body functional strength ($P < 0.01$, $\eta^2 = 0.63$) over time in both groups combined. These objective changes in muscle strength were not associated with improvements in self-reported physical function (Table 6). There were no statistically significant group x time interactions in any of the two disability scales used,

nor significant main effects for time in modified HAQ ($P = 0.80$, $\eta^2 = 0.00$) or advanced ADLs ($P = 0.47$, $\eta^2 = 0.02$).

There was a significant correlation between change in legs lean mass and change in knee extensors strength ($r = 0.45$, $P < 0.01$) but not between change in legs lean mass and change in lower body functional strength ($r = 0.12$, $P = 0.48$). Change in body mass measured by scale was highly correlated to change in body mass measured by DXA as the sum of total lean mass, fat mass and BMC (Figure 6), thus confirming the internal validity of our measurements.

Safety

Psychological status and parameters of disease activity and liver function at baseline and follow-up are shown in Table 6. There were no statistically significant group x time interactions in any of these measures (Table 6), and no significant main effects for time in psycholological status ($P = 0.56$, $\eta^2 = 0.01$), RADAI ($P = 0.69$, $\eta^2 = 0.00$), fatigue ($P = 0.47$, $\eta^2 = 0.02$), ESR ($P = 0.76$, $\eta^2 = 0.00$), bilirubin ($P = 0.13$, $\eta^2 = 0.07$), ALP ($P = 0.99$, $\eta^2 = 0.00$), AST ($P = 0.72$, $\eta^2 = 0.00$), and ALT ($P = 0.56$, $\eta^2 = 0.01$). Similarly, quantitative analysis of the adverse events questionnaires administered at baseline and follow-up revealed no significant between group differences in the incidence of negative side effects (Table 7).

During the monthly interviews, with the exception of the rash which occurred in one subject in the placebo group, patients did not report any side effects requiring discontinuation of treatment. However, a significantly higher proportion of subjects in the placebo group (67%) complained of gastrointestinal discomfort (e.g., bloated stomach, fullness) after taking the supplement compared to the HMB/GLN/ARG group (28%), $P = 0.02$. Two subjects (one in each group) had a significant increase in disease activity (i.e.,

flare-up) during the intervention period which was resolved with catabolic, short-term, high dose corticosteroids therapy (Roubenoff, Roubenoff et al. 1990).

DISCUSSION

This is the first study to investigate the efficacy and safety of a nutritional intervention for the treatment of cachexia in RA patients. Similar to previous randomized controlled trials in wasted HIV and advanced cancer patients (Clark, Feleke et al. 2000; May, Barber et al. 2002), we found that oral supplementation with a mixture of HMB/GLN/ARG significantly increases FFM in RA patients. Contrary to our expectations, however, the mixture of alanine, glutamic acid, glycine and serine used as placebo had a similar effect. These anabolic responses were evident in the arms and legs, our main outcome measure for efficacy, suggesting that both HMB/GLN/ARG and the isonitrogenous and isocaloric placebo stimulated skeletal muscle growth in RA patients. The analysis of FFM subcompartments also revealed that both dietary supplements significantly increased total body protein, whereas total BMC and TBW remained unchanged. As changes in body composition reflect the "area under the curve" of the organism's metabolic state (Roubenoff 1997), this significant increase in total body protein suggests that both treatments can overturn the negative nitrogen balance characteristic of RA (Roubenoff, Roubenoff et al. 1990).

We did not anticipate the anabolic response to placebo treatment as May et al. (May, Barber et al. 2002) found a loss of FFM in the cachectic cancer patients given the same mixture of alanine, glutamic acid, glycine and serine used in our study. A possible explanation for this discrepancy with our results is that simply increasing nitrogen and calorie intake does not reverse catabolism in highly catabolic patients and, therefore, the significant increase in FFM in the cachectic cancer patients treated with HMB/GLN/ARG

is due to its pharmacological properties. This hypothesis is somewhat supported by the observation that our RA patients gained a lower amount of FFM (61 g/week) compared to the more severely wasted patients with advanced malignancy (280 g/week) (May, Barber et al. 2002) or HIV (319 g/week) (Clark, Feleke et al. 2000) treated with HMB/GLN/ARG. It is important to note, however, that the results of the randomised controlled trial of May and colleagues are weakened by the very high rate of loss to follow-up (> 20%) which raises serious questions regarding the validity of their study regardless of sophisticated statistical analysis (Schulz and Grimes 2002). Additionally, there is strong evidence that a simple increase in nitrogen and calorie intake is effective in reversing loss of FFM in wasted cancer patients indeed (Fearon, Von Meyenfeldt et al. 2003). As Clark et al. (Clark, Feleke et al. 2000) used an isocaloric maltodextrin placebo, which does not control for increased nitrogen intake, their results in wasted HIV patients do not prove the pharmacological effects of HMB/GLN/ARG.

For the time being, we believe that the most likely explanation for the anabolic responses we observed in RA patients in response to oral supplementation with both HMB/GLN/ARG and the mixture of alanine, glutamic acid, glycine and serine is the substantial increase in nitrogen intake (from the equivalent of 1.0 g of protein/kg of body mass/day to 1.6 g/kg/day). Several studies have demonstrated that, within certain limits, increasing nitrogen intake improves nitrogen balance in catabolic patients when adequate energy is provided (Hoffer 2003). From the food-frequency questionnaire and compliance to treatment, we estimated an overall average nitrogen intake of 255 mg/kg of body mass/day and a non-protein energy intake of 21 kcal/kg of body mass/day in our depleted patients during the intervention period. Based on the equation developed by Radrizzani and colleagues (Radrizzani, Iapichino et al. 1986), a daily nitrogen balance of 18.3 mg/kg of body mass/day is predicted from these two variables. This value fits well with the

actual daily nitrogen balance calculated from change in total body protein in our patients (19.1 mg/kg/day). As energy balance, estimated from changes in body composition (Elia, Stratton et al. 2003), was only slightly positive (38 kcal/day) during the intervention period, we believe that these calculations strongly support our argument that an increased nitrogen intake is the main determinant of the anabolic responses we observed in RA patients supplemented with the two different oral amino acids mixtures. As our study did not have an unsupplemented (i.e., normal nitrogen intake) control group, it is theoretically possible that body protein accretion in our RA patients was due to other factors such as history, maturation, or reactive effects of testing. However, we think this is unlikely as a longitudinal study demonstrated that, over a period of six months, total body protein does not change in RA patients treated with different anti-inflammatory drugs (Kennedy, Boddy et al. 1979).

Our findings that supplementing a balanced diet with oral mixtures of mainly non-essential amino acids induced a significant increase in both arms and legs lean mass (a proxy measure of total body skeletal muscle mass) in RA patients contrast with the results of Volpi et al. (Volpi, Kobayashi et al. 2003) who demonstrated that consuming an additional 22 g of non-essential amino acids does not augment the positive muscle protein balance brought about by oral administration of 18 g of essential amino acids in healthy elderly adults. This inconsistency may be because the mixture used by Volpi and colleagues was well balanced while our dietary supplements contained much higher doses of either HMB, glutamine, and arginine, or alanine, glutamic acid, glycine, and serine, and suggests another possible explanation for our findings: not only HMB/GLN/ARG but also some of the amino acids contained in the "placebo" formula have pharmacological properties. For example, glycine is an important regulator of cellular hydration state and volume, cellular protein metabolism, inflammation, and resistance to stress (Schliess and

Haussinger 2002; Zhong, Wheeler et al. 2003). Interestingly, glycine infusion reduces protein turnover and induces a net anabolic effect because of a greater decrease in proteolysis than protein synthesis in healthy humans (Hankard, Haymond et al. 1996). Similarly, alanine has been shown to have anticatabolic effects in endotoxemic rats (Holecek, Skopec et al. 2000). Although the same study also showed that infusion of glycine does not have any positive effect in catabolic rats (Holecek, Skopec et al. 2000), and enteral supplementation with either glutamine or alanine fails to alter muscle protein metabolism in critically ill patients (Gore and Wolfe 2003), doubts remain on the appropriateness of using a mixture containing high doses of alanine and glycine as an inert control for the pure effects of increased nitrogen and calorie intake.

Whatever the mechanism, oral supplementation with both mixtures of amino acids reversed cachexia in RA patients. This positive effect was corroborated by a significant improvement in some measures of muscle strength. Although it could be argued that the increase in habitual physical activity reported by a third of patients in both groups caused these improvements in muscle strength, none of these patients commenced regular and intense physical training during the intervention period and the nature of activities undertaken were of insufficient intensity to provoke the muscle hypertrophy we observed. Despite the significant improvement in some objective measures of physical function, a decrease in disability was not reported by our patients. A lack of effect on the physical functioning scale of the Short Form 36 has also been reported by May et al. (May, Barber et al. 2002) in wasted patients with advanced cancer treated with the same two mixtures. Therefore, at present there is no evidence for a positive effect of 12 to 24 weeks of dietary supplementation with these oral amino acids on subjective measures of physical function in cachectic patients. Longer term studies using more specific and sensitive questionnaires are necessary to assess the impact of dietary therapy of cachexia on this

important clinical outcome, as a discrepancy between objective and subjective measures of physical function have been reported in RA patients (O'Connor, Kortman et al. 1999).

Similar to previous studies in different patient populations (Clark, Feleke et al. 2000; May, Barber et al. 2002), the mixture of HMB/GLN/ARG has been relatively well tolerated by RA patients with two thirds of the reported gastrointestinal complaints concentrated in the placebo group. This is probably related to the higher osmolarity of the placebo mixture (1000 mosm/L) compared to HMB/GLN/ARG (400 mosm/L), and might explain the higher acceptability of the latter. Both mixtures of oral amino acids, however, proved to be equally safe as they did not cause any serious side effects, exacerbate signs and symptoms of RA, alter liver function, or negatively affect psychological status.

In conclusion, the results of this study suggest that nutritional supplementation with HMB/GLN/ARG can safely reverse cachexia RA patients. Although the oral mixture of alanine, glutamic acid, glycine and serine has similar efficacy and safety profile, it is not well tolerated and it is not commercially available, both of which argue against its use in clinical practice. Future studies should confirm and extend our promising results on the anabolic and functional effects of increased nitrogen intake in RA patients using means other than crystalline amino acids (e.g., dietary supplementation with intact or partially hydrolysed proteins) and by having a normal nitrogen intake control group. The combination of dietary nitrogen supplements with exercise training to optimise their effects on physical function also warrants further investigations. Because most subjects in our study had low-to-moderate disease activity and only mild disability, it is possible that our findings are limited to this population and, therefore, future studies should include RA patients more severely affected by the disease.

Table 5
Effects of 12 Weeks Supplementation with HMB/GLN/ARG and Placebo on Body Mass and Composition in Rheumatoid Arthritis Patients

Variable	HMB/GLN/ARG (n = 18)	Placebo (n = 18)	P	η^2
Body mass, kg				
Pre	67.9 ± 13.4	75.3 ± 11.0		
Post	68.6 ± 14.5	76.1 ± 10.5		
Change	0.68 ± 0.31	0.82 ± 1.18	0.86	0.00
Arms lean mass, kg				
Pre	3.96 ± 1.67	3.98 ± 1.26		
Post	4.06 ± 1.74	4.10 ± 1.35		
Change	0.11 ± 0.20	0.12 ± 0.17	0.84	0.00
Legs lean mass, kg				
Pre	11.4 ± 3.3	12.6 ± 3.0		
Post	11.7 ± 3.5	12.9 ± 3.2		
Change	0.27 ± 0.59	0.30 ± 0.49	0.88	0.00
Total lean mass, kg				
Pre	40.0 ± 9.6	41.9 ± 7.9		
Post	40.7 ± 9.9	42.8 ± 8.0		
Change	0.61 ± 1.56	0.84 ± 0.70	0.57	0.01
Total BMC, kg				
Pre	2.30 ± 0.53	2.37 ± 0.49		
Post	2.30 ± 0.52	2.37 ± 0.49		
Change	0.0059 ± 0.0382	-0.0015 ± 0.0485	0.61	0.01

Table 5 cont.

Variable	HMB/GLN/ARG (n = 18)	Placebo (n = 18)	P	η^2
FFM, kg				
Pre	42.4 ± 10.0	44.3 ± 8.3		
Post	43.0 ± 10.3	45.1 ± 8.3		
Change	0.61 ± 1.55	0.84 ± 0.68	0.58	0.01
Total fat mass, kg				
Pre	24.5 ± 9.7	29.9 ± 10.3		
Post	24.5 ± 10.0	29.9 ± 10.0		
Change	-0.010 ± 1.943	0.021 ± 1.190	0.95	0.00
Trunk fat mass, kg				
Pre	12.2 ± 5.9	14.8 ± 6.2		
Post	12.0 ± 6.0	14.4 ± 5.7		
Change	-0.11 ± 1.21	-0.37 ± 0.87	0.46	0.02
Percent body fat, %				
Pre	36.1 ± 11.4	39.8 ± 10.4		
Post	35.7 ± 11.5	39.5 ± 10.3		
Change	-0.38 ± 1.46	-0.30 ± 1.08	0.84	0.00
ECW, L				
Pre	14.2 ± 3.2	15.1 ± 2.6		
Post	14.5 ± 4.0	14.9 ± 2.5		
Change	0.27 ± 1.40	-0.18 ± 1.46	0.35	0.02
ICW, L				
Pre	17.0 ± 5.0	17.3 ± 3.2		
Post	16.5 ± 4.1	17.7 ± 3.6		
Change	-0.48 ± 1.93	0.40 ± 0.96	0.09	0.08

Table 5 cont.

Variable	HMB/GLN/ARG (n = 18)	Placebo (n = 18)	P	η^2
TBW, L				
Pre	31.2 ± 8.1	32.4 ± 5.2		
Post	31.0 ± 7.7	32.6 ± 5.9		
Change	-0.21 ± 1.34	0.22 ± 1.99	0.46	0.02
TBW:FFM				
Pre	0.74 ± 0.07	0.74 ± 0.08		
Post	0.72 ± 0.08	0.72 ± 0.05		
Change	-0.012 ± 0.031	-0.013 ± 0.052	0.96	0.00

Note. Pretest and posttest data and change scores (pretest – posttest) are presented as means ± *SD*. The difference between groups' changes on each outcome variable was tested as the group x (time) interaction of a 2x(2) ANOVA. HMB/GLN/ARG = β-hydroxy-β-methylbutyrate, glutamine, and arginine; Placebo = alanine, glutamic acid, glycine, and serine; BMC = bone mineral content; FFM = fat-free mass; ECW = extracellular water; ICW = intracellular water; TBW = total body water.

Table 6
Effects of 12 Weeks Supplementation with HMB/GLN/ARG and Placebo on Muscle Strength, Disability, Psychological Status, Disease Activity and Liver Function in Rheumatoid Arthritis Patients

Variable	HMB/GLN/ARG (n = 18)	Placebo (n = 18)	P	η^2
Hand-grip strength, N				
Pre	169 ± 126	142 ± 103		
Post	181 ± 116	137 ± 87		
Change	12.6 ± 96.6	-5.0 ± 53.2	0.50	0.01
Elbow flexors strength, N				
Pre	183 ± 64	149 ± 66		
Post	180 ± 53	155 ± 55		
Change	-3.9 ± 29.6	5.6 ± 31.0	0.35	0.03
Knee extensors strength, N				
Pre	261 ± 62	237 ± 80		
Post	281 ± 58	248 ± 84		
Change	19.2 ± 48.9	11.6 ± 31.5	0.58	0.01
SST-30, repetitions				
Pre	10.9 ± 4.1	11.3 ± 3.8		
Post	13.8 ± 3.6	13.4 ± 3.8		
Change	2.9 ± 2.1	2.1 ± 1.8	0.21	0.05
Modified HAQ, 1-4				
Pre	1.5 ± 0.4	1.5 ± 0.3		
Post	1.4 ± 0.4	1.6 ± 0.4		
Change	-0.07 ± 0.35	0.04 ± 0.30	0.31	0.03

Table 6 cont.

Variable	HMB/GLN/ARG (n = 18)	Placebo (n = 18)	P	η^2
Advanced ADLs, 1-4				
Pre	2.3 ± 0.4	2.4 ± 0.4		
Post	2.2 ± 0.5	2.4 ± 0.5		
Change	-0.11 ± 0.48	0.01 ± 0.33	0.39	0.02
Psychological status, 1-4				
Pre	1.6 ± 0.5	1.6 ± 0.4		
Post	1.5 ± 0.4	1.7 ± 0.6		
Change	-0.11 ± 0.48	0.01 ± 0.51	0.45	0.02
Fatigue, 0-10				
Pre	3.9 ± 3.0	5.2 ± 1.7		
Post	3.1 ± 2.7	5.5 ± 2.9		
Change	-0.9 ± 2.3	0.3 ± 2.7	0.17	0.06
RADAI, 0-10				
Pre	2.8 ± 1.1	3.8 ± 1.4		
Post	3.0 ± 1.2	3.9 ± 1.4		
Change	0.15 ± 1.26	0.03 ± 1.46	0.81	0.00
ESR, mm/hour				
Pre	27.4 ± 22.6	22.7 ± 14.6		
Post	23.3 ± 19.4	25.4 ± 12.1		
Change	-4.1 ± 16.5	2.7 ± 8.2	0.13	0.07
Bilirubin, μmol/L				
Pre	8.3 ± 3.1	8.4 ± 4.3		
Post	8.6 ± 4.2	9.5 ± 5.6		
Change	0.3 ± 2.4	1.1 ± 2.9	0.39	0.02

Table 6 cont.

Variable	HMB/GLN/ARG (n = 18)	Placebo (n = 18)	P	η^2
ALP, μmol/L				
Pre	177 ± 65	183 ± 59		
Post	174 ± 65	186 ± 53		
Change	-3.4 ± 21.9	3.5 ± 27.3	0.42	0.02
AST, μmol/L				
Pre	17.6 ± 4.4	19.2 ± 6.6		
Post	17.9 ± 5.0	18.0 ± 7.5		
Change	0.4 ± 4.0	-1.2 ± 8.5	0.50	0.01
ALT, μmol/L				
Pre	17.4 ± 7.0	24.3 ± 10.5		
Post	18.9 ± 9.6	20.5 ± 13.5		
Change	1.6 ± 5.6	-3.8 ± 14.2	0.17	0.06

Note. Pretest and posttest data and change scores (posttest – pretest) are presented as means ± *SD*. The difference between groups' changes on each outcome variable was tested as the group x time interaction of a 2x(2) ANOVA. HMB/GLN/ARG = β-hydroxy-β-methylbutyrate, glutamine, and arginine; Placebo = alanine, glutamic acid, glycine, and serine; SST-30 = 30-s maximal sit-to-stand test; HAQ = health assessment questionnaire; ADLs = activities of daily living; RADAI = rheumatoid arthritis disease activity index; ESR = erythrocyte sedimentation rate; ALP = alkaline phosphatase; AST = aspartate aminotransferase; ALT = alanine aminotransferase.

Table 7
Effects of 12 Weeks Supplementation with HMB/GLN/ARG and Placebo on Incidence of Adverse Events in Rheumatoid Arthritis Patients

	HMB/GLN/ARG (n = 18)		Placebo (n = 18)		
Variable	Initial incidence, %	On treatment incidence, %	Initial incidence, %	On treatment incidence, %	P
Stomachache	6	17	28	28	0.31
Nausea/vomit	6	6	0	6	0.38
Dizziness	17	17	0	17	0.35
Coughing	17	17	33	33	0.55
Wheezing	11	22	33	22	0.29
Chest pain	6	11	6	0	0.40
Weakness	17	17	22	17	0.94
Increased headache	17	28	11	11	0.46
Decreased stress	0	0	17	22	0.07
Negative mood	17	6	22	17	0.70
Rash	11	0	0	6	0.23
Dry scalp/Hair	6	0	6	6	0.61
Dry skin	0	11	28	11	0.12
Nail changes	6	11	6	0	0.36
Ear pain	11	6	6	6	0.84
Increased libido	11	6	0	0	0.36
Decreased memory	6	0	11	17	0.15
Itching	22	0	33	67	0.13
Swelling	28	17	33	22	0.52
Diarrhea	6	0	6	6	0.61

Table 7 cont.

Variable	HMB/GLN/ARG (n = 18)		Placebo (n = 18)		P
	Initial incidence, %	On treatment incidence, %	Initial incidence, %	On treatment incidence, %	
Stiff joints	78	72	89	67	0.55
Nose bleeds	0	6	17	0	0.13
Heart burn	6	17	28	28	0.29
Numbness	0	6	11	6	0.40
Nasal congestion	6	11	44	28	0.06
Ringing in ears	17	17	33	39	0.53
Increased stress	11	6	11	22	0.49
Decreased libido	11	11	17	11	0.89
Constipation	6	0	6	17	0.26
Increase in memory	0	0	0	0	1.00
Decreased headache	0	6	0	0	0.32
Shortness of breath	6	17	28	28	0.34
Loss of appetite	11	6	6	11	0.72
Increase in appetite	17	0	11	17	0.27
Loss of energy	22	28	39	39	0.37
Increase in energy	6	6	0	6	0.61
Blood in urine	0	0	0	6	0.32
Blood in stool	0	0	0	0	1.00

Note. The effect of treatment on each sign and symptom was tested by the Cochran-Mantel-Haenszel statistic. HMB/GLN/ARG = β-hydroxy-β-methylbutyrate, glutamine, and arginine; Placebo = alanine, glutamic acid, glycine, and serine.

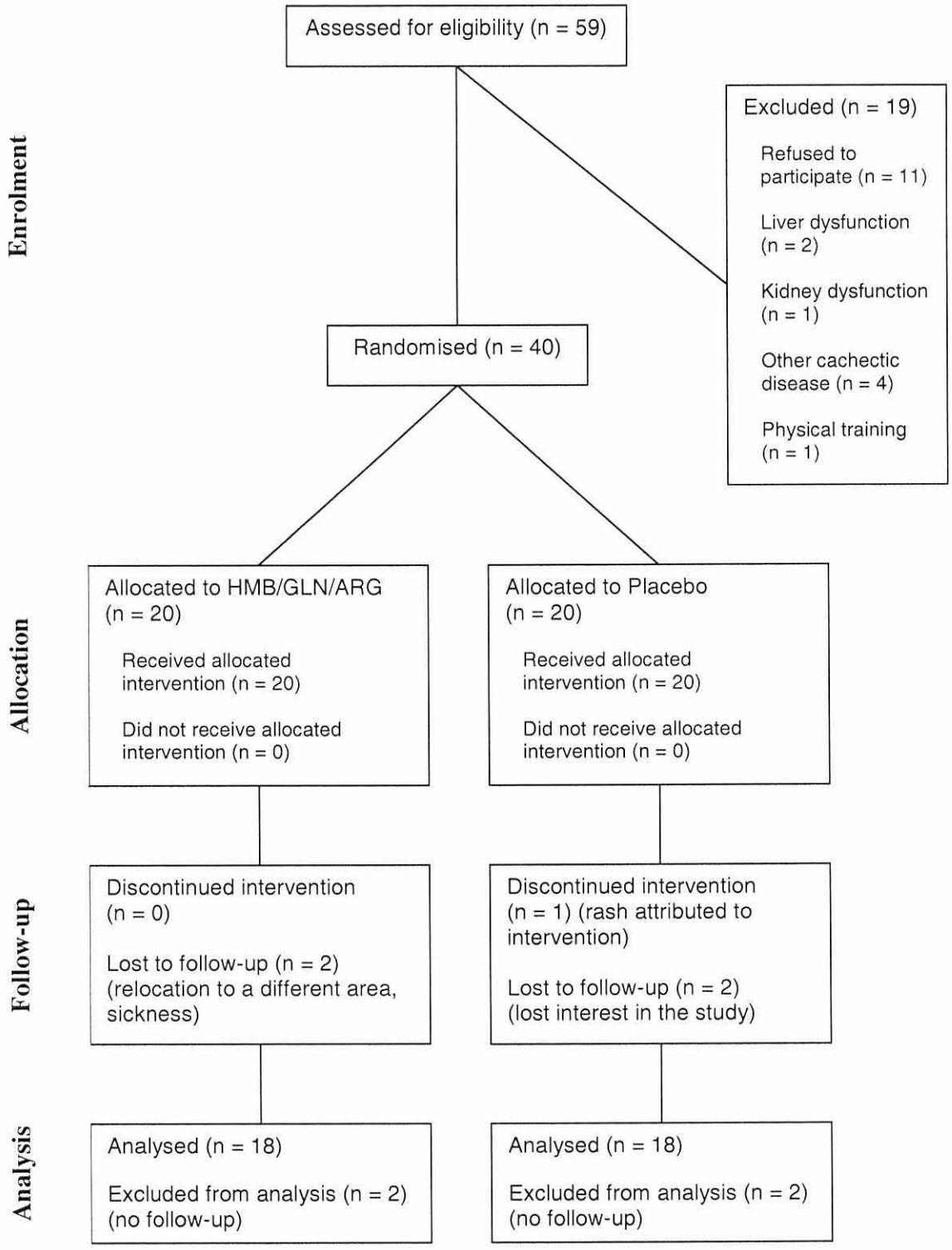

Figure 4. Flow of participants through each stage of the trial (HMB/GLN/ARG = β-hydroxy-β-methylbutyrate, glutamine, and arginine; Placebo = alanine, glutamic acid, glycine, and serine)

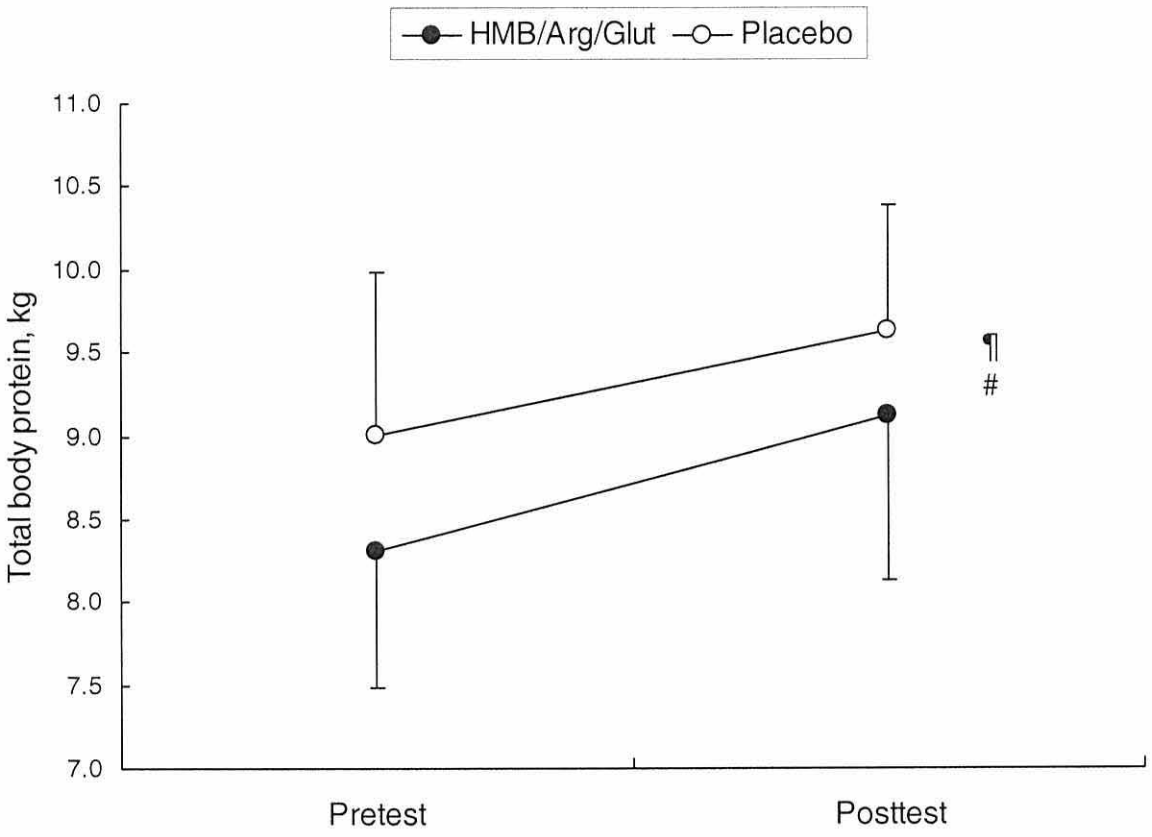

Figure 5. Effect of 12 week supplementation with β-hydroxy-β-methylbutyrate, glutamine, and arginine (HMB/GLN/ARG) and alanine, glutamic acid, glycine, and serine (placebo) on total body protein in rheumatoid arthritis patients. Data are presented as means ± SEM.
¶ Non significant group x time interaction; $P = 0.74$, $\eta^2 = 0.00$.
# Significant main effect for time; $P = 0.02$, $\eta^2 = 0.16$.

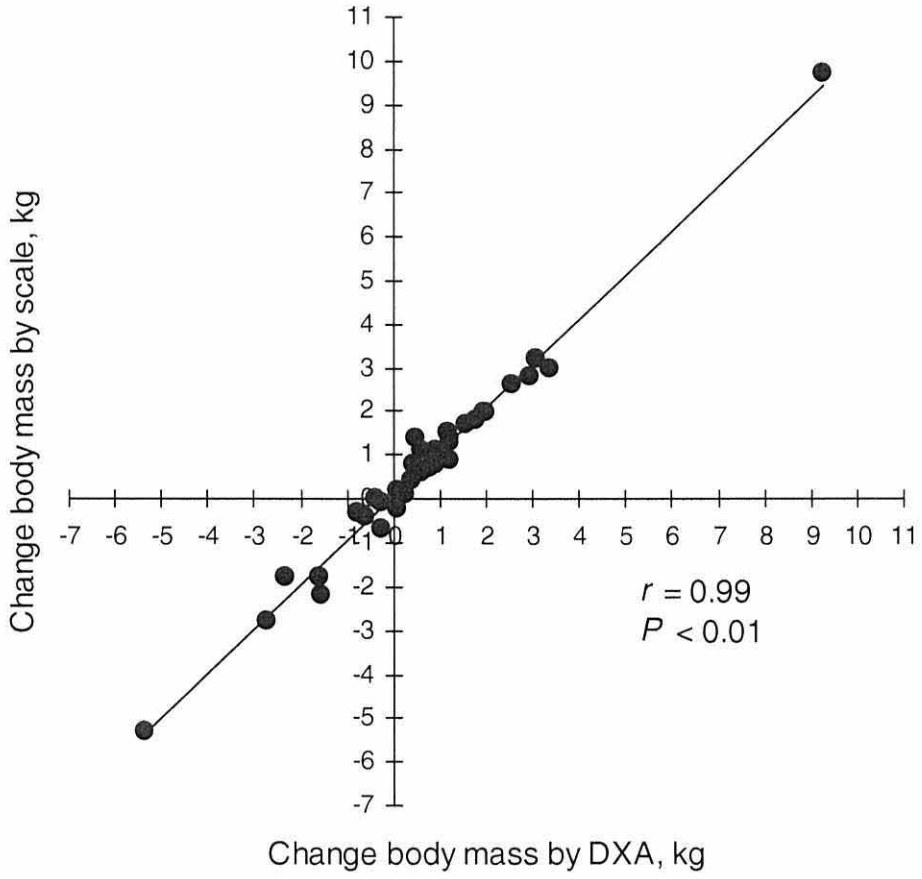

Figure 6. Association between change (posttest – pretest score) in body mass by scale and by dual energy X-ray absorptiometry (DXA) in rheumatoid arthritis patients supplemented for 12 weeks with β-hydroxy-β-methylbutyrate, glutamine, and arginine or alanine, glutamic acid, glycine, and serine (placebo).

CHAPTER 5

RANDOMISED CONTROLLED TRIAL OF ANTI-TUMOR NECROSIS FACTOR THERAPY WITH ETANERCEPT FOR THE PREVENTION OF CACHEXIA IN PATIENTS WITH EARLY RHEUMATOID ARTHRITIS

A PAPER SUBMITTED TO THE

AMERICAN JOURNAL OF CLINICAL NUTRITION

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INTRODUCTION

Rheumatoid arthritis (RA) is a chronic, often progressive, autoimmune disease of complex aetiology affecting 1% of the population and mainly characterised by inflammation and erosion of peripheral synovial joints (Silman and Pearson 2002). However, RA is a truly systemic disease complicated by significant alterations in protein and energy metabolism (Roubenoff, Roubenoff et al. 1990; Gibson, Poyser et al. 1991; Roubenoff, Roubenoff et al. 1994; Rall, Rosen et al. 1996; Walsmith, Vannier et al. 2003) and consequent muscle wasting despite normal food intake and little or no weight loss (Roubenoff, Roubenoff et al. 1994; Westhovens, Nijs et al. 1997; Roubenoff, Walsmith et al. 2002; Walsmith, Abad et al. 2004). This syndrome, named rheumatoid cachexia, severely affects more than 30% of RA patients (Roubenoff, Roubenoff et al. 1992; Munro and Capell 1997) and contributes to the poor outcome of this disease which typically features significant physical disability and reduced life expectancy (Collins, Dunn et al. 1987; Helliwell and Jackson 1994; Hernandez-Beriain, Segura-Garcia et al. 1996; Walsmith and Roubenoff 2002).

One of the main mediators of rheumatoid cachexia is thought to be tumor necrosis factor (TNF) (Walsmith and Roubenoff 2002). This inflammatory cytokine is found in high concentrations not only in the synovial fluid of affected joints but also in the serum of RA patients with active disease (Saxne, Palladino et al. 1988; Tetta, Camussi et al. 1990; Roubenoff, Roubenoff et al. 1992; Hasselgren 2000; Straub, Paimela et al. 2002) where it can directly influence the metabolism and function of many organs distant from the sites of inflammation, including skeletal muscles (Li and Reid 2001). *In vivo*, TNF can also induce skeletal muscle loss indirectly by reducing the systemic and local production of, bioavailability, and tissue sensitivity to anabolic mediators such as growth hormone (GH) and insulin-like growth factor(IGF)-I (Lang and Frost 2002), and

stimulating the production of catabolic hormones such as cortisol (Michie, Spriggs et al. 1988). Therefore, it is not surprising that in Lewis rats made arthritic using Freund's complete adjuvant a significant correlation was found between systemic TNF and the profound loss of body cell mass and weight characteristic of this animal model of RA (Roubenoff, Freeman et al. 1997). Similarly, transgenic mice overexpressing human TNF develop not only the articular features of RA, but also progressive and lethal wasting (Li and Schwarz 2003). In RA patients, Roubenoff and colleagues found a significant association between increased TNF production by circulating monocytes and the main features of rheumatoid cachexia, i.e. hypermetabolism (Roubenoff, Roubenoff et al. 1994), accelerated whole-body protein breakdown (Rall, Rosen et al. 1996) and reduced total body potassium (Walsmith, Abad et al. 2004) which is a proxy measure of body cell mass and total body skeletal muscle mass (Wang, Zhu et al. 2003). A significant correlation between increased local expression of TNF and reduced skeletal muscle protein synthesis in RA patients has also been reported (Walsmith, Vannier et al. 2003).

Overall, these findings suggest that TNF blockade should prevent or at least reduce cachexia in this population. This hypothesis is supported by preclinical studies in which treatment with anti-TNF agents was able to completely or partially prevent weight loss in various animal models of RA (Feige, Hu et al. 2000; Shealy, Wooley et al. 2002; Fathalla, Hamada et al. 2004). There is also a report of substantial weight gain in 87% of RA patients treated with TNF-blocking drugs over a period of 11 months (Fonseca, Canhao et al. 2002). However, this could simply reflect body fat accumulation because of improved appetite and general well-being, and not an anabolic response in skeletal muscle tissue. Therefore, the main aim of this study was to investigate the effects of anti-TNF therapy with etanercept (ETN) on body composition in patients with early RA. As TNF is thought to mediate accelerated loss of skeletal muscle in many other catabolic

conditions (Kotler 2000), this study could also prove to be a convenient human model for the investigating the potential efficacy of this anti-catabolic treatment in cachectic diseases such as cancer (Argiles, Moore-Carrasco et al. 2003).

SUBJECTS AND METHODS

Patients

Because of ETN availability constraints, the originally planned subjects number (50) and follow-up duration (12 months) had to be halved. Therefore, we selected 26 subjects over 18 years of age and with a diagnosis of RA according to the American Rheumatism Association 1987 revised criteria (Arnett, Edworthy et al. 1988), who were at high risk for accelerated loss of skeletal muscle, i.e. patients with early (less than six months history) and active disease (classified as a disease activity score on 28 joints [DAS28] > 3.2), and intact skeletal muscle mass defined as a height-adjusted appendicular muscle mass no more than two standard deviations below the mean of young adults (Baumgartner 2000). Potential participants were excluded from the study if they had previously received any disease-modifying anti-rheumatic drug (DMARD) (including biologics) or corticosteroid therapy, had a recent history of important infection, had any concurrent disease preventing safe participation in the required physical function tests (i.e., uncontrolled hypertension or musculoskeletal injury), had cognitive impairment or any other cachectic disease (i.e., cancer or chronic heart failure). Patients were also excluded if they were taking drugs or nutritional supplements known to affect skeletal muscle mass, or were participating in regular and intense physical training. Patients were screened by one of the investigators (GM) between July 2002 and October 2003 during outpatient clinics at the Rheumatology Department of Gwynedd Hospital. The study protocol was approved by the North Wales Health Authority Research Ethics Committee

and all volunteers gave their written informed consent to participate before commencing the study.

Study Design and Treatment

After baseline assessment (pretest), participants were randomly assigned to 24 weeks of treatment based on a computer-generated list of random numbers. The patients and the clinician (GM) responsible for their management were aware of treatment allocation. The investigators (SM and KC) involved in assessing body composition, physical function, disability and health-related quality of life (HRQoL) were, however, blinded to treatment assignment until after statistical analysis was completed. Patients were assessed again at week 12 (midtest) and 24 (posttest) of follow-up.

Treatment consisted of either ETN or methotrexate (MTX). Etanercept is a dimeric fusion protein consisting of the extracellular ligand-binding portion of the human 75 kilodalton TNF receptor linked to the Fc portion of human immunoglobulin G1. This TNF-blocking agent safely reduces disease activity and joint damage in RA patients and is approved for clinical use (Genovese and Kremer 2004). Etanercept was supplied by Wyeth Pharmaceuticals (Madison, NJ) in 25-mg single-use vials which were reconstituted with bacteriostatic water and injected subcutaneously twice a week as recommended by the manufacturer.

As irreversible erosion of articular cartilage and bone erosion occurs very early in the disease (Sharp, Wolfe et al. 1991), an untreated control group of RA patients would have been unethical and was not included in this study. Therefore, we compared ETN with MTX, the most common DMARD used for the treatment of early RA (Pincus, Yazici et al. 2003). Methotrexate is an antimetabolite capable of controlling disease activity and slowing joint damage through a variety of mechanisms (Kremer 2004).

Patients were started at a dose of 7.5 mg/week for a month. If deemed necessary, this dose was increased to a maximum of 15 mg/week in the second month and 20 mg/week in the subsequent four months. Patients on MTX also received the recommended folic acid supplementation (10 mg a week the day before taking ETN). Symptomatic use of non-steroidal anti-inflammatory drugs (NSAIDs) and analgesics, but no corticosteroids, was allowed in both groups. All participants were also instructed to maintain their habitual physical activity and dietary habits throughout the study.

Outcome Measures

Body mass and composition, physical function, disability and HRQoL were measured at baseline and during the follow-up tests by the same investigators (SM and KC) in the Bone Densitometry Laboratory in the School of Sport, Health and Exercise Sciences, University of Wales-Bangor, at approximately the same time of the day and in the same environmental conditions. Subjects presented at the lab fasted and were asked to void and remove all metallic objects. Subjects were instructed beforehand to avoid strenuous exercise in the 24 hours preceding testing and were questioned as to whether they had orthopaedic metal and silicone implants. During testing subjects were allowed to wear only socks, shorts, underwear (no bra) and a t-shirt. Disease activity was assessed monthly by the same experienced rheumatologist (GM). The primary endpoint for this clinical trial was no loss in *both* arms *and* legs (i.e., appendicular) lean mass by dual-energy X-ray absorptiometry (DXA), a proxy measure of total body skeletal muscle mass (Kim, Wang et al. 2002). As patients with long-term RA treated with MTX and other traditional DMARDs present with muscle atrophy, we hypothesised that anti-TNF therapy with ETN would be superior in preventing muscle wastage in patients with early RA compared to standard therapy.

Body Mass and Composition

Body mass was measured to the nearest 0.1 kg using a calibrated balance scale (Seca, Hamburg, Germany). Total and regional (left and right arm, left and right leg, trunk, head) body composition was assessed by DXA using a pencil-beam scanner (QDR1500, Hologic, Bedford, Massachusetts) which calculates the masses (g) of three different compartments: bone mineral content (BMC), fat mass and lean mass. The sum of total BMC and total lean mass corresponds to fat-free mass (FFM). Percent body fat was calculated as (total fat mass/total body mass by DXA) · 100. The procedures recommended by the manufacturer for whole-body examination (subject positioning, scanning and analysis with software version V5.72) were followed and the quality control procedure was performed daily.

Immediately after the DXA scan, intracellular water (ICW) and extracellular water (ECW) volumes (L) were estimated using bioelectrical impedance spectroscopy (BIS) (Hydra 4200, Xitron Technologies, San Diego, California), with total body water (TBW) being the sum of ICW and ECW. Bioelectrical impedance measurements were taken on the left side of the body, with the use of disposable electrodes and in accordance with a standard wrist-to-ankle protocol (Van Loan, Withers et al. 1993). At the time subjects were measured, they had been supine for approximately 20 min. The quality control procedure recommended by the manufacturer was performed before each measurement and the proximity of the DXA scanner had no effect on the validity and reliability of BIS (unpublished observations).

By combining DXA and BIS data, we were able to calculate FFM hydration (TBW/FFM) and total body protein, the latter estimated as total lean mass – TBW – 0.2305 · total BMC according to the model proposed by Fuller *et al.* (Fuller, Wells et al.

2001). For each of these calculations, TBW volume was converted into mass by assuming a water density of 0.99336 kg/L at a normal body temperature of 37°C.

Physical Function

After body composition assessment, subjects performed callisthenics for 5 min as general warm-up and then performed the following upper and lower body function tests:

1. Hand-grip strength. Maximal voluntary hand-grip strength was measured using a Grip-A dynamometer (Takey Kiki Kogyo, Japan). For this test, subjects were asked to stand erect holding the dynamometer parallel to their side with their dominant hand, dial facing away from the body and to squeeze the hand-grip without moving the arm. After two submaximal warm-up and familiarisation trials (50% and 75% of maximum effort), subjects were asked to perform three trials in which they exerted force maximally for approximately 5 s each time. Between all five trials (i.e., submaximal and maximal), 1 min rest was provided. Peak force produced during each of the three maximal trials was recorded in newtons and the best score noted for statistical analysis.
2. Arm curl test. After a practice trial, subjects were asked to curl a hand weight (5 pounds for women, 8 pounds for men) through full range of motion as many times as possible in 30 s while seated on a high-backed chair with their dominant arm parallel to their side.
3. Walking velocity. Subjects were asked to start from a standing position and walk at a fast pace for a distance of 50 feet. After a practice trial, three trials were performed with 1 min rest between them, and the best time, as measured by a manually-operated stopwatch, was used to calculate walking velocity in feet per minute.

4. Sit-to-stand test. After a practice trial, subjects were asked to sit-to-stand from a fixed chair (seat height 43.2 cm) as many times as possible in 30 s while keeping their arms folded across the chest.

As suggested by McNair *et al.* (McNair, Depledge et al. 1996), subjects were verbally encouraged during testing in order to attain maximum performance.

Physical Disability and Health-Related Quality of Life

Physical disability was quantified by the disability index of the full Health Assessment Questionnaire (HAQ), an RA-specific instrument which quantifies the degree of difficulty perceived by the patient in eight functional categories: 1) dressing and grooming, 2) arising, 3) eating, 4) walking, 5) hygiene, 6) reach, 7) grip, and 8) common daily activities. The overall score is expressed on a scale ranging from 0 (no physical disability) to 3 (severe physical disability) (Bruce and Fries 2003). Generic HRQoL was assessed using the 36 questions short-form health survey (SF-36) which measures eight multi-item dimensions: 1) physical functioning; 2) social functioning; 3) role limitations due to physical problems; 4) role limitations due to emotional problems; 5) mental health; 6) energy/vitality; 7) pain; 8) general health perception. Two overall summary scores can be gained from data from the eight domains: the physical component score and the mental component score which are expressed on the standard 0-100 scale, where higher scores indicate better health and well-being (Keller, Majkut et al. 1999).

Disease Activity

Disease activity was assessed by the DAS28. This is a composite measure of different clinical parameters ranging from 0 to 10 and includes the tender joint count (0-28), the swollen joint count (0-28), the erythrocyte sedimentation rate (ESR) (mm/hr) and general

health perceived by the patient measured on a visual analogue scale from 0 to 100 mm (DAS28 = 0.56 · tender 28 + 0.28 · swollen 28 + 0.70 · ln ESR + 0.014 · general health). Joint counts were performed by the same experienced rheumatologist (GM). The ESR was assessed using the Westergren's method in the Department of Biochemistry and Haematology of Gwynedd Hospital.

Other Measures

Height was measured to the nearest 0.5 cm using a wall-mounted stadiometer (Body Care, Warwickshire, UK). Body mass index (BMI) was calculated as body mass (kg)/height squared (m^2). All anthropometric measures were taken following standard procedures by the same trained investigators.

Habitual physical activity both at work and during leisure time was ranked from mainly sedentary (score = 1) to heavy and regular (score = 4), using the questionnaire proposed by Saltin *et al.* (Saltin and Grimby 1968), which gives a total habitual physical activity score ranging from 2 to 8. Appetite was also measured pre and post using a self-assessment numerical rating scale ranging from 0 to 10; 0 indicates *absolutely no appetite* and 10 indicates an *extremely good appetite* (Simons, Schols et al. 1999). Other required information (age, disease duration, current medications, etc.) was collected at baseline by structured interview and review of medical records. At follow-ups, all subjects were questioned if changes in habitual physical activity, diet, medications, and other aspects of their lifestyle had occurred during the study.

All patients were visited monthly by the same experienced rheumatologist (GM) who performed physical examinations, interpreted routine laboratory measurements and gathered reports of adverse events. An adverse event was defined as any unfavourable and unintended sign (including hepatic enzymes values higher than three times the

laboratory's upper normal limits), symptom, or disease which was not present at baseline or worsened during treatment.

Statistical Analysis

Unless otherwise noted, all data are presented as means ± *SD*. A two-way mixed analysis of variance (ANOVA) was used on each outcome measure (dependent variable). The independent variables included one between subjects factor, treatment, with two levels (ETN and MTX), and one within subjects factor, time, with three levels (pretest, midtest and posttest) with the exception of appetite that had only two levels: pretest and posttest. We used these ANOVAs to test the null hypothesis of no difference in change over time between the ETN and MTX group (treatment x time interaction), and the null hypothesis of no change over time in response to therapy (main effect of time). A significant treatment x time interaction was followed-up by one-way repeated measures ANOVAs, and then multiple pairwise comparisons using dependent *t* tests within each group. A significant main effect of time was followed-up by multiple pairwise comparisons using dependent *t* tests after pooling the data of both groups Effect size for both the main effect of time and the treatment x time interaction was calculated as eta squared (η^2), a measure of strength of relationship between the independent and dependent variable. Thresholds for small, moderate, large and very large effects were set at 0.01, 0.08, 0.26 and 0.50 respectively. Pearson product-moment correlation coefficient was used to express and test the significance of the relationships between measures of interest (pre-post change in body mass by scale with body mass by DXA; pre-post changes in arms and legs lean mass with pre-post changes in physical function, disability and HRQoL) after pooling the data of both groups. The null hypothesis of no difference between groups in the amount of FFM per kg of body mass gained, % body fat and body mass gained in patients who

had a significant increase in weight (> 3% of baseline body mass) during follow-up was tested using an unpaired *t* test. Significance was set at 0.05 (two-tailed) for all analyses which were conducted using the Statistical Package for the Social Sciences Version 11. A trend was defined as a *P* level between 0.06 and 0.10.

RESULTS

Subjects Characteristics and Treatment

We screened 39 patients. Six patients refused to participate and other seven were excluded for various reasons (Figure 7). The remaining 26 patients were randomly allocated to treatment with either ETN (n = 12) or MTX (n = 14). One subject withdrew as she lost interest in the study when allocated to MTX. Another patient in the MTX group was excluded from the final analysis as he violated the protocol by starting an intense and regular physical training program during the intervention period. Flow of participants is represented in Figure 7.

Although the number of subjects included in the final statistical analysis was relatively small, there were no important baseline differences between groups in gender distribution (75% females in both groups), age (ETN: 54 ± 11 years; MTX: 50 ± 15 years), height (ETN: 165 ± 7 cm; MTX: 163 ± 7 cm), BMI (ETN: 28 ± 7 kg/m^2; MTX: 28 ± 4 kg/m^2), body mass and composition, physical function, disability, HRQoL, and disease activity (Tables 8 and 9). All participants were sedentary (total physical activity score ETN: 2.9 ± 0.9; total physical activity score MTX: 3.5 ± 0.7) and Caucasians. Seven patients in each group were RF positive.

The MTX dose was escalated to 20 mg/week in all but two (17.5mg/week and 10 mg/week respectively) patients. Two subjects in the MTX group did not respond to treatment as defined by the European League Against Rheumatism response criteria for

RA (van Gestel, Prevoo et al. 1996). Similarly, three patients did not respond to treatment with ETN. The only significant adverse event occurred during the study was one skin reaction at the injection site in the ETN group which was mild and self limited. All patients included in the final analysis declared that no significant changes in their lifestyle had occurred during the study.

Effects on Appetite and Body Mass

There was a trend for a moderate increase in appetite in both the MTX (pretest: 7.3 ± 1.7; posttest: 8.2 ± 1.4) and the ETN group (pretest: 6.8 ± 1.6; posttest: 7.4 ± 1.5) (main effect of time: $P = 0.08$, $\eta^2 = 0.13$) but no significant treatment x time interaction ($P = 0.72$, $\eta^2 = 0.01$). The slight increase in body mass was not significant (main effect of time: $P = 0.26$, $\eta^2 = 0.12$) in either group (Table 8). Within each group there were six individuals who gained more than 3% of their baseline body mass over the six months follow-up period. The amount of weight gained by these subjects was not different between the MTX (3.3 ± 1.7 kg) and the ETN (4.4 ± 2.4 kg) groups ($P = 0.37$, $\eta^2 = 0.08$).

Effects on Body Composition

Body composition at baseline and follow-up tests is shown in Table 8. With the exception of arms lean mass, there was no statistically significant treatment x time interaction in any of the measured body compartments. Post-hoc tests on arms lean mass revealed no significant change over time within the MTX group ($P = 0.46$, $\eta^2 = 0.14$), but a significant increase at week 12 ($P = 0.01$, $\eta^2 = 0.44$) with no further significant change at week 24 ($P = 0.20$, $\eta^2 = 0.14$) was measured in the ETN group. Overall, there was a moderate main effect of time on arms lean mass within the ETN group ($P = 0.05$, $\eta^2 = 0.46$) and a significant increase between pretest and posttest ($P = 0.01$, $\eta^2 = 0.24$).

There were no significant main effects of time on legs lean mass ($P = 0.64$, $\eta^2 = 0.04$), total lean mass ($P = 0.65$, $\eta^2 = 0.04$), total BMC ($P = 0.20$, $\eta^2 = 0.14$), total FFM ($P = 0.68$, $\eta^2 = 0.04$), % body fat ($P = 0.78$, $\eta^2 = 0.02$), ICW ($P = 0.78$, $\eta^2 = 0.03$), TBW ($P = 0.78$, $\eta^2 = 0.02$), FFM hydration ($P = 0.46$, $\eta^2 = 0.07$) and total body protein ($P = 0.32$, $\eta^2 = 0.10$). Trends for an increase in total fat mass ($P = 0.06$, $\eta^2 = 0.24$) and trunk fat mass ($P = 0.09$, $\eta^2 = 0.21$), and a decrease in ECW ($P = 0.10$, $\eta^2 = 0.20$) were measured in both groups.

A sub-analysis of patients who gained weight over the six months follow-up period revealed a significant between group difference in the composition of the body mass gained (Figure 8), with patients treated with ETN gaining a significantly higher proportion of FFM than patients treated with MTX. Importantly, baseline percent body fat was similar between these two subgroups (ETN: 40.7 ± 7.9 %, MTX: 39.8 ± 8.7 %; $P = 0.86$, $\eta^2 = 0.00$).

Change in body mass measured by scale was highly correlated to change in body mass measured by DXA as the sum of total lean mass, fat mass and BMC (Figure 10), thus confirming the internal validity of our measurements. The significant change in arms lean mass did not correlate significantly with changes in physical function, disability or HRQoL (data not shown).

Effects on Disease Activity, Physical Function, Disability, and Health-Related Quality of Life

Disease activity at baseline and follow-up tests is shown in Table 9. There was no statistically significant treatment x time interaction in DAS28. However, there was a very large main effect of time on disease activity ($P < 0.01$, $\eta^2 = 0.76$). This was secondary to a very large decreases between baseline and week 12 ($P < 0.01$, $\eta^2 = 0.67$) and a further,

smaller improvements between week 12 and week 24 ($P < 0.01$, $\eta^2 = 0.32$) in both groups. Overall, there was a significant decrease in disease activity between pretest and posttest ($P < 0.01$, $\eta^2 = 0.75$) in response to both treatments.

Performance in the various physical function tests at baseline and follow-ups is shown in Table 9. The differences in performance between pretest and posttest are shown in Figure 9. There were no statistically significant treatment x time interactions in any of the measured functional variables. However, there were very large main effects of time on hand-grip strength ($P < 0.01$, $\eta^2 = 0.63$), arm curl test performance ($P < 0.01$, $\eta^2 = 0.69$), walking velocity ($P < 0.01$, $\eta^2 = 0.44$) and sit-to-stand test performance ($P < 0.01$, $\eta^2 = 0.70$). Hand-grip strength increased significantly between pretest and midtest ($P < 0.01$, $\eta^2 = 0.62$) but no further improvement was measured at week 24 ($P = 0.43$, $\eta^2 = 0.03$) in response to both treatments. The number of repetitions performed during the arm curl test significantly increased at week 12 compared to baseline ($P < 0.01$, $\eta^2 = 0.59$) and a smaller but still significant improvement in performance occurred between week 12 and 24 ($P = 0.01$, $\eta^2 = 0.25$) in both the ETN and the MTX group. Similarly to arm curl test performance, walking velocity increased significantly in response to both treatments between pretest and midtest ($P < 0.01$, $\eta^2 = 0.40$) and a trend for a small improvement was measured at week 24 ($P = 0.07$, $\eta^2 = 0.13$). Finally, in both the ETN and the MTX group, there was a very large increase in performance in the sit-to-stand test at week 12 compared to baseline ($P < 0.01$, $\eta^2 = 0.59$) and a moderate improvement between week 12 and 24 ($P = 0.01$, $\eta^2 = 0.24$).

Physical disability, as measured by the HAQ, decreased significantly over time (main effect of time: $P < 0.01$, $\eta^2 = 0.56$) (Figure 9) with no significant treatment x time interaction (Table 9). This decrease occurred over the initial 12 weeks of treatment ($P < 0.01$, $\eta^2 = 0.48$) with no further significant improvement apparent between week 12 and

24 ($P = 0.34$, $\eta^2 = 0.04$). Similarly, the physical component score of the SF-36 was significantly higher at midtest compared to pretest ($P < 0.01$, $\eta^2 = 0.47$) but no change occurred between week 12 and week 24 ($P = 0.70$, $\eta^2 = 0.01$). The overall increase in physical health over time was, however, highly significant ($P < 0.01$, $\eta^2 = 0.51$) with no significant differences between treatments (Table 9).

Similarly, there was no treatment by time interaction in mental health (Table 9) which improved moderately over time ($P = 0.04$, $\eta^2 = 0.27$). Most of the improvement occurred at week 12 ($P = 0.01$, $\eta^2 = 0.27$) with no further increase in the mental component score between midtest and posttest ($P = 0.63$, $\eta^2 = 0.01$). The difference in physical health score between pretest and posttest was large and highly significant ($P < 0.01$, $\eta^2 = 0.47$). The increase in mental health score was, as expected, much smaller but statistically significant ($P = 0.05$, $\eta^2 = 0.16$).

DISCUSSION

This is the first investigation on the effects of anti-TNF therapy on body composition in humans affected by a chronic disease characterised by TNF overexpression and cachexia. Contrary to our hypothesis, ETN was not superior to MTX in preventing muscle wasting in patients with early RA. Although there was a significant increase in arms lean mass in the ETN group, this occurred by week 12 and no further gains were observed at week 24. In addition, this effect was isolated and no significant increases in legs lean mass, total lean mass, FFM and total body protein were evident in this group. Therefore, we believe that the apparent anabolic effect of ETN in the upper limbs musculature is likely to be a type I error. If we make the reasonable assumption that early RA patients with active and untreated disease would have lost a significant amount of skeletal muscle mass over a

period of six months, both ETN and MTX seem to be equally effective in stopping the progress of cachexia in these patients.

A possible explanation is that anti-rheumatic therapy with both ETN and MTX has anti-catabolic properties. This hypothesis is supported by the results of a small metabolic investigation conducted by Rall and colleagues (Rall, Rosen et al. 1996) who observed that the accelerated whole-body protein breakdown characteristic of rheumatoid cachexia does not occur in patients treated with MTX. Experimental studies in the rat model of RA also demonstrated that MTX is effective in completely reversing the weight loss typically observed in these animals (Morgan, Baggott et al. 2001; Goodson, Morgan et al. 2003). The most likely mechanism for these anti-catabolic effects of MTX is its capacity to reduce systemic production and hence circulating levels of catabolic cytokines such as TNF, IL-1, IL-6 and interferon-γ (Zoico and Roubenoff 2002) in RA patients (Barrera, Boerbooms et al. 1996; Seitz 1999; Cutolo, Sulli et al. 2001; Gerards, de Lathouder et al. 2003). In addition, there is some evidence that MTX can suppress TNF-induced nuclear factor-κB (NF-κB) activation in a variety of cell lines (Majumdar and Aggarwal 2001). If this effect of MTX is also true for skeletal muscle cells, it could provide another explanation for the ability of this popular DMARD to prevent cachexia in early RA patients who are at high risk for accelerated loss of skeletal muscle. Indeed, the myofibrillar protein degradation induced by chronic TNF exposure in differentiated myotubes is dependent on NF-κB activation and consequent persistent up-regulation of ubiquitin-conjugating activity (Li, Schwartz et al. 1998; Li, Lecker et al. 2003), and this important signal transducer has been proposed as one of the main molecular targets for the treatment of muscle wasting associated with chronic diseases (von Haehling, Genth-Zotz et al. 2002; Guttridge 2004).

A second possible explanation for the results of our clinical trial is that the follow-up period was not long enough to demonstrate any differential effect of anti-TNF therapy with ETN versus standard therapy with MTX on body composition of RA patients with early and active disease. In fact, in another 6-month longitudinal body composition study of RA patients treated with either prednisolone or NSAIDs, no significant loss of total body nitrogen or other elements was demonstrated (Kennedy, Boddy et al. 1979). However, over a longer period of time (56 weeks), Westhovens (Westhovens 1999) could measure a significant 3% loss of total lean mass in 60 patients with early RA treated with standard therapy (NSAIDs, corticosteroids and various DMARDs including MTX). Similarly, numerous cross-sectional studies of patients with long-standing RA (average disease duration 10 years) treated with standard therapy (no biologics) demonstrate a significant 12% reduction in skeletal muscle mass compared to healthy, age-, gender-, and body size-matched controls (Helliwell, Coombes et al. 1984; Roubenoff, Roubenoff et al. 1990; Helliwell and Jackson 1994; Roubenoff, Roubenoff et al. 1994; Sambrook, Spector et al. 1995; Hernandez-Beriain, Segura-Garcia et al. 1996; Westhovens, Nijs et al. 1997; Krahenbuhl, Willer et al. 1999; Moriguchi, Terai et al. 1999; Gomez-Vaquero, Nolla et al. 2001; Rall, Walsmith et al. 2002; Roubenoff, Walsmith et al. 2002; Walsmith, Abad et al. 2004). This suggests that MTX and other drugs commonly used to treat RA are not totally effective in preventing rheumatoid cachexia in the long term. Therefore, it is possible that anti-TNF therapy with ETN might prove to have superior anti-catabolic effects compared to standard anti-rheumatic therapy when studied over a more prolonged period of time and this hypothesis should be investigated in future clinical trials.

Although the results of the primary analysis do not support the original research hypothesis, secondary analysis of those patients who gained a significant amount of weight (i.e., were in positive energy balance) during the follow-up period revealed an

interesting metabolic property of anti-TNF therapy with ETN which also deserves further investigation. Deliberate overfeeding induces weight gain in humans and, in healthy subjects with a body fat content similar to our RA patients, 30-40% of the body mass gained is FFM (Forbes 2000). In catabolic conditions such as sepsis, chronic infection, and cancer, however, this anabolic response to positive energy balance is impaired and when treated with hypercaloric feeding or appetite stimulants most of the body mass gained by these patients is fat with little or no gain in FFM (Cohn, Vartsky et al. 1982; Streat, Beddoe et al. 1987; Strang 1997; Kotler 2000). A similar phenomenon occurred in the six RA patients treated with MTX in which only 14% of the body mass gained was FFM. On the contrary, TNF blockade with ETN normalised the anabolic response to overfeeding and 44% of body mass gained by these other six patients was FFM rather than fat mass.

A possible explanation for this improved metabolic efficiency is provided by a recent study in which neutralisation of TNF by its antibody eliminated insulin resistance in skeletal muscle tissue but not in adipose tissue of aged Sprague-Dawley rats (Borst, Lee et al. 2004). However, more research into this is required as insulin resistance was measured only in term of glucose metabolism and treatment did not alter body weight, muscle mass or fat mass in these animals. Nevertheless, insulin, together with increased plasma concentrations of certain amino acids, is the main mediator of postprandial protein anabolism in humans (Prod'homme, Rieu et al. 2004) and specific restoration of its anabolic actions in skeletal muscle by anti-TNF therapy could be useful in diseases, such as RA, in which systemic inflammation causes, at least in part, insulin resistance (Dessein, Joffe et al. 2003) and muscle protein catabolism. Tumor necrosis factor blockade with ETN in RA patients might also improve the sensitivity of skeletal muscle

tissue to IGF-I (Frost, Lang et al. 1997), another hormone important for the regulation of the anabolic response to overfeeding (Forbes, Brown et al. 1989).

Although very interesting from a physiological and nutritional point of view, this novel metabolic property of ETN is not clinically relevant for the treatment of rheumatoid cachexia as most RA patients are not anorexic and present with excessive body fat. Therefore, the combination of hypercaloric feeding or appetite stimulants with anti-TNF therapy is not recommended in this population. Nonetheless, in underweight RA patients and patients affected by diseases such as cancer in which cachexia is complicated by profound anorexia and weight loss, this combination might prove to be clinically useful and should be investigated in specific clinical trials.

As in previous investigations in patients with early and active RA (Bathon and Genovese 2003; Klareskog, van der Heijde et al. 2004), ETN and MTX showed similar efficacy in terms of disease activity reduction and improvements in self-reported measures of disability and HRQoL. To our knowledge, however, this is the first study demonstrating a positive effect of both MTX and ETN on objective measures of physical function. This finding gives further support to the proposal of Escalante and colleagues (Escalante, Haas et al. 2004) who suggested the use of performance-based tests for measuring changes in functional limitations in clinical trials for the treatment of RA. This approach is based on a theoretical framework (Escalante and Del Rincon 2002) and should advance our understanding of the complex effects of anti-rheumatic drugs and other therapies on the disablement process in RA patients.

In summary, over a period of six months, there were no changes in body mass and composition in patients with early and active RA treated either ETN or MTX. Further studies are necessary to investigate whether these two agents have similar efficacy as anticatabolic treatments over more prolonged periods of time. The combination of ETN

with other DMARDs also warrants further investigations. Of particular interest is the combination of anti-TNF therapy with biological agents blocking the action of IL-1 as this strategy is more effective than either drugs alone for treating catabolism secondary to adjuvant arthritis (Bendele, Sennello et al. 1999; Bendele, Chlipala et al. 2000; Feige, Hu et al. 2000; Fathalla, Hamada et al. 2004). A recent large clinical trial of combination therapy with ETN and anakinra (a recombinant IL-1 receptor antagonist) proved, however, that this approach is not feasible in RA patients because it significantly increases the incidence of serious infections and other adverse events compared to ETN alone (Genovese, Cohen et al. 2004). On the contrary, the increasingly popular combination of ETN with MTX seems to be safe (Weinblatt, Kremer et al. 1999; Kremer, Weinblatt et al. 2003; Klareskog, van der Heijde et al. 2004) and it would be worth investigating its effects on body composition and metabolism in RA patients.

Further research is also necessary to confirm the apparent ability of TNF-antagonism to normalise the anabolic response to positive energy balance in patients with RA and, more importantly, with other cachectic diseases such as cancer complicated by profound anorexia and weight loss (Ramos, Suzuki et al. 2004). In these conditions, ETN might overcome the anabolic block which have frustrated clinical nutritionists over the years and randomised controlled trials of its combination with hypercaloric feeding or appetite stimulants are now justified.

Table 8
Effects of 24 Weeks of Treatment with either Etanercept or Methotrexate on Body Mass and Composition in Rheumatoid Arthritis Patients

Variable	Etanercept (n = 12)	Methotrexate (n = 12)	P	η^2
Body mass, kg				
Pre	76.4 ± 14.4	73.4 ± 18.9		
Mid	76.6 ± 14.6	73.7 ± 18.3		
Post	77.5 ± 16.1	74.5 ± 18.1	1.00	0.00
Arms lean mass, kg				
Pre	3.50 ± 1.68	3.52 ± 1.29		
Mid	3.73 ± 1.74	3.50 ± 1.27		
Post	3.84 ± 1.89	3.56 ± 1.29	0.05	0.25
Legs lean mass, kg				
Pre	12.3 ± 2.8	12.2 ± 3.0		
Mid	12.5 ± 3.0	12.1 ± 2.9		
Post	12.4 ± 3.1	12.4 ± 3.0	0.08	0.21
Total lean mass, kg				
Pre	41.3 ± 9.7	41.2 ± 8.5		
Mid	42.1 ± 9.6	40.9 ± 8.0		
Post	41.9 ± 10.5	41.3 ± 8.3	0.22	0.13
Total BMC, kg				
Pre	2.50 ± 0.47	2.32 ± 0.47		
Mid	2.47 ± 0.44	2.31 ± 0.46		
Post	2.47 ± 0.43	2.30 ± 0.47	0.68	0.04

Table 8 cont.

Variable	Etanercept (n = 12)	Methotrexate (n = 12)	P	η^2
FFM, kg				
Pre	43.8 ± 10.0	43.5 ± 8.9		
Mid	44.5 ± 10.0	43.2 ± 8.4		
Post	44.3 ± 10.9	43.6 ± 8.6	0.22	0.13
Total fat mass, kg				
Pre	31.7 ± 8.2	28.9 ± 13.8		
Mid	31.3 ± 7.5	29.2 ± 12.6		
Post	32.3 ± 8.5	30.0 ± 13.1	0.68	0.04
Trunk fat mass, kg				
Pre	16.7 ± 5.1	14.4 ± 8.9		
Mid	16.7 ± 4.5	14.9 ± 8.4		
Post	17.2 ± 5.2	15.3 ± 8.4	0.54	0.06
Percent body fat, %				
Pre	42.0 ± 6.8	38.6 ± 9.1		
Mid	41.3 ± 6.1	39.0 ± 8.8		
Post	38.7 ± 12.5	39.5 ± 8.9	0.25	0.12
ECW, L				
Pre	16.1 ± 3.6	15.0 ± 3.5		
Mid	15.8 ± 3.4	14.7 ± 2.9		
Post	15.5 ± 3.5	14.7 ± 2.9	0.63	0.04
ICW, L				
Pre	18.3 ± 5.4	18.2 ± 5.8		
Mid	18.6 ± 5.6	18.0 ± 5.1		
Post	18.7 ± 6.0	16.9 ± 3.8	0.55	0.06

Table 8 cont.

Variable	Etanercept (n = 12)	Methotrexate (n = 12)	P	η^2
TBW, L				
Pre	34.3 ± 8.8	33.2 ± 9.1		
Mid	34.4 ± 8.7	32.6 ± 7.9		
Post	34.2 ± 9.3	31.6 ± 5.5	0.73	0.03
TBW:FFM				
Pre	0.78 ± 0.05	0.76 ± 0.07		
Mid	0.77 ± 0.05	0.75 ± 0.07		
Post	0.77 ± 0.05	0.73 ± 0.09	0.96	0.00
Total body protein, kg				
Pre	6.3 ± 2.4	7.5 ± 2.4		
Mid	7.1 ± 2.1	7.7 ± 2.6		
Post	7.1 ± 2.1	8.3 ± 3.8	0.74	0.03

Note. Pretest, midtest and posttest data are presented as means ± *SD*. The significance level and effects size for each outcome variable refer to the treatment x (time) interaction by a 2x(3) ANOVA. FFM = fat-free mass; ECW = extracellular water; ICW = intracellular water; TBW = total body water.

Table 9
Effects of 24 Weeks of Treatment with either Etanercept or Methotrexate on Disease Activity, Physical Function, Disability, and Health-Related Quality of Life in Rheumatoid Arthritis Patients

Variable	Etanercept (n = 12)	Methotrexate (n = 12)	P	η^2
Disease activity score				
Pre	6.1 ± 0.7	5.8 ± 1.1		
Mid	3.8 ± 1.5	3.4 ± 1.2		
Post	3.2 ± 1.5	3.1 ± 1.4	0.53	0.06
Hand-grip strength, N				
Pre	100 ± 90	82 ± 79		
Mid	178 ± 107	158 ± 92		
Post	171 ± 123	179 ± 120	0.34	0.10
Arm curl test, reps				
Pre	10.8 ± 4.5	11.2 ± 4.2		
Mid	14.3 ± 5.0	16.1 ± 3.8		
Post	16.7 ± 7.0	17.7 ± 3.9	0.60	0.05
Walking speed, feet/min				
Pre	284 ± 125	320 ± 55		
Mid	343 ± 84	358 ± 47		
Post	345 ± 93	377 ± 54	0.23	0.13
Sit-to-stand test, reps				
Pre	7.2 ± 5.1	10.0 ± 2.0		
Mid	12.5 ± 4.2	13.5 ± 2.3		
Post	14.0 ± 4.4	14.7 ± 2.9	0.51	0.06

Table 9 cont.

Variable	Etanercept (n = 12)	Methotrexate (n = 12)	P	η^2
HAQ, 0-3				
Pre	1.9 ± 0.6	1.2 ± 0.7		
Mid	1.2 ± 0.8	0.6 ± 0.7		
Post	1.0 ± 0.9	0.6 ± 0.6	0.38	0.09
Physical health, 0-100				
Pre	28 ± 8	32 ± 11		
Mid	37 ± 13	44 ± 10		
Post	39 ± 12	43 ± 11	0.65	0.04
Mental health, 0-100				
Pre	43 ± 11	44 ± 15		
Mid	52 ± 9	50 ± 12		
Post	50 ± 14	50 ± 18	0.82	0.02

Note. Pretest, midtest and posttest data are presented as means ± *SD*. The significance level and effects size for each outcome variable refer to the treatment x (time) interaction by a 2x(3) ANOVA. HAQ = health assessment questionnaire.

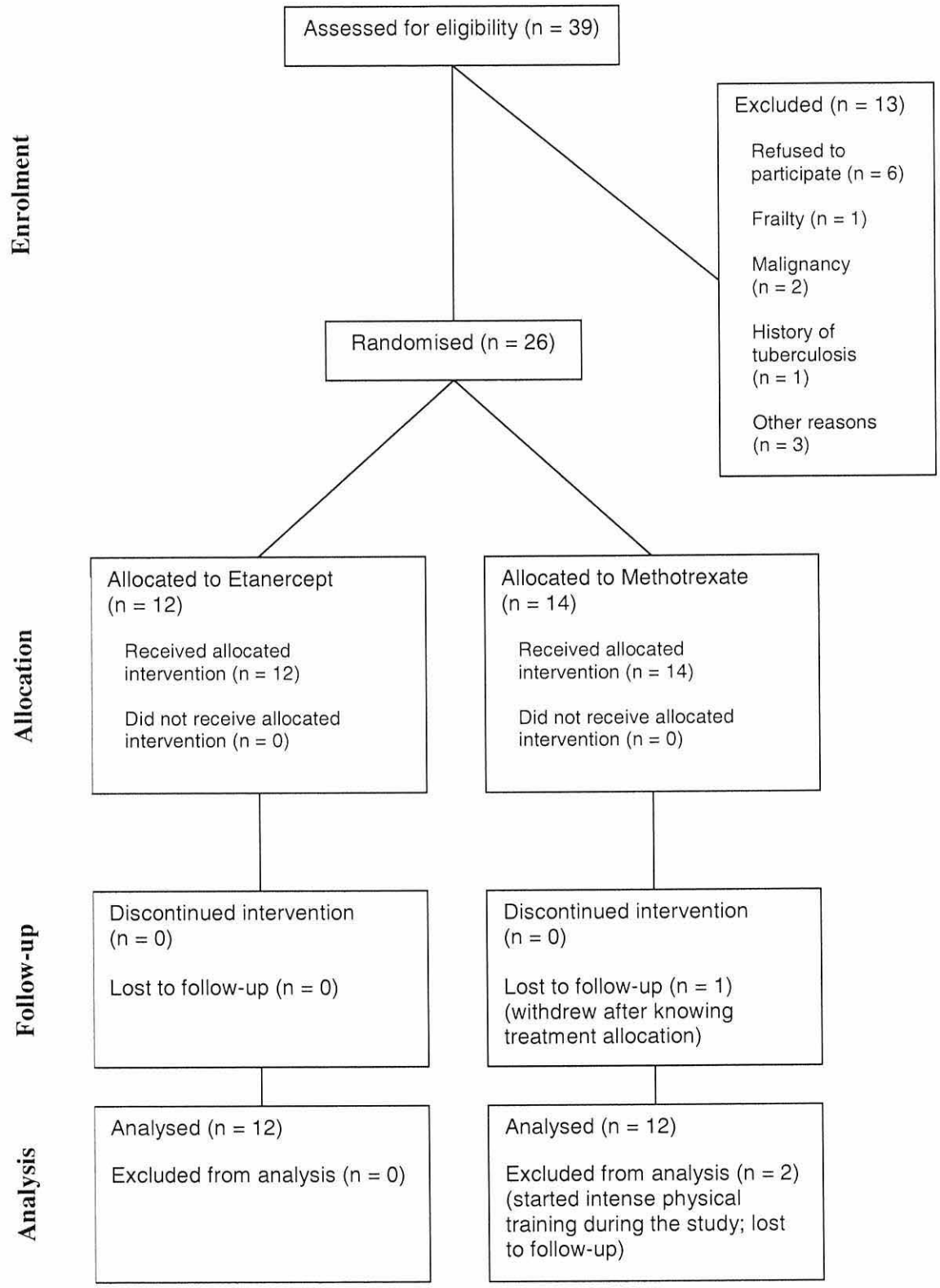


Figure 7. Flow of participants through each stage of the trial.

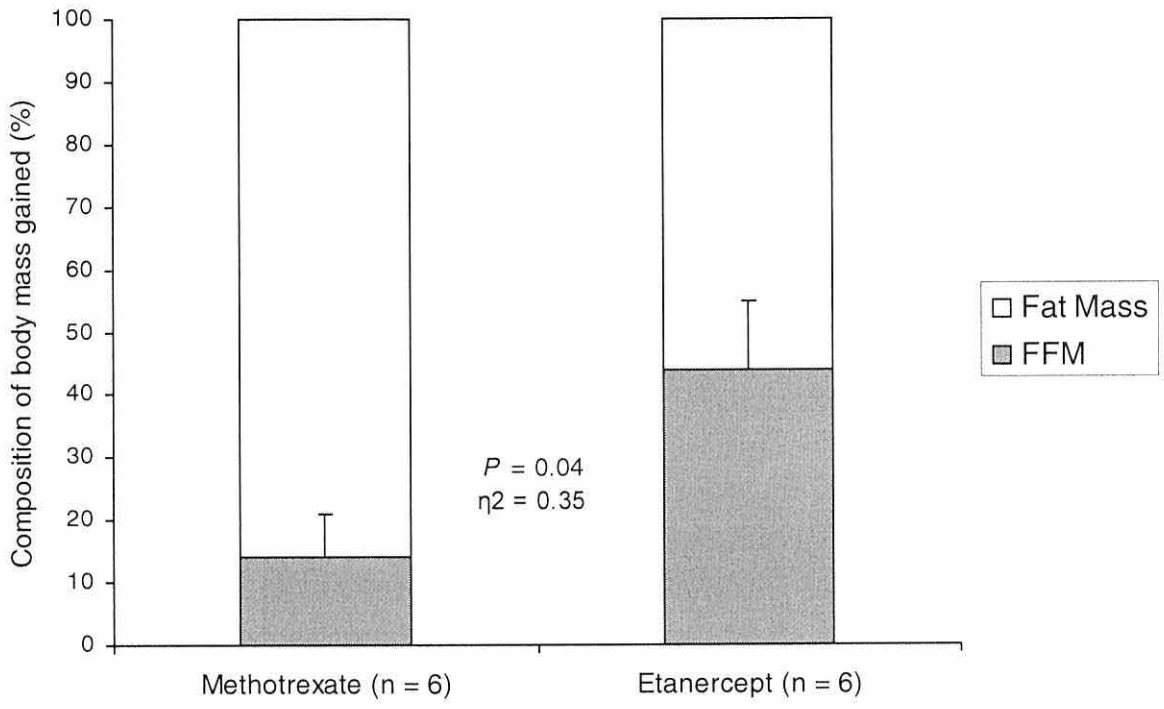


Figure 8. Effect of treatment with methotrexate or etanercept on the composition of the body mass gained (relative fat-free mass [FFM]) in the 12 subjects who had a significant increase in body weight over the six months follow-up period. Data are presented as means ± *SD* and analysed by unpaired *t* test.

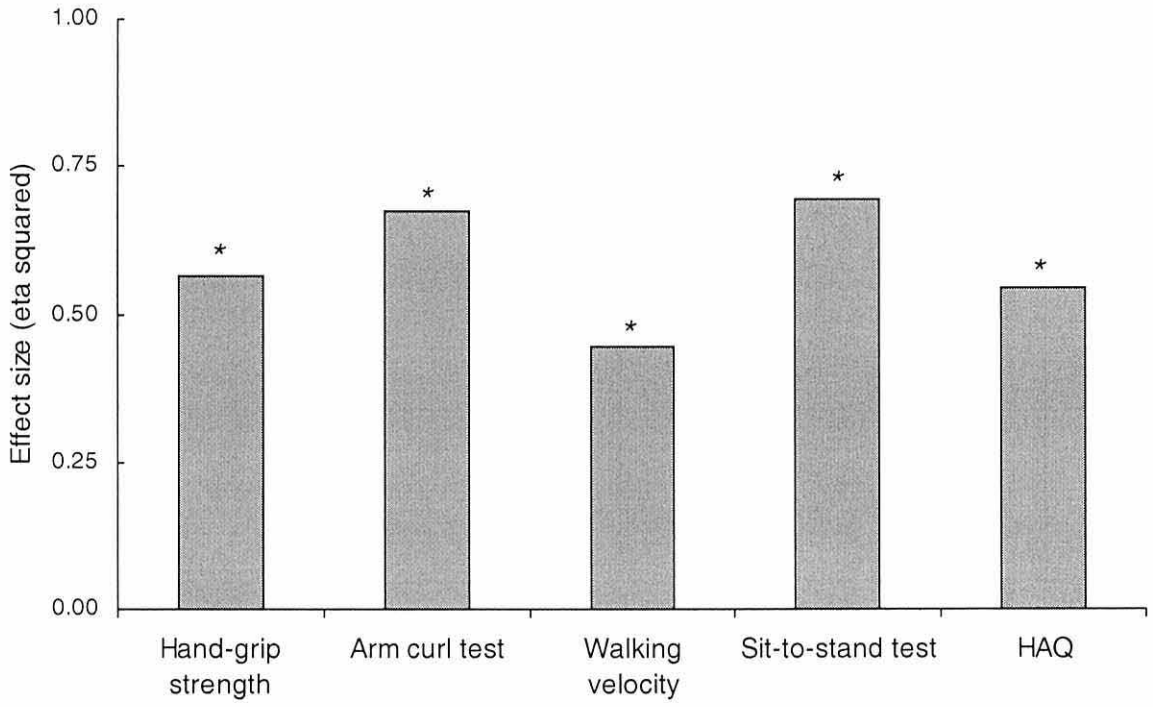


Figure 9. Difference between pretest and posttest in various measures of physical function and disability in response to 24 weeks of treatment with etanercept or methotrexate (pooled data) in early rheumatoid arthritis patients. HAQ = health assessment questionnaire.
* $P < 0.01$ for main effect of time by 2x(3) ANOVA.

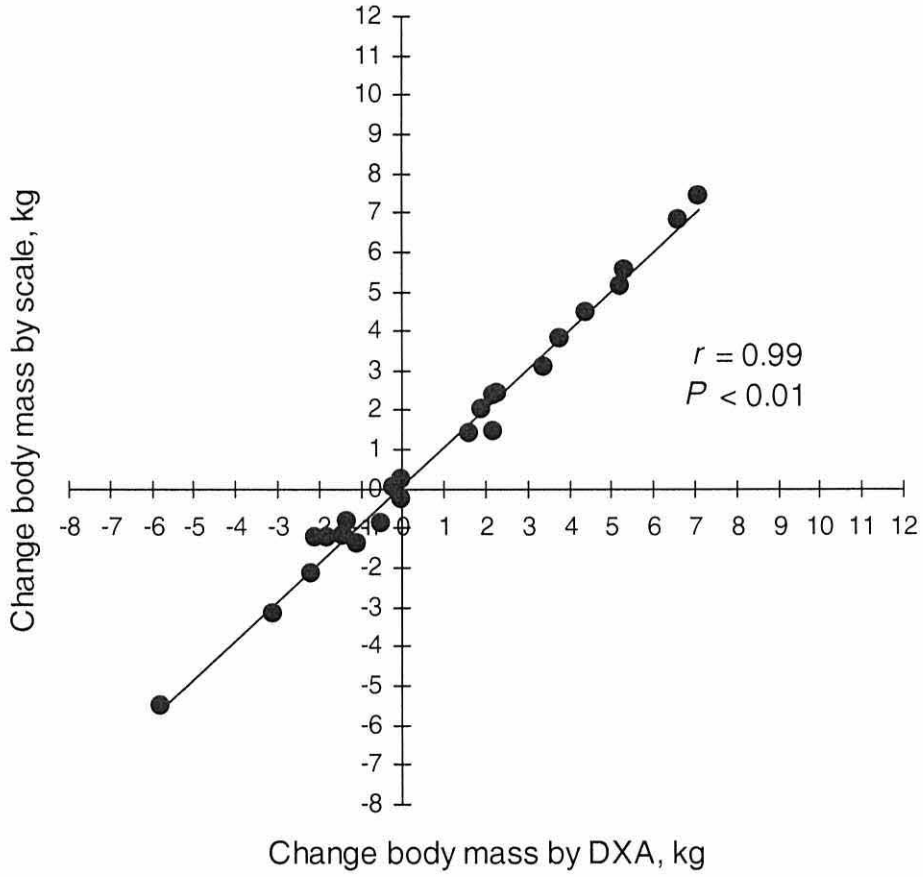


Figure 10. Association between change (posttest – pretest score) in body mass by scale and by dual energy X-ray absorptiometry (DXA) in rheumatoid arthritis patients treated for 24 weeks with either etanercept or methotrexate.

CHAPTER 6

GENERAL DISCUSSION

MAIN FINDINGS

Progressive resistance training with adequate intensity and volume reverses cachexia in rheumatoid arthritis (RA) patients. Although progressive resistance training (PRT) has been shown to be an effective and well-tolerated method of improving physical function in RA patients without exacerbating disease activity (Hakkinen 2004), a previous uncontrolled body composition study failed to demonstrate a significant increase in non-fat cellular body mass (the recommended primary endpoint of clinical trials for the treatment of cachexia (Bone 1999)), in a small group of RA patients (Rall, Meydani et al. 1996). Therefore, current recommendations to include PRT in the management of rheumatoid cachexia (Walsmith and Roubenoff 2002; Zinna and Yarasheski 2003) do not have an evidence base. In this thesis, I present the first evidence that PRT, when adequately prescribed, is effective in reversing muscle protein catabolism in RA patients. The 6% increase in skeletal muscle mass and total body protein found in my study is similar to the one measured in healthy subjects undergoing similar PRT programs (Yarasheski 2003) and rejects the hypothesis of resistance to the anabolic effects of PRT in this population. As the mode, intensity, duration and progression of training were similar to that prescribed by Rall and colleagues (Rall, Meydani et al. 1996), I believe the crucial factor for my positive results was the much higher total volume (576 versus 240 weight lifts per week) and this should be taken into account when implementing PRT with RA patients in clinical practice.

Dietary supplementation with a mixture of β-hydroxy-β-methylbutyrate, glutamine and arginine (HMB/GLN/ARG) does not have pharmacological effects in RA patients. Some authors have suggested that HMB, glutamine and arginine can modulate protein metabolism and that their combination in an oral formula is a safe and effective nutritional treatment for cachexia in chronic diseases (Clark, Feleke et al. 2000; May, Barber et al. 2002; Rathmacher, Nissen et al. 2004). Therefore, I tested the efficacy and safety of this commercially-available dietary supplement in RA patients. As hypothesised, HMB/GLN/ARG was able to stimulate muscle growth and protein accretion in RA patients and this anabolic response was corroborated by some improvement in objective measures of physical function. Contrary to my expectations, however, the nitrogen- and calorie-balanced mixture of other "metabolically inert" amino acids (placebo) had the same effects. These findings argue against any pharmacological effect of dietary supplementation with HMB/GLN/ARG over and above a simple increase in nitrogen intake (i.e., placebo) in RA patients. Although, both dietary supplements were shown to be safe in RA patients but HMB/GLN/ARG was better tolerated than placebo because of less gastrointestinal side effects. As compliance to any nutritional treatment limits its effectiveness (Bruce, Laurance et al. 2003), this apparently slight advantage of HMB/GLN/ARG over the placebo formula has important implications for clinical practice.

Increased nitrogen intake stimulates muscle growth and protein accretion in RA patients. Protein and energy intake is normal in RA patients (Roubenoff, Roubenoff et al. 1992; Roubenoff, Roubenoff et al. 1994; Morgan, Anderson et al. 1997; Gomez-Vaquero, Nolla et al. 2001; Roubenoff, Walsmith et al. 2002) and for this reason it is not thought to be an important cause of cachexia in this population. The results of my second study,

however, challenge this notion. Nutritional treatment with both HMB/GLN/ARG and the placebo mixture of alanine, glutamic acid, glycine and serine caused a significant increase in appendicular lean mass and total body protein in my patients who reported an adequate habitual protein intake. As both dietary supplements provided 7.19 g of nitrogen per day, I believe that the significant increase in total nitrogen intake (from the equivalent of 1.0 to 1.6 g protein/kg body mass/day) is the most likely explanation for the anabolic response I measured in these patients. This is consistent with nutritional studies in other catabolic conditions (Hoffer 2003) and suggests that altered whole-body protein metabolism (Rall, Rosen et al. 1996) and, possibly, augmented first-pass splanchnic extraction because of accellerated protein synthesis of certain acute-phase proteins in the liver (Duff 1994), increase the dietary protein requirements of RA patients. An alternative hypothesis is that glycine and alanine have anabolic properties themselves and doubts remain on the appropriateness of using a mixture containing high doses of these amino acids as a metabolically inert control for the effects of increased nitrogen and calorie intake. Nevertheless, this is the first ever study on the nutritional treatment of cachexia in RA patients (Akner and Cederholm 2001). Interestingly, the relative increase in appendicular lean mass induced by increased nitrogen intake (~ 3%) corresponds to about half of the muscle growth experienced by subjects training with high intensity resistance exercises three times a week over the same period of time (12 weeks) (see Appendix). This result is particularly impressive if we consider that PRT is considered the most effective treatment for cachexia in RA and other diseases (Walsmith and Roubenoff 2002; Zinna and Yarasheski 2003).

Patients with early RA treated with either anti-tumor necrosis factor (TNF) therapy with etanercept (ETN) or standard therapy with methotrexate (MTX) do not loose skeletal muscle mass over a period of six months. Several lines of evidence suggest that TNF and other catabolic cytokines are the main mediators of cachexia in RA and other catabolic conditions (Meguid and Pichard 2003) and, therefore, modulation of TNF bioactivity should be a very effective therapy for counteracting muscle wasting. Unfortunately, clinical trials of various TNF-blocking agents in RA patients have focused only on disease activity, joint damage, disability, and adverse events (Alldred 2001). There is also a report of substantial weight gain in 87% of RA patients treated with TNF-blocking drugs over a period of 11 months (Fonseca, Canhao et al. 2002). However, this could simply reflect body fat accumulation because of improved appetite and general well-being, and not an anabolic response in skeletal muscle tissue. In my third study I addressed this important question by conducting the first human study on the effects of chronic TNF-blockade on body composition. Contrary to what expected, ETN was not superior to standard therapy with MTX in preventing cachexia in patients with early RA as both groups of patients did not experience any accelerated loss of skeletal muscle over a period of six months. Although there was a trend for an increase in appetite in my patients, I could not confirm previous reports of general weight gain in cachectic patients treated with etanercept (Fonseca, Canhao et al. 2002; Steensma, Mesa et al. 2002).

Anti-TNF therapy with ETN normalise the anabolic response to overfeeding in patients with early RA. As changes in fat-free mass (FFM) can be influenced by changes in weight (Hughes, Frontera et al. 2002) and energy intake (Westerterp, Donkers et al. 1995), I conducted a sub-analysis of those RA patients (50%) who gained weight (i.e., were in positive energy balance) over the six months follow-up period (Study 3).

This revealed a significant difference between groups in the composition of the body mass gained, with patients treated with ETN gaining a significantly higher proportion of FFM (44%) than patients treated with MTX (14%). The anabolic response to overfeeding in patients treated with ETN was similar to the one expected in healthy individuals of similar body composition (Forbes 2000) whereas the little relative amount of FFM gained by patients treated with MTX is consistent with previous observations in patients with other catabolic disease treated with hypercaloric feeding or appetite stimulants (Cohn, Vartsky et al. 1982; Streat, Beddoe et al. 1987; Strang 1997; Kotler 2000). This novel finding suggests that the combination of ETN with nutritional treatment might overcome the anabolic block which have frustrated clinical nutritionists over the years.

Anti-rheumatic therapy with either ETN or MTX improves objective measures of physical function in patients with early RA. Another novel finding presented in my thesis is the positive effect of both MTX and ETN on objective measures of physical function in patients with early RA. A significant improvement in performance in all four tests employed, particularly the ones involving repeated muscular contractions, was evident at week 12 and sustained at week 24 and paralleled changes in disease activity. Although similar tests were used in the past as endpoints in clinical trials for the treatment of RA, in recent years they have been replaced by self-reported measures of physical function such as the disability index of the Health Assessment Questionnaire (Pincus, Callahan et al. 1989). However, a better understanding of the effects of treatment on the disability process in RA patients requires a proper theoretical framework and valid/reliable measures of *both* functional limitations *and* self-reported difficulties in activities of daily living (Escalante, Haas et al. 2004). My results provide evidence that

the objective measures of physical function implemented in Study 3 could serve this purpose as they are sensitive to changes in disease activity over time.

STRENGTHS AND WEAKNESSES OF THE RESEARCH PROGRAM

Internal Validity

Internal validity in controlled clinical trials refers to ensuring that the differences observed between groups of patients allocated to different interventions can, apart from random error, be attributed only to the treatment under investigation. Its main threats are 1) biased allocation to comparison groups (selection bias), 2) unequal provision of care apart from treatment under evaluation (performance bias), 3) biased assessment of outcome (detection bias), 4) biased occurrence and handling of deviations from protocol and loss to follow-up (attrition bias) (Juni, Altman et al. 2001).

In Studies 2 and 3, subjects were randomly assigned to treatment following recommended procedures and this controlled for selection bias. In Study 1, however, subjects in the training group were conveniently selected on the basis of their willingness and ability to take part in the demanding PRT program that was prescribed. Subjects in the control group were matched for age, gender and disease severity/activity, and other subject characteristics which might have had an effect on the outcome of the study (e.g., baseline body composition and functional status, dietary intake and habitual physical activity) were measured and did not differ between groups. Nonetheless, this approach can not control for other unrecognised potential confounding factors. For example, subjects more likely to participate in sports and leisure-time physical activities might have different genetic characteristics compared to sedentary subjects (Maia, Thomis et al. 2002). Nevertheless, spontaneous changes in body composition such as those measured in

the subjects who underwent the prescribed PRT program have not been reported in the literature and seem highly unlikely.

Another possible threat to the internal validity of Study 1 is performance bias as patients in the training group received, in addition to the treatment under investigation (PRT), my attention as their personal trainer 2/3 times a week. This might have had an effect on its own, especially on disability, psychological status and disease activity (Latham, Bennett et al. 2004). However, it could be argued that supervised PRT is a complex intervention in which interaction with the care provider is an integral part. There is also no evidence that increased personal attention can induce muscle growth, the primary endpoint of this clinical trial. A more serious threat to the internal validity of Study 1 is that I was also responsible for outcome assessment. Although every measure was taken to ensure objectivity and standardize testing procedures, being aware of treatment allocation might have caused me to unconsciously influence outcome assessment in favour of my research hypothesis. This argument is particularly valid for physical function measures and, partially, for regional body composition measures. However, the results of total body composition assessment can not be influenced by the operator and, therefore, detection bias is controlled for this major outcome. Patients' knowledge of treatment allocation might have also influenced self-reported measures and physical function tests performance. Study 2 was double-blind which controls for both performance and detection bias. Study 3, however, was only partially blinded: patients and the clinician responsible for their management and for assessing disease activity were aware of treatment allocation, while the assessors of body composition and physical function were not. Therefore, the internal validity of the primary outcomes was guaranteed in this study.

Protocol deviations and loss to follow-up did not occur in Study 1. In Study 2, attrition bias was minimal as loss to follow-up was only 10% and balanced across groups, and all remaining patients, regardless of their adherence to dietary supplementation, were included in the statistical analysis (Schulz and Grimes 2002). This intention-to-treat approach to data analysis was chosen for Study 2 not only to control for attrition bias, but because compliance to nutritional treatment has important implications for its efficacy in clinical practice (Bruce, Laurance et al. 2003). In Study 3, a different approach was used and two patients (both in the MTX group) were excluded from the final analysis: one subject withdrew from the study immediately after baseline assessment when allocated to MTX rather than ETN; the other violated the protocol by starting an intense and regular physical training program during the intervention period. As the main aim of this study was to investigate the physiological effects of TNF-blockade rather than its efficacy in clinical practice, an intention-to-treat analysis was not deemed appropriate.

So far I have discussed the internal validity of controlled experiments. Evidence for the anabolic and functional effects of increased nitrogen intake in RA patients in Study 2 is based, however, on an uncontrolled observation. This one-group, pretest-posttest, design is weak as some event other than treatment may have occurred during the intervention period (history). However, subjects participating in this investigation did not report significant changes in habitual dietary intake or other important protocol violations (e.g., taking anabolic drugs). Thirteen out of 36 participants reported an increase in habitual physical activity but none started an intense and regular physical training program during the intervention period. Therefore, I believe it is unlikely that the significant increase in total body protein is consequent to anything other than the substantial increase in dietary nitrogen intake that both oral amino acids mixtures ensured. This conclusion is also corroborated by the observation that average daily

nitrogen balance calculated from actual changes in body composition fits well with the that predicted from the average nitrogen and non-protein energy intake of my subjects using a mathematical model developed in another population of malnourished individuals (Radrizzani, Iapichino et al. 1986). Another rival hypothesis is maturation. However, I think this is highly unlikely as substantial losses of total body protein occur in patients with chronic diseases and aging individuals (Hansen, Raja et al. 2000). This is confirmed by the absence of any significant change in body composition within the control group of Study 1 (see Appendix). These patients were very similar to the ones included in Study 2 and were followed-up for the same period of time (12 weeks) using the same measurement methods. Therefore, they can provide an indirect control for Study 1. Also, baseline body composition assessment can not have any conceivable effect on body composition at follow-up. However, a testing threat to internal validity is possible for self-reported measures and, more importantly, objective measures of physical function and should be taken into account when interpreting the results of Study 2.

Finally, it could be argued that the symptomatic use of non-steroidal anti-inflammatory drugs (NSAIDs) and analgesics was a potential confounding factor in all studies as these agents have been shown to have some anti-catabolic effects in other cachectic conditions (McMillan, Wigmore et al. 1999; Barratt, Smith et al. 2002; Holte and Kehlet 2002). However, the presence of cachexia in RA patients with long-standing disease suggests that NSAIDs and analgesics are not very effective in this population (Walsmith and Roubenoff 2002). Additionally, this threat to internal validity is of particular concern for Study 3 rather than Studies 1 and 2 as no anabolic response to these drugs has been reported in the literature. Nonetheless, future studies of anabolic and anticatabolic interventions for the treatment of cachexia in RA patients should control and/or monitor the use of NSAIDs and analgesics.

External Validity

External validity refers to the generalisability of the results of a clinical trial to other patient populations, settings, treatment regimens and outcome measures (Juni, Altman et al. 2001). I believe that one of the major strengths of my doctoral research program is the variety of the therapeutic approaches investigated: two anabolic, non-pharmacological interventions (PRT and dietary supplementation with HMB/GLN/ARG) and one anti-catabolic, pharmacological intervention (anti-TNF therapy with ETN). I also included two different populations: patients with long-standing but well controlled RA, and RA patients with early and active disease. However, PRT and nutritional treatment with anabolic amino acids were tested only in RA patients with long-standing but well controlled disease, while the effects of TNF-blockade with ETN were investigated only in patients with early and active RA. Therefore, an interaction of selection bias and experimental treatment could prevent generalisation of the specific results of each study to RA patients with other disease characteristics. Similarly, the efficacy and safety of these interventions over longer periods of time and within different settings (e.g. primary care or home exercise) is not known.

Although I recognise these threats to the external validity of my doctoral research program, I believe, like others (Gosker, Wouters et al. 2000; Kotler 2000; Langhans 2002), that the pathogenic mechanisms driving cachexia in RA and other conditions are independent of the primary disease process and that, as such, a generalised therapeutic approach can be implemented. Hence, RA could serve as a convenient human model of chronic hypercytokinemic muscle wasting and lessons learned from clinical trials for the treatment of rheumatoid cachexia could be useful for the management of this metabolic complication in patients suffering from other cachectic diseases such as cancer, HIV-

AIDS, chronic obstructive obstructive pulmonary disease (COPD), congestive heart failure (CHF), and uraemia.

Measurement and Statistical Validity

Dual-energy X-ray absorptiometry (DXA) has become the method of choice for measuring the impact of different therapeutic regimens on body composition (Albanese, Diessel et al. 2003). The bioelectrical impedance spectroscopy (BIS) used in my studies, which is based on the 0/infinity-kHz parallel (Cole-Cole) model, is also a valid alternative to more problematic and expensive dilution techniques for assessing changes in changes in total body water (TBW) and its subcompartments (Gudivaka, Schoeller et al. 1999). A major strength of my doctoral research program was the combined use of these two different methods. Dual-energy X-ray absorptiometry measures three major components of the body at molecular level: fat mass, lean mass, and bone mineral mass (also called BMC). However, it cannot distinguish between the two molecular subcomponents of lean mass: water and protein. Studies in haemodialysis patients suggest that DXA accurately measure changes in the water content of the body as changes in lean mass (Stenver, Gotfredsen et al. 1995; Georgiou, Virvidakis et al. 1997). Although this confirms its robustness to violations of one of its main assumptions, a constant FFM hydration (Pietrobelli, Wang et al. 1998), it also suggests that changes in total lean mass cannot be interpreted as changes in protein mass without a concurrent measure of TBW. Because proteins are the functional components of lean mass, being able to make this distinction is crucial when assessing the efficacy of various anabolic and anticatabolic interventions in cachectic diseases. As patients with systemic diseases often suffer water retention (Sergi, Bussolotto et al. 1994), the ability of BIS to differentiate between intracellular and extracellular water also adds to the measurement validity of my studies. According to

current guidelines for measuring changes in body composition, DXA estimates of total body mass must accurately reflect both baseline and posttreatment body weight by scale (Lohman, Harris et al. 2000). This is illustrated for my studies in Figures 3, 6, and 10. Previous studies in other frail and catabolic populations have shown significant changes in body composition in response to either nutritional or exercise interventions over a period of 8 weeks (Fiatarone, Marks et al. 1990; Clark, Feleke et al. 2000). Therefore, the 12 weeks follow-up period chosen for Study 1 and 2 seemed appropriate. However, observational studies of RA patients treated with standard drugs have shown significant changes in body composition after 12 months but not six months (Kennedy, Boddy et al. 1979; Westhovens 1999). Therefore, the follow-up period of Study 3 might be inadequate to test the hypothesised superiority of anti-TNF therapy with ETN over standard therapy with MTX.

The objective and subjective measures of physical function and disease activity used in my studies are also well validated in RA patients or similar populations and reliable (Stucki, Liang et al. 1995; Stucki, Bruhlmann et al. 1998; Keller, Majkut et al. 1999; Bruce and Fries 2003; Escalante, Haas et al. 2004). For the first two studies a rheumatologist was not available for physical assessment. Therefore, I decided to use the Rheumatoid Arthritis Disease Activity Index (RADAI), a self-reported measure of global disease activity, joint pain, tenderness, and stiffness, rather than physician-reported outcome measures. This should not be considered a limitation as patient-reported outcome measures seem to be better than traditional measures of disease activity in discriminating active treatment from placebo in randomized controlled trials in RA (Strand, Cohen et al. 2004).

The assumptions of all statistical tests used for analysing the data included in this thesis were tested and met. Thanks to the high precision of both DXA and BIS (Ellis

2000), the sample size of my studies was large enough to prove statistical significance of relatively small changes in body composition, so type II errors were unlikely to have occurred. More concerning is the likelihood of a type I error when multiple outcomes are measured within a study. Although the classical view is to divide the desired alpha level by the number of separate statistical tests performed (Bonferroni's method), I believe this approach is too stringent for small-sized clinical trials like the ones included in my doctoral research program. Instead, I choose and declared in advance one or two primary endpoints (Feise 2002). When adequate, I have also used composite outcome measures (e.g., disease activity scores) and reported exact *P* values and eta sqaured, a standardised measure of effect size, so that the conscientious readers could make his/her own mind about the statistical and clinical significance of the results presented in this thesis.

CONCLUSIONS AND DIRECTIONS FOR FUTURE RESEARCH

Overall, the answer to my initial research question seems to be positive: readily available anabolic and anticatabolic interventions are safe, effective and well tolerated in RA patients. However, much more research is necessary before clear, evidence-based recommendations for the management of rheumatoid cachexia can be made.

Because of the methodological problems associated with non-random allocation to treatment, Study 1 needs to be followed by a Phase III trial. In fact, the useful data provided by the pilot study included in this thesis served as basis for a successful grant application to the Arthritis Research Campaign and the funded Phase III trial has recently commenced. Pending the results of this randomised controlled trial, high volume and intensity PRT should become the treatment of choice for rheumatoid cachexia as speculatively recommended by Roubenoff's group (Walsmith and Roubenoff 2002) and other authors (Zinna and Yarasheski 2003). Nonetheless, other studies are necessary to

assess the long-term safety of this intervention in terms of articular erosion of large joints and to investigate the effects on body composition of PRT programs more feasible in the long term such as that proposed by Hakkinen and colleagues (Hakkinen, Sokka et al. 2004). It is also necessary to assess the efficacy and safety of high intensity and high volume PRT in patients with more severe and active RA.

In agreement with clinical trials conducted in patients with other cachectic diseases (Clark, Feleke et al. 2000; May, Barber et al. 2002; Rathmacher, Nissen et al. 2004), the oral mixture of HMB, glutamine and arginine proved to be a well-tolerated and effective nutritional treatment of cachexia in RA patients. However, the results of my second study suggest that a simple increase in nitrogen intake via diet modifications or protein supplements might be equally effective. Controlled clinical trials of these cheaper nutritional interventions are urgent as dietary supplements like HMB/GLN/ARG are not provided free at point of delivery by the NHS. Further nutritional and metabolic studies should also assess whether protein requirements are increased above normal in RA patients. This is particularly relevant as many RA patients reduce or eliminate their intake of animal proteins in the attempt to control pain and other joint symptoms (Kjeldsen-Kragh 1999) and this could contribute to the development of cachexia in this population.

Although RA patients seem to respond well to the anabolic stimuli provided by PRT and increased nitrogen intake with oral amino acids, the results of this doctoral research program have not provided enough evidence to support the use of anti-TNF therapy with ETN in place of MTX for the management of muscle wasting in RA patients with early and active disease. Even if it is possible that anti-TNF therapy has superior anti-catabolic effects compared to standard disease-modifying anti-rheumatic drugs when studied over more prolonged periods of time, this remains only an attractive hypothesis. In the context of the anabolic stimulus provided by overfeeding, however, ETN seems

superior to MTX in facilitating conversion of excess energy into FFM rather than fat mass. Although not clinically relevant for the treatment of cachexia in most RA patients, who have increased adiposity and related cardiovascular complications (Watson, Rhodes et al. 2003), this novel property of ETN should be further investigated in well-designed nutritional studies. If confirmed by these studies, the ability of anti-TNF therapy to improve metabolic efficiency could be exploited in those situations characterised by systemic inflammation, anorexia and weight loss such as malignancy, HIV-AIDS, COPD, CHF, and chronic renal failure (Kotler 2000). In this context hypercaloric feeding or appetite stimulants are appropriate and their combination with TNF-blocking agents might solve the poor anabolic response who has frustrated clinical nutritionists over the years (Cohn, Vartsky et al. 1982; Streat, Beddoe et al. 1987; Strang 1997; Kotler 2000).

This finding also suggests that the best approach to cachexia management could be the combination of different interventions. Given the results of my doctoral research program, an obvious combination in RA patients would be PRT and dietary protein supplementation. However, this strategy has not been successful in other catabolic conditions (Fiatarone, O'Neill et al. 1994; Agin, Gallagher et al. 2001). Other interesting therapeutic combinations which, in my opinion, deserve specific clinical trials in RA patients are PRT and anabolic hormones (Bhasin, Storer et al. 2000; Lange, Andersen et al. 2002), and caloric restriction for weight loss with either PRT (Donnelly, Sharp et al. 1993), GH (Norrelund, Borglum et al. 2000) or anabolic steroids (Lovejoy, Bray et al. 1996). These last three combinations are particularly relevant to conditions like RA in which muscle atrophy is often combined with increased body fat (i.e., cachectic obesity).

A useful addition to the recommended outcome measures in clinical trials for the treatment of cachexia would be the investigation of the molecular, cellular, endocrine and metabolic mechanisms underlying the expected changes in body composition. This would

not only strengthen the evidence in favour of these anabolic and anticatabolic therapies, but also help us to understand the pathogenesis of rheumatoid cachexia. Similarly, assessment of "muscle quality" (muscle architecture and composition, single muscle fibre composition and function, neuromuscular activation and control) in response to PRT (Reeves, Narici et al. 2004; Trappe, Trappe et al. 2004) and other interventions would add considerable information to the simple measurement of "muscle quantity" with body composition techniques.

In conclusion, the results of my doctoral research program suggest that rheumatoid cachexia is a reversible systemic complication of RA. As cachexia is associated with impaired physical function and risk of premature death, further research and inclusion of these and other anabolic and anticatabolic therapies in the overall management of RA should improve the long term outcome of this disabling disease.

REFERENCES

Abe, T., Y. Kawakami, et al. (1997). "Effects of 20 days bed rest on muscle morphology." J Gravit Physiol **4**(1): S10-4.

Adams, G. R. (2002). "Invited Review: Autocrine/paracrine IGF-I and skeletal muscle adaptation." J Appl Physiol **93**(3): 1159-67.

Agin, D., D. Gallagher, et al. (2001). "Effects of whey protein and resistance exercise on body cell mass, muscle strength, and quality of life in women with HIV." Aids **15**(18): 2431-40.

Akner, G. and T. Cederholm (2001). "Treatment of protein-energy malnutrition in chronic nonmalignant disorders." Am J Clin Nutr **74**(1): 6-24.

Albanese, C. V., E. Diessel, et al. (2003). "Clinical applications of body composition measurements using DXA." J Clin Densitom **6**(2): 75-85.

Allan, C. A. and R. I. McLachlan (2004). "Age-related changes in testosterone and the role of replacement therapy in older men." Clin Endocrinol (Oxf) **60**(6): 653-70.

Alldred, A. (2001). "Etanercept in rheumatoid arthritis." Expert Opin Pharmacother **2**(7): 1137-48.

Allen, C., P. Glasziou, et al. (1999). "Bed rest: a potentially harmful treatment needing more careful evaluation." Lancet **354**(9186): 1229-33.

Alvarez, B., L. S. Quinn, et al. (2002). "TNF-alpha modulates cytokine and cytokine receptors in C2C12 myotubes." Cancer Lett **175**(2): 181-5.

Argiles, J. M., R. Moore-Carrasco, et al. (2003). "Catabolic mediators as targets for cancer cachexia." Drug Discov Today **8**(18): 838-44.

Arnett, F. C., S. M. Edworthy, et al. (1988). "The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis." Arthritis Rheum **31**(3): 315-24.

Arora, N. S. and D. F. Rochester (1982). "Effect of body weight and muscularity on human diaphragm muscle mass, thickness, and area." J Appl Physiol **52**(1): 64-70.

Arora, N. S. and D. F. Rochester (1982). "Respiratory muscle strength and maximal voluntary ventilation in undernourished patients." Am Rev Respir Dis **126**(1): 5-8.

Barratt, S. M., R. C. Smith, et al. (2002). "Multimodal analgesia and intravenous nutrition preserves total body protein following major upper gastrointestinal surgery." Reg Anesth Pain Med **27**(1): 15-22.

Barrera, P., A. M. Boerbooms, et al. (1996). "Effects of antirheumatic agents on cytokines." Semin Arthritis Rheum **25**(4): 234-53.

Basaria, S., J. T. Wahlstrom, et al. (2001). "Clinical review 138: Anabolic-androgenic steroid therapy in the treatment of chronic diseases." J Clin Endocrinol Metab **86**(11): 5108-17.

Bathon, J. M. and M. C. Genovese (2003). "The Early Rheumatoid Arthritis (ERA) trial comparing the efficacy and safety of etanercept and methotrexate." Clin Exp Rheumatol **21**(5 Suppl 31): S195-7.

Baumgartner, R. N. (2000). "Body composition in healthy aging." Ann N Y Acad Sci **904**: 437-48.

Bearne, L. M., D. L. Scott, et al. (2002). "Exercise can reverse quadriceps sensorimotor dysfunction that is associated with rheumatoid arthritis without exacerbating disease activity." Rheumatology (Oxford) **41**(2): 157-66.

Bendele, A., G. Sennello, et al. (1999). "Effects of interleukin 1 receptor antagonist alone and in combination with methotrexate in adjuvant arthritic rats." J Rheumatol **26**(6): 1225-9.

Bendele, A. M., E. S. Chlipala, et al. (2000). "Combination benefit of treatment with the cytokine inhibitors interleukin-1 receptor antagonist and PEGylated soluble tumor necrosis factor receptor type I in animal models of rheumatoid arthritis." Arthritis Rheum **43**(12): 2648-59.

Berry, J. K. and C. Baum (2004). "Reversal of chronic obstructive pulmonary disease-associated weight loss : are there pharmacological treatment options?" Drugs **64**(10): 1041-52.

Beutler, B., D. Greenwald, et al. (1985). "Identity of tumour necrosis factor and the macrophage-secreted factor cachectin." Nature **316**(6028): 552-4.

Bhasin, S., T. W. Storer, et al. (2000). "Testosterone replacement and resistance exercise in HIV-infected men with weight loss and low testosterone levels." Jama **283**(6): 763-70.

Bhasin, S., L. Woodhouse, et al. (2003). "Androgen effects on body composition." Growth Horm IGF Res **13 Suppl A**: S63-71.

Bird, H. A., L. Burkinshaw, et al. (1987). "A controlled trial of nandrolone decanoate in the treatment of rheumatoid arthritis in postmenopausal women." Ann Rheum Dis **46**(3): 237-43.

Boelens, P. G., R. J. Nijveldt, et al. (2001). "Glutamine alimentation in catabolic state." J Nutr **131**(9 Suppl): 2569S-77S; discussion 2590S.

Bone, H. G. (1999). "Summary remarks." J Nutr **129**(1S Suppl): 317S-318S.

Booji, A., C. M. Biewenga-Booji, et al. (1996). "Androgens as adjuvant treatment in postmenopausal female patients with rheumatoid arthritis." Ann Rheum Dis **55**(11): 811-5.

Boonen, A., J. van de Rest, et al. (1997). "How Renoir coped with rheumatoid arthritis." Bmj **315**(7123): 1704-8.

Bornstein, S. R., H. Rutkowski, et al. (2004). "Cytokines and steroidogenesis." Mol Cell Endocrinol **215**(1-2): 135-41.

Borst, S. E., Y. Lee, et al. (2004). "Neutralization of Tumor Necrosis Factor Alpha Reverses Insulin Resistance in Skeletal Muscle, but not Adipose Tissue." Am J Physiol Endocrinol Metab.

Breuille, D., L. Voisin, et al. (1999). "A sustained rat model for studying the long-lasting catabolic state of sepsis." Infect Immun **67**(3): 1079-85.

Bross, R., R. Casaburi, et al. (1998). "Androgen effects on body composition and muscle function: implications for the use of androgens as anabolic agents in sarcopenic states." Baillieres Clin Endocrinol Metab **12**(3): 365-78.

Bruce, B. and J. F. Fries (2003). "The Stanford Health Assessment Questionnaire: a review of its history, issues, progress, and documentation." J Rheumatol **30**(1): 167-78.

Bruce, D., I. Laurance, et al. (2003). "Nutritional supplements after hip fracture: poor compliance limits effectiveness." Clin Nutr **22**(5): 497-500.

Burns, C. P., S. Halabi, et al. (2004). "Phase II study of high-dose fish oil capsules for patients with cancer-related cachexia." Cancer **101**(2): 370-8.

Carbo, N., J. Lopez-Soriano, et al. (2001). "Interleukin-15 mediates reciprocal regulation of adipose and muscle mass: a potential role in body weight control." Biochim Biophys Acta **1526**(1): 17-24.

Carbo, N., J. Lopez-Soriano, et al. (2000). "Interleukin-15 antagonizes muscle protein waste in tumour-bearing rats." Br J Cancer **83**(4): 526-31.

Carroll, P. V. (2001). "Treatment with growth hormone and insulin-like growth factor-I in critical illness." Best Pract Res Clin Endocrinol Metab **15**(4): 435-51.

Castaneda, C., P. L. Gordon, et al. (2001). "Resistance training to counteract the catabolism of a low-protein diet in patients with chronic renal insufficiency. A randomized, controlled trial." Ann Intern Med **135**(11): 965-76.

Cegielski, J. P. and D. N. McMurray (2004). "The relationship between malnutrition and tuberculosis: evidence from studies in humans and experimental animals." Int J Tuberc Lung Dis **8**(3): 286-98.

Chai, J., Y. Wu, et al. (2003). "Role of ubiquitin-proteasome pathway in skeletal muscle wasting in rats with endotoxemia." Crit Care Med **31**(6): 1802-7.

Chang, R., R. Roubenoff, et al. (2003). "Work Group recommendations: 2002 Exercise and Physical Activity Conference, St Louis, Missouri." Arthritis Rheum **49**(2): 280.

Clark, M. A., L. D. Plank, et al. (1998). "Effect of a chimeric antibody to tumor necrosis factor-alpha on cytokine and physiologic responses in patients with severe sepsis--a randomized, clinical trial." Crit Care Med **26**(10): 1650-9.

Clark, R. H., G. Feleke, et al. (2000). "Nutritional treatment for acquired immunodeficiency virus-associated wasting using beta-hydroxy beta-methylbutyrate, glutamine, and arginine: a randomized, double-blind, placebo-controlled study." JPEN J Parenter Enteral Nutr **24**(3): 133-9.

Clark, W., P. Jobanputra, et al. (2004). "The clinical and cost-effectiveness of anakinra for the treatment of rheumatoid arthritis in adults: a systematic review and economic analysis." Health Technol Assess **8**(18): iii-iv, ix-x, 1-105.

Coblyn, J. S., N. Shadick, et al. (2001). "Leflunomide-associated weight loss in rheumatoid arthritis." Arthritis Rheum **44**(5): 1048-51.

Cohn, S. H., D. Vartsky, et al. (1982). "Changes in body composition of cancer patients following combined nutritional support." Nutr Cancer **4**(2): 107-19.

Collins, R., Jr., T. L. Dunn, et al. (1987). "Malnutrition in rheumatoid arthritis." Clin Rheumatol **6**(3): 391-8.

Costelli, P. and F. M. Baccino (2003). "Mechanisms of skeletal muscle depletion in wasting syndromes: role of ATP-ubiquitin-dependent proteolysis." Curr Opin Clin Nutr Metab Care **6**(4): 407-12.

Costelli, P., N. Carbo, et al. (1993). "Tumor necrosis factor-alpha mediates changes in tissue protein turnover in a rat cancer cachexia model." J Clin Invest **92**(6): 2783-9.

Crawford, B. A., P. Y. Liu, et al. (2003). "Randomized placebo-controlled trial of androgen effects on muscle and bone in men requiring long-term systemic glucocorticoid treatment." J Clin Endocrinol Metab **88**(7): 3167-76.

Cui, X. L., M. Iwasa, et al. (1999). "Effects of dietary arginine supplementation on protein turnover and tissue protein synthesis in scald-burn rats." Nutrition **15**(7-8): 563-9.

Cutolo, M., A. Sulli, et al. (2001). "Anti-inflammatory mechanisms of methotrexate in rheumatoid arthritis." Ann Rheum Dis **60**(8): 729-35.

Cutolo, M., B. Villaggio, et al. (2002). "Sex hormones and rheumatoid arthritis." Autoimmun Rev **1**(5): 284-9.

Daly, J. M., J. Reynolds, et al. (1988). "Immune and metabolic effects of arginine in the surgical patient." Ann Surg **208**(4): 512-23.

Danneskiold-Samsoe, B. and G. Grimby (1986). "The influence of prednisone on the muscle morphology and muscle enzymes in patients with rheumatoid arthritis." Clin Sci (Lond) **71**(6): 693-701.

Danneskiold-Samsoe, B. and G. Grimby (1986). "Isokinetic and isometric muscle strength in patients with rheumatoid arthritis. The relationship to clinical parameters and the influence of corticosteroid." Clin Rheumatol **5**(4): 459-67.

Danneskiold-Samsoe, B. and G. Grimby (1986). "Muscle morphology and enzymes in proximal and distal muscle groups of lower limb from patients with corticosteroid treated rheumatoid arthritis: the relationship to maximal isokinetic muscle strength." Clin Sci (Lond) **71**(6): 685-91.

Danneskiold-Samsoe, B. and G. Grimby (1986). "The relationship between the leg muscle strength and physical capacity in patients with rheumatoid arthritis, with reference to the influence of corticosteroids." Clin Rheumatol **5**(4): 468-74.

Darmaun, D., D. E. Matthews, et al. (1988). "Physiological hypercortisolemia increases proteolysis, glutamine, and alanine production." Am J Physiol **255**(3 Pt 1): E366-73.

De Bandt, J. P. and L. A. Cynober (1998). "Amino acids with anabolic properties." Curr Opin Clin Nutr Metab Care **1**(3): 263-72.

de Jong, Z., M. Munneke, et al. (2003). "Is a long-term high-intensity exercise program effective and safe in patients with rheumatoid arthritis? Results of a randomized controlled trial." Arthritis Rheum **48**(9): 2415-24.

de Palma, L., C. Chillemi, et al. (2000). "Muscle involvement in rheumatoid arthritis: an ultrastructural study." Ultrastruct Pathol **24**(3): 151-6.

D'Elia, H. F., L. A. Mattsson, et al. (2003). "Hormone replacement therapy in rheumatoid arthritis is associated with lower serum levels of soluble IL-6 receptor and higher insulin-like growth factor 1." Arthritis Res Ther **5**(4): R202-9.

Demay, M. (2003). "Muscle: a nontraditional 1,25-dihydroxyvitamin D target tissue exhibiting classic hormone-dependent vitamin D receptor actions." Endocrinology **144**(12): 5135-7.

Demir, H., F. Kelestimur, et al. (1999). "Hypothalamo-pituitary-adrenal axis and growth hormone axis in patients with rheumatoid arthritis." Scand J Rheumatol **28**(1): 41-6.

Denko, C. W., B. Boja, et al. (1996). "Growth factors, insulin-like growth factor-1 and growth hormone, in synovial fluid and serum of patients with rheumatic disorders." Osteoarthritis Cartilage **4**(4): 245-9.

Denko, C. W. and C. J. Malemud (2004). "The serum growth hormone to somatostatin ratio is skewed upward in rheumatoid arthritis patients." Front Biosci **9**: 1660-4.

Dessein, P. H., B. I. Joffe, et al. (2003). "Inflammation, insulin resistance, and aberrant lipid metabolism as cardiovascular risk factors in rheumatoid arthritis." J Rheumatol **30**(7): 1403-5.

Dhatariya, K. K. and K. S. Nair (2003). "Dehydroepiandrosterone: is there a role for replacement?" Mayo Clin Proc **78**(10): 1257-73.

Donnelly, J. E., T. Sharp, et al. (1993). "Muscle hypertrophy with large-scale weight loss and resistance training." Am J Clin Nutr **58**(4): 561-5.

Doran, M. F., C. S. Crowson, et al. (2002). "Frequency of infection in patients with rheumatoid arthritis compared with controls: a population-based study." Arthritis Rheum **46**(9): 2287-93.

Duff, G. W. (1994). "Cytokines and acute phase proteins in rheumatoid arthritis." Scand J Rheumatol Suppl **100**: 9-19.

Earthman, C. P., J. R. Matthie, et al. (2000). "A comparison of bioimpedance methods for detection of body cell mass change in HIV infection." J Appl Physiol **88**(3): 944-56.

Elia, M., A. Carter, et al. (1981). "Clinical usefulness of urinary 3-methylhistidine excretion in indicating muscle protein breakdown." Br Med J (Clin Res Ed) **282**(6261): 351-4.

Elia, M., R. Stratton, et al. (2003). "Techniques for the study of energy balance in man." Proc Nutr Soc **62**(2): 529-37.

Ellis, K. J. (2000). "Human body composition: in vivo methods." Physiol Rev **80**(2): 649-80.

Engelhart, M., J. Kondrup, et al. (1996). "Weight reduction in obese patients with rheumatoid arthritis, with preservation of body cell mass and improvement of physical fitness." Clin Exp Rheumatol **14**(3): 289-93.

Escalante, A. and I. Del Rincon (2002). "The disablement process in rheumatoid arthritis." Arthritis Rheum **47**(3): 333-42.

Escalante, A., R. W. Haas, et al. (2004). "Measurement of global functional performance in patients with rheumatoid arthritis using rheumatology function tests." Arthritis Res Ther **6**(4): R315-25.

Fathalla, B., K. Hamada, et al. (2004). "Effects of aging and cytokine blockade on inflammatory cachexia." Clin Exp Rheumatol **22**(1): 85-90.

Fearon, K. C., M. F. Von Meyenfeldt, et al. (2003). "Effect of a protein and energy dense N-3 fatty acid enriched oral supplement on loss of weight and lean tissue in cancer cachexia: a randomised double blind trial." Gut **52**(10): 1479-86.

Feige, U., Y. L. Hu, et al. (2000). "Anti-interleukin-1 and anti-tumor necrosis factor-alpha synergistically inhibit adjuvant arthritis in Lewis rats." Cell Mol Life Sci **57**(10): 1457-70.

Feise, R. J. (2002). "Do multiple outcome measures require p-value adjustment?" BMC Med Res Methodol **2**(1): 8.

Ferraccioli, G., P. Guerra, et al. (1995). "Cyclosporin A increases somatomedin C insulin-like growth factor I levels in chronic rheumatic diseases." J Rheumatol **22**(6): 1060-4.

Ferrando, A. A., C. A. Stuart, et al. (1999). "Inactivity amplifies the catabolic response of skeletal muscle to cortisol." J Clin Endocrinol Metab **84**(10): 3515-21.

Fiatarone, M. A., E. C. Marks, et al. (1990). "High-intensity strength training in nonagenarians. Effects on skeletal muscle." Jama **263**(22): 3029-34.

Fiatarone, M. A., E. F. O'Neill, et al. (1994). "Exercise training and nutritional supplementation for physical frailty in very elderly people." N Engl J Med **330**(25): 1769-75.

Figueras, M., S. Busquets, et al. (2004). "Interleukin-15 is able to suppress the increased DNA fragmentation associated with muscle wasting in tumour-bearing rats." FEBS Lett **569**(1-3): 201-6.

Fiori, M. G., S. Andreola, et al. (1983). "Selective atrophy of the type IIb muscle fibers in rheumatoid arthritis and progressive systemic sclerosis (scleroderma). A biopsy histochemical study." Eur J Rheumatol Inflamm **6**(2): 168-81.

Fischer, J. E. and P. O. Hasselgren (1991). "Cytokines and glucocorticoids in the regulation of the "hepato-skeletal muscle axis" in sepsis." Am J Surg **161**(2): 266-71.

Fonseca, J., H. Canhao, et al. (2002). "Weight gain associated with the administration of tumor necrosis factor alpha antagonists in patients with rheumatoid arthritis." Arthritis and Rheumatism **46**(9): S170-S170.

Forbes, G. B. (2000). "Body fat content influences the body composition response to nutrition and exercise." Ann N Y Acad Sci **904**: 359-65.

Forbes, G. B., M. R. Brown, et al. (1989). "Hormonal response to overfeeding." Am J Clin Nutr **49**(4): 608-11.

Fraker, D. L., M. J. Merino, et al. (1989). "Reversal of the toxic effects of cachectin by concurrent insulin administration." Am J Physiol **256**(6 Pt 1): E725-31.

Fransen, J., H. Hauselmann, et al. (2001). "Responsiveness of the self-assessed rheumatoid arthritis disease activity index to a flare of disease activity." Arthritis Rheum **44**(1): 53-60.

Fraser, D. A., J. Thoen, et al. (2000). "Reduction in serum leptin and IGF-1 but preserved T-lymphocyte numbers and activation after a ketogenic diet in rheumatoid arthritis patients." Clin Exp Rheumatol **18**(2): 209-14.

Frontera, W. R., V. A. Hughes, et al. (1991). "A cross-sectional study of muscle strength and mass in 45- to 78-yr-old men and women." J Appl Physiol **71**(2): 644-50.

Frost, R. A., C. H. Lang, et al. (1997). "Transient exposure of human myoblasts to tumor necrosis factor-alpha inhibits serum and insulin-like growth factor-I stimulated protein synthesis." Endocrinology **138**(10): 4153-9.

Fuller, N. J., J. C. Wells, et al. (2001). "Evaluation of a model for total body protein mass based on dual-energy X-ray absorptiometry: comparison with a reference four-component model." Br J Nutr **86**(1): 45-52.

Furmanczyk, P. S. and L. S. Quinn (2003). "Interleukin-15 increases myosin accretion in human skeletal myogenic cultures." Cell Biol Int **27**(10): 845-51.

Gabay, C. and I. Kushner (1999). "Acute-phase proteins and other systemic responses to inflammation." N Engl J Med **340**(6): 448-54.

Gallagher, D., E. Ruts, et al. (2000). "Weight stability masks sarcopenia in elderly men and women." Am J Physiol Endocrinol Metab **279**(2): E366-75.

Gelfand, R. A., D. E. Matthews, et al. (1984). "Role of counterregulatory hormones in the catabolic response to stress." J Clin Invest **74**(6): 2238-48.

Genovese, M. C., S. Cohen, et al. (2004). "Combination therapy with etanercept and anakinra in the treatment of patients with rheumatoid arthritis who have been treated unsuccessfully with methotrexate." Arthritis Rheum **50**(5): 1412-9.

Genovese, M. C. and J. M. Kremer (2004). "Treatment of rheumatoid arthritis with etanercept." Rheum Dis Clin North Am **30**(2): 311-28.

Georgiou, E., K. Virvidakis, et al. (1997). "Body composition changes in chronic hemodialysis patients before and after hemodialysis as assessed by dual-energy x-ray absorptiometry." Metabolism **46**(9): 1059-62.

Gerards, A. H., S. de Lathouder, et al. (2003). "Inhibition of cytokine production by methotrexate. Studies in healthy volunteers and patients with rheumatoid arthritis." Rheumatology (Oxford) **42**(10): 1189-96.

Giangregorio, L. M. and C. E. Webber (2003). "Effects of metal implants on whole-body dual-energy x-ray absorptiometry measurements of bone mineral content and body composition." Can Assoc Radiol J **54**(5): 305-9; quiz 270-1.

Gibson, J. N., N. L. Poyser, et al. (1991). "Muscle protein synthesis in patients with rheumatoid arthritis: effect of chronic corticosteroid therapy on prostaglandin F2 alpha availability." Eur J Clin Invest **21**(4): 406-12.

Gomes-Marcondes, M. C. and M. J. Tisdale (2002). "Induction of protein catabolism and the ubiquitin-proteasome pathway by mild oxidative stress." Cancer Lett **180**(1): 69-74.

Gomez-Vaquero, C., J. M. Nolla, et al. (2001). "Nutritional status in patients with rheumatoid arthritis." Joint Bone Spine **68**(5): 403-9.

Gonzalez-Alvaro, I., A. M. Ortiz, et al. (2003). "Increased serum levels of interleukin-15 in rheumatoid arthritis with long- term disease." Clin Exp Rheumatol **21**(5): 639-42.

Gonzalez-Cadavid, N. F. and S. Bhasin (2004). "Role of myostatin in metabolism." Curr Opin Clin Nutr Metab Care **7**(4): 451-457.

Goodson, T., S. L. Morgan, et al. (2003). "The energy cost of adjuvant-induced arthritis in rats." Arthritis Rheum **48**(10): 2979-82.

Gore, D. C. and R. R. Wolfe (2003). "Metabolic response of muscle to alanine, glutamine, and valine supplementation during severe illness." JPEN J Parenter Enteral Nutr **27**(5): 307-14.

Gosker, H. R., E. F. Wouters, et al. (2000). "Skeletal muscle dysfunction in chronic obstructive pulmonary disease and chronic heart failure: underlying mechanisms and therapy perspectives." Am J Clin Nutr **71**(5): 1033-47.

Guarnieri, G., R. Antonione, et al. (2003). "Mechanisms of malnutrition in uremia." J Ren Nutr **13**(2): 153-7.

Gudivaka, R., D. A. Schoeller, et al. (1999). "Single- and multifrequency models for bioelectrical impedance analysis of body water compartments." J Appl Physiol **87**(3): 1087-96.

Guttridge, D. C. (2004). "Signaling pathways weigh in on decisions to make or break skeletal muscle." Curr Opin Clin Nutr Metab Care **7**(4): 443-450.

Hadley, J. S. and C. J. Hinds (2002). "Anabolic strategies in critical illness." Curr Opin Pharmacol **2**(6): 700-7.

Hakkinen, A. (2004). "Effectiveness and safety of strength training in rheumatoid arthritis." Curr Opin Rheumatol **16**(2): 132-7.

Hakkinen, A., K. Hakkinen, et al. (1994). "Effects of strength training on neuromuscular function and disease activity in patients with recent-onset inflammatory arthritis." Scand J Rheumatol **23**(5): 237-42.

Hakkinen, A., P. Hannonen, et al. (2003). "Effects of concurrent strength and endurance training in women with early or longstanding rheumatoid arthritis: comparison with healthy subjects." Arthritis Rheum **49**(6): 789-97.

Hakkinen, A., T. Sokka, et al. (2004). "Sustained maintenance of exercise induced muscle strength gains and normal bone mineral density in patients with early rheumatoid arthritis: a 5 year follow up." Ann Rheum Dis **63**(8): 910-6.

Hall, G. M., D. Peerbhoy, et al. (2000). "Hip and knee arthroplasty: a comparison and the endocrine, metabolic and inflammatory responses." Clin Sci (Lond) **98**(1): 71-9.

Hamel, F. G., J. L. Upward, et al. (2003). "Inhibition of proteasome activity by selected amino acids." Metabolism **52**(7): 810-4.

Hankard, R. G., M. W. Haymond, et al. (1996). "Effect of glutamine on leucine metabolism in humans." Am J Physiol **271**(4 Pt 1): E748-54.

Hansen, R. D., C. Raja, et al. (2000). "Total body protein in chronic diseases and in aging." Ann N Y Acad Sci **904**: 345-52.

Hart, D. W., S. E. Wolf, et al. (2002). "Energy expenditure and caloric balance after burn: increased feeding leads to fat rather than lean mass accretion." Ann Surg **235**(1): 152-61.

Hasselgren, P. O. (1999). "Glucocorticoids and muscle catabolism." Curr Opin Clin Nutr Metab Care **2**(3): 201-5.

Hasselgren, P. O. (2000). "Catabolic response to stress and injury: implications for regulation." World J Surg **24**(12): 1452-9.

Haugeberg, G., R. E. Orstavik, et al. (2003). "Effects of rheumatoid arthritis on bone." Curr Opin Rheumatol **15**(4): 469-75.

Haugen, M., K. A. Homme, et al. (1999). "Assessment of nutritional status in patients with rheumatoid arthritis and osteoarthritis undergoing joint replacement surgery." Arthritis Care Res **12**(1): 26-32.

Hedstrom, M. (1999). "Hip fracture patients, a group of frail elderly people with low bone mineral density, muscle mass and IGF-I levels." Acta Physiol Scand **167**(4): 347-50.

Helliwell, M., E. J. Coombes, et al. (1984). "Nutritional status in patients with rheumatoid arthritis." Ann Rheum Dis **43**(3): 386-90.

Helliwell, P. S. and S. Jackson (1994). "Relationship between weakness and muscle wasting in rheumatoid arthritis." Ann Rheum Dis **53**(11): 726-8.

Hernandez-Beriain, J. A., C. Segura-Garcia, et al. (1996). "Undernutrition in rheumatoid arthritis patients with disability." Scand J Rheumatol **25**(6): 383-7.

Hoffer, L. J. (2003). "Protein and energy provision in critical illness." Am J Clin Nutr **78**(5): 906-11.

Holecek, M., F. Skopec, et al. (2000). "Effect of alanyl-glutamine on leucine and protein metabolism in endotoxemic rats." JPEN J Parenter Enteral Nutr **24**(4): 215-22.

Holte, K. and H. Kehlet (2002). "Epidural anaesthesia and analgesia - effects on surgical stress responses and implications for postoperative nutrition." Clin Nutr **21**(3): 199-206.

Hoshino, E., C. Pichard, et al. (1991). "Body composition and metabolic rate in rat during a continuous infusion of cachectin." Am J Physiol **260**(1 Pt 1): E27-36.

Howard, L. and C. Ashley (2003). "Nutrition in the perioperative patient." Annu Rev Nutr **23**: 263-82.

Hsieh, L. F., B. Didenko, et al. (1987). "Isokinetic and isometric testing of knee musculature in patients with rheumatoid arthritis with mild knee involvement." Arch Phys Med Rehabil **68**(5 Pt 1): 294-7.

Hughes, V. A., W. R. Frontera, et al. (2002). "Longitudinal changes in body composition in older men and women: role of body weight change and physical activity." Am J Clin Nutr **76**(2): 473-81.

Hulsmans, H. M., J. W. Jacobs, et al. (2000). "The course of radiologic damage during the first six years of rheumatoid arthritis." Arthritis Rheum **43**(9): 1927-40.

Hunter, G. R., D. R. Bryan, et al. (2002). "Resistance training and intra-abdominal adipose tissue in older men and women." Med Sci Sports Exerc **34**(6): 1023-8.

Hurson, M., M. C. Regan, et al. (1995). "Metabolic effects of arginine in a healthy elderly population." JPEN J Parenter Enteral Nutr **19**(3): 227-30.

Ibanez De Caceres, I., M. A. Villanua, et al. (2000). "IGF-I and IGF-I-binding proteins in rats with adjuvant-induced arthritis given recombinant human growth hormone." J Endocrinol **165**(3): 537-44.

Janssen, I., R. N. Baumgartner, et al. (2004). "Skeletal muscle cutpoints associated with elevated physical disability risk in older men and women." Am J Epidemiol **159**(4): 413-21.

Janssen, J. A., A. J. van der Lely, et al. (2004). "Is there a role of ghrelin in preventing catabolism?" J Endocrinol Invest **27**(4): 400-3.

Jaswal, S., H. C. Mehta, et al. (2003). "Antioxidant status in rheumatoid arthritis and role of antioxidant therapy." Clin Chim Acta **338**(1-2): 123-9.

Jawed, S., K. Gaffney, et al. (1997). "Intra-articular pressure profile of the knee joint in a spectrum of inflammatory arthropathies." Ann Rheum Dis **56**(11): 686-9.

Jikimoto, T., Y. Nishikubo, et al. (2002). "Thioredoxin as a biomarker for oxidative stress in patients with rheumatoid arthritis." Mol Immunol **38**(10): 765-72.

Joffe, I. and S. Epstein (1991). "Osteoporosis associated with rheumatoid arthritis: pathogenesis and management." Semin Arthritis Rheum **20**(4): 256-72.

Johansson, A. G., D. J. Baylink, et al. (1994). "Circulating levels of insulin-like growth factor-I and -II, and IGF-binding protein-3 in inflammation and after parathyroid hormone infusion." Bone Miner **24**(1): 25-31.

Jones, C. J., R. E. Rikli, et al. (1999). "A 30-s chair-stand test as a measure of lower body strength in community-residing older adults." Res Q Exerc Sport **70**(2): 113-9.

Juni, P., D. G. Altman, et al. (2001). "Systematic reviews in health care: Assessing the quality of controlled clinical trials." Bmj **323**(7303): 42-6.

Kalla, A. A., G. M. Brown, et al. (1992). "Nutritional status in rheumatoid arthritis. Effects of disease activity, corticosteroid therapy and functional impairment." S Afr Med J **82**(6): 411-4.

Kehlet, H. (2000). "Manipulation of the metabolic response in clinical practice." World J Surg **24**(6): 690-5.

Keller, S. D., T. C. Majkut, et al. (1999). "Monitoring health outcomes among patients with arthritis using the SF-36 Health Survey: overview." Med Care **37**(5 Suppl): MS1-9.

Kelley, D., A. Mitrakou, et al. (1988). "Skeletal muscle glycolysis, oxidation, and storage of an oral glucose load." J Clin Invest **81**(5): 1563-71.

Kennedy, A. C., K. Boddy, et al. (1979). "Whole body elemental composition during drug treatment of rheumatoid arthritis: a preliminary study." Ann Rheum Dis **38**(2): 137-40.

Kenny, A. M., B. Biskup, et al. (2003). "Effects of vitamin D supplementation on strength, physical function, and health perception in older, community-dwelling men." J Am Geriatr Soc **51**(12): 1762-7.

Kim, J., Z. Wang, et al. (2002). "Total-body skeletal muscle mass: estimation by a new dual-energy X-ray absorptiometry method." Am J Clin Nutr **76**(2): 378-83.

Kjeldsen-Kragh, J. (1999). "Rheumatoid arthritis treated with vegetarian diets." Am J Clin Nutr **70**(3 Suppl): 594S-600S.

Klareskog, L., D. van der Heijde, et al. (2004). "Therapeutic effect of the combination of etanercept and methotrexate compared with each treatment alone in patients with rheumatoid arthritis: double-blind randomised controlled trial." Lancet **363**(9410): 675-81.

Kotler, D. P. (2000). "Body composition studies in HIV-infected individuals." Ann N Y Acad Sci **904**: 546-52.

Kotler, D. P. (2000). "Cachexia." Ann Intern Med **133**(8): 622-34.

Kotler, D. P., A. R. Tierney, et al. (1989). "Magnitude of body-cell-mass depletion and the timing of death from wasting in AIDS." Am J Clin Nutr **50**(3): 444-7.

Kraemer, W. J., K. Adams, et al. (2002). "American College of Sports Medicine position stand. Progression models in resistance training for healthy adults." Med Sci Sports Exerc **34**(2): 364-80.

Krahenbuhl, S., B. Willer, et al. (1999). "Carnitine homeostasis in patients with rheumatoid arthritis." Clin Chim Acta **279**(1-2): 35-45.

Kremer, J. M. (2004). "Toward a better understanding of methotrexate." Arthritis Rheum **50**(5): 1370-82.

Kremer, J. M., M. E. Weinblatt, et al. (2003). "Etanercept added to background methotrexate therapy in patients with rheumatoid arthritis: continued observations." Arthritis Rheum **48**(6): 1493-9.

Kuhnke, A., G. R. Burmester, et al. (2003). "Bioenergetics of immune cells to assess rheumatic disease activity and efficacy of glucocorticoid treatment." Ann Rheum Dis **62**(2): 133-9.

Kvapil, M. (1993). "Energy expenditure at rest in patients with active rheumatoid arthritis and malnutrition." Vnitrni lekarstvi **39**(1): 31-37.

Kvien, T. K. (2004). "Epidemiology and burden of illness of rheumatoid arthritis." Pharmacoeconomics **22**(2 Suppl): 1-12.

Kyle, U. G., A. Piccoli, et al. (2003). "Body composition measurements: interpretation finally made easy for clinical use." Curr Opin Clin Nutr Metab Care **6**(4): 387-93.

Lang, C. H. and R. A. Frost (2002). "Role of growth hormone, insulin-like growth factor-I, and insulin-like growth factor binding proteins in the catabolic response to injury and infection." Curr Opin Clin Nutr Metab Care **5**(3): 271-9.

Lang, C. H., R. A. Frost, et al. (2002). "TNF-alpha impairs heart and skeletal muscle protein synthesis by altering translation initiation." Am J Physiol Endocrinol Metab **282**(2): E336-47.

Lang, C. H., C. Silvis, et al. (2003). "Endotoxin stimulates in vivo expression of inflammatory cytokines tumor necrosis factor alpha, interleukin-1beta, -6, and high-mobility-group protein-1 in skeletal muscle." Shock **19**(6): 538-46.

Lange, K. H., J. L. Andersen, et al. (2002). "GH administration changes myosin heavy chain isoforms in skeletal muscle but does not augment muscle strength or hypertrophy, either alone or combined with resistance exercise training in healthy elderly men." J Clin Endocrinol Metab **87**(2): 513-23.

Langhans, W. (2002). "Peripheral mechanisms involved with catabolism." Curr Opin Clin Nutr Metab Care **5**(4): 419-26.

Latham, N. K., D. A. Bennett, et al. (2004). "Systematic review of progressive resistance strength training in older adults." J Gerontol A Biol Sci Med Sci **59**(1): 48-61.

Laviano, A., M. Russo, et al. (2002). "Neurochemical mechanisms for cancer anorexia." Nutrition **18**(1): 100-5.

LeBlanc, A., C. Lin, et al. (2000). "Muscle volume, MRI relaxation times (T2), and body composition after spaceflight." J Appl Physiol **89**(6): 2158-64.

Lee, D. M. and M. E. Weinblatt (2001). "Rheumatoid arthritis." Lancet **358**(9285): 903-11.

Lee, R. C., Z. Wang, et al. (2000). "Total-body skeletal muscle mass: development and cross-validation of anthropometric prediction models." Am J Clin Nutr **72**(3): 796-803.

Lemmey, A., P. Maddison, et al. (2001). "Association between insulin-like growth factor status and physical activity levels in rheumatoid arthritis." J Rheumatol **28**(1): 29-34.

Li, P. and E. M. Schwarz (2003). "The TNF-alpha transgenic mouse model of inflammatory arthritis." Springer Semin Immunopathol **25**(1): 19-33.

Li, Y. P., Y. Chen, et al. (2003). "Hydrogen peroxide stimulates ubiquitin-conjugating activity and expression of genes for specific E2 and E3 proteins in skeletal muscle myotubes." Am J Physiol Cell Physiol **285**(4): C806-12.

Li, Y. P., S. H. Lecker, et al. (2003). "TNF-alpha increases ubiquitin-conjugating activity in skeletal muscle by up-regulating UbcH2/E220k." Faseb J **17**(9): 1048-57.

Li, Y. P. and M. B. Reid (2001). "Effect of tumor necrosis factor-alpha on skeletal muscle metabolism." Curr Opin Rheumatol **13**(6): 483-7.

Li, Y. P., R. J. Schwartz, et al. (1998). "Skeletal muscle myocytes undergo protein loss and reactive oxygen-mediated NF-kappaB activation in response to tumor necrosis factor alpha." Faseb J **12**(10): 871-80.

Llovera, M., N. Carbo, et al. (1996). "Anti-TNF treatment reverts increased muscle ubiquitin gene expression in tumour-bearing rats." Biochem Biophys Res Commun **221**(3): 653-5.

Llovera, M., F. J. Lopez-Soriano, et al. (1993). "Chronic tumour necrosis factor-alpha treatment modifies protein turnover in rat tissues." Biochem Mol Biol Int **30**(1): 29-36.

Lohman, T. G., M. Harris, et al. (2000). "Assessing body composition and changes in body composition. Another look at dual-energy X-ray absorptiometry." Ann N Y Acad Sci **904**: 45-54.

Lovejoy, J. C., G. A. Bray, et al. (1996). "Exogenous androgens influence body composition and regional body fat distribution in obese postmenopausal women--a clinical research center study." J Clin Endocrinol Metab **81**(6): 2198-203.

Madsen, O. R., C. Egsmose, et al. (1998). "Soft tissue composition, quadriceps strength, bone quality and bone mass in rheumatoid arthritis." Clin Exp Rheumatol **16**(1): 27-32.

Madsen, O. R., C. Egsmose, et al. (1999). "Influence of orthopaedic metal and high-density detection on body composition as assessed by dual-energy X-ray absorptiometry." Clin Physiol **19**(3): 238-45.

Magyar, E., A. Talerman, et al. (1977). "Muscle changes in rheumatoid arthritis. A review of the literature with a study of 100 cases." Virchows Arch A Pathol Anat Histol **373**(3): 267-78.

Maia, J. A., M. Thomis, et al. (2002). "Genetic factors in physical activity levels: a twin study." Am J Prev Med **23**(2 Suppl): 87-91.

Majumdar, S. and B. B. Aggarwal (2001). "Methotrexate suppresses NF-kappaB activation through inhibition of IkappaBalpha phosphorylation and degradation." J Immunol **167**(5): 2911-20.

Mantovani, G., A. Maccio, et al. (2003). "Cancer-related cachexia and oxidative stress: beyond current therapeutic options." Expert Rev Anticancer Ther **3**(3): 381-92.

Massardo, L., S. E. Gabriel, et al. (2002). "A population based assessment of the use of orthopedic surgery in patients with rheumatoid arthritis." J Rheumatol **29**(1): 52-6.

Matsumoto, T. and T. Tsurumoto (2002). "Inappropriate serum levels of IGF-I and IGFBP-3 in patients with rheumatoid arthritis." Rheumatology (Oxford) **41**(3): 352-3.

May, P. E., A. Barber, et al. (2002). "Reversal of cancer-related wasting using oral supplementation with a combination of beta-hydroxy-beta-methylbutyrate, arginine, and glutamine." Am J Surg **183**(4): 471-9.

McCann, S. M., M. Kimura, et al. (2000). "The mechanism of action of cytokines to control the release of hypothalamic and pituitary hormones in infection." Ann N Y Acad Sci **917**: 4-18.

McCarthy, D. O., P. Whitney, et al. (2004). "Indomethacin and ibuprofen preserve gastrocnemius muscle mass in mice bearing the colon-26 adenocarcinoma." Res Nurs Health **27**(3): 174-84.

McMillan, D. C., S. J. Wigmore, et al. (1999). "A prospective randomized study of megestrol acetate and ibuprofen in gastrointestinal cancer patients with weight loss." Br J Cancer **79**(3-4): 495-500.

McNair, P. J., J. Depledge, et al. (1996). "Verbal encouragement: effects on maximum effort voluntary muscle action." Br J Sports Med **30**(3): 243-5.

Meguid, M. M. and C. Pichard (2003). "Cytokines: the mother of catabolic mediators!" Curr Opin Clin Nutr Metab Care **6**(4): 383-6.

Meireles, S. M., L. M. Oliveira, et al. (2002). "Isokinetic evaluation of the knee in patients with rheumatoid arthritis." Joint Bone Spine **69**(6): 566-73.

Michelsen, C. B., J. Askanazi, et al. (1979). "Changes in metabolism and muscle composition associated with total hip replacement." J Trauma **19**(1): 29-32.

Michelsen, C. B., J. Askanazi, et al. (1982). "Effect of an anabolic steroid on nitrogen balance and amino acid patterns after total hip replacement." J Trauma **22**(5): 410-3.

Michie, H. R., D. R. Spriggs, et al. (1988). "Tumor necrosis factor and endotoxin induce similar metabolic responses in human beings." Surgery **104**(2): 280-6.

Mielants, H., M. De Vos, et al. (1991). "Intestinal mucosal permeability in inflammatory rheumatic diseases. II. Role of disease." J Rheumatol **18**(3): 394-400.

Minaur, N. J., R. K. Jacoby, et al. (2004). "Outcome after 40 years with rheumatoid arthritis: a prospective study of function, disease activity, and mortality." J Rheumatol Suppl **69**: 3-8.

Miro, O., E. Pedrol, et al. (1996). "Muscle involvement in rheumatoid arthritis: clinicopathological study of 21 symptomatic cases." Semin Arthritis Rheum **25**(6): 421-8.

Mirone, L., L. Altomonte, et al. (1996). "A study of serum androgen and cortisol levels in female patients with rheumatoid arthritis. Correlation with disease activity." Clin Rheumatol **15**(1): 15-9.

Moher, D., K. F. Schulz, et al. (2001). "The CONSORT statement: revised recommendations for improving the quality of reports of parallel-group randomised trials." Lancet **357**(9263): 1191-4.

Moots, R., A. Taggart, et al. (2003). "Biologic therapy in clinical practice: enthusiasm must be tempered by caution." Rheumatology (Oxford) **42**(5): 614-6.

Morgan, S. L., A. M. Anderson, et al. (1997). "Nutrient intake patterns, body mass index, and vitamin levels in patients with rheumatoid arthritis." Arthritis Care Res **10**(1): 9-17.

Morgan, S. L., J. E. Baggott, et al. (2001). "MTX affects inflammation and tissue destruction differently in the rat AA model." J Rheumatol **28**(7): 1476-81.

Moriguchi, M., C. Terai, et al. (1999). "Overestimation of renal function by estimating from serum creatinine concentration in patients with rheumatoid arthritis: Its relation to decreased lean body mass of extremities measured by dual-energy x-ray absorptiometry." Arthritis and Rheumatism **42**(9): 359 Suppl.

Morrissey, M. C., E. A. Harman, et al. (1995). "Resistance training modes: specificity and effectiveness." Med Sci Sports Exerc **27**(5): 648-60.

Moses, A. W., C. Slater, et al. (2004). "Reduced total energy expenditure and physical activity in cachectic patients with pancreatic cancer can be modulated by an energy and protein dense oral supplement enriched with n-3 fatty acids." Br J Cancer **90**(5): 996-1002.

Munro, R. and H. Capell (1997). "Prevalence of low body mass in rheumatoid arthritis: association with the acute phase response." Ann Rheum Dis **56**(5): 326-9.

Narici, M. and P. Cerretelli (1998). "Changes in human muscle architecture in disuse-atrophy evaluated by ultrasound imaging." J Gravit Physiol **5**(1): P73-4.

Natali, A., E. Toschi, et al. (2000). "Determinants of postabsorptive endogenous glucose output in non-diabetic subjects. European Group for the Study of Insulin Resistance (EGIR)." Diabetologia **43**(10): 1266-72.

Navegantes, L. C., R. H. Migliorini, et al. (2002). "Adrenergic control of protein metabolism in skeletal muscle." Curr Opin Clin Nutr Metab Care **5**(3): 281-6.

Neidel, J. (2001). "Changes in systemic levels of insulin-like growth factors and their binding proteins in patients with rheumatoid arthritis." Clin Exp Rheumatol **19**(1): 81-4.

Nissen, S., R. L. Sharp, et al. (2000). "beta-hydroxy-beta-methylbutyrate (HMB) supplementation in humans is safe and may decrease cardiovascular risk factors." J Nutr **130**(8): 1937-45.

Nissen, S. L. and R. L. Sharp (2003). "Effect of dietary supplements on lean mass and strength gains with resistance exercise: a meta-analysis." J Appl Physiol **94**(2): 651-9.

Nordemar, R., U. Berg, et al. (1976). "Changes in muscle fibre size and physical performance in patients with rheumatoid arthritis after 7 months physical training." Scand J Rheumatol **5**(4): 233-8.

Nordemar, R., L. Edstrom, et al. (1976). "Changes in muscle fibre size and physical performance in patients with rheumatoid arthritis after short-term physical training." Scand J Rheumatol **5**(2): 70-6.

Norrelund, H., J. Borglum, et al. (2000). "Effects of growth hormone administration on protein dynamics and substrate metabolism during 4 weeks of dietary restriction in obese women." Clin Endocrinol (Oxf) **52**(3): 305-12.

O'Connor, D., B. Kortman, et al. (1999). "Correlation between objective and subjective measures of hand function in patients with rheumatoid arthritis." J Hand Ther **12**(4): 323-9.

Orngreen, M. C., M. Zacho, et al. (2003). "Patients with severe muscle wasting are prone to develop hypoglycemia during fasting." Neurology **61**(7): 997-1000.

Orr, R. and M. Fiatarone Singh (2004). "The anabolic androgenic steroid oxandrolone in the treatment of wasting and catabolic disorders: review of efficacy and safety." Drugs **64**(7): 725-50.

Ostaszewksi, P., S. Kostiuk, et al. (2000). "The leucine metabolite 3-hydroxy-3-methylbutyrate (HMB) modifies protein turnover in muscles of laboratory rats and domestic chickens in vitro." Journal of Animal Physiology and Animal Nutrition **84**: 1-8.

Otero, M., R. Nogueiras, et al. (2004). "Chronic inflammation modulates ghrelin levels in humans and rats." Rheumatology (Oxford) **43**(3): 306-10.

Paddon-Jones, D., M. Sheffield-Moore, et al. (2003). "Hypercortisolemia alters muscle protein anabolism following ingestion of essential amino acids." Am J Physiol Endocrinol Metab **284**(5): E946-53.

Paget, J. (1873). "Nervous mimicry of organic diseases." Lancet **2**: 727-729.

Phillips, S. M., K. D. Tipton, et al. (1997). "Mixed muscle protein synthesis and breakdown after resistance exercise in humans." Am J Physiol **273**(1 Pt 1): E99-107.

Pichard, C., U. G. Kyle, et al. (2004). "Nutritional assessment: lean body mass depletion at hospital admission is associated with an increased length of stay." Am J Clin Nutr **79**(4): 613-8.

Pietrobelli, A., Z. Wang, et al. (1998). "Dual-energy X-ray absorptiometry: fat estimation errors due to variation in soft tissue hydration." Am J Physiol **274**(5 Pt 1): E808-16.

Pincus, T. and L. F. Callahan (1993). "The 'side effects' of rheumatoid arthritis: joint destruction, disability and early mortality." Br J Rheumatol **32 Suppl 1**: 28-37.

Pincus, T., L. F. Callahan, et al. (1989). "Self-report questionnaire scores in rheumatoid arthritis compared with traditional physical, radiographic, and laboratory measures." Ann Intern Med **110**(4): 259-66.

Pincus, T., C. Swearingen, et al. (1999). "Toward a multidimensional Health Assessment Questionnaire (MDHAQ): assessment of advanced activities of daily living and psychological status in the patient-friendly health assessment questionnaire format." Arthritis Rheum **42**(10): 2220-30.

Pincus, T., Y. Yazici, et al. (2003). "Methotrexate as the "anchor drug" for the treatment of early rheumatoid arthritis." Clin Exp Rheumatol **21**(5 Suppl 31): S179-85.

Plank, L. D. and G. L. Hill (2000). "Similarity of changes in body composition in intensive care patients following severe sepsis or major blunt injury." Ann N Y Acad Sci **904**: 592-602.

Podenphant, J., A. Gotfredsen, et al. (1996). "Comparison of body composition by dual energy X-ray absorptiometry to other estimates of body composition during weight loss in obese patients with rheumatoid arthritis." Scand J Clin Lab Invest **56**(7): 615-25.

Powanda, M. C. and W. R. Beisel (2003). "Metabolic effects of infection on protein and energy status." J Nutr **133**(1): 322S-327S.

Prod'homme, M., I. Rieu, et al. (2004). "Insulin and amino acids both strongly participate to the regulation of protein metabolism." Curr Opin Clin Nutr Metab Care **7**(1): 71-7.

Pu, C. T., M. T. Johnson, et al. (2001). "Randomized trial of progressive resistance training to counteract the myopathy of chronic heart failure." J Appl Physiol **90**(6): 2341-50.

Quinn, L. S., B. G. Anderson, et al. (2002). "Overexpression of interleukin-15 induces skeletal muscle hypertrophy in vitro: implications for treatment of muscle wasting disorders." Exp Cell Res **280**(1): 55-63.

Quinn, L. S., K. L. Haugk, et al. (1997). "Interleukin-15 stimulates C2 skeletal myoblast differentiation." Biochem Biophys Res Commun **239**(1): 6-10.

Quinn, L. S., K. L. Haugk, et al. (1995). "Interleukin-15: a novel anabolic cytokine for skeletal muscle." Endocrinology **136**(8): 3669-72.

Radrizzani, D., G. Iapichino, et al. (1986). "Main nitrogen balance determinants in malnourished patients." Intensive Care Med **12**(4): 308-11.

Raina, N. and K. N. Jeejeebhoy (1998). "Changes in body composition and dietary intake induced by tumor necrosis factor alpha and corticosterone--individually and in combination." Am J Clin Nutr **68**(6): 1284-90.

Rall, L. C., S. N. Meydani, et al. (1996). "The effect of progressive resistance training in rheumatoid arthritis. Increased strength without changes in energy balance or body composition." Arthritis Rheum **39**(3): 415-26.

Rall, L. C., C. J. Rosen, et al. (1996). "Protein metabolism in rheumatoid arthritis and aging. Effects of muscle strength training and tumor necrosis factor alpha." Arthritis Rheum **39**(7): 1115-24.

Rall, L. C., J. M. Walsmith, et al. (2002). "Cachexia in rheumatoid arthritis is not explained by decreased growth hormone secretion." Arthritis Rheum **46**(10): 2574-7.

Ramos, E. J., S. Suzuki, et al. (2004). "Cancer anorexia-cachexia syndrome: cytokines and neuropeptides." Curr Opin Clin Nutr Metab Care **7**(4): 427-434.

Rathmacher, J. A., S. Nissen, et al. (2004). "Supplementation with a combination of beta-hydroxy-beta-methylbutyrate (HMB), arginine, and glutamine is safe and could improve hematological parameters." JPEN J Parenter Enteral Nutr **28**(2): 65-75.

Reeves, N. D., M. V. Narici, et al. (2004). "Effect of resistance training on skeletal muscle-specific force in elderly humans." J Appl Physiol **96**(3): 885-92.

Reid, M. B. and Y. P. Li (2001). "Cytokines and oxidative signalling in skeletal muscle." Acta Physiol Scand **171**(3): 225-32.

Rennie, K. L., J. Hughes, et al. (2003). "Nutritional management of rheumatoid arthritis: a review of the evidence." J Hum Nutr Diet **16**(2): 97-109.

Rennie, M. J., A. Ahmed, et al. (1996). "Glutamine metabolism and transport in skeletal muscle and heart and their clinical relevance." J Nutr **126**(4 Suppl): 1142S-9S.

Ridker, P. M., J. E. Buring, et al. (2003). "C-reactive protein, the metabolic syndrome, and risk of incident cardiovascular events: an 8-year follow-up of 14 719 initially healthy American women." Circulation **107**(3): 391-7.

Ritchlin, C. T. (2004). "Mechanisms of erosion in rheumatoid arthritis." J Rheumatol **31**(7): 1229-37.

Robinzon, B. and M. Cutolo (1999). "Should dehydroepiandrosterone replacement therapy be provided with glucocorticoids?" Rheumatology (Oxford) **38**(6): 488-95.

Rofe, A. M., M. W. Whitehouse, et al. (1990). "Prevention of adjuvant-induced cachexia in rats by cyclosporin A." Immunol Cell Biol **68 (Pt 1)**: 63-9.

Rooyackers, O. E. and K. S. Nair (1997). "Hormonal regulation of human muscle protein metabolism." Annu Rev Nutr **17**: 457-85.

Rosenblatt, S., G. H. Clowes, Jr., et al. (1983). "Exchange of amino acids by muscle and liver in sepsis." Arch Surg **118**(2): 167-75.

Rosenthal, N. and A. Musaro (2002). "Gene therapy for cardiac cachexia?" Int J Cardiol **85**(1): 185-91.

Roth, S. M. and S. Walsh (2004). "Myostatin: a therapeutic target for skeletal muscle wasting." Curr Opin Clin Nutr Metab Care **7**(3): 259-63.

Roubenoff, R. (1997). "Inflammatory and hormonal mediators of cachexia." J Nutr **127**(5 Suppl): 1014S-1016S.

Roubenoff, R. (2003). "Exercise and inflammatory disease." Arthritis Rheum **49**(2): 263-6.

Roubenoff, R., L. M. Freeman, et al. (1997). "Adjuvant arthritis as a model of inflammatory cachexia." Arthritis Rheum **40**(3): 534-9.

Roubenoff, R. and J. J. Kehayias (1991). "The meaning and measurement of lean body mass." Nutr Rev **49**(6): 163-75.

Roubenoff, R., R. A. Roubenoff, et al. (1994). "Rheumatoid cachexia: cytokine-driven hypermetabolism accompanying reduced body cell mass in chronic inflammation." J Clin Invest **93**(6): 2379-86.

Roubenoff, R., R. A. Roubenoff, et al. (1992). "Rheumatoid cachexia: depletion of lean body mass in rheumatoid arthritis. Possible association with tumor necrosis factor." J Rheumatol **19**(10): 1505-10.

Roubenoff, R., R. A. Roubenoff, et al. (1990). "Catabolic effects of high-dose corticosteroids persist despite therapeutic benefit in rheumatoid arthritis." Am J Clin Nutr **52**(6): 1113-7.

Roubenoff, R., J. Walsmith, et al. (2002). "Low physical activity reduces total energy expenditure in women with rheumatoid arthritis: implications for dietary intake recommendations." Am J Clin Nutr **76**(4): 774-9.

Roubenoff, R. and I. B. Wilson (2001). "Effect of resistance training on self-reported physical functioning in HIV infection." Med Sci Sports Exerc **33**(11): 1811-7.

Rovensky, J., J. Bakosova, et al. (2002). "Somatotropic, lactotropic and adrenocortical responses to insulin-induced hypoglycemia in patients with rheumatoid arthritis." Ann N Y Acad Sci **966**: 263-70.

Ruchelman, H. and R. V. Ford (1962). "Inhibition of negative nitrogen balance by an anabolic agent (methandrostenolone) during corticosteroid therapy (dexamethasone) in rheumatoid arthritis." Metabolism **11**: 524-9.

Saltin, B. and G. Grimby (1968). "Physiological analysis of middle-aged and old former athletes. Comparison with still active athletes of the same ages." Circulation **38**(6): 1104-15.

Sambrook, P. N., T. D. Spector, et al. (1995). "Osteoporosis in rheumatoid arthritis. A monozygotic co-twin control study." Arthritis Rheum **38**(6): 806-9.

Sattar, N., D. W. McCarey, et al. (2003). "Explaining how "high-grade" systemic inflammation accelerates vascular risk in rheumatoid arthritis." Circulation **108**(24): 2957-63.

Saxne, T., M. A. Palladino, Jr., et al. (1988). "Detection of tumor necrosis factor alpha but not tumor necrosis factor beta in rheumatoid arthritis synovial fluid and serum." Arthritis Rheum **31**(8): 1041-5.

Schliess, F. and D. Haussinger (2002). "The cellular hydration state: a critical determinant for cell death and survival." Biol Chem **383**(3-4): 577-83.

Schulz, K. F. and D. A. Grimes (2002). "Sample size slippages in randomised trials: exclusions and the lost and wayward." Lancet **359**(9308): 781-5.

Seitz, M. (1999). "Molecular and cellular effects of methotrexate." Curr Opin Rheumatol **11**(3): 226-32.

Sergi, G., M. Bussolotto, et al. (1994). "Accuracy of bioelectrical impedance analysis in estimation of extracellular space in healthy subjects and in fluid retention states." Ann Nutr Metab **38**(3): 158-65.

Sharma, R. and S. D. Anker (2002). "Cytokines, apoptosis and cachexia: the potential for TNF antagonism." Int J Cardiol **85**(1): 161-71.

Sharp, J. T., F. Wolfe, et al. (1991). "The progression of erosion and joint space narrowing scores in rheumatoid arthritis during the first twenty-five years of disease." Arthritis Rheum **34**(6): 660-8.

Shealy, D. J., P. H. Wooley, et al. (2002). "Anti-TNF-alpha antibody allows healing of joint damage in polyarthritic transgenic mice." Arthritis Res **4**(5): R7.

Sheffield-Moore, M. and R. J. Urban (2004). "An overview of the endocrinology of skeletal muscle." Trends Endocrinol Metab **15**(3): 110-5.

Shibuya, K., H. Hagino, et al. (2002). "Cross-sectional and longitudinal study of osteoporosis in patients with rheumatoid arthritis." Clin Rheumatol **21**(2): 150-8.

Siegel, S. A., D. J. Shealy, et al. (1995). "The mouse/human chimeric monoclonal antibody cA2 neutralizes TNF in vitro and protects transgenic mice from cachexia and TNF lethality in vivo." Cytokine **7**(1): 15-25.

Sihvonen, S., M. Korpela, et al. (2004). "Death rates and causes of death in patients with rheumatoid arthritis: a population-based study." Scand J Rheumatol **33**(4): 221-7.

Silman, A. J. and J. E. Pearson (2002). "Epidemiology and genetics of rheumatoid arthritis." Arthritis Res **4 Suppl 3**: S265-72.

Simons, J. P., A. M. Schols, et al. (1999). "Weight loss and low body cell mass in males with lung cancer: relationship with systemic inflammation, acute-phase response, resting energy expenditure, and catabolic and anabolic hormones." Clin Sci (Lond) **97**(2): 215-23.

Soto, L., A. I. Martin, et al. (2001). "Cyclosporin a treatment is able to revert the decrease in circulating GH and IGF-I and the increase in IGFBPs induced by adjuvant arthritis." Horm Metab Res **33**(10): 590-5.

Spate, U. and P. C. Schulze (2004). "Proinflammatory cytokines and skeletal muscle." Curr Opin Clin Nutr Metab Care **7**(3): 265-9.

Steensma, D. P., R. A. Mesa, et al. (2002). "Etanercept, a soluble tumor necrosis factor receptor, palliates constitutional symptoms in patients with myelofibrosis with myeloid metaplasia: results of a pilot study." Blood **99**(6): 2252-4.

Stenver, D. I., A. Gotfredsen, et al. (1995). "Body composition in hemodialysis patients measured by dual-energy X-ray absorptiometry." Am J Nephrol **15**(2): 105-10.

Strand, V., S. Cohen, et al. (2004). "Patient-reported outcomes better discriminate active treatment from placebo in randomized controlled trials in rheumatoid arthritis." Rheumatology (Oxford) **43**(5): 640-7.

Strang, P. (1997). "The effect of megestrol acetate on anorexia, weight loss and cachexia in cancer and AIDS patients (review)." Anticancer Res **17**(1B): 657-62.

Straub, R. H., L. Paimela, et al. (2002). "Inadequately low serum levels of steroid hormones in relation to interleukin-6 and tumor necrosis factor in untreated patients with early rheumatoid arthritis and reactive arthritis." Arthritis Rheum **46**(3): 654-62.

Streat, S. J., A. H. Beddoe, et al. (1987). "Aggressive nutritional support does not prevent protein loss despite fat gain in septic intensive care patients." J Trauma **27**(3): 262-6.

Stucki, G., P. Bruhlmann, et al. (1998). "Isometric muscle strength is an indicator of self-reported physical functional disability in patients with rheumatoid arthritis." Br J Rheumatol **37**(6): 643-8.

Stucki, G., M. H. Liang, et al. (1995). "A self-administered rheumatoid arthritis disease activity index (RADAI) for epidemiologic research. Psychometric properties and correlation with parameters of disease activity." Arthritis Rheum **38**(6): 795-8.

Suchner, U., K. S. Kuhn, et al. (2000). "The scientific basis of immunonutrition." Proc Nutr Soc **59**(4): 553-63.

Svenson, K. L., T. Pollare, et al. (1988). "Impaired glucose handling in active rheumatoid arthritis: relationship to peripheral insulin resistance." Metabolism **37**(2): 125-30.

Templ, E., M. Koeller, et al. (1996). "Anterior pituitary function in patients with newly diagnosed rheumatoid arthritis." Br J Rheumatol **35**(4): 350-6.

Tengstrand, B., K. Carlstrom, et al. (2002). "Bioavailable testosterone in men with rheumatoid arthritis-high frequency of hypogonadism." Rheumatology (Oxford) **41**(3): 285-9.

Tetta, C., G. Camussi, et al. (1990). "Tumour necrosis factor in serum and synovial fluid of patients with active and severe rheumatoid arthritis." Ann Rheum Dis **49**(9): 665-7.

Toth, M. J., C. K. Sites, et al. (1999). "Hormonal and physiological correlates of energy expenditure and substrate oxidation in middle-aged, premenopausal women." J Clin Endocrinol Metab **84**(8): 2771-5.

Trappe, S., T. Trappe, et al. (2004). "Human single muscle fibre function with 84 day bed-rest and resistance exercise." J Physiol **557**(Pt 2): 501-13.

Truyens, C., F. Torrico, et al. (1995). "The cachexia associated with Trypanosoma cruzi acute infection in mice is attenuated by anti-TNF-alpha, but not by anti-IL-6 or anti-IFN-gamma antibodies." Parasite Immunol **17**(11): 561-8.

Van den Berghe, G. (2002). "Endocrinology in intensive care medicine: new insights and therapeutic consequences." Verh K Acad Geneeskd Belg **64**(3): 167-87; discussion 187-8.

van den Ende, C. H., F. C. Breedveld, et al. (2000). "Effect of intensive exercise on patients with active rheumatoid arthritis: a randomised clinical trial." Ann Rheum Dis **59**(8): 615-21.

Van Den Ende, C. H., T. P. Vliet Vlieland, et al. (2000). "Dynamic exercise therapy for rheumatoid arthritis." Cochrane Database Syst Rev(2): CD000322.

van Gestel, A. M., M. L. Prevoo, et al. (1996). "Development and validation of the European League Against Rheumatism response criteria for rheumatoid arthritis. Comparison with the preliminary American College of Rheumatology and the World Health Organization/International League Against Rheumatism Criteria." Arthritis Rheum **39**(1): 34-40.

Van Loan, M. D., P. Withers, et al. (1993). "Use of bioimpedance spectroscopy to determine extracellular fluid, intracellular fluid, total body water, and fat-free mass." Basic Life Sci **60**: 67-70.

van Marken Lichtenbelt, W. D. and H. A. Daanen (2003). "Cold-induced metabolism." Curr Opin Clin Nutr Metab Care **6**(4): 469-75.

Visser, M., D. J. Deeg, et al. (2003). "Low vitamin D and high parathyroid hormone levels as determinants of loss of muscle strength and muscle mass (sarcopenia): the Longitudinal Aging Study Amsterdam." J Clin Endocrinol Metab **88**(12): 5766-72.

Volpi, E., H. Kobayashi, et al. (2003). "Essential amino acids are primarily responsible for the amino acid stimulation of muscle protein anabolism in healthy elderly adults." Am J Clin Nutr **78**(2): 250-8.

von Haehling, S., S. Genth-Zotz, et al. (2002). "Cachexia: a therapeutic approach beyond cytokine antagonism." Int J Cardiol **85**(1): 173-83.

von Laue, S. and R. J. Ross (2000). "Inflammatory cytokines and acquired growth hormone resistance." Growth Horm IGF Res **10 Suppl B**: S9-14.

Walsmith, J., L. Abad, et al. (2004). "Tumor necrosis factor-alpha production is associated with less body cell mass in women with rheumatoid arthritis." J Rheumatol **31**(1): 23-9.

Walsmith, J. and R. Roubenoff (2002). "Cachexia in rheumatoid arthritis." Int J Cardiol **85**(1): 89-99.

Walsmith, J., E. Vannier, et al. (2003). "Patients with rheumatoid arthritis [RA] have low protein synthesis rates and elevated tumor necrosis factor-alpha (TNF-alpha) and transforming growth factor-(beta 1 (TGF beta) transcript levels in skeletal muscle." Arthritis and Rheumatism **48**(9): 159 Suppl.

Wang, Z., P. Deurenberg, et al. (1999). "Hydration of fat-free body mass: new physiological modeling approach." Am J Physiol **276**(6 Pt 1): E995-E1003.

Wang, Z., S. Heshka, et al. (2003). "Magnitude and variation of fat-free mass density: a cellular-level body composition modeling study." Am J Physiol Endocrinol Metab **284**(2): E267-73.

Wang, Z., M. P. St-Onge, et al. (2004). "Body cell mass: model development and validation at the cellular level of body composition." Am J Physiol Endocrinol Metab **286**(1): E123-8.

Wang, Z., S. Zhu, et al. (2003). "Whole-body skeletal muscle mass: development and validation of total-body potassium prediction models." Am J Clin Nutr **77**(1): 76-82.

Warren, R. S., H. F. Starnes, Jr., et al. (1987). "The acute metabolic effects of tumor necrosis factor administration in humans." Arch Surg **122**(12): 1396-400.

Watson, D. J., T. Rhodes, et al. (2003). "All-cause mortality and vascular events among patients with rheumatoid arthritis, osteoarthritis, or no arthritis in the UK General Practice Research Database." J Rheumatol **30**(6): 1196-202.

Wechsler, J. G., H. Wenzel, et al. (1984). "Nitrogen balance studies during modified fasting." Postgrad Med J **60 Suppl 3**: 66-73.

Weinblatt, M. E., J. M. Kremer, et al. (1999). "A trial of etanercept, a recombinant tumor necrosis factor receptor:Fc fusion protein, in patients with rheumatoid arthritis receiving methotrexate." N Engl J Med **340**(4): 253-9.

Westerterp, K. R., J. H. Donkers, et al. (1995). "Energy intake, physical activity and body weight: a simulation model." Br J Nutr **73**(3): 337-47.

Westhovens, R. (1999). Prognostic factors and outcome in rheumatoid arthritis. Division of Rheumatology, Department of Internal Medicine, University Hospitals. Leuven, K.U. Leuven: 112.

Westhovens, R., J. Nijs, et al. (1997). "Body composition in rheumatoid arthritis." Br J Rheumatol **36**(4): 444-8.

Wilder, R. L. (2002). "Neuroimmunoendocrinology of the rheumatic diseases: past, present, and future." Ann N Y Acad Sci **966**: 13-9.

Willer, B., G. Stucki, et al. (2000). "Effects of creatine supplementation on muscle weakness in patients with rheumatoid arthritis." Rheumatology (Oxford) **39**(3): 293-8.

Willett, W. C., L. Sampson, et al. (1985). "Reproducibility and validity of a semiquantitative food frequency questionnaire." Am J Epidemiol **122**(1): 51-65.

Wilson, A., H. T. Yu, et al. (2004). "Prevalence and outcomes of anemia in rheumatoid arthritis: a systematic review of the literature." Am J Med **116 Suppl 7A**: 50S-57S.

Wollheim, F. A. (2000). "Markers of disease in rheumatoid arthritis." Curr Opin Rheumatol **12**(3): 200-4.

Yarasheski, K. E. (2003). "Exercise, aging, and muscle protein metabolism." J Gerontol A Biol Sci Med Sci **58**(10): M918-22.

Yokoyama, M., Y. Noto, et al. (2000). "Hypothermia with acute renal failure in a patient suffering from diabetic nephropathy and malnutrition." Diabetes Metab **26**(2): 145-7.

Yoshikawa, M., T. Yoneda, et al. (2001). "Distribution of muscle mass and maximal exercise performance in patients with COPD." Chest **119**(1): 93-8.

Young, A. (1993). "Current issues in arthrogenous inhibition." Ann Rheum Dis **52**(11): 829-34.

Young, A., M. Stokes, et al. (1987). "Effects of joint pathology on muscle." Clin Orthop(219): 21-7.

Zamir, O., P. O. Hasselgren, et al. (1992). "Evidence that tumor necrosis factor participates in the regulation of muscle proteolysis during sepsis." Arch Surg **127**(2): 170-4.

Zhang, Y., G. Pilon, et al. (2000). "Cytokines and endotoxin induce cytokine receptors in skeletal muscle." Am J Physiol Endocrinol Metab **279**(1): E196-205.

Zhong, Z., M. D. Wheeler, et al. (2003). "L-Glycine: a novel antiinflammatory, immunomodulatory, and cytoprotective agent." Curr Opin Clin Nutr Metab Care **6**(2): 229-40.

Zhou, W., Z. W. Jiang, et al. (2003). "Role of NF-kappaB and cytokine in experimental cancer cachexia." World J Gastroenterol **9**(7): 1567-70.

Zierath, J. R., J. W. Ryder, et al. (1998). "Role of skeletal muscle in thiazolidinedione insulin sensitizer (PPARgamma agonist) action." Endocrinology **139**(12): 5034-41.

Zinna, E. M. and K. E. Yarasheski (2003). "Exercise treatment to counteract protein wasting of chronic diseases." Curr Opin Clin Nutr Metab Care **6**(1): 87-93.

Zoico, E. and R. Roubenoff (2002). "The role of cytokines in regulating protein metabolism and muscle function." Nutr Rev **60**(2): 39-51.

APPENDIX

Effects of 12 Weeks of Progressive Resistance Training in Rheumatoid Arthritis Patients (Training Group, n = 10)

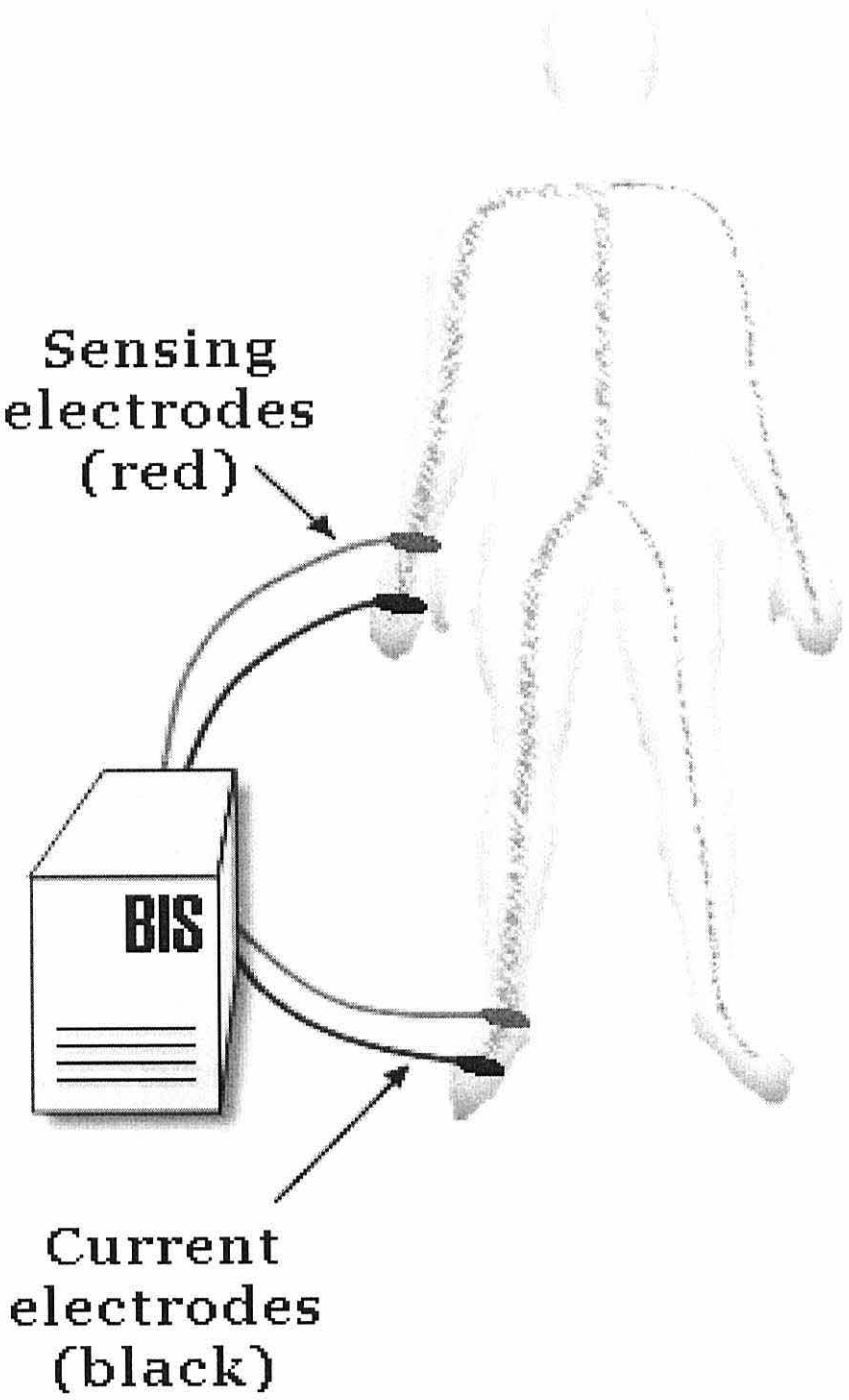
Variable	Pretest	Posttest	*P*	η^2
Body mass, kg	78.2 ± 14.6	78.6 ± 13.0	0.61	0.03
Arms lean mass, kg	4.26 ± 1.51	4.52 ± 1.59	< 0.01	0.74
Legs lean mass, kg	13.3 ± 3.2	14.1 ± 3.1	< 0.01	0.89
Total lean mass, kg	44.5 ± 9.4	45.4 ± 8.9	0.04	0.39
Total BMC, kg	2.58 ± 0.40	2.59 ± 0.42	0.14	0.23
FFM, kg	47.1 ± 9.7	47.9 ± 9.2	0.03	0.41
Total fat mass, kg	30.1 ± 9.9	29.5 ± 9.6	0.25	0.14
Trunk fat mass, kg	14.7 ± 6.5	14.0 ± 5.9	0.03	0.44
Percent body fat, %	38.6 ± 9.9	37.7 ± 10.1	0.61	0.03
ECW, L	15.5 ± 2.2	15.5 ± 1.9	0.77	0.01
ICW, L	18.6 ± 3.3	18.8 ± 3.5	0.57	0.04
TBW, L	34.1 ± 5.1	34.3 ± 5.2	0.44	0.07
TBW:FFM	0.73 ± 0.07	0.72 ± 0.06	0.14	0.23
Total body protein, kg	9.79 ± 5.29	10.42 ± 4.53	0.20	0.17
Hand-grip strength, N	187 ± 108	224 ± 115	0.14	0.23
Elbow flexors strength, N	171 ± 43	222 ± 63	0.01	0.52
Knee extensors strength, N	297 ± 85	347 ± 46	0.02	0.48
SST-30, repetitions	11.3 ± 2.8	15.7 ± 3.3	< 0.01	0.80
Modified HAQ, 1-4	1.3 ± 0.3	1.3 ± 0.2	0.41	0.08
Advanced ADLs, 1-4	2.1 ± 0.4	1.8 ± 0.3	< 0.01	0.58
Psychological status, 1-4	1.4 ± 0.3	1.4 ± 0.3	0.59	0.03
RADAI, 0-10	2.5 ± 1.1	2.0 ± 1.4	0.14	0.23
Fatigue, 0-10	4.4 ± 1.8	3.1 ± 2.1	0.56	0.04
ESR, mm/hour	18.8 ± 16.6	16.7 ± 8.9	0.56	0.04

Note. Pretest and posttest scores are presented as means ± *SD*. Differences between pretest and posttest were tested by one-way ANOVA for repeated measures. BMC = bone mineral content; FFM = fat-free mass; ECW = extracellular water; ICW = intracellular water; TBW = total body water; SST-30 = 30-s maximal sit-to-stand test; HAQ = health assessment questionnaire; ADLs = activities of daily living; RADAI = rheumatoid arthritis disease activity index; ESR = erythrocyte sedimentation rate.

Effects of 12 Weeks of Habitual Physical Activity in Rheumatoid Arthritis Patients (Control Group, n = 10)

Variable	Pretest	Posttest	*P*	η^2
Body mass, kg	79.0 ± 11.3	78.8 ± 11.9	0.77	0.01
Arms lean mass, kg	4.24 ± 1.77	4.22 ± 1.72	0.75	0.01
Legs lean mass, kg	12.9 ± 3.4	12.9 ± 3.3	0.96	0.00
Total lean mass, kg	43.8 ± 10.2	43.5 ± 10.1	0.10	0.27
Total BMC, kg	2.47 ± 0.52	2.48 ± 0.53	0.81	0.01
FFM, kg	46.3 ± 10.7	45.9 ± 10.5	0.10	0.27
Total fat mass, kg	31.6 ± 6.1	31.7 ± 6.4	0.91	0.00
Trunk fat mass, kg	16.9 ± 4.4	16.8 ± 4.7	0.77	0.01
Percent body fat, %	40.9 ± 7.8	41.1 ± 7.6	0.61	0.03
ECW, L	15.7 ± 2.9	15.7 ± 2.8	0.99	0.00
ICW, L	19.3 ± 4.5	19.0 ± 4.8	0.45	0.07
TBW, L	35.0 ± 7.2	34.7 ± 7.5	0.43	0.07
TBW:FFM	0.76 ± 0.05	0.76 ± 0.03	0.77	0.01
Total body protein, kg	8.27 ± 3.61	8.18 ± 3.12	0.77	0.01
Hand-grip strength, N	223 ± 133	204 ± 135	0.03	0.40
Elbow flexors strength, N	196 ± 87	194 ± 97	0.83	0.01
Knee extensors strength, N	312 ± 154	319 ± 147	0.65	0.02
SST-30, repetitions	12.2 ± 3.2	12.8 ± 2.7	0.22	0.16
Modified HAQ, 1-4	1.5 ± 0.6	1.3 ± 0.4	0.07	0.33
Advanced ADLs, 1-4	2.4 ± 0.6	2.3 ± 0.6	0.11	0.26
Psychological status, 1-4	1.6 ± 0.6	1.5 ± 0.3	0.49	0.06
RADAI, 0-10	2.8 ± 1.7	2.3 ± 1.5	0.10	0.27
Fatigue, 0-10	4.9 ± 3.2	4.4 ± 3.3	0.61	0.03
ESR, mm/hour	22.5 ± 17.6	20.9 ± 18.1	0.49	0.05

Note. Pretest and posttest scores are presented as means ± *SD*. Differences between pretest and posttest were tested by one-way ANOVA for repeated measures. BMC = bone mineral content; FFM = fat-free mass; ECW = extracellular water; ICW = intracellular water; TBW = total body water; SST-30 = 30-s maximal sit-to-stand test; HAQ = health assessment questionnaire; ADLs = activities of daily living; RADAI = rheumatoid arthritis disease activity index; ESR = erythrocyte sedimentation rate.



Standard wrist-to-ankle protocol for bioelectrical impedance spectroscopy

NORTH WALES HEALTH AUTHORITY
RESEARCH ETHICS COMMITTEE (WEST)

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Certificate of Confirmation of Ethics Approval

Name of Lead Researcher : Dr A Lemmey/Prof P Maddison

Title of Study : Effects of a nutritional mixture of ß-Hydroxy ß-Methylbutyrate (HMB), Glutamine and Arginine in Rheumatoid Arthritis patients

I confirm that all requirements have now been received for the study mentioned above. The research therefore has this Committee's full ethics approval.
(Approval from host institutions must be sought separately).

If, during the course of the study, there are protocol changes, serious adverse events, or major subject recruitment problems, you are required to notify the Committee as soon as possible .

It is also requested that you provide an annual interim report on the conduct and progress of the study, plus a final report within three months of completion .

The Committee wishes you every success with your research.

Signed : .....

Mr B Napier, Chairman .

Date : 16.5.00 .

cc Prof. Maddison.

ioneering research: Intensive exercise has both physical and emotional benefits

Given a new lease of life

the latest article on the ork of the North West ales NHS Trust we look at innovative research oject that offers hope to ose suffering from eumatoid arthritis.

RESEARCH project to investigate the ect of intensive exercise - Progressive sistance Training - on patients suffering m rheumatoid arthritis is to be nched by the North West Wales NHS ist in the autumn.

This follows a successful pilot project ich was undertaken with a group of ients in conjunction with the School of ort Health and Exercise Sciences at iversity of Wales, Bangor earlier this ır.

A trainee specialist, funded by the Ross search Fellowship, will lead the research oject for a total of 18 months initially.

Professor Peter Maddison, consultant umatologist at Ysbyty Gwynedd, said: /e are very much at the forefront in the earch into this. Progressive resistance ining has been found to be a very effec- e way of improving muscle function in rmal people as well as those who elderly and frail.

'Since one of the very early fea- es of rheumatoid arthritis is loss of uscle mass, the pilot project aimed find out whether intensive exercise ch as this could benefit people with s condition.

'In the past, rheumatologists have en concerned that too much exer- e might aggravate the condition t the difference in the 10 patients o took part in this three month ot project has been dramatic.

"Not only have they benefited ysically but it has also boosted their nfidence and morale."

The patients attended three 2 hour ssions per week for 12 weeks at the iversity gym.

They took part in repetitive exer- es using special machinery and ercised a number of muscle groups.

"They all reached the end of the programme and their sense of well being increased.

"There has also been an increase in their muscle bulk," said Professor Maddison.

"They have all continued to work out in the gym," he added.

One of the very early features of rheumatoid arthritis is loss of muscle. This happens quickly and is dramatic but it is difficult to detect because the body puts on fat to replace the muscle.

Two major factors contribute to muscle loss.

Firstly, when the muscle is inflamed, substances are produced which affect the muscle and contribute to the breakdown of the muscle

Secondly, normal mechanisms to maintain the muscle are reduced.

Ymddiriedolaeth GIG Gogledd Orllewin Cymru
North West Wales NHS Trust

DEALING WITH THE EFFECTS

THE effects of rheumatoid arthritis lead to a chain of reactions which is similar to the process which happens with a range of other conditions involving chronic inflammation.

These include Crohn's disease, chronic renal failure, chronic asthma and some cancers.

Findings from the impending research project at the Trust could therefore be useful in the treatment of these conditions as well as rheumatoid arthritis in the long term.

"The value of this research post will be to provide vital clinical support to develop this area and to extend the knowledge gained in other specialities," said Professor Maddison.

"It will involve a controlled study of Progressive Resistance Training in rheumatoid arthritis and to examine the extent of muscle loss in similar conditions.

"The post holder will be responsible for planning, developing and carrying out research but all members of the rheumatology team within the Trust, including doctors, a specialist nurse, physiotherapist and occupational therapist, will also be involved in the research process."

Creating a positive outlook

ONE who took part in the pilot project organised by Professor Maddison is Marian Ayrton who has suffered from rheumatoid arthritis for the past 20 years.

"I was involved in a car crash and suffered a whiplash injury at the time and I think that may have triggered the arthritis," she said. "I lost total movement in both my arms overnight and couldn't move. I have been on and off treatment, both conventional and alternative, ever since."

Mrs Ayrton, who lives in Benllech, is monitored regularly at Ysbyty Gwynedd and attends clinics run by Professor Maddison.

She decided to take up the offer to take part in the pilot scheme at the beginning of January and has not looked back since. "It's been a remarkable three months," she said. "I thought at the outset that I would never be able to do it. It was a struggle at first but I carried on and I'm now feeling so much better. Not only are my joints better - I can now do things like opening jars, picking up things, bending, kneeling - but I feel so much better in myself.

"Having rheumatoid arthritis is very limiting and since I had also suffered from cancer, I was quite low and unfit when I started on the intensive exercise. Now, I feel much more positive about life and have a lot more energy. It has given me a new lease of life."

On screen: Dr Andrew Lemmey and Sam Marcora study images of bone density provided by the whole body DEXA scanner.

Working as a team

DURING the past four and a half years, Dr Andrew Lemmey, a lecturer in Exercise Physiology at the School of Sport, Health and Exercise Sciences at Bangor University, has worked closely with Professor Peter Maddison, researching muscle wasting in relation to rheumatoid arthritis.

His main area of research in his native Australia - he gained his PhD in Adelaide - was growth hormone/insulin-like growth factor (IGF).

His research is currently focused on muscle wastage specifically in the field of rheumatoid arthritis and chronic renal failure and the effect of exercise on disease activity.

Sam Marcora, who hails originally from Milan, has been studying for a PhD in the department at the University for the past three years and has just been appointed to the post of lecturer in the department. A graduate in Physical Education with a Masters degree in Science Physiology, he supervised the training programme for the patients who took part in the pilot project.